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ÜBER DIE VERTEILUNG DES WUCHSSTOFFES IN KEIMSTENGELN UND WURZELN WÄHREND DER PHOTOTROPISCHEN UND GEOTROPISCHEN KRÜMMUNG

VON

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1. Einleitung.

Die Wuchsstofftheorie nimmt an, dass die phototropische und geotropische Krümmung der orthotropen Organe durch eine ungleiche Verteilung des zugeführten oder des in den betreffenden Organen vorhandenen oder neugebildeten Wuchsstoffes zu stande kommt. Die Grundlage dieser Theorie ist die Tatsache, dass der phototropische und geotropische Reiz in der Avenakoleoptile sich über einen Einschnitt fortpflanzen kann, woraus man schliessen musste, dass die Reizleitung mit einem Stofftransport verknüpft ist. Aus Einschnittversuchen wurde gefolgert, dass die Herabwanderung des Stoffes während der Krümmung vorzugsweise auf der Hinter- bzw. Unterseite stattfindet, und dass der betreffende Stoff eine Wachstumsbeschleunigung hervorruft. Später hat man versucht, die von der Vorder- und Hinterseite, bzw. Ober- und Unterseite der phototropisch oder geotropisch gereizten Organe abgegebene Wuchsstoffmenge separat zu bestimmen. Für die phototropische Reizung wurden derartige Versuche ausgeführt von Went (1928, Koleoptilspitze von Avena) und van Overbeek (1933, Hypokotyle von Raphanus), für die geotropische Reizung von Dolk (1929, Koleoptilspitze von Avena und Mais), Schmitz (1933, Gramineenknoten), Boysen Jensen (1933, Wurzelspitze von Vicia faba) Dijkmann (1934, Hypokotyle von Lupinus) und van der Laan (1934, Epikotyle von Vicia faba). Bei diesen Versuchen wurde der Wuchsstoff der bei-

den Seiten nach dem Verfahren von Went mit Agar oder Dextroseagar getrennt abgefangen. Eine Zusammenstellung der gewonnenen Ergebnisse findet sich in Tab. 1. Die Zahlen bedeuten die von den beiden Seiten abgegebene Wuchsstoffmenge in Prozenten der gesamten Wuchsstoffmenge.

Tab. 1.

	Phototropische Krümmung			Geotropische Krümmung				
	Koleoptilspitze von Avena (Went)	Hypokotyl von Raphanus (van Overbeek)		Koleoptilspitze (Dolk) Avena	Mais	Wurzelspitze von Vicia faba (Boysen Jensen)	Hypokotyle von Lupinus (Dijkman)	Epikotyle von Vicia faba (van der Laan)
Vorderseite .	32	37	Oberseite .	38	37.4	37 37.4	32.5	39—37
Hinterseite . .	68	63	Unterseite .	62	62.6	63 62.6	67.5	61—63

Es ergibt sich, dass während der phototropischen und geotropischen Reizung tatsächlich mehr Wuchsstoff von der Hinter- bzw. Unterseite als von der Vorder-, bzw. Oberseite abgegeben wird. Die Übereinstimmung zwischen den verschiedenen Versuchsreihen ist sehr befriedigend.

Der Nachweis einer ungleichen Wuchsstoffverteilung während der tropistischen Krümmungen ist von fundamentaler Bedeutung für die Wuchsstofftheorie. Weil nun die obigen Versuche alle mit abgeschnittenen Pflanzenorganen ausgeführt sind, weil ferner die Abfangungsmetode sich nicht auf alle Pflanzen anwenden lässt, habe ich untersucht, ob es möglich ist auch in intakten Pflanzenorganen eine ungleiche Wuchsstoffverteilung während der phototropischen und geotropischen Krümmung durch Extraktion und quantitative Bestimmung des Wuchsstoffes nachzuweisen. Ich hätte gedacht, dass es ziemlich leicht gewesen wäre, diese

Frage zu lösen. Das war indessen nicht der Fall. Es musste erst die Metode zur Wuchsstoffbestimmung in Pflanzen wesentlich verfeinert werden.

Bei der Ausführung der Versuche hat mir Frl. BETTY DYHRE-POULSEN treue Hilfe geleistet, wofür ich ihr auch an dieser Stelle bestens danken möchte.

2. Methodik.

Wie es in meiner Monographie »Die Wuchsstofftheorie« S. 20 erwähnt ist, habe ich schon seit mehreren Jahren versucht, den Wuchsstoff in Pflanzenteilen zu extrahieren und quantitativ zu bestimmen. Als Extraktionsmittel verwendete ich ursprünglich Alkohol; die alkoholische Lösung wurde eingedampft, der Rückstand in Wasser gelöst, mit $NaHCO_3$ zur alkalischen Reaktion versetzt und mit Äther ausgeschüttelt. Nachher wurde die wässerige Fraktion mit Essigsäure angesäuert und wieder mit der dreifachen Äthermenge ausgeschüttelt. Der letzte Ätherextrakt wurde eingedampft, der Rückstand in 0.3—1 ccm Wasser gelöst und mit der gleichen Menge 3 % igem Agar vermischt. Aus dieser Mischung wurden Würfelchen (Grösse 2×2×1 mm) herausgeschnitten und einseitig auf dekapitierte Avenakoleoptilen gesetzt.

Der Wuchsstoff lässt sich auch direkt mit Äther aus Pflanzenorganen extrahieren. Die nicht zerkleinerten Pflanzenteile können in einem Soxhletapparat mit 150 ccm Äther + 3 ccm 1 % iger Essigsäure (in ätherischer Lösung) in 3—5 Stunden extrahiert werden. Der Wuchsstoff geht dann in den Äther über und kann nach Abdampfen des Äthers bestimmt werden.

Bequemer ist es doch, wie THIMANN (1934) vorgeschlagen hat, Chloroform und Salzsäure zur Extraktion des Wuchs-

stoffes zu verwenden. Zwar könnte die Anwendung der Salzsäure, die eine Oxydation des Wuchsstoffes verhindern soll, Bedenken erwecken, weil einige Wuchsstoffe säureempfindlich sind. Ich habe aber bisher eine schädliche Wirkung der Säure nicht beobachtet. Das Pflanzenmaterial wird mit 60 g Chloroform + 1 ccm 2 n HCl in einem Mörter fein zerrieben, in einen Kolben übergeführt und die Nacht über hingestellt.

Es soll nun der Wuchsstoff aus der Chloroformlösung in eine genau feststellbare und möglichst kleine (um Pflanzenmaterial zu sparen) Agarmenge übergeführt werden. Ich gehe dabei in folgender Weise vor. 10 ccm von einem 1.5 $^0/_0$ igem, schwach saurem Agar werden auf eine heisse Glassplatte (10×10 cm) ausgegossen; die Agarplatte wird dann 1 mm dick. Nach dem Erkalten des Agars werden kleine, zirkelrunde Plättchen mit einer Grösse von 1 qcm herausgestanzt; diese werden auf einem eingefetteten Objektträger in dampfgesättigter Luft aufgehoben.

Ein aliquoter Teil der filtrierten Chloroformlösung wird abgewogen und in einem Becherglas eingedampft, der Rückstand wird mit 0.5 ccm peroxydfreiem Äther aufgenommen. Durch Zurückdrehung des Spindels der Spritze (Abb. 1, a) wird die wuchsstoffhaltige Ätherlösung in die Pipette (b) hineingesaugt. Ein Objektträger mit einem Agarplättchen (c_1) wird auf einem Wasserbad (Tp. 35—40°) gerade unter der Pipettenspitze angebracht. Durch Drehung des Spindels wird die Ätherlösung tropfenweise auf das Agarplättchen ausgepresst, indem gleichzeitig getrocknete Luft über das Plättchen durch d geblasen wird. Der Äther verdampft dann auf dem Plättchen. Nachher wird das Becherglas mit 0.3 ccm Äther gespült, und dieser in derselben Weise zum Verdampfen gebracht; die gesammte Wuchsstoffmenge, die

in der abgewogenen Chloroformlösung vorhanden war, ist nun in das Agarplättchen übergeführt worden; dieses wird mit einem kleinen Glase (c_2) bedeckt und gewogen. Der Objekt-

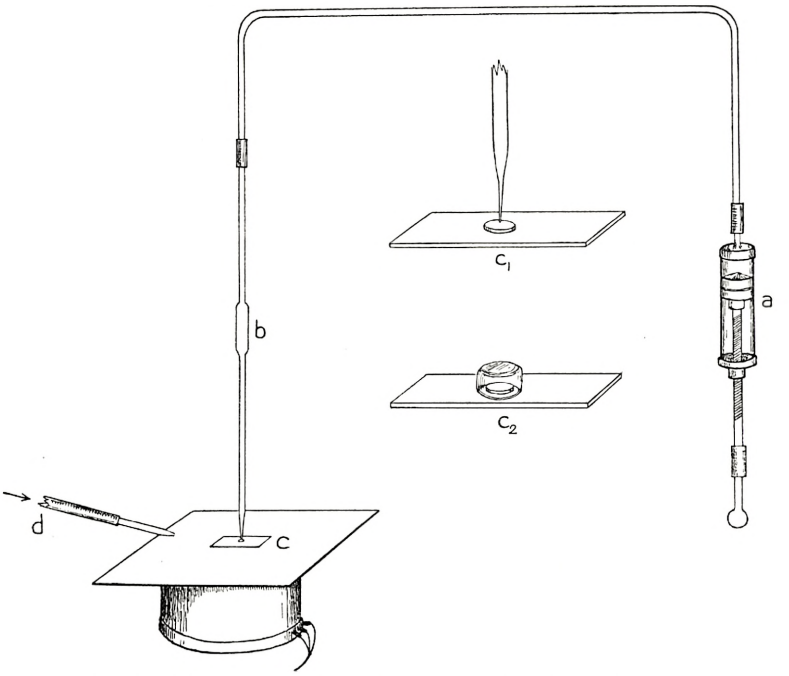

Abb. 1. Vorrichtung zur Überführung des Wuchsstoffes in eine genau feststellbare Agarmenge.

träger mit dem Plättchen wird die Nacht über in dampfgesättigter Luft hingestellt, damit der Wuchsstoff sich gleichmässig im Agar verteilen kann. Aus dem Agarplättchen werden dann mit planparallelen Messern Agarwürfelchen mit einer Grösse von $2 \times 2 \times 1$ mm herausgeschnitten, und diese einseitig auf dekapitierte Avenakoleoptilen gesetzt. Mit Hilfe der in Abb. 2 dargestellten Schablone[1] bestimmt man den Krümmungsradius und die Länge des gekrümmten

[1] Solche Schablonen können vom pflanzenphysiologischen Laboratorium der Universität Kopenhagen erhalten werden.

Teiles der Koleoptile, und berechnet dann die Wachstumsdifferenz »d« zwischen der konvexen und konkaven Seite (vgl. Boysen Jensen 1935 s. 21)[1]. Hieraus berechnet sich die Wuchsstoffmenge im Agarplättchen in WAE-Einheiten, indem man den d-Wert mit dem Gewicht des Agarplättchen multipliziert und durch 100 dividiert (1 WAE ist die Wuchsstoffmenge, die in 50 ccm Wasser + 50 ccm Agar gelöst, einen d-Wert von 1 hervorbringen kann). Weil man von der Extraktionsflüssigkeit nur einen aliquoten Teil benutzt, muss die gefundene Wuchsstoffmenge auf die gesamte Chloroformmenge umgerechnet werden. Wenn ferner das extrahierte Pflanzenmaterial getrocknet und gewogen wird, kann der Wuchsstoffgehalt in WAE-Einheiten pro 100 g Trockensubstanz berechnet werden[2].

Beispiel.

An 6 Vicia faba Pflanzen, im Grünhaus kultiviert, wurden die oberen 6 cm abgeschnitten und mit 60 g Chloroform + 1 ccm 2n HCl zerquetzt. Trockengewicht der Pflanzenteile 0.3341 g. Am nächsten Tage wurden 22 g Chloroform eingedampft, und der Rückstand in ein Agarplättchen mit einem Gewicht von 0.1010 g übergeführt; bei der Untersuchung des Agar-

[1] Die Reaktion ist sehr fein. Es genügt eine Wuchsstoffmenge von etwa 0.000004 mg im Agarplättchen um einen d-Wert von 1 hervorzurufen.

[2] Es kann zweifelhaft sein, ob man bei Untersuchungen über Wuchsstoffverteilung die Wuchsstoffmenge auf Trockengewicht oder Wassergehalt der Pflanzenorgane beziehen soll. Untersuchungen über den Trockensubstanzgehalt der beiden Seiten von phototropisch und geotropisch gekrümmten Phaseolusstengeln haben doch gezeigt, dass dieser in beiden Fällen ungefähr derselbe ist. Es wurde gefunden

Phaseolus, Trockensubstanz in Prozenten des Frischgewichtes

Phototropische Krümmung	6 Versuchsserien	Vorderseite 7.13	Hinterseite 7.17
Geotropische	— 11 —	Oberseite 7.24	Unterseite 6.95

Es geht hieraus hervor, dass es für vergleichende Versuche ziemlich gleichgültig ist, ob man den Wuchsstoffgehalt auf Trockensubstanz oder Wassergehalt berechnet.

plättchens auf Wuchsstoff, ergab sich ein d-Wert von 1,36; die Berechnungen gestalten sich dann folgendermassen.

Trockensubstanz des extrahierten Pflanzenmaterials	d (gefunden)	WAE $\left(\frac{d \cdot 0.1010}{100}\right)$	WAE pro 60 g	WAE pro 100 g Trocken-substanz
0.3441	1.36	0.00137	0.00375	1.09

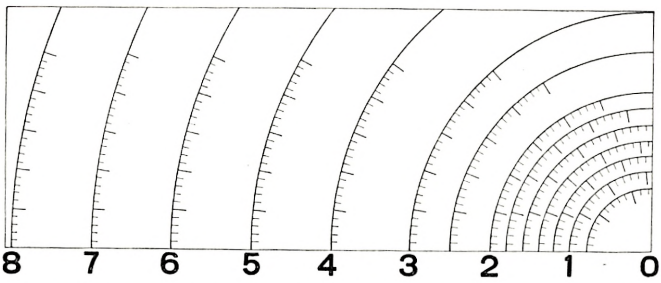

Abb. 2. Schablone zur Messung des Krümmungsradius und der Krümmungslänge.

Über die Genauigkeit der Bestimmung geben die folgende Versuche Aufschluss.

30 Vicia faba Stengel, 6 cm lang, wurden mit 80 ccm Chloroform + 2 ccm 2n HCl extrahiert; der Wuchsstoff in 8 ccm dieses Extraktes wurde in der oben beschriebenen Weise in ein Agarplättchen übergeführt. Es wurden 4 Versuche ausgeführt mit je zwei parallelen Bestimmungen; die erhaltenen d-Werte waren die folgenden

Tab. 2.

Vers.	1	2	3	4
d	1.08	1.17	1.12	0.97
	1.12	1.25	1.10	0.93

Übrigens lässt sich die Metode noch etwas weiter verfeinern, indem der Wuchsstoffgehalt eines Pflanzenteils in eine noch kleinere Agarmenge konzentriert werden kann.

Aus einem Agarplättchen werden 9 Würfelchen von der gewöhnlichen Grösse ($2 \times 2 \times 1$ mm) ausgeschnitten und eng einander auf einen Objektträger gelegt. Auf dieses Agarplättchen wird dann die ätherische Wuchsstofflösung getröpfelt. Das Gesamtgewicht dieser Würfelchen beträgt nur etwa 0.036 g. In diesem Falle soll man sich doch hüten, dass die Lösung sich nicht über den Kanten des Agarplättchens ausbreitet. Nachdem der Wuchsstoff sich gleichmässig in den 9 Würfelchen verteilt hat, werden diese alle auf Avenakoleoptilen gesetzt. Bei dieser Anordnung dürfte die Grenze der Wuchsstoffbestimmung erreicht sein.

Diese Methode genügt, die ungleiche Wuchsstoffverteilung in Pflanzenorganen, die sich schnell krümmen, nachzuweisen. Eine langsame Krümmung aber kann durch so kleine Unterschiede in der Wuchsstoffkonzentration der beiden Seiten hervorgerufen werden, dass diese sich kaum nachweisen lassen.

3. Ergebnisse.

a. Phaseolus multiflorus, geotropische Krümmung des epikotylen Stengels.

Lichtpflanzen. Die Pflanzen wurden im Grünhaus in Erde kultiviert; 24 Stunden vor dem Versuche wurden sie ins Dunkelzimmer gebracht, um eine möglicherweise vorhandene ungleiche Wuchsstoffverteilung auszugleichen. Die Länge der Versuchspflanzen betrug etwa 4—10 cm.

Durch Dekapitierungsversuche habe ich versucht zu entscheiden, an welcher Stelle der Wuchsstoff gebildet wird. Werden die zwei ersten Laubblätter, die Keimknospe und der obere Teil des epikotylen Stengels in einer Länge von 2—5 mm abgeschnitten, sinkt die Wachstumsgeschwindigkeit in wenigen Stunden fast bis auf Null, steigt dann wieder,

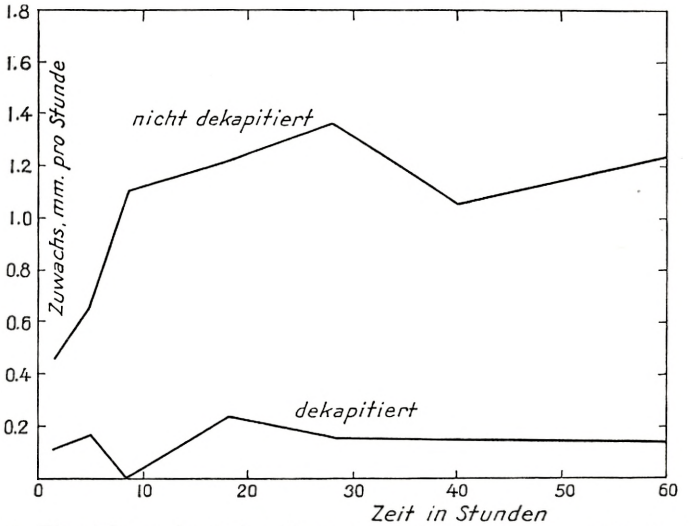

Abb. 3. Einwirkung der Dekapitierung auf die Wachstumsgeschwindigkeit bei Lichtpflanzen von Phaseolus, 40 mm. Die eine Hälfte der Versuchspflanzen wurde 1—2 mm unter der Insertionsstelle der beiden ersten Laubblätter dekapitiert, die andere Hälfte diente als Kontrolle.

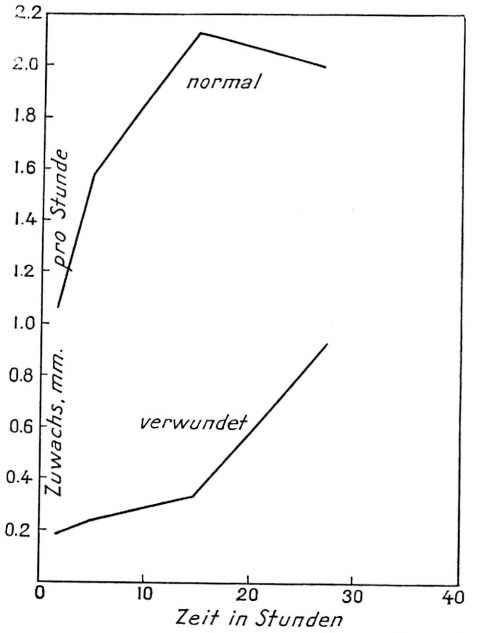

Abb. 4. Einwirkung eines Längsschnittes auf die Wachstumsgeschwindigkeit des epikotylen Stengels von Phaseolus, Lichtpflanzen, 40 mm lang. Die eine Hälfte der Versuchspflanzen wurde mit einem 2 mm langen medianen Längsschnitt versehen, die andere Hälfte diente als Kontrolle.

erreicht aber nur etwa den 10ten Teil des Wachstums einer nicht dekapitierten Pflanze (vgl. Abb. 3). Bei einzelnen Pflanzen kann doch der Anstieg der Wachstumsgeschwindigkeit bedeutend grösser sein. Das Ergebnis dieses Versuches ist doch nicht ganz leicht zu deuten. Es zeigt sich nämlich, dass ein medianer Längsschnitt von 2 cm, der den Wuchsstofftransport nicht beeinflussen kann, eine starke, aber vorübergehende Hemmung auf das Längenwachstum ausübt (vgl. Abb. 4). Diese Hemmung ist durch den Wundreiz verursacht. Man darf daher annehmen, dass die ursprüngliche Hemmung des Längenwachstums bei den dekapitierten Pflanzen durch den Wundreiz bedingt ist, die spätere Hemmung aber muss in anderer Weise erklärt werden.

Eine Untersuchung des Wuchsstoffgehaltes in dekapitierten Phaseolusstengeln zeigt, dass dieser schnell sinkt.

Tab. 3.

Wuchsstoffgehalt in dekapitierten Phaseoluspflanzen.

	Sofort	nach 5 Stunden	nach 24 Stunden	nach 48 Stunden
WAE pro 100 g Trockensubstanz.....	1.76	1.72	0	0

(Tab. 3). Nach 24 Stunden war überhaupt kein Wuchsstoff mehr nachweisbar. Es scheint daher, dass der Wuchsstoff in dem ersten Laubblattpaare oder in dem oberen Teil des epikotylen Stengels gebildet wird, und dass die spätere Wachstumshemmung in den dekapitierten Phaseolusstengeln durch Wuchsstoffmangel bedingt ist. Hierfür spricht auch, dass bei Zufuhr von Wuchsstoff die Wachstumsgeschwindigkeit bedeutend vergrössert wird (vgl. Tab. 4).

Gleichzeitig mit der Wachstumsabnahme in den dekapitierten Stengeln sinkt auch die geotropische Reaktionsfähig-

keit; auch diese kann durch Zufuhr von Wuchsstoff zu den dekapitierten Pflanzen vergrössert werden (vgl. Tab. 5).

Tab. 4.

Einwirkung des Wuchsstoffes auf die Wachstumsgeschwindigkeit der dekapitierten Phaseoluspflanzen.

Die Pflanzen wurden 5 mm unter der Insertionsstelle der Laubblätter dekapitiert und die Nacht über in trockener Luft belassen. Am nächsten Tage wurde eine frische Schnittfläche hergestellt und diese entweder mit Wasseragar oder Wuchsstoffagar (8 WAE in 100 ccm) bedeckt; die Pflanzen wurden in dampfgesättigter Luft hingestellt. Dauer des Versuches 24 Stunden.

		Wasseragar	Wuchsstoffagar
Zuwachs	7 Pflanzen	0.04	0.57
mm pro Stunde	6 Pflanzen	0.25*)	0.58

*) zwei von sechs Pflanzen wuchsen trotz der Dekapitierung weiter.

Tab. 5.

Einwirkung des Wuchsstoffes auf die geotropische Reaktionsfähigkeit der dekapitierten Phaseoluspflanzen.

Die Pflanzen wurden 5 mm unter der Insertionsstelle der Laubblätter dekapitiert und die Nacht über in trockener Luft belassen. Am nächsten Tage wurde eine frische Snittfläche hergestellt und diese mit Wasseragar oder Wuchsstoffagar (8 WAE in 100 ccm) bedeckt. Die Pflanzen wurden dann einige Stunden in waagerechter Lage in dampfgesättigter Luft belassen, und der d-Wert der geotropischen Krümmungen bestimmt. Die Zahlen bedeuten die d-Werte.

Anzahl Versuchspflanzen	Wasseragar	Wuchsstoffagar
je 8	0.83	2.2
»	0.97	2.17

Um die Wuchsstoffverteilung während der geotropischen Krümmung der Phaseolusstengeln zu untersuchen, ging ich in folgender Weise vor. Die Schale mit den Keimpflanzen wurde so gestellt, dass die Keimstengeln horizontal lagen. Die Reaktion fand im Dunkelzimmer statt und dauerte zwei Stunden. Die oberen 4 cm des Keimstengels wurden abge-

schnitten und durch zwei Längsschnitte in drei Teile zerlegt: einen oberen, mittleren und unteren Teil. Von diesen wurde die obersten und untersten Teile in der oben beschriebenen Weise mit Chloroform extrahiert und der Wuchsstoffgehalt bestimmt. Das Ergebnis findet sich in Tab. 6.

Tab. 6.

Wuchsstoffverteilung während der geotropischen Krümmung der Epikotylen von Phaseolus multiflorus, Lichtpflanzen.

In jedem Versuch wurden 20—30 Pflanzen 2 Stunden in waagerechter Lage belassen. Tp. 21—22°. Die oberen 4 cm des epikotylen Stengels wurden durch zwei Längsschnitte in 3 Teile zerlegt; die obersten und untersten Teile wurden zur Wuchsstoffbestimmung verwendet.

	Wuchsstoffgehalt (WAE pro 100 g Trockensubstanz)		Wuchsstoffgehalt in Proz. der gesamten Wuchsstoffmenge	
	Oberseite	Unterseite	Oberseite	Unterseite
$^{11}/_{9}$ 35	2.24	2.52	47	53
$^{12}/_{9}$ -	4.05	3.90	51	49
$^{13}/_{9}$ -	5.71	6.45	47	53
$^{16}/_{9}$ -	3.71	11.20	25	75
$^{19}/_{9}$ -	1.49	6.40	19	81
$^{20}/_{9}$ -	4.25	5.61	43	57
$^{21}/_{9}$ -	2.58	3.16	45	55
$^{24}/_{9}$ -	1.48	1.62	48	52
$^{25}/_{9}$ -	0.66	2.11	24	76
$^{26}/_{9}$ -	1.67	2.89	37	63
$^{27}/_{9}$ -	0.56	1.38	29	71
$^{28}/_{9}$ -	1.24	2.76	31	69
$^{1}/_{10}$ -	1.39	0.92	60	40
$^{2}/_{10}$ -	1.53	1.53	50	50
$^{3}/_{10}$ -	0.54	1.20	31	69
$^{7}/_{10}$ -	0.76	1.24	38	62
Durchschnitt	2.77		39	61

Es geht aus der Tabelle hervor, dass der Wuchsstoffgehalt der Versuchspflanzen sehr schwankend ist; er variiert von 1—7.5 WAE pro 100 g Trockensubstanz.

Im ganzen wurden 16 Versuchsreihen angestellt; in 13 Fällen war der Wuchsstoffgehalt am grössten an der Unterseite, in 2 Fällen enthielten Ober- und Unterseite ungefähr die gleiche Wuchsstoffmenge, und im einen Fall war der Wuchsstoffgehalt am grössten an der Oberseite. Im Durchschnitt enthielt die Oberseite 39 und die Unterseite 61 % der gesammten Wuchstoffmenge.

Dunkelpflanzen. Die Dunkelpflanzen wurden im Dunkelzimmer bei konstanter Temperatur (21°) in Sägemehl kultiviert. Auch bei den etiolierten Pflanzen ruft Dekapitierung Wachstumshemmung hervor; diese scheint im allgemeinen schwächer zu sein als bei den Lichtpflanzen.

Tab. 7.

Wuchsstoffverteilung während der geotropischen Krümmung der Epikotylen von Phaseolus multiflorus, Dunkelpflanzen.

In jedem Versuch wurden 20—30 Pflanzen 2 Stunden in waagerechter Lage belassen. Tp. 21—22°. Die oberen 4 cm des epikotylen Stengels wurden durch zwei Längsschnitte in 3 Teile zerlegt; die obersten und untersten Teile wurden zur Wuchsstoffbestimmung verwendet.

	Wuchsstoffgehalt (WAE pro 100 g Trockensubstanz)		Wuchsstoffgehalt in Proz. der gesamten Wuchsstoffmenge	
	Oberseite	Unterseite	Oberseite	Unterseite
$^{8}/_{10}$ 35	0.56	0.67	46	54
$^{9}/_{10}$ -	0.75	1.49	34	66
$^{10}/_{10}$ -	0.85	0.76	53	47
$^{11}/_{10}$ -	0.79	1.10	43	57
$^{12}/_{10}$ -	1.00	1.12	47	53
$^{13}/_{10}$ -	0.78	0.85	48	52
$^{16}/_{10}$ -	0.76	1.24	38	62
$^{17}/_{10}$ -	1 14	1.30	47	53
$^{18}/_{10}$ -	1.62	1.74	48	52
$^{19}/_{10}$ -	1.91	2.35	45	55
$^{22}/_{10}$ -	2.74	3.56	43	57
$^{23}/_{10}$ -	3.47	3.56	50	50
$^{25}/_{10}$ -	3.9	5.3	43	57
$^{26}/_{10}$ -	1.98	4.1	33	67
Durchschnitt	1.84		44	56

Die Versuche über die Wuchsstoffverteilung während der geotropischen Krümmung wurden in derselben Weise ausgeführt wie mit den Lichtpflanzen. Das Ergebnis der Versuche findet sich in Tab. 7.

Obwohl auch hier die Zahlen etwas schwanken, ist die Übereinstimmung zwischen den einzelnen Versuchen doch weit besser als bei den Lichtpflanzen. Es wurden 14 Versuchsreihen angestellt; in einem Falle war die Wuchsstoffkonzentration am grössten auf der Oberseite, in einem Falle gleich und in den übrigen 12 Fällen am grössten auf der Unterseite. Im Durchschnitt enthielt die Oberseite 44, die Unterseite 56 % der gesammten Wuchsstoffmenge.

b. Phaseolus multiflorus, phototropische Krümmung des epikotylen Stengels.

Es wurde auch die Wuchsstoffverteilung während der phototropischen Krümmung bei Phaseolus untersucht. Die Pflanzen wurden in Präparatengläsern (40 × 100 mm) in Sägemehl kultiviert. Es kamen teils Lichtpflanzen, teils Dunkelpflanzen zu Verwendung. Wenn die Epikotyle eine Länge von 4—10 cm erreicht haben, wurden sie einseitig mit 60 Luxmeter $2^1/_2$ bis 4 Stunden einseitig beleuchtet. Es wurde dann die Wuchsstoffkonzentration in der Vorder- und Hinterseite bestimmt.

Das Ergebnis findet sich in Tab. 8.

Bei den 4 letzten Versuchsreihen (vom 12—20 Dez.) wurde eine Samensorte, die Keimpflanzen mit einem abnorm hohen Wuchsstoffgehalt erzeugten, benutzt; diese sind daher nicht mitgerechnet worden. In den übrigen 12 Versuchen enthielt die Hinterseite in allen Fällen mehr Wuchsstoff als die Vorderseite, und zwar war der durchschnittliche Gehalt der Vorderseite 32 % und der der Hinterseite 68 %.

Der Unterschied zwischen den beiden Seiten schwankt doch beträchtlich.

Tab. 8.

Wuchsstoffverteilung während der phototropischen Krümmung der Epikotylen von Phaseolus multiflorus.

Lichtpflanzen. In jedem Versuch wurden 6—8 Pflanzen mit 60 Luxmeter $2^1/_2$ Stunden einseitig beleuchtet. Die oberen 2 oder 4 cm des epikotylen Stengels wurden durch zwei Längsschnitte in 3 Teile zerlegt; die vordersten und hintersten Teile wurden zur Wuchsstoffbestimmung verwendet. Dunkelpflanzen. Diese wurden 4 Stunden einseitig beleuchtet. Nur die obersten 2 cm unterhalb des Knies wurden zur Wuchsstoffbestimmung verwendet.

	Wuchsstoffgehalt (WAE pro 100 g Trockensubstanz)		Wuchsstoffgehalt in Proz. der gesamten Wuchsstoffmenge	
	Vorderseite	Hinterseite	Vorderseite	Hinterseite
Lichtpflanzen.				
$^{21}/_{11}$ 35.....	0	5.8	0	100
$^{23}/_{11}$ -	9.8	11.6	46	54
$^{26}/_{11}$ -	0	1.11	0	100
$^{28}/_{11}$ -	1.66	2.75	38	62
Dunkelpflanzen.				
$^{7}/_{9}$ 34.....	1.44	1.8	44	56
$^{15}/_{9}$ -	1.5	2.4	38	62
$^{21}/_{9}$ -	2.4	4.3	36	64
$^{5}/_{10}$ -	2.4	2.6	48	52
$^{20}/_{10}$ -	0	2.8	0	100
$^{3}/_{12}$ 35.....	3.6	4.5	44	56
$^{4}/_{12}$ -	3.5	4.6	43	57
$^{6}/_{12}$ -	4.9	5.8	46	54
$^{12}/_{12}$ -	14.5	57.5	—	—
$^{16}/_{12}$ -	22.9	23.4	—	—
$^{17}/_{12}$ -	16.8	13.4	—	—
$^{20}/_{12}$ -	15.6	25	—	—
Durchschnitt			32	68

c. Vicia faba, Windsor white, geotropische Krümmung des epikotylen Stengels.

Die Kulturmethode war dieselbe wie bei den Lichtpflanzen von Phaseolus. Wegen der eckigen Form der Stengel ist die Zerlegung des geotropisch gekrümmten Teils bedeutend

schwieriger als bei Phaseolus. Es wurden daher vor dem Versuch die Pflanzen aus der Erde herausgenommen und auf einer Korkplatte so befestigt, dass eine der flachen Seiten nach unten zeigte. Die Wurzeln wurden mit feuchtem Sägemehl bedeckt. Nach 4 Stunden wurden die oberen 4 cm des Stengels, wie oben beschrieben, in 3 Teile zerlegt, und die obersten und untersten Teile extrahiert. Das Ergebnis der Versuche ist in Tab. 9 zusammengestellt.

Tab. 9.

Die Wuchsstoffverteilung während der geotropischen Krümmung der Epikotylen von Vicia faba, Windsor white, Lichtpflanzen.

In jedem Versuch wurden 30 Stengel 4 Stunden in waagerechter Lage belassen, Tp. 18°. Nachdem die Endknospe abgeschnitten war, wurden die obersten 2 oder 4 cm des Stengels durch zwei Längsschnitte in 3 Teile zerlegt; die obersten und untersten Teile wurden zur Wuchsstoffbestimmung verwendet.

	Wuchsstoffgehalt (WAE pro 100 g Trockensubstanz)		Wuchsstoffgehalt in Proz. der gesamten Wuchsstoffmenge	
	Oberseite	Unterseite	Oberseite	Unterseite
$^{30}/_{1}$ 35	0.29	0.32	47	53
$^{8}/_{3}$ -	0.24	0.33	42	58
$^{9}/_{3}$ -	0.14	0.21	40	60
$^{11}/_{3}$ -	0.18	0.20	47	53
$^{12}/_{4}$ -	0.75	0.80	48	52
$^{13}/_{4}$ -	0.34	0.44	44	56
$^{29}/_{4}$ -	0.90	1.10	45	55
$^{14}/_{5}$ -	1.83	1.90	49	51
$^{15}/_{5}$ -	2.29	3.85	37	63
$^{16}/_{5}$ -	2.95	3.80	44	56
$^{20}/_{5}$ -	2.83	3.15	49	51
$^{21}/_{5}$ -	3.28	4.27	43	57
$^{22}/_{5}$ -	2.72	3.14	47	53
$^{23}/_{5}$ -	4.71	4.00	54	46
$^{24}/_{5}$ -	1.18	4.55	21	79
$^{25}/_{5}$ -	2.34	2.65	47	53
$^{27}/_{5}$ -	3.41	3.29	51	49
Durchschnitt	2.01		44	56

Es wurden im ganzen 17 Versuchsreihen durchgeführt. In 15 Fällen war der Wuchsstoffgehalt am grössten auf der Unterseite, in zwei Fällen ein wenig grösser auf der Oberseite. Im Durchschnitt enthielt die Oberseite 44 %, die Unterseite 56 % der gesamten Wuchsstoffmenge.

d. Vicia faba, Windsor white, geotropische Krümmung der Wurzeln.

Vor drei Jahren konnte ich zeigen, dass man aus Wurzelspitzen von Mais und Vicia faba Wuchsstoff mit Agar-Würfelchen, die 10 % Dextrose enthalten, abfangen kann, und dass die Wuchsstoffkonzentration von der Spitze ab nach oben abnimmt. Dieses Ergebnis ist von THIMANN (1934) bestätigt worden; durch Extraktion der Avena-Wurzeln mit Chloroform konnte gleichfalls Wuchsstoff in diesen Wurzeln nachgewiesen werden. Ferner verglich er die Wuchstoffmenge, die er mit Chloroform extrahieren konnte, mit derjenigen, die er mit Dextroseagar abfangen konnte, und fand, dass die erstere am grössten ist. Hieraus schliesst er, dass der Wuchsstoff nicht in der Wurzelspitze gebildet wird, sondern dass er durch polaren Transport von oben in die Wurzelspitze gelangt und dort angehäuft wird. Diese Anschauung stimmt zwar mit der Polaritätstheorie von WENT überein, steht aber sonst mit allem, was wir über die Bedeutung des Wuchsstoffes für die geotropische Krümmung der Wurzel wissen, im Widerspruch. Wir wissen, dass der geotropische Reiz in der Spitze perzipiert wird, dass man durch eine abgetrennte und wieder aufgesetzte Spitze eine geotropische Krümmung im oberen Teil der Wurzel hervorrufen kann, und dass man aus einer geotropisch induzierten Wurzelspitze mit Dextroseagar mehr Wuchsstoff von der unteren als von der oberen Hälfte abfangen kann.

Als Stütze für seine Anschauungen führt Thimann an, dass Dextrose notwendig ist, um Wuchsstoff aus der Wurzel abzufangen. Es soll durch die Dextrosezugabe zum Agar ein osmotischer Gradient errichtet werden, wodurch eine Rückwärtsbewegung des Wuchsstoffes entstehen soll. Thimann hat übersehen, dass es bei Wurzeln von Vicia faba möglich ist auch ohne Zusatz von Dextrose Wuchsstoff abzufangen; dies deutet darauf hin, dass auch unter normalen Bedingungen Wuchsstoff von der Wurzelspitze an die oberen Teile abgegeben wird.

Direkt lässt sich die Auffassung von Thimann durch die folgenden Versuche widerlegen. Es wurde der Wuchsstoff der 4 äussersten mm von 40 Vicia faba Wurzeln mit Chloroform extrahiert, und der Wuchsstoff wurde in ein Dextroseagarplättchen mit einem Areal von 1 qcm übergeführt. Würfelchen von diesem Plättchen riefen eine Krümmung, dessen d-Wert gleich 0.59 und 1.04 war, hervor. Der Wuchsstoff von einer Wurzel erzeugt somit einen d-Wert, der 0.015 und 0.026, im Durchschnitt 0.02 beträgt. In einem anderen Versuch wurden die äussersten 4 mm von 2 Vicia faba Wurzeln, je in zwei Zylinder mit einer Länge von 2 mm zerlegt. Diese Zylinderchen wurden mit dem basalen Ende nach abwärts, auf ein Dextroseagarplättchen durch etwa 20 Stunden gestellt; Würfelchen von diesen Plättchen gaben eine Krümmung, deren d-Wert 0.79, 0.82 und 1.0 betrug. Der Wuchsstoff von einer Wurzel ruft somit in diesem Falle im Durchschnitt eine Krümmung, die 0.43 beträgt, hervor. Im ersten Fall bestimmt man den augenblicklichen Wuchsstoffgehalt der Wurzel, im zweiten die Wuchsstoffmenge, die in 20 Stunden abgegeben wird; weil die letztere 21 mal so gross ist wie die erstere, muss man schliessen, dass die Wurzelspitze von Vicia faba imstande ist Wuchsstoff zu bilden.

Wenn der Wuchsstoff der Wurzelspitze von oben her zugeführt wird, dürfte man erwarten, dass eine Dekapitierung eine Anhäufung von Wuchsstoff über der Wunde hervorrufen müsste. Dies ist jedoch nicht der Fall; es findet im Gegenteil eine Abnahme statt. Dies geht aus Tab. 10 hervor. Die Versuche wurden in der Weise angestellt, dass die zwei äussersten mm abgeschnitten wurden. Es wurde dann der Wuchsstoffgehalt der folgenden 4 mm bestimmt, teils sofort, teils 5, 19 und 49 Stunden nach der Dekapitierung.

Tab. 10.

Einwirkung der Dekapitierung auf den Wuchsstoffgehalt der Wurzel.

Die Zahlen bedeuten WAE pro 100 g Trockensubstanz.

Sofort nach der Dekapitierung	nach 5 Stunden	nach 19 Stunden	nach 49 Stunden
3.0	1.0	0.37	0.26
2.36	1.46	0.52	—

Aus den beiden letzten Versuchsreihen geht wohl mit Sicherheit hervor, dass der Wuchsstoff der Wurzel in der Spitze gebildet und von dort aus in basipetaler Richtung transportiert wird.

Czaia (1935) nimmt an, dass in der Wurzel zwei Wuchsstoffströme vorhanden sind, teils ein basipetaler und teils ein akropetaler; die positiv geotropische Reaktion soll durch ein Gegenspiel dieser antagonistischen Wuchsstoffströme zustande kommen. Selbst wenn man annimmt, dass der Wuchsstoff für das Wachstum der Wurzel unentbehrlich ist, scheint es mir doch, dass es überflüssig ist einen akropetalen Wuchsstoffstrom zu postulieren, weil ja auch nach der Dekapitierung von der Spitze herrührender Wuchsstoff

in den oberen Teilen der Wurzel vorhanden ist. Es ist aber eine Frage, ob der Wuchsstoff für das Wurzelwachstum notwendig ist oder nicht. Es geht aus den in dieser Arbeit angeführten Wuchsstoffbestimmungen hervor, dass die Wuchsstoffkonzentration in der Vicia faba Wurzel und in epikotylen Stengeln von Phaseolus und Vicia faba in Prozenten der Trockensubstanz ausgedrückt sich innerhalb derselben Grenzen bewegt. Sicher ist es ferner, dass eine Vermehrung der Wuchsstoffkonzentration in der Wurzel eine Hemmung, in der Coleoptile und in dikotylen Stengeln dagegen eine Beschleunigung des Wachstums hervorruft. Die Abhängigkeit der Wachstumsgeschwindigkeit der Wurzeln und Stengeln von der Wuchsstoffkonzentration kann daher durch die Kurven c und d schematisch dargestellt werden (Abb. 5). Die Frage ist nun, ob die Kurve c sich in die Kurve a oder b fortsetzt. Im letzteren Fall ist der Wuchsstoff notwendig für das Wurzelwachstum, im ersteren dagegen nicht. Falls die letztere Auffassung, dass der Wuchsstoff auch für das Wachstum der Wurzel notwendig ist, die richtige ist, so erklärt sich die entgegengesetzte Reaktion der Wurzeln und Stengeln dem Wuchsstoff gegenüber dadurch, dass die Wurzel mehr empfindlich für Wuchsstoff ist, so dass die optimale Wachstumsgeschwindigkeit bei einer niedrigeren Wuchsstoffkonzentration erreicht wird als bei den Keimstengeln. Eine Konsequenz dieser Auffassung wäre es, dass bei Verminderung der Wuchsstoffkonzentration in der Wurzel, so dass dieselbe in dem Bereiche der b Kurve liegt, möglicherweise negativ geotropische Reaktionen entstehen könnten. Czaia hat tatsächlich solche Krümmungen bei dekapitierten Wurzeln von Pisum, Lupinus, Phaseolus und Lepidium beobachtet, dagegen nicht bei Vicia faba und Zea Mais. Ob diese Krümmungen wirklich

echt negativ geotropisch sind, ist schwer zu entscheiden; nach den Abbildungen zu urteilen, scheinen sie besonders bei Pisum hinter der Wachstumszone zu liegen. CZAIA betrachtet sie als Bestätigung seiner Hypothese über die Existenz eines akropetalen Wuchsstoffstromes; sie können aber

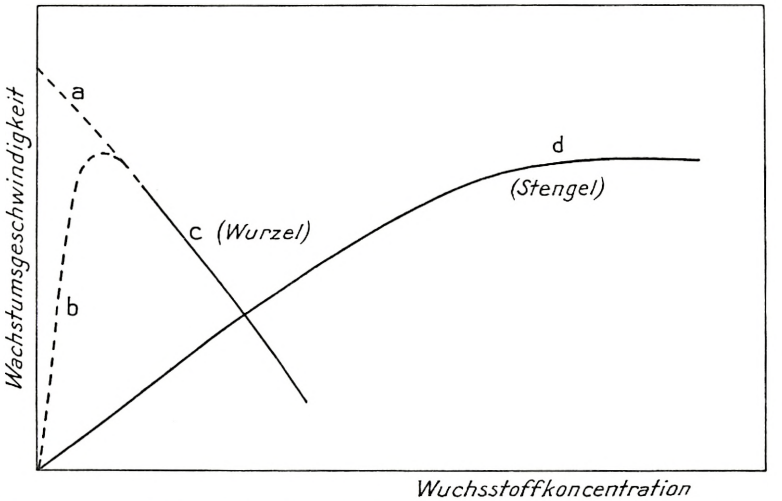

Abb. 5. Die Abhängigkeit der Wachstumsgeschwindigkeit von der Wuchsstoffkonzentration in Wurzeln und Stengeln (Schematisch).

nach Abb. 5 auch ohne diese Hypothese erklärt werden. Die Krümmungen der erythrosinbehandelten Wurzeln, die CZAIA abbildet, habe ich auch beobachtet, ja, man sieht sogar oft, wie CZAIA auch erwähnt, dass Pisumwurzeln, selbst wenn die Samen nicht behandelt sind, sich sofort nach dem Austritt aus dem Samen zurückbeugen. Ich habe immer geglaubt, dass diese Krümmungen traumatotroper Natur sind, und ich möchte an dieser Auffassung festhalten.

Die Versuche über die Wuchsstoffverteilung in der Wurzel während der geotropischen Krümmung wurden in folgender Weise ausgeführt. Die Wurzel wurden in eine dünne Glasröhre hineingesteckt und 4 Stunden in waagerechter

Lage in feuchtem Sägemehl bei 21° belassen. Nachher wurden in den ersten Versuchen 10 mm, in den letzten Versuchen 4 mm abgeschnitten und diese in eine obere und untere Hälfte zerlegt, die je mit 60 g Chloroform und Salzsäure extrahiert wurden. Die Ergebnisse finden sich in Tab. 11.

Tab. 11.

Die Wuchsstoffverteilung während der geotropischen Krümmung der Wurzel von Vicia faba, Windsor white.

In jedem Versuch wurden die Spitzen von 30—60 Wurzeln in ein enges Glasrohr eingeführt und $2^1/_2$ oder 4 Stunden in waagerechter Lage belassen. In den Versuchen vom $^{26}/_3$ bis $^{10}/_4$ wurden die äussersten 10 mm, in den übrigen Versuchen die äussersten 4 mm in eine obere und untere Hälfte zerlegt. Die beiden Hälften wurden zur Wuchsstoffbestimmung verwendet.

	Wuchsstoffgehalt (WAE pro 100 g Trockensubstanz)		Wuchsstoffgehalt in Proz. der gesamten Wuchsstoffmenge	
	Oberseite	Unterseite	Oberseite	Unterseite
$^{26}/_3$ 35	2.1	3.6	37	63
$^{27}/_3$ -	1.9	2.6	42	58
$^{28}/_3$ -	2.0	2.7	43	57
$^{29}/_3$ -	2.4	2.0	54	46
$^{6}/_4$ -	2.38	2.67	47	53
$^{8}/_4$ -	2.55	2.74	48	52
$^{9}/_4$ -	3.85	5.11	43	57
$^{10}/_4$ -	2.96	2.47	55	45
Durchschnitt	2.8		46	54
$^{15}/_4$ 35	2.74	4.11	40	60
$^{30}/_4$ -	1.50	2.41	38	62
$^{29}/_5$ -	3.74	4.60	45	55
$^{1}/_6$ -	4.25	5.55	43	57
$^{4}/_6$ -	5.85	7.20	45	55
$^{5}/_6$ -	7.40	9.10	45	55
$^{17}/_6$ -	3.65	4.97	42	58
$^{18}/_6$ -	4.15	4.95	46	54
$^{20}/_6$ -	4.46	5.36	45	55
$^{22}/_6$ -	2.54	3.84	40	60
$^{25}/_6$ -	1.85	3.21	37	63
$^{27}/_8$ -	2.84	6.80	29	71
Durchschnitt	4.5		41	59

In den Versuchen von 26. März bis 10. April wurden die äussersten 10 mm, in den übrigen Versuchen nur die äussersten 4 mm extrahiert. In beiden Fällen war die Wuchsstoffkonzentration am grössten auf der Unterseite, die Differenz zwischen Ober- und Unterseite war aber bedeutend grösser, wenn nur 4 mm zur Extraktion benutzt wurden. Mit dieser letzteren Versuchsanstellung wurden im ganzen 12 Versuchsreihen durchgeführt; in allen Fällen war die Wuchsstoffkonzentration am grössten auf der Unterseite. Durchschnittlich war auf der Oberseite 41 % und auf der Unterseite 59 % der gesamten Wuchsstoffmenge vorhanden.

4. Vergleichung zwischen Wuchsstoffverteilung und Krümmungsgrösse.

Die Versuche über die Wuchsstoffverteilung während der geotropischen Krümmung sind in Tab. 12 zusammengestellt.

Tab. 12.

	Phaseolus, Stgl. Lichtpflanzen	Phaseolus, Stgl. Dunkelpflanzen	Vicia faba, Stgl. Lichtpflanzen	Vicia faba, Wurzeln
Oberseite ...	39	44	44	41
Unterseite ...	61	56	56	59

Es ergibt sich somit, dass während der geotropischen Krümmung tatsächlich mehr Wuchsstoff in der Unterseite als in der Oberseite vorhanden ist sowohl bei Stengeln als bei Wurzeln. Es entsteht nun die Frage, ob der gefundene Unterschied in der Wuchsstoffkonzentration gross genug ist um die Entstehung der geotropischen Krümmungen zu erklären. Um diese Frage zu beleuchten, muss man sowohl die Wachstumsgeschwindigkeit als die in der Versuchszeit entstehende Krümmungsgrösse für jedes einzelne Pflanzen-

organ messen. Aus der Krümmungsgrösse berechnet man die Längendifferenz zwischen Ober- und Unterseite des gekrümmten Organs (d. h. den »d« Wert der Krümmung). Unter der Annahme, dass die Wachstumsgeschwindigkeit (d. h. das Wachstum der Mittelzone) während der Krümmung nicht verändert wird, kann man annähernd berechnen, wie gross die Wachstumsgeschwindigkeit der Ober- und Unterseite in Prozenten der normalen ist. Diese Zahlen findet sich in Tab. 13, Kolonne 3 und 4. Nimmt man ferner an, dass die gesamte Wuchsstoffmenge sich während der Krümmung nicht ändert, kann man aus den Zahlen in Tab. 12 berechnen, wie gross die Wuchsstoffkonzentration der beiden Seiten in Prozenten der normalen ist. Falls nun die Wachstumsgeschwindigkeit der Wuchsstoffkonzentra-

Tab. 13.

	Phaseolus, Epikotylen Lichtpfl.	Phaseolus, Epikotylen Dunkelpfl.	Vicia faba, Epikotylen Lichtpfl.	Vicia faba, Wurzel
Versuchszeit	2 Stunden	2 Stunden	4 Stunden	4 Stunden
Zuwachs	3.5	2.9	4.0	4.36
d	2.7	2.1	1.8	0.72
Zuwachs, Oberseite	61	64	77	109
Proz. des normalen Wachstums				
Zuwachs, Unterseite	139	136	121	91
Proz. des normalen Wachstums				
Wuchsstoffkonzentration, Oberseite	78	88	88	92[1]
Proz. der normalen Wuchsstoffmenge				
Wuchsstoffkonzentration, Unterseite	122	112	112	108[1]
Proz. der normalen Wuchsstoffmenge				

[1] Es wird hier die Differenz der Wuchsstoffkonzentration für eine Spitzenlänge von 10 mm benutzt.

tion proportional ist, könnte man erwarten, dass die Zahlen der Kolonnen 3 bis 6 in Tab. 13 einander entsprechen würden. Man sieht bald, dass das nicht der Fall ist. Bei den Wurzeln von Vicia faba ist eine gute Übereinstimmung vorhanden, in den übrigen Fällen ist der Unterschied zwischen der Wuchsstoffkonzentration der Ober- und Unterseite weit kleiner als man aus den Änderungen der Wachstumsgeschwindigkeit erwarten sollte.

Zur Erklärung der Tatsache, dass die Differenz der Wuchsstoffkonzentration in den beiden Seiten zu klein gefunden wurde, könnte man verschiedene Möglichkeiten heranziehen. Man konnte sich erstens denken, dass die Wuchsstoffbestimmung ungenau war. Obwohl die Differenzen zwischen den beiden Seiten in den verschiedenen Versuchsreihen bedeutend schwanken, so ist es doch zweifellos, dass die Durchschnittswerte einigermassen genau sind, was dadurch bestätigt wird, dass die Differenz der Wuchsstoffkonzentration bei allen drei Stengeln im grossen ganzen dieselbe und in allen drei Fällen zu klein gefunden wurde. Freilich könnten systematische Störungen vorhanden sein, die z. B. bewirken konnten, dass die Wuchsstoffkonzentration der Unterseite zu klein, oder die der Oberseite zu gross bestimmt wurde. Diese Störungen müssten dann aber unter dem Einfluss der Schwerkraft einseitig verteilt sein; vorläufig spricht nichts für eine solche Annahme. Zweitens könnte man sich denken, dass ausser dem Wuchsstoff auch andere Stoffe, die das Wachstum oder die Wuchsstoffwirkung beeinflussen könnten, unter der Einwirkung der Schwerkraft einseitig verteilt wurden. Man könnte z. B. an die Wasserstoffionenkonzentration denken. Ich habe den p_H Wert der Pressäfte von Ober- und Unterseite der Phaseolusepikotyle während der geotropischen Krümmung (d. h. etwa 2 Stun-

den nach der Horizontallegung) untersucht und keinen Unterschied gefunden (der p_H wert der Oberseite war 6.27, der der Unterseite 6.31). Wahrscheinlicher als die beiden ersten Annahmen ist wohl eine dritte, dass der Wuchsstoff innerhalb der Zelle verschieden verteilt und verschieden wirksam sein kann, so dass neben wirksamem, transversal verschiebbarem auch nicht wirksamer und nicht transversal verschiebbarer Wuchsstoff vorhanden ist. Hierauf hat schon vorher Bonner (1934) hingewiesen. Aus Versuchen über die Einwirkung von Säuren auf das Wachstum der Avenakoleoptile, schliesst er, dass der Wuchsstoff teils in aktiver, nicht dissoziierter Form, teils in nicht aktiver, dissoziierter Form vorkommt. Es ist doch auch möglich, dass die Verteilung der Wuchsstoffe innerhalb der Zelle der entscheidende Faktor für die Wirkung des Wuchsstoffes ist. Tatsächlich wissen wir über diese Verteilung nichts. Er kann in wässeriger Lösung im Zellsaft und im Protoplasma oder in den Lipoiden der Protoplasmahäute gelöst vorkommen. Ferner ist auch die Möglichkeit vorhanden, dass ein Teil des Wuchsstoffes an verschiedene Stoffe der Zelle gebunden und in dieser Weise inaktiviert werden kann. Hierfür sprechen verschiedene, noch nicht veröffentlichte Untersuchungen des hiesigen Laboratoriums.

Wenn nun aber in einem Pflanzenteil nur ein Teil des Wuchsstoffes transversal verschiebbar und wirksam ist, erklärt es sich leicht, dass die Wuchsstoffdifferenz zwischen Ober- und Unterseite kleiner als die beobachtete Wachstumsdifferenz sein kann. In den Keimstengeln ist anscheinend relativ viel nicht wirksamer Wuchsstoff vorhanden.

In Tab. 1 sind die mit Hilfe der Abfangungsmethode gefundenen Werte bezüglich der Wuchsstoffverteilung während der tropistischen Krümmungen zusammengestellt. Es ergibt

sich, dass diese bei der Epikotyle von Vicia faba mit den zu erwartenden Werten besser übereinstimmen, als die Werte, die bei den Extraktionsversuchen gefunden wurden. Auch dieses spricht dafür, dass man zwischen einem wandernden, aktiven und einem nicht wandernden und auch nicht aktiven Wuchsstoff unterscheiden muss.

5. Zusammenfassung.

1. Es wird eine neue Methode zur Wuchsstoffbestimmung in Pflanzenteilen beschrieben. Der Wuchsstoff wird nach THIMANN mit Chloroform extrahiert und mit Hilfe einer speziellen Apparatur in eine genau feststellbare Agarmenge übergeführt und mit Avena als Testobjekt quantitativ bestimmt.

2. Mit Hilfe dieser Methode wurde die Wuchsstoffverteilung während der geotropischen Krümmung der Epikotyle von Phaseolus und Vicia faba untersucht. Es zeigte sich, dass die Wuchsstoffkonzentration grösser auf der Unterseite als auf der Oberseite ist.

3. In entsprechender Weise ist während der phototropischen Krümmung der Epikotyle von Phaseolus die Wuchsstoffkonzentration am grössten auf der Hinterseite.

4. Es konnte gezeigt werden, dass der Wuchsstoff der Wurzel nicht von oben zugeführt wird, sondern dass er in der Wurzelspitze gebildet wird. Die Wuchsstoffmenge, die sich mit Agar in 20 Stunden abfangen lässt, ist nämlich etwa 20 Mal so gross, wie die Wuchsstoffmenge, die augenblicklich in der Wurzel vorhanden ist. Abschneidung der Wurzelspitze ruft eine Wuchsstoffabnahme im oberen Teil der Wurzel hervor. Die Annahme eines akropetalen Wuchsstoffstromes ist nach der Meinung des Verfassers nicht genügend begründet.

5. Während der geotropischen Krümmung der Wurzel ist die Wuchsstoffkonzentration am grössten in der Unterseite.

6. Während die Differenz zwischen der Wuchsstoffkonzentration der Ober- und Unterseite bei Vicia faba Wurzeln genügend gross ist um den Wachstumsunterschied der beiden Seiten während der geotropischen Krümmung zu erklären, ist sie bei den Epikotylen von Vicia faba und namentlich von Phaseolus weit kleiner als man aus den Wachstumsänderungen erwarten sollte. Es wird daher wahrscheinlich, dass der Wuchsstoff, wie auch Bonner angenommen hat, teils als wandernd und aktiv und teils als nicht wandernd und nicht aktiv vorkommt. Die Aktivität des Wuchsstoffes könnte von der Verteilung zwischen den verschiedenen Phasen innerhalb der Zelle abhängig sein. Möglicherweise kann der Wuchsstoff auch durch Bindung an andere Stoffe inaktiviert werden.

(Aus dem pflanzenphysiologischen Laboratorium der Universität Kopenhagen.)

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BY

LOUIS SIGURD FRIDERICIA
AND
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Some recent investigators have expressed doubts as to the retarding effect of vitamin A deficiency on the growth of bony structures. By measuring the length of the long leg bones of killed rats, some of which had been fed on adequate diet and some on diet deficient in vitamin A, E. J. QUINN, C. G. KING, and B. H. DIMIT (1929—30) and J. B. ORR and M. B. RICHARDS (1934) found that in the case of the vitamin A restricted animals these bones had increased in length relatively faster than the body had increased in weight. ORR and RICHARDS are of the opinion that the retardation of growth in vitamin A deficiency is a secondary consequence of the pathological conditions and of the diminished appetite in this avitaminosis and that no justification exists for assigning to vitamin A a specific influence on growth.

In order to investigate this problem in another way, we have measured the rate of growth of the incisors of two groups of albino rats, one of which was fed on an adequate diet and the other on diets deprived of vitamin A.

JOHN S. MARSHALL (1921) divided the growth process of the growing permanent incisors of rodentia into three stages, interstitial, extrusive, and curvative. The first deals with the formation of the concentric layers of dentine and, according to the above-mentioned writer, increases the thickness of the dentine stratum of rat teeth, on an average, about 0.01 mm. daily. The second deals with the extrusion,

or pushing out, of the tooth and is found by William H. F. Addison and J. L. Appleton (1915) to have an average rate of growth of 2.2 mm. per week for the upper, and 2.8 mm. per week for the lower, incisors of adult rats. The third deals with the curving of the incisors, which is caused by the more rapid growth of the labial side than of the lingual side of these teeth, the difference, according to Marshall, being an average of 0.7 mm. per week in rats.

The influence of diet on the extrusive growth of the incisors of rats has been examined by some investigators. Addison and Appleton (1915) found the same rate during feeding with hard food as during feeding with soft. B. Orban (1927) and William G. Downs (1931) varied the amounts of protein, fat, carbohydrates, and salts, in the diet, or gave a basal diet of wheat with the addition of various kinds of nutritive stuffs. H. J. Sedwick and B. S. Bibby (1933) examined the effect of pregnancy and of different diets. Fritz Egger (1925) found a slower rate of growth of the incisors with "completely vitamin free diet". Gilbert Dalldorf and Celia Fall (1930) experimented with guinea-pigs and found a deficiency of vitamin C to have a decidedly retarding influence on the rate of growth of the incisors.

There are no papers on the effect of vitamin A deficiency on tooth growth, but S. B. Wolbach and P. R. Howe (1925) mention that the rate of growth of the incisors of rats fed on a diet deficient in vitamin A was much slower than in rats fed on high protein diets, or diets deficient in the vitamin B complex.

In our experiments the extrusive growth of the lower incisors of albino rats has been measured.

Methods.

The extrusive growth of the incisors of rats can be ascertained either by clipping the exposed portion of one of these teeth every fifth day and measuring the length of the fragments (DALLDORF and FALL (1930)), or by marking the enamel and measuring the increase in distance between the mark and the gingival margin at certain intervals (as has been done by most other investigators). The two methods give different results, because fractured teeth grow faster than unfractured (ADDISON and APPLETON (1915), A. WILTON (1931), own measurements).

In our experiments we have made a fine transverse notch near the gingival margin on the labial side of one of the incisors and measured the distance between the mark and the margin once, or twice, weekly by means of a caliper gauge constructed especially for this purpose. The caliper gauge has two parallel needles which can be adjusted by a fine screw though still remaining parallel to one another. The head of the screw has a scale on which the distance between the needle points is read to an accuracy of 0.1 mm. During measurement, the mouth of the rat is kept open by introducing a finger into the diastema between the incisors and the molars.

The albino rats were of the laboratory stock, and were given the attention, feeding, and weighing, which is usual in vitamin experiments in this laboratory. Each cage contains one rat and is raised on legs in order to prevent coprophagy.

Series I. Adult rats on an adequate diet.

The standard error in estimations of the weekly extrusive rate of growth of the incisors of rats is not indicated

in the existing records. The adequate diet given in our experiments was:— Casein, 18 %. Rice starch, 54 %. Dried autolyzed brewer's yeast, 5 %. Butter fat, 15 %. Agar, 3 %. McCollum's salt mixture, No. 185, 5 %.

Table I. Extrusive growth of the incisors of rats, fed on an adequate diet.

	Weight	Average extrusive incisor growth per week	Standard deviation σ	Largest observed +deviation	Largest observed −deviation (in multiples of the standard deviation σ)
13 adult male rats ...	236—310 g.	2.65 mm.	0.22	$2.0 \cdot \sigma$	$2.5 \cdot \sigma$
12 — female — ...	152—226 g.	2.73 mm.	0.37	$2.1 \cdot \sigma$	$1.4 \cdot \sigma$
25 —	152—310 g.	2.69 mm.	0.30	$3.0 \cdot \sigma$	$2.0 \cdot \sigma$
9 young rats	47—272 g.	3.35 mm.	0.47	$3.1 \cdot \sigma$	$1.8 \cdot \sigma$

The weekly rate of growth of the incisors was measured in 25 adult rats, 13 males and 12 females, for 2—4 weeks. The results are seen in Table I. The average rate of growth was 2.7 mm. per week, with a standard deviation of 0.3 mm. for a single measurement. The difference between the average rates for males and females is 0.08 mm. As this is only 1.6 times the standard deviation in the 32—38 measurements for each sex respectively ($\sigma = 0.05$), the difference is without significance.

Series II. Young rats on an adequate diet.

It is not known whether the rate of the growth of the incisors is the same in young as in adult rats. As it is necessary to use young rats for experiments comparing changes in tooth growth, which are of dietary origin, with changes in the increase in weight, this matter must be cleared up.

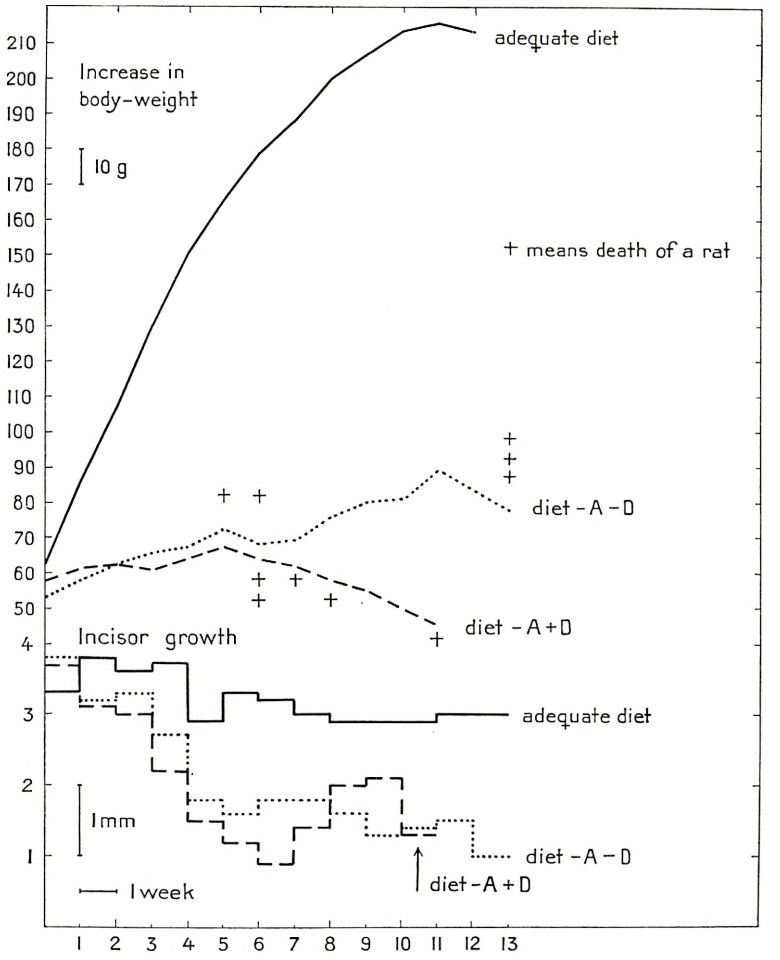
The adequate diet was the same as that used in Series I. The rate of growth of the incisors and the increase in body weight was measured in nine young rats belonging to two litters, 35 and 37 days old at the beginning of the experiment. The measurements were continued for 13 weeks, being made weekly for the first 8 weeks and twice during the last five.

At the outset, the young rats weighed 47—75 grm., on an average, 62 grm.; at the end, their weight was 138—272 grm. The average weekly rate of growth of their incisors was 3.35 mm., with a standard deviation of 0.47 mm. for a single measurement (see Table I). This standard deviation is considerably larger than that in Series I. As the rate of tooth growth is found to be larger in this series on young rats than in Series I on adult rats (3.35 mm. weekly as against 2.69 mm. weekly), the question as to whether the rate of tooth growth has decreased during the experiment with young rats must be gone into. This would explain why the standard deviation is larger in Series II than in Series I.

Table II. Extrusive growth per week in mm, of the incisors of young rats, fed on different diets.

Week no.	1	2	3	4	5	6	7	8	9	10	11	12	13
		3.6			3.3			3.0			3.0		
9 young rats fed on an adequate diet	3.3	3.8	3.6	3.7	2.9	3.3	3.2	3.0	2.9	2.9	2.9	3.1	3.1
5 — — - - - a diet — A + D	3.7	3.1	3.0	2.2	1.5	1.2	0.9	1.4	2.0	2.1	1.3		
5 — — - - - — — A — D	3.8	3.2	3.3	2.7	1.8	1.6	1.8	1.8	1.6	1.3	1.4	1.5	1.0
5 — — - - - — — B	3.3	3.4	3.1	3.1	2.9	2.0	2.5	2.4	1.8	1.5	1.9	1.8	

Curve A and Table II show that the average rate of growth of the incisors of the young rats has decreased



Curves A. Average increase in body weight and average weekly extrusive incisor-growth in young rats fed on an adequate diet and on diets deficient in vitamin A and vitamin A and D respectively.

during the course of the experiment. This can easily be seen if the first 12 weeks of the experiment are divided into 4 periods, each of 3 weeks. In these 4 periods the average weekly rates of growth of the incisors are respectively, 3.6 mm., 3.3 mm., 3.0 mm., 3.0 mm. Each period

represents 27 measurements and the standard deviation of its average is $\frac{0.47}{\sqrt{27}} = 0.09$. The decrease in the rates is, therefore, significant.

This decrease in the rate of growth of the incisors as the young rat grows older must be taken into consideration in the following experiments.

The rate of tooth growth in quickly growing young rats is greater than in those that grow more slowly. This is seen in Table III.

Table III.

	Average increase in weight per rat in 8 weeks	Average weekly rate of growth of incisors
Group 1. 3 rats	101 grm.	3.25 mm.
— 2. 3 —	148 —	3.38 —
— 3. 3 —	166 —	3.43 —

In experiments on young rats fed on other adequate diets a still more rapid growth of the incisors has been observed in this laboratory.

Series III. Young rats on diets deficient in vitamin A.

The diet was the same as that in Series I and II but with 15 % oxidised lard instead of 15 % butter fat. This diet is practically devoid of vitamin A and vitamin D. From the investigations of MAY MELLANBY (1928), and others, it is known that a deficiency of vitamin D influences the structure of growing teeth. We have therefore measured the effect of diets devoid of vitamin A and vitamin D (called: Diet $-A\ -D$) as well as those only devoid of vitamin A (called: Diet $-A\ +D$). Rats on the

diet —A +D received 5 drops of a pharmaceutical solution of irradiated ergosterol daily.

The rats used in all the following experiments are of the same initial age (5 weeks) as those in Series II.

Series III A. Diet —A +D. Five young rats weighing 55—62 grm. (average, 58 grm.) were used. Two died after 6 weeks, the other three after 7, 8, and 11 weeks, respectively. The average increase in weight is shown in Curves A, the average weekly growth of the incisors in Curves A and Table II.

Series III B. Diet —A —D. Five young rats weighing 45—56 grm. (average, 53 grm.) died after 5, 6, and 14 weeks, see Curves A and Table II.

Some of these rats lived longer than is usual in experiments with vitamin A free diets.

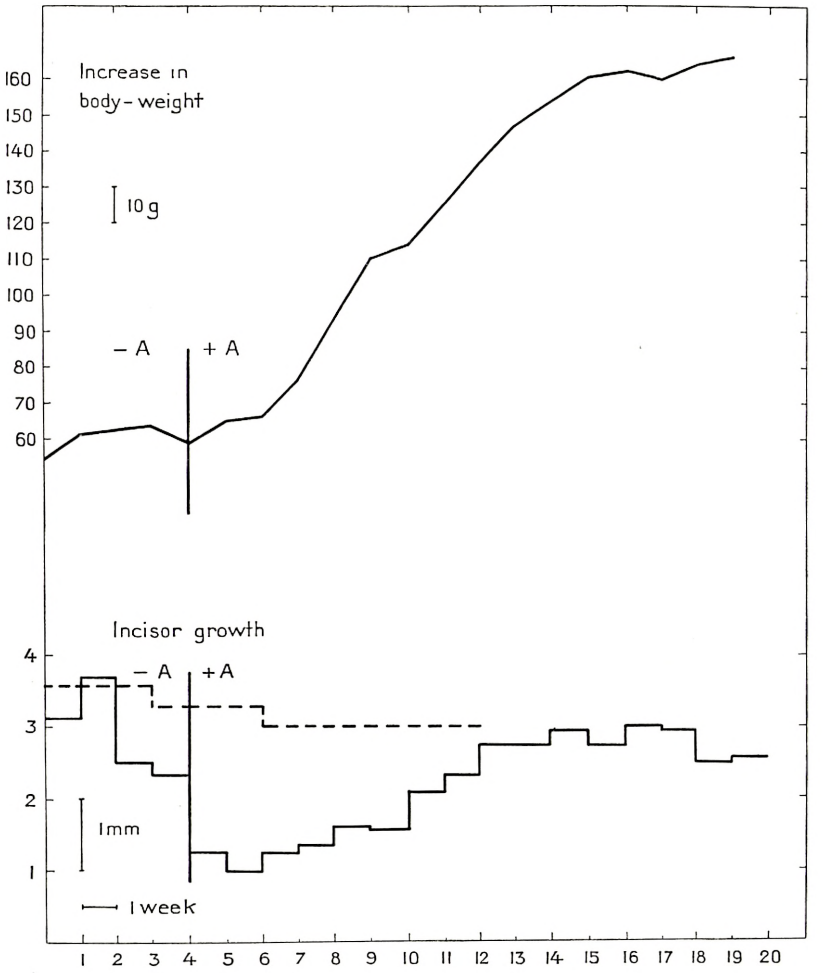
Curves A and Table II show that the weekly rate of growth of the incisors decreased from the fourth experimental week in both series. In the fourth week the average rate of growth of the incisors was 2.2 mm. in Series III A, and 2.7 mm. in Series III B, as against 3.7 mm. in young rats of the same age which had been adequately nourished. In the following weeks the rate of growth of the incisors further decreased to about 1.3—2.1 mm. weekly in the young rats on deficient diets. The difference between the weekly rate of growth of the incisors in the adequately nourished animals and in those nourished deficiently is 1.1 mm.—2.3 mm. from the 5th week and on. The standard deviation in the average measurements of five rats is less than $\frac{0.47}{\sqrt{5}}$ or 0.2 mm. This difference is therefore significant.

The retardation in the growth of the incisors is the same in Series III A as in Series III B. This means that the deficiency of vitamin A and the combined deficiency of vitamins A and D produce identical effects on this process. The growth of the incisor continues in young rats deprived of vitamin A in their food when the increase in weight has stopped and goes on until the death of the animals, but only at about half the normal rate.

Series III C. This experiment was performed in the same way as vitamin A estimations by the curative method, in order to see if the growth of the incisors could be restored by giving vitamin A again after having first been restrained by a deficiency of this vitamin.

Three young rats (weight 52—59 grm.) were fed on diet —A —D. In the fourth experimental week all three decreased in weight and exhibited incipient xerophthalmia. The average weekly rate of growth of the incisors decreased from 3.1—3.7 mm. to 2.4 mm. (see Curves B). Then a daily supplement of 0.2 grm. cod-liver oil was given. The avitaminotic symptoms disappeared and the body weight increased. But the weekly rate of growth of the incisors decreased still further during the first two weeks when cod-liver oil was given and reached the low value of 1.0 mm. The experiment was continued for 20 weeks. At the end, the rats weighed 155, 168, and 199 grm., and the growth of their incisors had regained an almost normal average weekly rate of 2.9—3.0 mm.

In this experiment an after-effect of the vitamin A deficiency was observed on the growth of the incisors. While a supply of cod-liver oil immediately cured the sore eyes and produced an increase in body weight in the A avita-



Curves B. Average increase in body-weight and average weekly extrusive incisor growth in young rats, fed during four weeks on a diet deficient in vitamin A, and then on an adequate diet (compared with the average weekly incisor growth in young rats, fed on an adequate diet all the time, shown in three-weekly periods).

minotic rats, the rate of the growth of their incisors further slowed down for two weeks after the supply of vitamin A had been resumed, after which restoration set in.

Series III D. A preliminary experiment was carried out on the effect of different suboptimal doses of vitamin A on the rate of growth of the incisors. Three groups, each of two rats, were used. The initial weight of the rats was 40—68 grm. From the beginning of the experiment the three groups received a daily supplement to the vitamin A free basal diet of 0.25 mg., 0.50 mg., and 1.00 mg., respectively, cod-liver oil (containing about 1500 internat. vitamin A units per grm.). In Table IV the average weekly rate of growth of the incisors for periods comprising three weeks each is tabulated for the three groups, and compared with the corresponding rates in young rats on an adequate diet (Series II), and on a diet devoid of vitamin A.

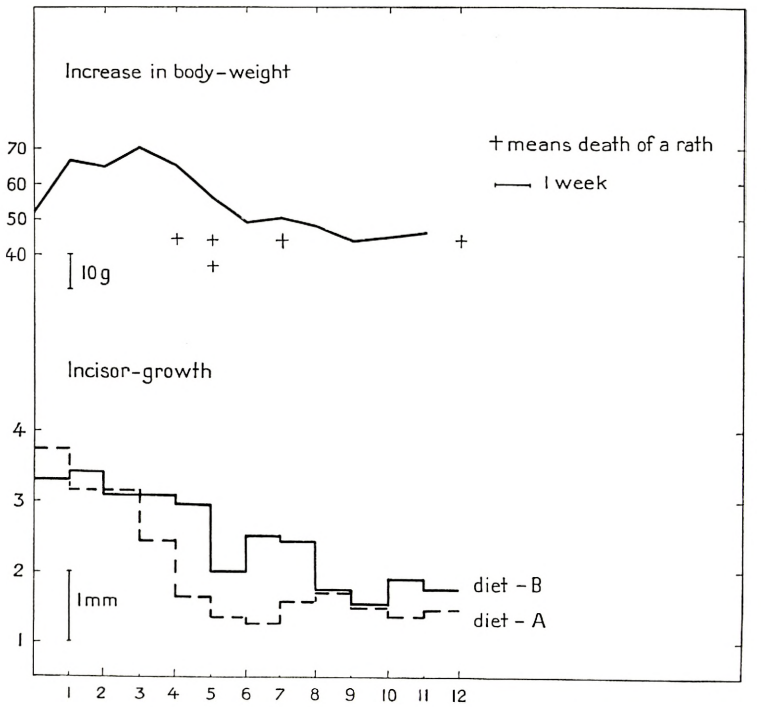
Table IV. Rate of growth of the incisors of young rats receiving different daily amounts of vitamin A.

	Adequate diet	Cod-liver oil per day			Diet devoid of vitamin A
		1.00 mg.	0.50 mg.	0.25 mg.	
1st— 3rd week..	3.6 mm.	3.5 mm.	3.5 mm.	3.3 mm.	3.3 mm.
4th— 6th -- ..	3.3 -	2.9 -	2.9 -	2.6 -	1.8 -
7th— 9th — ..	3.0 -	2.2 -	1.8 -	1.6 -	1.6 -
10th—12th — ..	3.0 -	2.0 -	1.5 -	1.2 -	1.4 -

The results are only provisional because each group comprised only two rats. It is seen that the depressing influence of the smaller doses on the rate of tooth growth is not apparent before the 7th—9th week of the defective feeding. Still, the results suggest the possibility of using measurements of the rate of growth of the incisors as a biological method for the quantitative estimation of vitamin A.

Series IV. Young rats on a diet deficient in the vitamin B complex.

In the preceding experiments deficiency of vitamin A was found to exert a depressing influence on the rate of



Curves C. Average increase in body-weight and average weekly extrusive incisor growth in young rats fed on a diet deficient in the vitamin B complex (compared with the average weekly incisor growth in young rats fed on a diet deficient in vitamin A).

growth of the incisors. In order to see whether this influence is specific to vitamin A deficiency, or is also found in other dietary deficiencies, an experiment with a diet devoid of the vitamin B complex was made.

This diet was the adequate food mixture used in Series I and II, but without the 5 % dried autolyzed yeast. The

casein was especially purified from content of the vitamin B complex.

Five young rats, weighing 44—58 grm. (average, 52 grm.) were used. Their body weight decreased after a few weeks and they died after 4, 5, 5, 7, and 12 weeks respectively. The average weight curve is seen in Curves C, and the average weekly rates of growth of the incisors in Curves C and Table II.

The curves and the table show that the weekly rate of growth of the incisors decreases from the 6th week of the experiment, being 2.0—2.5 mm. from the 6th to the 8th, and 1.5—1.9 mm. from the 9th to the 12th week, as against 3.0—3.3 mm. in young rats on an adequate diet. The decrease in the rate occurs later in this experiment than in the experiments concerning vitamin A deficiency (in the 6th week when the vitamin B complex is deficient, as against the 4th week when vitamin A is deficient).

Discussion.

A considerable decrease in the rate of growth of the incisor teeth of young rats fed on a diet devoid of vitamin A was found in Series III. This result agrees with the histological investigations of S. E. Wolbach and P. R. Howe (1933) who found an atrophy of the enamel organ followed by atrophy of the odontoblasts of the incisors of rats fed on a diet deficient in vitamins A and D. The atrophy finally involved the whole length of the enamel organ, including the basal formative end.

Series III A compared with Series III B shows that the presence or absence of vitamin D in a diet devoid of vitamin A is without influence upon the decrease in the rate of growth of the incisors effected by vitamin A deficiency.

The pushing out of the tooth, accordingly, seems to be dependent on processes not influenced by vitamin D.

An after-effect of vitamin A deficiency upon the rate of growth of the teeth is found in Series III C. The rate of growth is nearly normal again only about three months after the rat has again been given a sufficient supply of vitamin A, although the morphological re-establishment of the enamel organ is completed 19 days after vitamin A is given again (Wolbach and Howe (1933)). C. E. Bloch (1931) found no after-effect from severe vitamin A deficiency on the teeth of human beings.

Orr and Richards (1934) raised the problem as to whether vitamin A deficiency has a direct influence on the growth of bony structures or not. The experiments described in this paper cannot settle this question. A correlation between vitamin A deficiency and retardation of the rate of growth of the extrusive incisor teeth of rats has been proved, but the mechanism connecting these two phenomena has not been investigated. Wolbach and Howe (1933) described changes in the enamel organ of rats and guinea-pigs fed on a diet deficient in vitamin A, and regarded these changes as one of the primary consequences of vitamin A deficiency common to many epithelial organs. The changes in the enamel organ may be one of the factors retarding the growth of the tooth.

Deficiency in vitamin A is not the only dietary deficiency which is able to retard the rate of growth of the incisors of rats. In Series IV, deficiency of the vitamin B complex was found to have the same effect, though it was of later occurrence. Dalldorf and Fall (1930) observed the same effect in experiments on guinea-pigs fed on a diet deficient in vitamin C.

Summary.

1. The rate of extrusive growth of the incisor teeth is, on an average, 2.7 mm. per week in adult rats, and 3.3 mm. per week in young rats, fed on an adequate diet. The standard deviation for the single measurement was 0.30 mm. for adult, and 0.47 for young rats.

2. In young rats on a diet deficient in vitamin A, this rate decreases after three weeks to about 2.5 mm. and in the following weeks to about 1.5 mm.

3. It makes no difference to this effect whether the diet which is deficient in vitamin A contains vitamin D or not.

4. Different daily suboptimal amounts of vitamin A, when fed for periods of longer than 6 weeks, retard the rate of extrusive growth of the incisor teeth to different degrees.

5. When a sufficient supply of vitamin A is given to young rats, the rate of growth of whose incisors has been retarded by deficiency of vitamin A, an after-effect appears from the deficiency, the rate decreasing further during the first two weeks after the supply of vitamin A is resumed, in spite of the immediate effect of the supply on other avitaminotic symptoms in the animals. The rate of growth of the incisors is about normal again only after three months' adequate feeding.

6. A diet deficient in the vitamin B complex also depresses the rate of extrusive growth of the incisors of young rats, but the effect occurs later than in vitamin A deficiency.

From the University Institute of Hygiene, Copenhagen.

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DEN KINESISKE ULDHAANDSKRABBE (*ERIOCHEIR SINENSIS* M.-EDW.) I DANMARK

AF

AD. S. JENSEN

MED 3 TAVLER

DEUTSCHE ZUSAMMENFASSUNG

KØBENHAVN
LEVIN & MUNKSGAARD
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Under Verdenskrigen (1914—18) fangede Fiskerne af og til i Nedre-Elben Krabber, som senere har vist sig at være en i Nordkina hjemmehørende Ferskvandskrabbe, kaldet Uldhaandskrabbe (*Eriocheir sinensis* H. Milne-Edwards), fordi Klosaksene paa første Par Ben hos voksne Individer er tæt besat med lange Haar, som ligner Uld (jfr. Tavle II—III). Hvornaar Krabben er kommet ind i Tyskland, vides ikke; muligvis er den kommet ind i flere Omgange, i hvert Fald blev der allerede i 1912 i Aller, en Biflod til Weser, fanget et Eksemplar, der endnu opbevares.

Til at begynde med bemærkedes Uldhaandskrabben kun i Elben og Weser, hvis store Handelsbyer Hamburg og Bremen har livligt Samkvem med Østasien. Det er derfor sandsynligt, at Krabberne er kommet ind i nævnte Floder med Handelsdampere, som dér har udtømt Ballastvand, de ovre i Østen havde indtaget i deres Dobbeltbundtanke; Indstrømningsventilen i disse har en Diameter af 40 mm, saa at ikke alene Krabbens Larver, men ogsaa smaa Krabber med Lethed kan slippe igennem.

I de forløbne godt 20 Aar har Uldhaandskrabben erobret næsten hele Nordtyskland og Holland. Gaaende ud fra Elben og Weser, hvor den stadig tiltager i Antal, har den efterhaanden, især i Tiden efter 1925, bredt sig mod Øst til Oder og Weichsel, mod Vest til Ems og Rhinen, til disse Floders Tilløb og Kanalforbindelser samt til mange

Brakvande og afsides liggende Indvande, saa at Udbredelsen i Mellemeuropa ved Udgangen af 1932 strakte sig fra Holland i Vest til Øst-Preussen (Kurisches Haff og de Masuriske Søer) i Øst. Inden for disse Grænser findes den langs Kysterne af Nordsøen og Østersøen og i de store Vandløb fra Kysten højt op, i Rhinen saaledes mindst 500 km op og i Elben 700 km op (indtil Bøhmen). I sin ubetvingelige Vandretrang overvinder den alle Hindringer, som møder den i Form af Strømfald o. l., om ikke paa anden Maade saa ved at omgaa dem over Land. I størst Mængde forekommer den i Elben og Weser, særlig i deres nedre Løb; ved en forsigtig Beregning er man kommet til det Resultat, at i Elben neden for Hamburg er i Aaret 1931 over en Million Uldhaandskrabber kommet med i Fiskernes Redskaber.

Fra Undersøgelser i Tyskland ved man, at Uldhaandskrabben ikke kan yngle i Ferskvand; naar den skal forplante sig, maa den vandre ud til det brakke Vand i Flodmundingerne og til det salte Vand ved Havkysterne. Alle Krabber er oprindelig Havdyr, og skønt Uldhaandskrabben har vænnet sig til at opholde sig den største Del af sit Liv i Ferskvand, har den dog bevaret det af sin oprindelige Natur, at Forplantningen maa foregaa i Saltvand. Her gennemgaar Larverne deres Forvandling, og de unge Krabber stiger derpaa op i de ferske Vande og opholder sig dér, indtil de som voksne (kønsmodne) atter vender tilbage til Havkysten. Det menes, at de udlegede Krabber, som overlever Forplantningen, for det meste forbliver i Flodmundingernes brakke Vand.

Disse kortfattede, for Forstaaelsen af det efterfølgende nødvendige Oplysninger er uddraget af den i 1933 udkomne Monografi: »Die Chinesische Wollhandkrabbe (*Eriocheir si-*

nensis H. Milne-Edwards) in Deutschland«[1], forfattet af Dr. NICOLAUS PETERS og Dr. ALBERT PANNING, med et Tillæg om Udviklingen af Prof. Dr. W. SCHNAKENBECK. Dette Arbejde, hvortil der ogsaa i det følgende oftere vil blive henvist, behandler udførligt og fortrinligt alle Sider af Uldhaandskrabbens Naturhistorie og giver tillige en udmærket Oversigt over de talrige, i Litteraturen spredte Angivelser om Forekomst af Uldhaandskrabber i Tyskland.

Efter disse indledende Bemærkninger skal jeg gaa over til at gøre Rede for Uldhaandskrabbens Optræden her i Landet. Herom foreligger der talrige Notitser spredt i Dagspressen og i Specialtidsskrifter, derfor vanskeligt tilgængelige, ofte uden anden Oplysning end Lokalitetens Nævnelse og for Resten ingenlunde altid paalidelige.[2] Nærværende Skrift tilsigter at give en samlet Oversigt over de sikre Tilfælde (afsat paa Oversigtskortet Tavle I) samt en nærmere Omtale af dem, hvor særlige Forhold maatte opfordre dertil; med dette Formaal for Øje har jeg foretaget talrige Henvendelser paa de rette Steder og indhentet nærmere Oplysning om de angivne Fund. Tillige har jeg fra flere Sider faaet tilstillet Meddelelse om adskillige nye Forekomster.

Den første Uldhaandskrabbe paa dansk Omraade blev fanget i 1927 ved Vestkysten af Jylland, helt oppe Nord

[1] Zoologischer Anzeiger, Ergänzungsband zu Band 104. Leipzig.

[2] Jeg kan nævne, at Fund baade af Stankelbenskrabber (*Stenorhynchus rostratus*), Taskekrabber (*Cancer pagurus*) og Troldkrabber (*Lithodes maia*) har været kundgjort som værende af Uldhaandskrabber. For to Aar siden gik der saaledes gennem den svenske og danske Dagspresse en Meddelelse om, at en Fisker havde fanget to Uldhaandskrabber i Garn paa Westerflacket i Sundet Sydost for Øen Hveen, en Lokalitet, der vilde være særdeles bemærkelsesværdig. Om det ene af disse Eksemplarer har imidlertid Prof. BERTIL HANSTRÖM i Lund oplyst, at det er en Troldkrabbe (jfr. »Fauna och Flora« 1934, S. 275). Og paa Forespørgsel har Prof. HANSTRÖM meddelt mig, at ogsaa det andet Eksemplar, der er blevet undersøgt af Amanuensis LOVÉN, er en Troldkrabbe.

for Limfjorden, af Fisker Karl Josefsen, Lyngby Fiskerleje, der var skarpsynet nok til straks at se, at det var en Sjældenhed; derfor sendte han den til København til Dansk Fiskeriforening, i hvis Aarsberetning for Driftsaaret 1926—27 den lige er nævnet (S. 30)[1]. For at faa nærmere Oplysning om dette interessante Fund skrev jeg til Fisker Josefsen, der velvilligst meddelte mig, at Krabben blev fanget i Marts Maaned 1927 i Torskegarn ud for Lyngby Landingsplads. En Tilføjelse i hans Brev kan tjene til Belysning af denne Krabbearts Sejglivethed: »Det var en sjælden levedygtig Krabat; første Nat løb den væk; jeg gennemrodede hele Huset og fandt den omsider under Vaskekedlen, hvor den var helt forsvunden i Asken, men lige levende; jeg vaskede den i fersk Vand og satte den sidenhen i et Fad med Havvand, men der vilde den ikke være, og den faldt flere Gange paa Gulvet, men var lige levende«. Den var endnu i Live, da den flere Dage efter Fangsten kom til Dansk Fiskeriforening i København.

Denne Uldhaandskrabbe opbevares nu i Marinbiologisk Laboratorium, og takket være dets Leder, Dr. Å. Vedel-Tåning, har jeg haft Lejlighed til at undersøge den. Det er en stor Han; Spændvidden mellem det længste Benpars Spidser udgør 350 mm, Skjoldets Bredde 83 mm, dets Længde 78 mm.[2] Paa den forreste Del af Skjoldet og paa Klosaksene sidder mange smaa Balaner, af Cand. mag. K. Stephensen bestemt som *Balanus crenatus* Bruguière.

[1] Findes ogsaa omtalt af Peters (l. c. p. 67), hvor Lokaliteten dog angives som: »Lyngby in der Jammerbucht«, en Fejltagelse, der let kan ske, thi der ligger et Lyngby ved Jammerbugt; det er imidlertid ikke der, Krabben blev fundet, men ved et sydligere liggende Lyngby, Nord for Lodbjerg Fyr.

[2] Dette er hidtil det største danske Eksemplar. Det mindste er det S. 9 nævnte, i Køge Aa fangede Ekspl., hvor Spændvidden mellem det længste Benpar udgør 157 mm, Skjoldets Bredde 37 mm, dets Længde 35 mm.

Denne Forekomst paa Nordsøens aabne Kyst og hvor intet Vandløb udmunder, er ganske interessant, idet den danner den extreme Modsætning til Forekomsten i Ferskvand: Uldhaandskrabben er en udpræget euryhalin Dyreform, den kan leve i Vand, hvis Saltholdighed svinger fra $^{0}/_{00}$ til $^{33}/_{00}$.

Der skulde gaa $6^{1}/_{2}$ Aar, inden Uldhaandskrabben paany blev observeret i Danmark. Den 8. Oktober 1933 fangede Fisker Boy S. Petersen (Rudbøl) ved Højer Sluse paa Vestkysten af Sønderjylland to Krabber af en Slags, som han aldrig havde set før; den største knustes under Arbejdet, den mindste sendte han til Fiskeribiologen Mag. sc. C. V. Otterstrøm, der bestemte den som en Uldhaandskrabbe; det var en Han, hvis Skjold maalte 57 mm i Længderetningen og havde en Afstand af 300 mm mellem Spidsen af det længste Benpar. Beretningen om dette Fund har Mag. Otterstrøm offentliggjort i »Ferskvandsfiskeribladet« for 1. November 1933, S. 157.

Allerede sidst i samme Maaned fik Boy Petersen daglig et Par Stykker i Ruserne.

I 1934 fangedes der igen adskillige Uldhaandskrabber ved Højer Sluse. Fisker Boy Petersen var saa venlig paa min Anmodning i November Maaned at sende mig til Universitetets zoologiske Studiesamling to velvoksne Eksemplarer, en Han (Tavle II) og en Hun (Tavle III), der har følgende Maal:

	Spændvidden mellem Spidsen af det længste Benpar	Rygskjoldets Bredde	Rygskjoldets Længde
♀	330 mm	75 mm	70 mm
♂	315 -	69 -	63 -

I Ribe Aa fangedes i 1934 en Uldhaandskrabbe. Magister Otterstrøm meddeler herom i »Ferskvandsfiskeribladet«

for 1934, S. 164, at Isenkræmmer Otto Micheelsen, Ribe, den 13. Oktober sendte ham et Eksemplar, der var taget af Slusemester Ernst i en Aaleruse ved Digegravens Udløb i Kanalen lige Øst for Kammerslusen i Ribe Aa. Det var en Hun, hvis Rygskjold var 61 mm bredt og 52 mm langt.

I 1934 er Uldhaandskrabben naaet ind i Limfjorden. I Skive Fjord er der af Fisker Kristen Pedersen i Skive fanget to Eksemplarer, det første d. 15. Marts i Aaleruse, og det andet d. 16. Juni i Nedgarn paa 2 Meter Vand ca. 400 Meter Nord for Udløbet af Skive-Karup Aa, omtrent midt imellem Aaens Udløb og Skive Havn. Underretningen om disse Fangster skylder jeg Fiskeeksportør Bunger i Skive. Det sidstnævnte Eksemplar skænkede Fiskeren til Dansk Fiskeriforenings Samling, hvor jeg har haft Lejlighed til at se det (Samlingens No. 285); det er en Hun, hvis Skjold er 58 mm bredt og 53 mm langt.

Paa Østkysten af Jylland fangede Fisker P. Christensen en voksen Han (Skjoldets Bredde 70 mm, dets Længde 68 mm) d. 27. September 1934 i Aaleruse i Randers Fjord (Tjæreby Bredning), hvor Dybden var 2 m og Saltholdigheden ca. $^{20}/_{00}$. At dette Eksemplar blev tilvaretaget, skyldes ihærdige Bestræbelser fra den kendte Biolog i Randers, Hj. Ussing, som i »Randers Amtsavis« for 13. Oktober 1934 indgaaende har skrevet om denne Krabbe og givet gode Fotografier af den; han holdt den levende i længere Tid og beretter interessante Træk af dens Livsvaner.

Paa Sjælland er Uldhaandskrabben ligeledes observeret flere Steder i 1934. En voksen Han (Skjoldets Bredde 64 mm) fangedes d. 30. Juni ved Kalveboderne Syd for Slusen; Biologisk Station modtog Eksemplaret levende fra Fisker Rasmus Petersen iflg. Dr. H. Blegvad (»Dansk Fiskeritidende«, $^{31}/_{10}$ 1934).

I Køge Aa tog Maler KNUD SPARRE en ung Han (Skjoldets Bredde 37 mm, dets Længde 35 mm) d. 2. Juni i en Ruse, anbragt 1 km fra Aaens Munding, oppe i selve Køge By. SPARRE bragte den levende Krabbe til mig; den anbragtes i et Akvarie i Zoologisk Museum til Beskuelse for Publikum og holdt sig i Live i en Maaneds Tid; jeg fodrede den med Snegle (*Limnæer*) og Muslinger (*Dreissensia*), hvis Skaller den brød op og tømte for Indholdet. En større Han (Skjoldets Bredde 70 mm) fangedes i Begyndelsen af Oktober i Køge Aa mellem Klapbroen og Jernbanebroen, hvor den var gaaet i et Laksegarn og havde indfiltret sig saadan i Garnet, at en Kvadratmeter maatte klippes itu for at faa den fri. Den 1. November blev et tredje Eksemplar fanget i et Garn i Svajebassinet ved Udløbet af Køge Aa. Underretningen om de to sidst nævnte Eksemplarer skylder jeg Lærer LÆSSØE ENGBERG i Køge.

I Smaalandsfarvandet har Uldhaandskrabben ogsaa holdt sit Indtog i 1934. Dr. H. BLEGVAD meddeler herom (»Dansk Fiskeritidende« 21.11.24), at der d. 29. September blev fanget en usædvanlig stor Han i Ruse i Fladstrand, mellem Gaunø og Vejlø Skov; en Klosaks af dette Eksemplar indsendtes til Biologisk Station. Og en fuldvoksen Hun (Skjoldets Bredde 71 mm) blev d. 6. November fanget i en Gedderuse i Karrebæk Fjord ved den nordlige Pynt af Gaunø.

Efter at Uldhaandskrabben saaledes i 1934 havde vist sig forskellige Steder omkring Gaunø, og der i Foraaret 1935 yderligere var fanget to Eksemplarer ved Appenæs, kom der Efterretning om, at Uldhaandskrabben var vandret fra Fjorden op i Susaa. Herom har Bibliotekaren ved Næstved Centralbibliotek VALDEMAR HOLST velvilligst meddelt mig følgende: »Den 11. Oktober 1935 toges det første Eksemplar, en Hun, i Susaa saa langt oppe som oven for

Magle Mølle ved Næstved. Krabben blev fundet i et af Magle Mølles roterende Vandfiltre; den har sikkert levet i Omegnen af det Rørsystem, der fra Susaaen fører Vand til Papirfabrikken, er kommet for nær og er derefter blevet suget med Strømmen. Krabben havde mistet de tre midterste Benpar, hvilket intet Under er, naar man kender den store Hastighed, hvormed Vandfiltret roterer. En Uge senere blev en Han fundet samme Sted, og ligeledes med nogle Lemmer afrevet, deriblandt den højre Klosaks. For at komme oven for Papirmøllen maa disse Krabber enten være klatret over en med »Aaletrappe« forsynet ca. 2 Meter høj Stenvold, der danner et Stemmeværk, eller være gaaet ca. 12—15 Meter over Land, uden om Stemmeværket«. Hr. Holst var saa venlig at sende mig begge Eksemplarer fra Susaa[1], og jeg kan derfor angive følgende Maal for dem:

	Afstand imellem 3die Benpars Spidser	Skjoldets Bredde	Skjoldets Længde
♂	318 mm	70 mm	65 mm
♀	?	67 mm	65 -

[1] Ved Undersøgelse af disse lemlæstede Eksemplarer viste det sig, at samtlige manglende Ben (3 paa det ene og 6 paa det andet Eksemplar) er brækket af paa et ganske bestemt Sted, nemlig imellem 2. og 3. Led. Paa et ubeskadiget Lem er disse Led vokset ubevægeligt sammen, men paa det derved fremkomne Dobbeltled ses paa Sammenvoksningsstedet en fin Rille, hvor Chitinen er tynd, og proximalt herfor gaar tværs igennem Benet en Hinde. Ved Sammentrækning af en særlig Muskel kan Benet sprænges af paa Sammenvoksningsstedet, Hinden lukker da for Saaret paa den tilbageblevne Benstump, saa at Blødning forhindres. En frivillig Afkastning (Autotomi) af et Ben kan finde Sted, f. Eks. naar Krabben bliver grebet ved et Ben af en Fjende, eller naar et eller flere Ben kommer i Klemme paa anden Maade (i foreliggende Tilfælde vel i de roterende Vandfiltre). Senere vokser der paa Brudstedet et nyt Ben frem, tiltagende i Størrelse for hvert Skalskifte. Paa et af Eksemplarerne fra Susaa ses paa Brudfladerne et lille, knopformet Fremspring, Anlæg til det nye Ben. Selvamputation hos Uldhaandskrabber er iøvrigt allerede iagttaget og beskrevet af Dr. Panning (l. c. S. 54—58), der tillige har givet en Serie Figurer af Regenerationens successive Forløb (Fig. 23 A og B).

I Slutningen af Oktober fangedes endnu en Uldhaandskrabbe i Susaaen oven for Magle Mølle, ligeledes i et roterende Vandfilter; det er en Han, hvis Skjold maaler 70 mm i Bredde og 68 mm i Længde. Meddelelsen herom skylder jeg Lektor JOHS. FERDINAND, Herlufsholm, hvor Krabben opbevares i Skolesamlingen.

Lektor FERDINAND meddeler mig ligeledes, at en Fisker fra Appenæs midt i Oktober 1935 fik en Uldhaandskrabbe ved Gaunø; det var en Hun, Skjoldets Bredde 73 mm, dets Længde 68 mm; ogsaa dette Eksemplar opbevares i Herlufsholms Samling.

Et andet Tilfælde af Uldhaandskrabbens Forekomst højt oppe i en Aa paa Sjælland har Magister C. V. OTTERSTRØM henledt min Opmærksomhed paa. Det paagældende Eksemplar er taget i Køge Aa ca. 6 km vest for Køge, af Bestyrer HELGE HOLM HANSEN, Lellinge Fiskeri. Paa Forespørgsel har Hr. HOLM HANSEN meddelt mig, at Krabben er gaaet ad Aaen op i den derværende Sø og derfra ind i en Aalekiste neden for Søen; men for at komme op i Søen maa Krabben enten være kravlet op over et Stemmeværk, som er ca. 1,8 m højt, eller op over temmelig høje (ca. 5 m) og stejle Jordvolde. Dette Eksemplar er skænket til Marinbiologisk Laboratorium, og ved Dr. VEDEL TÅNING's Velvilje har jeg haft Lejlighed til at se det. Det er en Han, taget d. $^{10}/_{9}$ 1935; Skjoldets Bredde er 57 mm, dets Længde 52 mm, det længste Benpars Spændvidde 231 mm.

I 1935 har Uldhaandskrabben endvidere, iflg. skriftlig Meddelelse til mig fra Dr. H. BLEGVAD, vist sig baade paa Falster, Laaland og Fyen. Paa Falster fiskedes et Eksemplar (Skjoldets Bredde 70 mm, Benenes Spændvidde 300 mm) d. $^{19}/_{10}$ i Udløbet af Tingsted Aa (Vandløb Nr. 4); det forevistes levende i Nykøbing zoologiske Have. Paa Laaland

fangedes en Hun (Skjoldbredde 68 mm) d. $^{24}/_{9}$ i Nakskov Inderfjord. Paa Fyen toges d. $^{28}/_{9}$ en Han (Skjoldbredde 63 mm) i Stavis Aa, $^{1}/_{2}$ km fra Odense Kanal.

I 1935 toges d. $^{27}/_{5}$ en Hun (Skjoldets Bredde 73 mm) i den sydlige Del af Ringkøbing Fjord, iflg. skriftlig Meddelelse fra Dr. H. Blegvad.

Paa Østkysten af Sønderjylland fangedes d. $^{27}/_{11}$ 1935 af Fisker P. I. Hansen et Eksemplar i en Ruse ved Indsejlingen til Haderslev Fjord, nærmere bestemt paa Fjordens Nordside ved Ørbyhage. Underretningen om dette Eksemplar, der opbevares i Hajstrup Skole, skylder jeg Læreren, P. F. Gotthelf, der velvilligst sendte mig det til Eftersyn. Det er en Hun, der maaler 270 mm mellem Spidserne af det længste Benpar, Skjoldets Bredde 60 mm, dets Længde 55 mm.

Jeg har nu nævnet de sikre Fund af Uldhaandskrabber her i Danmark; men det kan med temmelig Sikkerhed siges, at Fangsterne kun udgør en ringe Del af det virkelige Antal Forekomster[1]. Desuden bliver de unge Uldhaandskrabber næppe erkendt som saadanne, da det karakteristiske Kendemærke, Klosaksenes Behaaring, kun er udviklet hos udvoksede Eksemplarer.

Da det var mig særlig magtpaaliggende at faa at vide, hvorledes Forholdene har udviklet sig ved Højer Sluse,

[1] Efter at Manuskriptet var afsluttet, meddelte Dr. H. Blegvad mig, at han fra Fisker Albert Nielsen, Kastrup, har modtaget en Klosaks af en voksen Uldhaandskrabbe, som fangedes levende d. 24. 3. 1936 ud for Badeanstalten Helgoland paa Amager, ca. 1000 m fra Land. Endvidere har jeg fra Dr. Bøje Benzon modtaget en Uldhaandskrabbe (♂, Skjoldets Bredde 68 mm, dets Længde 63 mm) fanget d. 22. 5. 1936 i Garn ved Dragør. Og Zoologisk Museum har fra fhv. Inkassator N. C. Christensen modtaget en Uldhaandskrabbe (♂, Skjoldets Bredde 42 mm, dets Længde 38 mm) fanget d. 28. 5. 1936 af Fisker Johansen i Aaleruse i Kalveboderne ved Nordsiden af Slusedæmningen til Amager (nogen Tid forinden havde samme Fisker fanget endnu et Eksemplar).

hvor der, som før sagt, allerede i 1933 og sidenhen i 1934 var fanget adskillige Uldhaandskrabber, henvendte jeg mig til Fisker Boy Petersen, der velvilligst imødekom Anmodningen og tilstillede mig efterfølgende Oplysninger:

»Uldhaandskrabben findes her endnu, og vi bliver vel heller ikke saa hurtig denne Gæst kvit i vore Farvande. Jeg kan med Bestemthed sige, at der fanges flere Uldhaandskrabber for hvert Aar her. Jeg regner med, at vi, der i Fællesskab fisker ved Højer-Sluse, i Efteraaret 1935 har fanget ca. 100 Krabber. Endnu kan vi jo ikke mærke, at den gør større Skade, men det er vel, fordi Antallet ligger saa lavt; men vi ved jo ikke, hvad de næste Aar bringer os.

Krabben drager stadig længere op ad Vidaaen, forbi Rudbøl efter Tønder til. Saa her ved Rudbøl fanger vi ogsaa af og til en enkelt. Et Eksemplar er fanget tæt inde ved Tønder, ved »Lægan«, en Kro et lille Stykke denne Side af Tønder[1].

Enkelte fanger vi hele Aaret rundt, men flest i September, Oktober og November; sidste Halvdel af Oktober fangedes de fleste«.

Den sidste Bemærkning af Boy Petersen, at de fleste Uldhaandskrabber fanges ved Højer Sluse, altsaa ved Vidaaens Udløb i Vesterhavet, i Maanederne September, Oktober og November giver Anledning til at formode, at man her staar overfor en Udvandring fra det ferske Vand til Havet udenfor for Forplantningens Skyld. Og jeg mener, at de Uldhaandskrabber, der er fanget oven for Stemmeværker i Susaa (Oktober) og i Køge Aa (September), ligeledes har været for Nedgaaende for Forplantningens Skyld.

[1] Ca. 14 km fra Udløbet af Vidaaen, naar man følger Aaens Bugtninger.

Det stemmer ogsaa godt med, at Udvandringen til Havet fra tyske Floders nedre Løb gaar for sig fra September til December (jfr. Peters l. c. p. 115).

Vilde man spørge, hvorfra Uldhaandskrabben er kommet til Danmark, maa Svaret lyde: Fra Tyskland.

Den danske Egn, som for Tiden synes at være stærkest inficeret med Uldhaandskrabber, nemlig Vidaaens Opland, ligger nær Grænsen mod Slesvig-Holsten. Her findes i Elbens Munding udstrakte Ynglepladser og Mængder af voksne Uldhaandskrabber, og højere oppe paa Vestkysten er der paavist ægbærende Hunner udfor Büsum. Herfra har Krabben bredt sig nordefter; iflg. Peters (l. c. p. 77) er den i 1928 paavist i Vadehavet ved Wyk paa Föhr og i 1931 ved Nordenden af Sylt, paa selve Kysten allerede i 1924 ved Oekholm og Bongsiel. Disse Steder ligger Højer Sluse saa nær, at Krabbens Indvandring hertil kun er, hvad man kunde vente.

Fra Elben er Uldhaandskrabben, iflg. Peters, trængt ind i Nord-Østersø-Kanalen om til Kielerfjord; endvidere op ad Østsiden af Holsten og Slesvig, hvor den i 1932 havde naaet Slien. Som en Fortsættelse af denne Udbredelse kan betragtes den tidligere (S. 12) omtalte Forekomst ved Indløbet til Haderslev Fjord i 1935.

Hvorledes Uldhaandskrabben er kommet højere op paa Jyllands Vest- og Østkyst, i Limfjorden og til Øerne, kan man kun udtale sig hypotetisk om. Om de voksne Krabber, der synes at holde sig til Kysternes Nærhed, kan vandre paa Bunden af Østersøen over til de danske Øer, kender man intet til, og der er andre Maader, hvorpaa Uldhaandskrabben kan spredes. I Tyskland har man iflg. Peters (l. c. p. 68) fundet et lille Eksemplar i en Spalte

i Roret af et Fartøj saa højt oppe i Elben, at Krabber af saa ringe en Størrelse ikke naturligt kan være naaet derop. Det kan derfor tænkes, at de mindre Handelsfartøjer, de saakaldte Everter, som i betydeligt Antal sejler Stykgods fra tyske Østersøhavne til Danmark, lejlighedsvis kan bringe Krabber med sig gemt i Bundbevoksningen eller andre Steder. Den Mulighed kan heller ikke afvises, at Kvaser med Dam (et stort i Fartøjet indbygget Hyttefad), der transporterer levende Fisk fra Danmark til tyske Havne, kan bringe Smaakrabber eller Krabbelarver i Dammen fra tysk Kyst tilbage til dansk Kyst. For en Transport af Uldhaandskrabber med Skibe kunde tale den Omstændighed, at disse Dyr især har vist sig ved eller nær ved Søkøbsteder (Ringkøbing, Skive, Randers, Odense, Nakskov, Nykøbing Falster, Næstved, Køge), men det kan ogsaa tænkes, at de ved disse Byer udmundende Aaer har lokket Krabberne netop hertil.

Der er endnu et Middel, hvorved Uldhaandskrabben kan spredes fra tyske til danske Kyster, nemlig ved Havstrømme. Langs Jyllands Vestkyst løber som bekendt en nordgaaende Strøm, og den kan føre Krabbens pelagiske Larver og svømmende Unger med sig fra Ynglepladserne i Elbens Munding. I denne Forbindelse kommer man uvilkaarligt til at tænke paa en Afhandling af C. H. Ostenfeld[1], hvori han paaviste, at en indo-pacifisk Diatomé *Biddulphia sinensis*, som sandsynligvis med et Skib, i dets Bundbevoksning eller Ballastvand, var indslæbt til Elbens Munding i September 1903, spredtes med Strømmen op langs Jyllands Vestkyst omkring Skagen og ned i Kattegat, hvortil den naaede allerede i November samme Aar.

[1] Meddel. fra Kommiss. for Havundersøgelser, Serie: Plankton, Bd. I, 6, 1908.

Endvidere har Cand. mag. Helge Thomsen, Hydrografisk Laboratorium, venligst tilladt mig at omtale nogle hidtil upublicerede Resultater af Forsøg med Strømflasker udsat i 1921 fra Horns Rev Fyrskib. De nedenfor anførte Strømflasker er de, der hurtigst har passeret de respektive Strækninger, hvilket naturligvis har størst Interesse i den foreliggende Forbindelse:

8 Strømflasker udsat i Januar genfundet 7-8 Døgn senere ved Thyborøn.

1 Strømflaske udsat i Marts genfundet 12 Døgn senere paa Vestkysten omtrent midt mellem Hirtshals og Skagen.

3 Strømflasker udsat i Marts er paa 14 Døgn drevet til den svenske Kyst ved Göteborg og nordefter.

Endvidere har Hr. Helge Thomsen meddelt mig, at Strømstyrken fra Elbmundingen til Horns Rev næppe er mindre end paa Strækningen Horns Rev og nordefter. Strømmen skulde saaledes let kunne gennemløbe Strækningen fra Elben til Skagen paa en Maanedstid.

Den af Ostenfeld paa Grundlag af *Biddulphia*'s Drift beregnede Strømhastighed viser sig saaledes ingenlunde at være overdrevet. Heraf følger igen, at der er rigelig Tid for Uldhaandskrabbens Larver (*Zoëa*- og *Megalops*-Stadierne), som iflg. Peters's og Schnakenbeck's Undersøgelser (l. c. p. 129 og 161) kommer frem i April og i Juli gaar over i Bundstadiet, til med Strømmen at spredes fra Elbmundingen mod Nord; men ogsaa de unge Krabber kan føres med Strømmen, idet de — indtil de har naaet en Panserlængde af 25 mm — kan svømme og saaledes hæve sig op fra Bunden og holde sig i de frie Vandmasser (jfr. Peters l. c. p. 92). Derved kan forklares Forekomsten af Uldhaandskrabber i Ringkøbing Fjord, ved Lyngby Nord for Thyborøn

og inde i Limfjorden ved Skive. Forekomsten ved Lyngby har iøvrigt allerede PETERS (l. c. p. 67) forklaret saaledes, »dass die Krabbenlarven mit der Strömung von dem Laichgebiet abgetrieben wurden und später die jungen Krabben vom Wege nach ihrem elterlichen Flussgebiet abirrten«.

Men ogsaa i Østersøen, i hvis vestlige Del (Eckernföhrde-Fjord, Kieler Fjord og Lübecker Bugt) der er fundet ynglende Uldhaandskrabber (jfr. PETERS l. c. p. 128), er Strømforholdene gunstige for Transport af Larver til danske Kyster. Da Overfladevandets Strømforhold i den vestligste Del af Østersøen i høj Grad er afhængig af Vindretningen og derfor meget varierende i Retning og Styrke[1], vil der, efter hvad Cand. mag. THOMSEN meddeler mig, fra et hydrografisk Standpunkt intet være til Hinder for, at Strømmen kan overføre Krabbelarver fra den sydvestlige Østersøkyst til vore sydlige Øer. Det Forhold, at Krabberne bl. a. er truffet paa de sydlige Kyster af de danske Øer, kunde ogsaa tale for, at deres Tilstedeværelse skyldes en Indvandring — som pelagiske Larver eller svømmende Unger — fra Sydvest.

Det vil heraf fremgaa, at Danmark ifølge sin Beliggenhed maa være ret udsat for at inficeres med Uldhaandskrabber fra Tyskland, idet de kan komme hertil dels aktivt ved Vandringer, dels passivt ved Fartøjer og Havstrømme. Af den S. 5—14 givne Oversigt og Kortet (Tavle I) over de sikkert konstaterede Tilfælde af Uldhaandskrabbens Optræden hos os fremgaar jo ogsaa, at den har vist sig paa mange og vidt spredte Steder. Man kunde derfor befrygte, at den efterhaanden vil blive til lignende Plage hos os som i Vest-Tyskland, hvor man klager over, at den beskadiger

[1] Jfr. R. KOHLMANN: Beiträge zur Kenntnis der Strömungen der westlichen Ostsee (Wiss. Meeresuntersuch. Abt. Kiel N. F. 8. Bd. 1905, p. 192 ff. — O. KRÜMMEL: Handbuch der Ozeanographie, 2. Bd., 2. Aufl., 1911, p. 642 ff.

Fiskernes Garnredskaber, æder Maddingen af Krogene, begnaver de fangne Fisk eller i Ruserne beskadiger dem med sine spidse Torne, ved Mængdeforekomst optræder som Konkurrent til Nyttefiskenes Næring (jfr. Peters l. c. p. 144 — 149) og endelig ved at grave Gange i Brinkerne udsætter dem for Nedbrydning (jfr. Peters p. 96—107). Og paa den anden Side er det hidtil ikke lykkedes at finde nyttig Anvendelse for Uldhaandskrabben, hverken til Føde for Mennesker eller Dyr.

Selv om Uldhaandskrabben har vist sig ret mange Steder her i Landet, er den dog intetsteds optraadt i saadan Mængde, at den kan have gjort Skade. Og saa længe der ikke her i Danmark er paavist en Bestand, der er kommet til Verden hos os, men det maa antages, at Individerne er kommet hertil fra Tyskland, vil der næppe være Fare for Masseoptræden. Hvis det derimod skulde vise sig, at Krabben kan yngle ved vore Kyster, vil der være Grund til Ængstelse, da Krabbens Formeringsevne er overmaade stor. Iflg. Peters (l. c. p. 138) frembringer en enkelt Hun, alt efter dens Størrelse, 270.000—920.000 Æg; men hidtil er der mig bekendt ikke i vore Farvande fundet nogen Uldhaandskrabbe med Æg.

Hvorvidt Uldhaandskrabben kan yngle i danske Farvande, beror først og fremmest paa Saltholdigheden. I 1933 beretter Dr. Peters (l. c. p. 132), at vel parrer Uldhaandskrabben sig saa højt oppe i Elbmundingen, at Saltholdigheden kun er 5.6—9.6 ‰, men at Hunnerne straks derefter opsøger det salte Vand længere til Søs. Senere har Dr. Peters oplyst, at en Saltholdighed af mindst 15 ‰ er nødvendig for Æggenes og Larvernes Udvikling[1]. Betragter

[1] Schleswig-Holsteinische Landeszeitung, 18. Marts 1936. — Rigtigheden af denne Angivelse har Dr. Peters venligst bekræftet for mig i et Brev af 22. April.

man nu Isohalinernes Forløb paa de Tavler (II—V), som Dr. J. P. JACOBSEN har udarbejdet for de danske Farvande[1], da finder man, at en Saltholdighed af 15 ‰ og derover findes, kort udtrykt, i Kattegat, Lille Bælt og den vestligste Ende af Østersøen, hvorimod den øvrige Østersø har Saltholdighed under 15 ‰. Heraf følger igen, som de hidtidige Erfaringer tyder paa, og som ogsaa Dr. PETERS fremhæver, at i Østersøen yngler Uldhaandskrabben kun i den allervestligste Del. Langs det østlige Jylland derimod vilde der være Mulighed for, at Uldhaandskrabbens Æg og Larver kunde bringes til Udvikling. Men Saltholdigheden er næppe den eneste Faktor, hvoraf Uldhaandskrabbens Forplantning afhænger. Ogsaa Havvandets Temperatur kan have Indflydelse, men til dennes Betydning for Uldhaandskrabbens Forplantning har vi endnu intet Kendskab. Det var jo tænkeligt, at Uldhaandskrabben for sin Forplantning kræver højere Temperaturer end de i de salte Dele af vore Farvande herskende, med andre Ord: at vel kan de voksne Uldhaandskrabber trives i vore Farvande, men at Temperaturen er for lav til, at de kan forplante sig.

Endnu en Betingelse for en Masseforekomst maa være til Stede, nemlig at vore Aaer er saa rige paa den for disse Krabber nødvendige Næring (Krebsdyr, Insekter, Larver, Orme, Snegle, Muslinger og Vandplanter), at den kan slaa til til at opretholde store Bestande af disse overmaade graadige Dyr. Selv vore største Aaer er jo for intet at regne mod Tysklands Floder.

Det er saaledes endnu et aabent Spørgsmaal, om Uldhaandskrabben i Danmark som nu nærmest vil være at

[1] Mittelwerte von Temperatur und Salzgehalt bearbeidet nach Hydrographischen Beobachtungen in Dänischen Gewässern 1880—1907. Meddel. fra Kommiss. for Havundersøgelser, Serie: Hydrografi, Bd. I, 10, 1908.

betragte som en interessant Mærkværdighed, eller om den som i det vestlige Tyskland vil udvikle sig til en virkelig Plage. Under alle Omstændigheder bør man opmærksomt følge Udviklingen her i Danmark, hvadenten den gaar i den ene eller i den anden Retning.

Trods Beretninger om, at denne asiatiske Indkomling skulde være i Tilbagegang i europæiske Vande, viser en saglig Undersøgelse, at det ikke er Tilfældet. Saaledes fangedes der i 1935 10.000 Centner Uldhaandskrabber i Elben (Peters, 1936). I Holland vokser Bestanden stadig[1]. Endvidere har Uldhaandskrabben i de senere Aar bredt sig til Belgien[2] og til Polen[3], og den er trængt ind i Rusland[4]. Uldhaandskrabben er ligeledes kommet til Sverige (paa Østkysten imellem ca. 56° og 61° N. B.) og Finland (ved Viborg og Åbo), hvor henholdsvis Professor Hanström[5] i Lund og Professor Luther[6] i Helsingfors har gjort Rede for dens Optræden. Til Norge synes den endnu ikke at være naaet, iflg. Prof. Aug. Brinkmann[7]. Derimod har den nylig vist sig i England, i Themsen ved London[8].

Jeg har ment, det kunde have sin Betydning at give denne Oversigt over Uldhaandskrabbens Indvandringshistorie i Danmark, idet den bør skrives, inden Kilderne til Oplysning herom ikke længere vil være at opspore.

[1] Y. P. Otto und L. F. Kamps, Zoologischer Anzeiger, 110. Bd., 1935, p. 109.
[2] S. A. Lestage, Pêche et Pisciculture, 42. Année, Bruxelles 1931, p. 247.
[3] W. J. Kulmatycki, Zoologischer Anzeiger, 106. Bd., 1935, p. 164.
[4] Iflg. Dr. Peters, l. c. 1936.
[5] Fauna och Flora, 29. Årg. 1934, p. 273.
[6] Memoranda Societatis pro Fauna et Flora Fennica, 10, 1934, p. 69.
[7] Naturen, 58. årg. 1934, p. 33.
[8] Nature, vol. 136, 1935, p. 673.

Zusammenfassung.

Die chinesische Wollhandkrabbe (*Eriocheir sinensis* M.-Edw.) in Dänemark.

Nach einer kurzen Einleitung über die Uebertragung nach Nord-Deutschland und Holland der in Nord-China heimatlichen Wollhandkrabbe und ihre Verbreitung in diesen Ländern (S. 3—5), wird über ihr Vorkommen in Dänemark berichtet (S. 5—14), (vergl. Tafel I):

1927.

(Auf der Karte mit + gezeichnet).

Jütland: Lyngby, ein Fischerdörfchen an der Westküste, nördlich vom Limfjord. 1 Expl.

1933.

(Auf der Karte mit △ gezeichnet).

Jütland: Højer Sluse an der Westküste Südjütlands. Mehrere Expl.

1934.

(Auf der Karte mit ○ gezeichnet).

Jütland: Højer Sluse. Mehrere Expl.

Ribe Aa, gerade östlich von der Kammersluse. 1 Expl.

Limfjorden, Skive Fjord nahe bei Skive-Karup Aa. 2 Expl.

Randers Fjord, Tjæreby Bredning. 1 Expl.

Seeland: Karrebæk Fjord. 2 Expl.
Køge Aa vom Ausflusse 1 km aufwärts. 3 Expl.
Kalveboderne südlich von der Schleuse. 1 Expl.

1935.

(Auf der Karte mit ● gezeichnet).

Jütland: Højer Sluse, etwa 100 Expl.
Rudbøl bei Vidaa, dann und wann ein einzelnes Exemplar.
Vidaa bei Tønder, etwa 14 km vom Ausflusse des Bachs. 1 Expl.
Ringkøbing Fjord. 1 Expl.
Haderslev Fjord, in der Mündung. 1. Expl.
Fünen: Stavis Aa, $^1/_2$ km vom Odense Kanal. 1 Expl.
Laaland: Nakskov Inderfjord. 1 Expl.
Falster: Tingsted Aa, Ausfluss. 1 Expl.
Seeland: Karrebæk Fjord. 3 Expl.
Susaa, oberhalb Maglemølle, etwa 5 km vom Ausflusse des Bachs. 3 Expl.
Køge Aa, bei Lellinge, 6 km vom Ausfluss. 1 Expl.

In Susaa und Køge Aa hat die Krabbe die Stemmenwerke passiert.

Dann werden (S. 14—17) die verschiedenen Weisen besprochen, in welchen die Wollhandkrabbe von Deutschland nach Dänemark gekommen sein mag, nämlich aktiv durch Wanderungen, passiv durch Fahrzeuge und Meeresströme.

Alle in Dänemark bis jetzt gefundene Wollhandkrabben waren erwachsene Individuen, aber kein eiertragendes Weibchen wurde bis jetzt gefunden. Man weiss deshalb noch nicht, ob die Wollhandkrabbe an unseren Küsten laichen und sich in beunruhigem Grade vermehren kann.

Die Bedingungen dafür, dass die Fortpflanzung der Woll-

handkrabbe in dänischen Gewässern (Salzgehalt und Temperatur) durchgeführt werden kann, werden diskutiert (S. 17—20).

Auch weiss man nicht, ob unsere relativ kleinen Wasserläufe Nahrung in genügender Menge darbieten, um grössere Mengen von diesen Krabben unterhalten zu können.

Es ist also noch eine offene Frage, ob die Wollhandkrabbe in Dänemark, wie jetzt, sozusagen als Kuriosum betrachtet werden soll, oder ob sie sich, wie in Deutchland, durch Massenvorkommen zu einer wirklichen Plage entwickeln kann.

Schliesslich wird (S. 20) die weitere Verbreitung der Wollhandkrabbe in Europa in den allerletzten Jahren erwähnt.

Meddelt i Mødet den 3. April 1936.
Færdig fra Trykkeriet den 13. Juli 1936.

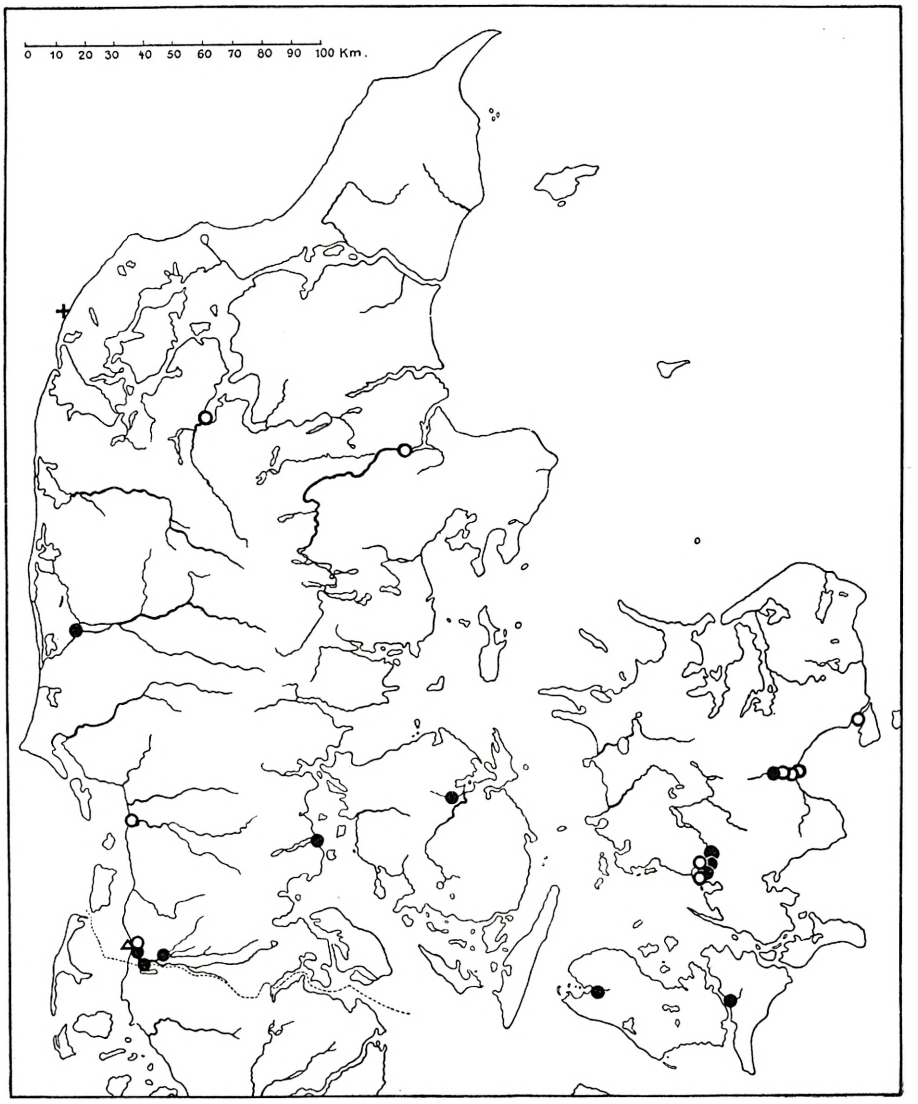
Tavleforklaring.

Tavle I. Oversigtskort over de sikre Fund af *Eriocheir sinensis* i Danmark.

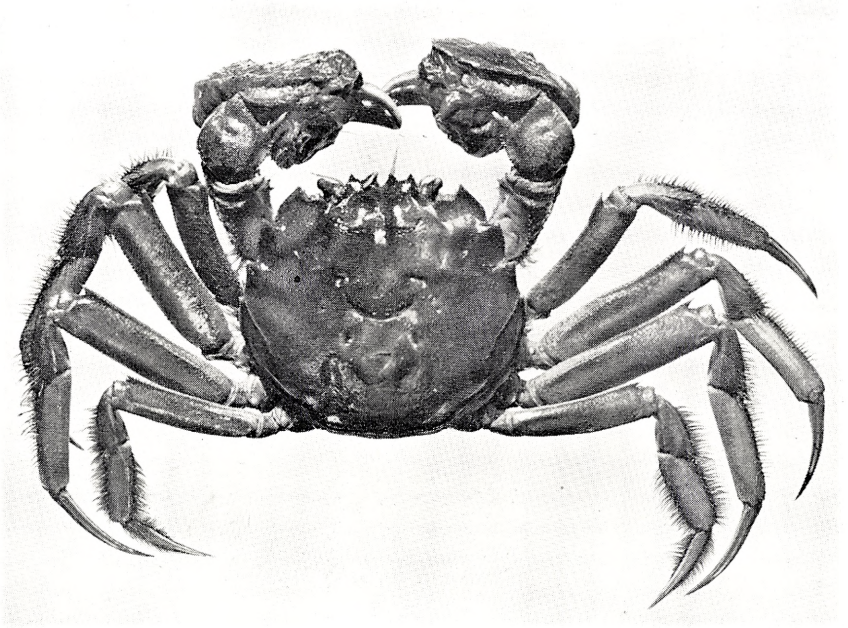
Tavle II—III. Uldhaandskrabber (*Eriocheir sinensis* M.-Edw.) fra Højer Sluse paa Vestkysten af Sønderjylland, November 1934. Lidt over halv Størrelse.

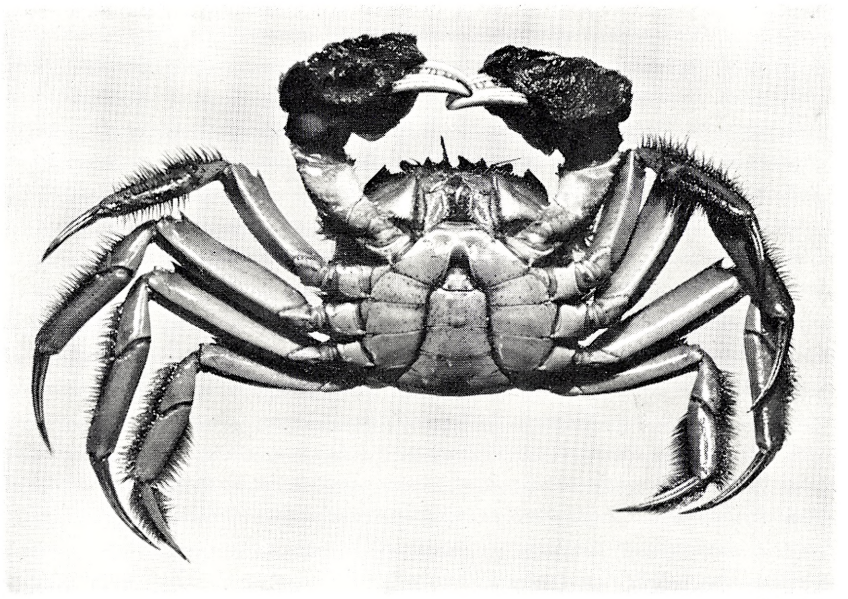
Tavle II. Han, fra Over- og Undersiden.

Tavle III. Hun, fra Over- og Undersiden.



De sikre Fund af *Eriocheir sinensis* i Danmark.
+ 1927. △ 1933. ○ 1934. ● 1935.





I. L. fot.

Eriocheir sinensis ♂.

I. L. fot.

Eriocheir sinensis ♀.

BIOLOGISKE MEDDELELSER

UDGIVNE AF

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ON A NEW BOTTOM-SAMPLER FOR INVESTIGATION OF THE MICRO FAUNA OF THE SEA BOTTOM

WITH REMARKS ON THE QUANTITY AND SIGNIFICANCE OF THE BENTHONIC MICRO FAUNA

BY

AUGUST KROGH AND R. SPÄRCK

KØBENHAVN
LEVIN & MUNKSGAARD
EJNAR MUNKSGAARD
1936

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While the benthonic macro fauna of the sea has in the last decades been subject to a series of investigations, especially by C. G. Joh. Petersen and his collaborators, we have until now only very little knowledge of the quantitative composition of the micro fauna of the sea bottom. This deficiency has certainly been caused by the lack of a suitable apparatus for taking samples for this purpose. Some years ago Th. Mortensen[1] (1925) described an apparatus for catching the micro fauna of the sea bottom; this apparatus has turned out to be very useful for collecting animals of several groups, as for instance free living *Nematoda*, *Ostracoda*, bottom *Copepoda*, *Cumacea* etc., as it is obvious from the investigations of Remane[2] (1933) in the Bay of Kiel, but it does not give any information regarding the quantity of the said micro fauna. As it may be of importance to our conception of the production and metabolism of the sea to know the rôle of the micro fauna in these processes we have been anxious to elaborate an apparatus for this purpose.

After several trials we have arrived at the construction shown in fig. 1 and 2. It consists of a brass frame which can be loaded with lead weights. Six arms carrying brass

[1] Th. Mortensen: An apparatus for catching the micro fauna of the sea bottom. (Vid. Medd. **80**, 1925).

[2] A. Remane: Verteilung und Organisation der Benthonischen Microfauna der Kielerbucht. (Wiss. Meeresunters. N. F. **21**, Abt. Kiel 1933).

tubes of 40 cm. length and 35 mm. inside diameter are provided. These arms are interchangeable and the apparatus can work with 2, 3, 4 or 6 according to the character of the bottom. Each metal tube carries a rubber valve allowing water to pass out. The bottom samples are taken into celluloid tubes of 50 cm. length, 28 mm. inside and 33 mm. outside diameter securely fastened in the metal tubes by means of rubber tubing. The right dimensions for these tubes have been adjusted by trial to conditions in Danish waters. Narrower tubes will give smaller samples and from wider tubes the bottom material is likely to drop out, especially when the sampler is lifted out of the water.

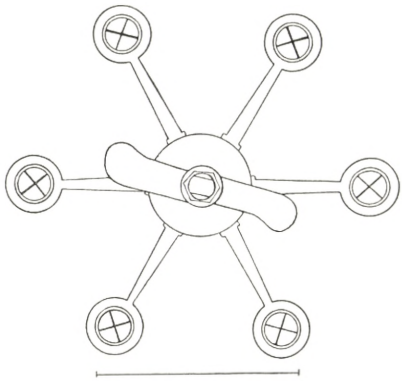

Fig. 1.

When the apparatus strikes the bottom the tubes penetrate to a certain depth. The load and the number of tubes should be so adjusted that this depth is at least 10—15 cm.

When the sampler gets on board the celluloid tubes are immediately stoppered and removed (the valves must be kept open during this process) and when fresh tubes are inserted the sampler is again ready for use.

Each sampling tube now contains a column of undisturbed bottom material. The upper ends are also closed and the tubes which must be kept in a vertical position can be brought to the laboratory. The bottom animals will keep alive for several hours at room temperature, but whenever possible they should be kept in a refrigerator at 2°—4°.

The sorting out of the micro fauna has been made in the following way. The water column has first been drawn from the tube by means of a siphon into flat dishes where it can be investigated with a binocular microscope. Then the column of bottom material is forced by means of a rubber piston to the upper end of the tube, and when it reaches about 1 cm. over the upper edge of the tube a slice of bottom material 1 cm. thick is cut off. In some cases it may perhaps be more practical to drive the material the other way out, which can be effected by applying air pressure to the column by means of a bicycle pump.

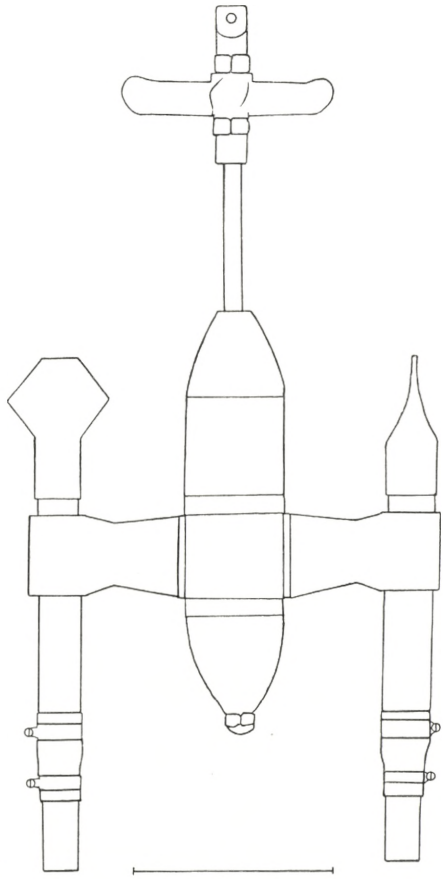

Fig. 2.

We have found that by far the greater part of the micro fauna is present in this upper layer of the bottom. This slice is washed into sea water and is then sifted through a sieve with a size of meshes of about 0.5 mm. By this process the larger specimens of the micro fauna are kept back. The water and bottom material going through this sieve are then examined in small glass dishes with a binocular microscope at a mag-

Table 1. Numbers and weights in grammes per sq. m. of of the harbour

Dates of collecting	26. VIII. 1935		2. IX. 1935		25. IX. 1935	
Names of groups	Number per sq. m.	Weight per sq. m.	Number per sq. m.	Weight per sq. m.	Number per sq. m.	Weight per sq. m.
Prosobranchiata (Hydrobia)	10600	6 g.	37000	22 g.	30000	18 g.
Opisthobranchiata	...	...	...	...	200	0.3 g.
Small Lamellibranchiata	800	0.8 g.	800	0.8 g.	800	0.8 g.
Isopoda	...	...	...	...	200	0.4 g.
Amphipoda	800	1.4 g.	600	1 g.	1000	1.7 g.
Copepoda	1600	0.2 g.	2600	0.3 g.	2200	0.3 g.
Polychæta	1800	2 g.	1200	1.2 g.	200	0.2 g.
Oligochæta	5800	5.8 g.	1200	12 g.	3600	3.5 g.
Nematoda	37000	3.7 g.	47000	4.7 g.	25000	2.5 g.
Turbellaria	...	...	1000	0.7 g.	1600	1 g.
Infusoria	600	0.6 g.	400	0.3 g.	200	0.1 g.
Foraminifera	...	...	...	...	...	...
Total ...	59000	20.5 g.	91800	43.0 g.	65000	28.8 g.

nification of about 20. Deeper than 1 cm. only *Polychæta*, *Oligochæta* and *Nematoda* are found in larger quantities. Therefore it is sufficient to strain the rest of the column through a sieve which will retain these forms. Before this sifting the bottom material is washed into fresh water; by this process the Nematodes uncoil so that they can be kept back in the sieve together with the larger animals.

In the last few years we have used this apparatus in a number of cases and in different parts of Danish waters. In most cases we have succeeded in getting suitable samples in localities where the bottom consisted of clay or mud; and in the autumn and winter 1935—36 we have made a more regular investigation at some localities in the Sound. In the following we shall give some main results of this investi-

the micro-fauna at a depth of 5.5 m. in the outer basin of Copenhagen.

10. XII. 1935		18. XII. 1935		23. I. 1936		27. I. 1936	
Number per sq. m.	Weight per sq. m.	Number per sq. m.	Weight per sq. m.	Number per sq. m.	Weight per sq. m.	Number per sq. m.	Weight per sq. m.
22600	13.5 g.	16000	11.6 g.	52000	31.0 g.	29000	18.0 g.
...	...	...	...	...	...	...	...
200	0.2 g.	200	0.2 g.	800	0.8 g.	1200	1.2 g.
...	...	...	...	400	0.5 g.	1600	2.0 g.
200	0.2 g.	800	1.0 g.	...	...	400	0.4 g.
1000	0.1 g.	3600	0.4 g.	2800	0.3 g.	7800	0.8 g.
200	0.5 g.	2200	6.0 g.	1400	2.8 g.	1000	1.7 g.
12600	13.0 g.	14000	14.0 g.	17000	17.0 g.	26000	26.0 g.
43000	4.3 g.	53600	5.4 g.	71000	7.0 g.	66000	6.6 g.
400	0.4 g.	1000	1.0 g.	400	0.4 g.	1000	1.0 g.
200	0.1 g.	...	...	...	...	200	0.1 g.
...	...	200	0.1 g.	1400	0.7 g.	200	0.1 g.
80400	32.3 g.	91600	39.7 g.	147200	60.5 g.	134400	57.9 g.

gation to show the applicability of the apparatus. The sorting out of the material from this investigation has been made by Mag. sc. HOLGER MADSEN, and the technique of sorting out described above is due to him.

The localities which have been specially investigated are the outer deep basin of the harbour of Copenhagen, where the samples have been taken at a depth of 5.5 m., and a locality in the Sound a little northwest of Middelgrunden, where the samples were taken at 17 m. At these two localities samples have been taken at intervals from the end of August 1935 to the end of January 1936, besides samples have been taken also from greater depths in the Sound, maximum 56 m. The results of this investigation are shown in tables 1—3.

Table 2. Numbers and weights in grammes per sq. m. of the micro-fauna at a depth of 17 m. in the Sound.

Dates of collecting	1. X. 1935		3. XII. 1935		5. I. 1936	
Names of groups	Number per sq. m.	Weight per sq. m.	Number per sq. m.	Weight per sq. m.	Number per sq. m.	Weight per sq. m.
Prosobranchiata	800	0.5 g.	...	...	1200	0.7 g.
Opisthobranchiata	800	0.8 g.	...	...	200	0.2 g.
Small Lamellibranchiata	200	0.2 g.	...	...	...	...
Small Echinocardium cordatum	200	0.2 g.	...	...	...	...
Holothurioidea	...	...	...	...	200	0.2 g.
Halacaridæ	200	0.1 g.	400	0.2 g.	2600	0.3 g.
Cumacea	200	0.3 g.	400	0.6 g.	...	...
Ostracoda	1400	1.5 g.	600	0.6 g.	1600	1.5 g.
Copepoda	14500	1.5 g.	13000	1.3 g.	13000	1.3 g.
Polychæta	1800	4.0 g.	1600	3.6 g.	6200	11.0 g.
Oligochæta	1000	1.0 g.	...	...	11000	11.0 g.
Nematoda	87000	8.7 g.	39000	3.9 g.	46000	4.6 g.
Kinorhyncha	400	0.1 g.	...	...	...	...
Turbellaria	1000	0.7 g.	1000	0.7 g.	3400	2.2 g.
Infusoria	...	...	800	0.6 g.	...	...
Foraminifera	...	...	200	0.1 g.	7000	3.5 g.
Total ...	109500	19.6 g.	57000	11.6 g.	52400	36.5 g.

The weighgings have been made in the following way: A number (5, 10 or 20) of the different size categories in each group has been weighed and "average weight" of the group has been determined in this way. The animals have been weighed in alcohol preserved condition: they have been taken out of the alcohol, superficially dried on filter paper and then weighed. To the weights found have been added 10 per cent. and the weight thus found is to be regarded as the weight of the living animal. According to the experience of the Danish Biological Station the alcohol weight augmented by 10 per cent. is equal to the weights of the living animal.

Table 3. Numbers and weights in grammes per sq. m. of the micro-fauna at depths of 26 and 54 m. in the Sound.

Date of collecting	7. X. 1935 depth 26 m.		7. X. 1935 depth 54 m.	
Names of groups	Number per sq.m.	Weight per sq.m.	Number per sq.m.	Weight per sq.m.
Small Lamellibranchiata	2200	2.2 g.	2800	2.8 g.
Ostracoda	1400	1.5 g.	...	...
Copepoda	2200	0.3 g.	...	...
Polychæta	1400	3.0 g.	2800	6.0 g.
Oligochæta	2200	2.2 g.	...	...
Nematoda	47000	5.0 g.	70000	7.0 g.
Turbellaria	400	0.3 g.	...	...
Infusoria	2200	1.0 g.	...	...
Foraminifera	177000	75.0 g.	2800	1.6 g.
Total	236000	90.5 g.	78400	18.4 g.

In the weights of the molluscs and Foraminifera are included the weight of the shells.

Regarding the distribution of the animals in the different tubes of the apparatus we can refer to table 4. It will be seen from this table that the variations from tube to tube are not greater than each tube will show which of the groups are really common. When we have chosen 6 tubes it is because in this way we can obtain a fairly large material of the rarer groups represented by one or two specimens in each tube.

In spite of the rather few samples of this preliminary investigation it is evident from the tables that the micro fauna on the sea bottom is composed of 50000 to about 200000 individuals per sq. m. Numerically it is consequently far greater than the macro fauna, as was to be expected.

Table 4. Number of specimens in each of the 6 tubes in a single sample (taken in the sound 27. XI. 1935 at a depth of 12 m.).

Name of groups	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Total
Opisthobranchiata	..	..	1	..	..	..	1
Small Lamellibranchiata	..	1?	..	..	..	1	2
Halacaridæ	..	..	2	1	..	..	3
Ostracoda	..	1	..	1	..	..	2
Harpacticoidæ, Cyclopoidæ	5	8	3	4	5	5	30
Polychaeta	..	1	1	2	3	1	8
Oligochaeta	..	1	1	2	..	..	4
Nematoda	47	40	9	41	19	24	180
Turbellaria	1	..	2	3	2	..	8
Infusoria	..	..	1	2	1	1	5
Foraminifera	..	20	20	1	..	5	46

Also regarding the weights the micro fauna proves to be of some importance. The weights vary from 11 to 90 gr./sqm. It seems that the weights are generally a little higher in the samples taken at 5.5 m. compared to those taken at greater depths. However a sample from 26 m. shows the highest value, but this is only due to the very great number of *Foraminifera*. In the papers of Petersen (1913) and Blegvad (1932)[1] we can find information concerning the macro fauna of the same regions. It appears from these papers that the quantity of the macro fauna at depths of about 5 to 10 m. off the Harbour of Copenhagen is 4—500 g., the number of specimens 7—800 on an average. At 5.5 m. the quantity of the micro fauna is 40 g. and 80000 individuals per sq. m. on an average. It seems thus that the micro fauna represents about 10 per cent. of the macro fauna in the said locality.

[1] In Rep. Dan. Biol. Stat. 21 & 37.

At greater depths in the Sound the macro fauna according to the investigations of PETERSEN seems to show quantities of about 200 g. per sq. m. The 3 samples from 17 m. show a micro fauna of 25 g per sq. m. on an average. Also in this case the micro fauna seems to represent about 10 per cent. of the macro fauna. It is likely that the oxygen consumption of the species making up the micro fauna is at least 3 to 4 times larger per g. than that of the macro fauna, and the importance of the micro fauna to the metabolism of the sea bottom must therefore be considered much larger than 10 per cent., probably about 40 per cent.

From the investigations of the fauna of the forest soil by BORNEBUSCH (1930)[1] it appears that in the soil rather big species, as f. inst. earthworms, constitute the greater part of the weight of living animals and consequently they also play the greatest rôle to the metabolism even if they occur in rather small numbers per sq. m. Also in the sea bottom it seems to be animals of the medium size as *Polychæta* and *Lamellibranchiata* whose individual weights are a few grammes, which are responsible for most of the metabolism, even if the rôle of the micro fauna is not quite unimportant. If we regard the micro fauna it is obvious from the tables that not the *Nematoda*, which in nearly all the samples are numerically by far the most predominant, but the *Oligochæta, Polychæta* and *Prosobranchiata* are the most important from a metabolism point of view.

Even if we do not intend at the present time to discuss more in detail the qualitative differences between the samples from the different depths we shall point out some conspicuous features.

In the samples from 5.5 m. small *Prosobranchiata* play a

[1] The Fauna of Forest Soil. Diss. Copenhagen.

very important rôle, further the occurrence of *Amphipoda*, *Isopoda* and many *Oligochæta* is characteristic of this depth. At medium depths (17 m.) the *Amphipoda* and *Isopoda* dissappear, the *Prosobranchiata* are quite insignificant and *Oligochæta* are of smaller consequence. At this medium depht *Copepoda* and *Polychæta* play a greater part and also *Halacaridæ*, *Cumacea* and *Ostracoda* are of a certain importance. At still greater depths the *Foraminifera* seem to be important, whereas the rôle of the other groups seems to be decreasing. The greatest number of species is found at a depth of 17 m. Even if we have not yet made any exact determination it can however be said at the present time that it is in part different species of *Nematoda* which occur at different dephts.

From the above mentioned we have arrived at the conclusion that by means of the described apparatus it will be possible to make thorough investigations of the micro fauna on soft bottom and to make out communities on a quantitative basis similar to the communities of the macro fauna described by Petersen. Further we believe that by means of this apparatus it will be possible to determine the importance of the micro fauna to the metabolism at different depths and in different localities in the waters.

(From the Laboratory of Zoophysiology, Copenhagen University).

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BY

R. SPÄRCK

KØBENHAVN
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INTRODUCTION

Our knowledge of the metabolism of the marine Invertebrata is rather poor. In the course of time a few species have been investigated; as regards the older literature I can refer to the book by KROGH (1915) and the paper by KESTNER (1922) in Winterstein's Handbuch. In recent years some other investigations on the metabolism of marine Invertebrata have been published as for instance the papers by BERKELEY (1921), BRUCE (1926), COLLIP (1921), FISHER, DUVAL & RAFFY (1933), MARSHALL, NICHOLS & ORR (1935), and the two papers by RAFFY from 1933, one containing a bibliography of the subject. But in spite of these publications investigations have hitherto not been made from the point of view of elucidating the relation of metabolism to the geographical distribution of the species investigated and to the normal condition of temperature of the same species. In the last few years the present author has made determinations of the consumption of oxygen of a series of common Lamellibranches. These investigations have been made partly in the Zoophysiological Laboratory of the University of Copenhagen, partly during a stay at the Zoological Station at Naples in 1930. Further, determinations by using the same method have been made by THAMDRUP (1935) in Danish waters, and by THORSON (1936) in arctic waters.

Summarizing these different investigations I think we have now the possibility of examining the hypothesis set forth by the present author (1926) to the effect that the relation between metabolism and temperature is one of the factors playing an important part in the competition among the species in nature, thus being of no small consequence to the regulation of the distribution of the animals.

I am much indebted to the Director of the Zoophysiological Laboratory of the Copenhagen University, Prof. AUG. KROGH, for laboratory facilities and kind advice during these investigations. Further I wish to acknowledge financial assistance received from the Carlsberg Foundation.

Method and Material.

The oxygen consumption has been determined by the method of WINKLER as described by BJERRUM (1903) and transformed in the Laboratory of Zoophysiology to a micro method by using a microburette. In most cases a number of animals, about 10, have been used in each single experiment for the purpose of checking out individual variations. The animals are placed into a glass bottle of a size varying from 100 to 2000 cc. according to the size of the animals of the experiment. The bottle is provided with a ground glass stopper. The bottle with the animals is put into an aquarium with sea-water of the temperature at which the determination is to be made. The content of oxygen of the water in the aquarium is determined. Then the plug is placed in the bottle, the latter is laid on the bottom of the aquarium so that the animals can be evenly distributed in the water which is now enclosed in the bottle. After a suitable time which depends on the relation between the oxygen

consumption of the animals and the water volume available, the bottle is taken out of the water and shaken. The plug is carefully taken out, and by means of a siphon a sample of water of 12 to 15 cc. is taken from the bottom of the bottle into the special sampling flasks, and the oxygen content is now determined again. The difference between the content of oxygen in the water of the aquarium at the beginning of the experiment and that of the water in the bottle at the end of the experiment is due to the consumption of the animals.

One or two of the special sampling flasks of 12 to 15 cc. capacity are filled from the bottom of bottle. To these 0.1 cc. of each of the WINKLER reagents is added, and after a suitable interval HCl is added and 5 or 2 cc. samples pipetted off for titration.

The duration of the single experiment has been varying, and for each species been determined by preliminary experiments with a view to the consumption of oxygen and the size of the animals. In most cases the duration of the single experiment has been a few hours. At very high temperatures only 1 or $^1/_2$ hour. Care has been taken that the content of oxygen in the bottles was never below 20 to 30 per cent. at the end of the experiment. Before the animals were taken into the experiments they have lived 24 to 48 hours in sea water of the same salinity and temperature as in the water of the experiment. As will be demonstrated later the consumption of oxygen at a certain temperature is not the same in cases when the animals have suddenly been transferred to water of that temperature, and in cases when they have lived for some time at that temperature. When each experiment has been finished the animals have been transferred to an aquarium with the same temperature and

salinity as that of the experiment. In most cases several experiments have been made with the same individuals of each species at different temperatures.

The consumption of oxygen is given in cubic cms. per kilo per hour. The animals of each experiment were weighed at the beginning of each series of experiments and again when it was finished. Then the animals were killed and all organic material was taken out of the shells, dried by means of filter paper and then weighed again. In the determination of the consumption of oxygen per hour and kilo these last weights of the animals without shells and the water between the shells have been used.

As far as can be seen the animals do not move in the experimental bottles. Moreover the animals have been narcotized in some cases by means of ethyl-uretane and hereby it has been proved that it is of no importance if the animals in these experiments are narcotized or not.

The material used in the experiments in Copenhagen has been procured partly in the Sound, partly in the Great Belt by the courtesy of the Danish Biological Station, which also has taken some material in other parts of the Danish waters. The material of the experiments in Naples has been procured through the fishermen of the Zoological Station at Naples. The following species have been investigated: In the Mediterranean *Pecten varius*, *P. flexuosus*, *Lima hians*, *L. inflata*, *Venus verrucosa*, *V. gallina*, *Loripes lacteus*, *Dosinia exoleta*, *Tapes decussatus*, *Tellina planata*, *Cardium edule*, *Mytilus edulis*, *Psammobia vespertina* and *Cardita trapezia*. In Danish waters *Ostrea edulis*, *Mytilus edulis*, *Cardium edule*, *Macoma baltica*, *Saxicava arctica*, *Astarte borealis*, *A. montagui*, *A. elliptica* and *Scrobicularia plana*. Further some *Ostrea edulis* and *Gryphaea angulata* from West France have been investigated.

THAMDRUP (1935) determined the oxygen consumption of a few of the above-mentioned species and further that of *Mya arenaria*. At last should be mentioned the determinations by THORSON (1936) of the oxygen consumption of a series of arctic species.

The oxygen consumption at different temperatures.

The results of the experiments mentioned above are shown in the graphs figs. 1–16. In these graphs the temperatures are set off on the abscissa and the consumption in cc. per hour and kilo on the ordinate. The intention of the experiments was not

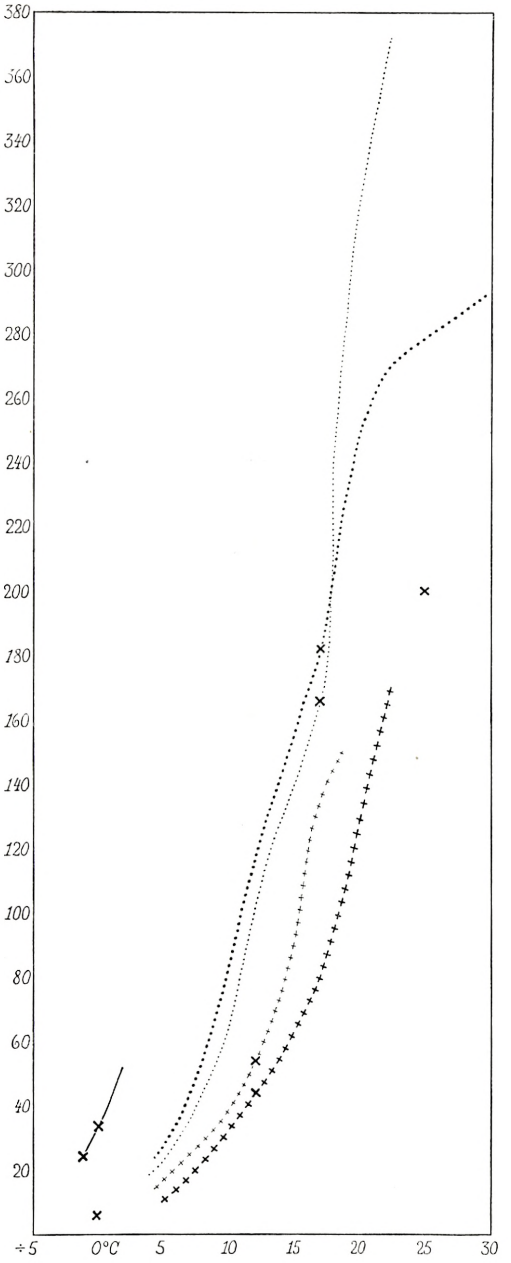

Fig. 1. Oxygen consumption of *Pectinidæ* and *Limidæ* in cc. per kilo and hour. *P. groenlandicus* the fully drawn line, *P. varius* the thick dotted, *P. flexuosus* the thick crossed, *L. inflata* the fine dotted, *L. hians* the fine crossed.

to determine the metabolism from a physiological point of view, but to find out the consumption at different temperatures under otherwise normal conditions to get an idea of the actual requirements of oxygen of the different species in nature. I admit that these results are obtained under experimental conditions, which are not altogether natural, so that they can only give an approximate idea of the amount of the consumption. But I am of the opinion that nevertheless these approximate results are sufficient for the purpose of the present investigation.

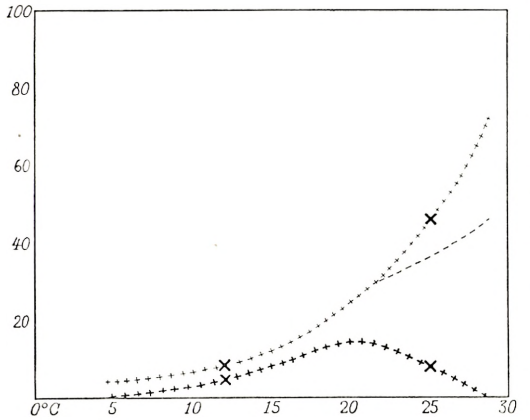

Fig. 2. *Cardita* (the fine crosses) and *Dosinia exoleta* (thick crosses). The broken line shows the consumption by suddenly transferring to high temperature.

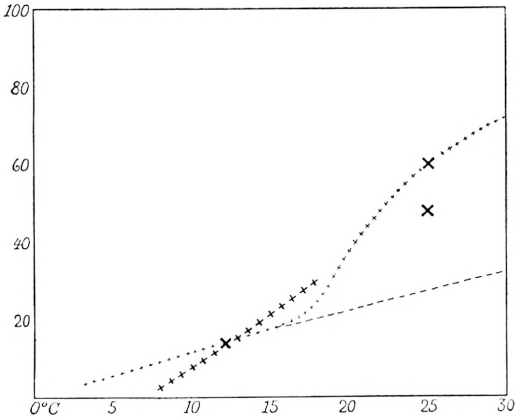

Fig. 3. *Tapes decussatus* (thick crosses) and *Loripes lacteus* (fine crosses). Broken line as above.

From the graphs it is obvious that the Lamellibranches investigated have a rather different consumption and further a considerably different relation between temperature and this consumption. The differences are very marked and more-

over they coincide with other features so that they cannot be due to chance or experimental errors. The Lamellibranches

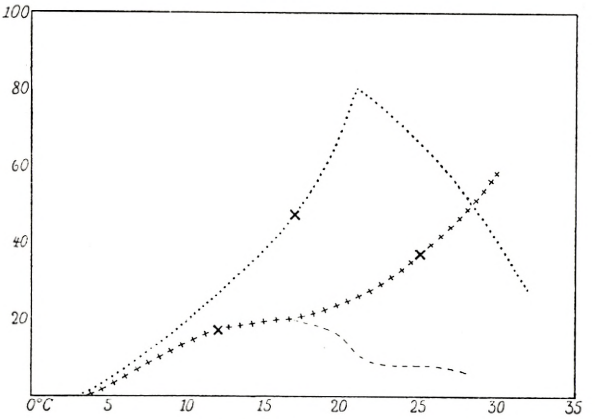

Fig. 4. *Venus gallina* (dotted) and *V. verrucosa* (crosses). Broken line as fig. 2.

can as regards their consumption of oxygen be divided into 3 groups. One group comprising the investigated species of the families *Pectinidæ* and *Limidæ* (fig. 1) has a fairly high consumption, and the increase of the consumption of oxygen at rising temperatures is very considerable. The arctic[1] species, *Pecten groenlandicus*, which lives at temperatures

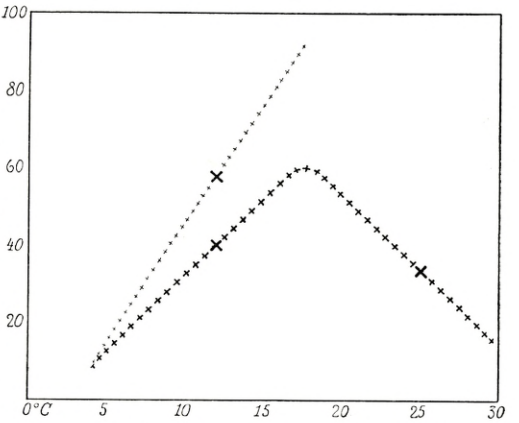

Fig. 5. *Tellina planata*, young (small crosses) and bigger (thick crosses) specimens.

[1] In all the graphs the arctic species is shown by fully drawn lines, that of boreal species by dotted lines, and that of mediterranean by crossed lines. The two larger crosses on the graphs show the limits of the "normal" temperature of the species.

constantly below zero or at least only a few degrees centigrade above zero, has a consumption at $\div 1°$ C. of about

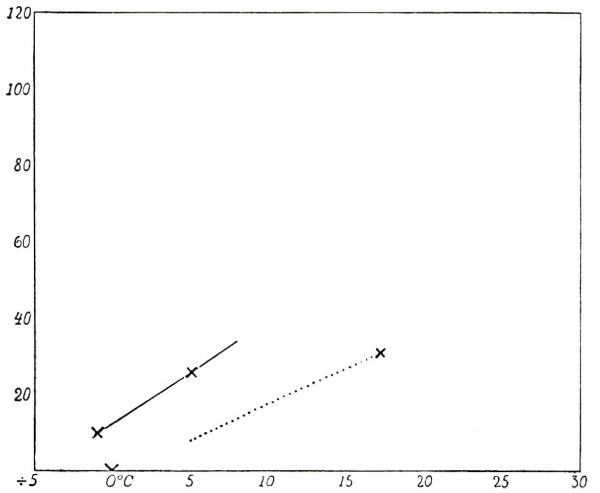

Fig. 6. *Astarte borealis* at Greenland (fully drawn) and Denmark.

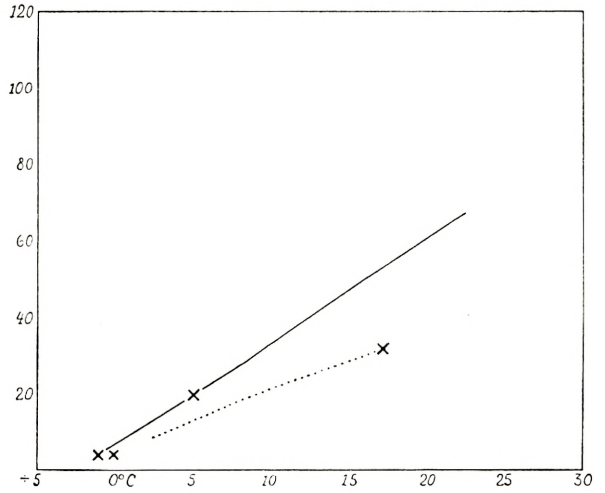

Fig. 7 *Astarte elliptica* at Greenland (fully drawn) and Denmark.

25 cc. per kilo and hour, and already at a few degrees above zero the consumption is about 50 to 60 cc. And it has not been possible to keep this species alive in experiments at

temperatures above 8 to 10° C. Turning to the boreal-mediterranean species *Pecten varius* it will be seen that this species

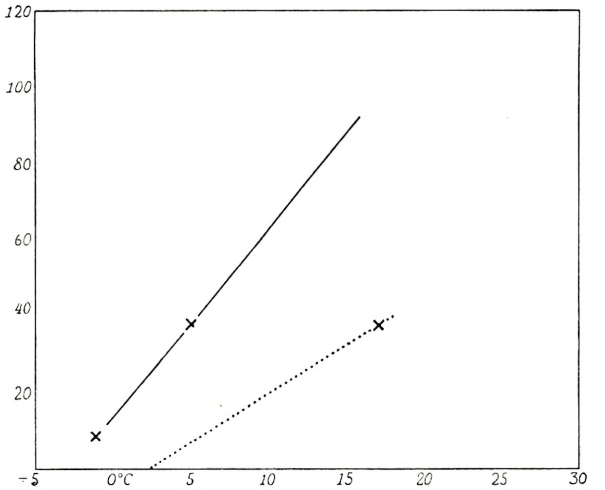

Fig. 8. *Astarte montagui* at Greenland (fully drawn) and Denmark.

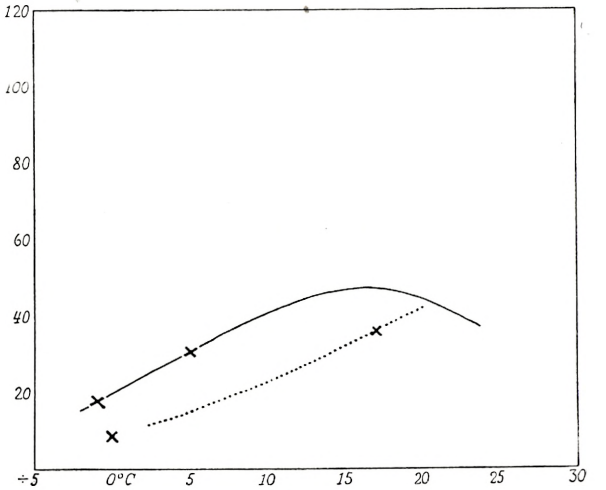

Fig. 9. *Mya truncata* at Greenland (fully drawn) and *M. arenaria* at Denmark (dotted).

has a consumption of about 25 cc. at 5° C. increasing to about 250 cc. at a little above 20° C. and to about 300 cc. at 30° C.

This species lives normally at temperatures between 10 to 20° and at these temperatures may be regarded when at rest as having an oxygen consumption of about 120 to 150 cc. Finally the mediterranean species *Pecten flexuosus* at 5° has an oxygen consumption of about 10 cc. increasing to about 200 cc. between 20 and 25°, and the two *Lima* species *L. inflata* and *L. hians* show quite the same type of consump-

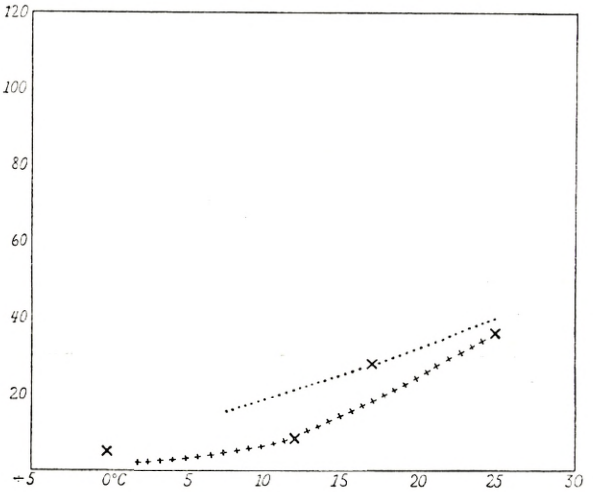

Fig. 10. *Ostrea edulis* (dotted) and *Gryphaea angulata* (crosses).

tion and temperature relation using about 20 cc. at 5° and increasing very steeply to 2—300 at 20°.

Quite different is the oxygen consumption in another much larger group comprising species of *Tellinidæ*, *Astartidæ*, *Veneridæ* and further *Cardita*. As will be seen from the graphs figs. 2—9 all these Lamellibranches at temperatures about zero have a very low consumption. In the mediterranean species only some few cc. per kilo and hour, in the arctic about 10 cc. And further the increase is very slow at rising temperatures. In most species of this group, especially the mediterranean ones, the consumption does not reach

more than 20 to 60 cc. even at temperatures of 20 to 30° C.

Between these two groups, one with a consumption steeply increasing with the temperature and attaining very

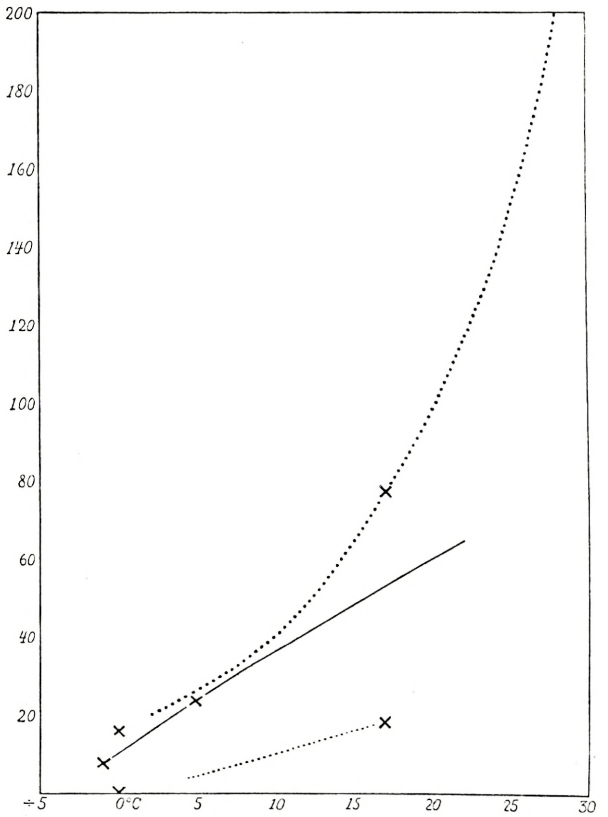

Fig. 11. *Macoma baltica* (dotted, the upper in spawning time) and *M. calcaria* (fully drawn).

high values before fatal temperatures are reached and the other with a very low consumption slowly increasing with the temperature we have an intermediate group consisting of the *Mytilidæ* and *Cardiidæ*, and also *Saxicava* which have a medium consumption of oxygen increasing rather steeply

with the temperature but not so markedly increasing as in the *Pectinidæ* and *Limidæ*.

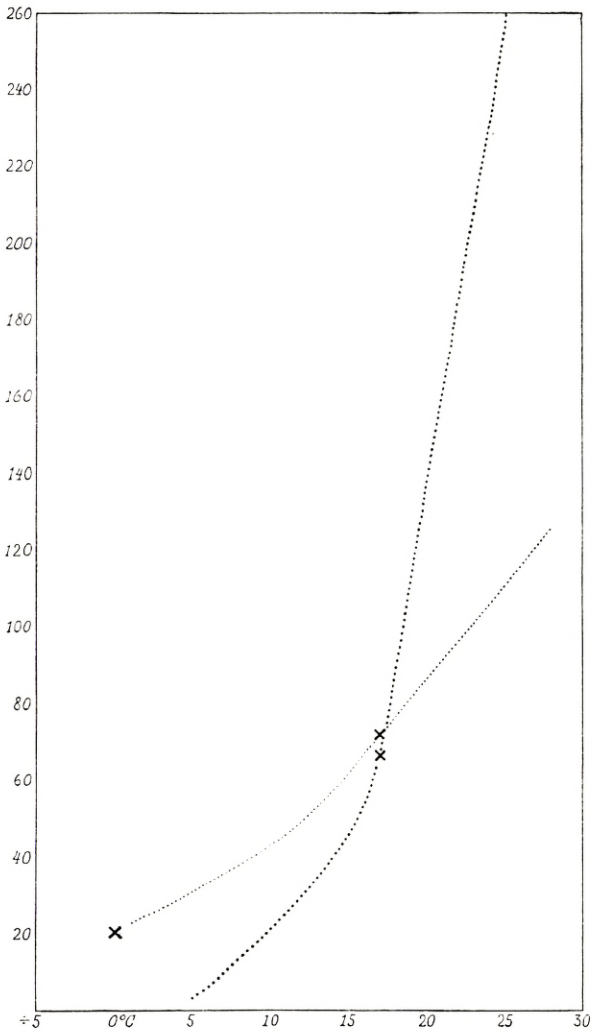

Fig. 12. *Scrobicularia plana.*

It is obvious that these differences in the type of oxygen consumption in the three groups may be related with the different ecology of the species of the families in these

groups. The first group (the *Pectinidæ* and *Limidæ*) consists of species which among the Lamellibranches are the only

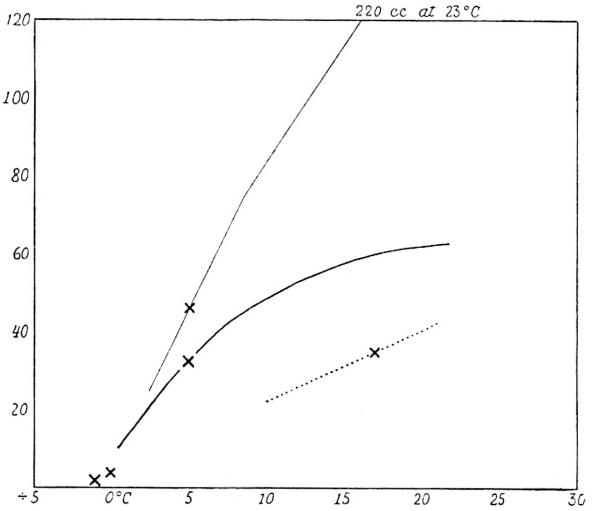

Fig. 13. *Saxicava arctica* at Greenland (fully drawn lines) and Denmark (dotted).

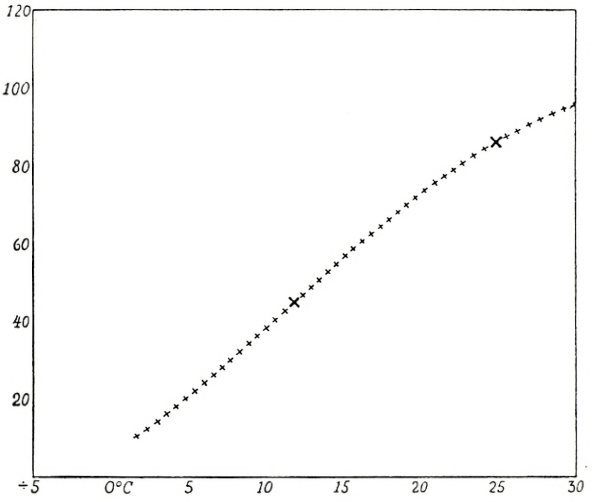

Fig. 14. *Psammobia vespertina.*

ones which are able to move rapidly, it being well known that they can swim fairly well. In contradistinction to the

families of the first group are the members of the second group practically without the possibility of moving over a greater distance. The species of the second group are all living buried into the bottom material, and they are practically sedentary. If food is lacking or scarce a *Pecten* or

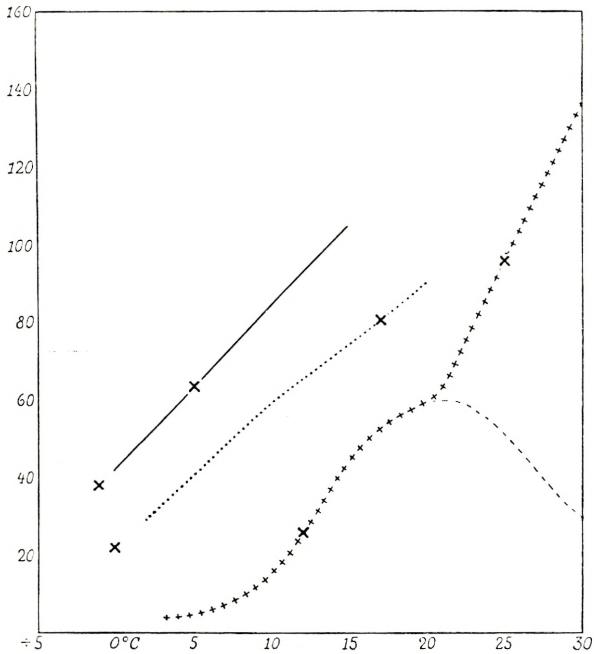

Fig. 15. *Modiolaria laevigata* at Greenland (fully drawn), *Mytilus edulis* at Denmark (dotted) and in the Mediterranean (crosses).

Lima specimen is able to move to other places in search of it. But a *Mya, Tellina, Venus* etc. must in such a case remain on the place where it lives, it can only move some few metres, and try to get through the period of scarcity by reducing its consumption. It is a well known fact that it is rather difficult to keep *Pecten* alive for a longer time in aquaria and specially above the water, whereas the most Lamellibranches of the group with a low oxygen consumption can be kept

for months in aquaria and for hours and days in moist air. The intermediate group consists of species of the epifauna,

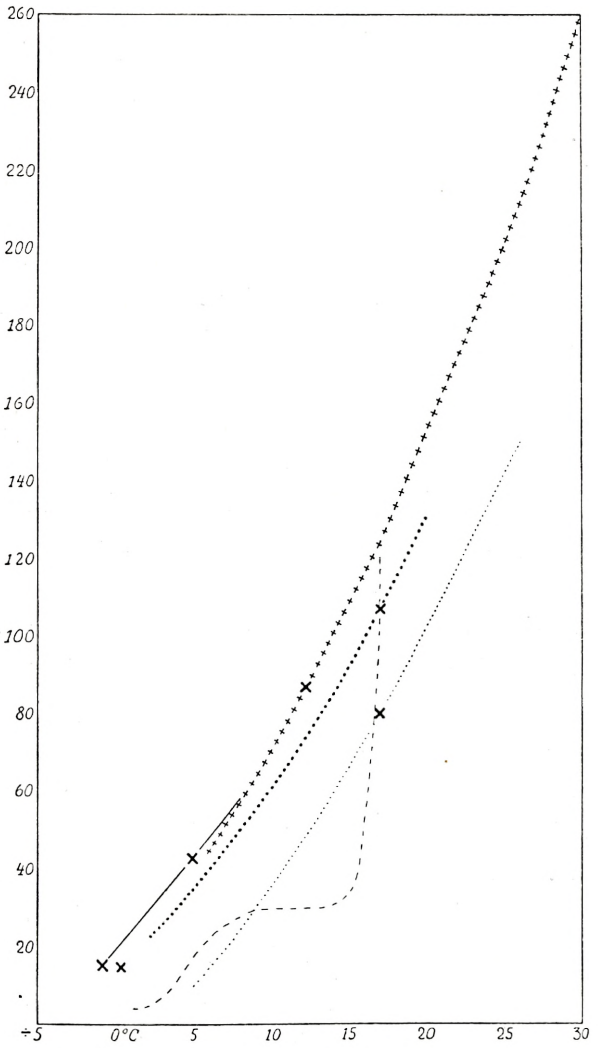

Fig. 16. *Cardium ciliatum* at Greenland (fully drawn), *C. edule* at Denmark (dotted) and in the Mediterranean (crosses, in spawning time).

or of species with a certain possibility of moving as *Cardium* and *Psammobia*. The species of the epifauna as for

instance the *Mytilidæ* and *Saxicava* do not live buried down into the bottom material in calm waters, they are sitting on rocks, stones, vegetation etc. more or less above the bottom material, and their possibility of obtaining food must be regarded as much better than that of most *Tellinidæ, Veneridæ* etc.

As it is obvious from the above mentioned facts there seems to be a marked relation between the type of oxygen consumption especially its relation to temperature and the ecology of the species. Species with a high consumption in which there is a steep increase with the temperature are also by moving and swimming able to get the sufficient food for this high metabolism, and only species with a low oxygen consumption and a slow increase with temperature have been able to live buried down in the bottom material, whereas they cannot compete in localities where the food conditions are good enough to allow the species of the epifauna with a higher metabolism and higher food requirements to live. I am therefore of the opinion that it is justifiable to say that the relation between oxygen consumption and temperature characteristic of each species is an important factor in the competition among the species in nature.

Even if considered more in detail this result seems to be correct. If we are looking at *Scrobicularia plana* (fig. 12) it appears that this Lamellibranch has a peculiarly steep increase of the oxygen consumption with the rising temperature in spite of the biology of the species. *Scrobicularia* is allied to the *Tellinidæ*, but nevertheless the oxygen consumption at a temperature of 25 to 30°C. is about 200 cc., and still the species lives quite as the other *Tellinidæ, Veneridæ* etc. with their low consumption of oxygen. As pointed out some years ago by the present author (1926 p. 282) *S. plana* has a re-

markable distribution indicating that the food problem may be of special importance to this species. It occurs near the mouth of rivers, in the innermost parts of some shallow water inlets and fjords, where there is a considerable production, and finally *S. plana* is characteristic of tidal coasts with the great renewal of water. All these three types of localities are of such a nature that they may be considered to offer exceptionally good food conditions for a sedentary Lamellibranch buried down in the bottom. And a glance at the graph fig. 12 corroborates this view in that the oxygen consumption of *Scrobicularia plana* is of quite another type than that of most other Lamellibranches buried down in the bottom. This explains the peculiar distribution of this species. Also *Macoma baltica* and *Macoma calcaria* seem to differ to a certain degree from the normal type of the *Tellinidæ* (fig. 11), at least at the spawning time during which the consumption of oxygen is rather high. And *Macoma calcaria* has a comparatively high oxygen consumption compared with several other arctic bottom Lamellibranches. In this connection it should be pointed out that just these two species are characteristic of the shallow water of the boreal and arctic waters, and especially *Macoma baltica* is restricted in most waters to a belt of a few meters' depth, only in the Baltic where the majority of their competitors are lacking is it found at greather depths. C. G. Joh. Petersen (1918) has explained this phenomenon in the way that the cause of the greater bathymetrical distribution of *M. baltica* in the Baltic is the lack of enemies, especially *Asterias rubens*, and this may perhaps also be partly responsible for this distribution. I think however that the relatively high oxygen consumption of *M. baltica*, at least in periods, presumably accounts for the explanation that it is the lack of competitors which

is the cause of the greater bathymetrical distribution of this species in the Baltic. In some waters, for instance in the Greenland and the North Atlantic fjords, *Macoma calcaria* has a more restricted bathymetrical distribution than in the North Russian seas and the Spitsbergen waters, and this difference may perhaps also be due to the same cause.

Also among the epifaunistic Lamellibranches there is an exception, the oysters *O. edulis* and *G. angulata* (fig. 10). They have a comparatively low oxygen consumption of the same category as that of the Lamellibranches which live buried down in the bottom. This may certainly be explained in the way that the oysters are absolutely sedentary and cannot move at all for which reason it must be considered of importance to these species in the competition to have an oxygen consumption which rises slowly with temperature.

It should be pointed out that differences in the consumption of oxygen may be expected according to age of the investigated animals and according to the season of the year. Bruce (1926) has shown that there is a remarkable increase in the oxygen consumption at the time of spawning. This has also proved to be the case in several of the Lamellibranches investigated here, for instance in *Macoma baltica* and *Cardium edule,* which have a higher consumption of oxygen in spring and summer than in the autumn, whereas *Astarte borealis* which is an autumn spawner has a higher oxygen consumption in the autumn. When the consumptions of oxygen are compared care must be taken that animals from the spawning season are not compared with animals from another season, and further that the animals are of the same size category, since small individuals have a somewhat higher consumption calculated per unit weight.

As mentioned above there is a difference in the relation

between temperature and oxygen consumption in cases where the animals are suddenly transferred to a higher or lower temperature and in cases where the consumption has been determined after a period of acclimatization. If the animals are suddenly transferred from a higher to a lower temperature, the oxygen consumption will decrease considerably at the beginning and then rise a little (figs 2, 3, 16). When they are transferred suddenly to a much higher temperature the consumption will also decrease in some cases, but in most cases the consumption will increase again after a period of acclimatization so that we will get a normally rising curve.

In some cases however an acclimatization to the high temperature is of no effect (fig. 2, 4, 5 and 9), and it is remarkable that three species in the Mediterranean, *Tellina planata*, *Venus gallina*, *Dosinia exoleta*, have a decrease of oxygen consumption at temperatures about 20° C, i. e. temperatures which in summer time occur in the waters in which they live. I think that this peculiar phenomenon can be explained in the way that these species live buried down in the bottom so that they are able to avoid to a certain degree the high temperatures of the water.

The zoogeographical importance of the relation between temperature and oxygen consumption.

If the view set forth in the above, namely that the said relation is an important factor in the competition among the species, is correct, it seems likely that a certain geographical relation of the different types of oxygen consumption can be demonstrated. If we look on the graphs figs. 1—16 it is obvious that there is such a relation. Some years ago

the present author put forth the hypothesis that species of the Arctic could be expected to have a relatively high oxygen consumption, whereas species of waters with a high temperature could be expected to have a relatively low consumption of oxygen. If we are looking at the graphs it will be seen that the consumption of oxygen of the arctic species (the fully drawn lines) is relatively high. The arctic *Pecten groenlandicus* has a considerably higher oxygen consumption at about 0° C. than that of the boreal-mediterranean *Pecten varius* and the mediterranean *Pecten flexuosus*, and the latter has the lowest consumption of oxygen. If we look at the graph fig. 15 it will be seen that the consumption of oxygen of the boreal *Mytilus edulis* is higher than that of the mediterranean *M. edulis* f. *galloprovincialis*, and the arctic *Modiolaria laevigata* has a still higher consumption. The boreal-mediterranean *Venus gallina* has a higher consumption of oxygen than the more southerly distributed *Venus verrucosa* (graph fig. 4). Further *Mya truncata* has a greater consumption than the southerly *Mya arenaria*, *Macoma calcaria* a greater consumption than *Macoma baltica* and the common European oyster, *O. edulis*, consumes more oxygen than *Gryphaea angulata* of which it is shown that it is able to supplant *O. edulis* in several localities.

All this seems to show that there exists a relation between the type of oxygen consumption and the geographical distribution of the species to the effect that among allied species that species which has the most northerly distribution also has the relatively highest oxygen consumption when compared at the same temperature. In this connection we touch upon the problem of the influence of temperature on the distribution of the species. It is a well known fact that there is a correlation between temperature and

the geographical occurrence of the different species. This correlation is to a large extent due to the influence of the temperature on the reproduction and the larval development as it is has been shown in a series of cases. But the present investigations seem to me to indicate that the effect of the temperature also may to a certain degree be due to its influence on the oxygen consumption. If an animal with a relatively high oxygen consumption (arctic type) is transferred to a water with a temperature of about 20° C. the metabolism will rise rapidly so that the need for food will increase in such a degree that the animal will run the risk of dying from starvation. Whereas the influence of temperature on the reproduction and larval development in most cases limits the species towards the north, it is not unlikely that it is the effect of temperature on the metabolism which limits the distribution of the arctic animals towards the south. So-called sthenothermic animals may, at least in some cases, be regarded as animals with an arctic type of oxygen consumption.

If we regard the oxygen consumption of the same species at different localities within its area of geographical distribution there are indications of a certain difference, geographically related, of the type of oxygen consumption within the same species. As appears from graph fig. 15 it may be seen that *Mytilus edulis* of Danish waters has a distinctly higher oxygen consumption than that of *Mytilus edulis* from the Mediterranean. The different *Astarte* species seem in Greenland to have a higher oxygen consumption than that of the same species in Danish waters. It must be considered probable that also regarding the type of oxygen consumption we may find within the species geographically located physiological races. Runnström (1927, 1930 and

1936) has demonstrated that races of a similar character may be found regarding the reproduction and breeding time of several marine invertebrates.

On all the graphs the minimum and maximum temperatures to which the particular species are exposed in the locality where they live are marked with a cross. It will be seen that the arctic species at the low temperatures at which they normally live have an oxygen consumption of about 20 to 30 cc. in most cases, which is somewhat but not essentially lower than the consumption of most of the boreal and mediterranean species at the temperatures at which they live. And there are several mediterranean species which have an oxygen consumption at their "normal" temperature which is even lower than that of the arctic species. They may perhaps be explained by the poor food conditions in the Mediterranean. If the oxygen consumption found in marine invertebrates by other investigators is compared with the consumption found in this investigation it seems that the oxygen consumption of the Lamellibranches is comparatively low. Only the *Pectinidæ* and *Limidæ* have a consumption of the same size category as has been found by the same method by the present author in some pelagic fishes and Crustacea and in the Oyster larvae. This is perhaps due to the fact that most Lamellibranches are to be regarded as almost sedentary.

Summary of results.

1. It has been shown that among marine Lamellibranches several types of relation between oxygen consumption and temperature exist.

2. There is a marked correlation between the type of oxygen consumption and the ecology of the species, so that species which can move or are living above the bottom have a relatively higher consumption than that of the species living more or less sedentary in the bottom.

3. Further, differences within the above mentioned ecological types of oxygen consumption can be stated. These last named differences are related to the geographical distribution of the species, a relatively high consumption being characteristic of the arctic species, a relatively low one of the mediterranean species.

4. It is likely that an important influence of the temperature on the distribution of marine animals is due to the effect of temperature on the metabolism, and thereby on the food problem and competition among the species.

5. Even within the same species locally different races n this respect seem to exist.

(From the Laboratory of Zoophysiology, University of Copenhagen & the Zoological Station, Naples.)

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II. *CENTROSPERMAE*

VON

O. HAGERUP

KØBENHAVN
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VORWORT

Die hiermit vorliegende Abhandlung ist eine direkte Fortsetzung meiner zwei 1933 und 1934 erschienenen phylogenetischen Arbeiten. Wie ich dort mittels Serien von Mikrotomschnitten durch kontinuierliche Reihen von Entwicklungsstadien der Blüten die Koniferenzapfen und die *Gnetales*-Blüten untersuchte, so habe ich an dieser Stelle nach der nämlichen Methode die Centrospermen-Blüten untersucht. Auch die massgebenden Gesichtspunkte sind dieselben wie in den beiden vorhergehenden Arbeiten; besonders sei hervorgehoben, dass die Integumente + Funiculi als Makrosporophylle aufgefasst werden; die Begründung dieser Annahme liegt in der Abhandlung über die Koniferen (1933) S. 24—36 vor.

Das Untersuchungsmaterial sammelte ich selbst, teils in Tropisch-Afrika, teils in dem Botanischen Garten der Kopenhagener Universität. Wenn keine speziellen Angaben gemacht werden, stellen die Figuren keine Schemata dar, sondern sind mit Hilfe eines Zeichenapparates nach einem Präparat gezeichnet. Der Übersicht halber habe ich jedoch den verschiedenen Figuren eine gleichartige Schraffierung gegeben; es ist dadurch ein Subjektivismus eingeführt worden, der ebenfalls der kritischen Bewertung des Lesers unterzogen werden muss.

Ich erlaube mir, auf diesem Wege dem »Carlsberg-Fond«, dessen wertvolle Unterstützung die Durchführung meiner Arbeit erst ermöglichte, meinen verbindlichsten Dank abzustatten.

Die Übersetzung ins Deutsche besorgte Herr Adjunkt A. Rossen mit üblicher Tüchtigkeit. Die Herren Professor, Dr. L. Kolderup Rosenvinge und Professor, Dr. K. Jessen haben meine Arbeit durch kritische Winke gefördert, wofür ihnen herzlich gedankt sei.

1. Einleitung: Die Probleme.

Die vorliegende Arbeit bezweckt, einen Beitrag zur Lösung der Frage, von welchen der niedrigeren Archegoniaten die heute auf der Erdkugel lebenden Angiospermen abstammen, zu liefern.

Dies wichtige phylogenetische Problem ist in jüngster Zeit wieder mit Nachdruck angeregt worden; es grenzt ja auch an die Diskussion über die Morphologie der Coniferen-Zapfen. Die heute noch herrschende Unsicherheit ist dadurch hervorgerufen, dass man nach Untersuchungen und Erörterungen, die über ein Jahrhundert umspannen, immer noch nicht weiss, was das Gynöceum der Angiospermen eigentlich ist. An dieser Stelle müssen neue Untersuchungen folglich einsetzen; denn Homologieschlüsse vom *Cycas*-Sporophyll führten nicht zu befriedigenden Ergebnissen; trotzdem herrschen in der modernen Botanik auf diesem Gebiete noch derartige Anschauungen vor. Mit dieser Arbeit wird der Versuch gemacht, zu beweisen, dass die *Cycas*-Homologien in gewissen Fällen falsch sind, und, dass der langwierige Stillstand in der Erforschung phylogenetischer Fragen in wesentlichem Masse durch sie hervorgerufen ist.

In jüngster Zeit haben jedoch eine Reihe von Forschern gewagt, die Allgemeingültigkeit der *Cycas*-Theorien zu bezweifeln. Unter diesen sind Saunders, der von anatomischen Gesichtspunkten ausgeht, Thomas, der das Problem

paläontologisch, Gregoire, der es organogenetisch angreift, u. a. m. Wenn man auch nicht die Anschauungen dieser Forscher in ihrer Gesamtheit billigen kann, so gebührt ihnen doch die Ehre, erstmalig an der unbedingten Gültigkeit der *Cycas*-Homologien gezweifelt zu haben. Die meiner Meinung nach besten Untersuchungen sind jedoch von J. M. Thompson ausgeführt worden; obwohl wir verschiedene Methoden benutzten, gelangten wir dennoch ungefähr zu denselben Schlussfolgerungen. Thompson untersuchte die Organogenie des Gynöceums und stellte fest, dass der Fruchtknoten von *Scitamineae* ein krugförmiger Stengel (»crater«) mit dazugehörigen Blättern ist.

Meine Untersuchungen begannen mit *Coniferae* und führten über *Gnetales* zu *Piperales* und *Juglandales*; es wurden vorwiegend organogenetische und teratologische Methoden benutzt. In folgenden werde ich die von den Koniferen ausgehende phylogenetische Linie wieder aufnehmen und untersuchen, ob sich dieselbe auch bei anderen Angiospermen fortsetzt. Als Ausgangspunkt für diese Untersuchung wähle ich eine der am höchsten organisierten Koniferen, und zwar *Juniperus*.

2. *Juniperus.*

Der phylogenetische Ausgangspunkt unserer Untersuchung wird am besten mit Hilfe einiger Schemata über die Organogenie des *Juniperus communis*-Gynöceums festgehalten, wie sie in den Hauptzügen in nebenstehenden Figuren 1—6 dargestellt ist. Ich habe sehr gründlich diese Entwicklung an allwöchentlich eingesammelten kontinuierlichen Serien von Entwicklungsstadien untersucht und gelangte zu folgenden sicheren Ergebnissen: der Same (in den Fig. ist das Integument getüpfelt, der Nuzellus schwarz

gezeichnet) wird nicht an der »Zapfenschuppe«, sondern als gewöhnliches Blatt an der (wagerecht schraffiert gezeichneten) Achse der Blüte angelegt. Die »Zapfenschuppe« ist also ein »falsches Fruchtblatt«, d. h. ein steriles Blatt,

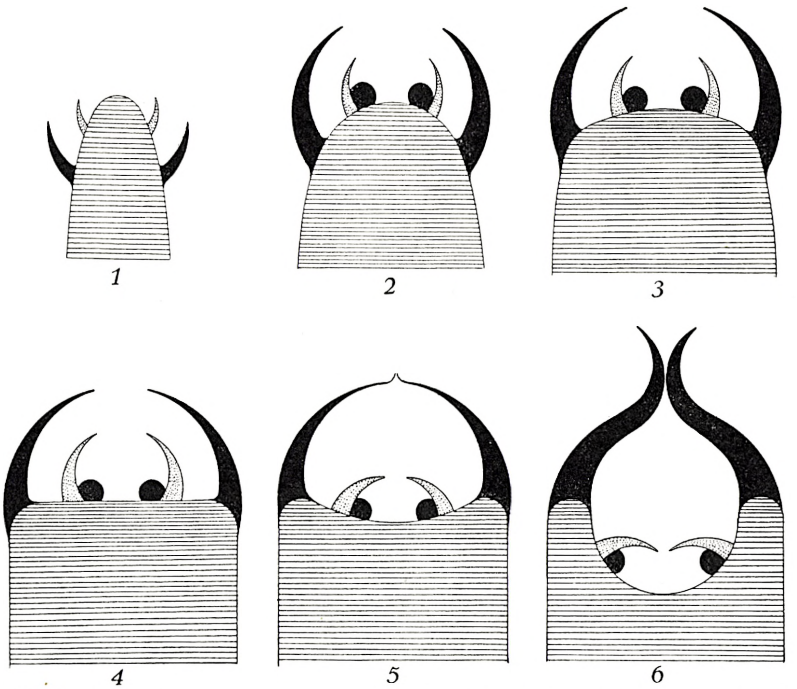


Fig. 1—6. *Juniperus communis*. Schemata über die Entwicklungsgeschichte des Gynöceums. Blütenachse wagerecht schraffiert; Integumente getüpfelt; falsche Fruchtblätter schwarz. Plazentation ursprünglich zentral, zuletzt parietal in einem Stengelkrug; die reife Frucht ist angiosperm (Fig. 6). Vgl. im übrigen den Text.

das den Samen umgibt und beschützt (Vergl. Hagerup, 1933); es trägt denselben aber nicht (er sitzt z. B. nicht den Rändern des Blattes auf), wie mehrere der früheren Forscher angenommen haben.

Von ganz besonderem Interesse sind jedoch die Änderungen, denen die Form der Blütenachse unterzogen wird:

ursprünglich (Fig. 1) hat sie eine kegelförmige Spitze, ganz wie der Vegetationspunkt eines gewöhnlichen vegetativen Zweiges. Den oberhalb der falschen Fruchtblätter befindlichen Teil der Blütenachse müssen wir als Plazenta bezeichnen, da er die Samenanlagen trägt, genau wie die Plazenta bei *Caryophyllaceae* und *Primulales* z. B.

Sobald die Integumente angelegt sind, geschieht nun das Merkwürdige, dass die Blütenachse ihr Wachstum im axilen Teil der Spitze einstellt, dagegen aber nicht im peripherischen Teil des Vegetationspunktes (»Toral growth« von M. L. Thompson). Am kräftigsten wird das Längenwachstum der Achse in der ringförmigen Zone, der die »Karpellen« ansitzen, fortgesetzt, welches die in Fig. 1—6 dargestellten interessanten Formänderungen veranlasst: der Stengel wird an der Spitze immer flacher (Fig. 1—3) und ist im Bestäubungsstadium (Fig. 4) fast wagerecht »abgeschnitten«. Zuletzt bewirkt die ringförmige Vegetationszone, dass die falschen Fruchtblätter auf den Rand des krugförmigen Stengels, der die Samen nun völlig umschliesst, emporgehoben werden (Fig. 5—6).

Mit anderen Worten: das *Juniperus*-Gynöceum besass anfänglich eine Zentral-Plazenta, die aber während der Ontogenie derartig umgestaltet wird, dass die Samen zuletzt eine parietale Stellung im Innern des krugförmigen Stengels einnehmen. Diese beiden Stellungen, die bei den höheren Phanerogamen wohlbekannt sind, bilden also nicht so scharfe Gegensätze, wie man annehmen könnte, sondern gehören eben zusammen, indem sie von einander entwickelt und abgeleitet werden können.

Fügt man dann noch hinzu, dass das *Juniperus*-Gynöceum bekanntlich nach beendigtem Blühen die Samen völlig umschliesst (Fig. 5—6), so leuchtet es ein, dass man mit

gutem Grund Angiosperm-Gynöceen nachforscht, die dem *Juniperus*-Gynöceum so nahe kämen, dass man auf eine nähere Verwandtschaft schliessen könnte.

Am günstigsten wäre es, wenn man eine grössere systematische Einheit von Phanerogamen finden könnte, die zweifellos mit einander verwandt wären, aber dennoch verschiedenartige Gynöceen hätten, so dass man in Erfahrung bringen könnte, wie dieselben teils von einander und teils von einer Zentral-Plazenta, die ja den ursprünglichsten Typus bei *Juniperus* bildete (Fig. 1—3), abgeleitet werden.

Auch Scheidewände gibt es im Gynöceum von *Juniperus*. Bei dieser in phylogenetischer Beziehung interessantesten aller Gymnospermen finden wir also mehrere entscheidende Merkmale, die auf gewisse Angiosperm-Gynöceen hindeuten. Unter letzteren wollen wir denn zunächst solchen nachforschen, die eine Zentral-Plazenta, die ja wie schon erwähnt bei *Juniperus* ursprünglich war, besitzen. Eine derartige Zentral-Plazenta findet sich typisch u. a. bei *Primulales* und *Centrospermae*. Da sie aber bei *Primulales* gleichgeartet ist, habe ich diese Reihe nicht zum Untersuchungsobjekt gewählt.

Innerhalb der *Centrospermae* gibt es dagegen fast alle möglichen Plazentationsverhältnisse. Da ferner die zahlreichen Typen untereinander nahe verwandt (v. Wettstein 1935) — und auch zugleich mit *Cactales* verwandt sind, so werden wir typische Vertreter der hauptsächlichsten, zu den beiden genannten Reihen gehörigen Familien auswählen und zunächst die Entwicklungsgeschichte des Gynöceums betrachten, um darauf mit *Juniperus* Vergleiche anzustellen.

3. *Caryophyllaceae.*

Von den ca. 12 Familien, die gewöhnlich den *Centrospermae* zugezählt werden, sind nur die *Caryophyllaceae* hinsichtlich der Entwicklungsgeschichte der Blüten einigermassen erforscht. Die besten Untersuchungen über diese Frage sind von Payer (1857) und Lister (1883) angestellt worden; die Arbeiten dieser Forscher sind von Rohrbach (1868), Schaefer (1890), Kraft u. a. ergänzt worden.

Aber die wertvollen klassischen Arbeiten des vorigen Jahrhunderts sind auf Grund der primitiven technischen Hilfsmittel jener Zeit so mangelhaft, dass man sich nicht mit Literaturstudien begnügen kann, wenn man sich ein befriedigendes Bild von z. B. den ersten Stadien in der Entwicklungsgeschichte der Plazenta und der Fruchtblätter oder der Ovula machen will. Deshalb kann man auch keine sichere Stellung einnehmen zu der in den meisten modernen Lehrbüchern allgemein angeführten Theorie, nach welcher die zentrale Plazenta (Fig. 28) aus den verwachsenen Rändern der »Fruchtblätter« gebildet sein sollte. Und diese Theorie sollte wiederum dazu dienen, die Homologien mit den *Cycas*-Fruchtblättern zu retten, die man dann als allgemeingültig auffassen könnte.

Falls die erwähnte Theorie richtig wäre, so müsste die Entwicklungsgeschichte zeigen, dass die Fruchtblätter zuerst angelegt würden; erst später solle dann die Plazenta als Abschnitt der Fruchtblätter entstehen. Die nebenstehenden Zeichnungen wollen die Angaben der älteren Literatur ergänzen und die Frage zu entscheiden suchen, ob die erwähnten *Cycas*-Homologien richtig oder falsch sind.

Als erstes Beispiel wähle ich *Tunica prolifera* (Fig. 7—13). Der Übersicht halber ist die Blütenachse in sämtlichen

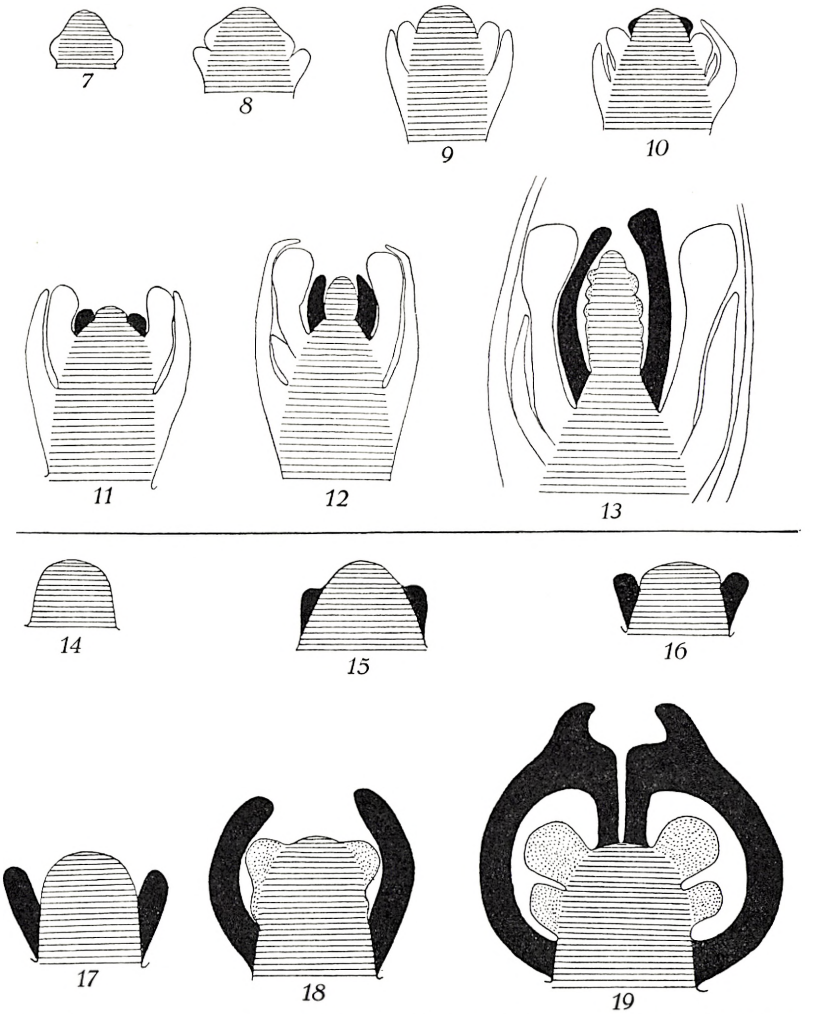


Fig. 7—19. *Caryophyllaceae*. Fig. 7—13. Entwicklungsgeschichte der Blüte (Längsschnitte) von *Tunica prolifera*. × 75. Fig. 14—19. Entwicklungsgeschichte des Gynöceums von *Spergula arvensis*. × 120. Wagerechte Schraffierung bezeichnet ungefähr den Verlauf des Stengels; Samenanlagen getüpfelt; Karpellen schwarz. Vgl. den Text.

Figuren wagerecht schraffiert. Wie man sieht, ist diese Achse überall kegelförmig, sie bildet neue Blätter unterhalb

der Spitze und erinnert durchaus an den Vegetationspunkt eines gewöhnlichen vegetativen Zweiges.

Es gibt jedoch eine Eigenschaft, die für die Morphologie der Blüten von sowohl dieser als vieler anderer Caryophyllaceen von entscheidender Bedeutung ist, und zwar die, dass beinahe alle Internodien kraft einer interkalaren Vegetationszone durch ihre Basen wachsen. Dieser Umstand ist oft ganz unmittelbar an den älteren vegetativen Teilen der Pflanzen wahrzunehmen (z. B. bei *Cerastium* und *Stellaria*); in den Blüten veranlasst ein derartiges sonderbares Wachstum, dass z. B. der jüngste Wirtel von Staubblättern innerhalb der Vegetationszone des Internodiums unter den ältesten Staubblättern angelegt wird (Obdiplostemonie). In entsprechender Weise wird oft die Krone später als die Staubblätter angelegt (Fig. 9—10).

Fig. 7 zeigt uns eine ganz junge Blüte (von *Tunica*), die aus einer Achse besteht, die nur Kelchblätter trägt. Darauf werden (Fig. 8) Staubblätter entwickelt; das Achsenende setzt sein Wachstum fort und ragt weit über die Staubblätter empor (Fig. 9), bevor die Fruchtblätter noch angelegt sind. Verfolgt man die Entwicklung weiter, so sieht man, dass es das oberste Ende des Stengels (ɔ: der Blütenachse) ist, welches sich zur Plazenta entwickelt. Die Plazenta ist vor den Fruchtblättern da und kann daher nicht aus den »verwachsenen Rändern« derselben gebildet sein; denn die Plazenta kann doch nicht ein Teil von noch nicht existierenden Blättern (Fruchtblättern) sein. Wie man sieht, widersprechen die *Cycas*-Homologien hier den tatsächlichen Verhältnissen und sind daher falsch.

Die folgenden Figuren (14—41) zeigen uns, dass auch die Gynöceen anderer Gattungen ungefähr dieselbe Entwicklungsgeschichte durchmachen. Besonders bei *Spergula*

(Fig. 15), *Arenaria* (Fig. 21), *Cerastium* (Fig. 25) und *Stellaria* (Fig. 29, 30) ist es deutlich erkennbar, dass die Fruchtblätter unterhalb der Spitze der Plazenta, die wiederum die Spitze der Blütenachse bildet, angelegt werden.

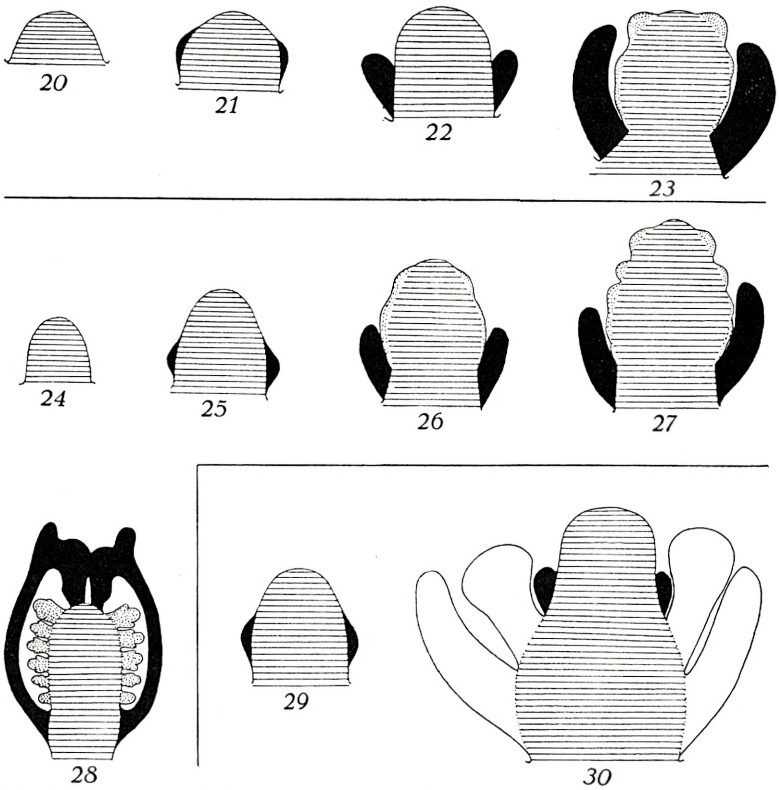


Fig. 20—30. *Caryophyllaceae*. Entwicklungsgeschichte von Gynöceen (Längsschnitte). Karpellen schwarz; Samenanlagen getüpfelt; Stengel schraffiert. Fig. 20—23. *Arenaria serpyllifolia*. × 180. Fig. 24—28. *Cerastium semidecandrum*. × 100 (Fig. 28, × 50). Fig. 29—30. *Stellaria media*. × 180. Vgl. Text.

Verfolgen wir sodann das Schicksal der Vegetationspunkte der Blüten, indem wir den Blick über die Figuren (7—41) gleiten lassen, so sehen wir, dass der Vegetationspunkt noch eine Weile die Bildung von Blattanlagen fort-

setzt. Aber die Stellung derselben nimmt sich sonderbar aus, indem sie dadurch bedingt ist, dass auch die Internodien der Plazenta interkalar an der Basis wachsen. Deshalb entsteht an der Plazenta eine ähnliche »Obdiplostemonie« wie bei den Staubblättern; und die Längsschnitte lassen deutlich erkennen, dass die Blätter der Plazenta so angelegt werden, dass die ältesten oben und die jüngsten unten zu stehen kommen. Die Anzahl der Orthostichen an der Plazenta ist meistens doppelt so gross wie die der Fruchtblätter; sind z. B. 5 Karpellen vorhanden, so werden oft 10 senkrechte Reihen von Blattanlagen an der Plazenta gebildet, wie entsprechendermassen auch die weiter unten stehenden Blätter der Blüte 10 senkrechte Reihen bilden. Die Blattanlagen an der Plazenta entwickeln sich auch hier später zu Samenanlagen.

Unter der kräftigen Nahrungszuführung zu den jungen Samenanlagen werden senkrechte Leitbündel in der Plazenta entwickelt, die längs den Orthostichen anschwellen. Die Samen stehen deshalb später auf ähnlichen erhöhten Leisten (Samenleisten) wie z. B. die Dornen an einem kugelförmigen Kaktus. Diese senkrechten Wülste sind es, die man als die Ränder der Fruchtblätter aufgefasst hat; eine Auffassung, die deshalb sehr naheliegend ist, weil die Ränder der Fruchtblätter längs der erhöhten Leisten an die Plazenta befestigt sind. Die Entwicklungsgeschichte — und Querschnitte — beweisen aber, dass die Samenleisten von den Plazenten und nicht von den Fruchtblättern gebildet sind.

Schliesslich veranschaulichen uns die Fig. 38—43 die Entwicklung des Gynöceums bei zwei Arten (*Scleranthus*, *Paronychia*), wo jede Blüte nur einen Samen besitzt. Auch hier werden die Fruchtblätter als laterale Organe an der

Spitze der Blütenachse angelegt. Diese Plazenta entwickelt nur einen pseudoterminalen Samen; übrigens weisen diese Pflanzen nichts prinzipiell Neues auf; sie sind nur der Verallgemeinerung wegen in die Untersuchung mit einbe-

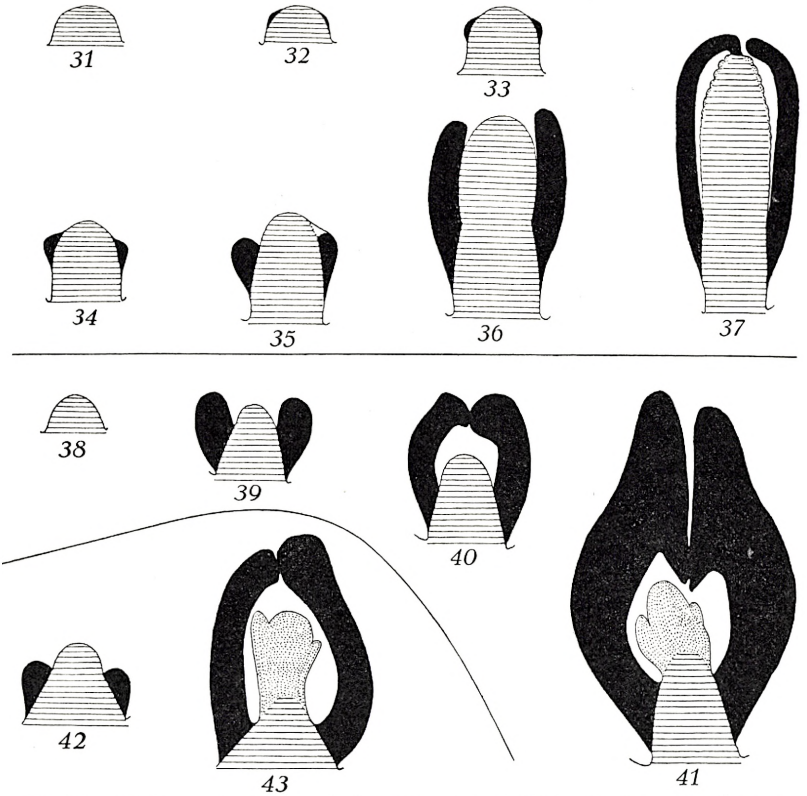


Fig. 31—43. *Caryophyllaceae*. Entwicklungsgeschichte von Gynöceen (Längsschnitte). Blütenachse schraffiert; Samenanlagen getüpfelt; Karpellen schwarz. Fig. 31—37. *Spergularia media*. × 100. Fig. 38—41. *Schleranthus perennis*. × 180. Fig. 42—43. *Paronychia polygonifolia* DC. × 180. Vgl. den Text.

zogen. Wir haben nämlich jetzt Vertreter der verschiedenen Hauptgruppen der Caryophyllaceen untersucht und eine solche Übereinstimmung zwischen den Gynöceen der verschiedenen Gattungen festgestellt, dass wir uns schon be-

rechtigt glauben dürfen, die Auswahl für hinreichend umfangreich anzusehen, um eine Generalisierung der Ergebnisse vorzunehmen. Der Übersicht halber werden die Resultate in zwei schematische Figuren (161, 162) zusammengefasst. Die Erörterung des morphologischen Wertes der Samenanlage wird auf später verschoben; vorläufig fassen wir sie als ein Blatt (Makrosporophyll) auf.

4. *Chenopodiaceae.*

Schon Payer hat einige wenige Stadien der Entwicklungsgeschichte des Gynöceums dieser Familie untersucht. Wir begnügen uns damit, seine Untersuchungen durch ein paar Typen zu ergänzen; denn der Bau des Gynöceums ist offenbar innerhalb der gesamten Familie sehr gleichgeartet, und es schliesst sich ausserdem den schon im obigen beschriebenen einsamigen Caryophyllaceen ganz nahe an.

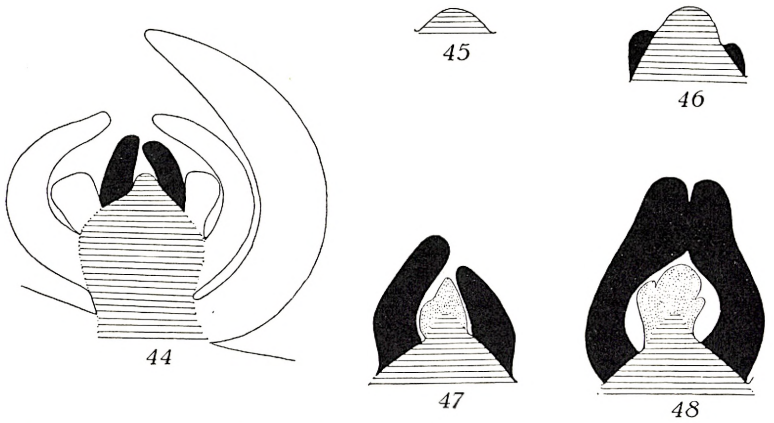


Fig. 44—48. *Chenopodiaceae.* Karpellen schwarz; Schraffierung bezeichnet die Blütenachse; Samenanlagen getüpfelt. Fig. 44. Blüte von *Chenopodium nitrariaceum* F. Muell (Längsschnitt). × 30. Fig. 45—48. Entwicklungsgeschichte des Gynöceums von *Rhagodia nutans* R. Br. × 100. Vgl. im übrigen den Text.

Fig. 44 stellt einen axilen Längsschnitt durch eine ganze Blüte von *Chenopodium* dar (Deckblatt rechts). Die näheren Einzelheiten in der Entwicklung des Gynöceums (bei *Rhagodia*) zeigen uns die Fig. 45—48. Die Karpellen werden auch hier (Fig. 46) als laterale Blätter unmittelbar unter dem Vegetationspunkt der Blütenachse (Fig. 45) angelegt. Bald darauf wird der Same als pseudoterminales Blatt am Stengel — und nicht an einem der »nach innen gebogenen Ränder der Fruchtblätter« — angelegt. Es gibt nämlich gar keine Scheidewände im Fruchtknoten, und der Same kann folglich nicht derartigen Scheidewänden ansitzen. Es wird also auch hier durch die Entwicklungsgeschichte bewiesen, dass die Plazenta nicht aus den Rändern der Fruchtblätter gebildet ist, — und auch nicht mit Teilen von Fruchtblättern bekleidet ist, die die Stengelspitze hinauf-»verschoben« wären. Auch bei den Chenopodiaceen lassen sich die *Cycas*-Homologien demnach nicht aufrechterhalten.

5. *Amaranthaceae.*

Payer hat eine zu dieser Familie gehörende Art (*Celosia margaritacea*) untersucht, eine der wenigen Amaranthaceen, deren Fruchtknoten mehrere Samen besitzen. Dieselben sitzen einer zentralen Plazenta, die Payer richtig als einen Stengel auffasst, an. Ich habe eine *Celocia*-Art (*C. nitida* Vahl) untersucht; sie ist wie die von Payer untersuchte gebaut. Abbildungen waren deshalb überflüssig, zumal da sich schon bei Payer sehr schöne Illustrationen finden.

Das Gynöceum von *Celocia* gleicht also stark denjenigen der Caryophyllaceen, unterscheidet sich von ihnen aber dadurch, dass (wie bei den Primulaceen) Scheidewände fehlen. Man ersieht folglich, dass das Vorhandensein der

zentralen Plazenta nicht durch die Existenz von Scheidewänden bedingt ist; und die Entwicklungsgeschichte beweist

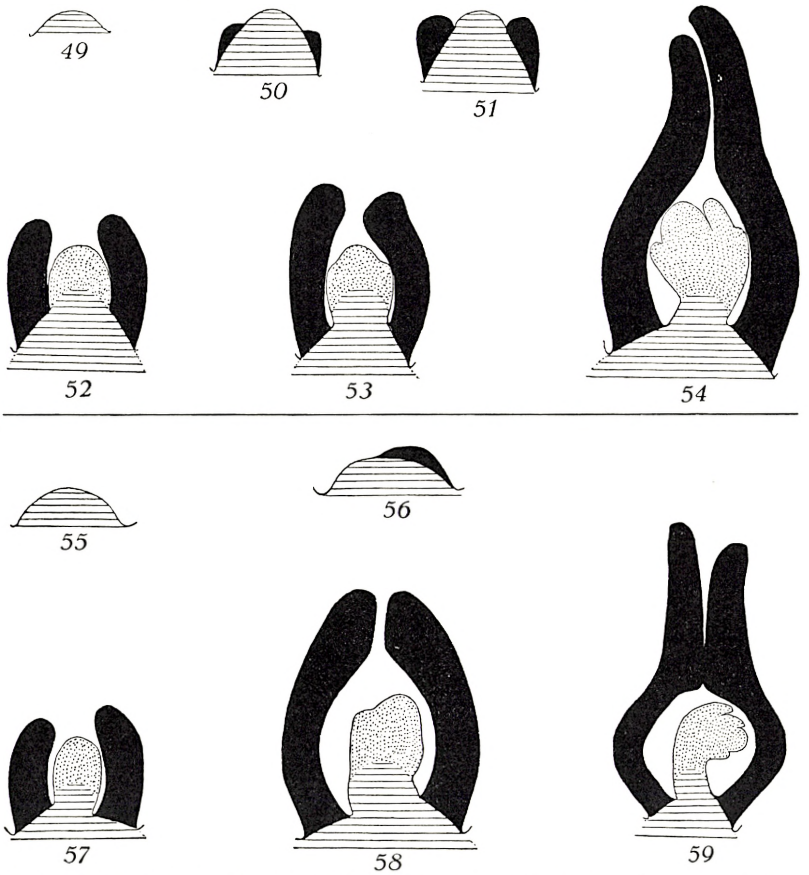


Fig. 49—59. *Amaranthaceae*. Entwicklungsgeschichte des Gynöceums (in Längsschnitten). Karpellen schwarz; Stengel schraffiert; Samenanlagen getüpfelt. Fig. 49—54. *Amaranthus blitum*. × 100. Fig. 55—59. *Aerva tomentosa*. × 100. Vgl. im übrigen den Text.

auch hier (vgl. z. B. die Figuren bei Payer), dass die Plazenta keinen hinzugewachsenen Teil der Karpellen umfasst.

Die meisten anderen der zu dieser Familie gehörigen Gattungen besitzen nur einen Samen im Fruchtknoten. Eine Untersuchung der Entwicklungsgeschichte (vgl. Fig. 49—59)

ergibt, dass das Gynöceum in ähnlicher Weise wie bei den einsamigen Caryophyllaceen und Chenopodiaceen gebaut ist: die Fruchtblätter werden auch hier in der Nähe der Spitze der Blütenachse angelegt (Fig. 49—50). Das letzte Blatt, welches dem Vegetationsscheitel entspriesst, wird zur Samenanlage, die also auch hier nicht dem Rande irgendwelchen Fruchtblattes aufsitzt.

Payer hat noch andere der einsamigen Arten untersucht und eine Entwicklungsgeschichte festgestellt, die derjenigen vollständig analog ist, die die nebenstehenden Figuren (49—59) veranschaulichen, und deren Hauptzüge schematisch auf Fig. 161 dargestellt sind.

6. *Phytolaccaceae.*

Diese Familie ist von besonderem Interesse, weil sie Arten umfasst, deren Fruchtblätter frei sind (Apocarpie), während wiederum die Karpellen anderer Arten völlig oder teilweise verschmolzen sein können, wie es bei den Caryophyllaceen der Fall ist. Dass alle diese verschiedenen Gynöceen demselben nahen Verwandtschaftskreis — sogar derselben Gattung (*Phytolacca*) — angehören, berechtigt uns zu der Erwartung, dass sie auch morphologisch von einander abzuleiten sind. Die nebenstehenden Figuren zeigen, dass dies auch tatsächlich zutrifft.

Schon Payer untersuchte zwei Arten *Phytolacca*, die mehrere unter sich freie Fruchtblätter besitzen; und ferner zwei Vertreter für Gattungen, deren Gynöceum nur ein Karpell hat. Wir begnügen uns deshalb an dieser Stelle mit einer ähnlichen Auswahl von Untersuchungsobjekten, die die Beobachtungen Payers ergänzen sollen.

Fig. 60 zeigt uns denjenigen Teil der Blütenachse, der

sich oberhalb der jungen Anlagen zu Staubblättern (die auf Fig. 60 nicht gezeichnet sind) befindet. An diesem Achsenende treten bald die ersten zarten Anlagen zu einem Karpell auf (Fig. 61), und bald umgibt ein ganzer Wirtel

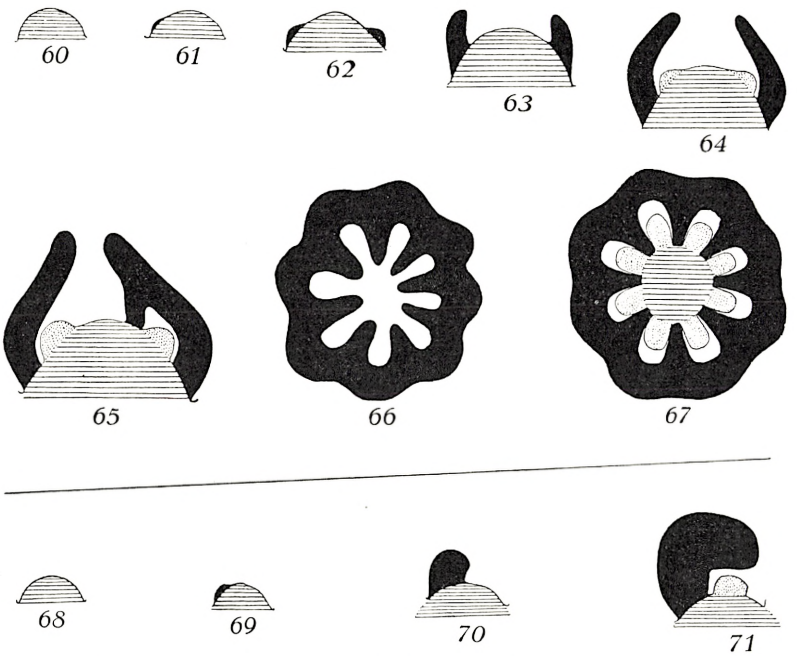


Fig. 60—71. *Phytolaccaceae*. Entwicklungsgeschichte des Gynöceums. × 75. Fig. 60—67. *Phytolacca octandra* in Längsschnitten (Fig. 60—65) und Querschnitten durch Spitze (Fig. 66) und Basis (Fig. 67). Fig. 68—71. *Trichostigma peruvianum*; Längsschnitte. Stengel wagerecht schraffiert; Samenanlagen getüpfelt; Karpellen schwarz. Vgl. Text.

von Karpellen die Spitze der Blütenachse. In der Literatur (z. B. bei Goebel) findet man oft die Angabe, dass der Vegetationspunkt von den Karpellanlagen ganz »verbraucht« und zugedeckt wird, so dass er seine Funktion als Vegetationspunkt einbüsst. Dass dies für *Phytolacca* nicht zutrifft, erhellt aus den Fig. 62—65. Ein grosser Teil der zentralen Teile des Vegetationspunktes liegt zwischen den

jungen Karpellen (Fig. 62—63), und der Vegetationspunkt setzt sein Wachstum noch ein Weilchen fort und bildet sogar noch einen ganzen Wirtel von Blattanlagen (auf Fig. 64 getüpfelt). Auch diese Blätter »verbrauchen« nicht den gesamten Vegetationspunkt; merkwürdigerweise alternieren sie nicht mit den Karpellen, sondern stehen gerade über denselben (Fig. 67). Während der Weiterentwicklung der Blüte (Fig. 65—67) geschieht nun das Sonderbare, dass die Karpellen ihre Ränder zu kahnförmigen Deckblättern zusammenbiegen, die je eine der unmittelbar drüber stehenden Samenanlagen umschliessen. Die so eingeschlossenen Blätter entwickeln sich zu Samenanlagen, und dieselben sitzen folglich nicht den Karpellen, sondern dem Stengel als ganze, selbständige Blätter an.

Die Figuren 68—71 zeigen uns, dass die Entwicklung des Gynöceums der einsamigen Arten in entsprechender Weise vor sich geht, wie es oben für ein Karpell von *Phytolacca* beschrieben wurde. Ferner untersuchte ich noch *Rivinia humilis*, die auch von PAYER abgebildet wurde. Seine Beobachtungen entsprechen wieder völlig den meinigen.

Wir werden also zu der Auffassung geführt, dass sowohl in apokarpen als synkarpen Centrosperm-Gynöceen die Karpellen nur als sterile Blätter zu betrachten sind, die die Samen weder »tragen« noch hervorbringen, sondern nur umgeben und beschützen.

7. *Nyctaginaceae.*

Die Entwicklung des Gynöceums ist von PAYER bei nur einer *Oxybaphus*-Art untersucht worden. Ich habe eine Untersuchung von *Bougainvillea spectabilis* und von zwei

Boerhaavia-Arten angestellt; die untenstehenden Fig. 72—80 stellen einige der wichtigsten Stadien in der Entwicklungsgeschichte ihres Gynöceums dar. Die untersuchten Gattungen erinnern teils stark an einander und teils auch an die einsamigen Phytolaccaceen. Und systematischen Beschreibungen zufolge gibt es innerhalb der Familie kaum mehr als einen Gynöceum-Typus.

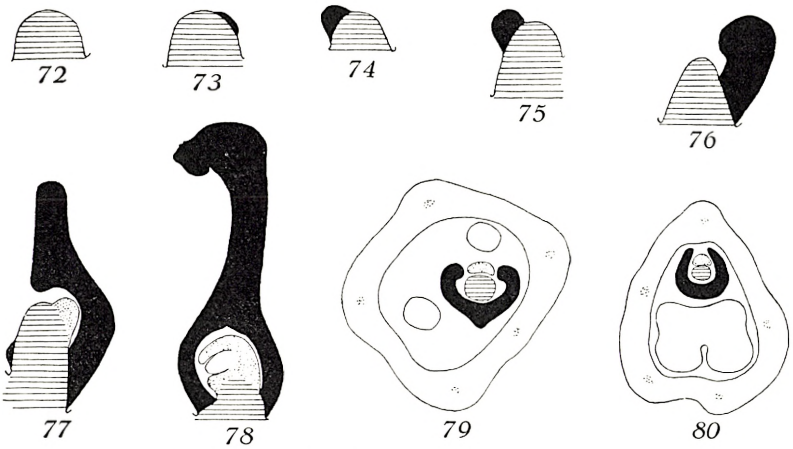


Fig. 72—80. *Nyctaginaceae. Boerhaavia.* Entwicklungsgeschichte des Gynöceums. Fig. 72—78. *B. repens*; Längsschnitte. × 150. (Fig. 78, × 75). Fig. 79. Querschnitt durch Blüte von *B. verticillata.* × 70. Fig. 80. Querschnitt durch Blüte von *B. repens.* × 80. Karpellen schwarz; Samenanlagen getüpfelt; Stengel schraffiert. Vgl. übrigens den Text.

Fig. 72 stellt eine Zeichnung des über dem Androeceum sitzenden Teiles der Blütenachse dar. An demselben kommt später das Fruchtblatt zur Anlage (Fig. 73), welches im Laufe seines fortgesetzten Wachstums das Achsenende käppchenförmig umschliesst (Fig. 79—80). Der so eingeschlossene Vegetationspunkt bringt noch ein Blatt zur Entwicklung (Fig. 77, 79, 80), welches zu einer Samenanlage wird, die einer zentralen Plazenta vom selben morphologischen Wert wie z. B. bei den Caryophyllaceen aufsitzt.

8. *Aizoaceae.*

Zur Lösung der in Frage stehenden Aufgabe ist diese Familie von ganz besonderer Bedeutung, weil sie Formen mit scheinbar ganz verschiedenen Gynöceen enthält. Diese Pflanzen sind jedoch zweifellos nahe mit einander verwandt und haben ferner auch deutliche Beziehungen zu anderen Familien innerhalb der Gruppe der Centrospermen. Wir müssen daher auch mit sowohl phylogenetischer als »morphologischer Verwandtschaft« zwischen den verschiedengebauten Gynöceen rechnen, welches denn auch durch die Entwicklungsgeschichte bewiesen wird; dies verdeutlichen die umstehenden Figuren (81—103).

Erstens gibt es eine Gattung, *Gisekia*, deren Fruchtblätter nicht verwachsen sind; sie bildet eine deutliche Brücke zu den Phytolaccaceen (denen sie auch oft zugezählt wird); und die Entwicklungsgeschichte ihres Gynöceums (Fig. 153—157) erinnert völlig an diejenige des Gynöceums von *Phytolacca* (Fig. 60—65): bei beiden Pflanzen bilden die Karpellen nur ein Involucrum von sterilen Bracteen, die die darüber stehenden Sporophylle (Samenanlagen) umgeben.

Bei der Mehrzahl der übrigen Gattungen der Familie ist das Gynöceum wie bei den Caryophyllaceen gebaut: es findet sich nämlich eine zentrale, aus der Spitze der Blütenachse gebildete Plazenta.

Schon Payer hat die Entwicklungsgeschichte des Gynöceums bei 3 Gattungen (*Mollugo*, *Trianthema*, *Mesembryanthemum*) verfolgt; später hat auch Eichler in seiner kurzen, knappen Art den Bau des Gynöceums von *Mesembryanthemum* beschrieben. Wir begnügen uns deshalb mit den nebenstehenden Typen, die die auf primitiver Technik beruhenden Mängel in den Beobachtungen der älteren Forscher ergänzen wollen.

Die Figuren 81—87 zeigen die Entwicklung des Gynöceums bei zwei der Arten mit zentraler Plazenta. Wie bei den im vorhergehenden besprochenen Familien ist auch

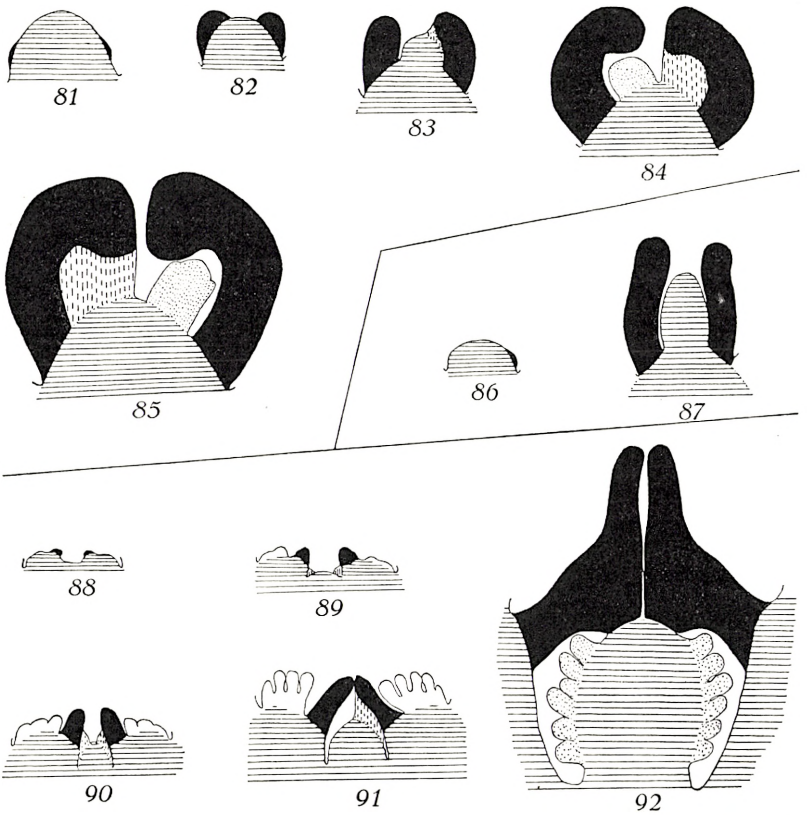


Fig. 81—92. *Aizoaceae.* Entwicklungsgeschichte von Gynöceen. Stengel wagerecht schraffiert; Karpellen schwarz; Scheidewände senkrecht schraffiert; Samenanlagen getüpfelt. Fig. 81—85. *Limeum pterocarpum* (Gay). × 120. Fig. 86—87. *Trianthema crystallina* (Forsk.). × 120. Fig. 88—92. *Mesembryanthemum cordifolium.* × 45. Vgl. Text.

hier die Plazenta aus dem obersten Achsenende und nicht aus den ein Stückchen unterhalb desselben angelegten Fruchtblättern gebildet (Fig. 81, 86). Die Samenanlagen werden als die obersten Blätter am Achsenende entwickelt.

Die interessanteste sämtlicher Gattungen ist jedoch *Mesembryanthemum*, weil sie bekanntlich sowohl Arten mit zentraler Plazenta als auch solche mit parietaler Plazenta umfasst.

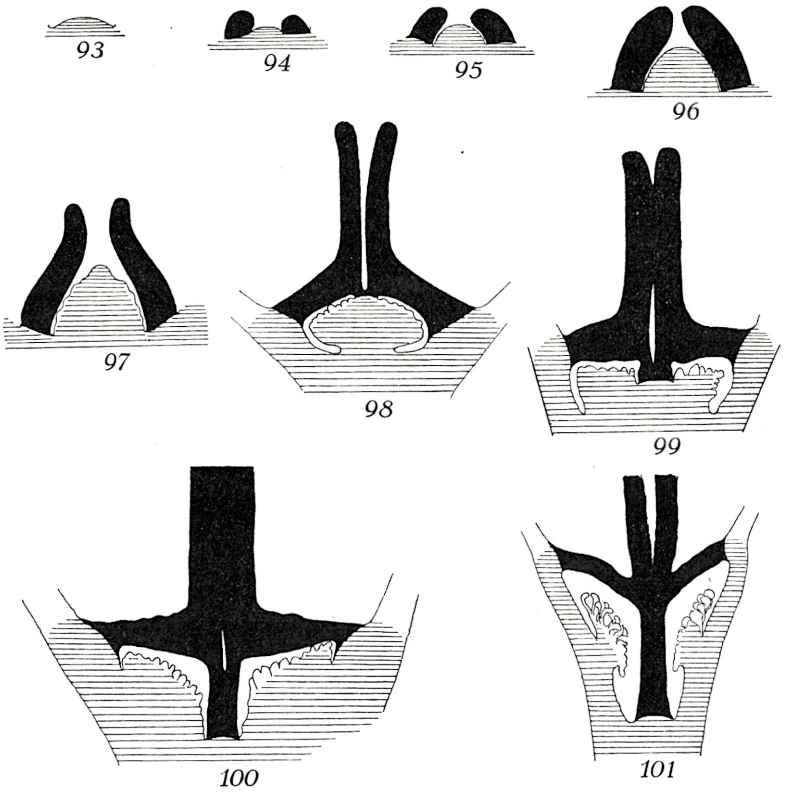


Fig. 93—101. *Mesembryanthemum hispidum*. Entwicklungsgeschichte des Gynöceums. Plazentation ursprünglich zentral, später — nach Bildung des Stengelkrugs — parietal. Karpellen schwarz; Stengel wagerecht schraffiert. × 45 (Fig. 101, × 15). Vgl. im übrigen den Text.

Das Ovarium ist bei den untersuchten Arten unterständig und besitzt Scheidewände (die in den Fig. durch senkrechte Schraffierung gekennzeichnet sind). Auf Fig. 88—92 verfolgen wir die Entwicklung bei *M. cordifolium*; beobachtet man das Achsenende, so sieht man, dass es ähn-

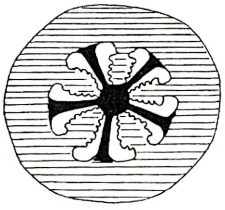
liche Formänderungen durchmacht wie oben (Fig. 1—6) für *Juniperus* beschrieben und zuletzt krugförmig wird. Am Rande des Kruges stehen Blütenhülle und Staubblätter, an der Innenseite die Karpellen, die gleichsam einen Deckel bilden und den Krug oben zuschliessen. Die oberste Spitze der Achse setzt indessen das Wachstum fort und bildet eine zentrale Plazenta, die in der Mitte des Stengelkrugs emporragt (Fig. 89—91); und an dieser Sprossspitze werden zahlreiche Blätter angelegt, die sich zu Samenanlagen entwikkeln (Fig. 92).

Fig. 101 zeigt uns den anderen Gynöceentyp, der durch seine parietalen Samenanlagen von den Arten mit zentraler Plazenta prinzipiell verschieden zu sein scheint. Aber die Entwicklungsgeschichte deckt auf, wie dies sonderbare Verhältnis entstanden ist: die ersten Stadien in der Entwicklung der Blüte sind genau dieselben wie z. B. bei den Caryophyllaceen und den oben abgebildeten Aizoaceen. Das Achsenende wird zur Plazenta, die also da ist (Fig. 93), bevor die Fruchtblätter angelegt werden (Fig. 94). Die Spitze der Blütenachse wächst weiter und nimmt eine krugförmige Gestalt an, ganz wie es bei der vorigen Art und bei *Juniperus* der Fall war.

Wenn die Samenanlagen zum Vorschein kommen, ist die Plazenta kegelförmig wie bei den Caryophyllaceen; bald beginnt sie aber die Form zu ändern, u. zw. auf die sonderbare Weise, die auf den Fig. 97—101 dargestellt ist: das Längenwachstum wird eingestellt, statt dessen tritt eine Verdickung ein; besonders die Ränder wachsen stark. Dies bewirkt, dass die Vegetationszone der Plazenta in einem gewissen Stadium der Entwicklung (Fig. 99) ungefähr wagerecht ist; darauf erfolgt eine Versenkung (Fig. 100) und zuletzt nimmt sie eine krugförmige Gestalt an (Fig. 101).

Das Gynöceum besteht also im unteren Teil aus einem krugförmigen Stengel, der sowohl am Rande (Perianthium und Staubblätter) als an der Innenseite (Ovula) Blätter trägt.

Diese sonderbare parietale Plazentation lässt sich auch



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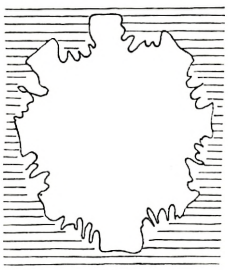

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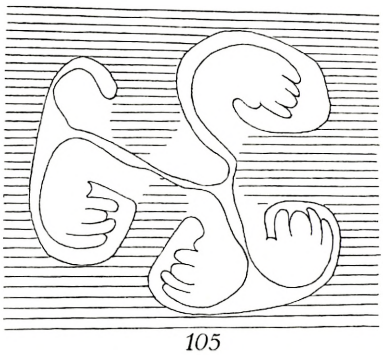

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Fig. 102—103. *Mesembryanthemum hispidum*. Querschnitte durch älteres Gynöceum in der Nähe der Basis (Fig. 102) und der Spitze (Fig. 103). × 15. Karpellenränder (Scheidewände) schwarz. Fig. 104. *Phyllocactus sp.* Querschnitt. Parietale Plazentation im Stengelkrug. × 15. Fig. 105. *Rhipsalis capillariformis*. Querschnitt. Die Samen sitzen Wülsten an, die von der Innenseite des Stengelkrugs gebildet sind. × 65. Stengel wagerecht schraffiert. Vgl. Text.

durch Querschnitte durch Basis (Fig. 102) und Spitze (Fig. 103) des Ovariums veranschaulichen. Man sieht deutlich, dass die Samenleisten den Rändern falscher Scheidewände ansitzen, die erhöhte Teile der Innenseite des Stengelkruges, und also nicht aus Teilen der Fruchtblätter ge-

bildet sind. Die Figuren 104 u. 105 zeigen, dass genau dasselbe bei den dargestellten Vertretern für *Cactaceae* der Fall ist.

9. *Cactaceae.*

Wir haben im vorhergehenden gesehen, dass es innerhalb der Aizoaceen Formen mit einer zentralen Plazenta gab; in der Gattung *Mesembryanthemum* änderte sich das Gynöceum während der Organogenie der Blüte so, dass die Samenleisten zuletzt der Innenseite eines Stengelkruges ansitzen. Im folgenden werden wir nachweisen, dass das Gynöceum bei den Cactaceen in den Hauptzügen wie bei *Mesembryanthemum* gebaut ist, weshalb wir mit v. Wettstein *Cactaceae* zu den Centrospermen zählen und sie nicht als selbständige Ordnung aufrechterhalten. Bei v. Wettstein (S. 663) finden wir sogar eine *Peireskia* abgebildet, die eine zentrale Plazenta wie die übrigen Centrospermen hat.

Die Entwicklung des Gynöceums bei *Opuntia vulgaris* hat Payer schon untersucht. Wir wählen zwei andere Gattungen, um die Untersuchungen Payers zu ergänzen. Auf den Figuren 106—114 (*Rhipsalis*) ist die Entwicklung der ganzen Blüte von den jüngsten Stadien an in Längsschnitten dargestellt; um einen Überblick über die Organogenie zu gewinnen, ist es von besonderer Bedeutung, die Formänderungen, die die Blütenachse durchmacht, zu verfolgen. In sämtlichen Figuren ist die Blütenachse wagerecht schraffiert, während die Karpellen schwarz gezeichnet sind.

Während der jüngsten Entwicklungsstadien (Fig. 106) besteht die Blüte nur aus einer Achse; unter dem Achsenende werden allmählich Blätter angelegt, aus denen sich später Perianthium und Andröceum entwickeln. Ursprünglich war

das Achsenende kegelförmig, wird aber — unmittelbar vor der Anlage des Andröceums — ungefähr wagerecht »abge-

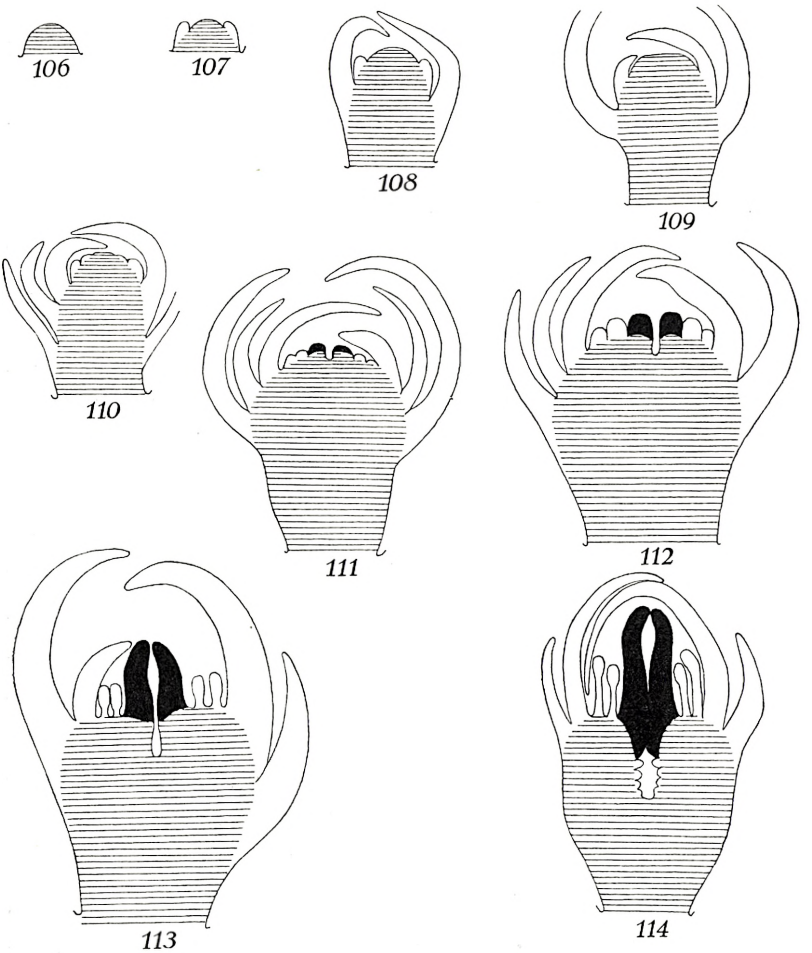

Fig. 106—114. *Rhipsalis capillariformis*. Entwicklungsgeschichte der Blüte (in Längsschnitten). Stengel wagerecht schraffiert; Karpellen schwarz. Bildung des Stengelkrugs zu bemerken. × 75. Vgl. Text.

schnitten« (Fig. 109). Sobald die Karpellen zutage treten, stellt der axile Teil des Vegetationspunktes das Wachstum ein; dagegen wächst das Achsenende stark an den Rändern,

und zwar an den Stellen, wo die ältesten Blütenblätter befestigt sind (Fig. 111—114). In entsprechender Weise wie oben für *Juniperus* und *Mesembryanthemum* nachgewiesen ist die ältere Blüte (Fig. 114) aus einem Stengelkrug (= dem »crater« J. M. Thompsons) gebildet, dessen Rand Perianthium und Andröceum ansitzen, und der oben von den gegen einander gebogenen und mit einander verwachsenen Griffeln zugeschlossen wird.

Der am Boden des Stengelkruges befindliche Vegetationspunkt der Blüte fährt fort, Blätter zu bilden, die sich an der Innenseite des Stengelkruges in senkrechten Reihen (Samenleisten, Fig. 104—105) anordnen, so dass die jüngsten dem Vegetationspunkt am nächsten und folglich zuunterst stehen.

Bei *Rhipsalis* ist das Lumen des Stengelkruges ganz eng, und ein Blick auf die Figuren macht es begreiflich, dass ältere Forscher haben annehmen können, dass die Samen an der Innenseite des Kruges an den »abwärtsgewachsenen Rändern« der Karpellen sässen. Oder der Krug könnte aus den unten verwachsenen Karpellen gebildet sein, wodurch die *Cycas*-Homologien »gerettet« wären.

Dass derartige Annahmen falsch sind, zeigt uns wieder die Entwicklungsgeschichte der *Epiphyllum*-Blüte (Fig. 115—122): denn hier ist deutlicherweise der Stengelkrug schon gebildet, bevor die Karpellen noch da sind, und sogar, bevor das Andröceum angelegt ist (Fig. 118—119); und logischerweise kann der Krug nicht aus Organen aufgebaut sein, die noch nicht existieren. Dass die zentralen Teile der Blüte ein Stengel sind, geht aus den Fig. 115—117 hervor; diese zeigen uns eine Achse mit einem kegelförmigen Vegetationspunkt, an deren Seiten Blätter entstehen. Wie üblich wird jetzt ein Stengelkrug mit versenktem Vegetations-

punkt gebildet, dessen eingesperrte Blattanlagen zu Samenanlagen werden (Fig. 122, 126), die durch die Karpellen, die an der Innenseite des Krugrandes entstehen, von der Umwelt abgesondert werden (Fig. 120, 121).

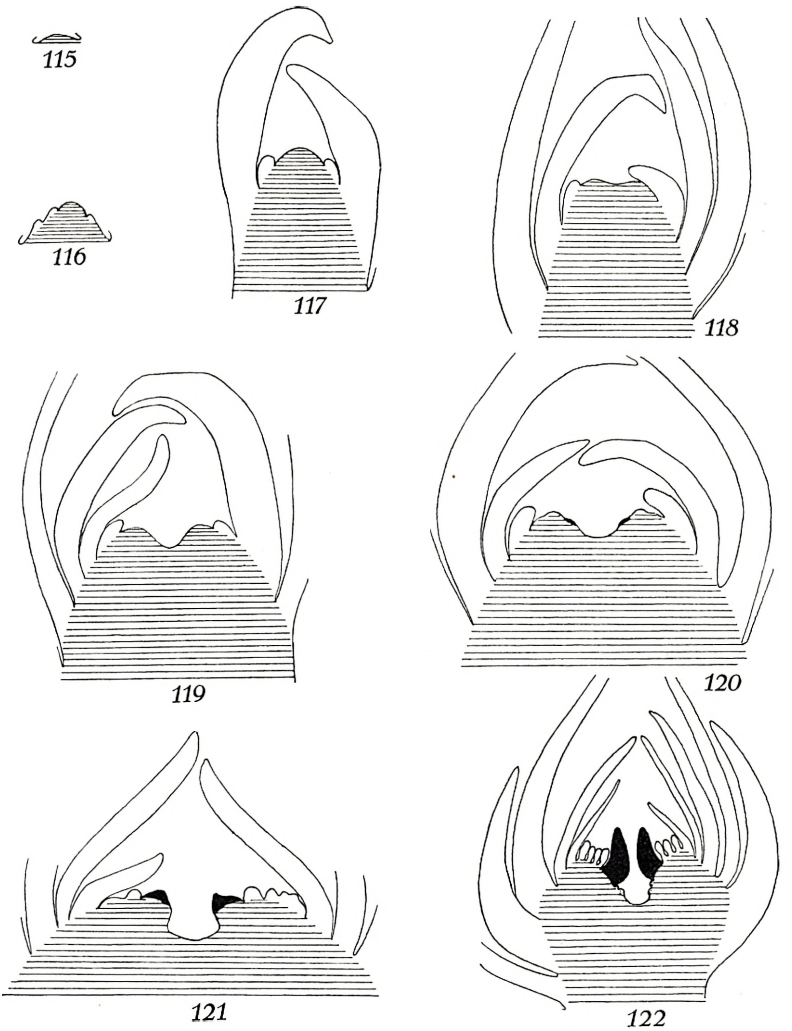

Fig. 115—122. *Epiphyllum truncatum*. Entwicklungsgeschichte der Blüte. Längsschnitte zeigen die Bildung des Stengelkrugs. Karpellen schwarz; Samenanlagen getüpfelt; Stengel schraffiert. Fig. 115—121, × 50. Fig. 122, × 15. Vgl. übrigens den Text.

Der Verallgemeinerung halber geben uns die Fig. 123—125 noch einige Stadien der Blütenentwicklung bei *Phyllocactus*. Auch hier wird ein geräumiger Stengelkrug ge-

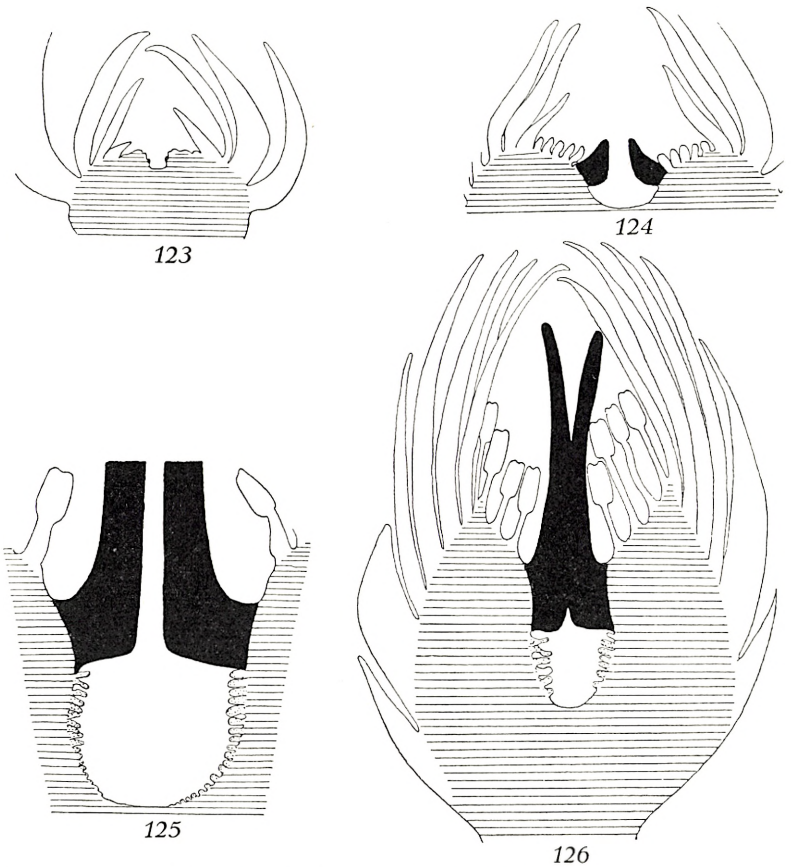

Fig. 123—125. *Phyllocactus sp.* Entwicklungsgeschichte der Blüte in Längsschnitten. Karpellen schwarz; Samenanlagen getüpfelt; Stengel schraffiert. × 15. Fig. 126. *Epiphyllum truncatum.* Längsschnitt durch junge Blüte. × 15. Vgl. Text.

bildet, der an seinem Rande das Andröceum und einige Blätter des Perianthiums trägt; an der Aussenseite des Kruges stehen auch Perianthium-Blätter. Die Karpellen

sind auch hier nur sterile Blätter, die den Blättern (Samenanlagen) an der Innenseite des Kruges als Hülle dienen.

Die merkwürdige Kaktus-Blüte (Fig. 127, 128) weist in den Hauptzügen also denselben Bau, wie wir ihn bei *Mesembry-*

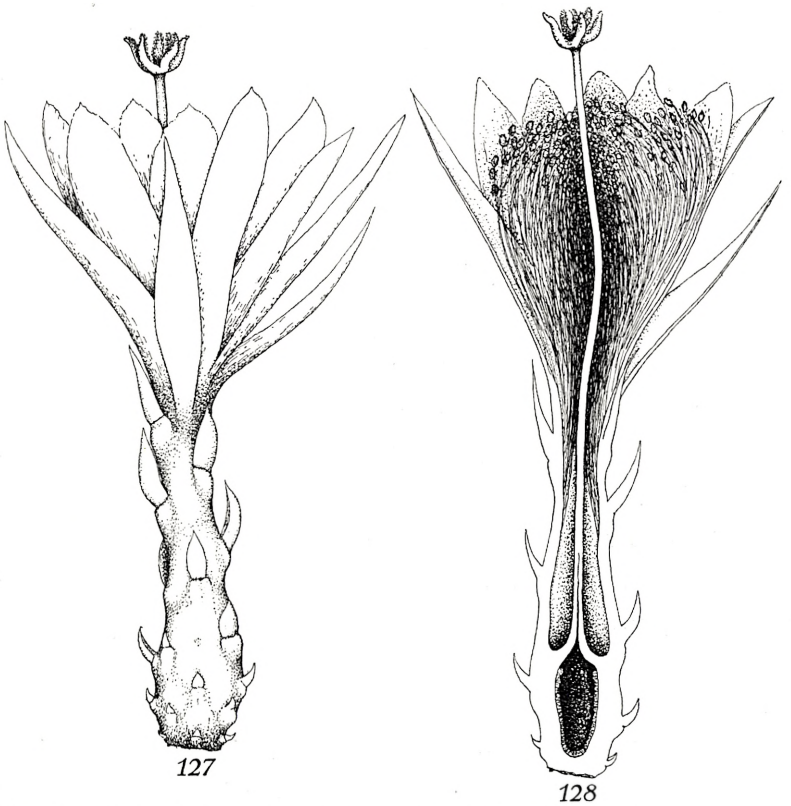

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Fig. 127—128. *Phyllocactus sp.* Blüte. Fig. 128. Längsschnitt, zeigt den röhrenförmigen Stengelkrug mit schuppenförmigen Blättern an der Aussenseite, Perianthium am Rande und Staubblättern, Karpellen und darunter Makrosporophyllen (= Samenanlagen) im Innern. Vgl. Fig. 165 u. den Text.

anthemum vorfinden, auf; aber bei der abgebildeten *Phyllocactus* z. B. ist der Stengelkrug so tief, dass er röhrenförmig genannt werden muss. Dass diese Röhre nicht aus verwachsenen Blättern gebildet, sondern ein Stengel ist, beweist —

ausser der Entwicklungsgeschichte — auch der Umstand, dass er Blätter trägt (Fig. 127—128), und zwar sowohl an der Aussenseite (wo sie schuppenförmig sind) als auch an der Innenseite, wo sie oben als Staubblätter, darunter als Griffel und ganz unten als Samenanlagen entwickelt sind.

Es ist ferner noch an den oft wiederholten Versuch zu erinnern, wo man den Fruchtknoten als Steckling benutzt; aus demselben kann sich dann eine neue Pflanze entwikkeln. Das Schema Fig. 165 veranschaulicht uns also den Bau der Kaktusblüte.

10. *Portulaccaceae.*

Die im obigen besprochenen Familien gaben namentlich zu Untersuchungen von Gynöceen mit zentraler oder parietaler Plazentation Anlass; und es wurde dadurch nachgewiesen, dass diese beiden Stellungen von einander abzuleiten sind. Bei den Portulaccaceen gibt es noch einen Gynöceen-Typus, der zugleich innerhalb anderer Gattungen der höheren Phanerogamen weit verbreitet ist, und der deshalb besonderes Interesse beanspruchen darf, wenn es den Versuch gilt, die an dieser Stelle nur innerhalb einer Reihe gemachten Beobachtungen zu generalisieren.

Dieser neue Gynöceentyp (den *Portulacca* aufweisen kann), zeichnet sich dadurch aus, dass zwar ein Stengelkrug da ist, dass es aber nur die äusserste Spitze der zentralen Plazenta ist, die eine krugförmige Gestalt besitzt. Bei der Untersuchung des Gynöceums von *Portulacca* ist es zunächst von Wichtigkeit festzustellen, dass alle anderen untersuchten Vertreter der Familie wie die Caryophyllaceen gebaut sind; das Gynöceum hat eine einfache zentrale Plazenta, woran die Scheidewände befestigt sind. Von diesem gewöhnlichen Typus lässt sich nun das Gynöceum von

Portulacca ableiten, wie es aus einer Untersuchung der Entwicklungsgeschichte erhellt.

Wir fangen mit der Untersuchung des einfachsten Typus von Gynöceen an, und auch hier können wir uns wieder

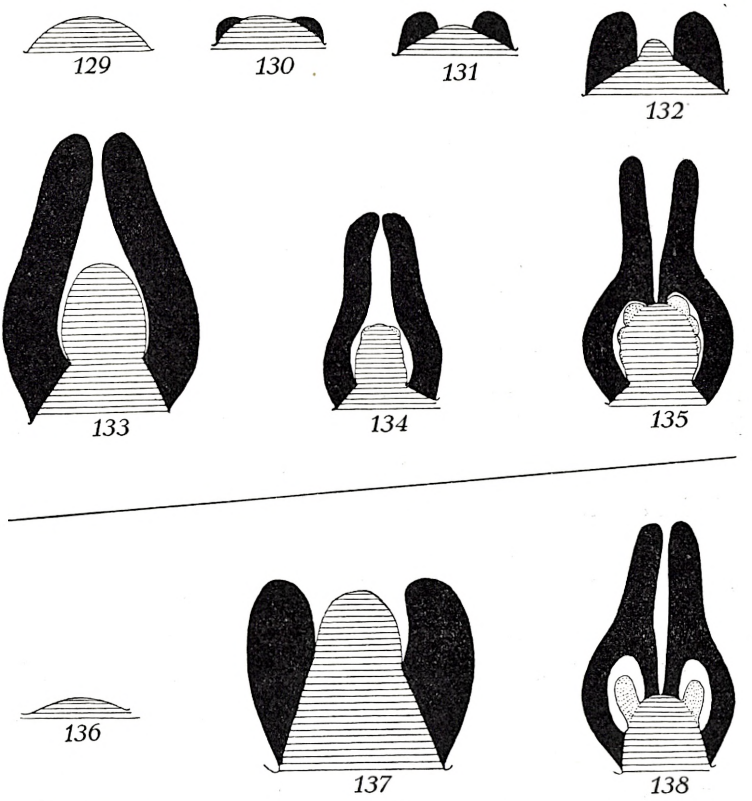

Fig. 129—138. *Portulaccaceae*. Entwicklungsgeschichte des Gynöceums. Stengel schraffiert; Karpellen schwarz; Samenanlagen getüpfelt. Fig. 129—135. *Talinum cuneifolium* Willd. Fig. 129—133, × 160; Fig. 134—135, × 80. Fig. 136—138. *Claytonia sibirica* L. Fig. 136—137, × 200; Fig. 138, × 75. Vgl. den Text.

mit ganz wenigen Typen begnügen, die nur dazu dienen sollen, die schönen Beobachtungen Payers zu ergänzen. Payer untersuchte *Portulacca*, *Calandrinia*, *Montia* und *Talinum*. Aber auch diese Untersuchungen — wie die schon

früher erwähnten — sind mit Fehlern behaftet, die dadurch hervorgerufen sind, dass er die Entwicklung der innerhalb der geschlossenen Karpellen befindlichen Teile nicht genau hat verfolgen können; namentlich trifft dies für die ersten Entwicklungsstadien der Samenanlagen und deren Platz ein, wo nur gute Schnittserien befriedigende Resultate ergeben.

Die Fig. 129—138 zeigen uns einige der wichtigsten Entwicklungsstadien von zwei Arten mit einfacher zentraler Plazenta. Wie gewöhnlich innerhalb der Centrospermen ist die Plazenta der zuerstangelegte Teil des Gynöceums (Fig. 129, 136). Die Karpellen werden unmittelbar unterhalb der Plazenta angelegt (Fig. 130), die ihr Wachstum zwischen die Karpellen hinauf fortsetzt (Fig. 132, 133). Die jüngsten Blattanlagen entwickeln sich zu Samenanlagen, die von den Karpellen umschlossen werden.

Bei *Portulacca* sind die ersten Entwicklungsstadien des Gynöceums genau wie bei den anderen untersuchten Portulaccaceen: die Fruchtblätter decken nicht das Achsenende zu (Fig. 139, 140), sondern dasselbe ragt frei zwischen ihnen empor (Fig. 141). In einem frühen Zeitpunkt der Entwicklung macht aber die junge Plazenta ähnliche sonderbare Formänderungen (Fig. 142—146) durch, wie oben für die Gynöceen von *Juniperus* (Fig. 1—6), *Mesembryanthemum* und *Cactaceae* beschrieben, Änderungen, die zur Bildung eines Stengelkrugs an der Spitze der Plazenta führen. Das Lumen dieses Krugs ist jedoch keine einfache Röhre, sondern ein tiefer, enger Spalt (Fig. 146), von wo nach jedem Hohlraum des Fruchtknotens noch je ein Spalt führt. Die Form dieser Plazenta lässt sich am besten an einer Serie von Querschnitten durch eine Blüte untersuchen, wie die Fig. 147—149 es veranschaulichen. Fig. 147 zeigt uns einen

Schnitt durch das Gynöceum unmittelbar über der Plazenta; man bemerke, dass die (senkrecht schraffierten) Ränder der Fruchtblätter nicht an einander heranreichen und nicht an der Innenseite verdickt sind.

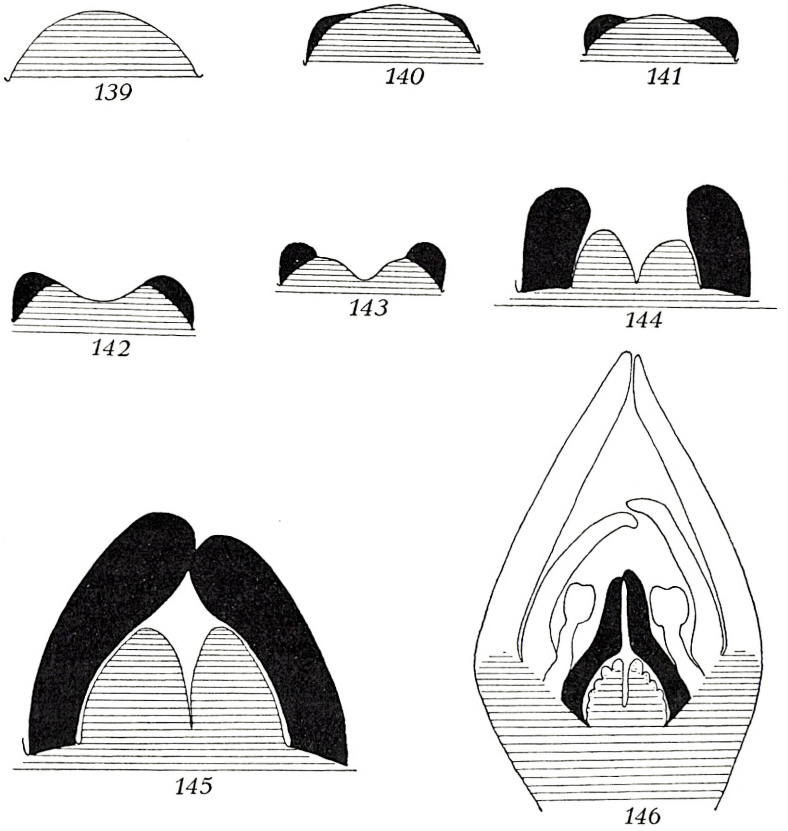

Fig. 139—146. *Portulacca oleracea*. Entwicklungsgeschichte des Gynöceums (in Längsschnitten). Stengel schraffiert; Karpellen schwarz; Samenanlagen getüpfelt. × 250. Fig. 146. Längsschnitt durch eine junge Blüte. Spitze der Plazenta gespalten. Vgl. Fig. 164 u. den Text.

Unterhalb der Spalte in der Plazenta ist der Querschnitt derselben ungefähr zirkulär (Fig. 149), und an ihrer Oberfläche sieht man die ersten zarten Anlagen zu Blättern (= Samenanlagen, getüpfelt). Trifft der Schnitt dagegen

den oberen Teil der Plazenta (Fig. 148), so zeigt es sich, dass ihre Spitze in der Längsrichtung in mehrere Läppchen gespalten ist. Vergleicht man die Längs- und Querschnitte, wird man einsehen, dass die Plazenta eine ähnliche Gestalt besitzt wie der Stengel (Cupula), der bei *Fagus* die Früchte umgibt.

Bildungen wie z. B. die auf Fig. 148 dargestellten machen die allgemein angeführte Theorie verständlich, nach welcher die Samenanlagen den geschwollenen Rändern der Frucht-

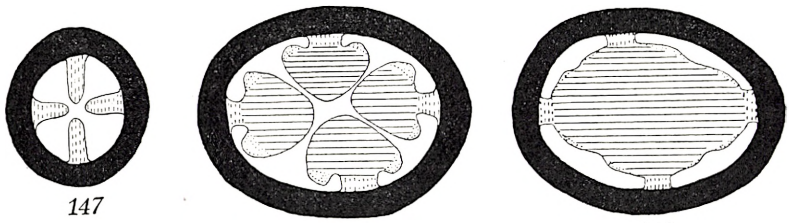

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Fig. 147—149. *Portalacca oleracea*. Querschnitte durch junges Gynöceum: in der Nähe der Spitze (Fig. 147), durch den gespaltenen Teil der Plazenta (Fig. 148) und an der Basis (Fig. 149). Karpellen schwarz; Scheidewände senkrecht schraffiert; Stengel wagerecht schraffiert; Samenanlagen getüpfelt. × 75. Vgl. im übrigen die Fig. 146 u. 164 und den Text.

blätter ansitzen sollten. Dass auch diese *Cycas*-Homologie falsch ist, zeigen sowohl die Entwicklungsgeschichte als auch ein Vergleich mit den übrigen Mitgliedern der Familie und der Gattung. (Dass die Spalte der Plazenta dazu dienen können, die Pollenröhren zu den Samenanlagen hinzuleiten, habe ich mit Sicherheit bei einigen *Bicornes* festgestellt, deren Plazenta bei sämtlichen untersuchten 35 Arten in ganz entsprechender Weise wie bei *Portulacca* gespalten ist.)

Die Läppchen, die die Samenanlagen tragen (Fig. 148), könnten auch den Anschein erwecken, als wären sie selbständige Blätter (vgl. z. B. Fig. 145), aber die Fig. 139—143

zeigen, dass sie aus der Vegetationszone selber entstehen, deren gesamte Masse als direkte Fortsetzung des Stengels in sie aufgeht; sie sind demnach mit der Spitze der zentralen Plazenta bei den übrigen Portulaccaceen und Caryophyllaceen homolog. Dass zwischen der Basis und der gespaltenen Spitze der Plazenta kein grösserer morphologischer Unterschied besteht, kommt auch dadurch zum Vorschein, dass die Samen oben und unten an der Plazenta in derselben Weise angelegt werden. Das merkwürdige Gynöceum von *Portulacca* ist von grosser Bedeutung für die phylogenetische Auffassung der Blüte bei vielen anderen Angiospermen, die eine ähnliche, an der Spitze gespaltene und mit den Scheidewänden des Fruchtknotens verwachsene Plazenta besitzen und die deshalb das Aussehen gewinnen, als ob sie »geschwollene Ränder« hätten, die die Samenanlagen trügen.

11. Kritische Zusammenfassung.

1. Plazenta und Karpellen.

Mit Hinblick auf den phylogenetischen Endzweck unserer Aufgabe wollen wir jetzt versuchen, die im obigen gegebene bunte Sammlung von Beobachtungen so kurz und übersichtlich wie möglich zusammenzufassen. Wir lassen deshalb alle Fragen, die nicht von direkt phylogenetischer Bedeutung sind, fort; so sind z. B. die Scheidewände des Gynöceums im vorhergehenden nur oberflächlich behandelt worden, weil die Samen ihnen nicht ansitzen (Fig. 151); sie finden sich jedoch z. B. (senkrecht schraffiert) auf den Fig. 83—91 u. 147—149. Der Einfachheit halber haben wir in der Regel auch nicht die Blütenteile unterhalb des Gynöceums gezeichnet. Die Fig. 7—13, 106—114 u. 115—122

stellen jedoch Zeichnungen von ganzen Blüten dar, um uns zu zeigen, dass die Blütenachse sich durch das Gynöceum fortsetzt und die Plazenta bildet. Dass die Plazenta nichts von den Fruchtblättern enthält, geht —

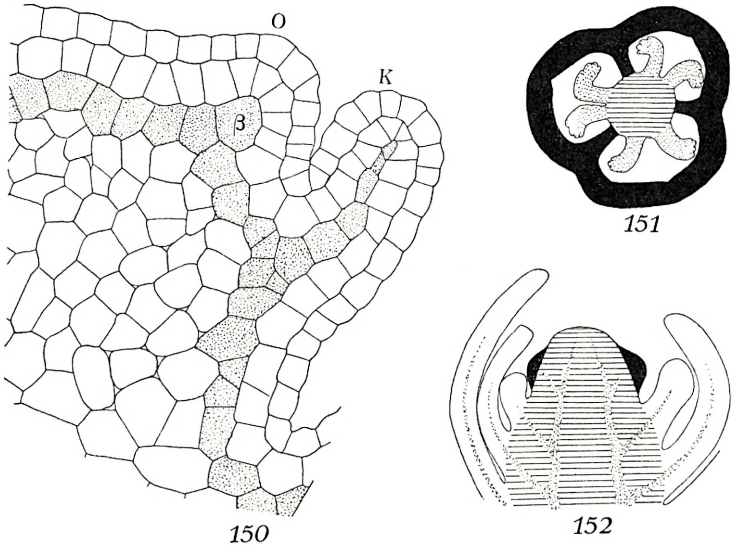


Fig. 150. *Gisekia pharnaceoides* L. Rechte Hälfte der Vegetationszone eines jungen Gynöceums mit junger Samenanlage (O). Dieselbe entsteht nicht am Karpell (K), sondern in der drittäussersten (getüpfelten) Schicht des Stengels, deren Zellen (bei β) in O eindringen. Die Samen werden also als selbständige Blätter (Sporophylle) am Stengel angelegt. × 600. Fig. 151. *Talinum cuneifolium* Willd. Querschnitt durch Fruchtknoten, welcher zeigt, dass die Samen (getüpfelt) nicht den Karpellen (schwarz), sondern dem Stengel (schraffiert) aufsitzen. × 50. Fig. 152. *Arenaria serpyllifolia*. Längsschnitt durch eine ganze Blüte zeigt, dass die Plazenta eine direkte Fortsetzung des Stengels bildet. Gefässbündel getüpfelt; Karpellen schwarz. × 270. Vgl. Text.

ausser aus der Entwicklungsgeschichte (Fig. 153—157) — auch daraus hervor, dass sie ihre Leitbündel nicht aus den Karpellen empfängt (Fig. 152); das Leitbündelsystem setzt sich ununterbrochen von der Blütenachse durch die Plazenta weiter fort.

Wie schon von GOEBEL und anderen Forschern betont und bewiesen, muss man jedoch mit Schlussfolgerungen aus dem Verlauf der Leitbündel sehr vorsichtig sein, da derselbe physiologisch und nicht morphologisch bedingt sein kann. (Vergl. SAUNDERS und EAMES).

Die Hauptergebnisse der Entwicklungsgeschichte sind am kürzesten mit Hilfe der umstehenden Figuren (153—157), die die Organogenie des Gynöceums bei einem typischen Vertreter der Centrospermen (*Gisekia*) darstellen, zusammenzufassen: die Spitze der Blütenachse ist es, die zur Plazenta wird; und da diese vor den Karpellen (Fig. 153) da ist, kann die Plazenta folglich nicht — wie gewöhnlich angenommen wird — Teile der Karpellenränder enthalten.

Der Vegetationspunkt wird nicht ganz von den Karpellen zugedeckt (Fig. 154) und auch nicht »verbraucht«; die Karpellen sind laterale Organe (Blätter), zwischen denen das Achsenende (= Plazenta) hervorragt und sein Wachstum sowohl in Länge als Breite fortsetzt. Auch die Karpellen wachsen weiter und werden zu einem Involucrum von sterilen, kahnförmigen Blättern (Fig. 156, 157), das die darüber sitzenden Samenanlagen, die nicht den Rändern der sterilen Karpellen, sondern der Plazenta (d. h. der Blütenachse) ansitzen, umgibt.

Um das Verhältnis zwischen Karpell und Samenanlagen noch eingehender zu veranschaulichen, zeigt uns Fig. 150 einen stark vergrösserten Schnitt durch eine junge Samenanlage (O) von *Gisekia*. Links auf Fig. 150 sieht man die obere rechte Hälfte der Vegetationszone der Plazenta. Der Schnitt hat ferner noch ein Karpell (K) getroffen, und man sieht, dass dieses Blatt (K) aus den drei äussersten Zellen-

schichten des Stengels (von denen auf Fig. 150 die innerste getüpfelt ist) gebildet ist. Diese dritte Zellenschicht lässt sich leicht den ganzen Schnittrand herum verfolgen, und sie erstreckt sich schon mitten ins Karpell (K) hinein und wird mit Hilfe der Zelle β, die die beiden davor liegenden Zellenschichten schon gleichsam emporgehoben hat, in die

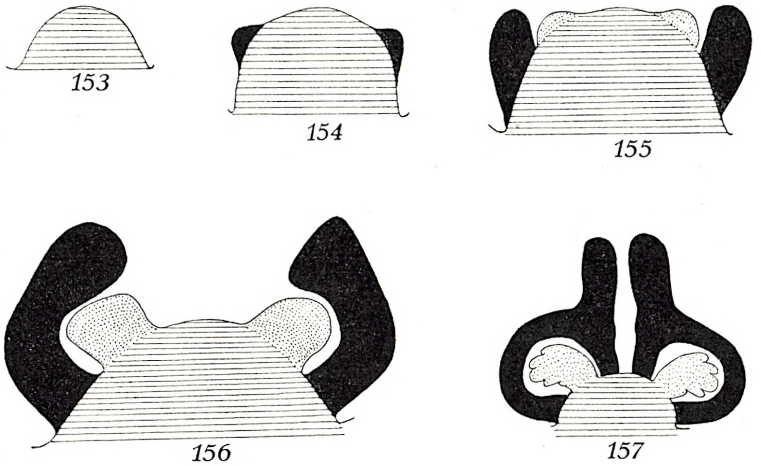


Fig. 153—157. *Gisekia pharnaceoides*. Entwicklungsgeschichte eines typischen Centrosperm-Gynöceums: Plazenta = Achsenende (schraffiert). Die Karpellen (schwarz) tragen nicht die (getüpfelten) Samenanlagen, welche selbständige, Karpellen und Laubblättern homologe Blätter (Makrosporophylle) sind. Fig. 153—156, × 200. Fig. 157, × 70. Vgl. im übrigen den Text u. Fig. 162.

Samenanlage (O) hineinwachsen. Der Same (O) wird also am Stengel (d. h. an der Plazenta) angelegt, genau wie das Karpell. Das will folglich heissen: als ein selbständiges Blatt, das deutlicherweise nicht am Karpell entsteht. Dies trifft auch für alle anderen, an dieser Stelle untersuchten Centrospermen zu.

2. Die Samenanlagen.

Was die Samenanlagen morphologisch gesehen eigentlich sind, ist die nächste Hauptfrage, wenn es gilt, die Abstammung der Angiospermen aufzudecken. Dieses Problem wurde schon im vorigen Jahrhundert eifrig diskutiert, und alle denkbaren Möglichkeiten wurden erwogen. Zum grossen Teil war es die Unvollkommenheit der Technik, die die grossen Morphologen der Vergangenheit das eigentliche Wesen der Samenanlagen nicht erkennen liess. Heutzutage begnügt man sich oft mit den bekannten *Cycas*-Homologien, und diese treffen, wie es aus den obigen Ausführungen hervorgeht, jedenfalls für die Centrospermen nicht zu. Die ältere Literatur ist indessen so oft und ausgiebig besprochen worden, z. B. von EICHLER (1875), WORSDELL (1904), DE HAAN (1920) u. a. m., dass eine nochmalige Darstellung nur eine Wiederholung bedeuten würde; wir begnügen uns deshalb mit einem Hinweis auf die Arbeiten der genannten Forscher.

Die besten der älteren Untersuchungen (z. B. die von CRAMER, 1864) fussen auf Studien über Vergrünungen; jedoch auch diese Untersuchungen sind recht unzuverlässig, da es schwierig sein kann, ein missgebildetes Organ mit einem normalen zu homologisieren.

Das wichtigste Hilfsmittel zur Beseitigung dieser Unsicherheit bietet uns die moderne Paläontologie, indem sie nachgewiesen hat, dass mindestens innerhalb zweier verschiedener Gruppen von Archegoniaten Samen entstanden sind. Von diesen gibt es in den rezenten Cycadeen Nachkommen der Pteridospermen. Ferner habe ich schon in früheren Arbeiten nachzuweisen versucht, dass *Coniferae* und *Gnetales* von den *Lycopodiinae* (*Lepidospermae*) abstammen.

Hieraus folgt, dass nicht sämtliche Samenanlagen unter sich homolog sind, und dass die Gymnospermen polyphyletisch sind; da die Angiospermen von den letzteren abstammen, sind sie also ebenfalls polyphyletisch. Dies beweist wiederum, dass man nur nach gründlichen Untersuchungen und mit grösster Vorsicht von einer Gruppe von Angiospermen verallgemeinernde Schlüsse auf andere Gruppen ziehen darf. Und man darf auch nicht ohne weiteres von den Centrospermen z. B. auf andere Pflanzen schliessen.

Dass die meisten Samenanlagen dennoch ungefähr gleich aussehen, mag darauf beruhen, dass sie denselben Aussenbedingungen unterworfen sind, indem sie sich bei schlechten Raumverhältnissen im Dunkeln eingesperrt entwikkeln. Und wie z. B. fliegende oder schwimmende Geschöpfe oft an den Organen, die am meisten vom Aufenthaltsmedium des Tieres beeinflusst sind, einander ähnlich werden, so ergeht es auch den im Fruchtknoten eingesperrten Organen. Ferner ist auch noch zu bedenken, dass die genannten Ahnen der Angiospermen (als Archegoniaten) unter einander verwandt sind, welches wiederum in gewissen Übereinstimmungen im Bau der Fortpflanzungsorgane zum Ausdruck kommt.

Wir können deshalb die von M. L. Thompson benutzte Ausdrucksweise: »The state of flowering known as angiospermy« zur unsrigen machen; auch Gymnospermie bezeichnet, genau wie Angiospermie es tut, nur ein Stadium in der phylogenetischen Entwicklung verschiedener Pflanzengruppen. Wir müssen also damit rechnen, dass einige Angiospermen von den Cycadeen abstammen, während andere Nachkommen von *Coniferae* und *Gnetales* sind.

Dass mehrere Forscher festgestellt haben, dass bei gewissen Pflanzen die Samenanlagen Stengeln, bei anderen

wiederum Blättern (Karpellen) ansassen, kann uns deshalb auch nicht überraschen. Diese anscheinend scharfen Widersprüche werden demnach ausgeglichen: beide Möglichkeiten sind vorhanden, und die eifrigen Diskussionen wurzelten in dem Dogma, dass alle Samenanlagen homolog sein sollten (Čelakowský, Strasburger, Eichler); dass dies falsch ist, haben also erst paläontologische Forschungen der jüngsten Zeit bewiesen.

Vielleicht gehören *Rosales* und *Saxifragales* zu dem Phylum, welches die Cycadeen fortsetzt? M. L. Thompson fasst das Karpell der Leguminosen als ein Phyllocladium auf, was jedoch anderseits von Newman in Frage gestellt wird. Besonders sei hier auf die schönen Untersuchungen von Troll und Eber hingewiesen. Troll u. Eber haben u. a. mit Gruppen von Monocotyledonen (*Helobiae*) gearbeitet, die »laminale Plazentation« aufweisen können. Man könnte annehmen, dass solche Karpellen vom *Cycas*-Sporophyll abgeleitet werden könnten, wenn nicht andere *Helobiae* Samenanlagen besitzen, die der Blütenachse ansitzen (Eber) und deshalb selbständige Blätter wie bei den Centrospermen sind. Es ist deshalb zu untersuchen, ob nicht einige der Gynöceen von *Helobiae* in entsprechender Weise wie bei *Phytolacca* gebaut sind, wo das Karpell nur ein steriles kahnförmiges Blatt ist, welches die Samen nicht trägt, sondern nur zudeckt.

Nur das von den Coniferen ausgehende Phylum ist der Gegenstand der Untersuchungen gewesen, die in sowohl dieser als in meinen beiden früheren Arbeiten mitgeteilt sind. Unser phylogenetischer Ausgangspunkt ist hier *Juniperus*, diejenige unter den Koniferen, die den Angiospermen am nächsten steht; sie besitzt nach der Bestaubung ein geschlossenes Gynöceum, das in den Haupzügen wie die Gynöceen

von *Gnetales*, *Juglandales* und *Piperales* gebaut ist. Den obigen Ausführungen zufolge gehören auch die Centrospermen diesem Formenkreis an.

Dass das Integument der Koniferen ein selbständiges Blatt (Sporophyll) ist, haben wir schon früher zu beweisen versucht (1933, S. 24—36); diese Auffassung fusste auf organogenetischen und teratologischen Untersuchungen. Da ferner auch Lanfer zahlreiche Missbildungen abgebildet hat, werden wir uns mit nebenstehenden Figuren von *Juniperus communis* begnügen, die Übergänge zwischen röhrchenförmigen Integumenten und sterilen, nadelförmigen Blättern darstellen.

Die Blütenachse trägt über den drei (auf den Fig. schwarz gezeichneten) »Karpellen« drei Samenanlagen (fein getüpfelt), die also — wie u. a. die Entwicklungsgeschichte es beweist — nicht den Karpellen aufsitzen. Ausnahmsweise trägt die zentrale Plazenta über den normalen 3 Samenanlagen noch 2—3 Blätter (grob getüpfelt). In dem auf Fig. 158 gezeichneten Gynöceum sind die drei überzähligen Blätter alle als sterile Laubblätter entwickelt; aber Fig. 159 zeigt, dass eines von ihnen (a) einen rudimentären Nuzellus haben kann, der von einem röhrchenförmigen, dem nadelförmigen sterilen Blatte a auf Fig. 158 homologen Integument umgeben ist. Und schliesslich zeigt uns Fig. 160 ein Gynöceum, wo das eine Blatt, b, welches normal (Fig. 159) als Integument (Megasporophyll) entwickelt ist, zu einem sterilen, nadelförmigen Blatt geworden ist. Es gibt bei den Koniferen alle möglichen Übergänge zwischen röhrchenförmigen Integumenten und schuppen- oder nadelförmigen sterilen Blättern, worüber z. B. Lanfer nähere Aufschlüsse gibt.

Es sind bei den Centrospermen viele Vergrünungen

gefunden worden, und zwar besonders innerhalb der Caryophyllaceen und da wiederum am häufigsten bei *Stellaria media*, *Agrostemma githago*, *Dianthus caryophyllus* u. a. Auch das Gynöceum ist oft in sehr verschiedenartiger Weise missgebildet; z. B. kann die Plazenta durch den Fruchtknoten hindurchwachsen und zu einem kürzeren oder längeren Zweig werden. Die auf diese Weise in die Luft hinausgeführten Samenanlagen machen verschiedene

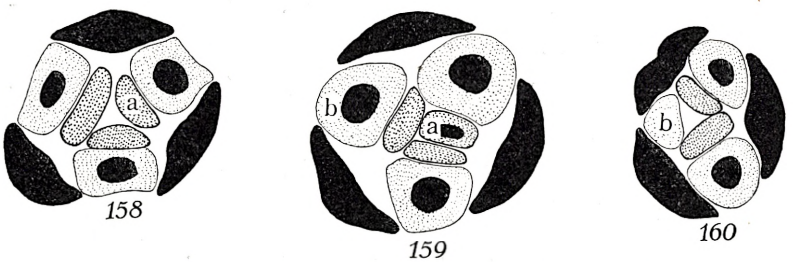


Fig. 158—160. *Juniperus communis*. Querschnitte durch Gynöceen, die Übergänge (Vergrünungen) zwischen Samenanlagen (Makrosporophyllen) und Blättern (a u. b) veranschaulichen. Innerhalb der 3 Karpellen (schwarz) stehen ein äusserer (fein getüpfelter) und ein innerer (grob getüpfelter) Wirtel von Blättern, die bald fertil (Integumente), bald steril und nadelförmig sind. Vgl. im übrigen den Text.

sonderbare Änderungen (»Oolysen«) durch; sie können z. B. eine Ähnlichkeit mit Karpellen annehmen (vgl. MASTERS Fig. 179, 180). In anderen Fällen können die umgebildeten Samenanlagen solchermassen gestaltet sein, dass sie Petalen oder grünen Laubblättern ähneln. Die meisten in Gärten gezogenen Nelken weisen ja solche missgebildeten Blüten auf, wo die meisten Blätter Petalen ähneln. Die reichhaltige Literatur über diese Missbildungen ist von PENZIG zusammengestellt und so gut bearbeitet worden, dass ein Hinweis auf seine Arbeit und auf die Figuren bei MASTERS genügen wird.

Was den Funiculus betrifft, ist nur zu erwähnen, dass

er bei *Coniferae*, *Gnetales*, *Juglandales* und *Piperales* (samt *Polygonales*) fehlt; bei diesen ist das Integument (= Megasporophyll) sitzend, und der Nuzellus steht an dessen Basis. Bei den Centrospermen und den meisten anderen Angiospermen gibt es dagegen einen deutlichen Funiculus, welches nur besagen will, dass das Sporophyll gestielt ist, und dass der Nuzellus an dessen Spitze steht. Im Vergleiche hierzu sei noch erwähnt, dass auch das Mikrosporophyll bei den Coniferen meistens ungestielt, bei den Angiospermen aber gestielt ist.

Das äussere Integument war bei *Gnetum* ein selbständiges Blatt, wogegen es bei *Taxus* kein Blatt, sondern ein Organ »sui generis« war. Wir müssen deshalb die Möglichkeit in Betracht ziehen, dass nicht alle Integumente der Angiospermen unter sich homolog sind. Diese Frage greift jedoch über den Rahmen dieser Untersuchung hinaus, und wir verweisen auf De Haan. Der Übersicht halber unterlassen wir ebenfalls Untersuchungen über die vielen anderen Einzelheiten im Bau des Gynöceums (z. B. ob das innere oder das äussere Integument die Lamina des Sporophylls ist).

Wir betonen deshalb noch einmal in aller Kürze, dass wir das Makrosporophyll (= Funiculus + Integument) als ein schildförmiges Blatt (Schlauchblatt) auffassen, wie es von Troll meisterhaft beschrieben worden ist.

3. Phylogenie des Gynöceums.

Im obigen haben wir versucht, die Aufschlüsse über den Bau des Centrosperm-Gynöceums, die für dessen Verständnis notwendig sind, zu geben; wir werden dieselben nun phylogenetisch verwerten, indem wir Vergleiche mit den

Gynöceen von *Coniferae* und *Gnetales* anstellen. Die Tatsachen haben uns gezwungen, die üblichen *Cycas*-Homologien aufzugeben; sie sind für sämtliche besprochenen Pflanzen falsch. Wir ersetzen sie durch die neue Auffassung vom Centrosperm-Gynöceum, die in ne-

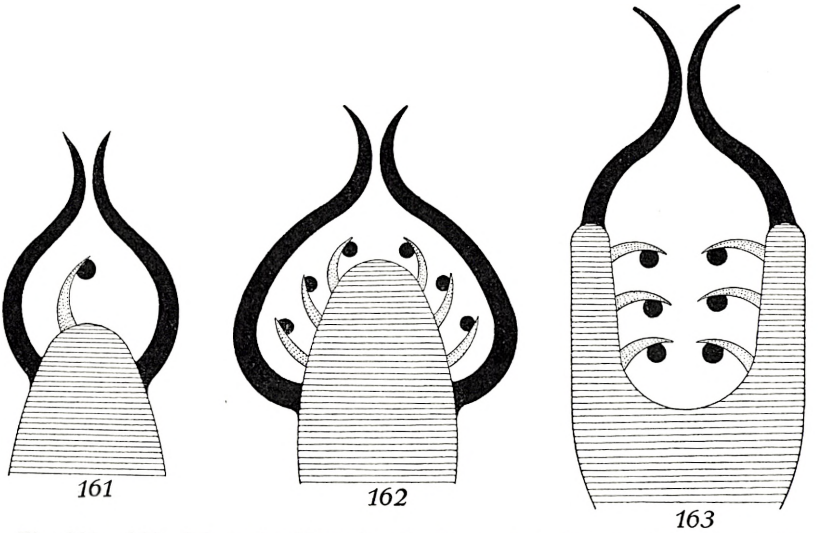


Fig. 161—163. Schemata über die Gynöceen bei den *Centrospermae*. Die Karpellen (schwarz) sind sterile Blätter, die die Makrosporophylle (= Samenanlagen, getüpfelt) umgeben. Plazenta (wagerecht schraffiert) ist die Spitze der Blütenachse. Nuzellus (schwarz) = Makrosporangium. Fig. 161. Die einsamige Zentral-Plazenta ist in sämtlichen Familien (z. B. *Chenopodiaceae*) vertreten. Fig. 162. Die mehrsamige Zentral-Plazenta gibt es nur in wenigen Familien (*Caryophyllaceae, Portulaccaceae. Aizoaceae* und *Amaranthaceae*). Fig. 163. Parietale Plazentation in einem Stengelkrug (dessen Bildung auf Fig. 1—6 dargestellt ist) gibt es bei *Mesembryanthemum, Cactales* (vgl. Fig. 165) und *Juniperus* (Fig. 6). Vgl. den Text.

benstehenden Schemas (Fig. 161—165) zum Ausdruck kommt. Es ist ferner leicht ersichtlich, wie sehr diese Centrosperm-Gynöceen an den *Juniperus*-Fruchtknoten erinnern, so wie dieser in den Schemas (Fig. 1—6) dargestellt ist, indem wir daran festhalten, dass eine Samenanlage

einem Makrosporophyll von ähnlichem Typus wie das der *Lycopodiinae* homolog ist.

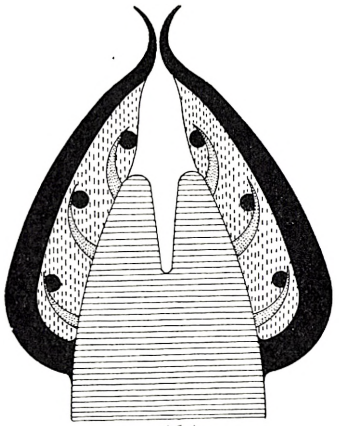
Die erwähnten Gynöceen bestehen sämtlich aus einem kurzen Achsenende mit Blättern. Ursprünglich war die Achse des Gynöceums kegelförmig; behält sie diese Form, so bildet sie eine zentrale Plazenta (Fig. 162), ganz wie im jungen *Juniperus*-Fruchtknoten (Fig. 2), bei den Caryophyllaceen, vielen Aizoaceen, Portulaccaceen usw.

Wenn die zentrale Plazenta nur einen Samen entwickelt, erhalten wir das auf Fig. 161 dargestellte Gynöceum, welches sich bei den vielen einsamigen *Juniperus*-Arten, *Taxus*, samt bei einigen Caryophyllaceen, vielen Amaranthaceen, Nyctaginaceen, Chenopodiaceen, vielen Phytolaccaceen u. a. m. vorfindet.

Schliesslich kann das Ende der Blütenachse die Gestalt wechseln, so dass es infolge eigentümlicher Wuchsprozesse (Fig. 1—6) krugförmig — wie ein eingestülpter Handschuhfinger — wird (Fig. 6, 163, 165). Da die Blätter des Achsenendes (= Samenanlagen) in Übereinstimmung mit den erwähnten Formänderungen ihre Stellung ändern müssen, erhält das Gynöceum in diesem Falle parietale Plazentation. Dies ist bei *Juniperus communis* (Fig. 6), *Cactaceae* (Fig. 165) und vielen *Mesembryanthemum*-Arten der Fall.

Bei *Portulacca* (Fig. 164) gibt es eine spezielle Form von zentraler Plazenta, die sich dadurch auszeichnet, dass sie an der Spitze gelappt ist; und jedes Läppchen ist an eine der Scheidewände (auf Fig. 164 senkrecht schraffiert) festgewachsen. Ein Querschnitt durch ein solches Gynöceum (Fig. 148) könnte den Eindruck erwecken, als sässen die Samen »den geschwollenen Rändern der Fruchtblätter« an. Sowohl die Entwicklungsgeschichte als auch ein Vergleich mit den übrigen Portulaccaceen zeigen aber, dass diese

»geschwollenen Ränder« Teile der Blütenachse sind. Dieses Gynöceum ist von ganz besonderer Bedeutung, wenn man verstehen will, wie man die *Cycas*-Homologien als für sowohl *Centrospermae* als auch viele andere Pflan-



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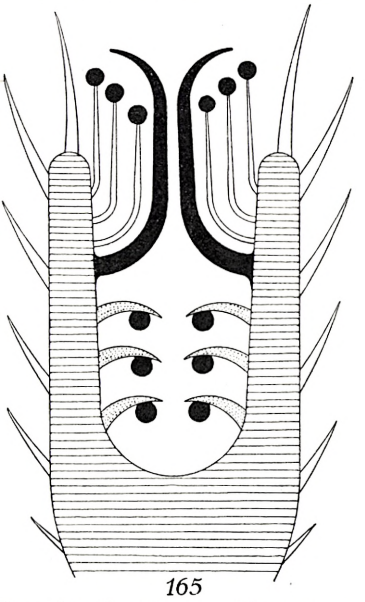

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Fig. 164. *Portulacca*. Schema über den Bau des Gynöceums. Plazenta an der Spitze gespalten; im übrigen wie bei den *Caryophyllaceae* (Fig. 162). Scheidewände senkrecht schraffiert. Vgl. den Text. Fig. 165. *Cactaceae*. Schema über den Bau der Blüte. Der Stengelkrug (wagerecht schraffiert) trägt sowohl aussen als innen Blätter. Die Karpellen (schwarz) sind neutrale Blätter, die Makrosporophylle (= Integumente, getüpfelt) und Mikrosporophylle (schwarze Spitze) von einander trennen. Der Stengelkrug trägt am Rande Perianthium und an der Aussenseite Schuppenblätter. Vgl. den Text.

zengruppen mit ähnlichen Gynöceen zutreffend hat generalisieren können.

Wir warnen energisch vor der Annahme, dass das Gynöceum aller anderen Angiospermen in ähnlicher Weise wie bei *Centrospermae* (und *Juniperus*) gebaut sei. Eine derartige Generalisierung ist nur nach einer gründlichen Untersuchung jeder Reihe für sich vorzunehmen. Zum Zweck

einer vorläufigen Orientierung habe ich einzelne Stichproben verschiedener anderer Ordnungen untersucht, wie z. B. *Fagales*, *Juglandales*, *Primulales*, *Personatae*, *Ericales*, *Rubiales*, *Myrtales*, *Columniferae*, *Tricoccae*, *Polygonales*, *Synandrae* u. a. m., die mit noch anderen von J. M. Thompson, Troll, Payer u. a. untersuchten Typen zusammen vermuten lassen, dass das von *Coniferae* ausgehende Phylum

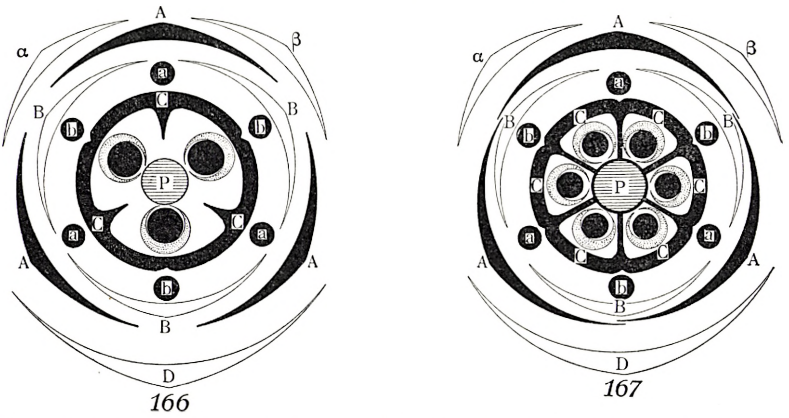

Fig. 166—167. Diagramme, die die Ähnlichkeit der Blüten von *Juniperus communis* (Fig. 166, nach Renners Angaben zwitterig gezeichnet) und eines Vertreters der Angiospermen (Fig. 167, *Empetrum hermaphroditum*) veranschaulichen. Bei beiden Pflanzen ist die Plazenta die Spitze der Blütenachse (P), deren oberste Blätter Makrosporophylle (= Samenanlagen, getüpfelt), sind, die wiederum von sterilen Hochblättern (= Karpellen, C) umgeben werden. b, innerer Wirtel von Staubblättern; a, äussere Staubblätter, B, Krone; A, Kelch. α u. β, Vorblätter. D, Deckblätter.

noch zahlreiche andere grosse Gruppen von Angiospermen umfasst.

Juniperus schliesst sich *Gnetales* eng an, wo die Samen wie bei den Angiospermen am Boden eines aus den stark zusammengewachsenen »falschen Fruchtblättern« (= Karpellen) gebildeten Kruges sitzen, der oben nur eine ganz enge Öffnung besitzt. Der Unterschied zwischen diesen Gymnospermen und z. B. *Myrica* ist nur der rein morphologi-

sche, von welchem Blatt das Pollen aufgefangen wird. Nach der Bestaubung sind sowohl *Juniperus* als *Gnetales* angiosperm, und ihre reifen Samen sind ganz umgeben von zusammengewachsenen »falschen Fruchtblättern«, d. h. sterilen Blättern (= Karpellen), die die Samen nicht tragen, sondern nur als ein beschützendes Involucrum dieselben umgeben.

Wir haben in dieser Arbeit ferner den Nachweis zu führen versucht, dass das Gynöceum der Centrospermen (Fig. 161—165) in den Hauptzügen wie der Fruchtknoten von *Juniperus* (Fig. 2, 6) gebaut ist, eine Ähnlichkeit, die auch durch die Diagramme Fig. 166—167 veranschaulicht wird. Und man darf mit gutem Recht annehmen, dass diese neue Auffassung der genannten Gynöceen uns allmählich ermöglichen wird, den Ausgangspunkt für die phylogenetische Entwicklung eines erheblichen Teiles der jetzigen höheren Pflanzenwelt unserer Erde zu finden.

12. Resumé.

1. Die vorliegende Arbeit bezweckt, die Organogenie und Morphologie des Gynöceums der Centrospermen (und Kakteen) klarzulegen, um darauf die ermittelten Resultate phylogenetisch zu verwerten.

2. Die Haupttypen von Gynöceen innerhalb der Gruppe der Centrospermen wurden mit Hilfe von Schnittserien durch kontinuierliche Reihen von Entwicklungsstadien untersucht. Obige Figuren (161—167) veranschaulichen die wichtigsten Ergebnisse dieser Untersuchungen, und zwar kurz gefasst folgende:

3. Der zuerst angelegte Teil des Gynöceums ist die Plazenta (Fig. 153), die nur aus der Spitze der Blü-

tenachse gebildet ist; die Samen sitzen keineswegs irgendwelchem angewachsenen Teil der Karpellenränder an.

4. Erst später werden die Karpellen (Fig. 154) als laterale Blätter an der Plazenta angelegt; und bald entwickeln sie sich zu kahnförmigen sterilen Blättern, die nur die Samen umgeben und beschützen, sie aber nicht tragen. Die Karpellen sind deshalb am richtigsten als falsche Fruchtblätter zu bezeichnen, indem sie nicht den *Cycas*-Sporophyllen homolog sind.

5. Durch merkwürdige Wuchsprozesse (vgl. Fig. 1—6) gewinnt der Stengelteil des Gynöceums (bei *Mesembryanthemum* und *Cactaceae*) die Gestalt eines eingestülpten Handschuhfingers. Die ursprünglich zentrale Plazenta (Fig. 162) wird dadurch in einen Stengelkrug mit parietalem Samen verwandelt (Fig. 163).

6. Bei *Portulacca* (Fig. 164) ist die zentrale Plazenta an der Spitze gespalten; die dadurch gebildeten Stengelläppchen sehen aus, als stellten sie die »geschwollenen Ränder« der Karpellen dar.

7. Bei *Juniperus* (Fig. 1—6) und *Gnetales* ist das Gynöceum in den Hauptzügen ganz wie bei den Centrospermen gebaut: auch hier gibt es eine zentrale (oder parietale) Plazenta, und diese ist die Spitze der Blütenachse selbst. Unterhalb des Vegetationspunktes derselben werden falsche Fruchtblätter entwickelt, die die Samen nicht tragen, sondern sich nur zu einem Involucrum von sterilen Blättern entwickeln, die die Samen umgeben und beschützen.

8. Die Samen werden sowohl bei *Juniperus*, *Gnetales* als *Centrospermae* als selbständige Blätter (= Makrosporophylle) an den Seiten des Achsenendes (d. h. der Plazenta) angelegt (Fig. 155). Sie sind bei allen genannten Pflanzen unter einander und auch mit dem Sporophyll bei *Lycopodiinae* homolog.

9. Der wichtigste morphologische Unterschied zwischen den Gynöceen von z. B. *Juniperus* und den Angiospermen ist nur der, ob das Pollen an dem einen oder dem anderen Blatte keimt. Nach der Pollination werden sowohl *Juniperus* als *Gnetales* angiosperm. Bei *Gnetales* ist die Hauptmasse des Samens zwischen die ganz geschlossenen falschen Fruchtblätter versenkt. Bei *Myrica* und anderen Angiospermen besitzt das Gynöceum oben eine ähnliche winzige Öffnung wie bei *Gnetales* und ist also gewissermassen (vor der Blüte) gymnosperm.

10. Ein Vergleich zwischen den wichtigsten Hauptzügen der respektiven Gynöceen deckt, wie die Fig. 1—6 u. 161—167 uns zeigen, eine so grosse Ähnlichkeit auf, dass unserer Meinung nach auch die Centrospermen (mit *Juglandales* und *Piperales* zusammen) zum selben Phylum wie *Coniferae* und *Gnetales* gehören. Und unter den Centrospermen finden wir doch beinahe alle möglichen Gynöceen-Typen, sowohl oberständige als unterständige, sowohl zentrale als parietale Plazenta und ausserdem noch sowohl apocarpe als syncarpe Karpellen.

11. Einige der Hauptstadien der genannten Entwicklungslinie sind: *Lycopodiinae* (*Lepidospermae*) → *Coniferae* (*Juniperus*) → einige Angiospermen.

12. Für sämtliche erwähnten Pflanzen sind die *Cycas*-Homologien falsch. Wir ersetzen sie durch die in den Fig. 1—6 u. 161—167 ausgedrückte Auffassung vom Gynöceum. Diese Umwertung des Gynöceums wirft neues Licht auf die wichtigste und schwierigste phylogenetische Frage: die Abstammung der Angiospermen.

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RHYTHMIC DIURNAL VARIATIONS IN THE OESTROUS PHENOMENA OF THE RAT AND THEIR SUSCEPTIBILITY TO LIGHT AND DARK

BY

AXEL M. HEMMINGSEN AND NIELS B. KRARUP

WITH 4 TABLES AND 4 FIGURES AND 1 TABLE IN THE TEXT

KØBENHAVN
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CONTENTS.

1. Introduction.[1]

a. Diurnal rhythm of biological processes. A number of biological processes, notably metabolic, are subject to rhythmic variations synchronous with the natural astronomical alternation of day and night.

A classical example is the body temperature, which in man is lowest about 4—5 o'clock and highest about 18—19 o'clock.

In monkeys the curve of body temperature has been reversed by GALBRAITH and SIMPSON (1903; cited from LINDHARD, 1910) by reversing the daily light and dark periods.

Whereas human social life in civilized countries prevents complete reversal of night and day, in regard to factors other than light, so that even night-watches retain the normal curve of body temperature, LINDHARD (1910) in the polar night of North-East Greenland succeeded in reversing the curve of body temperature in 28 members of the Denmark Expedition by delaying bed-time once 4 hours and then 8 hours. He concludes that the curve of tem-

[1] Abstracts of the main conclusions have been read before the XV International Physiological Congress in Leningrad, 1935 (Summaries of Communications, p. 150), Dansk Naturhistorisk Forening (Danish Natural History Association, Copenhagen), 1935, and Biologisk Selskab (Biological Society, Copenhagen), 1935.

perature variations is determined by work and mode of living.

Other examples may be quoted in which, at least at first glance, the explanation appears less simple (cp. also Jores, 1935, and Müller, 1936).

Thus Hatlehol (1924) studied the blood-sugar curve of fasting diabetics and found that in heavy diabetes as a rule the blood-sugar fell in the course of the day, but rose during the night ("paradoxical rise").

The variation in regard to meals, muscular activity, and sleep, did not explain these rhythmic variations.

Pfaff and Balch (1897), et al., have found in patients carrying a biliary fistula, that the secretion of bile is subject to rhythmic diurnal variations. The same is true of the excretion of urea and urobilin. Also the diuresis is subject to such variations.

Attention has been directed to these phenomena particularly through the works of Forsgren (for references see his book from 1935). He has been engaged in studying the function of the liver, and from his publications it appears to be beyond doubt that, at least in rabbits and mice (cp. Ågren, Wilander, and Jorpes, 1931) the function of the liver is rhythmic, deposition of glycogen alternating with bile secretion; or, as Forsgren puts it, a phase of assimilation alternating with a phase of dissimilation in a 24 hours rhythm, and completely independently of, say, food consumption.

On the basis of Forsgren's studies, and in amplification of Hatlehol's work, Möllerström (1930) has found in non-fasting diabetics rhythmic diurnal variations in the blood-sugar and the excretion of sugar in the urine, apparently independent of exogenous factors.

Quite apart from their scientific importance, these rhythmic variations have a considerable practical bearing, e. g. in interpreting various functional tests and in the treatment of diabetics with insulin.

The underlying cause of these various rhythmic variations is unknown so far.

As regards the domain of sexual phenomena, a number of authors, on the basis of extensive statistical investigations, agree that deliveries in women are most frequent by night (JORES, 1935, p. 621—622); but it has received but little attention that also in the sexual phenomena of certain animals there are rhythmic diurnal variations.

Through our observation that the oestrous phenomena in the rat is subject to such rhythmic diurnal variations, an experimental basis is available for the study of their affectibility by various external factors.

Before passing on to a closer examination of this, it will be necessary to mention some details regarding the oestrous phenomena, which are necessary for the proper understanding of the following.

b. The periodicity of oestrous phenomena. In female mammals the sexual desire is generally restricted to definite periods of the mating season, the so-called periods of oestrus.

If no conception takes place during oestrus, the latter may recur a certain number of times (polyoestrous species), or there is no recurrence until the next mating season (monoestrous species).

In the wild state animals appear to have as a rule one mating season a year; but as well the number of mating seasons as the number of recurrences of oestrus (oestrous cycles) during a single mating season, may increase under

the influence of domestication. Thus, species which seem to be monoestrous in the wild state may become polyoestrous when domesticated.

The interval between the oestrous periods within a mating season is called the dioestrous interval. The non-breeding period of the year when the generative organs are at rest, is called the anoestrous period, which in many mammals occupies the greater part of the year.

The number of recurrences of oestrus within a mating season and the length of the oestrous cycles in polyoestrous mammals, varies from species to species. In cattle and in the mare the length is 3 weeks; in primates, 1 month; in the guinea pig, 16 days.

Detailed descriptions of oestrus and its associated phenomena are to be found in the works of HEAPE (1901), MARSHALL (1922), and PARKES (1929).

The laboratory rat, with which the experiments reported on in this paper, are primarily concerned (albino Wistar stock), is, like the laboratory mouse, polyoestrous with an oestrous cycle of 4—5 days (LONG and EVANS, 1922).

These laboratory forms breed practically all the year round, although there seems to be most pregnancies in the spring and summer time.

WANG (1923, 1924) and SLONAKER (1924) have shown that the spontaneous muscular activity of female rats with free access to revolving wheels, exhibits marked rhythmic changes in close relation to the oestrous cycle, the day of maximum activity coinciding with the day of oestrus.

The time relations of heat and anatomical oestrous changes in the female rat were characterized by LONG and EVANS (1922) as follows. The oestrus is preceded by the stage of prooestrus during which the follicles of the ovary

are growing large, the uterus becomes distended with fluid and the smear of the vaginal content shows epithelial nucleated cells only. Toward the end of this stage the rat may be in heat. In the stage of oestrus the follicles have attained their largest size, the eggs may maturate, uterus reaches its greatest distention, and then regresses; the vaginal smear consists of cornified cells only and the animal is in heat. In the next stage, metoestrus I, ovulation takes place, the vaginal smear consists of an abundant mass of cornified cells, and the animal has ceased to show signs of heat. Then follows the stage of metoestrus II, characterized by young corpora lutea, eggs in the oviduct, small follicles and invasion of leucocytes into the vaginal lumen. In the dioestrous interval corpora lutea continue to grow, while the other portions of the genital tract are in a stage of relative quiescence, and the vaginal smear consists of leucocytes, epithelial cells, and mucus.

The periodicity of the oestrous phenomena in the female rat can thus be conveniently studied in the intact animals by three methods: first, by recording the spontaneous muscular activity by means of revolving wheels to which the animals have free access, second by means of mating tests, which of course in the end is the most important, and third by vaginal smears.

c. The relation of oestrus to day and night, according to previous authors. The spontaneous muscular activity of the rat is confined to the nightly hours (Richter, 1922), both on the oestrous and the non-oestrous days of the 4—5 days oestrous cycle (Slonaker, 1925). According to Ishii (1922, p. 313) and Hemmingsen (1933, p. 128—129), in the female rat on the day of oestrus heat is strongest toward midnight; and there seemed also (Hemmingsen,

1933, p. 129) to be a correlation between the cyclic vaginal changes and the time of the day, although the association was not so consistent as in the case of the heat symptoms.

Young, Myers, and Dempsey (1933), and Dempsey, Myers, Young, and Jennison (1934), have reported that also in the guinea-pig heat, occurring at intervals of 16 days, is predominantly nocturnal, that it shifts with sundown, and that when the guinea-pigs are confined in a dark room, the tendency to heat at night is lost.

d. Starting points. In seeking to decide whether oestrogenic substances of various kinds were actually oestrus-producing, i. e. capable of evoking mating instincts in spayed rats, the nocturnal occurrence of these instincts made it necessary to make the injections with due allowance for the length of the reaction time (abt. 24 hours) so as to produce the oestrous phenomena at night. On the basis of the observed apparent association between the oestrous phenomena and the natural day-night rhythm, the wish naturally arose to try to shift the oestrous phenomena 12 hours by artificial reversal of day-light and dark, so as to enable inspection for mating instincts to be confined to the usual working hours, still under these new conditions making the injections with due allowance for the length of the reaction time.

To keep the animals in continuous dark, seemed too unbiological for practical purposes. It might, moreover, perhaps tend to disperse the occurrence of heat over the 24 hours, as found for the guinea-pig by Dempsey, Myers, Young, and Jennison (1934), rather than to confine it to the convenient day-time. In preliminary experiments, continuous dark appeared to bring all oestrous phenomena in

the rat to a standstill. Our evidence on this point is, however, open to the objection that the numbers used were too small, and the results, therefore, inconclusive from a quantitative standpoint.

As regards the theoretical side of the problem, it appeared to us to be of considerable interest, if it would be possible, after having established the existence of the rhythm, to influence it by varying an external factor, as for instance light. Most biological phenomena so far recognized as rhythmic in mammals, have not been subject to such an experimental test, because they have been either studied in man, whose day-night habits and illumination cannot easily be reversed, or in animals, which as a rule have been killed at the investigation, thus interrupting the rhythm.

Light was chosen as the external factor to be varied, not only because of its obvious rôle in determining the natural day and night, but also because it is known to stimulate the internal secretion of the gonads irrespective of its influence on muscular activity (BISSONNETTE, 1932, and several later authors; see discussion on p. 33—36). That the nocturnal activity of forest mice can be reversed by reversal of day and night, was shown by JOHNSON (1926).

We were also prompted to make our experiments by the fact that the reproductive activity of plants is under the influence of day-length (GARNER and ALLARD, 1920; see SCHICK, 1932, MAXIMOW, 1929, or MÜLLER, 1934).

The fact that the female rats are very active at the time of the night when they usually experience the strongest symptoms of heat, and that the daily amounts of muscular activity exhibits periodic fluctuations with maxima on the days of oestrus, made it natural to expect that if the muscular

activity were shifted by reversing the periods of darkness and light, the heat symptoms would be shifted exactly to the same extent.

At first, this might appear to follow merely from the well-known nocturnal habits of the rat. The point at issue would be whether the anatomical changes were also shifted, these being from a critical point of view more directly indicative of the action of oestrin within the organism and thus of the ovarian activit .

e. Object of this paper. The object of the present investigation was thus: first, to establish definitely for the female rat that under the natural alternation of day and night not only the oestrous phenomena under direct nervous influence, the mating instincts and the spontaneous muscular activity, but also the cyclic anatomical changes of the genital tract, as recognized by the vaginal smear, are preferably confined to certain hours of the natural day-night rhythm; second, to show that all these three groups of oestrous changes, the mating instincts, the spontaneous muscular activity, and the anatomical changes, are shifted 12 hours if an artificial day-night rhythm is established by exposing the animals to light in the night and to darkness in the day-time.

A description in detail of these three groups of oestrous phenomena in the normal female rat and the technique employed in studying them, was given by one of us (Hemmingsen, 1933, p. 113—139 and 192—200) with due reference to previous authors. A recapitulation in brief appears to be desirable here, with a special view to minor modifications of technical details.

2. Technique in recording the oestrous phenomena of the rat.

A. The spontaneous muscular activity.

The spontaneous muscular activity is recorded by means of revolving wheels in the form of wire drums, which the rats may at will enter from a small nest box attached, and which by means of an automatic device continuously register on a kymograph the number of revolutions made in either direction, say every hour, also during the night. By a cyclometer connected to the revolving wheel the diurnal number of revolutions was also read off as a check of the continuous register on the kymograph. The cages and technical devices employed are shown in figs. 1 and 2.

The wheels and cages are constructed on the basis of the description of RICHTER and WANG (1926). The automatic, continuous register was devised in collaboration with Dr. H. C. HAGEDORN and Mr. BØRGE CLAUSEN.

B. The mating instincts.

STEINACH (1894, 1911, 1912, 1913) and SAND (1918) called much attention to the psycho-sexual phenomena in regard to experimental studies on rats and guinea pigs. As already pointed out by SAND (1918, p. 58; see also HEMMINGSEN, 1933, p. 108—109, and 119) the criteria of female heat used by STEINACH are inconclusive. SAND (1918, p. 56—58; and personal correspondence), who among his studies on the sexual characters of mammals has investigated, in extensive experimental series, as well the male as the female psycho-sexual character, also in rats, first points out that this character of course is strongest at oestrus, which in guinea pigs and rats is present essentially un-

changed from about February to about November. In the other months it decreases somewhat, without completely disappearing, but in these months it may be difficult to obtain safe responses.

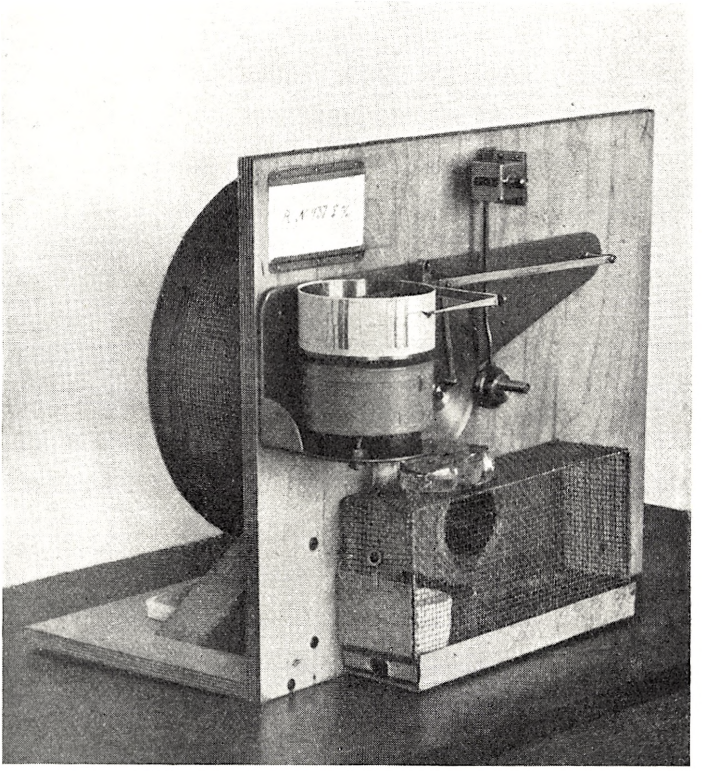

Fig. 1. Activity cage and antomatic register (cp. fig. 2).
The circular opening leads to the drum which is partly visible.

According to Sand female rats are often rather passive, but allow themselves to be pursued by males until copulation. Female rats in real oestrus directly approach the male and exhibit lordosis sub actu; the so-called "tail reflex" (vertical position of the tail of the female during pursuit), is sometimes observed, but not as a constant trait. Sand's experiments were made with piebald laboratory rats

of mixed origin; they were made both in day-light and by night at faint artificial light. SAND stated the conclusion (1918, p. 57—58) that the females rarely display clear symptoms of sexual desire.

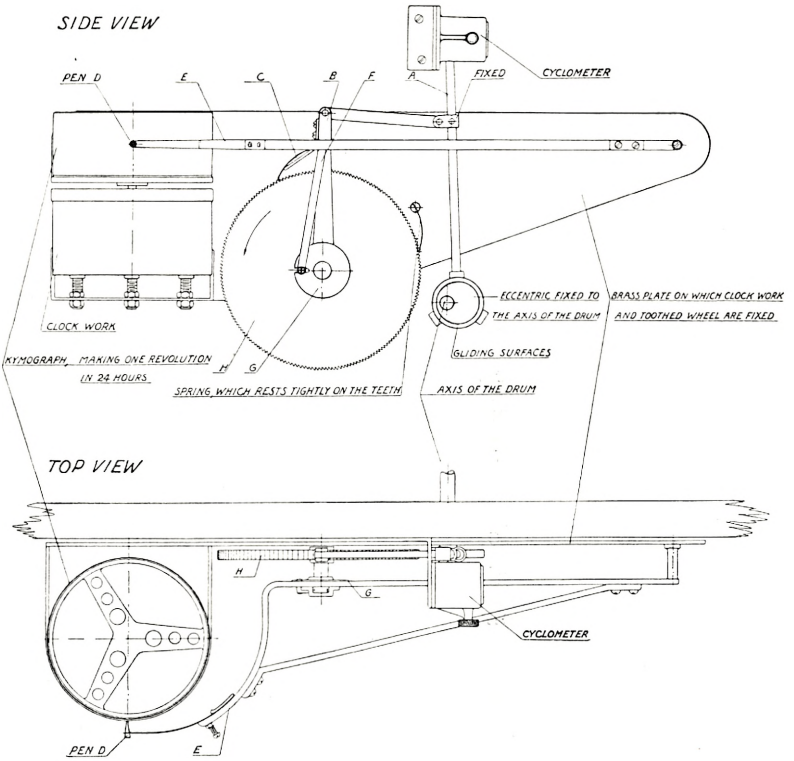

Fig. 2. Automatic register.

When the axis of the drum revolves, *A* moves up and down. Thereby at each revolution *B*, through the spring *C*, pushes the toothed wheel one tooth in the direction of the arrow. As there are 200 teeth, the pen *D* writes one peak on the kymograph for each 200 revolutions of the wheel (the distance from the lower to the upper position of the pen on the kymograph corresponding to 100 revolutions). The kymograph makes one revolution in 24 hours. A typical record is visible on the kymograph in fig. 1.

B moves from side to side behind *E* and *F*, and thus is not fixed directly to *E* and *F*. *G* and *H* are fixed together. *B* is penetrated by the axis of the toothed wheel between *G* and *H*.

Sand's studies on this point appear to have appeared only in Danish; readers interested in Sand's works on other points are referred to his reviews in English (1919, 1923), in French (1921), and in German (1925—32, 1933).

Later studies notably by Long and Evans (1922, p. 71—72), Stone (1922), Hemmingsen (1933), Ball (1934), and Møller-Christensen (1935), have revealed, however, that in the rat the sexual desire in the female rat is very frequently displayed and is characterized by still more reliable symptoms (a peculiar quivering of ears and body, darting hops, spontaneous lordosis) than those observed by earlier workers.

Whereas Steinach, Heinlein, and Wiesner (1925), in attempting to induce sexual receptivity in spayed rats with ovarian extracts, employed the insufficient criteria of Steinach, later Kun (1934), at Steinach's laboratory, in repeating these attempts, improved the technique so far as the observation of lordosis sub actu is concerned. Yet he did not include the above mentioned still stronger and most reliable heat symptoms (the peculiar ear quivering, darting hops, spontaneous lordosis). Kun, though on p. 316 he quotes Hemmingsen (1933), seems not to have realized that besides lordosis sub actu strong reliable symptoms (the peculiar ear quivering, darting hops) had already been produced by Hemmingsen (1933) in spayed rats with ovarian extracts.

The inability of earlier eminent workers to recognize the characteristic and frequent heat symptoms in the female rat may be due either to the inferior average quality of laboratory rats in regard to general state of health, as compared with the ideal rat strains of more recent years, or to the fact that heat in the female rat and guinea pig is

predominantly a nocturnal phenomenon, which can be observed only every fourth or fifth night.

In the present study the 12 types of mating behaviour described by HEMMINGSEN (1933, p. 119—123) have been adopted for relative measurements of the degree of sexual receptivity. The reader is referred to the photographs published in the paper quoted (figs. 1—15, p. 120—122), which illustrate the behaviour of the rats at the various oestrous stages. Since the arguments leading to the establishment of these 12 types have already been set forth in the communication referred to, it will be sufficient to refer the reader for further details to the earlier investigation.

The inspection for the degree of sexual receptivity is made as follows: The female is removed from its cage and placed in a box measuring 30 × 30 × 50 cm. At inspections made during dark periods a faint electric bulb illuminates the interior of the box. Before males are introduced, it is ascertained whether the characteristic quivering of the ears (type 12 a) or spontaneous lordosis (type 12 b) is observed immediately after the female has been placed in the box, or possibly still some time after; it may be aroused, for instance, by knocking at the outside of the box. If this is not the case, the back or vaginal region of the female is tickled by one or two fingers. This must, just like the inspection for behaviour of type 12, be made in the cage and not on the table, where the animal may be distracted by the unusual surroundings. If lordosis is evoked in this way (type 11), and often this does not occur until the vaginal region is stimulated, it is, as far as our memory goes, under normal conditions practically always accompanied by quivering of the ears (we have a few observations in constant light of type 11 without quiverings). If quivering only,

but not lordosis, is evoked, the behaviour is recorded as type 10. If this stimulation produces no symptoms, a non-aggressive male is introduced. If now the female curves her back in lordosis, even though the male has not yet smelled at her vagina or attempted to mount her, the behaviour is recorded as type 9. Type 9 will probably always be accompanied by ear vibrations.

If the female only quivers her ears without exhibiting lordosis, the type is No. 8. If no symptoms are experienced, an aggressive male is introduced. If now the female quivers her ears when the male smells at her, type 7 is recorded. If there is no ear-quivering until the female is mounted, the behaviour is either of type 6, namely when lordosis is also present, or type 5, namely if only quivering and not lordosis is seen, in spite of mounting. If the response to mounting is lordosis alone, quivering being absent, the type is No. 4. If this response is a reluctant one with only slightly pronounced lordosis, it is recorded as behaviour of type 3. If neither quivering nor lordosis is produced, even after several mountings, the female is out of heat, and the behaviour is recorded as type 2. If, in spite of several attempts at mounting, the male does not succeed in achieving any mounting, the behaviour is recorded as type 1. If the males happen to make no approaches at all, the designation 0 (zero) is used, which actually merely means that the type is below 7. If the characteristic darting hops are seen, the letter d is attached to the type number.

As pointed out in the quoted paper by Hemmingsen (1933), the response of the female may vary at one and the same inspection if the above described tests are repeated, notably in the direction of increasing excitement on the part of the female, due to stimulation from the approaches

and mountings of the male. The types of sexual behaviour recorded in this paper are always based on the first response observed, by testing for the degrees in the order 12—1, as described in the preceding, and they thus form the best possible basis for comparison between the stage of sexual excitement at different hours.

It is a matter of fact that the use of the mating instincts in studies on oestrus, for instance in the experimental production of oestrus by oestrogenic compounds, represents a very rapid and convenient, let alone reliable, method of ascertaining whether a female rat is in oestrus or not.

It is, in all these respects, superior to the vaginal smear method, as described in the following paragraph. An apparent disadvantage is that in experimental studies with oestrogenic compounds larger doses are required to produce mating instincts in spayed rats than to produce vaginal cornification. Yet, it is not out of the question that a reliable assay method might be developed involving the use of the mating instincts. Another disadvantage is the nocturnal occurrence of the mating instincts. This disadvantage can, however, be disposed of, as the following pages will show.

C. Vaginal smears.

The vaginal samples were collected with a rustless steel-wire loop, which was heated in a flame after each sample. The smears were placed on a slide, usually several smears on each slide, and each smear received a running number. The smears were collectively fixed in 96 p. c. alcohol and stained with hematoxylin and eosin. The smears were studied and filed without a cover glass. They are still in existence.

The various stages of the oestrous cycle, as determined from vaginal smears, have been designated by the following letters:

D = dioestrus or the dioestrous interval (leucocytes in the vaginal smear; some nucleated epithelial cells, or cornified non-nucleated cells, or mucus, may also be present).

P = pro-oestrus (nucleated epithelial cells in the vaginal smear, but no cornified elements, and no leucocytes).

P-O = transition from P to O (both nucleated epithelial cells and cornified non-nucleated epithelial cells in the vaginal smears. No leucocytes).

O = oestrus (cornified non-nucleated epithelial cells in the vaginal smear. No nucleated epithelial cells, and no leucocytes).

M = metoestrus (cornified non-nucleated epithelial cells and leucocytes).

The reasons for throwing the stages oestrus and metoestrus I, as defined by Long and Evans, together into one stage O were given in the previous communication.

The method of vaginal smears has been of paramount importance in the study of oestrin, and practically all assay work is made by means of it. On the whole it is a convenient method, but it should be remembered that there are intermediate stages between all those mentioned, and it may, therefore, in the case of a single smear, be difficult to decide with certainty to which stage it belongs. In addition, a cornified smear does not a priori warrant the conclusion that the animal is in real oestrus (heat). A cornified vaginal smear may be found for instance during vitamin A deficiency even in spayed females (Evans and Bishop, 1922; Evans, 1928; Reiss and Perény, 1928), in normal females with deficient sexual activity when out of heat (Hemmingsen,

1933), and in spayed rats after frequent smear collection (WADE and DOISY, 1935; EMERY and SCHWABE, 1936). It should thus never be trusted as sole criterion.

3. Technique of illumination.

Usually the observations were started in one of the ordinary animal rooms under the normal day-night alternation of the season. After a number of oestrous cycles of 4 or 5 days, as recognized from regular periodic activity curves, had been observed, the animals, still remaining in their cages, were transferred to the light-proof experimental room, from which all day-light could be shut out. The door was opened only in entering the room for inspections, feeding, and cleaning. The daily feeding in this room was always made at the same hour irrespective of whether the light was on or off at that hour. The room was ventilated by the same system of canals as ventilate the other animal rooms.

In this room the animals were observed during some oestrous cycles under artificially established equinoctial conditions, with equal length of day and night, but still with the light period covering mainly the day time; and the dark period covering mainly the night time.

The light was automatically switched on and off at the proper hours by means of an electrical time-switch in the form of a contact-clockwork like those used on a large scale for tariff-shifting.

The artificial light used for this, and later for reversing the dark and light periods, was given from an Osram-Vitalux bulb of 500 watts, which like the sun gives a continuous spectrum with wave-lengths ranging from 270 to 4000 $\mu\mu$.

The percentage of ultraviolet rays is said to be the same as in sun-light. The rats were so placed that they could hide against the direct light, which was further somewhat tempered by a veil.

There was a tendency for the temperature of the room to rise 1°—2° C during the first hours of the periods of illumination and then to fall again during the first hours of the dark periods.

4. Preliminary observations concerning the influence of reversal of alternating light and dark 12 hour periods on the muscular activity.

a. Albino females. So much labour is imposed in carrying out vaginal inspections and mating tests at frequent intervals, that a preliminary series of experiments were made with 3 females in which only the muscular activity was studied. Since the muscular activity could be automatically registered, inspection of the animals once every 24 hours was sufficient.

Fig. 3 illustrates the results obtained with one of these rats. The others behaved much the same.

It is obvious from fig. 3 that the spontaneous muscular activity is predominantly confined to the dark periods; both under the natural alternation of day and night and after some time of exposure to light in the night and to darkness in the day-time.

That the effect of light and darkness is not an immediate and direct one, is obvious from the observation that the adjustment to the new rhythm takes place gradually, the new rhythm beginning to display its effects at a time when the effects of the old rhythm have not yet entirely vanished.

In the other two rats this overlapping of the old and the new rhythm was not marked.

The observations reveal individual differences. Thus, the maximum of muscular activity need not fall about midnight but may occur at individually different hours, as for instance in fig. 3 consistently about 20 o'clock. This individuality subsists during the gradual transition from the old to the new activity rhythm, and still after the

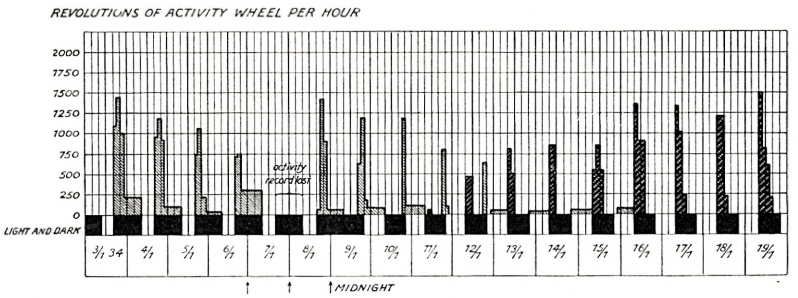

Fig. 3. Spontaneous muscular activity of a female albino rat before and after reversal of light and dark.

establishment of the new rhythm, so that after the reversal of light and dark periods the maximum of activity is at about 8 o'clock, the shift thus being just about 12 hours.

In other individuals studied later, the maximum run has been observed to shift gradually under constant day-night alternation, so that a maximum which in the beginning was before midnight, later occurred after midnight; or the converse. Instances of one maximum before and another one after one and the same midnight have also been recorded.

b. Brown females. In order to exclude the possibility that the nocturnal occurrence of the observed phenomena and the observed effect of reversing the periods of darkness and light, were dependent on the albinism of the strain of

rats used, one similar experimental series was made with three wild brown female rats. These were too wild for mating tests and vaginal smears to be made; only the muscular activity could be recorded. The effect on the activity of reversing the dark and light periods in the case of the brown rats was essentially the same as for the albinos.

An individual trait in one of these brown females before reversal was the consistent habit of displaying some, if not much, activity about noon.

c. Albino males. Experiments with albino males have given the same results as the experiments with albino and brown females. It should be mentioned, however, that among a number of males used for an entirely different purpose, we have observed two individuals which exhibited the main part of their activity in the natural forenoon. For reasons irrelevant in this connection, no reversal experiments could be made with these individuals, and later searching for individuals with this particular habit, was unsuccessful.

5. Main experimental series. Shifting muscular activity, sexual receptivity, and vaginal oestrous changes, 12 hours by reversal of alternating light and dark 12 hour periods.

The main experimental series included not only observations on the muscular activity, but also frequent examinations of the sexual receptivity and the vaginal smear in 6 females before and after reversal of the light and dark periods. Although two of these six rats came into constant oestrus after the reversal (continuous vaginal oestrous stages and heat of types 5—11); and though in the other four some few cycles, when considered individually, gave an unclear impression as regards the shift of the vaginal

Table 1.

The cyclic oestrous changes in the sexual receptivity of rat No. 3947 before and after reversing artificially established equinoctial dark and light periods. The various degrees of sexual receptivity are designated as described in the text (p. 17—18). The dark periods are underlined. The dark periods in which the oestrous maxima of muscular activity occur, are doubly underlined.

Date 1935	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22
17—20/2												1		10	11	11				11		9	4	3				1	1	1	1	1
21—24			1	1	1	1	1				1		7	11	11				7	8	6	4		2			1		1		2
25—28			1	1	1	1	1				1	1	7	11	11				10	10	10	1	3				1	1	1	2	2
1— 4/3			1	1	1	1	2				1	1		11													1		0	1	1
5— 8			1	1	1	1	2				1	1	5		11				10	10	7	3	2				0	1	1	2	2
9—12			1	1	1	0													1	2	2	2	2				1	1	1		2
13—16			1	0	2	0	2				1	0	0	2	2				1	1	1	0	2				0	0	1	0	
17—20											0	1	1	0	2				1	0	1	0	0				0	2	0	1	0
21—24			1	0	2	0	0				1	1		0	0				1	2	2	2									
25—27					*						11	12	12	11	10				1	1	1	2	1				2	1	1	2	0
28—31			1	2	2	2	0				11	12	12	11	11				1	2	2	2									
1— 4/4			1	0	0	2	2				11	12	12	11	12						1	2	2				1		2		0
5— 8			1		2		0				11	12		12						1		1					1	1	2	2	2
9—12			1	2	2	2	2				11	12	12	12	11				0	1	1	1	2				1	1	1	1	2
13—16			1	2	1	0	2							12					1									0			
17—20				2							12									1								1			
21—24				1								12								2								1			
25—28				2								12								2									1		
29/4— 2/5			0	2	2	2	2				11	12	12	11	11				1	1	2	2	2				2	2	1	2	1
3 — 6			0	0	0	2	0				12	12	12	12						2			2				1	0	0	0	2
7 —10			1	0	0	0	2				11	12	12	12	11				2	2	0	0	0				0	0	0	0	0
11			0	0	0		0																								

*) A blank day is here inserted.

Table 2.

The cyclic oestrous changes in the vaginal smear of rat No. 3947 before and after reversing artificially established equinoctial dark and light periods. The various vaginal stages are designated as described in the text (p. 20). The dark periods are underlined. The dark periods in which the oestrous maxima of muscular activity occur, are doubly underlined.

Date 1935	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22
17—20/2												D		P	O	O				O		O	O	M			M	D	D	D	D
21—24			D	D	D	D	D					D-P		P	O	O				O	O	O	O		M		D		D		D
25—28			D	D	D	D	D					P	P-O	P-O	O	O				O	O	O	O	O			M	M	D	D	D
1— 4/3			D	D	D	D	D					P	P		O												M		D	D	D
5— 8			D	D	D	D	D					D-P	P	P-O		O				O	O	O	O	M			D	D	D	D	D
9—12			D	D	D	D									D					D	D	D	D	D			D	D	D		D
13—16			D	D	D	D	D					D	D	D	D	D				D	D	D	D	D			D	D	D	D	
17—20												D	D	D	D	D				D	D	D	D	D			D	D	D	D	D
21—24			D	D	D	D	D					D	D		D	D				D		D	D								
25—27							*					P	P	P	P-O	P-O				M	D	D	D	D			D	D	D	D	D
28—31			D	D	D	D	P					P-O	O	O	O	O				O	O	O	D								
1— 4/4			D	D	D	D	P					P-O	O	O	O	O					O	M	D	D			D		D		D
5— 8			D		D		P					P-O	O		O						O		D				D	D	D	D	D
9—12			D	D	D	P-O	O					O	O	O	O	O				O	O	O	O	M			M	M	M	M	M
13—16			D	D	D	P	P-O								O					O											
17—20																																
21—24																																
25—28																																
29/4— 2/5			D	D	D	D	D					P	P-O	O	O	O				O	O	O	M	D			D	D	D	D	D
3— 6			D	D	D	D	D					D	O	O	O						O		M				D	D	D	D	D
7—10			D	D	D	D	D					P	P-O	O	O	O				O	O		O	M			M	M	M	D	D
11			D	D	D		D																									

*) A blank day is here inserted.

Table 3.

The cyclic oestrous changes in the sexual receptivity of rat No. 3950 before and after reversing artificially established equinoctial dark and light periods. The various degrees of sexual receptivity are designated as described in the text (p. 17—18). The dark periods are underlined. The dark periods in which the oestrous maxima of muscular activity occur, are doubly underlined.

Date 1935	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22
18—21/2				0		0	1	1				0		1	11	11				10	8	9	6	6				1	1	1	1	2										
22—25				1		1	1	2				1	1	6	11		10			10		10		2				1	1	1	2	2										
26/2— 1/3				1	1	1	1	2				1	1	10	10	11				10	10	10	10	6				1	1	1	0	2										
2— 7				1	1		1													10		10	11	11				10	10	10	7	2			1	1	1	2			1	1	1	2	1
8—12				1	1	1						7	10	10	10													1	1	1	2	0			1	1	1	2						
13—18				1	1	0	1	1				1	1	5	10	11				10	10	10	10	10				0	1	1	0										1	1	1	1	1
19—23				10	6	1	1	6				12	12	12	12	10				1	2	1	0	0				1	1		0	0			1	1	1	1						
24—28												10	12	12	12	11				1	1	1	0	2				1	1	1	2	2			1	1	2	2	0					
29/3— 2/4				1	5	4	0	1				12	12	12	12													1	2	1	1	1			1	0	1	1	0					
3— 7						1	2	2				12		12		10				1		1		1				1	1		0					1		1						
8—12				1	1	1	1	0				12	12	12	11	11				5	1	1	1	0				1	2	7	1	2			1	1	1	2	2					
13—17				1	6	6	5	5							10					1									1							1								
18—22					1								12								5								1							1								
23—27					1								12								1								1							2								
28/4— 2/5							1					11	12	12	12	11				5	1	0	1	2				0	5	1	1	1			1	1	1	2	1					
3— 7				1	1	0	1	0				12	12	12	12						1		0					1	1	2	2	2			1	1	2	1	2					
8—11				1	1	2	5	5				12	12	12	12	10				1	0	0	0	0				0	2	0		1												

Table 4.

The cyclic oestrous changes in the vaginal smear of rat No. 3950 before and after reversing artificially established equinoctial dark and light periods. The various vaginal stages are designated as described in the text (p. 20). The dark periods are underlined. The dark periods in which the oestrous maxima of muscular activity occur, are doubly underlined.

Date 1935	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22	0	3	6	9	12	15	18	22
18—21/2				D		D	D	D				D		D	D-P	P-O				O	O	O	O	O				D	D	D	D	D										
22—25				D		D	D	D				P	P	P-O	O	0				O		O		O				M	D	D	D	D										
26/2— 1/3				D	D	D	D	D				D	D	P-O	P-O	P-O				O	O	O	O	O				D	D	D	D	D										
2— 7				D	D		D													O		O	O	O				O	M	M	M	D			D	D	D		D	D	D
8—12				D	D	D						O	O	O	O								M					D	D	D	D	D			D	D	D		D		
13—18				D	D	D	D	D				D	P	P	P	P-O				O	O	O	O	M				M	D	D	D									D	D	D	D	D
19—23				O	O	O	O	O				O	O	O	O	O				D	D	D	D	D				D	D		D	D			D	D	D	D				
24—28												O	O	O	O	O				M	D	D	M	M				D	D	D	D	D			D	D	D	D	D			
29/3— 2/4				D	P	P	P-O	P-O				O	O	O	O													O	M	M	D	D			D	D	D	D	D			
3— 7					D		D	P-O	P-O			O			O		O			O		D		D			D	D		D					D		D				
8—12				D	D	D	D	P-O				P-O	P-O	O	O	O				O	M	M	M	M				M	M	M	M	D			D	D	D	D	D			
13—17				P	P-O	P-O	O	O							O					M																						
18—22																																									
23—27																																								
28/4— 2/5													P	P	P	P				P-O	D	D	D	D				D	D	D	D	D			D	D	D	D	D			
3— 7				D	D	D	D	D				D	P	P	P						D			D				D	D	D	D	D			D	D	D	D	D			
8—11				D	P	P	O	P-O				P-O	O	O	O	O				O	M	D	D	D				D	D	D		D										

changes, the observations on the mating instincts, and the vaginal stages on these four as a whole, left no doubt about the positive effects of the reversal. The results obtained with two of these females are recorded in tables 1—4. The two others showed essentially the same picture[1].

A typical experiment is also illustrated in fig. 4.

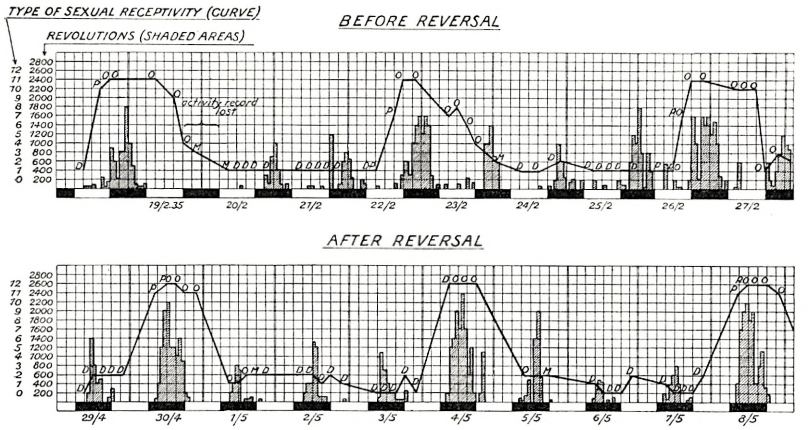

Fig. 4. The relation between the vaginal oestrous stages, the degree of sexual receptivity, and the spontaneous muscular activity, in a female rat before and after reversal of darkness and light. The unit of the abscissae is 3 hours, the thick vertical lines representing midnight. The black areas below the abscissae represent the dark periods. The adjacent letters represent the stage of the vaginal smear.

In order to make the dark periods in which the oestrous maxima of muscular activity occur, fall in vertically corresponding columns in tables 1—4, allowance had to be made for the occurrence of oestrous cycles differing in length from the typical length of 4 or 5 days. Therefore, either blank days have been inserted, or extensions made to the right side of the tables. The underlinings, representing the dark

[1] Readers interested in the details can obtain the entire material through the authors or the University Library, Copenhagen.

periods, and the dates in column 1, will guide the reader on this point.

It is seen from fig. 4 that under the natural alternation of darkness and light the spontaneous activity is confined to the night, both on the days of oestrus with maximum activity, which are every fourth or fifth day, and on the other days. The figure shows also, as do the tables, that in general the vaginal stage of oestrus starts between noon and midnight, and that the sexual excitement reaches its maximum in the night. The lower part of the figure and the tables show that the time relations of the same phenomena after establishing the artificial day-night rhythm have shifted correspondingly.

With regard to the muscular activity the results of the reversal were essentially the same as in the preliminary series. All 6 females had normal oestrous activity cycles before being transferred to the experimental room on January 18th 1935. Under the equinoctial conditions in the experimental room these cycles became still more regular. The total amount of muscular activity became somewhat depressed for some few cycles after the frequent examinations recorded in tables 1—4 were started on February 18th, but then it rose again to the previous level. The total period of observation extends roughly from November 1934 to May 1935.

Beside the thorough main experimental series in point, successful reversal experiments involving less frequent examinations, have been made later with 10 other normal females. As none of these responded with continuous oestrus, the chance of obtaining successful reversals may well be assumed to be larger than the main experimental series appears to show.

It has thus been firmly established that in the female rat not only the oestrous phenomena under direct nervous influence, the spontaneous muscular activity and the mating instincts, but also the cyclic vaginal oestrous changes, are associated with certain hours of the natural day-night rhythm, the maximum of oestrus being about midnight; and that all these three groups of oestrous changes are shifted 12 hours if an artificial day-night rhythm is established by artificial day-night reversal.

6. The effect of constant light.

Four females whose oestrous phenomena had been "reversed" (not those four in the main experimental series, but some of the others in which, besides the muscular activity, only one daily mating test, in the middle of the dark period, had been recorded), and two which had been under the normal day-night alternation in an ordinary room, were all transferred to constant light in the experimental room. In all cases the effect was a marked stimulation of oestrus. The females were examined daily at 10 and 22 o'clock for types of sexual excitement above No. 10. One retained a four days oestrous rhythm, but within each oestrous cycle the sexual excitement (types 10—12) was exhibited on 2—3 days instead of only on one day. The others came into more or less constant heat (types 10—12). One of these later came into a four days oestrous rhythm, but still with prolonged heat periods. In all animals the type of behaviour was mostly No. 11. The experiment in constant light covered two months.

The muscular activity was on the average reduced. It

was in each rat still confined to certain hours, and there was a strong tendency for the activity to be displayed by all the rats during the same hours.

The period of activity was each day delayed on the average about two hours, so that after the elapse of about 12 days, and again after 24 days, it occurred again at the original time. The observations on this point cover 1 month. In our opinion this phenomenon is to be interpreted as an inherent 24 hours rhythm which, owing to the depressing influence of light, is delayed so long as it is possible for the animal to suppress it. It is as if the rat organism wishes to store the activity for a coming dark period, but as no such period comes, it cannot suppress the rhythmic 24 hours activity more than about two hours. It is thus not to be regarded as equivalent to a steady 26 hours rhythm.

The striking feature with the observations on the effect of constant light, is that light stimulates oestrus, although under an alternating day-night rhythm oestrus is predominantly nocturnal. The observations are consistent with the stimulating effect of light recorded by previous authors (Bissonnette and others, see discussion, p. 33 seq.), and add interesting evidence to support the conclusion that the normal day-night alternation is a most important factor in controlling the oestrous cycle and its associated phenomena.

7. The effect of alternating light and dark periods of 8 hours instead of 12 hours.

It follows from the relation of the oestrous phenomena to day and night, that the length of an oestrous cycle must always be a whole number of days. Average figures like

4.6, 4.8, or 5.4 days (LONG and EVANS, 1922, p. 45—46), never really occur. The typical, and by far most frequent, length is 4 days. It seems probable that a minimum time is required for the processes of one single oestrous cycle to be run through. Oestrous cycles of 2 and 3 days have been recorded, as well in rats with multiple ovaries (FRIEDMAN and NICE, 1930) as in otherwise normal rats, at least as recognized by vaginal smears (e. g. HEMMINGSEN, 1933, p. 117—118, and previous authors there cited). Thus, concurrently with the immediate object of this paper, the question naturally arises whether other rhythms may be substituted for the natural rhythm of 24 hours, and whether, with shorter rhythms, this would result in correspondingly shorter oestrous cycles than the normal of 4 days. In view of the observed vaginal cycles of 2 and 3 days, a 16 hours rhythm would not seem an unreasonable starting point in a preliminary experiment. It was decided also here in the preliminary experiments to confine the observations to the muscular activity.

A 16 hours rhythm was instituted by switching the light on and off at 8 hours intervals. With 3 adult females it appeared impracticable in this way to modify the 24 hours activity rhythm. Even after 2 months a struggle was still going on between the tendency to display the activity in the dark periods and the tendency to have one pronounced period of activity once every 24 hours (see table 5, col. 4—6).

The next experiment was to place breeding couples under the influence of such alternating 8 hour periods of light and dark, in order to prevent their offspring, even from their first origin at the time of their fertilization in their mother's body, from ever experiencing any 24 hours light-darkness rhythm. Even in these young animals the rivalling

Table 5.

The muscular activity of female rats under a 16 hours rhythm (alternation of light and dark periods every 8 hours).

1	2	3	4	5	6	7	8	9
O'clock	Light (l) or dark (d)	Date	Number of revolutions of revolving wheels of adult female rats having been under the 16 hours rhythm from 19/1-34.			Date	Number of revolutions of revolving wheels of young female rats under the 16 hours rhythm from their birth. Their parents under 16 hours rhythm from 5/2-34.	
			Rat 3400	Rat 3244	Rat 3450		Rat 3554	Rat 3555
0— 8	l	18/3-34	0	3	0	15/4-34	303	34
8—16	d		8610	1776	1018		1278	762
16—24	l		8	0	629		} 287	} 790
0— 8	d	19	2	3	913	16		
8—16	l		1489	25	30		426	30
16—24	d		1331	670	8245		791	1087
0— 8	l	20	0	0	72	17	0	0
8—16	d		6736	2537	263		1386	358
16—24	l		8	91	204		290	355
0— 8	d	21	5	2	321	18	153	173
8—16	l		53	0	8		715	10
16—24	d		3271	2350	5967		520	568
0— 8	l	22	0	1	112	19	26	65
8—16	d		5077	478	9		369	110
16—24	l		0	7	155		1833	1107
0— 8	d	23	8	79	1921	20	175	419
8—16	l		387	9	0		984	118
16—24	d		4834	401	4712		1147	644
0— 8	l	24	2	0	41	21	780	47
8—16	d		4267	1239	3		554	836
16—24	l		4	9	73		814	92
0— 8	d	25	1	82	8580	22	154	281
8—16	l		346	61	62		71	38
16—24	d		4353	4853	933		360	191

influences of the dark, on one hand, and the endogenous 24 hours periodicity in activity, on the other hand, were still evident (see table 5, col. 8—9).

These preliminary results deterred us from making further experiments on this line. Their possible theoretical bearing will receive consideration in the discussion to follow (p. 42—45).

8. Discussion.

A. Practical side of results.

The results reported have a practical side and a theoretical side. First a few words regarding the practical side.

The present investigation has taught us that if one is interested merely in ascertaining whether or not a female rat has normal oestrous cycles, the labour involved may be reduced to merely tickling her with a finger daily at about 22—24 o'clock; or, if day and night are reversed, at about 10—12 o'clock. That this is of practical importance in all studies involving observations on the oestrous cycle in the rat, goes without saying.

So far all studies of the various purified oestrogenic compounds, α, β, γ ketohydroxyoestrin (theelin), trihydroxy-oestrin (oestrinhydrate, theelol), equilinin, hippulin, etc., have been confined to the vaginal smears, more on account of the convenience of this method, than on account of the conviction it carries in ascertaining whether or not a certain compound is actually oestrus-producing. The study of the actual oestrus, the heat symptoms, the mating instincts, has been almost entirely neglected. In the case of the rat, one cause of this has been the nocturnal occurrence of the heat symptoms, which makes the studies troublesome. This communication shows how to shift the occurrence of heat to

the natural day-time. We have already made use of this practical side of our results, in demonstrating amongst other things (see the following communication) that the synthetic oestrogenic compound of Cook, Dodds, Hewett, and Lawson (1934), 9:10-dihydroxy-9:10-di-n-propyl-9:10-di-hydro-1:2:5:6-dibenzanthracene besides vaginal cornification, produces mating instincts and also enhanced muscular activity in spayed female rats.

B. Theoretical side of results.

a. Importance of shift in anatomical phenomena. As to the theoretical side, it is evident from the results that all the cyclic oestrous phenomena, not only the sexual behaviour and the spontaneous muscular activity, but also the structural vaginal changes characteristic of oestrus, are controlled by the alternation of day and night. All these three groups of oestrous phenomena are known to be controlled through the action of oestrin (see Hemmingsen, 1933, who also refers to previous authors). It seems an obvious conclusion that also the internal cyclic structural changes in the reproductive organs, both those that are controlled by oestrin, viz. the growth and distention of uterus, and the cyclic changes in the probable site of origin of oestrin, the ovary, have been shifted together with the phenomena here studied, so that the mutual correlations of all these anatomical cyclic changes become the same as under the natural alternation of day and night.

The criticism might be urged that normal oestrous periodicity of 4—5 days may be exhibited in the absence of ovarian structural changes, as shown by Parkes (1927) in X-ray-treated mice. In our rats the ovaries were excised

at different oestrous stages, after the reversal had been established for weeks. Their structure corresponded with the stage of oestrus, as recognized by the three external criteria studied. The observations were only 4 and thus much too few to be related to light and dark in the experiments. It would, however, be highly unreasonable to think that the time-relation of these structural ovarian changes, in relation to light and dark periods before reversing these periods, should have been retained after the reversal.

The experimentum crucis would be, of course, to kill rats and study their ovaries and uteri at various stages before and after reversal of day and night. This would require an extensive independent experimental series, which to us has appeared unwarranted.

b. The relation of light to oestrus according to previous authors. It was mentioned in the introduction that the influence of light on the gonads through the mediation of the anterior lobe of the pituitary has been established by previous authors.

Is the rôle of light in these previous investigations and in the observations recorded in this paper, of identical nature? They have in common the influence of light on the occurrence of oestrus; but is the resemblance more than superficial?

It is necessary before passing on to a discussion of this question, to recapitulate briefly these earlier investigations which are much concerned with the relation of light to seasonal sexual periodicity.

That light may play a rôle as regards the occurrence of oestrus, has been definitely shown by BISSONNETTE (1932), who was able to induce oestrus during the anoestrous

period of the ferret by increasing the daily light ration in the autumn. Artificial prolongation of the day time was effective. Later experiments indicated that increased light intensity would also be effective. Results of similar nature have been obtained with starlings, crows, canaries, and junco finches (Rowan; Bissonnette; Bissonnette and Wadlund; see Bissonnette, 1933), voles (Baker and Ranson, 1932 a), mourning doves (Cole, 1933), ducks (Benoit, 1934, 1935 d), and Japanese pet domesticated birds (Miyazaki, 1934). That additional illumination increases winter egg-production in the domestic fowl, is a fact to which poultry farmers have long attested (cp. Baker and Ranson, 1932 a, p. 320). Prolongation of the daily light period has been practiced from ancient time in Japan to make pet birds begin to sing their mating songs as early as possible in winter (see Miayzaki, 1934)[1].

In starlings the long-waved red light is the effective part of the spectrum (see Bissonnette, 1933). According to Marshall and Bowden (1934) in the ferret heat rays and the near infra-red (wave-length 750 $\mu\mu$) are comparatively inactive. The effect begins with the red radiation (wave-length 650 $\mu\mu$) and extends to the near ultraviolet (wave-length 365 $\mu\mu$).

That, in these cases, light and not temperature is the responsible factor, is evident also from other control experiments.

According to Ludwig and Ries (1931) the activity of oestrin is augmented by red radiation, and according to Küstner (1933) and Nikolaew (1935) the time taken for an Aschheim-Zondek test for pregnancy, can be essentially shortened if the urine or the mice are exposed to red light. One feels, however, the desirability of the publication of

[1] Bissonnette's recent review (1936) adds further examples.

more experimental details in regard to this alleged direct effect of light upon the hormones.

ROWAN maintained that, at least in birds, the effect of light on sexual activity is mediated through increased muscular exercise. BISSONNETTE was able to disprove this.

For pertinent information and references the reader is referred to BISSONNETTE's reviews (1933, 1936).

The well-known effects of anterior lobe pituitary substances on the gonads, together with experiments by HILL and PARKES (1933) on ferrets and by BENOIT (1934) on ducks, lead to the assumption that the stimulating effect of light on the gonads is mediated through the anterior lobe of the pituitary gland. That in the ferret the light rays react upon some part of the head, was shown by BISSONNETTE in experiments with hooded ferrets. HILL and PARKES (1933) showed that the induction of oestrus in the anoestrous female ferret is inhibited by hypophysectomy, and BENOIT (1934), working with hooded ducks with or without perforation in the hoods for the eyes, found that light influences the development of the testes through the eyes, and that the content of gonadotropic substances, as tested by implantations into immature mice, was much larger in ducks acted upon by light than in non-illuminated control ducks.

In later experiments (BENOIT, 1935 b, c) he found that the effect through the eyeholes of hooded ducks was present also in blinded ducks, and discussed the possibility of the mediation of the effect in such animals either through direct stimulation of the cut nerve or through capability of the red rays to reach the hypophysis through the orbital "window".

It might seem at first glance an obvious working hypothesis to assume that the beginning of the mating season

in animals like the ferret, vole, starling, and others with mating seasons in the spring, is influenced by the increasing duration of day-light, whereas in other animals like some species of deer such as the red deer and fallow deer in this country and certain varieties of sheep which have their mating season in the autumn, the beginning of the mating season is determined by decreasing daily duration of light.

As the following will show, this hypothesis is too simple and should perhaps be modified so as to place more weight on the annual rhythm of light (p. 45—52).

c. *Comparing the influence of light on oestrus in our experiments with previous investigations.* Now, we turn again to the results of the present study, in order to compare them with the previous observations quoted.

One of the most striking experiences in this study is the observation that constant light stimulates vaginal cornification and heat, whereas under a 24 hours light-dark rhythm these phenomena are not associated with the light but with the dark periods.

It seems hardly questionable that the effect of constant light in stimulating oestrus in the rat is directly comparable with the effect of light in producing oestrus during the anoestrous period of species with a definite mating season. On the other hand, quite obviously the rôle of the light in controlling, through the day-night alternation, the oestrous cycle of the rat, on the one part, and in stimulating oestrus in the ferret, starling, duck, vole, and in the rat in constant light, on the other part, are different problems. In the rat, during the day-night rhythm, oestrus is associated with dark rather than with light. In the ferret, starling, duck, or vole, and in the rat when exposed to constant illumination, oestrus is produced by light.

The apparent paradox that additional light stimulates oestrus both in the ferret and in the rat, whereas oestrus at least in the rat is in the dark, calls for an extension of the present study to a non-nocturnal species. The difficulty is to find a small, suitable, polyoestrous, non-nocturnal form. The squirrel has been recommended to us by Professor Zoologiae AD. S. JENSEN. Squirrels, which seem to be monoestrous in our latitudes, are polyoestrous in Southern Europe and Algiers (HEAPE, 1901, p. 17; MARSHALL, 1922, p. 38) and may perhaps in captivity be made so also here. However, the question whether in non-nocturnal forms heat is actually confined to, or preferably experienced in, the light, appears to have received from zoologists but little, if any, direct study.

d. Possible mediation of the observed effects of the day-night rhythm through the anterior lobe of the pituitary. If the stimulating effect of constant light is mediated through the anterior lobe of the pituitary; is this true also with regard to the influence of the day-night rhythm on the oestrous phenomena?

The theory has been put forward (MOORE and PRICE, 1930, 1932; MOORE, 1931; BROUHA and SIMONNET, 1931; MØLLER-CHRISTENSEN, 1935) that the periodicity of oestrus arises from an interplay of the anterior pituitary and the ovary. It is assumed that the follicle-stimulating hormone of the anterior pituitary stimulates the production of oestrin, which in turn produces the oestrous phenomena. Further that oestrin inhibits the production of the follicle-stimulating hormone. Thus, the stimulus to a certain oestrous period is not applied until the inhibitory effect of the preceding oestrus upon the pituitary has vanished.

It has, in fact, been found that the content of gonadotropic

hormone of the anterior lobe of the pituitary exhibits rhythmic changes in relation to the oestrous cycle (Smith and Engle, 1929; Wolfe, 1931).

It may not be out of place, therefore, to suggest the possibility that the influence of the day-night rhythm on the oestrous phenomena of the rat may be mediated by the anterior lobe of the pituitary, and thus, that the primary effect of the reversal of light and dark may be to shift the cyclic changes in the anterior lobe.

Hill and Parkes (1934) have expressed the view that the oestrous cycle is normally due to some inherent rhythm of the anterior pituitary and occurs independently of external factors. The results of the present investigation may not be inconsistent with an inherent rhythm of the pituitary, but shows that the oestrous cycle, irrespective of whether or not it depends on a pituitary rhythm, must be dependent on at least one external factor, viz. light.

It is known from the observation of oestrous phenomena on the first two days following ovariectomy, that in the normal female rat the stimulus to oestrus operates about 24—36 hours before oestrus starts, and that it has a certain duration, perhaps about 12 hours (cp. Hemmingsen, 1933, p. 142—148). Thus the shift produced in the present study must probably mean a corresponding shift in the time-relations of this pituitary stimulus.

e. Observations on continuous oestrus. It requires notice that out of the 6 females employed in the main experimental series in which day and night were reversed, 2 came into constant heat, which lasted one month until they were killed.

In these two rats the stage of the oestrous cycle, at which the day-night reversal in the form of duplication of a night

period was undertaken, happened to coincide with the stage when the stimulus to oestrus must be assumed to have been acting; whereas in the others the reversal occurred at an earlier stage of the cycle. One could imagine that there had been a prolongation of the stimulus due to the artificial prolongation of the night when the stimulus occurred, and that this has brought the wave-like sequence of phenomena out of order. Experiments with ten females (already referred to in the preceding) which were "reversed" at the stage in question, failed to confirm this hypothesis.

In the two rats in point the strength of this constant heat showed a tendency to being higher in the dark periods than in the light. On autopsy their ovaries were found to be filled with follicles with a diameter of up to 0.6 mm. Corpora lutea were absent.

Apart from these two females, and the prolonged heat in the 6 females placed in constant light, continuous heat in female rats including the strong symptoms of sexual receptivity (above type No. 7), has been previously described by HEMMINGSEN (1933, p. 152—155), in one of the rats with transplanted ovaries, and by MØLLER-CHRISTENSEN (1935) as a consistent phenomenon in normal female rats connected in parabiosis with male or female castrates.

In MØLLER-CHRISTENSEN's experiments hyperfunction of the anterior pituitary of the castrated partner was the probable cause of the constant heat of the normal partner. In the present experiments the constant heat of the two rats exposed to "reversed" conditions may perhaps be explained as due to a stimulating effect of the artificial light on the gonads through the pituitary gland in the two most sensitive rats. This would be an effect of similar nature to the effect of light in the experiments of BISSONNETTE and in the present

experiments with constant light. The light in our experiments was on the whole probably stronger than the day-light to which rats are usually exposed.

There are other possibilities, for instance that the frequent collections of vaginal smears or the frequent sexual stimulation in the mating tests without the natural satisfaction in the form of actual insemination has a tendency to produce follicular hyperfunction. This is no probable explanation, however, as in the experiments with constant light there was no such frequent stimulations, and yet heat was prolonged with resulting continuous heat.

The absence of corpora lutea in all the cases of constant heat here discussed, and the presence of either a few large cystic or numerous smaller follicles, implies that the exhibition of continuous heat in these animals has been unaccompanied by ovulation. In some species (e. g. ferret, rabbit) heat is normally continuous until copulation takes place (MARSHALL, 1904, HEAPE, 1905; cited from PARKES, 1929, p. 53—54), leading by mediation through the pituitary (FEE and PARKES, 1929) to ovulation and formation of corpora lutea. Copulation during the continuous heat, with insertion of vaginal plugs, as well in the experiments of MØLLER-CHRISTENSEN (private communication, not stated in his book) as in the two "reversed" rats of this study (twice in the one rat, once in the other) did not lead to pseudopregnancy or pregnancy, the heat being still continuously exhibited for a long time after these copulations. Similar studies were not made with those exposed to constant light.

f. The relation of muscular activity to heat. At the inception of our studies we were inclined to expect that the culmination of heat at night would turn out to coincide

with the hour of maximum muscular activity. It will, however, be seen from tables 1—4 that high degrees of heat may start by day long before the animals have begun to display their nightly activity and continue during the subsequent light period. In addition to this, the occurrence of strong heat in constant light in the absence of enhanced muscular activity supports the contention of BISSONNETTE, in contrast to that of ROWAN (see BISSONNETTE, 1933), that the influence of light on the sexual phenomena is not mediated through an increase of the muscular activity.

g. The relative superiority of the rhythm over the single light and dark periods. There are still other observations of strong symptoms of heat and oestrous vaginal stages in the light periods. Thus, in the first days after the reversal of light and dark, the oestrous periods which would be expected to occur in the dark periods, now changed into light periods, occur at the expected time irrespective of the fact that they are now exhibited in light. Not until about 10 days after the reversal has the new oestrous rhythm become established.

All these facts show that the direct action of darkness does not immediately and directly produce the manifestations of oestrus. But once a certain rhythm of darkness and light has become established, the oestrous phenomena centre about the middle of the dark periods. It is the day-night rhythm as such, and not the single day and night, that determines the time relation of the oestrous phenomena to the diurnal rhythm.

Observations on the effect of similar inverse illumination on diurnal rhythms in insects and plants, are analogous to those here recorded, in so far as the old rhythm asserts itself for some time after the new external rhythm of light

and dark has become established (see Bünning, 1935 a, p. 600).

h. Do rhythms of 24 hours take up an exceptional position in biology? The apparent stubbornness on the part of the rats in retaining the 24 hours rhythm of activity under a 16 hours light-dark rhythm raises the question:

To what extent have the possibilities of modification of biological rhythms connected with the alternation of night and day, been limited by the fact that in the course of at least the last million years (Quaternary period) living organisms have been affronted with practically no variations (less than 1—2 p. c.) in the time taken for the globe to make one revolution (G. H. Darwin, 1879, 1908)?

According to Beling (1929) and Wahl (1932, 1933) attempts to train bees to seek their food at definite hours (1—3 times daily) succeeded only when the rhythm was 24 hours. The biological significance of this lies in the opening at restricted hours of certain flowers which the bees visit, or diurnal rhythmic variations in the availability of pollen and nectar (Parker, 1926; Wahl, 1933; and Kleber, 1935).

Ants and termites, on the other hand, can be trained to seek their food at rhythms other than 24 hours (Grabensberger, 1933). This seems to be most easy with forms whose food does not depend on the diurnal periodicity of flowers, although their sleep is said to depend on a 24 hours rhythm even in the absence of diurnal variations in light and temperature (Müller, 1931, p. 378).

Bünning (1935 a, p. 608) has adduced evidence to show that the diurnal periodicity in emergence of *Drosophila* imagines from the puparia, as observed also by Bliss (1926), Bremer (1926), and Kalmus (1935), and known also

from other insects, is an inherent 24 hours rhythm, which is still retained after keeping this species through several generations in a 16 (8 dark—8 light) hours or 36 (18 dark—18 light) hours rhythm, or in constant faint light. Such flies kept in constant dark or faint illumination apparently lose this periodicity, but one single stimulus in the form of intense illumination for some hours is sufficient to bring about the 24 hours rhythm in emergence in spite of subsequent constant conditions.

Similar evidence of an inherent 24 hours rhythm was obtained respecting rhythmic diurnal movements in plants (BÜNNING, 1932).

Some plants can be made to exhibit other rhythms, e. g. of 12 hours, but nevertheless change spontaneously to a 24 hours rhythm when placed in constant light or dark (see JOST, 1923, p. 375—382).

There seems for plants to be some unknown internal or external factor which tends to keep them in a 24 hours rhythm (beside JOST, and BÜNNING, see STOPPEL, 1926).

All these rhythms, both those of bees and ants (GRABENSBERGER, 1934; KALMUS, 1934) and those of plants and *Drosophila* (BÜNNING, 1932, 1935; KALMUS, 1935), can be modified by influences on the metabolism. The rhythm becomes shorter if the metabolism is raised and longer if the metabolism is reduced. The question whether the changes in temperature necessary to modify the 24 hours rhythm, are always so large that this rhythm will remain unaffected under the environmental temperatures in which the respective rhythms are of biological significance, appears to deserve further investigation.

Many marine animals living in the tidal region of the sea shore have a tidal rhythm, which is retained for some time

in aquaria in the absence of any such external rhythm; even the long 14 days rhythm of neaptide and springtide may be recognized in such aquaria (see Hoffmann, 1926, p. 653—655). The daily tidal rhythms are, of course, not 24 hours rhythms, but correspond to the lunar day. Whether these animals can artificially be taught other rhythms than the tidal rhythm, is not discussed in the work referred to.

It is obvious from these examples that some of these diurnal rhythms in lower organisms are not bound to follow the 24 hours rhythm, whereas other of these rhythms are.

That in the rat a 16 hours rhythm of muscular activity cannot be established, may be due either to an inherent rest-activity rhythm of 24 hours (this to us seems the most probable) or to the impracticability of repeatedly establishing oestrous cycles essentially shorter than 4 days, so that the inherent oestrous periodicity with its maximum of muscular activity at oestrus interferes with the influence of the light-dark rhythm on the muscular activity. In the end this would mean that the oestrous periodicity is bound to obey the controlling 24 hours rhythm. Experiments with males or castrates may elucidate this point further.

Whether the various diurnal rhythms which seem to be correlated with the daily rest-activity rhythm of higher animals including man (see introduction), can be modified so as to deviate from the 24 hours rhythm, regularly through reasonably long periods of time, to us appears questionable. We have obtained from the rats the impression that there is a very strong tendency to a 24 hours rest-activity rhythm, which can be suppressed only for a limited period of time (cp. p. 28). But we would expect these various diurnal rhythms, including the rhythm in liver function, to be

susceptible to shifts, including reversals, of the daily light-dark 24 hours rhythm.

It is striking that out of the diurnal rhythms dealt with in the preceding paragraph, those known to be dependent on the alternation of day and night (bees, *Drosophila* plants, rats) can be shifted at will by shifting the determining external or diurnal light rhythms, so long as the actual length of the rhythm is maintained; whereas it seems impossible in the organisms in point to establish rhythms of lengths other than the natural.

Further, such ants as besides seeking other kinds of food also visit flowers, appear not as readily to accept rhythms different from 24 hours as forms which never seek food in flowers (Grabensberger, 1933).

We quite realize that a generalization of this statement cannot be made, and we regard the statement as a tentative suggestion, which may lead to a final scrutiny of the question in the hands of others. No doubt numerous instances can be collected of diurnal rhythms suitable for further work (examples: Welsh, 1935; Young, 1935; Park, 1935).

i. Comparing the seasonal and diurnal types of sexual rhythmicity. As already pointed out, the recurrence of short oestrous cycles in polyoestrous species like the rat or mouse, with or without a restricted mating season, is not comparable to the seasonal recurrence of mating seasons in species like the monoestrous ferret, or the polyoestrous vole.

It might appear, therefore, that the seasonal sexual periodicity of animals with long periods of sexual quiescence should be kept quite distinct from the periodic phenomena within the oestrous cycle of polyoestrous species, and that neither of these two types of periodicity could throw any light on the other.

In our opinion, however, it may be profitable to suggest, by analogy, a tentative hypothesis about the rhythmic nature of the seasonal sexual periodicity on the background of the observations on the oestrous day-night rhythm recorded in this paper.

There are some traits with the seasonal and diurnal rhythms under discussion, that appear to be of related nature, as for instance, on the one hand, the two facts that in the ferret the initial mating season changes are evident histologically already in December, before the amount of day-light has ceased to decrease (Allanson, 1932), and that in the rat the initial oestrous phenomena, both as regards anatomy and behaviour, may start already during the daily light periods; and, on the other hand, the two facts that in the ferret the onset of the mating season in the spring cannot be prevented by hooding the ferrets or keeping them in total darkness $23^1/_2$ hours per day from the end of January onwards (Hill and Parkes, 1933), and that in the rat an oestrous period, which is about to start cannot be prevented manifesting itself during an artificial light period at the time when the organism from preceding experience, would expect darkness.

In keeping the ferrets in total darkness from January onwards there was actually on the average a lag in the times of onset of oestrus, but it is evident that the effect of seasonal changes in the length of day-light on seasonal sexual periodicity, is no immediate and direct one. This applies to the ferret. In other species, e. g. the duck (Benoit, 1935 a) the effect may be direct.

Hill and Parkes (1933) infer from the experiments just quoted that the increasing day-light in the spring is not a factor influencing the normal sexual periodicity of the

ferret. The question is however, whether the annual rhythm of alternating decreasing and increasing day-lengths may not be in the end an important controlling factor for the seasonal reproductive periodicity. Nor as regards the diurnal rhythmic changes in the rat the effect of daylight is an immediate and direct one. The diurnal internal sexual rhythm will not adapt itself to the new external rhythm until this has been repeated, or so to speak reinforced, for some time.

Observations on the seasonal rhythm of various species brought to their antipodal hemisphere, appear to represent a striking analogy with the gradual adaptation of the female rat to the new light-dark rhythm, as illustrated for instance in fig. 3 of this paper.

Thus, instances are known of reindeer brought from the northern to the southern hemisphere (OLSTAD, 1930, p. 11—12; the same mentioned by DEGERBØL, 1935, p. 26), and ponies brought from the southern to the northern hemisphere (cited from MARSHALL and BOWDEN, 1934, p. 420), which in their new locality first came on heat at the same time of the year as in their native country, i. e. at a different season; but subsequently adjusted their sexual periodicity to the new conditions.

Several other examples are known of a similar adjustment to an antipodal hemisphere. Whether it is always a "lagging" adjustment, we have not been able to ascertain; but probably it is, at least often, so. Such examples might be quoted as regards several species brought from the southern hemisphere to Zoological gardens in Europe or North America, e. g. penguins (private communication by Dr. TH. MORTENSEN), geese and black swans from Australia, sheep from South Africa (cp. MARSHALL, 1922, p. 24). Also

ferrets shift their mating season when they are brought from the northern to the southern hemisphere (ZUCKERMAN, cited by BISSONNETTE, 1935).

From the following statement by MARSHALL (1922, p. 24, cp. also p. 45) one gets the impression (if we have not misunderstood the statement) that some observations on camels form an exceptional example in this connection: "..... whereas the occurrence of breeding in any one country or locality is closely connected with the climatic conditions and the periodicity of the seasons in that country, this rule does not hold invariably. For while the sheep in South Africa breed in April and May (the South African autumn), thus following the seasons (since sheep breed ordinarily in autumn in this country), the camels in the Zoological Gardens in London experience rut in the early spring, or at approximately the same time as the breeding season of the wild camels in Mongolia". Since London and Mongolia are both on the northern hemisphere, to us it seems that if the camels represent an exception it is one of those that test the rule.

The seasonal and diurnal periodic phenomena in point thus have in common, as far as we know, the somewhat lagging adjustment of an inherent internal sexual rhythm to an external rhythm, which in the latter case is, and in the former case may be, a rhythm of varying amounts of light.

It should be noted, that apart from the obvious fact that the seasonal sexual rhythms under consideration are not directly comparable with the day-night association of the 4 or 5 days oestrous rhythm in the rat, there seems to be one further point where the analogy between these two forms of sexual rhythm cannot be carried through: The seasonal sexual rhythm in the ferret, though it can be only slightly influenced in the form of a slight delay in the spring, can be definitely influenced in the autumn, whereas in the rat the prolongation of a dark period does not bring about

an immediate occurrence of oestrous phenomena. This difference, however, must be attributed to the fact that light and not dark stimulates oestrus in the rat, and need not affect the impression that in both groups of rhythmic phenomena we have to do with related underlying biological mechanisms: the mechanisms of adjustment to shifts in rhythmic environmental factors.

It seems not far-fetched to imagine that these mechanisms are related to the mechanisms operating in the form of conditioned reflexes, of which many types bear witness to the existence of a sort of biological clockwork of the nervous system. A similar point of view was taken with regard to the vanishing 4—5 days rhythm in spontaneous muscular activity occurring after ovariectomy or after discontinuation of periodic oestrin injections (HEMMINGSEN, 1933, p. 200—202 and 211—214).

Apart from the seasonal changes in light, seasonal changes in other ecological factors, like type and quantity of food, temperature, and rain, may undoubtedly, under certain conditions, control the breeding time, not least in tropical countries (see discussions by BISSONNETTE, 1932b, 1936, and MARSHALL and BOWDEN, 1934).

One cannot exclude a combined influence of light with other factors like food and temperature, even in the cases that are apparently determined to a great extent by light.

In some animals light or absence of light may not play any direct rôle in stimulating oestrus. In hedgehogs (ALLANSON and DEANESLY, 1934) changes characteristic of the mating season can be produced in winter merely by keeping the animals in the laboratory. In ground squirrels (MOORE, SIMMONS, WELLS, ZALESKY, and NELSON, 1934) daily addition of light failed to produce observable effects,

whereas females kept continually for several months in cold (and darkness), with more or less normal hibernation, exhibited sexual development at any time of the year. Craig-Bennett's study on sticklebacks (1931) stresses the absence of a light effect, but shows the importance of relative changes in temperature. In field mice (Baker and Ranson, 1932 a, b, 1933) sometimes one factor seems to act, sometimes another.

But, even in animals where factors other than light, entirely or partially, determine the mating season, the influence of the seasonal rhythmic changes of such other factors, or complex of factors, may have to be considered under essentially the same aspects, only in the arguments substituting for the annual rhythm of light the annual rhythm of the complex of determining factors.

Even the mysterious lunar rhythm among marine crustacea, sea urchins, and polychætes, notably the palolo worms (see Hoffmann, 1926, p. 655—658) may have to be considered from this view-point, for instance in explaining that these worms may experience their breeding phenomena at the usual time of the year in aquaria in the apparent absence of the usual lunar influences.

Besides the observations of this study other similar observations of previous authors concerning the gradual adjustment of biological rhythms in insects and plants to variations in external rhythms, may be cited in favour of the views here advanced (see Bünning, 1935, p. 600).

The efficiency of light in causing in certain species the seasonal periodicity of reproduction, may have become evolved as an adaptation to those seasonal environmental conditions which are correlated with the amount of daylight through the seasonal light variations, in the sense that

the rhythmic day-light variations advertise in due time the organisms of the approach of the environmental conditions to which the manifestation of the whole sequence of breeding phenomena have been adapted.

The insertion of long intervals between the mating season and ovulation as in bats or a long quiescent period following the first segmentations of the fertilized ovum as in roedeer and badger, must be secondary phenomena which have been caused by gradually changing environmental conditions. No matter whether such intervals are inserted or not; and no matter what factors determine the length of the gestation period in general (for discussion see MARSHALL, 1922, p. 29—30), it is plain a priori that the mating season must be so placed that the young are produced at an auspicious time.

The annual rhythm of decreasing and increasing daily light rations, would appear to be a most reliable clockwork for any species to trust, in seeking to achieve this end.

By higher plants this clockwork is actually trusted to achieve a similar purpose. Thus, in higher plants the duration of day-light determines the time of flowering. In some plants short days favour early flowering; in others, long days, according to the natural habitat of the respective plants (GARNER and ALLARD, 1920; see MAXIMOW, 1929, SCHICK, 1932, and MÜLLER, 1934).

Likewise, according to MARCOVITCH (1924) the appearance of sexual forms in plant lice and their migrations to alternate hosts, are controlled by the length of the day.

In conclusion, we do not wish anything more than to suggest that all the apparently conflicting observations, in respect of the relation of light to seasonal sexual rhythms, may be brought under one point of view covering all the

ground, by assuming that in the animals where light is an important determining factor there is an inherent tendency to an internal reproductive annual rhythm, and that the external seasonal light rhythm controls at what time of the year the breeding season occurs. On shifting the external rhythm there is some lag on the part of the internal rhythm in adjusting itself to the external rhythm.

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9. Summary.

1. Under the normal alternation of day and night not only the oestrous phenomena under direct nervous influence, viz. the spontaneous muscular activity, as recorded by activity cages, and the mating instincts, but also the cyclic anatomical changes of the vaginal epithelium are

preferably confined to certain hours of the natural day-night rhythm. Heat and activity are at their maximum in the dark.

2. All the three groups of oestrous phenomena mentioned, are shifted 12 hours if an artificial day-night rhythm is established by exposing the animals to light in the night and to darkness in the day-time.

3. The convenience in using, for all studies on oestrus in the rat, the reliable psychical symptoms of heat instead of the less reliable vaginal changes, is emphasized. It is an added advantage that they can be studied by day, if the animals are placed in a dark room which is illuminated at night.

4. Reversal of light and dark periods produces a shift in muscular activity not only in albino females, but also in brown females, and in males.

5. A rhythm involving the alternation of 8 hours of dark and 8 hours of light, does not abolish the 24 hours activity rhythm, even in rats kept under these artificial conditions from before birth.

6. Constant light stimulates heat and vaginal cornification, although the muscular activity is somewhat depressed.

7. An attempt is made to consider the influence of light on the seasonal sexual rhythm of some animals with a restricted mating season, on the one part, and the effect of the day-night rhythm on the oestrous cycle of the rat, on the other part, under one point of view, suggesting the theory that in both cases the fundamental fact is the somewhat lagging adjustment of an inherent internal sexual rhythm to an external rhythm of varying light rations.

(From Nordisk Insulinlaboratorium, Copenhagen, Denmark.)

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BY

AXEL M. HEMMINGSEN AND NIELS B. KRARUP

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1. Introduction.

Recent years have seen the discovery of a number of chemically well-defined oestrogenic compounds (see e. g. Dodds, 1935; Dodds and Lawson, 1936). The vaginal smear method has made this possible; but, on the other hand, it seems to have been the object of scanty interest, if any, to what extent these compounds are capable of inducing the essential oestrous phenomena, the mating instincts; let alone the rise in spontaneous muscular activity characteristic of oestrus. Both these groups of oestrous phenomena can be induced with more crude follicular extracts (see Hemmingsen, 1933, p. 164—183, and p. 203—214). One cause of the omission has been the nocturnal occurrence of the heat symptoms in the laboratory species employed for these studies. In the previous communication (Hemmingsen and Krarup, 1937) we have shown how to shift the occurrence of heat in the rat to the natural day-time by exposing the animals to light in the night and to dark in the day.

On spayed rats under such "reversed" conditions we have tested three of the compounds in point for ability to induce mating instincts and enhanced spontaneous muscular activity, viz. the crystalline preparations theelin (ketohydroxyoestrin, or estrione) and theelol (trihydroxyoestrin, or estriol) of Parke, Davis & Co., and the synthetic oestrogenic compound 9 : 10-dihydroxy-9 : 10-di-n-propyl-9 : 10-dihydro-1 : 2 : 5 : 6 dibenzanthracene of Cook, Dodds, Hewett,

and Lawson (1934), which was kindly placed at our disposal through Professor Dodds and Dr. Hagedorn. According to Wolfe (1936) the latter substance prevents the structural changes which occur in the anterior hypophysis of the female rat following castration.

2. Technique.

The technique of reversing the illumination and of recording the vaginal smears, the mating instincts, and the spontaneous muscular activity, was exactly as in the previous communication, to which the reader is referred. Vaginal smears and mating tests were made daily every 3 hours from 9 to 21 o'clock (dark period from 6—18) both before the injections from the day of ovariectomy, and after the injections.

3. Description of experiments.

4 of the normal female rats used in the previous investigation were spayed. After ovariectomy 10 days were allowed to pass in order to obtain assurance that all the oestrous phenomena were abolished.

Two of the rats were then injected one day every hour from 9—22 o'clock with 0.4 c. c. of a solution of theelol containing 50 rat units per c. c. It was prepared by dissolving commercial theelol Parke, Davis & Co., into 0.9 p. c. NaCl, containing 0.025 n NaOH (according to Melchiona, 1931, p. 653). Each of these two rats thus received 20 rat units of theelol per injection, i. e. 280 rat units in total.

These large amounts were given because in earlier experiments (see Hemmingsen, 1933, p. 181) 100—200 times the dose giving vaginal effect in some insensitive rats only give mating responses of type 3.

Both rats reacted normally as regards the vaginal smear, but only one of them showed mating reactions above No. 2 (responses of types 5, 6, and 7, were observed on the day after the injection).

The two others were injected in the same way with the same number of theelin units. Theelin was available in commercial vials containing 50 rat units per c. c. The result was similar. Both rats showed vaginal response, but only the one showed mating reactions above 2 (types 5 and 6 were observed on the two days following the injections). 3 days afterwards when the vaginal effects had vanished in all 4 animals, those two which had not shown mating responses, were again injected with the same total dose of 280 rat units with the modification that double the amount was injected every two hours from 10—22 o'clock. The result was still negative, a vaginal response being obtained without any mating reactions. Obviously these two animals were less sensitive than the two others. The positive mating responses in the other two rats showed that both theelin and theelol are capable of producing mating instincts.

We wished to confine ourselves to the sole object of deciding this, and therefore no further experiments were made with theelin and theelol.

On the same day as these two insensitive animals were injected the second time with theelin and theelol, those two which had shown positive mating responses, were now injected with the synthetic oestrogenic compound obtained through Prof. Dodds, and at the same hours, i. e. every second hour from 10—22 o'clock; each time with 0.5 c. c. of a sesame oil solution containing 4.3 mg per c. c., i. e. 30 mg in total. A rest, which would not readily dissolve, was

mixed thoroughly with the oil in a mortar. According to Professor Dodds oil is the only medium in which the substance can be taken up. The sites of injection of these oily injections were closed with collodium.

It is impossible to state in any exact way the strength of the oily solution in terms of rat units. We can only say that in a preliminary assay experiment a total amount of 0.1 mg in one spayed rat and 0.05 mg in two spayed rats injected over 48 hours at 9, 18, 9, 15, 22 and 9 o'clock (method of Allan, Dickens, and Dodds, 1930) gave a positive vaginal response, whereas 0.025 mg injected in the same way in one spayed rat, was negative.

Smears were taken in these preliminary tests at frequent intervals (every four hours) on the day when an effect was to be expected. By the same method Dodds and his coworkers have seen full vaginal cornification in 50 p. c. of the injected rats with 0.025 mg (personal communication).

The total dose injected in the two rats which received 0.5 c. c. every two hours from 10—22 o'clock, thus was approximately 600 times as large as the lowest dose observed in the preliminary tests to give vaginal response. The previous experience quoted above and the negative results with 280 rat units of theelin and theelol in two of the 4 injected rats, dictated these large doses.

These two rats came into constant heat varying from types No. 6 to 11. After some days when heat had been observed to be continuous, the inspections were confined to noon, i. e. in the middle of the dark periods. When it had lasted one month, the two animals were killed. Their uteri were not distended.

The two other rats which had shown negative mating responses to the injections of theelin and theelol, were in-

jected 10 days after the last of these injections, when the vaginal smear had been D for about a week, with a total amount of 15 mg of the synthetic compound, given in three injections at 10, 13 and 16 o'clock. The one came into constant heat varying between types 4 and 11, until it was killed after this condition had lasted for 3 weeks (inspections daily at noon). Its uteri were enormously distended. The other one, however, only reached mating response of type 3, but not until on the fifth day after the injection. It was, therefore, 8 days after the injection, again injected with about double this amount, i. e. 30 mg, in three injections at 10, 13 and 16 o'clock. Thereafter it exhibited heat of type 4 every day (inspections at noon) until it was killed 11 days after the last injection. Its uteri were moderately distended. This animal obviously was less sensitive than the others.

No doubt the protracted effect of the synthetic product must be attributed to its slow absorption from the oily medium in which it is dispersed, and which in itself remains for long periods under the skin.

About the capability of the synthetic product to produce mating instincts there can be no doubt.

In all four animals the vaginal smear during the continuous heat was often invaded by numerous leucocytes.

In conformity with previous observations (e. g. HEMMINGSEN, 1933, p. 200—203) the spontaneous muscular activity was considerably reduced by ovariectomy, and its normal oestrous periodicity vanished. The injections of theelin and theelol affected the muscular activity only in the two positive cases, where small peaks in the activity curves were observed. The injections of the synthetic product, however, caused a considerable rise in all four animals as the foll-

owing table shows. There was no regular periodicity in this enhanced activity, which showed a gradual rise during the continuous heat lasting until the animals were killed.

Table 1.

Spontaneous muscular activity.

(Average daily number of revolutions of activity wheel).

Rat No.	From ovariectomy to injection of synthetic compound.	From injection of synthetic compound until killed (i. e. during constant heat).
3947	1090	3620
3949	497	5060
3950	1190	6400
3951	1230	3870

Discussion.

The existence of a great number of chemically well-defined compounds known to influence the genital tract of females, has raised the question of the specificity of the female sex hormones (see for instance Dodds, 1934—35).

It is natural to ask whether this relative lack of specificity extends beyond the mere anatomical effects.

The work involved in testing all the compounds known to produce the vaginal effect, also for mating instincts and enhanced spontaneous muscular activity would be large, but the present successful induction of mating instincts and enhanced spontaneous muscular activity with three rather widely differing compounds shows that actually the relative lack of specificity in regard to the vaginal effect, applies also to phenomena of primary biological significance in regard to oestrus and mating. In our opinion this should be the object of as much attention, or still more, than the lack of specificity of the mere vaginal reaction.

Summary.

Not only the vaginal smear changes, but also reliable mating instincts of high degree, and enhanced spontaneous muscular activity, are produced by injections into spayed female rats of theelin and theelol, and the synthetic oestrogenic compound 9 : 10-dihydroxy-9 : 10-di-n-propyl-9 : 10-dihydro-1 : 2 : 5 : 6-dibenzanthracene of Cook, Dodds, Hewett, and Lawson (1934).

Beside Dr. H. C. Hagedorn, Mr. Børge Clausen, Mr. Anker Nielsen, and Miss I. L. Bisgaard, who have helped us in the same respects as mentioned in the preceding paper, we wish to thank Professor E. C. Dodds for providing samples of the synthetic di-n-propyl-benzanthracene compound.

(From Nordisk Insulinlaboratorium, Gentofte, Denmark.)

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In a recent letter to Nature[1] we communicated the results of some experiments on the metabolism of phosphorus using a radioactive phosphorus isotope as indicator. What follows is a more detailed description of some of our experiments, carried out chiefly on rats but partly also on human subjects.

Principle of the method used.

Disregarding hydrogen, the only element which is ever met with in a nuclear state (as a proton) in chemical reactions, isotopes do not separate to a measurable extent during chemical or biochemical processes. It follows from this inseparability that when a known amount of radioactive phosphorus is added to, for example, 1 mgm. of phosphorus the presence of the former will always indicate the presence of the latter. We can thus distinguish for example between the phosphorus atoms taken in with the food (to which we add some radioactive phosphorus) and those already present in the system. The use of isotopic indicators is not dependent on an absolute inseparability of isotopes by chemical methods. We know indeed that minute separations almost always occur. It is sufficient that, within the analytical accuracy claimed, no separation takes place.

[1] O. Chievitz and G. Hevesy, Nature, **136**, 754, 1935.

Phosphorus has only one stable isotope ^{31}P but we can prepare unstable radioactive isotopes of phosphorus having atomic weights of 30 and 32; the latter has a half-life of about a fortnight and is very suitable for use as an indicator. It was used by us in many experiments of different kinds.

Preparation of radioactive phosphorus.

Radioactive phosphorus $^{32}_{15}P$ can be prepared from chlorine or from sulphur under the action of fast neutrons, or from ordinary phosphorus under the action of slow neutrons; the nuclear reactions are:

$$^{35}_{17}Cl + ^{1}_{0}n = ^{32}_{15}P + ^{4}_{2}He$$

$$^{32}_{16}S + ^{1}_{0}n = ^{32}_{15}P + ^{1}_{1}H$$

$$^{31}_{15}P + ^{1}_{0}n = ^{32}_{15}P$$

Using neutrons liberated from mixtures of radium and beryllium, ^{32}P can be prepared most conveniently from sulphur. We found it advisable to use carbon disulphide instead of the elementary sulphur used by Fermi and his colleagues in their original experiments. About 10 litres of carbon disulphide were exposed to neutrons from radium-beryllium mixtures and a fortnight later the carbon disulphide was distilled off. The residue contained the radioactive phosphorus formed, along with some of the decomposition products of carbon disulphide. The residue was oxidized and the phosphoric acid obtained converted into the phosphate compound wanted. We used chiefly sodium radiophosphate in our experiments. The weight of the radiophosphorus produced is extremely minute; using a source containing 100 mgm. of radium, less than 10^{-10} gm. of radiophosphorus

is obtained. By adding a suitable quantity of sodium phosphate to the sodium radiophosphate solution we obtain the "radioactive" ("labelled") sodium phosphate desired.

To concentrate the radiophosphorus obtained by neutron bombardment of carbon disulphide other methods besides that outlined above were used. A very convenient way to prepare nearly pure radiophosphorus is the following. Under the action of the radiation some decomposition of the carbon disulphide takes place and a partly orange-coloured precipitate is formed which settles on the glass walls. This slight precipitate contains a large part of the radioactive phosphorus formed. The precipitate is possibly identical with the red sulphur described by MAGNUS as far back as 1854, which was found to consist of a mixture of sulphur and organic sulphur compounds. We are engaged on the investigation of this precipitate.

In a third method of preparation the phosphorus formed was removed from the carbon disulphide solution by shaking the latter with diluted (20:1) nitric acid.

Determination of the radioactive sodium phosphate.

The radioactivity of the samples of blood, bones, etc. to be analysed is in most cases too feeble to be measured even by means of a very sensitive electroscope. GEIGER-MÜLLER counters, much more sensitive instruments, are therefore utilised for measuring purposes. We use for the most part tubes having an available surface of about 1.5 cm.^{2}. The sample to be measured must accordingly be spread over about the same area. The β-rays emitted by the radio phosphorus are fairly penetrating and are not appreciably weakened when an aluminium dish of 1.5 cm.^{2} surface is

filled to a depth of a few millimeters with a bone sample weighing 100 mgms. We want to know what percentage of the radioactive phosphorus taken is to be found after a certain time in, for example, the bones. The procedure is as follows. We take a solution of active sodium phosphate, use 99 per cent. of it for feeding the animal and keep 1 per cent. as a "standard". We kill the animal, separate a bone sample, ignite it, and measure its activity. Should the latter be, for instance, half as large as that of the standard which is measured simultaneously, then we can conclude that 0.99×0.5 per cent. of the active phosphorus atoms eaten are actually present in the bone sample investigated. Although the β-radiation from radioactive phosphorus atoms is not appreciably weakened in penetrating through 100 mgms. of bone ash, we can entirely eliminate the possible error due to this absorption by adding 100 mgms. of calcium phosphate to the standard solution; this has the same absorbing power as the bone sample. It is advisable to make the standard as similar to the sample to be measured as possible. In dealing with urine, faeces, muscles, liver etc. we first destroyed the organic matter by one of the usual methods; in several cases, however, these were replaced by treatment with fuming nitric acid. Then calcium phosphate and calcium oxide were added if necessary to make the sample more similar in its composition to our standard preparation and finally the sample was ignited.

To demonstrate the utility of the isotopic indicator method we will first consider the problem of the origin of the phosphorus in the faeces.

Origin of the phosphorus in the faeces.

Chemical analysis enables us to determine the phosphorus content of the excreta but not to decide to what extent the phosphorus found in the faeces is undigested material and what fraction of it is phosphorus having its origin in the organism. The investigations described in this paper have revealed that a fairly rapid interchange takes place between the phosphorus present in the different bodily organs and that present in the blood. A part of the latter finds its way, when the digestive fluids are formed, into the intestinal tract and is thus added to the faeces. The following experiment permits us to distinguish between food phosphorus and that originating from the blood. We add a known amount of radioactive phosphorus to the diet and determine what percentage of the latter is to be found in the faeces. In a separate experiment we inject a known amount of radioactive phosphorus (sodium phosphate) into the blood and determine what part of this phosphorus appears in the faeces. The combination of the two results enables us to determine what part of the phosphorus found in the faeces is due to incomplete digestion of the food eaten.

In Table I the amount of radioactive phosphorus eliminated through the kidneys and the gut is given for the case of a patient fed on a normal hospital diet to which 0.5 mgm. of labelled sodium phosphate was added. Within 5 days 21.7 per cent. of the phosphorus was eliminated in the urine and 15.5 per cent. in the faeces. Similar results were obtained in other cases. Table II shows the results obtained when the radioactive phosphorus was injected into the blood of the same patient. Within 5 days 20.5 per cent. was lost through the kidneys and 2.5 per cent. through the gut.

Table I.

Radioactive phosphorus given to human subject per os.

Number of days after taking P	Diuresis in gm.	Percentage of original radioactive P in 1 gm. of the urine ash	Percentage of original radioactive P in total urine
0—1	1880	1.23	11
1—2	1800	0.31	2.8
2—3	1620	0.31	2.8
3—4	1670	0.26	2.4
4—5	1540	0.29	2.7
5—6	1860	0.25	1.8
			in total faeces
0—1	..	..	0
1—2	..	..	7.0
2—3	..	..	5.6
3—4	..	..	1.8
4—5	..	..	1.1
5—6	..	..	0

Table II.

Radioactive phosphorus injected into the blood of a patient.

Number of days after injection	Diuresis in gms.	Percentage of original radioactive P in 1 gms. of the ash	Percentage of original radioactive P in total urine
0—1	1650	0.78	12.5
1—2	1510	0.20	3.1
2—3 3—4	1850	0.15	2.9
4—5	850	0.16	1.6
5—6	1450	0.13	2.2
7—8	800	0.09	0.6
8—9	2000	0.10	1.8
		in 1 gms. of the ash	in total faeces
0—1	..	0.085	0.24
1—2	..	0.11	1.37
2—3	..	0.072	0.37
3—4	..	0.072	0.56
4—5	..	0	0

Thus about $^1/_8$ of the phosphorus atoms eliminated from the blood pass through the gut. By combining the above results it follows that of the phosphorus found in the faeces about 20 per cent. was not undigested material but was phosphorus which had already had a share in building up the organism and had left it by entering the digestive liquids and thus getting into the faeces.

In the case above, 22.3 per cent. of the rad. P left through the kidneys within 6 days and in other cases values varying between 20 and 25 per cent. were obtained.

In carrying out experiments like those described above, the most satisfactory procedure would be to replace by radioactive labelled phosphorus atoms the normal phosphorus present in all the foodstuffs administered. By bombarding the material in question with a strong source of slow neutrons we could turn some of the phosphorus atoms into radioactive phosphorus; but such a process always leads to a disruption of the molecular bonds of the phosphorus atoms which become activated and so to a destruction of the chemical compound. We must therefore content ourselves with adding inorganic radioactive phosphate to the food consumed and try to obtain a mixture of radioactive inorganic phosphate and food as uniform as possible. In our experiments carried out with human subjects the sodium radiophosphate was administered in a large volume of milk. Milk contains 0.0795 per cent. of inorganic phosphorus and about half that amount (0.036 per cent.) of phosphorus in organic form. Although the latter does not exchange with the atoms of the inorganic radioactive phosphate, the bulk of the phosphorus (0.0795 per cent.) reaches a state of kinetic equilibrium with the radioactive phosphate added and becomes radioactively indicated. During the

digestion process the 0.036 per cent. will be set free from its molecular binding and only at this stage will it have an opportunity to become thoroughly mixed (in an atomic sense) with the radioactive phosphate atoms. While, as has already been mentioned, it would be preferable in investigating phosphorus metabolism to utilize food in which all the phosphorus atoms are labelled, it is not probable that the information obtained with such material would be noticeably different from that obtained in the experiments described in this paper. The blood plasma, where the phosphorus eaten first arrives, contains mostly inorganic phosphorus; we must therefore assume that it is primarily phosphate ions that are involved. Furthermore experience shows that the retention of phosphorus does not depend on the form in which the phosphorus is present[1] in the food, on whether it is present as inorganic and thus exchangeable phosphate or as non-exchangeable. Ducks reared on diets containing phosphate only in inorganic form matured normally and laid 85 to 795 eggs during the first summer.[2] About 15 per cent. of the phosphorus present in meat, more than half that present in milk, and the greater part of that present in vegetables, i.e. the bulk of the phosphorus eaten, is present in inorganic and thus exchangeable form.

Rats are inclined to eat their offspring and they could easily be fed on young rats born by a mother fed on radioactive phosphorus, but the chief source of phosphorus would in this case, too, be inorganic phosphorus, namely that present in the skeleton.

[1] M. Speirs and H. C. Sherman. J. Nutrit. **11**, 216, 1936.
[2] G. Fingerling, Biochem. Z. **38**, 448, 1911.

Elimination of phosphorus by rats.

We carried out numerous experiments with rats which were fed on a normal diet to which radioactive phosphorus was added. In some cases we added 0.1 mgm. or less in the form of sodium phosphate dissolved in a few drops of water which was then soaked up by a small piece of bread given to the animal. The average of several experiments gave a total excretion of 26 per cent. through the kidneys and of 32 per cent. through the gut. In some other experiments calcium phosphate was administered, mixed with butter, which was given to the rat on a small piece of white bread. The result of such an experiment is seen in Table III, which contains the results of the analysis of the urine and the faeces collected during 19 days. The urine was concentrated by evaporation, treated with fuming nitric acid, and ignited; a known fraction of the ash obtained was then introduced under the Geiger counter. 19 days later the rat, which weighed 256 gms., was killed, the corpse was treated with fuming nitric acid to destroy organic compounds, the fatty residue was treated with conc. sulphuric acid, and then ignited in an electric oven. 50.2 per cent. of the

Table III.

1.5 mgm. radioactive calcium phosphate added to normal diet of adult rat.

Number of days after taking rad. P	Percentage of original rad. P in the urine	Percentage of original rad. P in the faeces
0— 3	11.4	13.1
3— 7	3.9	4.7
7—10	2.7	2.4
10—13	1.8	0.93
13—16	1.3	1.1
16—19	1.2	1.8[1]
Total	22.3	24.0

[1] Faeces contaminated by urine.

phosphorus given was found in the ashes, which were to a large extent composed of calcium phosphate, and had a total weight of 5.84 gm.

In some cases we added large amounts of calcium phosphate containing active phosphorus to the diet. When for example 18 mgm. of phosphorus as calcium phosphate were given — this corresponds to about four times the phosphorus present in the normal diet — 41 per cent. of the active phosphorus was eliminated through the gut in the course of 19 days and only about 10 per cent. through the kidneys. Furthermore an analysis of the active phosphorus content of the corpse and the excreta revealed that when large amounts of phosphorus were added to the diet the animals would eat only part of it, however, carefully it was administered. We decided thereforet to study the effect of the intake of large amounts of phosphorus on dogs.

The phosphorus atoms absorbed have ample opportunity to enter into kinetic exchange with the phosphate ions present in muscles, bones, and other organs and also to a certain extent to enter organic molecules and replace the phosphorus atoms present there. Many of the last mentioned processes are dependent on enzymatic action. The rate at which the active phosphorus enters the blood corpuscles, the particulars of this process, and the distribution of the radioactive phosphorus between the blood and the different organs were investigated by Professor Lundsgaard and one of us and the results will be published shortly.

Phosphorus exchange in adult rats.

A preliminary investigation revealed the following distribution in adult rats killed three weeks after eating the radioactive phosphate administered in the form of 0.5 mgm. sodium phosphate added to the normal diet.

Table IV.

Distribution of rad. P in adult rats killed 3 weeks after eating it.

	p. c. rad. P
Urine	26.3
Faeces	31.8
Skeleton	24.8
Muscles and fat	17.4
Liver	1.7
Brain and Medulla	0.1
Kidneys and Pancreas	0.1

In interpreting the results obtained it is convenient to compare the radioactivity of equal weights (say 100 mgms.) of the ashes, of the bones, the teeth, the liver, and so on. These all contain about the same percentage of phosphorus (17 per cent., 17 per cent., 16 per cent.); the phosphorus content of the ash of the blood is rather different, but as was stated above the behaviour of the active phosphorus in the blood was not investigated to any great extent in the course of this work.

In a series of experiments we gave the same amount of radioactive phosphorus to 6 rats. One pair of rats was killed after one week, a second pair after two weeks, and a third pair after three weeks. The results are seen in the following table.

Table V.

Animal killed weeks after eating rad. P	p. c. of rad. P found	
	in the skeleton	in the incisors
1	34.2	2.1
1	35.3	2.1
2	32.2	2.8
2	27.2	2.1
3	24.6	2.8
3	25.4	2.7

The weights of the different skeletons vary to an appreciable extent; the weights of the animals were 225, 210, 200, 215, 235, and 220 gms. before, and 220, 205, 200, 205, 235, and 220 gms. resp. after the experiment. In comparing the rad. P content of different organs of the same rat we are independent of the assumption that all the rad. P given was actually eaten by the animal, though we are not, when we compare the rad. P content of organs from different rats. The greater rad. P content of the bones of the animals killed after the lapse of only a week cannot, however, be due chiefly to such a reason as this, because in that case the rad. P content of the incisors would also be appreciably higher in the case of rats killed after the lapse of one week. This is not the case, as can be seen from the figures in Table V. We must therefore conclude that the rad. P taken up by the bones, and in exactly the same way all the phosphorus taken up by the bones, has a certain chance of being lost again. Indeed a uptake of phosphorus atoms by the bones of an adult rat can only be explained by a corresponding

Table VI.

	p. c. of rad. P taken, present in 100 mgms. of ashes	weight of ashes of the organ in mgms.	p. c. of rad. P taken, present in the total ashes
a) rat killed after 1 week.			
Bones	0.8	4300	34.3
Molars	0.2	100	0.2
Incisors	1.3	253	3.3
Liver	3.2	103[1]	—
b) rat killed after 2 weeks.			
Bones	0.7	4200	29.5
Molars	0.2	100	0.2
Incisors	1.9	215	4.1
Liver	2.0	210	4.2

[1] The weight of the ashes of the liver was found to be very variable.

process in the opposite direction. Another example of the decrease in the active phosphorus content of the bones with time is seen in Table VI.

While the bones show a decrease in their rad. P content with time and the molars no change to within the accuracy of experiments, the incisors show a marked increase. The incisors of adult rats show a very pronounced growth. The discussion of their behaviour is therefore better postponed and will be dealt with in the next chapter, where experiments on young rats are described.

The results of an experiment carried out with two rats both killed after 5 days time are seen in Table VII.

Table VII.

	p. c. rad. P taken found in 100 mgms. of ashes	
	I	II
Bones	1.3	1.4
Molars	0.24	0.34
Incisors	2.4	2.3
Liver	2.7	1.7
Muscles	1.7	1.8
Brain	0.46	0.58

As is seen from the above figures the muscles show a somewhat large content of rad. P than the bones. The active P content of the brain ash is decidedly lower. To ascertain if the phosphorus atoms present in the brain phosphatides are also replaced by active P atoms, the brain was treated with 6 per cent. trichloracetic acid solution. By this means all the acid soluble phosphorus was removed. The operation was carried out with great care. After igniting the filtrate and residue, the activity of both fractions was measured. We found both fractions to be active, the activity of the phosphatide fraction being about $^1/_3$ of that of the

trichloracetic acid extract. We are engaged in following up this point in greater detail, using more trustworthy methods of separation.

Exchange of phosphorus by growing rats.

The uptake of phosphorus shown by different organs of rats about 2 weeks old is seen in Table VIII. The rats were killed three days after being fed with radioactive phosphorus added to their normal diet.

Table VIII.

	Rat I (weight 27 gms.)		Rat II (weight 24 gms.)	
	weight of ashes in mgms.	p. c. of rad. P taken present in 100 mgms. of ashes	weight of ashes in mgms.	p. c. of rad. P taken present in 100 mgms. of ashes
Bones (Leg)	65.4	10.5	59	10.9
Incisors	—	5.8	—	5.8
Molars	39.4	2.9	33.8	2.6
Muscles	—	11.0	—	—
Blood	—	2.8	—	2.6

Focussing our attention first on the bones we notice that 100 mgms. of ash contain more than ten times as much radioactive phosphorus as was found in the case of adult rats. The high radioactivities of the bones are due to the fact that in this case an appreciable part of the bones are actually grown from blood of high radioactive phosphorus content; a rapid formation of new cells takes place, in whose building up radioactive phosphorus participates.

A very conspicuous difference is found between the active phosphorus content of the molars of rapidly growing and of adult rats, the great difference being due primarily to the low exchange values in the latter. The brain as a whole was found to contain 0.5 per cent. of the active phosphorus taken by the animal.

The ratio between the rad. P content of the muscles and the bones is nearly unity in the case of the young rats, while in adult rats the muscles show a higher rad. P content.

When we compare the radioactive phosphorus content of the bones of growing rats, we find for example more activity in 100 mgms. of the ashes of the bones of animals killed after one week and than in those killed after two weeks. This is due chiefly to the fact that the phosphorus atoms present in the bone at a certain time will soon be found in an entirely different part of the growing skeleton, and will also have a certain chance of leaving the skeleton entirely. If we want to obtain information on the latter point we must compare the "radioactive" phosphorus contents of whole skeletons. We carried out such experiments, comparing the whole of the leg material. Five very young rats having a total weight of only 25 gms. were fed on their normal diet plus some radioactive phosphorus (0.50 mgms. each). Two were killed 2 days later and three 65 days later. 10 mgms. of the ashes of the leg bones of animals killed after 2 days contained 8.4 times as much radioactive phosphorus as that of rats killed after 65 days. The active phosphorus atoms were in fact distributed all through the greatly increased amount of bone tissue; the leg bones increased in the course of 63 days to about ten times their original weight, as can be seen from Table IX. When we compare the radioactive phosphorus content of the total bone material of the legs the difference between the rats killed after 2 days and after 65 days is much less; the difference still present is due to the loss of phosphorus atoms by the bone material. The phosphorus atoms which were present in the bone for a while and left it again will be found chiefly in the excrements but to a small extent also in some of the organic compounds

building up the organism, which are formed slowly. In the course of two months about one-third of the phosphorus atoms originally present left the skeleton entirely.

A comparison of the behaviour of the active phosphorus present in the incisors with that in the bones is difficult in view of the rapid using up and replacement of the incisors. Prof. Holst, Prof. Krogh and one of the writers of this paper are at present engaged on an investigation of the exchange of phosphorus in the incisors on different lines.

Table IX.

Period between taking of radioactive P and killing	Weight of bone ash (legs) in mgms.	p. c. of radioactive P present
2 days	65.4	7.4
2 „	59.0	7.5
65 „	440	4.1
65 „	514	5.1
65 „	613	5.5

Uptake of phosphorus in pregnant rats and in human placenta.

In Table X the result of the investigation of adult normal and pregnant rats is seen. Those designated I were killed after a lapse of one week, those marked II after two weeks.

As can be seen from the above figures the different organs of the pregnant rats took up less rad. P than normal rats the difference being found at least partly in the foetus and placenta. In the first rat, which was in an advanced stage of pregnancy, the foetus and still more the placenta had a high content of rad. P, higher than any organ of the mother. We find here again a very conspicuous illustration of the difference between the taking up of P through an exchange

Table X.

	Normal rat p. c. of rad. P taken present in 100 mgms. ashes	Pregnant rat p. c. of rad. P taken present in 100 mgms. ashes
I Bones	0.78	0.49
II Bones	0.74	0.52
I Incisors	1.3	1.2
II Incisors	1.9	1.7
I Molars	0.21	0.12
II Molars	0.23	0.16
I Liver	2.0	1.6
II Liver	1.94	1.0
I Foeta	—	2.7
II Foeta	—	0.54
I Placenta	—	4.0
II Placenta	—	2.3

process and through actual growth, the latter being much more effective in introducing rad. P into the tissue. An appreciable part of the foetus has actually been built up by utilising blood of rad. P content and has correspondingly a high content of the latter. This is still more the case for the rapidly growing placenta, the latter also being subject to a very thorough blood circulation. In the case of the second animal pregnancy occurred at a much later date than the intake of rad. P. The foetus was nourished by blood poor in rad. P, and correspondingly the rad. P content of the foeta was much less. Whereas in the first case the weight of the ash of all foeta was 345 mgms., in the second case it was only 52 mgms., the weight of the placenta ash being 43 and 12 mgms. respectively.

We also had an opportunity to witness what was a comparatively very high rad. P content for the placenta of a human subject; as much as 0.095 per cent. was found in the ash of the placenta, which weighed 133.8 mgms. We

can estimate the total ash which the patient in question should give on ignition as 2800 gms. The weight of the placenta ash thus amounted to less than $^1/_{20\,000}$ of the total ash, while the rad. P content was as much as $^1/_{1000}$ of the total amount of rad. P given, showing a concentration of rad. P in the placenta ash more than twenty times as great as that in the average ash of the body. One might try to explain the high rad. P content of the placenta by its high blood content. That this explanation fails is seen, however, from the following. The ash of the placenta was found to weigh 133.8 mgms. and the ash of about 5 ccs. of blood would weigh the same. But as early as 8 hours after the injection of rad. P such a volume of blood was found to contain less than $^1/_{10\,000}$ of the latter[1], and after the lapse of a few days — when the placenta were removed — still less. The high rad. P content of the placenta cannot therefore be due to their blood content. No activity could be detected in the ash of the few weeks old foetus removed in the course of an operation, but the weight of this amounted to only a few mgms.

Uptake of phosphorus by rachitic rats.

We carried out a set of experiments on two months old rachitic rats, which had been used by Fredericia and Gudjonson in their experiments on the effect of vitamin A and D deficiency on rickets. The rats were fed before and during the experiments on a diet free from or poor in vitamins A and D. The weights of the animals before the

[1] In the case of another subject we found 1 cc. of blood to contain 0.0027 per cent. of the phosphorus injected after the lapse of 12 hours, the blood particles containing 11 times as much active phosphorus as the plasma.

Table XI.

Killed	p. c. from the rad. P taken found in 100 mgms. ashes				Weight in mgms.			
	Bones	Incisors	Molars	Liver	Bones[1] (legs)	Incisors	Molars	Liver
1 week....	4.2	3.8	0.7	3.2	358	100	72	135
1 „	4.2	3.8	1.1	5.9	329	105	76	103
2 weeks...	3.0	4.1	0.9	5.0	403	113	55	86
2 „ ...	3.5	3.7	0.9	5.0	361	96	64	84
3 „ ...	2.7	5.0	1.4	1.8	313	115	69	168
3 „ ...	2.2	3.6	1.1	1.2	419	109	57	145
3 „ ...	2.9	4.3	0.9	1.8	422	115	81	205

experiment were 89, 83, 85, 93, 90, 95, and 103 gms. The results are seen in Table XI.

The above figures show no outstanding difference as compared with normal rats of the same age. We are engaged in carrying out further experiments on rats with rickets.

General Considerations.

The rapid entrance of the labelled phosphorus into the bone is in no way puzzling. If solid calcium phosphate, one of the chief constituents of the bone, is in contact with the solution containing labelled phosphate ions a rapid distribution of the latter takes place between the surface of the solid phase and the liquid phase, as was seen from the following experiment. 3.950 gms. freshly precipitated $Ca_3(PO_4)_2$ were shaken with 5 ccm. of water saturated with $Ca_3(PO_4)_2$ at room temperature and containing an infinitely small amount of labelled sodium phosphate. After lapse of four hours 84.1 per cent. of the labelled phosphate ions were found in the solid phase and only 15.9 per cent. in the solution. The calcium phosphate of the bone tissue

[1] The weight of the total skeleton is obtained by dividing the figures obtained for the legs by 0.26.

being in a very intimate contact with the blood stream, i.e. with cells containing labelled phosphate, a similar exchange to that described above will take place between the unlabelled phosphate of the bone and the labelled phosphate present in the liquid phase.

Beside the above mechanism we have to consider two others yust as important. During growth, the bone tissue formed will be built up from labelled phosphorus as long as the blood stream contains the latter.

Finally we have to envisage a third possibility, namely the entrance of labelled phosphorus into the bone through a constant break-down of the bone tissue already formed and the formation of new tissue in the case of adult animals as well.

The following examples may help to make the three ways of entrance of the labelled atoms into the bone easier to understand.

1) When solid salts are in contact with labelled ions of the solution within a short time a distribution equilibrium of the labelled ions between the surface layer of the solid and the solution will take place, as is seen for example in the experiment described above. This phenomenon was studied extensively by Paneth and his collaborators[1] in the case of lead salt which were shaken with solutions containing labelled (radio- active) lead ions.

2) If we deposit for example lead electrolytically from a solution containing labelled lead ions, the metallic deposit will be a labelled one, just as the bone grown from blood containing labelled phosphorus will contain labelled phosphorus.

3) In investigating the exchange between metallic lead

[1] F. Paneth and W. Vorwerk, Zs. phys. Chem. **101**, 445, 480, 1922.

and a solution of labelled lead ions, or vice versa, we find[1] a different behaviour to that described above in the case of lead salts. The exchange in the case of metal is not restricted to the uppermost atomic layer of the lead surface; many atomic layers are involved in the exchange process. This is due to the fact that the lead actually goes into solution from certain parts of the surface, while lead ions are discharged, at other parts. This is a much more effective process in bringing about an exchange between the lead atoms in the solid and in the liquid phase than that observed in the case of solid salts where only the uppermost atomic layer is involved (within any reasonable time) in the exchange process. The entrance of labelled phosphorus into the bone will also be much facilitated if it is not only the uppermost phosphate layer that is involved in the exchange process; if in fact the bone is destroyed at certain places and rebuilt at others. In view of the important enzymatic actions[2] going on in the bone tissue such a reversible breakdown process will easily occur.

Summary.

By adding radioactive phosphorus (phosphate) to the diet of rats, the metabolism of the phosphorus atoms taken in with the diet can be followed up in the animal body. An appreciable part of the phosphorus taken finds its way not only in growing but also in adult animals into the bones, teeth, muscles, and different bodily organs.

In growing animals it was found that the atoms already present at an early stage of the formation of the skeleton

[1] G. Hevesy, Phys. Zs. **16**, 52, 1915.

[2] R. Robison: The significance of phosphoric esters in metabolism, New York 1932.

become equally distributed in the course of time over the different parts of the skeleton and other organs demonstrating thus the dynamical nature of the building up of bone tissue. Some of the phosphorus atoms present in the bones leave the skeleton for good, being eliminated through the kidneys or the bones or becoming located in other organs of the body.

The replacement of individual phosphorus atoms by other phosphorus atoms also takes place in the bone tissue of adult animals including that of the teeth.

It was ascertained that about one-seventh of the phosphorus found in the faeces of a human subjects is due to material which has entered the intestines through the digestive juices after being located in the blood stream or in the organs of the body for a shorter or longer time.

We would like to express our best thanks to Professor Niels Bohr for the radium used and to him and to Professor August Krogh for the kind interest he has taken in this work and to express our thanks to Miss Hilde Levi, Mrs. Teichert and Mr. Høffer-Jensen for their very efficient help in the experiments described.

The Finsen Institute and
the Institute of Theoretical Physics, Copenhagen.

Færdig fra Trykkeriet den 22. Marts 1937.

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BY

TH. MORTENSEN

WITH 4 PLATES

KØBENHAVN
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A few months ago Mr. K. FEIFEL, Stuttgart, wrote me a letter informing me that in the course of his investigations of the fauna of Foraminifera in the Jurassic deposits of Schwaben, Germany, he had come across a number of fragments of Echinoderms, among which a good deal of pedicellariæ of sea-urchins, saying that, if I would care to look them over and see whether they might be of interest, he would be glad to send me the material collected. On my reply that I would like very much to see his material he sent me a number of slides containing pedicellariæ and some other rests of Echinoids — isolated plates of tests, pieces of lanterns, and spines, etc. The marvellous state of preservation of most of the pedicellariæ roused my interest; it seemed probable that it might be possible, at least partly, to ascertain to which of the Echinoids known from the corresponding deposits they must belong, so I wrote to Mr. FEIFEL asking him to look out for more of these Echinoid remains in the washings from which he used to collect his Foraminifera. So he did, and in the course of the next few months he sent me several other samples of Echinoderm remains, among which I found quite a number different forms of pedicellariæ, the more important of which I shall describe in the present paper.

No doubt, it would be possible to do some valuable work also on the isolated plates of tests and lanterns and

spines of sea-urchins sent me, seing particularly what Bather has made out of similar material in his "Triassic Echinoderms of Bakony"; but that would require very much more time than I could possibly spare, and I must therefore confine myself mainly to the pedicellariæ, and only the more notable and characteristic ones. A single type of Cidarid spines, is so interesting that I must describe and figure it; it represents beyond any doubt a new species of *Anaulocidaris*. Mention may also be made of the sample figured in Pl. I. Fig. 6. It is a piece of the sucking disk of a tubefoot, exactly resembling the corresponding pieces in recent sea-urchins. They are so closely alike in the various recent forms that there is no possibility of ascertaining to which genus of the fossil forms it belongs, beyond the fact that it is of some Regular Echinoid, outside the Cidarids, in which the sucking disks are not so typically developed. Several such pieces are found in the collected material.

Another conspicuous type represented by various "species" in Mr. Feifel's material is the terminal plate of seastars. I shall confine myself to figuring a few species (Pl. I. Figs. 1—5); some specialist in fossil seastars might well be able to make a good deal out of these plates.

Remains of Ophiurids are very scarce in the material sent me; this fact, however, does not mean that they are scarce in the deposits studied by Mr. Feifel. On the contrary, Ophiurid vertebræ, ventral, dorsal, and lateral plates are, as Mr. Feifel informs me, very numerous in all the deposits, in many of them even so numerous as to be a nuisance. I do not regret to have not received all these Ophiurid remains. It seems questionable, whether a study of fossil Ophiurid vertebræ will lead to very valuable results; at least, it will be a very troublesome study. The

shape of the vertebræ changes greatly from the base to the end of the Ophiurid arms, without, on the other hand, being very characteristically different within the divers genera or even families — apart from the curious vertebræ with the closed ventral groove characteristic of the family of the Trichasterids (cf. my "Studies of Indo-Pacific Euryalids". Vid. Medd. Dansk Naturhist. Forening. 96. 1933. p. 3). In any case, a study of the fossil Ophiurid vertebræ will require a careful comparison with the vertebræ of a great number of representatives of the families of recent Ophiurids, before results of real value can be attained.

I was delighted to find in the material sent me by Mr. FEIFEL an extraordinarily fine piece of an Ophiurid arm, with a couple of hook-shaped spines preserved in situ (Pl. I. Fig. 10.). Nothing quite comparable is known to me among recent Ophiurids. The shape of the arm-joints proves that it is no Euryalid; it rather recalls an Ophiolepidid. The absence of dorsal plates recalls *Ophiomusium* (in which hook-shaped arm-spines do sometimes occur, e. g. *Ophiomusium spinigerum* Mrtsn.), but I do not think it can have anything with *Ophiomusium* or any other Ophiolepidid(?) to do. The hook-shaped spine represented in Pl. I. Fig. 11 recalls those of some Euryalids, e. g. *Astrothamnus*, but it may just as well belong together with the form of which the arm joints are preserved.

As already said, the preservation is in general remarkably fine. Moreover, I find that the mounting of the smaller forms in Canada balsam means a great improvement. The finest microscopical details often become quite distinct, so that these about 150 million years old small parts look almost as if they were taken directly from living specimens.

A very important paper has recently been published by H. L. Geis: "Recent and Fossil Pedicellariæ" (Journ. of Paleontology. X. 1936. pp. 427—448), describing a number of different kinds of pedicellariæ from the Carboniferous (Pennsylvanian) deposits of the United States. I shall have to criticize some of his conclusions in the following, but otherwise I agree with him that the study of fossil pedicellariæ, even if found isolated, not attached to the tests of the sea-urchins, may yield important results not only from a taxonomic, but possibly also from a stratigraphical point of view. Whereever deposits are found which are in such condition that they can be washed out, there may be prospects of finding these delicate organs well preserved. It will be sure to pay the trouble.

Dr. Geis gives a review of what has hitherto been done in the study of fossil pedicallariæ — but he is not quite up to date, not knowing my paper "Notes on some Fossil Echinoids" (Geol. Magaz. LXXI. 1934), which contains a chapter "On some Fossil Echinoid Pedicellariæ", with Plate XXII. That he does not mention the finds of pedicellariæ of fossil Echinoids recorded in Part II of my Monograph of the Echinoidea (*Acrosalenia*, *Trochotiara*) is more excusable, as that work could hardly have been in hands before his paper was sent to press. (I may mention here that also Part III of my Monograph of the Echinoidea will contain observations on the pedicellariæ of some fossil Echinoids.)

For the eventual identification of the fossil pedicellariæ it was, of course, very important to know which Echinoids have been found in the corresponding deposits. On my request Mr. Feifel sent me such list, worked out by Dr. Berckhemer, Vorstand. d. Geolog.-Paleontolog. Abtei-

lung d. Staatlichen Naturaliensammlung, Stuttgart, for which I am greatly indebted to the two named gentlemen.

The following list of the deposits from which the material comes is likewise due to Dr. BERCKHEMER and Mr. FEIFEL.

1) Lias α — Tone der Psilonoten-Schichten —, Sulzgrieser Kelter bei Esslingen a. N.;
2) Lias α — Tone der Angulaten-Schichten —, Uhlbach bei Stuttgart;
3) Lias β —, Ober-Esslingen;
4) Lias δ — Zone des Amaltheus costatus —, Gewand Enzenhart bei Nürtingen a. N.;
5) Lias ζ —, Probegrube der Reichsautobahn bei Holzmaden, Kreis Kirchheim u. T.;
6) Lias ζ —, Heiningen, Kreis Göppingen;
7) Dogger ε — Parkinsoni-Schichten —, "Erkenberg" bei Neidlingen, Kreis Kirchheim u. T.;
8) Malm α — Impressa-Mergel —, Reichenbach i, T., Kreis Geislingen;
9) Malm α — Schwammfacies —, "Lochengründle" bei Balingen;
10) Malm γ — Tonfacies —, Steige von Beuren n. Erkenbrechtsweiler, Kreis Nürtingen a. N.;
11) Malm δ — Schwammfacies —, "Bosler" bei Gruibingen, Kreis Göppingen;
12) Malm ζ — Cement-Mergel —, Sotzenhausen (Steinbrüche der Portlandcementfabrik Blaubeuren, Gebrüder SPOHN A. G.);
13) Malm ζ — Cement-Mergel —, Gerhausen (Steinbrüche der Portlandcementfabrik Blaubeuren, Gebrüder SPOHN A. G.).

Pl. I. Figs. 1—5. **Terminalia of Asteroids.** Lias α, Uhlbach; Malm δ, Bosler; Malm γ, Beuren.

Quite a number of different sorts of Asteroid terminalia are found in the material sent me, several of them highly characteristic. I have only figured three of them, wishing only to call the attention of specialists in fossil Asteroidea to these characteristic plates, which, according to Mr. Feifel, are well represented in all the various deposits. No doubt a careful study of them would give interesting results. The great number of characteristic forms found in this fossil material leads also to the suggestion that the terminalia of the recent sea-stars, which have up till now hardly received any attention at all, may deserve to be taken into consideration; it is probable that they would prove to offer characters of classificatory value. Also the fossil forms have scarcely been touched; a single form "*Astropecten Pichleri*" v. Wöhrmann is described and figured by Bather in his "Triassic Echinoderms of Bakony" p. 235. Pl. XIII. 435—437.

Pl. I. Fig. 7. **Valve of rostrate pedicellaria.** Malm α. Reichenbach.

This very simple form of pedicellariæ can scarcely be anything but a rostrate pedicellaria of some Irregular Echinoid; certainly nothing like it is known from any Regular Echinoid. There are some small teeth along the distal edge of the blade, as usual in rostrate pedicellariæ of recent forms.

The following Irregular Echinoids are recorded from the Malm α—δ of Württemberg: *Collyrites carinata* Leske, *C. capistrata* Goldf., *C. bicordata* Klein; *Dysaster granulosus* Münster, *D. bicordatus* Klein; *Holectypus depressus* Leske, *H. orificiatus* Loriol. None of these have any near relations

among recent Echinoids, so it is impossible to say to which of them this type of pedicellaria belongs, but the hint herewith given as to its being either of a Collyritid or of a Holectypid is already of some value and may lead to further discoveries of the pedicellariæ of these important, wholly extinct types of Irregular Echinoids.

Pl. I. Figs. 8—9. **Valves of tridentate pedicellariæ.** Malm *α*, Lochengründle; Malm *δ*, Bosler.

None of the larger samples of this very fine and interesting type of pedicellariæ are complete, but they must have reached a size of up to c. 4—5 mm length of head, and thus have been quite conspicuous organs, and apparently quite active defence weapons of the sea-urchin to which they belonged. Fig. 8, representing the terminal part in side view, shows that any small organism caught by these pedicellariæ would have little chance of escaping unhurt or alive. In contradiction to the elaborate and apparently powerful terminal part stands, however, the small size of the basal part and particularly the cavities lodging the adductor muscles, so that the strength of these pedicellariæ would seem not quite in correspondance with the size and elaborate shape of the valves.

The shape of the terminal part recalls a tennis racket; it is flat on the outer side and entirely smooth. The "shaft" is elegantly curved. The valves are found in different sizes, down to c. 0.5 mm length.

This type of pedicellariæ recalls the one figured on Pl. XXII, fig. 4 of my "Notes on some Fossil Echinoids" (Geol. Magaz. LXXI. 1934) and referred (Op. cit. p. 406) with rather great probability to the genus *Pelanechinus*, a nearly identical form of tridentate pedicellariæ being described from *Pelanechinus corallinus* by GROOM ("On

some new features in *Pelanechinus corallinus*". Quart. Journ. Geol. Soc. XLIII. 1887), and we may then reasonably assume the form described here to belong to some, unknown, Echinoid allied to *Pelanechinus*. Among recent Echinoids pedicellariæ of this type are unknown.

Pl. I. Fig. 12. **Tridentate pedicellaria of a Cidarid.** Malm ζ, Sotzenhausen.

Only a single fragmentary specimen of this pedicellaria is found in the material received. Evidently, it has been quite a large one, some 5 mm long. It can hardly be doubtful that this is a tridentate pedicellaria of some Cidarid, similar forms being found in the genus *Phyllacanthus* (cf. Monograph of the Echinoidea I. Pl. LXXXVIII). Judging from the large size of this pedicellaria it must have been from a large Cidarid. From Malm ε and ζ are known no less than 10 species of *Rhabdocidaris* and 3 of *Diplocidaris*. As *Rhabdocidaris* is a near relation of the recent *Phyllacanthus* it may well be suggested that the pedicellaria here figured belongs to one of the *Rhabdocidaris*-species. It is, of course, also possible that it rather belongs to *Diplocidaris*; but as none of the recent forms are nearly related to *Diplocidaris*, we cannot have any idea of what the pedicellariæ of this genus looked like. Thus from our present knowledge we must conclude that this tridentate pedicellaria belonged to a *Rhabdocidaris*-species.

Pl. I. Fig. 13. **Periproctal spine of a Salenid.** Malm ζ, Sotzenhausen.

This little spine has all the appearance of being a periproctal spine of some Salenid, these spines in recent Salenids having a more or less similar characteristic, irregular shape. Mr. Feifel found it attached to a small irregular polygonal plate, which likewise has all the appearance

of a periproctal plate of a Salenid. The only Salenid known from the said deposit is *Pseudosalenia aspera* (Ag.), of the family Acrosalenidæ. Although no recent Acrosalenid exists, there is no reason to doubt that they would resemble the true Salenids in regard to their periproctal spines, and it is thus very probable that this spine does really belong to *Pseudosalenia aspera*.

Pl. I. Fig. 14. **Valve of tridentate pedicellaria.** Malm ζ, Sotzenhausen.

There is no possibility of ascertaining to which Echinoid this striking form of tridentate pedicellaria belongs. The only pedicellaria of recent sea-urchins recalling it is that of *Echinolampas sternopetala* A. Ag. & H. L. Clark, figured by H. L. Clark in his "Hawaiian and other Pacific Echini". Echinoneidæ .... Spatangidæ (Mem. Mus. Comp. Zool. XLVI. 1917, Pl. 144. fig. 22). This is, however, a much smaller form, the valves c. 0.6 mm long, whereas the present, fragmentary valve must have been about 2 mm long (the point and the lower part of the base are broken away). It may be very tentatively suggested that it may belong to one of the (three) *Holectypus*-species known from this deposit. At any rate, it is so highly characteristic that it may easily be recognized by future researches, so I have found it desirable to figure it. That it must have been a no less effective defense-weapon than the form described above, Pl. I. Figs. 8—9, is evident, and it is interesting to witness the inventive power of nature in devising two so widely different apparatus for the same purpose, both highly elaborate.

Pl. II. Figs. 1—2, and 6. **Globiferous and ophicephalous pedicellariæ of Hemipedina.** Lias α; Sulzgrieser Kelter; Lias ζ, Holzmaden; Malm ζ, Sotzenhausen and Gerhausen.

I hardly ever expected that the globiferous pedicellariæ of fossil Regular Echinoids would be found with their delicate terminal fangs in complete state of preservation. Thus the globiferous pedicellariæ figured by Geis (Op. cit. Pl. 60. figs. 22—27) entirely lack the terminal part, and accordingly do not give more information than that globiferous pedicellariæ (— if indeed they are really globiferous —) existed already in some Echinoid of the Carboniferous period. As it is particularly the globiferous pedicellariæ which are of so great importance for the classification of some of the families of Regular Echinoids of the Order Camarodonta (the families Echinidæ, Toxopneustidæ, and Echinometridæ), besides the Cidarids, it was, of course, a serious drawback that the fossil forms, of which these pedicellariæ were not likely to be made known, could not with certainty be referred correctly to the family, in spite of the fact that they can be identified to both genus and species — exactly as it was the case with the recent forms before the structure of the globiferous pedicellariæ was taken into consideration in classification. The finding of the globiferous pedicellariæ here figured gives us hope that by and by we may find also these structures preserved in such fossils as belong to one or other of the above named families, and that their true position can thus be ascertained.

The globiferous pedicellariæ here figured, with their three terminal fangs preserved complete, are so perfectly like those of the recent Pedinids of the genus *Cœnopedina* that there cannot be any doubt that they likewise belong to a Pedinid. Similarly the ophicephalous pedicellaria, fig. 6, with the very characteristic double series of teeth along the margin of the blade, very closely resembles those of *Cœnopedina*, and as no other recent Echinoid is known

to have such ophicephalous pedicellariæ we may conclude with certainty that this pedicellaria likewise belongs to some Pedinid. *Hemipedina nattheimensis* (Quenst.) and *H. calva* (Quenst.) being the only Pedinids known from the deposits in which these pedicellariæ were found, it can be regarded as certain that they must belong to one of these species.

Pl. II. Fig. 3. **Miliary spine of Collyrites** (?). Malm *α*, Reichenbach.

The spine here figured so closely resembles those peculiar small spines of which the fascioles of Spatangoids are composed that one is tempted to say, it must be such one. However, none of the Echinoids recorded from the Malm have fascioles, such being known only in Spatangoids, which do not appear before the Cretaceous. Recalling that a kind of primitive fascioles are found in some of the Meridosternata, with clavulæ very closely resembling the one here figured (cf. my "Ingolf" Echinoidea. II. Pl. XI. 42, a clavula of the Pourtalesiid, *Echinosigra paradoxa* Mrtsn.), it would seem not unreasonable that these very small spines — which occur, evidently, in good numbers, several samples being found in the material at hand — may belong to *Collyrites* (or *Dysaster*), the spines of which, judging from the minute size of their tubercles, must have been very small.

The size of these spines is only c. 0.4—0.5 mm. They have not all of them a quite so elaborately formed terminal part as the one figured, but they are all of them of the same main type. They are remarkably well preserved, some of them showing the original microscopical structure almost as clearly as do the clavulæ of recent Spatangoids.

Pl. II. Figs. 4—5. **Ophicephalous pedicellariæ.** Malm *α*, Reichenbach.

This kind of ophicephalous pedicellariæ of which both a complete head and several isolated valves and stalks, in an exquisite state of preservation, are found in the material from Malm *α*, Reichenbach, recalls very much the ophicephalous pedicellariæ figured by Geis (Op. cit. Pl. 59. 28—34), from the Carboniferous (Graham formation) of Texas, taken by him to indicate the existence already in this palæozoic period of Irregular Echinoids, though such have otherwise not been found earlier than the Jurassic period.

This is an exceedingly improbable assumption, and the isolated plate shown in Geis Pl. 59.39, and regarded as a further indication of the existence in this carboniferous deposit of some unknown Irregular Echinoid, is certainly so indistinct and unidentifiable that it cannot be of any value at all in this connection. H. L. Clark has informed Dr. Geis that in his opinion this ophicephalous pedicellaria would rather belong to some Pedinid, but Geis thinks it much more like the ophicephalous pedicellariæ of *Pourtalesia*, and reproduces (Pl. 58.15—17) my figures of the ophicephalous pedicellariæ of *Pourtalesia Wandeli* ("Ingolf" Ech. II. Pl. XI. 13, 14, 18) and bases thereupon the said assumption. Even if there is no proof that this ophicephalous pedicellaria belongs to some Pedinid as suggested by Clark — and Pedinids are not known either from the palæozoic formations — we need not at all draw the conclusion that either Pedinids or Irregular Echinoids were already present in the Carboniferous. In Echinothurids ophicephalous pedicellariæ of a somewhat similar type occur (cf. Monograph of the Echinoidea II. e. g. Pl. LXXV. 9, 10, 16, 18, of the genus *Tromikosoma*, one of the more primitive of Echinothurids). As Echinothurids are, without doubt, (in

my opinion at least), derived from Lepidocentrids, the only reasonable suggestion is that the ophicephalous pedicellaria of GEIS belongs to some Lepidocentrid, a family so well represented in the Carboniferous period.

As for the ophicephalous pedicellaria represented here, Pl. II. Fig. 4—5, it is evidently not of a Pedinid, as it has not the characteristic double series of teeth along the edge of the blade. It has much the appearance of belonging to some Irregular Echinoid, which would then be either *Collyrites*, *Dysaster*, or *Holectypus*, the only Irregular Echinoids known from the same deposit. But it is also quite possible that it belongs to some Echinothurid. Till now no Echinothurids are known from these deposits, but the existence of some Echinothurid in these same deposits is proved by the pedicellariæ described below (p. 16).

Pl. II. Fig. 7. **Ophicephalous pedicellaria.** Lias β. Ober-Esslingen.

There are several valves of this very small pedicellaria, only c. 0.2 mm long, partly in exquisite state of preservation; not only the fine marginal teeth are distinct, but even the original holes in the calcareous substance of the blade. The edge of the blade is remarkably thick. The irregular top above the edge is not distinct on all the valves, and it is uncertain whether it is somewhat serrate.

Only *Acrosalenia minuta* Buckman and *Diademopsis Quenstedti* Desor are recorded from Lias β, from which fact it might be concluded that this pedicellaria would belong to one of them. As for *Acrosalenia* the ophicephalous pedicellaria of *A. hemicidaroides* Wright figured in the Monograph of the Echinoidea II. fig. 377.d (p. 640) is so different from the present form that it is hardly thinkable that they could both belong to the same genus. *Diademopsis*,

so closely related to *Hemipedina*, might be expected to have a similar form of ophicephalous pedicellariæ as has the latter genus, with the very characteristic narrowing of the lower part of the blade into a sort of "stalk", and also with the characteristic double series of marginal teeth. It is therefore not very likely that this pedicellaria belongs to any of the two forms. If *Magnosia* or *Polycyphus* were found in the Lias deposits, I would believe it to belong to one of these genera, but they are not known from older deposits than the Bathonian. For the present we can only say that this pedicellaria must belong to some (evidently small) Regular Echinoid. As it is a very characteristic and easily recognizable form, we may hope that by future finds it may be disclosed to which Echinoid it belongs.

Pl. II. Figs. 8—9. **Tridentate pedicellariæ of Echinothurids.** Malm ζ, Sotzenhausen.

Tridentate pedicellariæ of the type represented in these figures are of common occurrence in Echinothurids, in the genera *Arœosoma* and *Asthenosoma* (cf. Monograph of the Echinoidea II. Pl. LXXVII; "Ingolf" Echinoidea. I. Pl. XIII. 27; XIV. 1, 5), but are not known in other Echinoids. We may then be safe in concluding that these pedicellariæ must belong to some Echinothurid and see herein the proof of the existence of some Echinothurid, probably related to *Arœosoma*, in the Malm period. That no such Echinoid has hitherto been recorded from any period below the Cretaceous (apart from the aberrant *Pelanechinus*) need not trouble us, since the loose connection of the skeletal plates renders the preservation of fossil tests of Echinothurids very exceptional. Isolated plates of Echinothurids, on the other hand, may well be expected to occur; no such plates, however, are present in the material sent me.

It is a matter of great satisfaction to have proved herewith the existence of Echinothurids in the Malm. If, as I think it certain, the Echinothurids are derived from the Lepidocentrids, they must, of course, have existed also in the long period between the Cretaceous and the Palæozoic eras. The present find begins to fill the gap. The still older pedicellaria (from the Bajocian) figured in my "Notes on some Fossil Echinoids" (Geol. Magaz. LXXI. Pl. XXII, 5, 6, and 8) may not improbably also belong to Echinothurids, as I have suggested there (p. 405), but it is less certain than is the case with the two pedicellariæ here represented.

Pl. II. Fig. 10. **Rostrate pedicellaria of Irregular Echinoid.** Malm *α*, Reichenbach.

The valve here figured strikingly recalls the rostrate pedicellariæ of various Irregular Echinoids, whereas nothing very like it is known in the Regular Echinoids. It is therefore rather safe to conclude that it must belong to one of the Irregular Echinoids known from the Malm. A rather similar form is figured by H. L. Clark in the "Hawaiian and other Echinoids". Echinoneidæ . . . Spatangidæ, Pl. 144,14 from *Echinolampas Alexandri* de Loriol. It may then not unreasonably be suggested that the present form belongs to *Holectypus* — but it can be no more than a suggestion for the present. Very similar rostrate pedicellariæ are found e. g. in *Aceste* and *Hemiaster*, but no true Spatangoid being known before the Cretaceous, these must be excluded.

Several other forms of pedicellariæ are found in the material at hand, among which some small globiferous pedicellariæ of Cidarids, but none of them are so characteristic that any reasonable suggestion can be made as to the Echinoids to which they belong, or that they would be

recognizable with certainty if found in other localities or formations. I have therefore thought it preferable to leave them out of consideration. But the forms mentioned above offer a considerable interest and indicate that further collecting of such micro-material may lead to very valuable results.

Anaulocidaris tuberculata n. sp.

Pl. II. Figs. 11—16.

The divers spines represented here, from Malm ζ, Sotzenhausen, undoubtedly represent a new species of the genus *Anaulocidaris*. The adoral "spatuliform" and "remiform" spines (Pl. II. 11—15) differ from those of the other two species of the genus known till now, *A. Buchi* (Münster) and *A. testudo* Bather[1], in being coarsely tuberculate on their aboral, flattened side, those of the other two species being smooth, or at most finely granulated (the var. *granulata* Bather of *A. Buchi*; cf. Bather. Triassic Echinoderms of Bakony, p. 168). In the smallest of these spines, no doubt those nearest the peristome, the shaft is developed into a broad oblique plate on top of the short, distinctly striated neck; in the larger, subambital spines the shaft becomes gradually more straight and less widened. Only a single of the flattened ("trulliform") aboral spines is found in the material at hand (Pl. II. Fig. 16). The acetabular cavity is round, not transversely elliptical as in *A. testudo*. The figures here given make, I think, a more detailed description superfluous. No plates identifiable as belonging to the test of this species are found.

The largest of these spines (fig. 11) is only 3 mm long,

[1] The *Anaulocidaris Faurai* of Lambert is, in my opinion, no true *Anaulocidaris*; cf. Monograph of the Echinoidea. I. p. 67.

which indicates that this species must have been a very small one, a real pygmy.

The other species of *Anaulocidaris* being triassic, it is of very considerable interest to find now that the genus did survive till the upper part of the Jurassic period.

It is a curious fact that the Cidarid spine figured in side view in the diagrammatic figure 12 in BATHER's Triassic Echinoderms of Bakony (p. 135) resembles a spine of *Anaulocidaris tuberculata* so much that one might think, BATHER had drawn it from one of these latter. Of course, it is only a very curious coincidence, BATHER's figure being no doubt constructed after a "*Cidaris alata*" spine, as represented in Pl. XI of his eminent work.

Holothurians.

(Pls. III—IV).

A considerable number of Holothurian spicules, belonging to several distinct forms, are found in the material sent me by Mr. FEIFEL. Several of these are identical with those figured by *A.* Issler in his "Beiträge zur Stratigraphie und Mikrofauna des Lias in Schwaben" (Palæontographica. Bd. 55. 1908. Taf. VII.), whereas others are evidently unknown and, being very characteristic and easily recognizable, deserve to be described and figured. Also I have thought it desirable to give drawings of some of the species represented by ISSLER in photographic figures, which do not show all details very clearly.

I beg to say that it is not my intention to go into a critical study of the rather extensive literature dealing with fossil remains of Holothurians, the more so as this literature is only partly accessible to me. (A very careful review of this literature is given by C. CRONEIS and J. MCCORMACK in their

paper "Fossil Holothurioidea" (Journ. of Paleontology. 6. 1932). All the forms figured by Issler I am trying to identify.

Pl. III. figs. 1—2. **Wheels of Myriotrochus.** Malm *α*, Reichenbach.

Several of these wheels are in an exquisite state of preservation, showing all the structural details almost as clearly as if they were taken from the skin of recent Holothurians. They vary very considerably in size; the two figures, drawn in the same magnification, represent the extremes, but there are all intermediate sizes. That the larger wheel has an undulating margin, the smaller one not, does not mean a specific difference. It is a difference due to size, and also in the smallest wheels there may be a slight undulation of the margin.

This form of wheels, evidently the same as represented in Issler's figures 363 and 365, so closely resembles those of the recent genus *Myriotrochus* that it seems beyond doubt they prove the existence of this genus at least so far back as Jurassic times.

Pl. IV. Figs. 1—2. **Wheels of Chiridota.** Lias *β*, Ober-Esslingen; Lias *δ*, Enzenhart.

These wheels likewise are present in good numbers, partly in very fine state of preservation. Probably the two figures represent two different species, as indicated by the considerable difference in size and number of serrations along the inner edge of the rim of the wheel. The difference in the number of spokes is of no specific importance, since the number varies from 6 to 10 in what is decidedly the same species. Also in the recent forms a similar variation occurs. It is probably this type of wheels which is represented in Issler's figure 364.

These wheels correspond so closely with the wheels of the recent genus *Chiridota* that there can be no doubt they do belong to this same genus, proving thus its existence already in the Lias period.

Pl. III. Figs. 9—15. **Spicules of Synaptids.** (*Ancistrum Issleri* Croneis). Lias β and δ; Dogger ε; Malm α.

Especially in Dogger ε, Erkenberg, these spicules are found in great numbers. They vary very much in size, as seen in Pl. III. 9—14, all from the said deposit.

It is hardly probable that all these hooks from the deposits of Lias β to Malm α belong to one and the same species; but it is not possible to distinguish different species of them in view of their great variation.

This type of spicules has been designated by the name of *Ancistrum* (Etheridge) Smith, and it may be correct to keep this name; they resemble the hooks of the recent Synaptids *Tæniogyrus* and *Scoliodota* to a rather striking degree, but differ from these latter in having the one end closed completely so as to form an eye, whereas in the recent forms it is only inrolled but not quite closed. But it cannot be doubted that the *Ancistrum* spicules belong to Synaptids closely related to the said recent genera. Such spicules are known already from the Lower Carboniferous of Scotland.

It is a curious fact that no anchors or anchor plates of Synaptids are found in the material sent me by Mr. Feifel. This does not mean that the genus *Synapta* (sensu latiori) had not yet appeared in the Jurassic period, since an anchor of a *Synapta* from the Jurassic Scyphia limestone of Streitburg was figured by v. Münster in his "Beiträge zur Petrefactenkunde" 6. 1843. Taf. IV. 9. On the other hand,

the fact that no wheels referable to the genus *Protocaudina* of Croneis are found either may indicate that this genus, so richly developed in the Carboniferous, became extinct before the end of the Paleozoic; at least, Spandel's *Chiridota geinitziana* from the Zechstein formation, designated by Croneis & McCormack (Op. cit. p. 132) as *Protocaudina geinitziana*, certainly does not rightly belong within that genus.

Pl. III. Figs. 4—5. **Spicules of Chiridota** (?). Malm *α*. Reichenbach.

These large spicules, more than 1 mm long, so strikingly recall those of *Chiridota Stuhlmanni* Lampert figured p. 677, fig. 12.6 of S. G. Heding's paper "Über die Synaptiden d. Zoologischen Museums Hamburg" (Zool. Jahrb. Syst. Bd. 51. 1931) that there can scarcely be any doubt that they must likewise belong to the genus *Chiridota*, together with the wheels Pl. IV. 1—2[1].

Pl. III. Figs. 6—8. **Spicules of Holothurians.** Lias *ζ*. Heiningen.

The spicules here figured recall very much the rods from the tentacles of *Stichopus* (cf. e. g. fig. 22. f., p. 329, of my Echinoderms of New Zealand and the Auckland-Campbell Isl. III—V. Papers from Dr. Th. Mortensen's Pacific Expedition. XXIX. Vid. Medd. Dansk Naturhist. Foren. Bd. 79. 1925). Also the C-shaped bodies figured by Croneis & McCormack, Op. cit. Pl. 21. figs. 24—28, are no doubt of the same nature.

Pl. III. Fig. 3. **Spicule of Holothurian.** Lias *δ*, Enzenhart.

Nothing very like this spicule is known from recent

[1] I am indebted to Mr. Heding for calling my attention to these spicules of *Chiridota Stuhlmanni*, figured by him, as also to the "spectacle"-like spicules of *Cucumaria frauenfeldi* mentioned below.

Holothurians. It is not even possible to say with certainty whether it is from an Aspidochirote or a Dendrochirote Holothurian or perhaps from a Molpadid. We can only say with a fair degree of certainty that it is a Holothurian spicule, and as it is easily recognizable, it is well worth figuring. It is clearly the same as ISSLER's Tab. VII. fig. 379, which is only simply pentagonal, not so distinctly five-radiate as the one here figured; I have, however, also specimens which are simply pentagonal. They are rather thick and complicately built, as shown in the figure.

It is scarcely to be doubted that the form figured by TERQUEM & BERTHELIN (Etude microscopique des Marnes du Lias. Mém. Soc. Géol. France. Pl. VIII. a—b) under the name of "*Ophiotrix*" is in reality the same as the Holothurian spicule here mentioned.

Pl. III. Figs. 16—19. **Spicules of Holothurian.** Lias *α*, Sulzgrieser Kelter.

Numerous finely preserved specimens of this type of spicules are found in the said deposit. They are evidently identical with those figured in ISSLER's Tab. VII. figs. 359—360. It is impossible to say whether they belong to a Dendrochirote or an Aspidochirote Holothurian; they may perhaps be a primitive sort of buttons of a *Holothuria* (they recall to a no small degree the buttons of the tubefeet in some *Holothuria* species).

Pl.IV. Fig.3. **Spicule of Holothurian.** Lias *β*. Ober-Esslingen.

This spicule so closely resembles that of *Staurocucumis Liouvillei* (Vaney) figured in fig. 7. c, p. 376 of Sv. EKMAN's "Holothurien d. deutschen Südpolar-Expedition" (1927) that it seems rather safe to say it must belong to a closely related Dendrochirote Holothurian. Only two specimens are found in the material sent me.

Pl. IV. Figs. 4—5. **Spicules of Dendrochirote Holothurians.** Lias β, Ober-Esslingen.

There is no doubt that the two types of spicules here represented both belong to some Dendrochirote Holothurians; as to the genus to which they belong nothing can be said with certainty — but they may both very well be of the genus *Cucumaria*. Fig. 4 to some degree recalls Issler's figures 369 and 370, but it is not at all certain that it is the same species, I am even not at all convinced that these figures are really Holothurian spicules and not perhaps rather Foraminifera; at least, I have been unable to convince myself of the Holothurian structure of some specimens apparently identical with Issler's figures.

The very simple spicule represented in Pl. IV. 5 recalls the more or less fragmentary spicules from the Carboniferous of the United States represented under the name of *Ancistrum*? on Pl. 20.29—45 of Croneis & McCormack's paper. That the present form from the Lias of Germany is not identical with any of those from the Carboniferous of the United States is clear, but, on the whole, these simple spicules are so little characteristic that it is quite hopeless to try to distinguish species of them. That they have nothing to do with the Synaptid *Ancistrum* is certain; there can be no doubt that all these simple plates belong to Dendrochirote Holothurians.

In the material at hand there are several specimens, often fragmentary, which resemble the one represented in fig. 5, more or less. Some of them are not simple as the one figured, but more or less complicate, cushion shaped. But as they will hardly be recognizable with any reasonable degree of certainty, I have not thought it desirable to figure all these various forms.

Pl. IV. Figs. 6—9. **Spicules of Holothurians.** Lias β, Ober-Esslingen; figs. 6—7 also from Lias δ, Enzenhart.

The two types of spicules here represented both very probably belong to Dendrochirote Holothurians. Several forms of spicules recalling figs. 6—7 are known from recent Holothurians, e. g. the one figured by Sv. Ekman in his Report on the Holothurians of the Swedish Antarctic Expedition, 1925, p. 76, fig. 15. c (of *Cucumaria crocea* Lesson). Figs. 8—9 are to some degree recalled by the

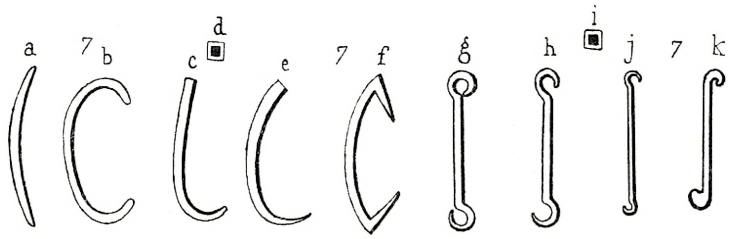

Fig. 1. *Uncinulina polymorpha* Terquem. From Terquem. Recherches sur les Foraminifères du Lias. (Pl. VII. fig. 7. a—k.). × 10.

curious spicules of *Phyllophorus incompertus* Théel ("Challenger" Holothurians. II. Pl. V. fig. 8. b). But an almost identical form of "spectacle"-like spicules is found in *Cucumaria frauenfeldi* Ludwig (cf. H. L. Clark. Echinoderm Fauna of S. Africa. Ann. S. African Mus. XIII. 1923. p. 414). There can thus be no doubt that this remarkable spicule likewise belongs to a *Cucumaria* related to the said species.

Both the forms of spicules here represented are designated by Issler as *Uncinulina polymorpha* Terquem, and also the form represented in Pl. III. figs. 16—19, as well as a couple of *Ancistrum* spicules are likewise referred to *Uncinulina polymorpha* (Issler. Tab. VII. figs. 346—362). It is perfectly clear that the forms here mentioned represent four

different species, so it is inadmissible to designate them all by the same name, *Uncinulina polymorpha* — it is even doubtful whether any of them are identical with any of the forms represented under the name *Uncinulina polymorpha* by Terquem (Recherches sur les Foraminifères du Lias. II. Mém. Ac. Imp. Metz. XLII. 1862. Pl. V. fig. 7. a—k.). Of these forms, reproduced here in fig. 1, the figs. g—k recall my Pl. IV. figs. 8—9; but it is very unlikely that Terquem would have represented them with the "eyes" not closed, so I do not think they could be identical. If we want to keep the name *Uncinulina*, a type should be selected. The figs. a—e are so generalized forms that they cannot very well come into consideration as types, and with regard to figs. g—k some uncertainty remains, whether they are quite accurately drawn. Remains fig. f, which is a very unusual, but apparently highly characteristic form. Accordingly I select this as the lecto-holotype[1].

Pl. IV. Fig. 10. **Spicule of Holothurian.** Lias β. Ober-Esslingen.

Of this highly characteristic spicule, of which a couple of specimens are found in the material at hand, it cannot be said with certainty whether it belongs to an Aspidochirote or a Dendrochirote Holothurian. It has some resemblance to the quadriradiate spicules of *Staurocucumis*, but not so much that it could reasonably be referred to that genus. Anyhow, it is very easily recognisable and therefore may prove of importance, also from a stratigraphical point of view.

[1] I am greatly indebted to Dr. Leslie Bairstow, British Museum, Natural History, for information about Terquem's work, to which I had no access, and particularly for copies of the original figures of *Uncinulina polymorpha*.

The central prominence is a simple spire, about half the length of an arm.

Pl. IV. Fig. 11. **Spicule of Holothurian** (*Cucumaria Feifeli* n. sp.). Lias ζ, Heiningen.

This very unusual type of spicule I beg to dedicate to the collector of all this marvellous material, naming it *Cucumaria Feifeli*. There is scarcely any doubt that it is from a Dendrochirote; whether strictly of the genus *Cucumaria* is, of course, not quite so certain, nothing very similar being known from any recent form. As there is only a single specimen at hand, it is uncertain whether it is always threeradiate. It has much resemblance to such arenaceous Foraminifera as *Rhabdammina* or *Astrorhiza*, but the microscopical structure proves definitely that it is a Holothurian spicule. It makes the impression of being hollow; at least it is rather thick, not flat.

Pl. IV. Fig. 12. **Spicule of Holothurian** (*Cucumaria proteus* n. sp.). Lias ζ, Heiningen.

This again is a very unusual type of spicule, also represented by only a single specimen in the material at hand. The peculiar irregular shape, recalling an Amoeba (— it is perfectly preserved, the irregular outline not due to fracture of any kind —) seems to show that it must be referred to the genus *Cucumaria*.

It may still be added that of Issler's figures of "Echinodermenreste" I have seen nothing like figs. 380 and 381; they look much like sections of spines of sea-urchins, and I think it very doubtful whether they are really wheels of Holothurians. Figs. 382, 386, and 387 are scarcely Echinoderm remains, as also holds good of figs. 367—368.

Fig. 371 probably is a Holothurian spicule, perhaps identical with my Pl. III. figs. 4—5 and fig. 372 no doubt is an incomplete *Ancistrum*-spicule (cf. Pl. III. fig. 9). Fig. 373 is a spicule of a Dendrochirote of the type mentioned under Pl. IV. fig. 5. Finally figs. 383—385 are either braces from the lantern of Echinoids, as suggested by Croneis & McCormack (Op. cit. p. 128), or vertebræ of Ophiuroids; particularly 385 seems rather certainly to be an Ophiuroid vertebra.

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Plate I.

Figs. 1—5. Terminalia of Asteroids. 1—2. From Malm δ. Bosler; dorsal (1) and ventral side (2). 3—4. From Malm γ. Beuren; dorsal (3) and ventral side (4). 5. From Lias α. Uhlbach; dorsal side. 1—4 × 35; 5 × 30.

— 6. Part of sucking disk of a Regular Echinoid. Malm ζ. Sotzenhausen. × 80.

— 7. Valve of rostrate pedicellaria of an Irregular Echinoid; from the inside. Malm α. Reichenbach. × 120.

— 8—9. Tridentate pedicellaria of a Regular Echinoid, apparently allied to *Pelanechinus*. Malm α. Lochengründle. 8. Distal part of the blade in side view; 9. distal part of the blade, and the basal part, from the inside. × 35.

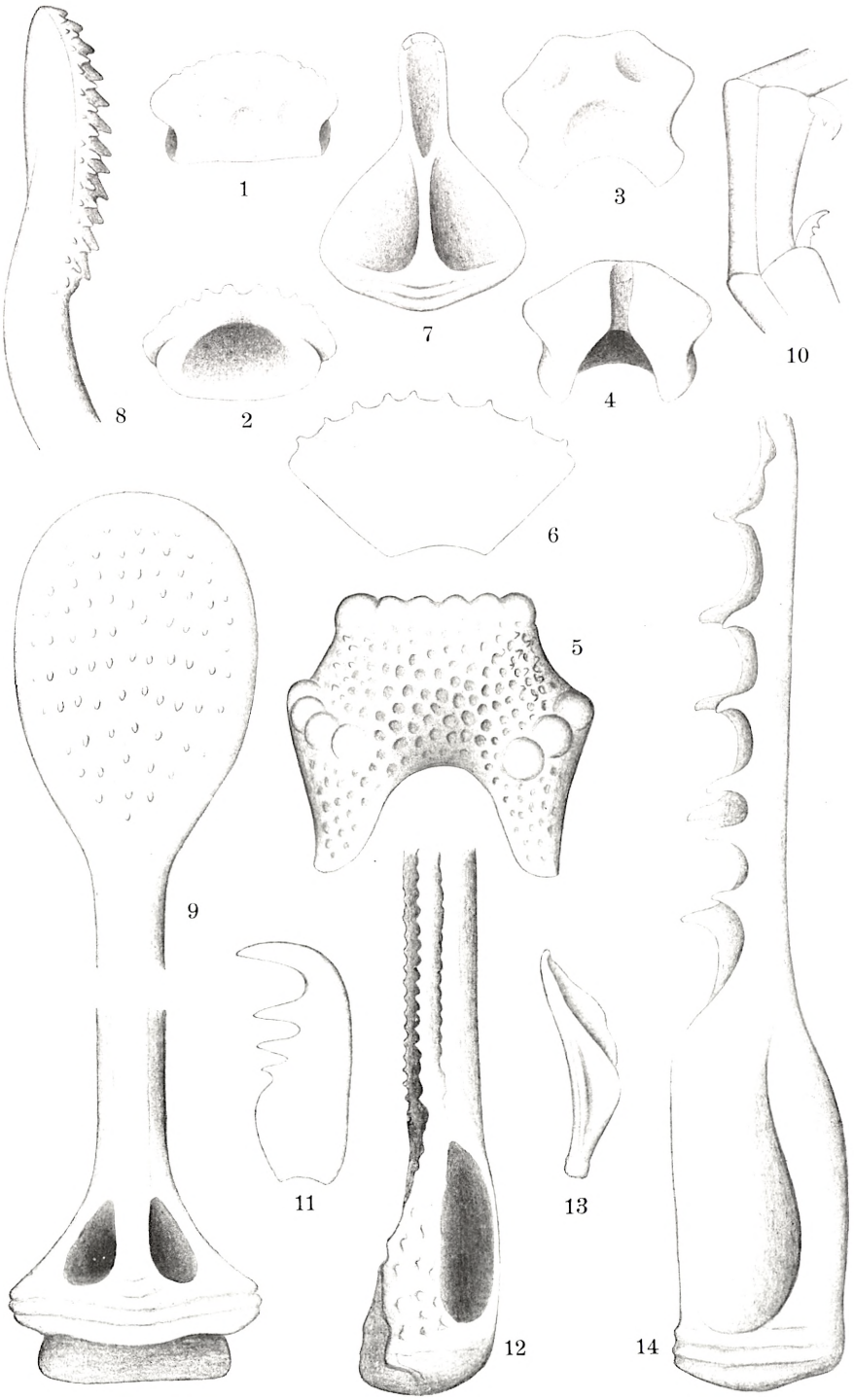
— 10. Part of arm of an Ophiuroid, with hook-shaped arm-spines preserved in situ. Malm ζ. Sotzenhausen. × 35.

— 11. Hook-shaped arm-spine of Ophiuroid, probably the same as fig. 10. Malm ζ. Gerhausen. × 95.

— 12. Basal part of valve of tridentate pedicellaria of a Cidarid, probably *Rhabdocidaris*; in side view. Malm ζ. Sotzenhausen. × 30.

— 13. Periproctal spine of a Salenid. Malm ζ. Sotzenhausen. × 35.

— 14. Valve of tridentate pedicellaria, in side view. The lower part of the base reconstructed on free hand; the point of the valve lacking. Malm ζ. Sotzenhausen. × 80.



Th. M. Del. E.H.P.

Plate II.

Figs. 1—2. Valve of globiferous pedicellaria of *Hemipedina*, from the inside (1) and in side view (2). Malm ζ. Sotzenhausen. × 80.

— 3. Spine of an Irregular Echinoid. Malm α. Reichenbach. × 230.

— 4. Ophicephalous pedicellaria, head and stalk, of an Irregular Echinoid(?). Malm α. Reichenbach. × 120.

— 5. Valve of the same sort of ophicephalous pedicellaria as fig. 4, from the inside. × 230.

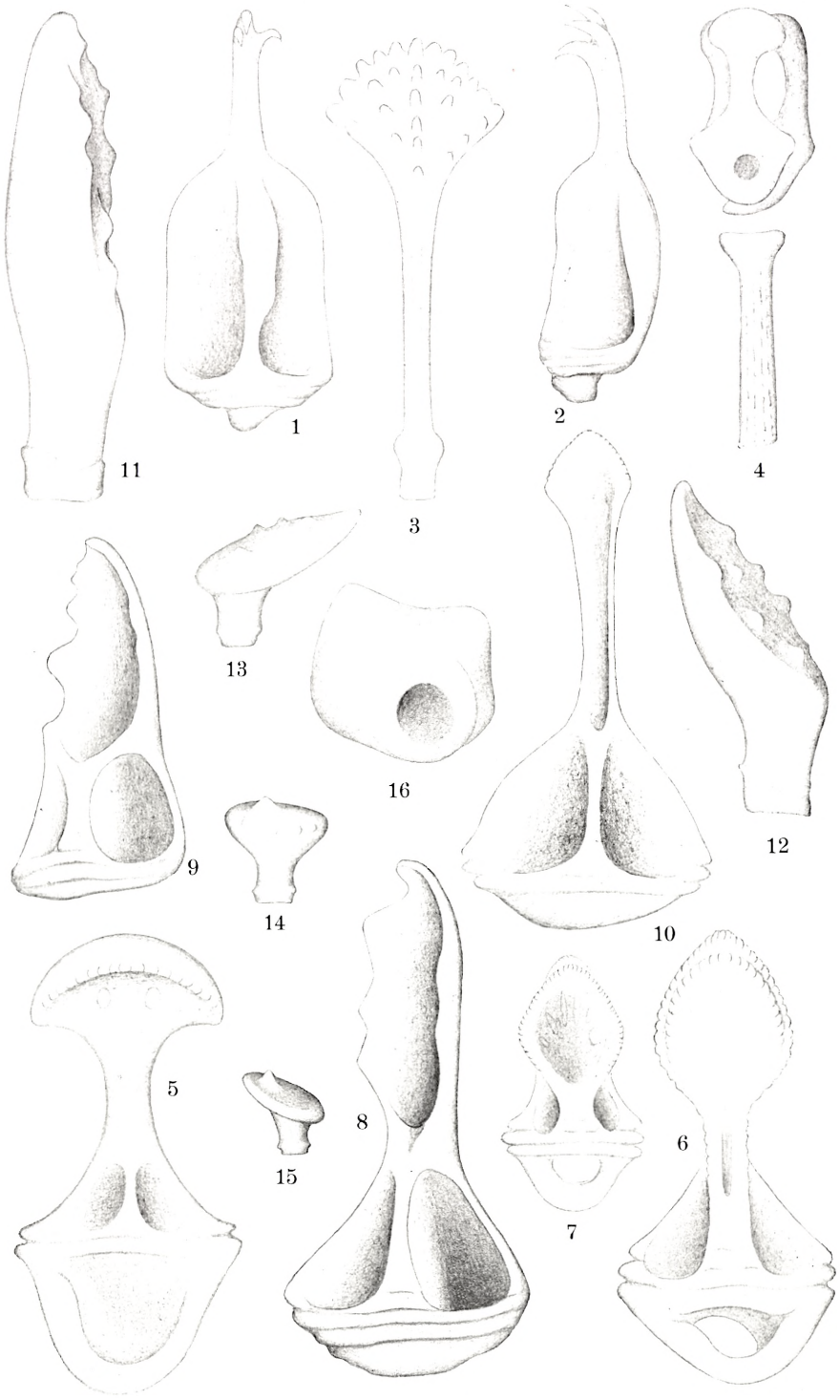
— 6. Valve of ophicephalous pedicellaria of *Hemipedina*, from the inside. Malm ζ. Sotzenhausen. × 95.

— 7. Valve of ophicephalous pedicellaria from Lias β, Ober-Esslingen. × 200.

— 8—9. Valves of tridentate pedicellariæ of Echinothurids, in half side view. Malm ζ. Sotzenhausen. × 80.

— 10. Valve of rostrate pedicellaria of *Holectypus*(?); from the inside. Malm α. Reichenbach. × 230.

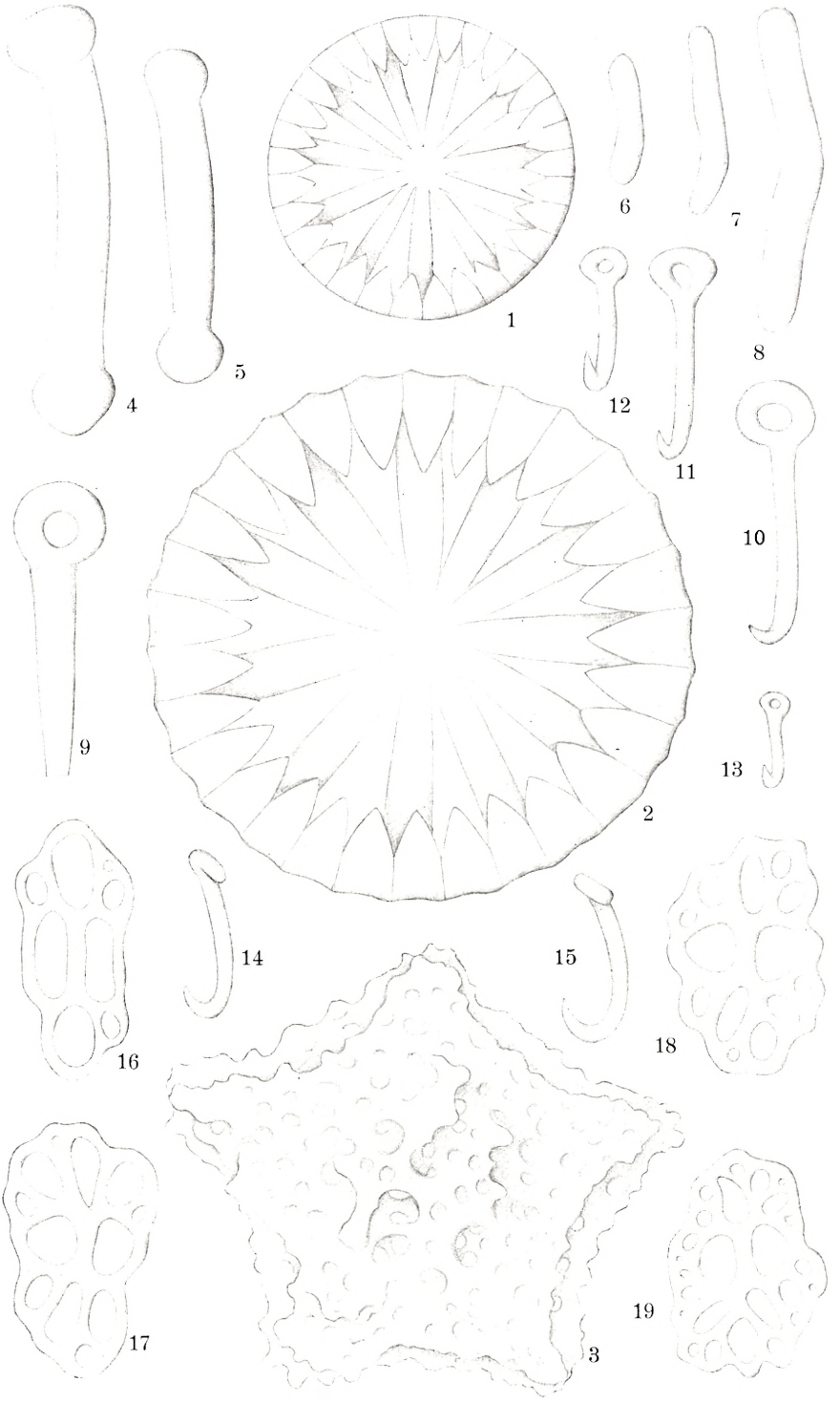
— 11—16. Spines of *Anaulocidaris tuberculata* n. sp. Fig. 16 seen from the under-side. Malm ζ. Sotzenhausen. × 22.



Th. M. Del. E.H.P.

Plate III.

Figs. 1—2. Wheels of *Myriotrochus*. Malm *α*. Reichenbach. × 200.
— 3. Spicule of Holothurian. Lias *δ*. Enzenhart. × 200.
— 4—5. Spicules of *Chiridota* (?). Malm *α*. Reichenbach. × 45.
— 6—8. Spicules from tentacles of Holothurians. Lias *ζ*, Heiningen. × 45.
— 9—15. Spicules of Synaptid (*Ancistrum*). 9—14. Dogger *ε*, Erkenberg; 15. Malm *α*. Reichenbach. × 45.
— 16—19. Spicules of Holothurian. Lias *α*. Sulzgrieser Kelter. × 80.



Th. M. Del. E.H.P.

Plate IV.

Figs. 1—2. Wheels of *Chiridota*. 1. Lias β. Ober-Esslingen; 2. Lias δ, Enzenhart. × 200.

— 3. Spicule of Dendrochirote Holothurian. Lias β. Ober-Esslingen. × 200.

— 4—5. Spicules of Dendrochirote Holothurians. Lias β. Ober-Esslingen. 4. × 180. 5. × 200.

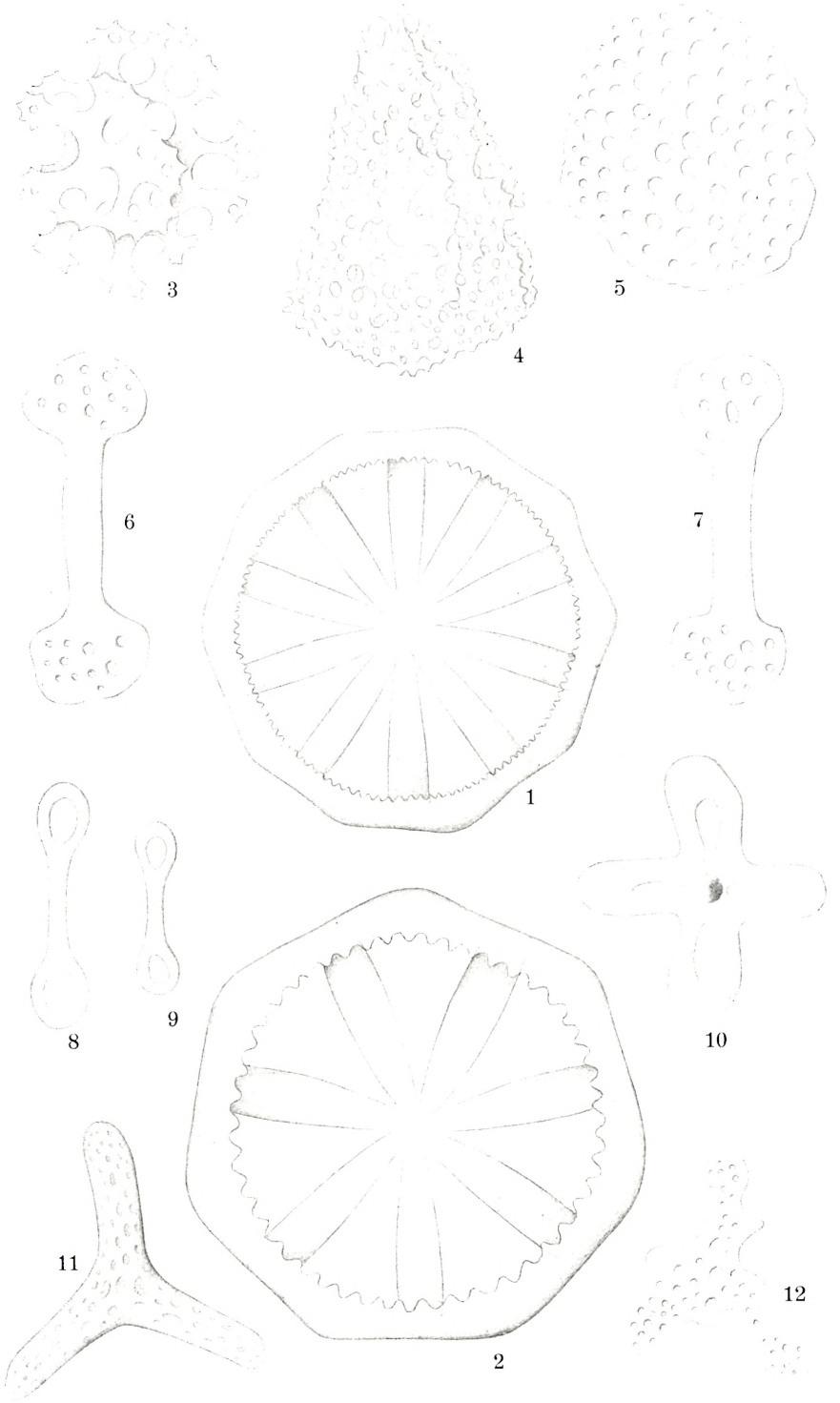
— 6—7. Spicules of Dendrochirote Holothurian. Lias δ. Enzenhart. × 90.

— 8—9. Spicules of Dendrochirote Holothurian. Lias β. Ober-Esslingen. × 80.

— 10. Spicule of Holothurian. Lias β. Ober-Esslingen. × 180.

— 11. Spicule of Dendrochirote Holothurian, *Cucumaria Feifeli* n. sp. Lias ζ. Heiningen. × 90.

— 12. Spicule of Dendrochirote Holothurian, *Cucumaria proteus* n. sp. Lias ζ. Heiningen. × 90.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BY

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PREFACE

The main purpose of this paper is to elucidate the benthic distribution of the *Corethra* larvae in a Danish lake, Esrom Lake; the relation between their benthic and their limnetic behaviour; and the cause of their diurnal migration. A number of other biological particulars concerning the *Corethra* are added. These studies on the *Corethra* of Esrom Lake were carried out simultaneously with a quantitative investigation of the rest of the bottom fauna of that lake. For purposes of comparison it proved convenient to make a few observations on *Corethra* larvae in other localities as well, namely in Frederiksborg Castle Lake and in Sorte Dam near Hillerød, and to carry out some experiments on the phototaxis of the larvae. The studies on the *Corethra* having thus become comparatively comprehensive and independent, they are here published separately, while the remaining investigations from Esrom Lake will appear later.

The work was carried out at the Freshwater Biological Laboratory of the University of Copenhagen, to whose chief, Professor C. WESENBERG-LUND, I wish to express my cordial thanks for his constant interest in my work and for the helpful way in which he has sought to further it. For financial aid granted by the Carlsberg Foundation I beg to offer my respectful thanks. Finally I likewise tender respectful thanks to the Rask-Ørsted Foundation for a grant of funds which has rendered possible the translation into English of this paper by Miss ANNIE I. FAUSBØLL, whom I thank for her careful work.

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The Species in Esrom Lake.

According to EDWARDS' systematic revision of the *Culicidae* (1932) the genus *Corethra* belongs to the subfamily *Chaoborinae*, and among others comprises the following species:

Chaoborus Lichtenstein (= *Corethra* Meigen):

Subgenus *Chaoborus* s. str.

obscuripes van der Wulp
crystallinus de Geer
Syn. *fusca* Staeger
Syn. *plumicornis* Fabricius
flavicans Meigen

Subgenus *Schadonophasma* Dyar & Shannon

nyblaei Zetterstedt

Subgenus *Sayomyia* Coquillett

pallidus Fabricius

Of these 5 species *S. nyblaei* is Northern, whereas the other 4 are natives of Central Europe. While the imagines of these species have been determinable after MARTINI (1931) and others, it has only lately been possible, by means of a paper by FRITZ PEUS (1934), to identify the larvae belonging to them. Considering the important part played by the *Corethra* larvae in limnological literature, and not least on account of the biological difference which — as will be

shown in the sequel -- exists between larvae from different localities, Peus deserves great credit for rendering possible a determination of the species of the larvae.

Already long before Peus, Wesenberg-Lund (1914, p. 13) had described morphological and ecological differences between two *Corethra* larvae, of which the one was found in ponds, the other in large lakes. Peus points out that even though it was not then possible to establish that the corresponding imagines were of different species Wesenberg-Lund's data for the two kinds of larvae are so excellent that his pond form is now seen to be *C. obscuripes* and his lake form *C. crystallinus*. These species, then, are recorded as larvae from Danish localities.

We shall now concider the morphological characters of the larvae in Esrom Lake, partly in order to determine the species, partly to ascertain whether this lake form shows deviating characters. Since the *Corethra* larvae live in localities from less than 1 to more than about 20 metres' depth and in lakes of a very different nature, it is reasonable to suppose that there may be some variation in them.

For distinguishing between the species of the *Corethra* larvae Peus (1934, p. 643) uses the mandibles and the so-called "knifeblades" ("Messerhaare"), i. e. hairshaped or flat appendages attached to the underside of the head in front of the labrum. Meinert, too, (1886, p. 401) as well as Wesenberg-Lund (1914, p. 17) has used the form of the knifeblades for the characterisation of the larvae.

In the larvae from Esrom Lake the knifeblades are flat, leafshaped, fairly slender, and provided with teeth on the anterior side (fig. 1). Knifeblades of this shape show that the larvae belong either to *crystallinus* or to *flavicans*, in which the knifeblades, according to Peus, resemble each

other both in their fundamental form and in their range of variation.

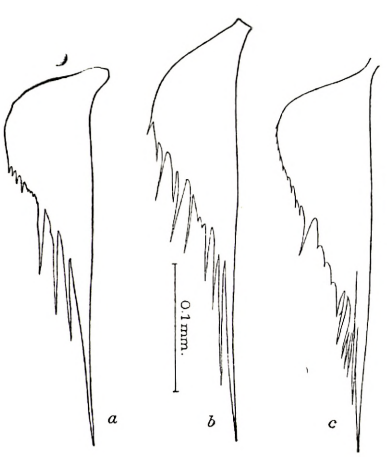
The mandibles are armed with strong teeth, 3 main teeth and 1 subordinate tooth, as also with a group of strong bristles, forming a fan, which can be unfolded or folded (fig. 2). In the larvae from Esrom Lake the subordinate tooth, which is No. 2 from behind, is placed between the 1st and 3rd main tooth, the subordinate tooth being displaced towards the 3rd tooth, though not actually issuing from it. In recently caught individuals the teeth of the mandibles are a rather deep black in their outermost third; the colour fades in preparations. The number of bristles in the fan were found to be 11 or 12; variation may occur in the same individual; the number may, for instance, be 11 on one side, 12 on the other.

Owing to the fact that the subordinate tooth is placed between the 1st and 3rd tooth, and not so much displaced as to be on the side of the 3rd tooth, the *Corethra* larva from Esrom Lake must, according to PEUS' list of determinations (p. 646), be referred to *C. flavicans*. It deviates from the typical *flavicans*, which has about 15 bristles on the mandibles, in having the above-mentioned 11—12 only, and thus comes near to *crystallinus*, which has about 10. In respect of the rather dark colour of the mandibular teeth also, the Esrom Lake form occupies an intermediate position between *crystallinus*, whose teeth are very dark, and the typical *flavicans*, whose teeth are dark at the points only.

Since the *Corethra* larvae from Esrom Lake deviate somewhat from the typical *flavicans* larvae, it will be of interest to obtain confirmation of the correctness of the determination by an examination of the pupa and the imago.

The pupae of *C. crystallinus* and of *C. flavicans* have the

same form of respiratory tubes. The difference between the two pupae is in the caudal swimming fan (Peus, 1934, p. 656). In the form from Esrom Lake the swimming fan



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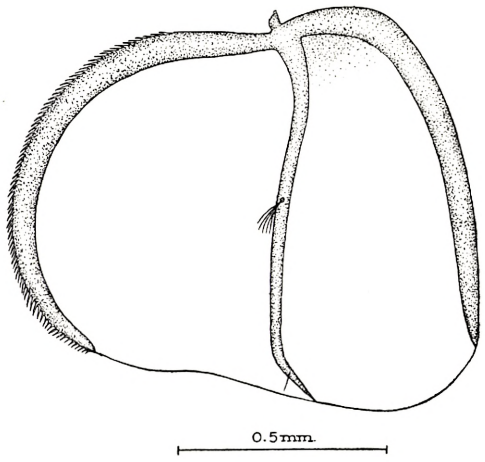

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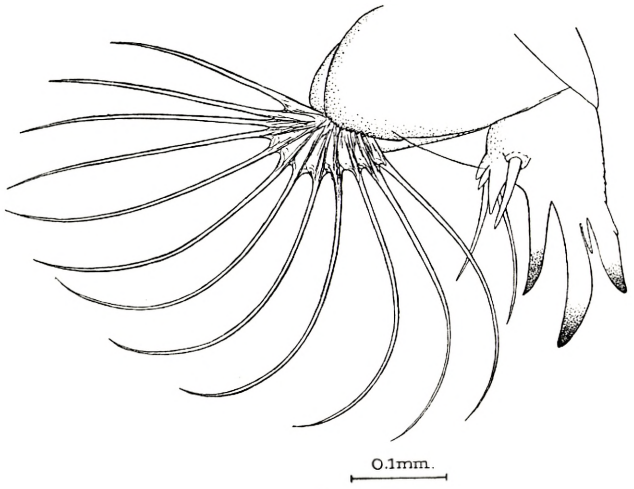

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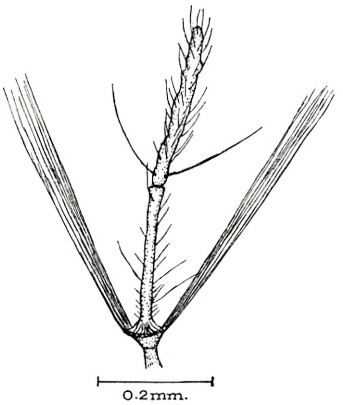
Fig. 1. Various forms of the knifeblade-shaped appendage in the larva of *Corethra flavicans* from Esrom Lake. Fig. 2. Mandible of a larva of *Corethra flavicans* from Esrom Lake. Fig. 3. Caudal leaf of pupa of *Corethra flavicans* from Esrom Lake.

has the main characters of *flavicans* (fig. 3), the outer and the inner rib being greatly thickened, and distinctly thicker than the slender median rib. The outer rib (to the right in the figure) is somewhat shortened so that the membrane



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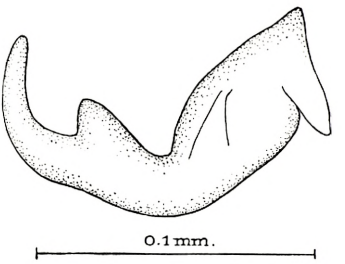

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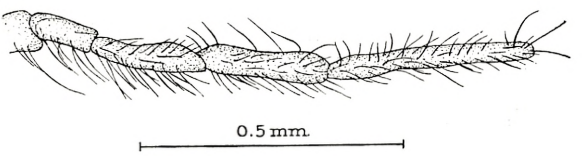

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Fig. 4. Palpus of *Corethra flavicans* from Esrom Lake. Fig. 5. The outermost joint of the antenna of *Corethra flavicans* ♂ from Esrom Lake. Fig. 6. Genital sclerite of *Corethra flavicans* ♂ from Esrom Lake.

belonging to it projects somewhat beyond its tip. The outer rib is always smooth at the distal end. But in a couple of less important characters the pupa from Esrom Lake deviates from the *flavicans* type: the group of bristles on the median rib seems as a rule to be placed somewhat proximally to the middle (the typical position is "medial oder etwas distal"). Further, the inner rib is provided with teeth

in the outermost three-fourths of its length, whereas the typical *flavicans*, according to Peus' figure, has teeth on the outermost half only. The principal characters in the pupae, however, show plainly enough — as in the larvae — that the species in Esrom Lake must be *flavicans*. If a great number of pupae from the large heaps washed up on shore are examined, it is seen that they all belong to this species.

If, finally, the imagines from Esrom Lake are examined, the result obtained is confirmed. The length of the last segment of the palpus is about 85 % of the length of the third and fourth segments (fig. 4), a character which *C. crystallinus* and *C. flavicans* have in common. It further appears that the antenna in the male (fig. 5) has a last segment that is almost just as long as the last but one, but thicker than the latter. Further, the genital sclerite of the male is almost devoid of a head (fig. 6). These characters show conclusively that the species must be *C. flavicans*. (Compare the description in Martini, 1931, p. 56, and his figure 77.) The determination of the imagines was made not only on specimens taken by the lake, but also on animals hatched in aquaria from larvae taken from the lake, and Professor Martini and Dr. Eckstein have kindly confirmed it.

Hence Peus' view (1934, p. 642) that the lake form found by Wesenberg-Lund must be referred to *C. crystallinus* does not hold good in all cases, and at any rate not for the species in Esrom Lake.

The Species in Frederiksborg Castle Lake and in Sorte Dam.

In order to throw light on the biology of the *Corethra* larvae from Esrom Lake, certain comparisons were made with the biological characters of larvae from localities with shallower water, as Frederiksborg Castle Lake and Sorte Dam near Hillerød. For the same reason morphological characters, too, were examined, and the forms of the two last-mentioned localities determined as to species with the following result:

The species from Frederiksborg Castle Lake must also be referred to *Corethra flavicans*, because the knifeblades in the larva are of the typical form; the mandibular teeth of a light colour; and especially because the subordinate mandibular tooth is placed between the first and the third tooth, and the caudal swimming fan of the pupa has the ribs typical of *flavicans*. But as in the species from Esrom Lake, so also in the species of Frederiksborg Castle Lake, there is not the typical number of mandibular bristles — about 15 (according to PEUS 1934, p. 646) — but 10—12 only. The other characters mentioned are, however, quite sufficient to show that the species from Frederiksborg Castle Lake is *C. flavicans*. An examination of the imagines confirms this.

In respect of PEUS' remark in the above-cited passage that the variability of the number of bristles seems to be very small, and that so far he has found 15 bristles only in *C. flavicans*, it must be pointed out that the observations from Esrom Lake and Frederiksborg Castle Lake render necessary a modification of both these statements. We know now that in *flavicans* the number of bristles may vary from

10—12, and according to Peus it may also be 15; the variability in this respect must therefore be said to be rather considerable. This is worth noting, because Peus thinks it possible that the number of the bristles may be of biological importance with respect to the nutrition. For the group of bristles on each mandible forms a kind of basket which prevents prey carried towards the mouth from escaping sideways. He thinks it possible that the *crystallinus* larva with 10 mandibular bristles prefers larger organisms as nourishment, and that these may be retained in the coarser meshes of the basket. The remaining species — *pallidus* with 13, *obscuripes* with 14—15, and *flavicans* with 15 bristles — should then prefer organisms of smaller average size as food. To this we must reply that since the number of bristles in *flavicans* may vary as described above, it will not be possible to point out such a nutrimental biological

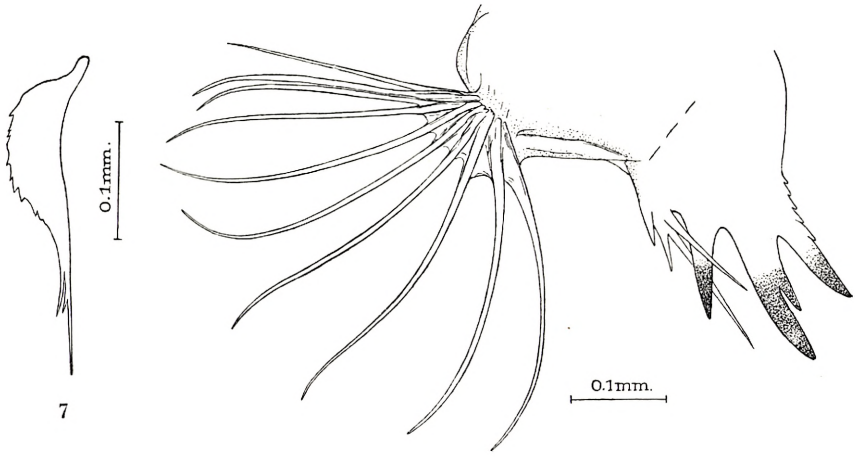

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Fig. 7. Knifeblade-shaped appendage of *Corethra crystallinus* from Sorte Dam. Fig. 8. Mandible of *Corethra crystallinus* from Sorte Dam.

difference between all populations of this species and *crystallinus*, but those that have 10—11 bristles must approximate to *crystallinus* in this respect. Within the species *C. flavicans*, on the other hand, among populations with a varying number of bristles, a biological difference may be

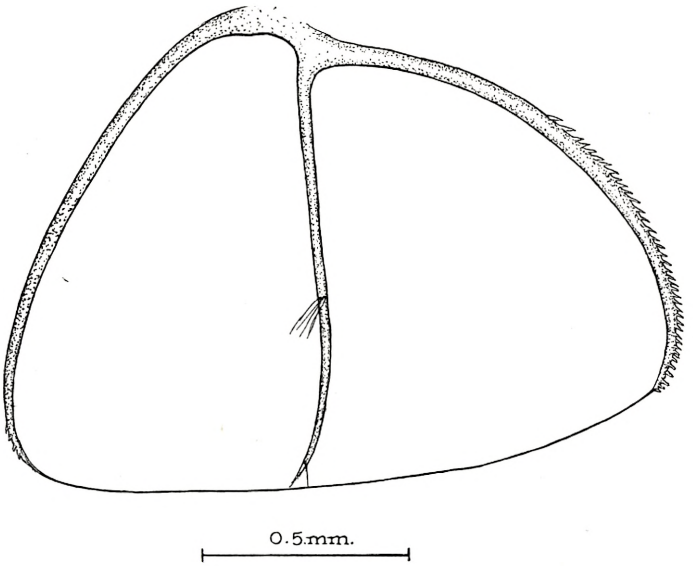
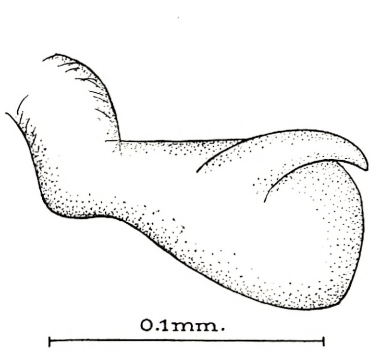


Fig. 9. Caudal leaf of pupa of *Corethra crystallinus* from Sorte Dam

conceived to be present similar to that which PEUS thinks likely between the different species.

In Sorte Dam the stock of *Corethra* larvae turned out to belong to 2 species. The one, which seems to be the largest and most yellowish, has knifeblades (fig. 7) which are slender, leaf-shaped and provided with teeth on the anterior side; occasionally the teeth are poorly developed. The mandibles (fig. 8) are characteristic in having the subordinate tooth placed right on the side of the largest tooth. The teeth are very dark over about two-thirds of their length. In the fan-shaped group of bristles the number of

bristles is usually 10; occasionally 11 were found, and once 12 rays. After the metamorphosis, the pupa proved to have a caudal swimming fan (fig. 9) the outermost rib of which (to the left in the figure) is just as thin as the median one and almost as long; it is provided with faintly defined teeth at the distal end. The inner, strongly curved rib armed with teeth is only slightly thicker than the others. The group



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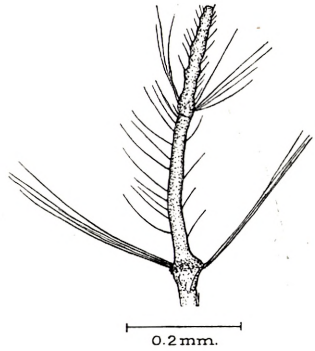

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Fig. 10. Genital sclerite of *Corethra crystallinus* ♂ from Sorte Dam.
Fig. 11. The outermost joint of the antenna of *Corethra crystallinus* ♂ from Sorte Dam.

of bristles on the median rib is placed slightly distally to the median point. In the imagines the male has a genital sclerite (fig. 10) with a distinctly swollen head and a short hook arising far from the top of the head. The last segment of the antenna is about two-thirds of the length of the last segment but one (fig. 11). — All these characters show that this species is *C. crystallinus* (cp. Peus, 1934 and Martini 1931). Martini characterises the species as very variable. As an example we may state that the number of bristles, as mentioned above, is occasionally 11 or 12 instead of the typical 10.

The other species found in Sorte Dam is *C. flavicans*; it accords with PEUS' description. However, only individuals with 13—14 mandibular bristles were observed, while PEUS states about 15 to be typical. The larvae of *C. flavicans* are more transparent than the somewhat yellowish *crystallinus* larvae present at the same time. — Since *C. flavicans*, as previously mentioned, was found both in Esrom Lake which is fairly clear and 22 m deep, in Frederiksborg Castle Lake which is $3^1/_2$ m deep, strongly eutrophic, and rich in *Cyanophyceae*, and in Sorte Dam, which is barely 1 m deep, whose bottom is covered with leaves, and whose water is, yellowish with humous substances, the species is thus able to live in highly differing environments. This is further emphasised by a comparison with the localities in which PEUS (1934, p. 663) found it. His localities for *C. flavicans* are river forests, and meadows along the Rhine, Oder, and others rivers. He states that this species has its optimum in the inundation area of large rivers and adds that it is remarkable that the larvae occur exclusively in the periodical pools found in the inundation zone proper. These waters arise from the inundation of the river, partly directly when it overflows its banks, partly because the river at high water causes a rising of the groundwater in its vicinity, which may then come to the surface as pools. The time when the pools are filled is mostly in the spring. PEUS thinks, however, that *C. flavicans*, though having its optimum in the inundation areas of rivers, is not exclusively restricted to this environment; from findings at Drewenz See (East Prussia) he supposes that it may also occur in pools at the shores of lakes with a fluctuating water level. The Danish localities and PEUS' localities in conjunction show that *C. flavicans* has a much larger power of adaptation to the dif-

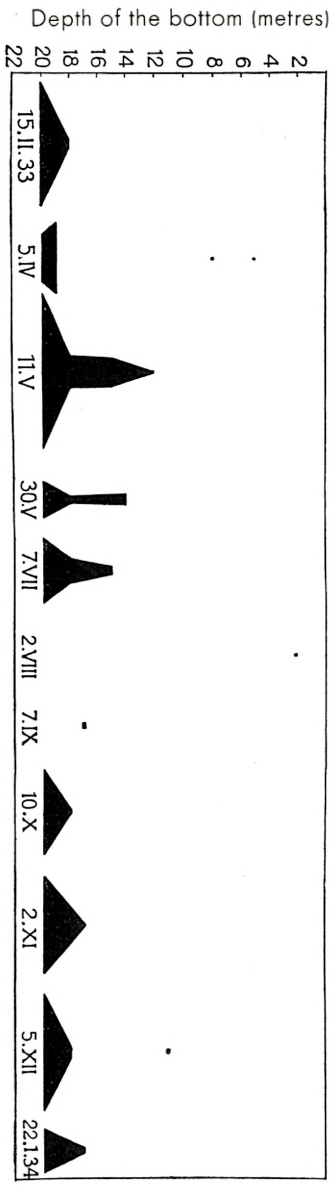

Fig. 12. Seasonal variation of the benthic distribution of *Corethra flavicans* from Esrom Lake.

ferent environments than Peus could know from his findings alone, and in accordance herewith its biological variation is very considerable.

Benthic Distribution in Esrom Lake.

The benthic distribution of the *Corethra* larvae was investigated by means of a Birge-Ekman bottom sampler. A series of samples was taken along a line extending into the lake in a direction approximately at right angles to the shore line. Along this line samples were taken at 7 stations, viz. at depths of about 2, 5, 8, 11, 14, 17 and 20 m; the samples from 20 m were taken in midlake. At each of the 7 stations, 2 samples were always taken. Altogether the sampling line was investigated on 12 occasions in the course of a little more than a year.

The distribution of *C. flavicans* along the sampling line

is very characteristic (fig. 12). It appears that, apart from the swarming period in the middle of the summer, it always occurs in quantity at a depth of 20 m, the average number at that depth being about 1290 per sq. m. This very considerable number on the deep bottom of the lake is reduced very rapidly as we pass towards land; at the foot of the slope of the shore, in the 16—18 m zone, only few individuals are found, and at 12—15 m the species is rarely met with, or at any rate in very small numbers only. In still shallower water it is as a rule absent. On a few occasions a single individual was found in shallow water, for instance on the 5/IV 33. Since, as shown in the figure, it occurs so rarely, the animals found there have possibly been carried away by the current from their normal area of distribution.

The benthic distribution of the larvae of *Corethra flavicans* in Esrom Lake seems to be very peculiar, when considered in conjunction with the fact that the *Corethra* larvae may live even in very shallow-watered localities, and particularly when it is noted that the very same species which was found in Esrom Lake occurred in quantity in Frederiksborg Castle Lake which is fully 3 m deep, Sorte Dam which is barely 1 m deep, and in pools in the inundation area of rivers. An indication towards the explanation of the peculiar benthic distribution in Esrom Lake is afforded by some bottom samples from Møllebugt at the southern end of the lake. Here, on the 23/XI 35, at a depth of 2 m, were found 4 larvae of *C. flavicans* in a double sample, and in two double samples 3 individuals at 5 m. Even though the number of individuals was not great, yet it may be said that anything like this could hardly have been found along the sampling line. The cause of this difference is probably to be found in differences in the quality of the bottom. Along

the sampling line the bottom is fairly hard in shallow water because it is sandy, and even though the content of detritus increases with the depth, it is still rather hard on most of the slope, owing to the presence of many fragments of shells. This seems to be the reason why *C. flavicans* is "uferscheu" there. In Møllebugt the bottom consists of a gyttja with coarser or finer plant remains, and in this fairly soft bottom a good deal of *Corethra* larvae can live, which is the reason why it can be found in shallow water in that part of the lake.

On the assumption that the softness of the bottom is a factor important in determining the horizontal distribution of the *Corethra* larvae, it will be more readily understood that there is such a difference in the statements regarding the depths at which the *Corethra* larvae are found. Thus Lang (1931, p. 46), in contrast with what was found along the sampling line in Esrom Lake, states that *Corethra* larvae have an extensive benthic distribution; in Lake Stråken they have been found at depths of 2,0—11,6 m. Eggleton (1931 a, p. 254) found *Corethra punctipennis* to be one of the most numerous organisms in the profundal zone, "at times in very large numbers"; frequently there is a limited number in the sublittoral zone in Douglas and Third Sister Lakes; it has not been taken in the littoral zone except when washed ashore by heavy onshore night winds. Lundbeck (1926, pp. 222 and 456) found the *Corethra* larvae in Kleiner Ukleisee at depths of from 0—4 to 12—16 m, in Ihlsee at depths of from 4—8 to over 20 m, and in Garrensee at depths of from 0—4 to over 20 m; his figures show distinctly, however, that the larvae are found in smaller numbers in shallower water where the bottom is probably harder. Lundbeck considers it probable that it is the amount of

Copepoda which determines the distribution of the *Corethra*. GUNNAR ALM (1922, p. 30) found *Corethra* larvae in the 1.5—6.0 m zone in Yxtasjön, and in the greatest quantity in the deepest places. ALM expressly emphasises this, but he likewise remarks that it is difficult to say why *Corethra* avoids the shallow-watered areas, since otherwise it often occurs in abundance in small shallow ditches. For the present no better explanation can probably be given than that the softness of the bottom at any rate has some influence on the horizontal distribution of the *Corethra* larvae in a lake. It will presumably be recognised that there is something correct in this, when the relation between the benthic and the pelagic occurrence of the *Corethra* larvae is more fully elucidated (p. 50). Probably light plays some part too. As will be shown later on, the *Corethra* larvae from Esrom Lake show a strong negative phototaxis (p. 70), and this must have the effect of making them "uferscheu".

The demonstration of negative phototaxis in the larvae of a large lake and the consequences thereof indicated above, makes it necessary to abandon LUNDBECK's view entirely (1926, p. 183). He thinks that since the *Corethra* larvae originally belong to smaller bodies of water, and thence have made their way to deep lakes, it is rather improbable that they, which previously lived in illuminated ponds, should suddenly have become photophobic. Hence he cannot explain their distribution as a flight from the light.

The average number of *Corethra* larvae found at a depth of 20 m in Esrom Lake — about 1300 per sq. m — is very considerable. Much larger numbers have, however, been found by JUDAY (1922, p. 472) in Lake Mendota. At a station with a depth of 20—23 m he found that the numbers ranged from approximately 18,000 to 30,000 individuals

in the daytime from November to April. These very high figures are even surpassed by the observations of Eggleton (1931 b, p. 364), who in Douglas Lake found populations of *Corethra* larvae of nearly 4,000 per sq. m of the bottom, and in Third Sister Lake a maximum of about 71,000 per sq. m, an enormously dense population.

Periodicity.

The main features of the periodicity of *C. flavicans* in Esrom Lake in 1933 will already appear in part from fig. 12, which shows that in the spring many larvae that had wintered were found in the bottom samples. As late as the beginning of July the number is considerable, still many of the individuals have now pupated and afford evidence that the swarming period must begin at this time, that is to say, rather late. It lasts for the greater part of July and the whole of August. The bottom samples from the 2/VIII contained no *Corethra* larvae at all, but only a pupa from the littoral zone. As late as the beginning of September only some few larvae of the new generation were found, and not until October was the new stock present in quantity in the bottom ooze and henceforward was constantly to be found in the subsequent autumn and winter samples.

Confirmation of the data as regards the swarming of the imagines and a close determination of it are afforded by observations from Esrom Lake in 1934 and 35. Combined they show the following facts. At the beginning of June larvae that have wintered and are about 10.9—11.6 mm in size occur; as yet (1/VI) they show no signs of metamorphosis and are present in quantity in the bottom ooze. At the close of the month (31/VI) a couple of pupae may be found in each bottom

sample, and many larvae now show signs of incipient metamorphosis. This proceeds rapidly. At the beginning of July the number of individuals in the bottom has greatly decreased, and nearly half of the individuals left are now pupae and the rest larvae which are about to pupate (2/VII); unlike what was previously the case, these individuals occur principally in the upper 5 centimetres of the mud, as shown by means of a stratification bottom sampler. Shortly after the first pupa-exuviae may be found washed up on the shores of the lake (7/VII), but the imagines do not as yet form large swarms. As early as the 13/VII, nearly all the individuals in the bottom samples have been transformed into pupae, and only a few are still in process of changing. Soon after (15/VII) large swarms may be observed on the shores of the lake, both over the water, near it, and, for instance, 1 kilometre from the shore. In damp or windy weather the bushes at the lakeside afford a shelter for thousands of animals. About at this time the first egg masses appear near the shore, floating at the surface of the water, especially where *Potamogeton* comes up to it. As yet (13/VII), however, the egg masses are not particularly numerous, but if the weather be favourable, they increase rapidly in number during the following days, and the imagines form large clouds. An examination of the population at the end of July 1934 showed that all the larvae were then (24/VII) transformed into pupae. In 1935 they had also been changed into pupae, and these, apart from some few exceptions, had ascended and been transformed into imagines (25/VII). During the time that followed *Corethra* could not be demonstrated in the bottom of the lake (9/VIII, 22/VIII), whereas the pupa-exuviae and imagines were very conspicuous on and by the shores of the lake. During the first half of August,

however, the swarms rapidly decreased in numbers, and soon only some few individuals were seen. The people living near the shore know this second period with swarms of gnats very well and are aware that, even if they are conspicuous, they are scarcely as dominant as the spring swarms of *Chironomus Liebeli-bathophilus*. The ratio between the numbers of the two animals in the bottom confirms this general observation.

The above-mentioned data for the hatching of *C. flavicans* in Esrom Lake fully confirm Wesenberg-Lund's observations (1914, p. 13); he has seen the same sudden hatching, the large swarms of imagines, the large quantities of pupa-exuviae, eggs etc., as I have seen, and precisely in the same weeks. Since Wesenberg-Lund's observations have been published in Danish I have taken the liberty of describing my own observations here, but for the same reason I shall later quote several of his observations in some detail.

When the larvae of *C. flavicans* leave the bottom of Esrom Lake in the latter half of July, the oxygen conditions at the bottom of the lake are growing unfavourable. In the water over the bottom there are then about 2.5 c. c. of O_2 per litre, and the oxygen content decreases rapidly in the ensuing time. In the bottom itself it must be still less. At the same time the temperature of the bottom water has risen to about 8°, and the respiration conditions are thus very nearly the worst in the year. The large larvae escape life in this environment, being hatched at that very time. It seems natural to ask, therefore, whether the hatching precisely at this point is an ecological necessity for the species, or at any rate an advantage. It is hardly necessary for the species to leave the bottom owing to lack of oxygen, for, as will be shown later on, the young *Corethra* larvae of the next generation

appear in the bottom about the 1st of September, at which time there are still poorer respiration conditions than when the former generation left the bottom. This does not seem to indicate that the *Corethra* larvae are especially susceptible to slight amounts of O_2, hence it is hardly a necessity for the large larvae to leave the bottom owing to the lack of O_2. Possibly, however, it is a biological advantage to them for the metamorphosis from larvae to pupae to take place while the amount of O_2 present in the hypolimnion is as yet not too small. — The supposition that the *Corethra* larvae are not highly susceptible to fluctuations in the amount of O_2 is also supported by observations on their vertical wanderings in Esrom Lake in the course of the twenty-four hours. It appears from these that the larvae wander from the hypoliminion poor in O_2 to the epilimnion rich in O_2 and back again. Thus the larger or smaller amount of the oxygen does not seem to be of any great importance for the *Corethra* larvae.

The literature has several statements, which do not entirely agree, about the relation of the *Corethra* larvae to the amount of O_2 in the water. Frankenberg (1915, p. 533) has shown that *Corethra* larvae can live for several weeks in boiled water. He states that the oxygen requirement of the *Corethra* larvae is very low compared with that of other insects, and that its metabolism is very much reduced because it so rarely moves. The extensive vertical wanderings of the *Corethra* larvae (see p. 54), however, renders this explanation improbable.

According to Thienemann (1920, p. 54) *Corethra* may occur in lakes with 2—3 c. c. of O_2 per litre or less, and it is absent from lakes with a comparatively high oxygen content, for instance in the *Tanytarsus* Lakes in Eifel. If,

therefore, it is found in company with red *Chironomus* larvae, Thienemann thinks that it is a sign of a very low O_2 content in the hypolimnion of the lake in question in the summer. Alm (1923, p. 172) remarks that in many lakes *Corethra* occur exclusively in the deeper parts where there is a lack of oxygen. He thinks that this occurrence is due to the fact that elsewhere the animals are exposed to a lively competition, and that they are able to tolerate a low O_2 content. In any case, their absence from the other parts of the lake cannot be due to the fact that they cannot live in water rich in oxygen, for they may be encountered in an environment rich in O_2. Several investigators state that *Corethra* larvae may occur in bottom water entirely or almost entirely free from oxygen and containing H_2S (Thienemann 1913, p. 245, 1923, p. 40, Birge and Juday 1911, pp. 36, 103, Juday 1921, p. 272). Valle (1927, p. 77) has investigated the occurrence of the *Corethra* larvae in relation to the oxygen content of the water in a series of Finnish lakes and arrives at the result that the larvae are not strictly limited to lakes, the oxygen content of which is low in the profundal region, nor does it occur in all the observed lakes poor in oxygen. Lang (1931, p. 49) found *Corethra* larvae in all the lakes he examined except one, in spite of the fact that the oxygen content of these was high. He does not think, therefore, that the *Corethra* larvae can be used as indicators of a low oxygen content. — The sum of these investigations would seem to show, then, that the *Corethra* larvae are fairly indifferent to the smaller or larger amount of oxygen contained in the water, and this agrees with the observations in Esrom Lake. Their absence from certain lakes must therefore be due to other causes, for instance to a lack of nourishment.

The periodicity of *Corethra flavicans* in Frederiksborg Castle Lake does not seem to follow quite the same course as in Esrom Lake, notably there does not appear to be so sudden and brief a hatching of imagines. It is possible that the imagines already appear at the Castle Lake in June; on the 7/VII, at any rate, 15—20 % of the larvae taken had been transformed into pupae. At the close of July, the swarming imagines are seen everywhere by the shore, but on the 3/VIII there were still many larvae and pupae in the lake. Throughout the month of August and as late as the 10/IX swarms of imagines may still be seen at sunset, hence the hatching seems to extend over a long period. The fairly long swarming time is perhaps connected with another phenomenon. The larvae in Frederiksborg Castle Lake vary a good deal in size in the middle of the summer, whereas those in Esrom Lake are all of the same length. On the 3/VIII larvae of *C. flavicans* measuring 11.9 mm occurred in Frederiksborg Castle Lake; they were individuals just about to pupate. But besides these there were others measuring about 7.1 mm only, and likewise intermediate sizes. Since larvae that have just been hatched grow up very quickly, there is a certain degree of probability that the small larvae which at the beginning of August are 7 mm, have been hatched from eggs laid the same year. If they had originated from eggs dating from the previous year, they would probably, in the course of the spring and the summer, have attained the full size of the species.

If larvae are about 7 mm in size at the beginning of August, it should not be excluded that they might have time to pupate and metamorphose into imagines in the same year, that is to say, in the same year in which they

must be presumed to have been hatched from the first eggs produced by the species. Frankenberg (1915, p. 551) has shown that the development in aquaria with plenty of nourishment and at optimum temperatures takes two months. In order to ascertain whether the larvae measuring 7 mm could undergo their metamorphosis in the same year, a number of larvae of this size were placed in aquaria and fed plenty of *Daphnia* and plankton from Frederiksborg Castle Lake. They grew rapidly, and on the 29/VIII several pupae and a few imagines were found in the aquaria and at the surface of the water. In the course of September all the larvae had attained full size and a large number of them metamorphosed into pupae and these again into imagines; the room in which the aquaria were kept was not heated. If it is really the case that the eggs from which the animals were derived were laid in the same year, the second generation had then been hatched. And it happened at a time when imagines might still be found flying in the open in small swarms near the shores of the lake. I can only suppose, therefore, that something similar may happen in nature, at any rate in years when the late summer is mild. In the aquaria, it is true, the larvae had plenty of food, but the amount of plankton in Frederiksborg Castle Lake is also decidedly so plentiful that no difference can be supposed to occur, on that account, between the experiment and conditions in nature. However, the second generation which thus, we may suppose, could occur under favourable circumstances in nature, intermixed with the last swarms of the first generation, can hardly comprise any very large amounts of individuals.

As controls for the above-mentioned aquarium experiments eggs of *C. flavicans* from Esrom Lake were placed

in aquaria in July-August. In the course of the autumn several of them were induced to change into pupae and imagines. Hence there is no doubt that *Corethra* larvae can be brought to full metamorphosis in the same year that they are laid as eggs, if only the conditions are favourable enough. This they are certainly not in so large a lake as Esrom Lake; probably the amount of food is not so plentiful as it was in the aquaria, and at any rate the temperatures in the hypolimnion are not nearly so favourable as the room temperature of the aquaria. On the other hand, Frederiksborg Castle Lake, which is particularly rich in plankton and warm right through, provides a more favourable environment for a rapid development; since the first larvae of the year are brought into the world there somewhat earlier, there is a probability, as previously mentioned, that some of them can attain metamorphosis in the same year, the weather being favourable.

The possibility that 2 generations of *Corethra* may occur has already been discussed by MEINERT (1886, p. 406), who writes on this subject: "It (*Corethra*) winters as a half-grown or full-grown larva, and the imago most frequently appears from the close of April to June; but already in the first days of spring, after mild winters as early as before the end of March, in the captive state, imagines will appear from pupae taken as larvae in the open in the same year, before the coming of spring. From the coming of the spring proper imagines continue to appear until late in the autumn, in captivity until the last days of November, and in captivity some few winter as pupae. At the close of September and the beginning of October imagines seem to appear in larger numbers, and perhaps we may put 2 generations a year, a first or main generation from the close of April to the

beginning of June, and a second or weaker generation 4 months after this, without the two generations being sharply marked off from one another, however." — As will be seen, Meinert's view of the possibility of 2 generations receives support from the observations from Frederiksborg Castle Lake that larvae of different sizes occur in the summer, and from the aquarium experiments; the main generation, however, is decidedly not as early in this locality as Meinert states.

On studying the periodicity of *Corethra* in three experimental ponds, in the forest near Frederiksdal, Wesenberg-Lund (1914, p. 8) arrived at the result that the larvae disappear from the ponds at the close of May when pupation takes place; the pupal stage lasts but a short time, only a few days; in the course of about 8 days nearly all the larvae of the ponds have been transformed into pupae. From about the 20th May to about the 15th June the ponds harboured neither pupae nor old larvae. In the first half of June the tiny larvae appear; by the end of June they have attained about half the full-grown size, and are full-grown already at the close of August. From August until May, when pupation takes place, the ponds contain full-grown larvae only; neither pupae nor small larvae were ever observed at that time; further, it must be pointed out that the larvae of the pond were at all times all of the same size, and that the pupae were never seen outside the period May 15th to June 1st. Hence Wesenberg-Lund concludes that in the case of these ponds there can be no doubt that *Corethra plumicornis* has mostly only one generation a year, the imagines of which appear at the close of May and the beginning of June. Scattered observations in numerous bogs in North Sealand also seem to show that the process of development indicated is the normal one, at any rate in certain years.

After citing the observations adduced by MEINERT in support of his conjecture of 2 generations of *Corethra*, WESENBERG-LUND remarks that these, taken in conjunction with his own observations, would seem to show that the periodicity may differ; the weaker autumn generation hardly develops in all localities, and scarcely every year. Experience shows that many freshwater insects normally have only one generation in Denmark, but in warm summers they can develop one more generation. — The presumed difference between the process of development in different localities is undoubtedly correct. This is confirmed by the investigations in Esrom Lake, where it is quite certain that no autumn generation of imagines is hatched. And it is probably right, too, that the autumn generation hardly attains development every year in places where it is at all possible; or else *Corethra* larvae of mutually differing sizes as demonstrated in the Castle Lake would more often be taken in the summer. Occasionally larvae belonging to different generations may indeed be found simultaneously. In small ponds draining into Kobberdam, Hellebæk, *Corethra* larvae were thus taken on the 21/VI which were partly full-grown, partly quite small; some few animals of intermediate sizes were also found.

FRANKENBERG (1915, p. 508) has examined *Corethra* larvae — *C. plumicornis*, now *C. crystallinus* — from a pool near Fregeteich, Leipzig. He states that it winters in the larval stage; the larvae which have wintered under the ice pupate in March and April. Already at the beginning of May he found egg-laying females (1st generation), and the generation deriving from them emerged from the pupal stage towards the close of June and immediately laid eggs (2nd generation). But whether the larvae which appear in this

pool at the beginning of July attain pupation in the same year, Frankenberg does not know.

Muttkowski (1918, p. 407) suggests the possibility of 3 generations of *Corethra* in one summer in Lake Mendota. Rawson (1930, p. 50), however, points out that an exactly defined minimum abundance of the larvae was only found in August in Lake Mendota. In Lake Simcoe the minimum was found in late July. Since this minimum indicates the maximum emergence, he thinks that it is doubtful if the early and late generations are of any significant numbers.

A full understanding of how much the periodicity of *Corethra flavicans* may vary, can only be obtained, however, when the observations from the Danish localities are compared with Peus' observations on the species from the inundation areas of German rivers (1934, p. 664). In pools along the banks of the Oder Peus found *flavicans* larvae in enormous quantities. Adult larvae and pupae were found at the close of May in the first days of June. It may happen that the complete desiccation of some pool brings the life of the larvae and the pupae to a close before metamorphosis can take place. They may then be found as a thick gelatinous mass at the bottom of the dried up pool. Normally the imagines emerge at the close of May, and from that time to the beginning of June they attain their maximum abundance. The number then decreases rapidly, and in the beginning of July the flying animals have quite disappeared. Now Peus, who, as was mentioned, does not know *C. flavicans* from localities such as the Danish ones, thinks that the brief space of time in which the pools are filled with water excludes the possibility that the life cycle of *C. flavicans* can take the same course as that of the other *Corethra* species. Normally the pools are dry from the early summer

to the next winter or spring. But the flying period of the imagines, as far as the majority of the individuals is concerned, is over with the month of June. Hence it is impossible, PEUS thinks, for oviposition to take place in the way it usually does in the rest of the *Corethra* species, viz. at the surface of the water. Further *C. flavicans* cannot winter as a larva, the hydrography of its localities simply forbidding it. PEUS therefore surmises that the egg-laying takes place in a similar way to that of the *Aëdes* species occurring in company with the *Corethra* larvae in the pools. The eggs are not laid in a gelatinous mass but perhaps singly at the bottom of the desiccated pool, and there await hatching till the next spring when the water fills the pool and attains a suitable temperature. Direct observations on the oviposition are, however, not at hand yet, and the conjecture as to the special periodicity is based mainly on the hydrography of the localities where PEUS found *C. flavicans*. But conditions in the localities are so well-known that of the main result that emerges in regard to the biology of *C. flavicans* PEUS can write: "Nach alledem hat das Larvenstadium also eine viel kürzere Dauer als bei den anderen *Chaoborus*-Arten" (1934, p. 666).

Even though it would be particularly desirable for PEUS' observations to be supplemented by direct observations on egg-laying, the hatching-time of the larvae etc., there can hardly be any doubt that his view of *C. flavicans*' biology in the inundation pools is correct. But now that we know, in addition, that *C. flavicans* lives in lakes such as Esrom Lake and Frederiksborg Castle Lake and has quite another periodicity there, we shall, on the whole, arrive at quite another idea of its biology than must be entertained by PEUS. *C. flavicans* is not a species with a highly deviating

and specialised biology, and with a periodicity adapted to the brief space of time in which the inundation pools are entirely filled with water. On the contrary, the species possesses an unusually large range of biological variation, which makes it possible for it to inhabit such different localities as shallow inundation pools only filled with water for a couple of months, and Esrom Lake which is 20 metres deep; and it shows a widely differing periodicity corresponding to the nature of the locality. Whether this is due solely to a great power of adaptation in *C. flavicans*, or whether this specific name possibly covers several species or subspecies is not known (compare Eckstein 1936 p. 484).

The Pupa.

If vertical hauls from the bottom to the surface are made in the daytime with coarse-meshed plankton bags in Esrom Lake at the time when the pupae are common, no animals will be taken. Hence they are not in the free water layers in the day, but can then be taken with the bottom sampler. At night, on the other hand, vertical hauls will give an abundance of pupae in the free water layers; for instance on the 22/VII at 11 o'clock p. m., vertical hauls from a depth of 1 m gave 1 pupa, from 5 m, 4 pupae, from 10 m, 50 pupae, from 15 m, 60 pupae. Thus the ascent of the pupae to the surface does not take place throughout the 24 hours, but only in the night. This being so, I suppose the ascent cannot be passive in all its phases; for then it might be expected to be evenly distributed over the 24 hours. Since the pupae are only found in the free water layers in the night, it is most reasonable to suppose that about the time the sun sets they work their way actively up through the

mud, after which the ascent through the water layers takes place. At 11 p. m. only some few individuals have reached the upper water layers; the majority are standing at about 10—15 metres' depth. If the net is dragged along the surface at this juncture, half under and half over the water, no pupae or imagines are taken. It must be supposed, therefore, that the pupae about to be hatched do not reach the surface until later in the night.

Some laboratory observations on the lifespan of pupae would seem to show that they have a life-time of several, though not very many, days. It is probable, therefore, that some of the pupae which may be found in the night in the free water layers again work their way down to the bottom at daybreak. This would also seem to be indicated by the fact that only a small part of the pupae mentioned above, which had been taken at 11 o'cl., were hatched the next night in the laboratory.

The *Corethra* pupae are preyed upon by the eel. An examination of the stomach contents of some eels during the hatching period seemed to show that they constitute by far the greater part of the food of the eel during this period, which is short, it is true. Some of the eels had empty stomachs; the following examples will show the food contained in the stomachs of the others. a: 2.1 g, consisting of 1 *Chironomus bathophilus* larva, some indeterminable chitin, several hundred *Corethra* pupae. b: 2.8 g, consisting of 1 *Corethra* larva, hundreds of *Corethra* pupae; further 2—3 score *Echinorhynchus* larvae. c: 0.5 g, consisting of 3 *Chironomus bathophilus* larvae, 1 *Pisidium*, some chitinous parts of insects and hundreds of *Corethra* pupae. At this time (12/VII) *C. bathophilus*, whose larvae are indeed small just now, does not seem to play any prominent part as a food.

The *Pisidia*, too, are few in number. The *Corethra* pupae, on the other hand, had been taken in such large numbers by the eels that they must be supposed to be of some importance to these, and conversely the persecution to which *Corethra flavicans* is thus exposed probably means a perceptible loss of individuals to the species.

Since the *Corethra* larvae are so hyaline, they might be supposed not to be worth much as food after the transformation to pupae. Undoubtedly they are valuable, however, for Juday (1921, p 285) has made a chemical analysis of the larvae of *C. punctipennis*, and showed that 67 % of the dry weight is crude protein and 9.5 % fat. The percentage of crude protein is remarkably high, in comparison herewith the larvae of *Chironomus tentans* yielded a much smaller percentage of crude protein, namely 46 %. The fat percentage, too, 9.5 % of the dry sample, is rather high. Together the crude protein and the fat constituted more than 76 % of the dry matter. Hence Juday draws the conclusion that from the standpoint of quality this large proportion of these two excellent food materials gives the larvae of *Corethra punctipennis* a very high rank as a source of food material for other organisms. Something similar must then be supposed to apply to other species of *Corethra*.

During the metamorphosis from pupa to imago the respiratory neck-tubes of the *Corethra* pupae have a special function which Wesenberg-Lund (1914, p. 11) has pointed out. At the moment when the pupa is about to be transformed and the cephalothorax bursts in the median line, the two air-filled neck tubes are spread sideways and lie like two air-containers on the surface of the water; this stabilises the whole of the pupa-skin from which the imago emerges, and on which it usually remains for some seconds.

However rapidly the change to imago takes place, it is, none the less, a critical moment in the life of the animal; during these few seconds the neck-tubes no doubt play a certain part as floating bladders and stabilisation factors. Precisely because the *Corethra* metamorphose in the middle of lakes

Fig. 13. From the shore of Esrom Lake in the month of July. Apparently the shore is sandy, but in reality the whole margin consists of washed up pupa-exuviae of *Corethra flavicans* with a small admixture of pupa-exuviae of *Chironomus Liebeli-bathophilus*.

and ponds under rather rough conditions, the strong stabilisation of the pupa-skin is probably of more importance than in the other species.

On quiet mornings during the hatching periods the pupa-exuviae may be found floating in small groups dispersed over the surface of the lake. If the surface is smooth the imagines too may be seen resting there, while those which have not succeeded in emerging float about, dead, at the surface. If a strong breeze rises the whole mass is carried in to the shore, and sooner or later a margin of washed up

material, 1 m wide, is formed, which in some places may be 1 decimetre deep. Over long stretches it looks as if the lake had a sandy beach (fig. 13), but there is barely one

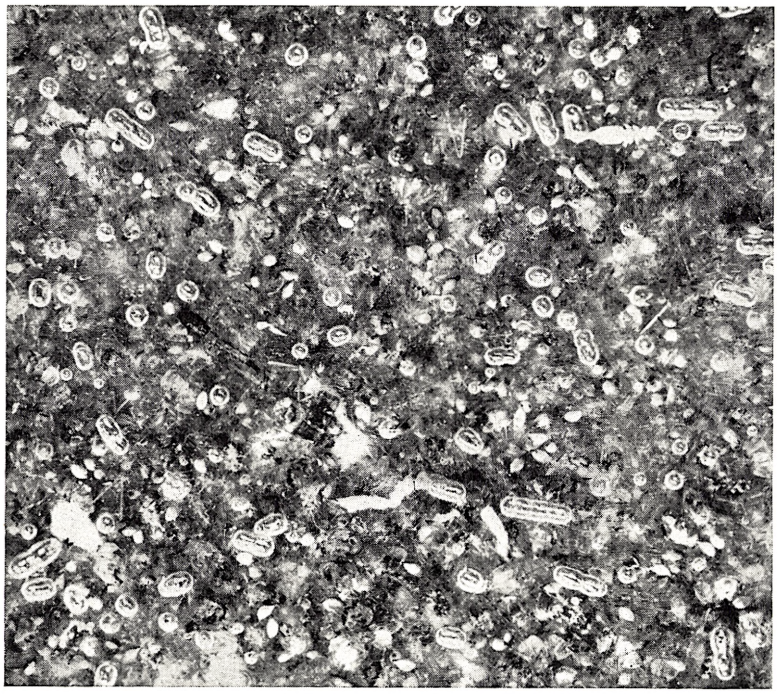

Fig. 14. The pulpy washed up mass forming the margin of Esrom Lake in July. The bulk of it consists of pupa-exuviae of *Corethra flavicans*; the small light-coloured bodies are the breathing tubes of the pupae. The pupa skins often contain one or several air-bubbles which have helped to carry the skins in to the shore.

grain of sand in the margin. The pulpy mass consists chiefly of the exuviae of *Corethra* pupae (fig. 14), but thousands of dead or moribund imagines may also be found in or on it. Here and there close inspection will show that old decaying pupa-exuviae of *Chironomus bathophilus* constitute part of the mass.

Imagines.

The swarms of imagines are largest about the middle of July. The animals are then seen at all times of the day, but especially on quiet evenings, at sundown, when they form

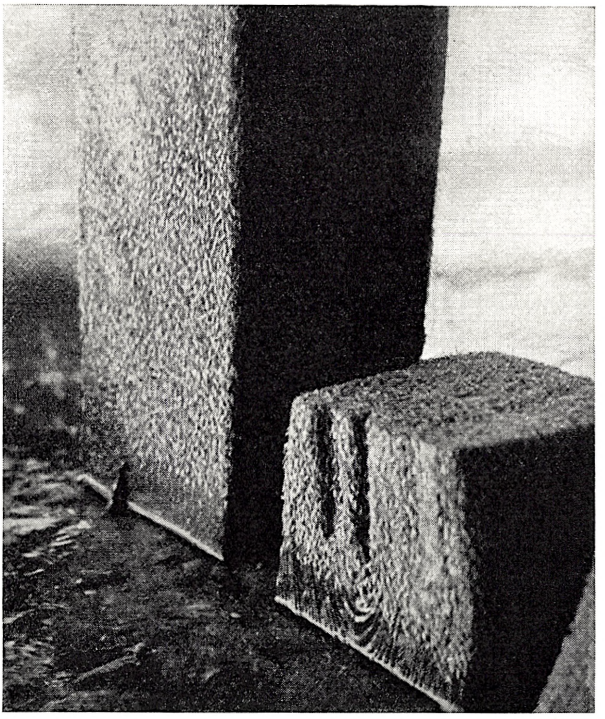

Fig. 15. Bridge piles covered with a layer of the imagines of *Corethra flavicans*; from the beginning of August.

large clouds. In strong sunlight they often sit by myriads on the shady side of poles, on the underside of leaves etc.

In the course of the latter half of July the swarms diminish in magnitude, but as late as the 31/VII large swarms were still observed, especially in the shady avenues along the lake. It is evident, however, that the swarming period has culminated. For dead and moribund animals form

layers on the margins of washed up material along the lake, fill cobwebs by hundreds, or sit languid on the ground of garden walks. Bridge piles etc. are covered by a carpet of them (fig. 15) and among the dead animals there are many which are overgrown with fungi, which especially grow up as a fatty mass around the thorax. Mr. N. F. Buchwald has kindly informed me that among these fungi there occur *Cladosporium herbarum*, a *Penicillium*, and an *Entomophthora* species. The latter species, which is related to fly mould (*Empusa muscae*), may perhaps have helped to kill the gnats, which have then become covered with the other saprozoic fungi.

On the imagines and on the washed up accumulations of pupa-exuviae, a good many sixlegged larvae of Hydracnids may be found. They have been studied by Wesenberg-Lund (1918, p. 22), who has shown that they belong to *Diplodontus despiciens* (O. F. M.); he observed that many of the hatched gnats carried numerous larvae, usually attached to the thorax. On the walls of pupae exuviae too ran millions of small red sixlegged larvae; here and there the walls were red with larvae. Undoubtedly the larvae of *Diplodontus*, when the gnats sought the littoral region to pair, and for egg-laying, mounted onto them and were carried away with them. This took place in the first days of August. The parasitic stage can by no means have lasted more than 14 days and normally only some few days. After a thorough examination of them, Wesenberg-Lund has proved that the gnats were infested with numerous sixlegged larvae, quite similar to those which he had found upon the accumulated masses along the shore. — In 1934 and 35, I, too, saw the sixlegged, parasitic *Diplodontus* larvae on the imagines of *Corethra* at Esrom Lake and on the pupa-

exuviae on the shore; though never in any great number, and at any rate not by millions. The short swarming period of *Corethra* at the close of July and in the first days of August in fact render it likely that the parasitic Hydracnid larvae are not every year fortunate enough to appear in such large numbers just at the right moment. — That *Corethra* may occur as host to larvae of *Diplodontus despiciens* has been experimentally confirmed by VIETS (1924, p. 324).

The Eggs.

The eggs are deposited in the well-known discs (see e. g. WESENBERG-LUND, 1914, p. 14). WESENBERG-LUND already remarks that it seems as if the egg-laying either takes place in the littoral region, or the eggs are carried in to the shore by the waves or the wind. He supposed that the first was the case, and this I can fully confirm. Oviposition takes place near the shore, with especial frequency in shallow water, where *Potamogeton pectinatus* and masses of filiform algae touch the surface. The female generally settles on these, but she may also alight directly on the water. In the course of 5—10 minutes she lays the eggs which are quite white when they are laid and only gradually assume a dark-brown colour. The eggs first deposited are pushed out towards the edge of the egg-heap by the ensuing ones. The jelly which surrounds the eggs prevents these from sinking to the bottom and likewise causes them to adhere slightly to parts of plants and to each other. They form a conspicuous dark coating on the water in those areas where the *Potamogeton* species reach the surface (fig. 16). There can be no doubt that the egg-masses are chiefly deposited in the littoral zone. Far out towards mid-lake, however, egg-heaps may also be found drifting at the surface. Some of them

may possibly have been laid there. Most of them, however, have probably been carried out there from the shore by the current, for they are frequently found together with floating fragments of plants from the littoral zone. In any

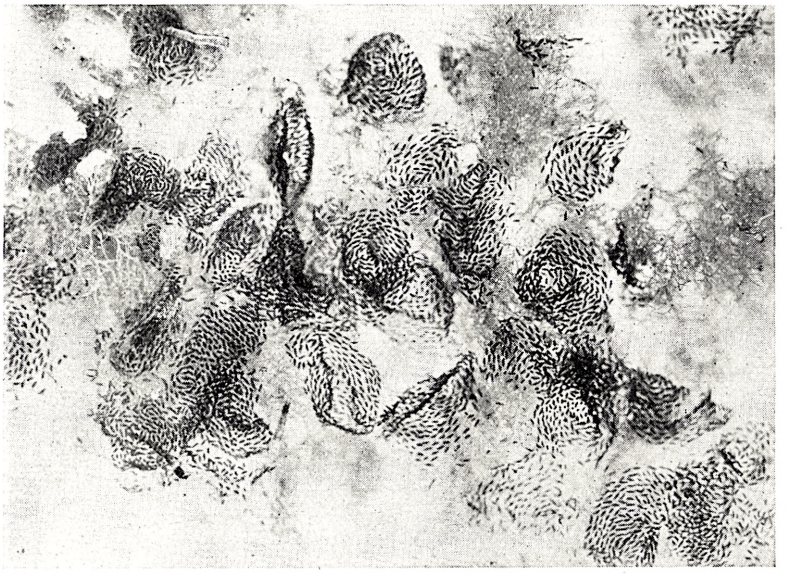

Fig. 16. Egg clumps of *Corethra flavicans* floating at the surface of the lake and adhering to algal filaments. × 2.5.

case, by far the greater part of the eggs are found by the shore and are hatched there.

Thus the eggs of *C. flavicans* behave differently in Esrom Lake to the eggs of *C. punctipennis* which, according to Juday (1921, p. 274), sink to the bottom in aquaria, and also sink to the bottom when laid in the open, at the surface of Lake Mendota. Mud from that lake, from depths of 23 m and 18 m, was sifted through meshes fine enough to remove all larvae; the sifted mud was then placed in 2 aquaria. In the course of 5—7 days small *Corethra* larvae appeared in both aquaria, by which it was thus proved that mud

from both stations contained eggs. Whether such eggs can develop in Nature in the bottom of the lake, is not known, however, for the lack of oxygen in the hypolimnion in the summer may possibly prevent or check the development. Mud from the bottom of Esrom Lake (20 m) was placed in an aquarium on the 7/VIII in order to ascertain, in a similar way, whether it contained *Corethra* eggs. It turned out not to harbour any. This further confirms the supposition that the hatching in this lake takes place at the surface only.

Oviposition probably occurs fairly soon after the emergence of the imagines, perhaps only a few hours later. In small aquaria, where isolated specimens of *Corethra* pupae are hatched, it may happen that young females, shortly after the emergence, lay eggs without having been fertilised. Since, as is well known, parthenogenesis occurs in other *Diptera* (e. g. in certain *Chironomids* and in *Miastor*) it could not at the outset be regarded as quite excluded that parthenogenetic eggs of *Corethra* might be able to develop. In the cases under observation, however, they always perished, and quite the same thing has been observed by FRANKENBERG (1915, p. 544).

The duration of the developmental period of the fertilised eggs has been investigated in the laboratory. Some females of *Corethra* were caught at Esrom Lake and kept in captivity, during which they laid eggs. Some of the egg heaps were set aside at a temperature of about 17.9—19.4° C, others at about 16.3° C. In the former case the period of development proved to be about 67 hours, in the latter about 85 hours. Since these temperatures are pretty near the temperatures in the littoral zone during the development of the eggs, we must reckon with a developmental period in nature of approximately that duration, i. e. about $2^1/_2$—$3^1/_2$ days.

The periods indicated with the temperatures belonging to them, would seem to show that small differences in temperature, in the case of temperatures of the size stated, greatly influence the duration of the developmental period; the data must therefore be said to agree very well with Juday's

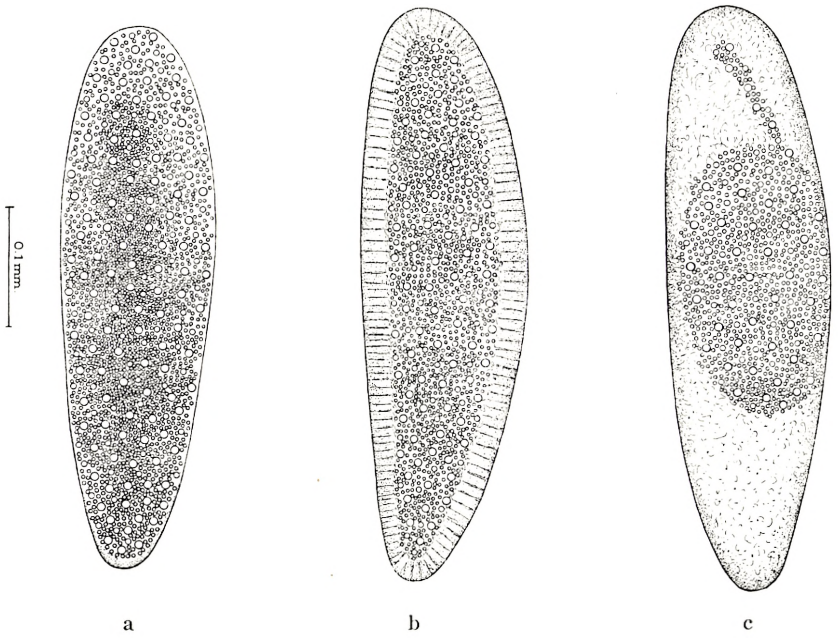

Fig. 17. Development in the egg of the larva of *Corethra flavicans*.

statements (1921, p. 274) of a developmental period of 48 hours at about 21—24° C. Frankenberg (1915, p. 544) gives a period of 3—4 days for the development of the eggs. According to Wesenberg-Lund (1914, p. 9) the egg-stage, from observations in the laboratory, hardly lasts more than 8—10 days, whereupon the young larvae appear. Compared with the periods given above, this time is very long, but since the temperature, as previously mentioned, plays a very great part, it probably applies to low temperatures of the water.

The newly laid, white egg (fig. 17 a) is quite filled with a uniform mass, which is drop-like; at the edges it is fairly clear and transparent. After 8 hours, a superficial cleavage has taken place, and the egg is now over the whole surface covered by high cells placed vertically to it (fig. 17 b). The

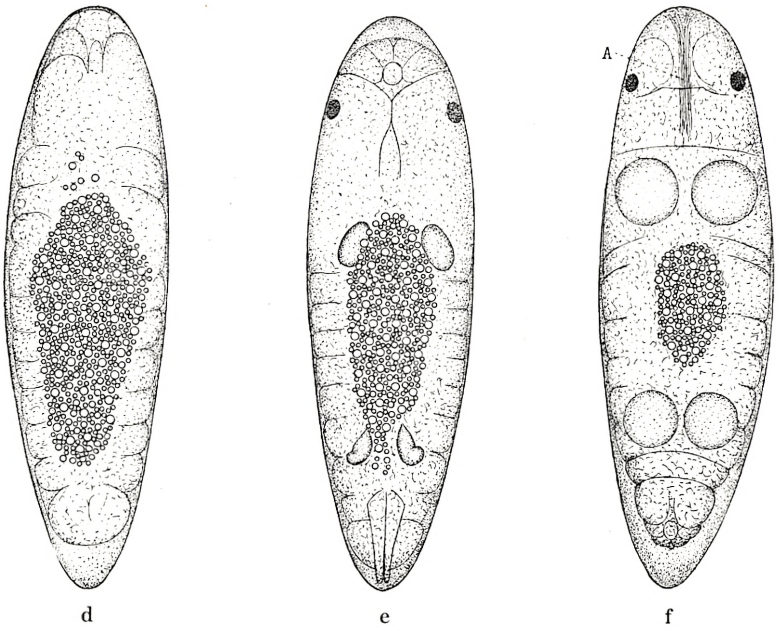

Fig. 17.

inside is filled with a uniform mass of yolk. After 14 hours (fig. 17 c), the yolk is partially dissolved and absorbed in the superficial cells; these have turned greyish and their cell borders are no longer visible. The greater part of the yolk is gathered in a large dark lump in the middle of the egg. When the egg has grown 28 hours old (fig. 17 d), a faint segmentation is observable, and the yolk has grown considerably smaller. A further development of the segmentation can be observed in the egg when 34 hours old

(fig. 17 e), and now different organs or incipient organs can readily be distinguished: in the head the incipient antennae and mouth parts and eyes; in the thorax and the abdomen the two pairs of tracheae, which are still small but plainly

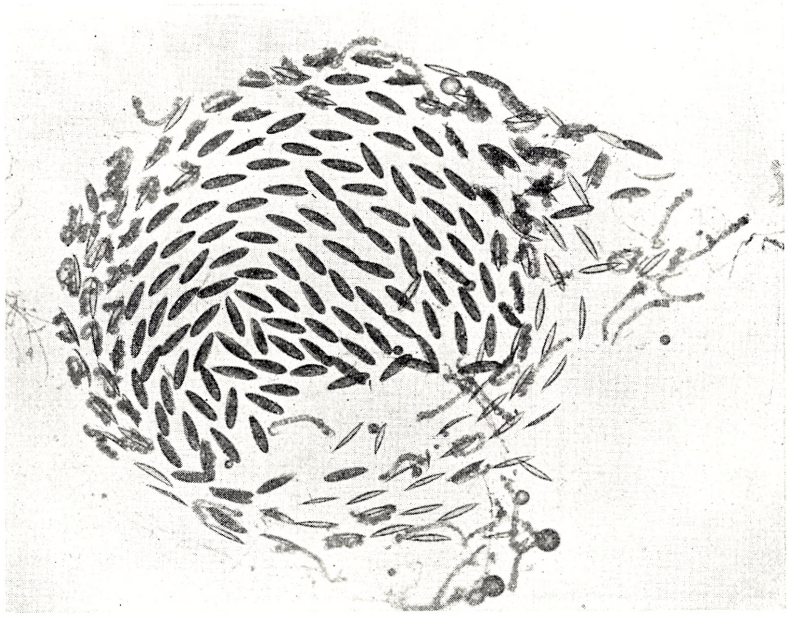

Fig. 18. Egg clumps of *Corethra flavicans*; the eggs at the edge are hatching. August 10. × 13.

shaped as bladders, not as tubes; finally the beginnings of the segments of the swimming fan. After 57 hours (fig. 17 f) there is a larva in the shell which is almost ready to emerge. The antennae (A) are now much better developed; the tracheal bladders have grown much bigger; as far as I have been able to see, it is correct that at this time they are filled with a fluid (Meinert, 1886, p. 40). The yolk has now been reduced to a small lump in the middle of the animal;

in the quite young, free-swimming larva a couple of drops of it are still left. At the same time as the egg in fig. 17 f was drawn, other eggs belonging to the same egg mass were hatched.

The hatching of the eggs in a heap usually first takes place in the periphery, where the oldest eggs are to be found (fig. 18). In the figure a number of larvae are seen emerging from the eggs; several of the outer eggs are already empty, while all the eggs in the centre still contain larvae. MEINERT (1886, p. 40) states that the animals come out backwards. This I have not been able to see. The egg cracks lengthwise, and then recalls a canoe in appearance. The median part of the larva is first pushed out, while it long remains attached to the egg by the head and tail. By much wriggling it tries to free itself until it at last succeeds in disengaging the ends. The development of the egg, as sketched above, took place at a temperature of about 17—19° C.

The Young Larvae.

Through the above-mentioned investigations on the place in which the eggs are chiefly deposited, and through experiments with a negative result on the hatching of larvae from mud taken from the middle of the lake, it was rendered probable that in Esrom Lake the hatching of larvae takes place exclusively at the surface of the water by the shore. An investigation of the horizontal distribution in the lake of the young larvae at the moment they emerge from the eggs confirms this. On drawing a plankton bag horizontally through the water on the 27/VII 33 over the different depths of the sampling line, in each place over a stretch

of 25 m, the following numbers of larvae were found in the free water layers:

Depth	1 m (by the shore)	2 m	5 m	8 m	11 m	14 m	17 m	20 m (in mid-lake)
Number of individuals	Several hundred	2	1	1	5	5	9	4

In vertical hauls from a depth of 17 m at the foot of the slope and in mid-lake, 0—4 individuals were found per dredge haul. From these plankton samples it appears that the newly hatched larvae already at the close of July, when the swarming has culminated, were found dispersed all over the lake in the free water layers, from the shore right out into mid-lake. But it likewise appears that the number in which they occurred in mid-lake was as yet very low compared with the enormous swarms by the shore. These show that it is there that the hatching takes place. The larvae which, already at that time, were spread over the lake had probably been carried out there by the current — several times during the past 1—2 weeks a strong wind had swept over the lake. Such a transportation, in the main passive, of young larvae from the shore, can in fact readily be understood, because precisely these, as will be shown on p. 84, possess a pronounced positive phototaxis, and therefore for a time will keep above in the light and in the most agitated parts of the water masses, in the epilimnion. Hence the positive phototaxis appears to be of very considerable biological importance to the species.

It has been demonstrated by Frankenberg (1915, p. 549) that newly hatched larvae can fill their tracheal bladders with air even at an excess pressure of 56 cm Hg. He regards

the demonstration of this as important and in that connection reminds the reader of WESENBERG-LUND's observation of *Corethra* larvae at a depth of 30 m in our largest lakes. Even though FRANKENBERG's demonstration is theoretically important, it must, however, be remarked that in a natural environment the young larvae cannot, on account of their being hatched by the shore, be supposed to have to fill their tracheal bladders for the first time at a excess pressure of any significance.

The larvae from the 27/VII mentioned above had just been hatched and they were only about 1.75 mm big. The growth takes place with extreme rapidity. On the 9/VIII the majority of the larvae in the lake were about 4.1 mm long, and only a small number were somewhat shorter. In the course of 13 days the size had been more than doubled. The larvae had now already left the shallow water; none at all were taken with the plankton net at the lower stations of the sampling line; only in mid-lake at about 10 metres' depth or still deeper were the larvae found swimming in the free water-layers. Bottom samples from the 20 m level showed that as yet there were no *Corethra* larvae in the mud of the bottom. The same thing is shown by fig. 12 p. 16 for the whole sampling line on the 2/VIII; the individual at the 2 m level is a pupa of the previous generation. On the 22/VIII the larvae could not be found in the bottom either. Fig. 12 shows that in 1933, as late as the 7/IX, there were only some few individuals to be found in the bottom samples, and also that the animals taken came from deep water. On the 12/IX a series of samples were taken along the whole sampling line, and now *Corethra* larvae were at last found in abundance in the bottom, even in mid-lake. Hence the animals must be

assumed to make their way into the bottom about the middle of September.

The distribution over the bottom at the various depths on the 12/IX is shown in fig. 19 to the right, and for comparison the average benthic distribution is given to the left. It will be seen that as soon as the young larvae again appear in the bottom samples, they have quite the same characteristic distribution as have the larger larvae on the average at the other seasons of the year, that is to say, there is by far the greatest number of individuals at a depth of 20 m, a small number on the deepest-lying parts of the slope, and none in shallow water; only the young larvae do not as yet occur in such a large average number as is otherwise the case.

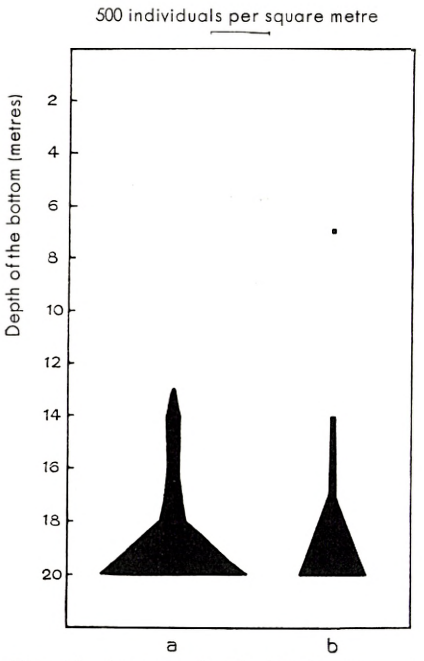

Fig. 19. Average depth distribution of *Corethra flavicans* (a) and the first depth distribution observed after the swarming period (b).

A recurrence of the *Corethra* larvae, in which they are first found freely in the water near the shore, then scattered over the lake and pelagically but not in the bottom and not near the shore, and finally, in the middle of September, in the bottom ooze, with quite the same benthic distribution as the older larvae, is characteristic of the species in Esrom Lake. J. Lundbeck (1926, p. 222) thinks that he has found

something different; he states that the young larvae first live in the bottom in the flat zones and then, with increasing size they also go down into the deeper-lying parts of the bottom. He gives some figures from Kleiner Ukleisee, which lend support to the supposition of such a descent. He points out himself, however, that a descent in the summer of this species is in entire contrast with that ascent of many bottom animals which takes place precisely at the same time. He infers that various factors must cause this, and in the case of *Corethra* consoles himself with the conjecture that perhaps it is a nutrition-biological dependency asserting itself. It is by no means clear, however, why such a dependency should cause the animals to change their habitat from the bottom in shallow water to the bottom in deep water. The investigations in Esrom Lake show that the descent surmised by LUNDBECK at any rate does not take place everywhere, but the young larvae are dispersed pelagically over the lake, and when they recur in the bottom, they have the benthic distribution peculiar to the species. — Some observations by C. JUDAY, on the other hand, agree well with the observations from Esrom Lake. In Lake Mendota JUDAY (1921, p. 277) found that young larvae of *Corethra punctipennis* occur in the free water layers in the daytime; they occupy the lower water during the daylight hours instead of the mud, being found in the lower part of the mesolimnion and in the hypolimnion. Thus the behaviour of the young larvae is very different in the daytime from that of the fullgrown individuals, which are found in the bottom of the lake in the daytime. It appears that the young larvae inhabit the lower water in the daytime instead of the mud, until they are approximately one third grown or perhaps a little larger.

In Esrom Lake the rapid growth of the young larvae is continued in the late summer and in the autumn. On September 3rd there are already larvae about 10.5 mm in length. In October a large number having an average length of 10.8 mm were measured. This size is not very much less than that of the larvae next year immediately before pupation. The average size of the larvae well on in June and about the 1st July was thus found to be 11.2 mm. The circumstance that the *Corethra* larvae attain almost full size already in the autumn is in good agreement with the fact that in certain localities — though not in Esrom Lake — they may possibly have time to produce an autumn generation; their rapid growth to full size is essential to this.

The Relation between the Benthic and the Limnetic Behaviour of the *Corethra* Larvae.

The profusion in which the *Corethra* larvae occur in the bottom samples from Esrom Lake somewhat astonished me, since the pronounced plankton characters of the animal — its hyalinity, its tracheal bladders, and its swimming fan — combined with its well known pelagic occurrence in ponds made me regard it as a typical plankton animal which only occasionally or under certain circumstances was found in or near the bottom, besides pelagically. It was, however, well known to me that of recent years it had frequently been mentioned in bottom investigations, but the relation between its benthic and its limnetic behaviour seems not to be fully elucidated (cp. Valle 1927, p. 75, Lang 1931, p. 46), and is evidently also somewhat different from one lake to another. Therefore it was decided, at the same time as the bottom was investigated, to try to throw some light on the behaviour of the *Corethra* as

a plankton animal. It then turned out that larvae of *C. flavicans* did not occupy the free water layers at all in the daytime in Esrom Lake, apart from the pelagic occurrence of the youngest stages in August-September. Since this result is rather remarkable for an organism with such pronounced plankton characters, I have assured myself of its correctness by numerous hauls with a plankton bag at different seasons of the year, at different hours of the day, and under different weather conditions. As documentary evidence I shall cite various extracts from my notes on these plankton hauls:

8/XI 33. 9—10 a. m. 8 vertical hauls with a plankton bag from the 3, 6, 10, 12, 19, and 20 m levels gave *0 Corethra*. From 19 m and 20 m 2 samples were taken from each level. The one from 20 m had touched the bottom and got a couple of Chironomids but no *Corethra*.

13/XI 33. 10 a. m. 6—7 horizontal hauls with a dark plankton bag, i. e. only visible with difficulty, with lead weights attached; the plankton bag was dragged over the bottom, but took no *Corethra*. Only one haul, which touched the bottom and took up mud, contained many *Corethra* individuals. 2 vertical samples: *0 Corethra*.

4/XII 33. 11 a. m. Several vertical samples from the 20 m level: *0 Corethra*. Horizontal samples: *0 Corethra*.

23/I. 34. 6 p. m. 3 vertical hauls from the 5 m level, and 3 from the 10 m level gave *0 Corethra*. 1 vertical haul from 20 m gave 1 *Corethra*.

1/VI 34. 9—10 a. m Several vertical hauls from the 20 m level: *0 Corethra*. Long horizontal hauls both at 5 m and near the bottom: *0 Corethra*. Only when the net goes into the bottom and takes Chironomids etc., do the samples contain *Corethra* too.

31/VI 34. 3 p. m. Horizontal hauls with a plankton bag at 2 m, at about 10 m, and still deeper: *0 Corethra*. Vertical samples from 20 m: *0 Corethra*.

24/VII 34. 6 p. m. 3 vertical samples from 20 m: *0 Corethra*.

12/IX 34. 10 a. m. — 1 p. m. Vertical and horizontal samples at various depths: *0 Corethra*.

These as well as many other examples show that *Corethra* larvae were not found in the free water layers in the daytime. This applies to the pupae too; for in the investigation on the 24/VII the animals were practically all changed into pupae. Only when the net came too near the bottom and took up bottom material, were *Corethra* larvae or pupae taken too.

A certain possibility that the *Corethra* larvae might stand immediately above the bottom, for instance only 1—2 dm removed from it, cannot be quite excluded, despite the result of the plankton hauls described above; for the vertical hauls cannot take animals so near the bottom. And the horizontal hauls can only as a rare exception, by a lucky chance, be carried quite near the bottom without at the same time touching it. And in that case it cannot be decided whether the larvae have been in the bottom or in the water layer just above it. In order to ascertain whether the *Corethra* larvae besides being in the bottom, should also be present in the water layers 1—2 dm above it, some bottom samples were taken with a Birge-Ekman bottom sampler in such a manner that they only went a very short way into the bottom but were partly filled with water, and such bottom samples were compared with normal ones. If the *Corethra* larvae in the daytime stand just above the bottom, the less deep-going bottom samples will take more larvae than the normal samples. The less deep-going bottom samples gave the following number of *Corethra* individuals per single sample: 1 and 2 (8/XI 33), 2 (22/XI 33), 1 and 2 (5/XII 33). Since the normal bottom samples contain on an average 29 individuals per single sample, the less deep-going samples have taken much fewer animals, that is to say, the *Corethra* larvae hardly stand

directly above the bottom in the daytime, but by far the greater part, at any rate, are found in the bottom.

This result is in good agreement with an observation made by K. S. BARDENFLETH (BARDENFLETH & EGE, 1916, p. 37), who writes on this subject that in the month of July when he made some observations in Esrom Lake with the apparatus of Dr. C. G. JOHS. PETERSEN for quantitative examination of the bottom fauna, he always found the lake form of *Corethra* larva abundant in the bottom samples from the deeper parts of the lake (the samples were taken at depths of 20—25 m), the number of the larvae varying from 1500 to 9000 per sq. m. In order to decide whether these larvae were caught by the apparatus on its way down through the water, or if they were really living in the mud on the bottom, BARDENFLETH let the apparatus go down to a depth of 1 m above the bottom, where he made the closing mechanism function. When closing, the apparatus will sink another metre, and when taken up it contained 40 larvae together with a small quantity of mud, having thus just touched the surface of the bottom. At the same station, however, when quite filled with mud, it contained 931 larvae. According to BARDENFLETH, this would seem to indicate that these larvae, as distinct from the pond form, do not swim freely in the water, but live in or immediately above the muddy surface of the bottom. — The difference between the number of individuals taken when the PETERSEN bottom sampler only just touches the bottom, and when it is carried right down into it, is so considerable that it appears to me to show decidedly that the *Corethra* larvae are chiefly found in the bottom in the daytime; and this result quite accords

with the tests with the Birge-Ekman bottom sampler. That the numbers found by Bardenfleth are greater than my average (p. 19) may be due to decrease of the population, but also to difference in the methodics.

Though Wesenberg-Lund (1908, p. 513, 1914, p. 12) accepted the view then current that the *Corethra* larva is the only insect larva so far known which has "quite emancipated itself from the bottom and the shore and found a home in the pelagic region", and found this readily understandable on account of its structural features, he had, however, already made an interesting observation on its occurrence which points in the same direction as the above. He showed that it was present in most of our large lakes (1904, p. 157), with especial frequency in Hald Lake and Esrom Lake, and expressly remarks hereof that it is only taken in large quantities when the net is drawn horizontally below a depth of 20 m.

Since *Corethra* larvae were not normally to be found in the free water layers of Esrom Lake in the daytime, a series of nightly excursions were made to the lake, in order to ascertain its presence, if possible, after dark. By means of horizontal plankton hauls its pelagic occurrence was always ascertained without exception. By means of vertical hauls from 1, 5, 10, 15, and 20 m — as a rule three hauls from each depth — with a coarse-meshed plankton bag an approximate expression for the number of individuals in the different water layers was found. Fig. 20 shows the vertical distribution ascertained in this way at various points of time after dark. The figure shows that

1) near sunset and near sunrise only few *Corethra* larvae were found in the free water layers, and none near the surface (23/I and 19/XII);

2) at certain hours well on in the night *Corethra* larvae were found in all the water layers of the lake from the surface to close to the bottom (22/XI);

3) near midnight *Corethra* larvae were found in the upper water layers, but not in water layers near the bottom. A very marked maximum was then demonstrated in the upper zone 1 m deep. There was then several times as many

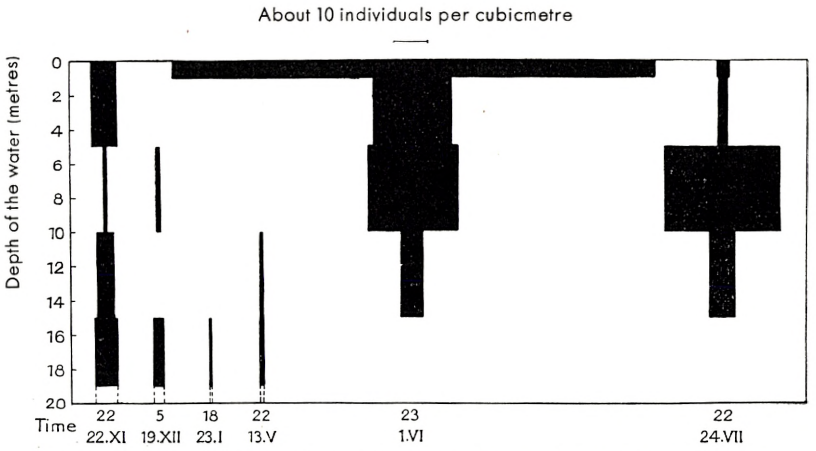

Fig. 20. Vertical distribution in the water layers of *Corethra flavicans* at night in the middle of Esrom Lake.

individuals in this water layer as were present in the corresponding deeper layers. The individuals that have wandered right up to the surface from a depth of 20 m will then, in the course of a few hours, be exposed to a considerable change of pressure — from about 3 to about 1 atmosphere (1/VI).

4) the numerous occurrence in the upper free water layers about midnight was also demonstrated, when practically all the population had been changed into pupae (this was the case on the 24/VII).

In the case where the greatest number of individuals was found to have migrated to the upper layers, viz. on

the 1/VI near midnight, a calculation shows that about 400 larvae are present in the whole column of water below 1 sq. metre of the surface. But since the average number of individuals occupying 1 sq. metre of the bottom is about 1300, it is impossible that all individuals can have ascended. It is possible perhaps that they have not all contrived to do so as yet, but that it will happen later on. It is also possible that they do not all ascend every night, and that perhaps is the most probable. Or the ascent and descent might take place at slightly different times of the night and happen so quickly that all the animals are not found in the pelagic region at the same time. Bottom samples taken simultaneously with the vertical plankton hauls from the 22/XI at 10 p. m. showed that the amount of larvae which as late as this after dark were found in the bottom was approximately the normal number also found in the daytime. — In Lake Mendota where Juday (1921, p. 277) has studied the vertical migration of *C. punctipennis* in the night, he found that the bottom contained a good many larvae in the night too; it contained from one half to two thirds as many at night as were there in the daytime.

To supplement the observations on the limnetic and benthic distribution of *C. flavicans* in Esrom Lake the following observations on the corresponding distribution of the same species in Frederiksborg Castle Lake are given. On the basis of an abundance of plankton samples taken there in the daytime at different seasons of the year and for different purposes, it can be stated with certainty that in most months *Corethra flavicans* cannot be taken in the free water layers in the daytime; some few observations from midsummer only, to be dealt with below, form an exception. At night, on the other hand, it has always without

exception been possible to take *Corethra* larvae limnetically. Hence there is a vertical migration of *Corethra* larvae in the lake.

In order to throw light on this migration in Frederiksborg Castle Lake plankton samples were taken at various hours of the day from the 2/VIII to the 3/VIII, 1935. The

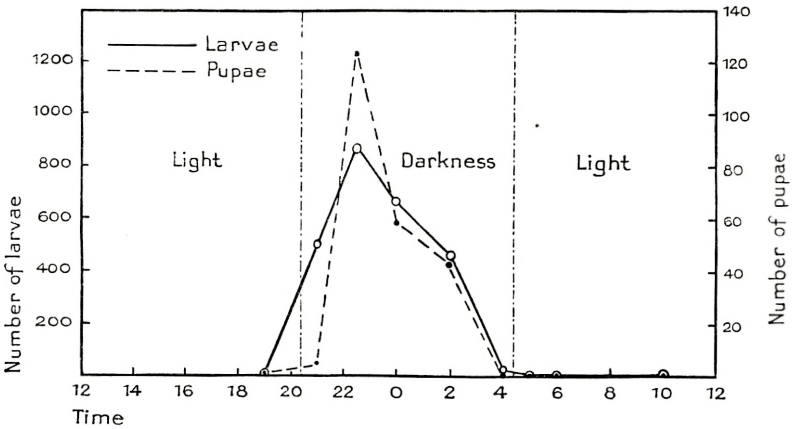

Fig. 21. Number of larvae and pupae of *Corethra flavicans* taken at the surface of Frederiksborg Castle Lake at various times of the day.

samples were taken in mid-lake with a coarse-meshed plankton bag drawn horizontally through the water over approximately 25 m at about 1 metre's depth below the surface. In each case 2 such samples were taken, in which the number of *Corethra* larvae as a rule agreed well. Only at the "critical" periods (at 9 p. m. and 4 a. m.) was there a good deal of difference between the two contemporaneous samples, the reason being probably that one sample was taken somewhat deeper down than the other; this circumstance at this time, when the upward and downward migrations are especially lively, may be of importance and seems to indicate that the larvae mostly

migrate in a body. — The result of the investigation, in which the larvae and pupae were counted separately, will be found in fig. 21. This shows that a migration to the upper layers only takes place at sunset. The light fades considerably earlier in the deeper layers of the lake, but as late as 7 o'clock p. m. no ascent had taken place. It did not take place until sundown. Samples taken just before, and at midnight show the largest number at the surface. After that it decreases, and already at sunrise the animals have almost entirely disappeared from the surface. In the daytime they are totally absent from the upper water layers.

The number of the pupae was counted separately and their curve has a similar form to that of the larvae. The shape of the pupa curve might perhaps be interpreted to mean that the pupae ascended from sunset to about midnight and were then gradually hatched in the course of the night, so that for that reason their number diminishes at the surface towards the morning. That probably is the case for many of the pupae. But since the life of a pupa is longer than 24 hours (see p. 33), this cannot apply to all, and some of the pupae therefore — like the larvae — probably descend when the day begins to break.

On the supposition that the larvae, when they ascended in the night, would leave few or no individuals in the bottom, but that many would be found in it in the daytime, a series of samples were taken with the bottom sampler simultaneously with the above-mentioned series of plankton samples. The result was that in 18 bottom samples distributed over various hours of the day the total of *Corethra* larvae taken was 1. This was astonishing considering the amount found in the bottom samples from other seasons of the

year. The difference between such samples will appear from the following examples.

Average per double sample (500 sq. cm) of *Corethra* larvae in Frederiksborg Castle Lake.

20/III	9/VII	3/VIII	16/X
81	20	0	116

As will be seen from this, the bottom of Frederiksborg Castle Lake harbours no *Corethra* larvae in midsummer; in July already the number is small. In the spring and the autumn, on the other hand, there is a large number of animals in the bottom. The fact that there are no *Corethra* larvae in the bottom (or practically none) in August cannot, as might perhaps be supposed, be due to the hatching of the animals at that period. This must certainly be assumed to have reduced the number of animals, but as shown by the aforementioned contemporaneous plankton samples, there is still a very plentiful population left. Only this does not, when it leaves the upper water layers in the daytime, migrate right down into the bottom so that it can be taken with the bottom sampler, as is the case in the spring and the autumn. In August it is found in the daytime in the water just above the bottom. If deep plankton samples are taken from just over the bottom, larvae and pupae may be taken by hundreds, while bottom samples below and plankton samples above give no result whatever.

As to the reason why the *Corethra* larvae occupy the bottom in the daytime in the spring and the autumn, and the zone just above the bottom in the summer in Frederiksborg Castle Lake, it seems natural to put forward two possibilities, which perhaps exert a combined influence. In the summer there will probably, owing to the high temperatures

prevailing in the shallow lake (Berg and Nygaard, 1929, p. 239), be a great lack of O_2 in the decidedly muddy bottom, which may possibly prevent the *Corethra* larvae from going right down into the bottom at this time. Another factor which undergoes great changes in the summer months is the intensity of the light in the lake. On account of the strongly eutrophic character of the lake an unusually marked *Cyanophyceae* maximum occurs in July-September. On calm days a strong waterbloom will be present. The transparency of the lake is then very slight; thus in August a transparency of 35 cm only was measured with a white porcelain disc. In the spring and autumn, at any rate, the transparency may be 2—3 times as great. Since now some *Corethra* larvae, as will be discussed below, are susceptible to light in rather a high degree and prove to be negatively phototactic, it is possible that it is the light which drives the larvae into the bottom at those seasons when the water is fairly transparent. But well on into the summer, when the strong *Cyanophyceae* maximum occurs, the amount of light in the water layers just above the bottom grows so slight that the *Corethra* larvae can quite well occupy this zone in the daytime (cp. Valle, 1930, p. 488). — Finally there is the possibility that the reaction of the larvae to the light differs under different conditions, for instance, different food conditions. See the later experiments p. 79.

According to the literature, the limnetic distribution of the *Corethra* larvae over different seasons of the year and different times of the day, and the relation of the larvae to the bottom, varies much from lake to lake. So far the causes of this biological variation must be said to be almost unknown, but it is, indeed, only of late years that investigators have taken an interest in these phenomena. As late as 1914

WESENBERG-LUND (p. 16) could justly say that while the plankton of clear lakes perform vertical migrations at night, and ascend to the surface, no deep-lake *Corethra* larvae have as yet ever been met with there; nor, as he points out, has any search ever been made for them at that time.

FRANK E. EGGLETON (1931 b, p. 364) found *Corethra punctipennis* limnetically in Douglas Lake in the deep water layers, but not in the upper zones. In some years no larvae were found above the 20 m level, in other years they were found higher up, and could constantly be caught at levels below 10 m. Douglas Lake has several fairly independent depressions, and the vertical distribution of the *Corethra* larvae in these depressions differs, the maximum of the larvae occurring at very different depths. In Third Sister Lake EGGLETON also found *Corethra punctipennis* larvae in the free water layers and the larvae occurred at noticeably higher levels than in Douglas Lake. In Third Sister Lake the larvae were usually present up to depths of 5 metres, were frequently found 2 or 3 metres below the surface, and at times were taken even within the 0—1 metre station during the day. EGGLETON also found a seasonal variation in the limnetic distribution of the *Corethra* larvae in this lake.

In Lake Mendota the vertical distribution of *Corethra punctipennis* has been studied by JUDAY (1921, p. 275). He found that full-grown larvae were never found in the water in the daytime; both on cloudy and on clear days the full-grown larvae left the water layers. JUDAY has also shown that a vertical migration of the larvae takes place at night, the ascent reaching 23.5 m. Thus the observations from Lake Mendota show great similarity to the corresponding observations from Esrom Lake. The considerable vertical migration in Lake Mendota would take

place with great rapidity, viz. in the course of about an hour. The full-grown *Corethra* larvae ascended from the bottom at sunset and they entered the bottom by the end of the first half hour after sunrise. It should be noted that the downward migration was not due to direct sunlight, since it began at least half an hour before sunrise.

That the limnetic and the benthic distribution of *Corethra* larvae differ widely and are a complicated phenomenon is shown by further observations by Juday in Devils Lake, Wisconsin. He found there that the behaviour of the full-grown larvae of *C. plumicornis* Fabricius deviates in the daytime from that of *C. punctipennis* in Lake Mendota. The larvae in Devils Lake were substantially equally divided between the water and the mud on a bright morning when the water was very transparent. In other words, the day distribution of the larvae of *C. plumicornis* was practically the same in Devils Lake as the nocturnal distribution of the larvae of *C. punctipennis* in Lake Mendota.

A study of the hydrostatic mechanism of the *Corethra* larvae does not come within the scope of this work; but in connection with the extensive vertical migration shown to be peculiar to these larvae, and the consequent great variation in the pressure to which these animals are subjected, it will be natural to explain briefly how — according to the physiological works at hand — the adjustment to the varying pressures is supposed to take place. Details relating to this problem are to be found in works by Krogh (1911), Frankenberg (1915 and 1928b), Bardenfleth and Ege (1916), Akehurst (1922), Damant (1924) and Holst-Christensen (1928).

As is well known, the *Corethra* larvae are able to adjust themselves to variations in the flotation conditions. Krogh

was the first to show that if the animals are subjected to increased or reduced pressure, they are at first respectively too heavy or too light, but after some time they again float quite normally. This demonstration of a power of compensation has subsequently been confirmed by others. If a floating larva is weighted down by a ring of tinfoil, it will at first be too heavy, but after 8 days it again floats normally and keeps afloat for a long time in spite of its weight (FRANKENBERG, 1928b, p. 237). Under natural conditions a quite similar adjustment takes place. After a meal of small Crustaceans the *Corethra* larvae are a little too heavy, but shortly after again float normally (FRANKENBERG 1915, p. 560).

As an explanation of the *Corethra* larvae's power of adjustment to various pressures two hypotheses have been put forward, one by KROGH (1911) and the other by FRANKENBERG (1915). KROGH has analysed the air content of the tracheal bladders and found that normally it consists of about 16% O_2 and 84% N_2. Since this composition is not altered by adaptation to increased pressure, the readjustment cannot take place by means of an O_2 secretion as in the swimming bladder of fishes. Nor would this be possible, for KROGH has further shown that a levelling by diffusion easily takes place between the content of the tracheal bladder and its surroundings. KROGH therefore supposes that the tracheal bladders act like the ballast tanks of a submarine boat. If the animal becomes too heavy, water is pumped out of it, and if it becomes too light, it is pumped in, until equilibrium with the water is restored.

FRANKENBERG arrives at another view. He points out that the tracheal bladder of the *Corethra* has a relatively rigid wall, stiffened by the chitin lists usual to tracheae.

It is not completely rigid, however, but elastic; upon change of pressure it will yield to a certain extent, but again assumes its old size when the original pressure is restored. Chiefly on the basis of this elasticity of the tracheal bladder Frankenberg put forward the following hypothesis on its regulation mechanism. Upon excess pressure the tracheal bladder is diminished in size. Its wall, on account of its elasticity, tends to increase the volume of the bladder again. This gives rise to an under-pressure in the tracheal bladder, and air must be diffused into the tracheal bladder until the tension in the tracheal wall has disappeared and the bladder has been restored to its previous volume. In under-pressure on the larva the case is reversed: the bladder expands, but owing to its elasticity it tends to contract again, its air-content being thus subjected to a certain pressure. This has the effect of diffusing air out of the bladder, until the tension in the wall has disappeared, and the bladder is restored to its previous size.

As a regulation mechanism when the *Corethra* larvae have been exposed to increase of weight owing to absorption of nourishment, there will, according to Frankenberg (1915, p. 560 and 1928b, p. 237), occur an increase in the volume of the tracheal bladders due to imbibition.

We shall not here discuss the two hypotheses, but refer the reader to the aforementioned works by Damant, Frankenberg, Holst-Christensen, and to Jacob's report (1935). From a biological point of view there will, however, be occasion to offer the following remarks: The variations in pressure to which the *Corethra* larvae have been subjected in the experiments of Krogh and Frankenberg are rather small compared with the variations in pressure to which we now know the larvae are exposed during their vertical

migrations in waters of the depth of Esrom Lake. As already mentioned, the larvae in this lake migrate from a pressure of about 3 atmospheres (20 m) to about 1 atmosphere and back again, whereas in the above-mentioned experiments the animals were at most subjected to a pressure of about 2 atmospheres and usually less. On this subject KROGH (1911, p. 187) writes: "My experiments have been carried out only to pressures of + 1 atmosphere corresponding to a depth of 10 m below the surface of the water, but *Corethra* larvae, possibly belonging to other species, have been found to live near the bottom in lakes of much greater depth." KROGH realised, then, that in Nature *Corethra* larvae could be exposed to much larger pressures (cp. WESENBERG-LUND, 1914, p. 13) than employed in the experiments, but he could not at that time know that in Nature the animals are exposed to the above-mentioned great variations in pressure owing to their vertical migrations. Now that this is known, the correct explanation of the hydrostatic mechanism of the *Corethra* larvae should therefore be able to explain how the larvae can adjust themselves to variations in pressure of such a size, and likewise how such variations in pressure from 3 atmospheres to 1 atmosphere can be tolerated in the course of a very short time. The latter explanation is required on account of the rapidity with which the vertical migrations take place in Nature. JUDAY (1921, p. 275) states that a vertical migration covering 23.5 m may take place in about an hour in Lake Mendota.

Even with the relatively low pressures employed in the afore-mentioned experiments, it has been seen several times that the larvae could not tolerate the change. KROGH, for instance, states (1911, p. 187) that in experiments with larvae taken near the surface in a pond, he has found that,

when the pressure is raised to + 70 to 80 cm Hg, many animals fail to react in the normal way. They remain at the bottom, and, when the pressure is again reduced to the normal, they are still too heavy. A microscopical examination then discloses the fact that the air has disappeared from the sacs and they have become filled with a fluid; the animals die in a few days. It is possible that such injuries occur because the larvae have been exposed too suddenly to the changed pressure. Possibly they are due to the fact that in these cases pond forms were used for the experiments. At any rate, lake forms, according to observations in Nature can tolerate much greater variations in pressure, when these occur in not too short a period. For that very reason it is of considerable interest to note that lake forms, unlike pond forms, in experiments by Bardenfleth and Ege, have proved able to tolerate a pressure of about 4 atmospheres; after 2—$2^1/_2$ hours such larvae can attain equilibrium again. The explanation is that the tracheal bladders of the lake form are much more thick and therefore much more resistent to pressure than those of the pond form (Bardenfleth and Ege, 1916); the observations on the different thickness I can fully confirm.

A possibility of error in the study of the *Corethra* larvae from deep lakes may in my experience lie in the fact that a large and sudden change of temperature may have a destructive effect on the regulation of their equilibrium. Upon the sudden transference from lake water with a temperature of about 4° to room temperature in the aquarium it may happen that the animals lose their equilibrium and cannot — even in the course of several weeks — regain it. The disturbance shows itself in that they become too

light and so gather at the surface; only by strenuous swimming motions can they penetrate a little way downward into the water after which they are again carried passively to the surface. FRANKENBERG (1915, p. 524 and 538, 1927 a, p. 133) has also observed that *Corethra* larvae which for some reason or other have been injured, become too light. He supposes that this is due to an increase in the tracheal bladders owing to an imbibition of their walls. — That it is a sudden great change in the temperature which may injure *Corethra* larvae from deep lakes, and not a great change of pressure, appears from observations on larvae likewise transferred from the lake into the laboratory but placed there at a low temperature (5—10°). They all regained their equilibrium.

Experiments on the Phototaxis of the *Corethra* Larvae.

Since, according to the observations mentioned above, the reaction of the *Corethra* larvae to light in their diurnal migrations, seems to have an important bearing on their behaviour in Nature, the following experiments were made on such reactions. These experiments, however, will not be able to furnish the final explanation of the vertical distribution of the animals but can merely yield a contribution towards it. For, as will indeed appear from the above, factors other than the light, for instance the age of the larvae and specific differences, are concurrent causes of their reactions.

If *Corethra flavicans* larvae from Esrom Lake are kept in an aquarium, the bottom of which is covered by several centimetres of soft gyttja, it will be noticed that a number of the larvae have disappeared during the daytime; they have made their way into the mud. If the aquarium is shaken,

not a few come darting out of the mud, and if the larvae are now observed for some time, a number of them will again be seen to bury themselves. This is done by quick wriggling movements. First the head is pushed down, and after beating once or twice with the swimming fan, which still projects, the rest of the animal disappears. Often they burrow in a couple of jerks, pausing a moment, with the

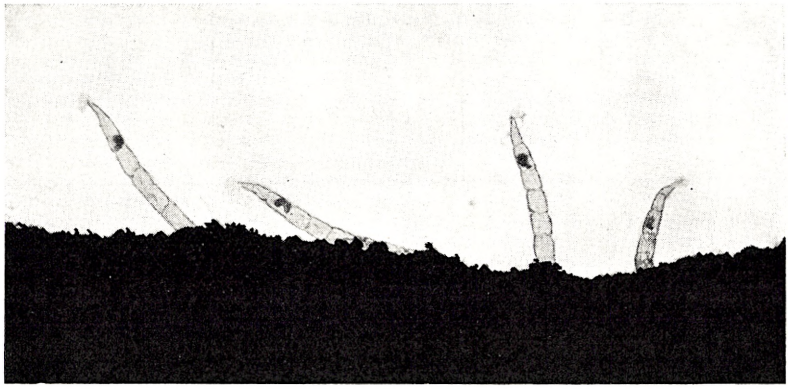

Fig. 22. *Corethra larvae* burrowing in the mud.

head and the anterior part of the body buried in the mud, while the rest projects obliquely into the water (fig. 22). The pause, however, merely lasts for a few seconds. In other cases they burrow without pausing, and in a very few moments the larvae have disappeared in the mud. Hence, despite their special morphologic and anatomic plankton characters, these larvae, by their inclination to seek cover under certain circumstances, show in their behaviour a biological similarity to many other diptera larvae; perhaps the *Corethra* larvae must thus be supposed to have preserved an original feature of their biology. The voluntary burrowing of the *Corethra* larvae in the bottom

of aquaria was already noticed by WESENBERG-LUND (1914, p. 17).

Now and then it is stated that *Corethra* larvae "rest upon the bottom" or that "they lie upon the bottom ooze during the daytime" (RAWSON, 1930, pp. 47 and 49). Observations such as the above show that they can bury themselves in the bottom ooze in the laboratory. Investigations with a stratification bottom sampler in Esrom Lake show that in Nature, too, they can bury themselves in the bottom; thus the idea that they lie on the bottom ooze is hardly correct.

The migration of *Corethra* larvae in aquaria, from the free water layers to the bottom and back again, has been utilised to study the bearing of the light on the migration under different conditions. Aquaria with a layer of gyttja at the bottom and a certain number of larvae have been set aside for several days, sometimes for some weeks, and the number of larvae in the water has been counted at various times of the day. The aquarium must be narrow so that the count can be made with sufficient certainty. The results from the days of the experiment have been summed up in a graph (e. g. fig. 23). In this the various hours of the day are plotted against the number of *Corethra* larvae observed moving freely in the water at these hours. The observations are marked by a cross. As a common expression of these a curve has been plotted which gives the average of the observations.

The curve has been constructed by means of smoothing according to the method of the successive media. By this method the mean is calculated of the 3 first observations a, b, c, in this way the first point of the smoothing curve is obtained. Then the mean of the three next observations

b, c, d, is calculated and the next point of the smoothing curve is obtained, and so on. The points of the smoothing curves are given as small circles.

Experiment 1. 20/XI—1/XII 33. About 40 larvae of

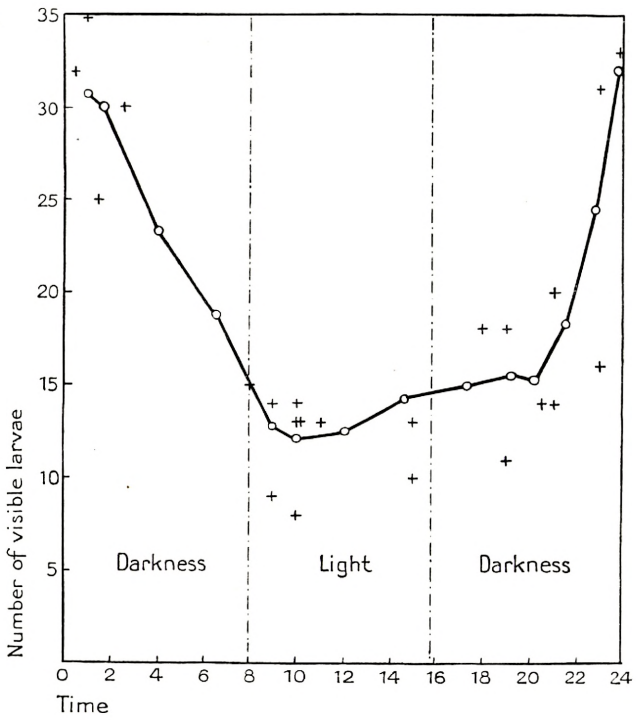

Fig. 23. Diurnal migration in an aquarium of *Corethra flavicans* from Esrom Lake. The aquarium has been exposed to the changes of the daylight in the course of the day.

Corethra flavicans from Esrom Lake were placed in an aquarium with a mud bottom. The aquarium was put in a cellar at a temperature of about 12—14°. It was exposed to the fluctations of the daylight in the course of the 24 hours.

The result of the experiment is shown in fig. 23, from which it appears (1) that there is at least always a small

number of larvae in evidence, (2) that few larvae are out in the water in the daytime, (3) that the greatest number of larvae are to be observed about midnight (late night observations are, however, lacking), and (4) that there

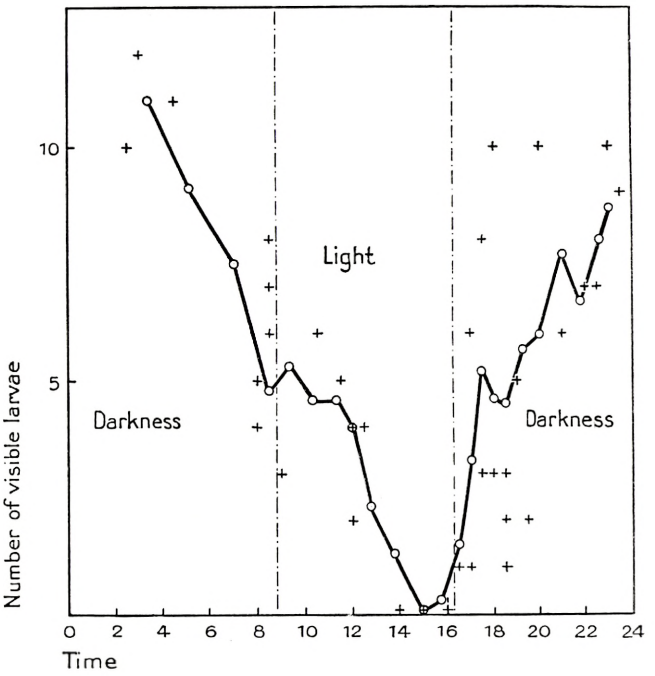

Fig. 24. Diurnal migration of *Corethra flavicans* in an aquarium. The larvae were taken at the surface of Esrom Lake.

consequently is a diurnal migration in the aquarium of *Corethra flavicans* larvae.

Experiment 2. 19/XII 33—10/I 34. In Experiment 1 the animals were not hydrostatically equilibrious, but had to prevent a slow rising by strokes of the swimming fan. Even after many days they were not equilibrious. Presumably the hydrostatic apparatus had been somewhat injured by the quick heating, when the animals were taken into

the laboratory. At any rate, the diurnal migration might be supposed to be affected by the lack of hydrostatic equilibrium. The experiment was therefore repeated with *Corethra* larvae which had been brought to a hydrostatic equilibrium. After being caught at the surface of Esrom Lake in the night, they were not taken into the laboratory but placed in an aquarium out-of-doors, at a temperature of about 1—5° C. 12 larvae were lodged in the aquarium.

The result of the experiment will appear from fig. 24 which confirms the above-mentioned observations on the diurnal migrations; the latter are in this case even more conspicuous. Thus several times in the day no larvae at all were seen or only a single one was in evidence, while several times in the night nearly all the animals were observed in the water.

Experiment 3. 16/XII 33—10/I 34. After *Corethra* larvae exposed to the light of day and the darkness of night have been seen to perform diurnal migrations under these conditions, the question arises whether it is the daylight that regulates their diurnal migration. In order to obtain a reply to this question, the aquarium with the animals from Experiment 1, after this experiment was over, was covered with a black cloth, the animals being thus completely in the dark for 24 hours. Only when it was to be ascertained how many larvae were out in the water was the cloth removed, and a count taken as quickly as possible.

The result is seen in fig. 25. This shows (1) that at all times of the day and night some individuals were out in the water, but that the number varied a great deal. More than half of the individuals were never to be seen. (2) The varying number of visible individuals shows that an upward

migration and a downward migration of the *Corethra* larvae constantly takes place in the dark. (3) But the quite irregular occurrence now of many, now of few individuals,

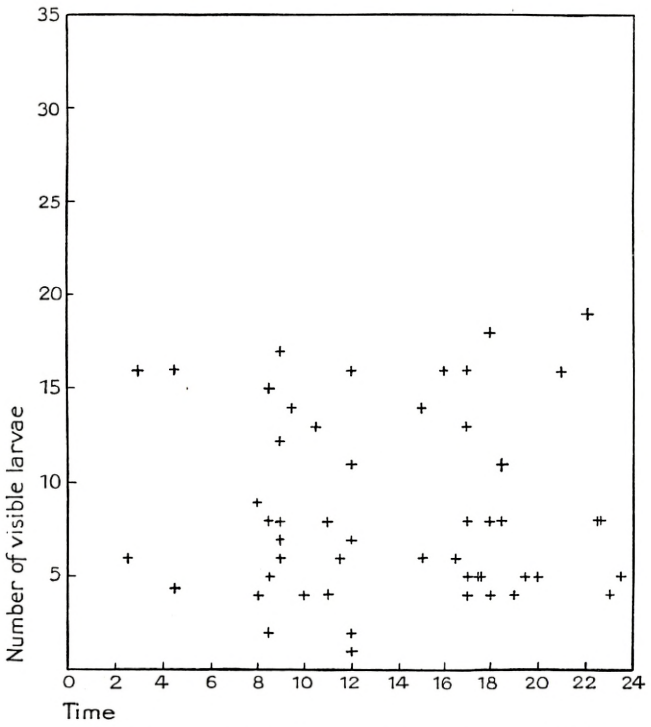

Fig. 25. The figure shows the number of larvae of *Corethra flavicans* swimming freely in the water layers of an aquarium at various times of the day. The aquarium has been covered with a black cloth. The number varies irregularly.

shows that no regular diurnal migration is observable in the dark.

It might perhaps have been expected at the outset that all the *Corethra* larvae, about 40 individuals, would have come out into the dark water when no light whatever prevented them; this they did not do. Or they might all have been expected to remain at the bottom. The fact

that now many, now few, animals were in evidence in the water would seem to indicate that the *Corethra* larvae possess the tendency to migrate vertically to such a degree that they need no stimulus — changing light and darkness — for the tendency to manifest itself. But on the other hand the lack of regularity in the vertical migration in the dark with the regular migration during the change from daylight to darkness (figs. 23 and 24), shows that it is the light which is the regulating factor in the diurnal migrations of the larvae of *Corethra flavicans.*

Experiment 4. 26/I 34—13/II 34. A further demonstration of the causal significance of the light for the diurnal migrations of *Corethra* larvae from Esrom Lake was made in an experiment in which an aquarium with larvae was covered with a black cloth in the day and lighted by strong electric light at night. Thus the illumination of the animals was the opposite of what was natural. The darkness in which the larvae were left in the daytime was only interrupted for brief periods when the cloth was removed to make the count. Temperature 12—14° C.

The result of this experiment appears in fig. 26. This shows that the natural diurnal migration may be reversed, so that many larvae appear in the water in those hours of the day which have been artificially changed into "night", and conversely, few or none appear in the night which has been changed by illumination into an artificial "day". This further confirms that it is the light which determines the diurnal migration for these *Corethra* larvae.

Experiment 5. 28/XI—25/XII 33. Pond forms of *Corethra* are found in the daytime in the free water layers of the ponds. Such larvae might therefore be expected to show quite a different reaction to the influence of the light

than the larvae of *C. flavicans* from Esrom Lake. In order to confirm this conjecture 35 *Corethra* larvae from Sorte Dam, Hillerød, were placed in an aquarium at a temperature of 12—14° C. and were observed as usual. The aquarium was exposed to the ordinary fluctations of the daylight.

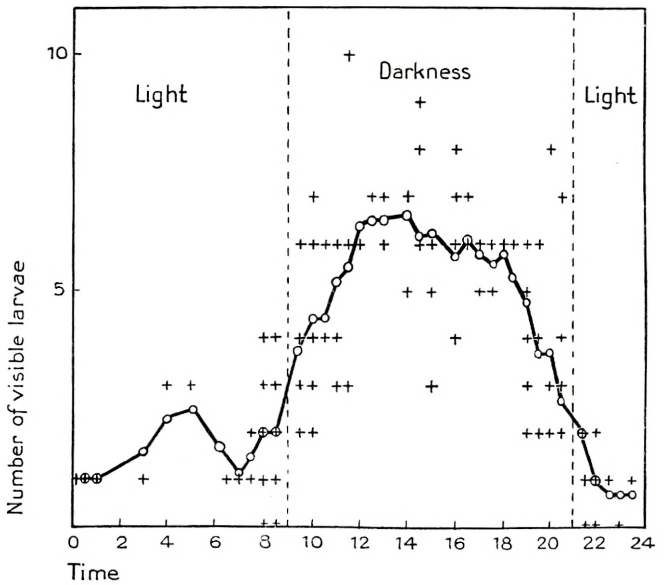

Fig. 26. Diurnal migration of *Corethra flavicans* in an aquarium placed in the dark in the daytime and in the light at night.

In Sorte Dam the larvae can always be taken in the water in the daytime with a net.

The result of the experiment is seen in fig. 27, which shows that the *Corethra* larvae from Sorte Dam performed no diurnal migrations whatever. They showed no reaction to the fluctuations in the intensity of the light. The fact that the full 35 larvae were not found in each count is due to the difficulty of carrying through the count with precision, and to the pupation of 1—2 larvae during the experiment.

At the time when this experiment was made, it was not possible to carry through a determination of the species of the larvae in the experiment. After the appearance of PEUS' work (1934), the larvae could, as previously mentioned,

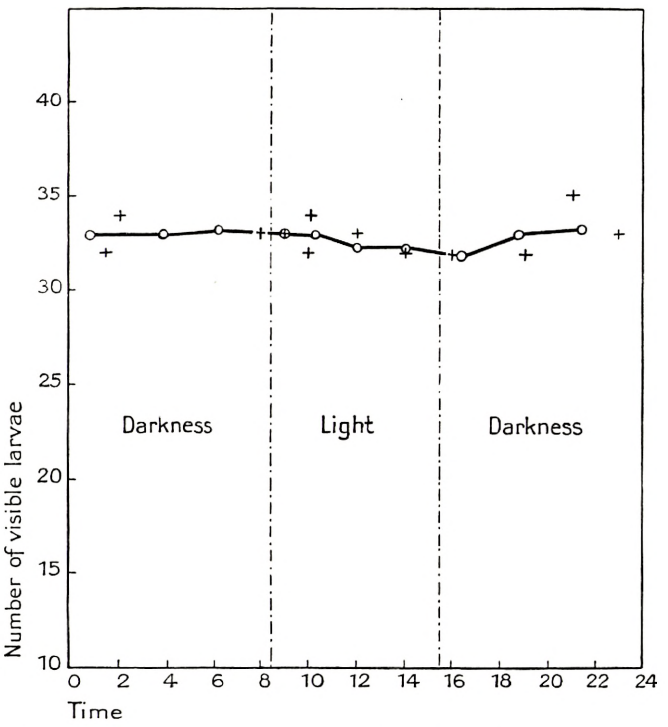

Fig. 27. The curve shows that there is no diurnal migration of larvae of *Corethra* from Sorte Dam. The aquarium has been exposed to the changes of the daylight in the course of the day.

be determined. It then turned out that Sorte Dam, as stated on p. 11, contained 2 species of *Corethra*, *C. crystallinus* and *C. flavicans*. The experiment was therefore repeated on the 15/IV—19/IV 35, in order to ascertain whether one — and which — or both species performed no diurnal migration. For the repeated experiment 22 larvae from

Sorte Dam were used; the result was as shown above; they showed no reaction whatever to the change from day to night, but 20—22 individuals were always present in the water. Then the larvae were isolated, and on determining the species of the larvae, or of the pupae or imagines into which they had been metamorphosed, or of both, it was found that 17 of the larvae belonged to *flavicans* and 5 to *crystallinus*.

This result is rather remarkable and may suggest certain considerations. Thus about $^3/_4$ of the larvae in Sorte Dam were *flavicans* larvae, that is to say, they belonged to the same species which inhabits Esrom Lake. But the larvae from Sorte Dam were unaffected by the light and showed no diurnal migration in experiments, whereas the larvae from Esrom Lake were negatively phototactic and performed diurnal migrations in experiments and in Nature. The same species, *C. flavicans*, thus reacts in one way from one locality, and in another way from another. In other words, we have here come across a biologic (ecologic) variation. This phenomenon, interesting in itself, might well prompt further investigations of such problems as what is the cause of such a biological variation. Is it due to the environment which is so different in the two localities, and are the two populations of *C. flavicans* in Esrom Lake and Sorte Dam thus only phenotypically different? Or is the biological difference hereditary, and is the species divided into subspecies differing biologically? In this connection it will be worth while to recall the morphological variation of the species which was mentioned on p. 11. And is the different phototaxis of any biological importance for the 2 populations? — Such questions, which require a special investigation, it has not hitherto been possible to answer.

The experiments at hand have merely been of a preliminary character. But the questions show how little known a comparatively familiar animal such as the *Corethra* larva is in respect of causal ecological conditions.

It was found by Valle (1930, p. 488) that larvae of *Corethra* "haben sich nicht auffallend lichtscheu gezeigt" (cp. Lundbeck, 1926, p. 183, Lang, 1931, p. 49). Experiment 5 shows that such a view may be right in some cases, but the other experiments prove that it has not a general validity.

Experiment 6. 17/III—8/IV 35. By means of further experiments with *C. flavicans* larvae from Esrom Lake some more knowledge of their phototaxis was gained. When the diurnal migration of the larvae in the lake was mentioned, it was pointed out that at night only a fraction of the larvae at a time had been found to migrate upwards into the water layers; more thorough investigations by Juday (1921, p. 276) showed the same thing. Hence there might possibly be a lack of uniformity within the population, certain individuals showing a greater tendency to migrate than others. A comparison of the migrations mentioned in fig. 23, of the larvae which were caught in the bottom of the lake, with those shown in fig. 24, whose corresponding larvae had been caught at the surface of the lake, would also seem to show that the tendency to migrate is strongest in the latter. In order to try whether there was really such a difference in the susceptibility of the larvae to the influence of the light, 2 simultaneous experiments were made in the usual way, the larvae being exposed to the natural change of light from day to night. The two aquaria were treated the same in every respect, but in the one there were placed 20 larvae taken in the bottom of Esrom Lake, and in the other 20 larvae taken in the night at the surface of the lake.

The result of the experiments was that the larvae in the two aquaria showed the usual diurnal migration with quite the same degree of intensity; hence benthic and pelagic larvae must be assumed to have the same susceptibility to the influence of light. The fact that only a fraction of the larvae in Nature at a certain time take part in the great vertical migration cannot then be caused by dissimilar biological characters in the larvae, but must be due to some other cause.

Experiment 7. 27/IV—7/V 35. In experiment 1—6 the *Corethra* larvae had not as a rule been fed in the experimental period, but only afterwards. It had then occasionally been observed that the reaction of the larvae to the influence of light seemed to have changed after the consumption of food. In order to verify this conjecture an experiment was made in which the vertical migration of some larvae which had been fed very plentifully was compared with that of larvae which were not fed during the experiment. The larvae — *C. flavicans* from Esrom Lake — which were to be fed were placed in 2 aquaria, 25 in each. To the larvae in aquarium I plenty of *Crustacea* plankton was fed, consisting chiefly of *Cyclops*; those in aquarium II were fed plenty of *Diaptomus castor*. Finally 25 larvae were placed as controls in aquarium III and were not fed at all. The aquaria were alike; at the bottom they had a layer of gyttja several contimetres deep, and they stood beside each other in a window exposed to the changes of light during the day and the night.

The result of the experiment will appear from fig. 28 which shows the migration in aquarium I, and fig. 29 which shows the migration in aquarium II. In both figures the migration of the controls in aquarium III is given for

comparison. From fig. 28 it appears that there are constantly about 20 larvae in the water, even in the daytime. From fig. 29 it will be seen that there are 14—18 animals in the water in the daytime and a few more at night. The

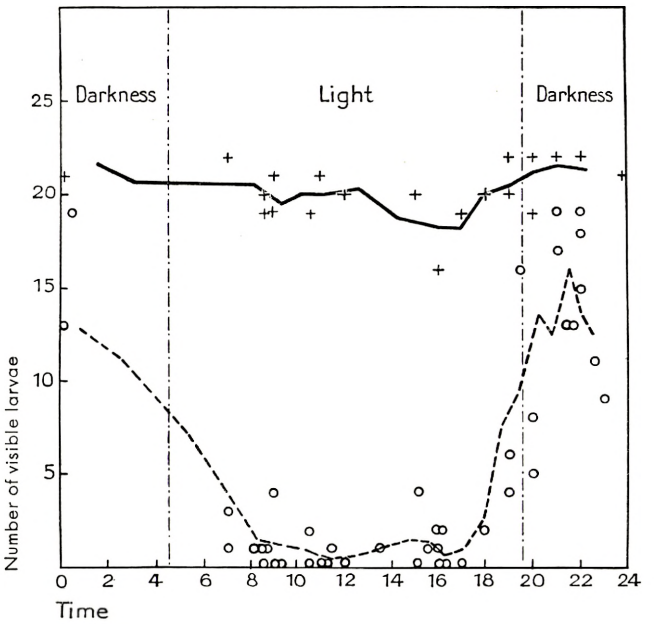

Fig. 28. The continuous curve shows the absence of a diurnal migration among larvae of *Corethra flavicans* fed on plankton (*Cyclops*). The dotted curve shows the diurnal migration of control animals not fed. The aquaria were exposed to the changes in the daylight in the course of the day.

controls show the usual thing: there are no animals or hardly any in evidence in the daytime, but a large number have migrated upwards in the night. In other words, the experiment shows that the catching of abundant prey may greatly affect the diurnal migration of *Corethra* larvae, either entirely stopping it (aquarium I) or much reducing it (aquarium II). And the change is evidenced by the fact

that the larvae which have taken plenty of food are not so negatively phototactic that they bury themselves in the mud in the daytime.

Thus, according to this experiment, the importance of

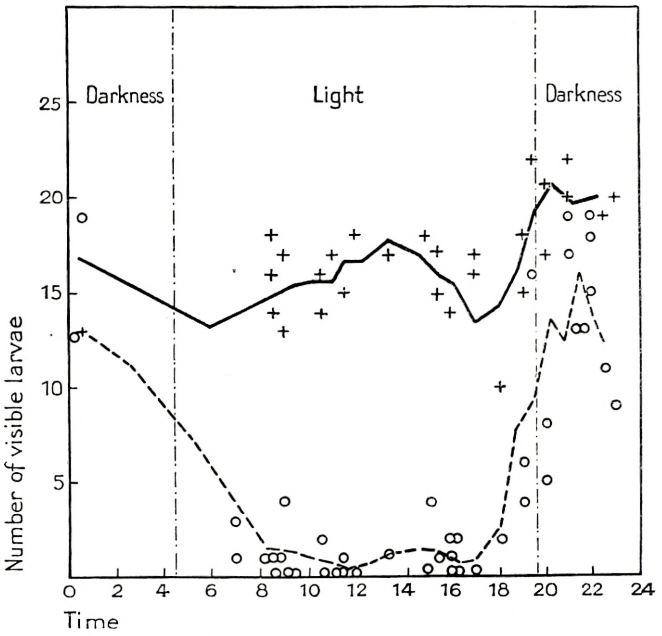

Fig. 29. The continuous curve shows the approximate absence of a diurnal migration among larvae of *Corethra flavicans* fed with *Diaptomus sp.* The dotted curve shows the diurnal migration of controls not fed. The aquaria have been exposed to the changes in the daylight in the course of the day.

the amount of food consumed for the phototactic reaction of the *Corethra* larvae would seem to be very considerable. This is not without interest. It opens up a possibility of another explanation of the seasonal variation in the diurnal migrations of the *Corethra* larvae than that so far offered. As mentioned on p. 59, it has been shown that the *Corethra* larvae in Frederiksborg Castle Lake in midsummer occupy

the water layer immediately above the bottom in the daytime, whereas in spring and autumn they are found in large numbers in the bottom itself. Various potential causes of such a change in the vertical distribution of the larvae have previously been pointed out. To these must now be added the possibility that the reaction of the larvae to light may change under different conditions of nutrition. In the summer their negative phototaxis may, then, possibly be reduced, on account of their larger consumption of food, and consequently they remain in the daytime in the water layer near the bottom, not penetrating into it. It is clear that this explanation of the cause does not exclude the possibility of those previously given, but may quite well supplement them.

This experiment on the bearing of the amount of nourishment on the vertical migration was confirmed by several others.

Experiment 8. 18/VII—23/VII 35. Besides the amount of nourishment there seem to be other factors which affect the vertical migrations of the larvae. This applies, for instance, to the age and size of the larvae. If young, newly hatched larvae are kept in an aquarium in the usual manner, in order to find out whether they perform vertical migrations, it turns out that their reaction is quite different from that of the adult larvae. Fig. 30 shows the result of such an experiment with 25 newly hatched larvae of *C. flavicans* from Esrom Lake. At the beginning of the experiment the larvae had only a size of about 1.7 mm. The figure shows that the young larvae, unlike the majority of the adults, are in the water even in the day; no diurnal migration at all was ascertained for these young larvae in this experiment. (The fact that the full 25 larvae were not found

in each observation may be due to an error of observation; it is very difficult to count the tiny hyaline larvae without overlooking some.)

Thus the reaction of the newly hatched larvae is similar

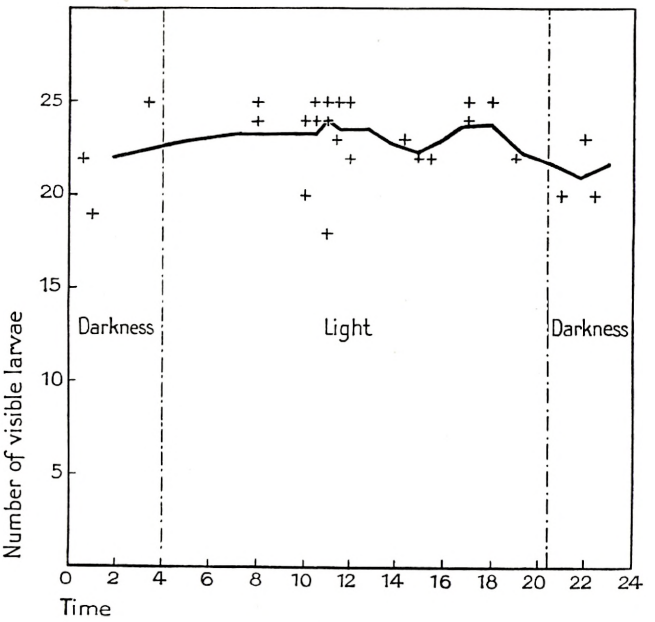

Fig. 30. The curve shows that there is no diurnal migration of newly hatched larvae of *Corethra flavicans* from Esrom Lake. The experiment was made in July. The aquarium has been exposed to the changes in the daylight in the course of the day.

to that shown by larvae which have consumed a plentiful supply of food (cp. fig. 30 with figs. 28 and 29). It is therefore not without interest to recall in this connection that the young larvae, when they leave the egg, may still have inside them some drops which are the remains of the yolk mass of the egg (see p. 44). Thus it is possible that the reason why the young larvae react like the old ones which

have consumed a plentiful supply of nourishment is that they have a reserve stock of food, brought from the egg; the remain of the yolk mass disappears later on.

Experiment 9. 23/VII—27/VII 33. If young larvae are kept in vessels, a number of them will migrate towards the light in the vessels; this applies especially to newly hatched larvae. If half of a long aquarium, e. g. the left part, is covered with a dark cloth, and newly hatched *Corethra* larvae placed in it, the larvae will nearly all collect in the uncovered, right part of the aquarium illuminated by the daylight. Hence they are positively phototactic. The dark part of the aquarium is nearly empty, only some few of the small larvae are seen there.

At night, when the uncovered right part of such an aquarium is also in the dark, the larvae disperse evenly over the covered and uncovered parts. Thus there is a certain migration in the aquarium in the course of the 24 hours, owing to the influence of the light on the larvae. The diurnal migration in this experiment is, however, horizontal, unlike the diurnal migration of the adult larvae in Nature. And the newly hatched larvae in the experiment seek the daylight. The larvae in this experiment, like the others in experiment 8, were only about 1.7 mm.

Positive phototaxis in young larvae has previously been mentioned by Frankenberg (1915, p. 547), who, however, only observed a strong positively phototactic reaction in larvae which had not yet filled their tracheal bladders with air; larvae with the tracheae filled with air no longer showed a phototactic reaction in his experiments. Frankenberg calls these phototactic conditions strange and says that perhaps the phototaxis is intended to carry the larvae to higher water levels richer in oxygen. Since Franken-

BERG's *Corethra* larvae were derived from a small pool, it is very likely that they have soon lost their phototaxis. As we know, pond forms have no phototaxis (Experiment 5, p. 75). But the young larvae from Esrom Lake at any rate retain their positive phototaxis for some time — probably for several days — and its biological significance is no doubt another than that pointed out by FRANKENBERG, viz. that of keeping the larvae in the upper water layers, so that they may spread from the hatching zone by the shore and be carried into the deep water in midlake by the circulating currents.

For comparison with the reactions of the young larvae some adult larvae and some pupae (from Frederiksborg Castle Lake) were placed under observation in a similar aquarium half covered by a dark cloth. The result for the full-grown larvae was exactly the opposite of that for the young larvae. In the daytime the adult larvae all or nearly all migrated to the covered, dark part of the aquarium; hence they were negatively phototactic. In the night, when it was equally dark throughout the aquarium, they dispersed evenly over the whole of it. Hence there arose, in this case too, a horizontal diurnal migration, but the difference manifests itself in the fact that the adult larvae seek out the dark half of the aquarium, whereas the young larvae migrate to the illuminated part. The experiment was confirmed by repetition.

Experiments with pupae gave the same results as with full-grown larvae. The negative phototaxis of the pupae was, if possible, even more pronounced. In the daytime they went to the darkest corners of the covered half of the aquarium.

Has the Phototaxis of the *Corethra* Larvae any Bearing on their Diurnal Migrations in Nature?

On reviewing the above-mentioned experiments it appears that *Corethra* larvae which are not newly hatched have a very conspicuous negative phototaxis. It likewise appears that owing to this character diurnal migrations may occur in aquaria which bear a great resemblance to the migrations observed in Nature. It therefore seems natural to assume that it is a negative phototaxis which causes the diurnal migrations in Nature, even though certain factors (nourishment, age) may modify it or cause it to cease.

C. JUDAY (1921, p. 278) has, in fact, considered this idea after observing that larvae of *C. punctipennis* give a prompt negative reaction to light. But he rejects it, thinking it hardly probable that the extensive depth migration of the larvae in Lake Mendota, even including a descent into the mud, is a simple light phenomenon. The rejection of this potential explanation is motived by JUDAY by the following observations and conclusions.

The transparency of the water in Lake Mendota is usually low in summer; a white disc 10 centimetres in diameter generally disappears from view at a depth of two metres to about four metres at this season of the year, which indicates that the light is cut off rather rapidly by the upper strata of water. A pyrlimnimeter has been used to determine the rate at which the sun's energy is cut off by the upper strata of the lake. The results obtained with this instrument indicate that the intensity of the illumination at a depth of 23 metres on a clear day, between 11:00 a. m. and 1:00 p. m., is about equal to that produced by full

moonlight at the surface of the lake. During the early forenoon and late afternoon, as well as on cloudy days, the illumination is much smaller than this. For some time before sunset, the bottom stratum must be substantially in total darkness, yet the observations show that the emergence of the larvae from the mud is very closely correlated in time with the setting of the sun.

One more objection is added by JUDAY who remarks that not only does the illumination in the bottom water become very small in the late afternoon, but there is a further protection from light afforded by the bottom ooze, in which the larvae remain concealed during the day. To what depth the larvae penetrate the loose mud is not known, but in the laboratory they readily burrow down to a depth of a centimetre or more. The dim light which reaches the bottom in the deeper portions of the lake can penetrate the ooze only to a very slight extent at most, even during the brightest part of the day, and this raises the very interesting question as to what stimulus causes the larvae and pupae to emerge from the mud so promptly and regularly about the time of sunset. No definite data bearing on this point have yet been obtained.

As to this last objection by JUDAY to the possible significance of the light for the diurnal migration it must be remarked that it is based on the quite correct fact that the *Corethra* larvae may be concealed in the mud in the daytime. JUDAY, when writting the above, was unable to know to what depths the larvae descend into the mud of the lake; but now it is shown by means of a stratification bottom sampler that they probably penetrate several centimetres into the bottom of Esrom Lake. Variations in the dim light which reaches the bottom can therefore hardly be felt by

the larvae when they are buried in the mud. Hence I too think it improbable that the decreasing light should be the stimulus which makes the larvae emerge from the mud. This view receives further support from the result of Experiment 3 (p. 72); for this experiment showed that in complete darkness there were now many, now few *Corethra* larvae in the water. Hence the *Corethra* larvae possess the tendency to ascend to such a degree that it even manifests itself under these conditions, and thus it is not the varying intensity of the light that makes them come out. It must be another, unknown, stimulus which makes now a few, now many larvae emerge from the mud.

But this does not mean, of course, that the presence or absence of light cannot be a regulating factor in the migrations of the larvae which for some unknown reason have emerged from the mud in greater or less numbers. On the contrary, the above-mentioned experiments seem to me to show so plainly the regulating effect of variations in light on the diurnal migration of *Corethra* larvae in aquaria that it must be considered highly probable that something similar is the case in Nature.

However, I do not on that account deny the value of Juday's first-mentioned objection that the absorption of light in lake water is so high that the illumination near the bottom is extremely small, especially during the early forenoon and the late afternoon, and that it is therefore difficult to imagine that so slight an amount of light can make its influence felt. The facts to which Juday refers are no doubt quite correct. But it must be noted that so far nothing is known about the size of the threshold value which the light must have in order to cause a reaction in *Corethra* larvae. It is possible that this threshold value is

also very low, and that the animals react vigorously to its influence. There are problems here, that call for treatment, and the solution of them might supplement the above-mentioned preliminary experiments on the phototaxis of the larvae. As long as we do not know the size of the threshold value for light-intensity which may affect *Corethra* larvae, and so cannot compare this threshold value with the small light-intensity found in the water layers near the bottom of the lake, so long we cannot either reject the possibility that it may be the variations of the light which are the regulating and determining factor in the diurnal migrations of the *Corethra* larvae. And, as previously mentioned, such a possibility receives strong support from the experiments on the phototaxis of the *Corethra* larvae. —

Nourishment of the Larvae.

It is a well-known fact that *Corethra* larvae are voracious beasts of prey which especially catch and swallow small Crustaceans. Their voracity has often been mentioned by earlier authors (Leydig, 1851, p. 449; Rymer Jones, 1867, p. 100; Weismann, 1866, p. 7; Pouchet, 1872, p. 224, and Meinert 1886, p. 409 etc.) and has subsequently been frequently confirmed. The *Corethra* larvae in Esrom Lake being benthic in the daytime and partly limnetic at night, it would seem probable at the outset that they can only during the latter time secure food amongst the plankton Crustaceans. In order to ascertain whether the *Corethra* larvae took nourishment within some limited part of the 24 hours only, the alimentary canal of the animals was examined at various hours, both in animals taken in the daytime in the bottom, and in such as had been caught

in the night in the free water layers. The larvae were partly examined while alive shortly after being caught, and partly in the conserved state. Whether they were caught at one time or another, however, the numerous samples showed that in a great many of them the alimentary tract was empty, and the rest, whose intestine contained a yellowish liquid, could be taken both benthically and limnetically, and at any time within the 24 hours. Thus no definite time for the consumption of food could be established.

When a *Corethra* larva has not taken any food for some time, its empty alimentay canal is very hyaline, and the cells of its wall clear. After the consumption of food the alimentary canal is filled with a yellowish or reddish fluid (cp. Meinert, 1886, p. 409), and the walls of the intestine shortly afterwards become opaque with the nourishment absorbed.

The consumption of the food is not difficult to observe in aquaria. If some *Corethra* larvae have starved for some days, and some zoo-plankton is then put into the aquarium, larvae may shortly afterwards be seen with Daphnids or *Copepoda* in the mouth parts; some of the prey projects freely from the mouth. After a little while a great number of larvae have the anterior part of the alimentary canal (the pharynx) from the mouth to a point on a level with the anterior pair of tracheal bladders filled with prey (fig. 31). The prey as a whole passes no further. At the point on a level with the tracheal bladders there is a closing mechanism which only allows liquid nourishment to pass into the intestine. Chitinous parts are retained in the anterior part. The figure shows a larva which has swallowed two *Copepoda*. The first one has passed as far down the intestine

as is possible, it is already partly dissolved. The last one has not yet passed wholly into the mouth.

That the chitinous parts do not pass right down into the intestine appears not only from the fact that the latter always only contains the yellowish liquid nourishment, but it can also be shown by isolating in small vessels *Corethra* larvae which have just swallowed some *Copepoda*. Some time after the isolation of such larvae, the chitinous skeleton

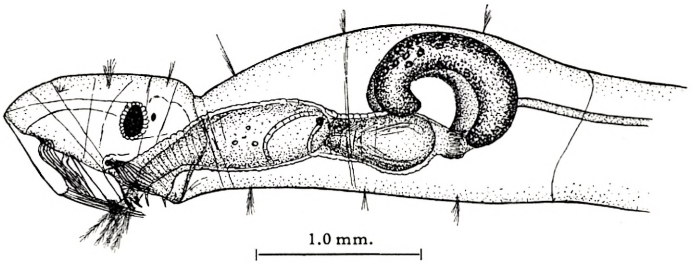

Fig. 31. *Corethra* larva which has swallowed 2 *Cyclops* for one of which there is not quite room enough in the anterior intestine.

of the *Copepoda* may then be found at the bottom of the vessels. After the contents of the *Copepoda* in the liquid state have passed down into the intestine of the larva, the rest, the chitinous skeleton, is brought up.

Since the researches of LEYDIG (1851, p. 443) it is known that the anterior part of the intestinal canal, the pharynx, is important in a physiological respect, a partial digestion of the food taking place there. In the procedure of vomiting the pharynx may be everted, and hang out of the mouth like a long tongue (WEISMANN, 1866, p. 9); later it can be drawn in again. If *Corethra* larvae are exposed to pressure the pharynx is also everted. The same thing may happen if larvae are conserved in a very strong formalin. FRANKENBERG (1927 a, p. 127) states that it may happen

that the animal is not able to draw in the pharynx again, so that it continues to hang out of the mouth. This seems especially to happen when the eversion has taken place with such violence that the thin oesophagus, the link between the pharynx and the intestine proper, is torn to pieces. But otherwise the drawing in of the pharynx occurs by the *Corethra* larva "eating" its own pharynx; for the larva conveys the pharynx into its body by exactly the same movements of the mouth parts which it uses when prey is swallowed. The normal vomiting of the chitinous remains through the pharynx takes place according to Leydig by a partial or even a complete eversion of the pharynx. According to Frankenberg (1927 b, p. 76; 1928 a, p. 58) the pharynx retains its normal position and the vomiting is due to peristaltic movements.

In addition to Daphnids and small *Copepoda* the *Corethra* larvae are also able to catch *Diaptomus*, as has previously been mentioned under Experiment 7 (p. 79). Of still larger animals I have on one occasion seen a larva catch a *Leptodora kindtii*. Meinert (1886, p. 409) gives some examples of how *Corethra* may catch rather large prey. He has, for instance, seen them swallow various larvae of *Diptera*, such as a *Dixa* larva which was nearly half the size of the *Corethra*; the *Dixa* filled the whole anterior part of the *Corethra's* alimentary canal, and partially hung out of the mouth. He has also seen a *Corethra* larva swallow another adult larva, the head of the latter being in the anterior part of the former's intestine, and in this part there were besides a *Cypris* and parts of another *Corethra* larva. — Meinert thinks that large Daphnids such as *D. pulex* and Cyprids constitute the chief food of *Corethra*. This is probably correct for *Corethra* larvae living in ponds. For larvae in-

habiting such large lakes as Esrom Lake the *Copepoda* of the plankton probably form the greater part of their food (cp. LUNDBECK, 1926, p. 184; LANG, 1931, p. 50).

Summary.

It is shown that the *Corethra* species in Esrom Lake is *C. flavicans*. The same species has been found in Frederiksborg Castle Lake and Sorte Dam near Hillerød; in the latter locality *C. crystallinus* has also been found. A study of the chief characters (figs. 1—11) showed a certain morphological variation in the larvae and pupae.

The Danish localities of *C. flavicans* and the German localities (shallow inundation pools) found by PEUS (1934) together show that the species has a much larger range of biological variation than could formerly be known from the German findings alone. The larvae show a widely differing periodicity corresponding to the nature of the localities.

The benthic distribution of the *Corethra* larvae in Esrom Lake is characterised by a numerous occurrence at 20 m (about 1290 individuals per sq. m) and a rapid reduction in numbers towards land (fig. 19, p. 48).

At Esrom Lake the swarming period is in July and the beginning of August. The eggs (figs. 16—18, pp. 40—44) are deposited near the shore, especially in shallow water, where *Potamogeton pectinatus* and masses of filiform algae touch the surface. A recurrence of the *Corethra* larvae, in which they are first found freely in the water near the shore, then scattered over the lake and pelagically, but not in the bottom and not near the shore, and finally in the bottom ooze, is characteristic of the species in Esrom Lake.

The relation between the benthic and the limnetic behaviour of the *Corethra flavicans* larvae in Esrom Lake, may be described thus: The larvae do not occupy the free water layers at all in the daytime (apart from the pelagic occurrence of the youngest stages in August-September). The larvae hardly stand directly above the bottom in the daytime, but by far the greater part, at any rate, are found in the bottom.

Near sunset and near sunrise only few *Corethra* larvae were found in the free water layers, and none near the surface. At certain hours well on in the night the larvae were found in all the water layers of the lake from the surface to close to the bottom. Near midnight the larvae were found in the upper water layers, but not in the water layers near the bottom (fig. 20, p. 55).

The numerous occurrence in the upper free water layers about midnight was also demonstrated, when practically all the population had been changed into pupae.

A migration of *C. flavicans* to the upper water layers in the night has also been demonstrated in Frederiksborg Castle Lake, for larvae as well as pupae (fig. 21, pag. 57). In most months the larvae of *C. flavicans* cannot be taken in the free water layers of this lake in the daytime, but in the bottom. In midsummer, however, they are found in the daytime in the water just above the bottom.

Experiments on the phototaxis of the *Corethra* larvae yielded amongst other things the following results: There is a diurnal migration of *C. flavicans* larvae from Esrom Lake in an aquarium exposed to the fluctuations of the daylight; few larvae are out in the water in the daytime, the greatest number of larvae are to be be observed about midnight (figs. 23, 24, pp. 70—71).

In an aquarium left entirely in the dark it turned out that an uppward migration and a downward migration of the *Corethra* larvae constantly took place, but no regular diurnal migration is observable (fig. 25, p. 73).

An aquarium was covered with a black cloth in the day and lighted by strong electric light at night. Many *Corethra* larvae appeared in the water in those hours of the day which had been artificially changed into "night" and conversely, few or none appeared in the night which had been changed into an artificial day (fig. 26. p. 75). Thus the natural diurnal migration may be reversed.

The conclusion drawn from these experiments is that it is the light which determines the diurnal migration of these *Corethra* larvae; they are negatively phototactic.

In experiments pond forms of *Corethra* from Sorte Dam (*C. flavicans* and *C. crystallinus*) performed no diurnal migration whatever (fig. 27, p. 76). The same species, *C. flavicans*, thus reacts in one way from one locality (Esrom Lake) and in another way from another locality (Sorte Dam).

The fact that only a fraction of the *C. flavicans* larvae in Esrom Lake at a certain time take part in the vertical migration, cannot be caused by dissimilar biological characters in the larvae; in experiment benthic and pelagic larvae showed diurnal migration with quite the same degree of intensity.

The reaction of the *C. flavicans* larvae from Esrom Lake to the influence of light is changed after the consumption of food. The catching of abundant prey may greatly affect the diurnal migration, either entirely stopping it (fig. 28, p. 80) or much reducing it (fig. 29, p. 81).

The age or size of the *C. flavicans* larvae affects the

reaction to the influence of light. Young larvae (size about 1.7 mm) showed no diurnal migration at all in the usual experiment (fig. 30, p. 83). They are positively phototactic.

On reviewing the above-mentioned experiments and the observations in Nature it seems natural to assume that the negative phototaxis is a contributory cause of the diurnal migration in Nature of the *Corethra* larvae; certain factors (nourishment, age) may modify it or cause it to cease.

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TRÆK AF SPÆTTERNES BIOLOGI

AF

AD. S. JENSEN

KØBENHAVN
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EJNAR MUNKSGAARD
1937

Printed in Denmark.
Bianco Lunos Bogtrykkeri A/S.

Denne lille Afhandling er udarbejdet paa Grundlag af nogle Iagttagelser, som jeg har haft Lejlighed til at anstille i Naturen, og hvorved jeg har kunnet gaa visse Angivelser i Litteraturen kritisk efter.

I Aaret 1894 offentliggjorde Professor COLLETT en lille Afhandling med Titel: »Spetterne og Telegrafstolperne«[1], øjensynlig forfattet ikke paa Grundlag af egne Iagttagelser, men paa Oplysninger, som han havde indhentet hos Telegrafintendanturen.

I Afhandlingen berettes, at i Norge borer de større Spættearter, især Grønspætten (*Picus viridis*), i mindre Grad Sortspætten (*Dryocopus martius*), ofte Huller i Telegrafstolperne. Disse Huller anbringes næsten altid henimod Stolpens Top, og der kan være indtil et Dusin i hver Stolpe; en Del af Hullerne er ganske smaa, andre noget dybere, og enkelte danner ligefrem en Gang gennem Stolpen. Der gøres nærmere Rede for Forekomsten og Omfanget af denne ejendommelige Spættevirksomhed i Landets forskellige Egne. Medens nogle Distrikter synes at gaa ganske fri for Spætternes Angreb, har andre vist sig særlig udsatte. Det synes, som om enkelte Stolper eller Stolper paa visse bestemte Strækninger lokker Spætterne til. Ved

[1] Naturen, 18. Aargang, p. 154—156.

en Gaard ovenfor Mandal viste saaledes en Stolpe sig helt gennembrudt af et Hul, 10—12 cm vidt; man fyldte dette Hul med en Træprop, men det følgende Aar var ogsaa denne angrebet; Stolpen blev ombyttet med en ny, men Aaret efter fandtes ogsaa denne at være angrebet. Paa en 55 km lang Strækning fra Mandal til Kvinesdalselven fandtes ialt 24 angrebne Stolper, men disse var fordelt paa 4 Grupper, mellem hvilke alle Stolper var urørt. Imprægnering med Kobbervitriol beskyttede ikke Stolperne, ejheller Maling eller Tjæring. Saavel friske og nymalede som gamle Stolper angribes.

Collett afviser Tanken om, at Hullerne i Telegrafstolperne skulde være frembragt under Spætternes Søgen efter Føde eller Indretning af Rugeplads. Spætterne er for øvede i at spore Insekter til, at de skulde lade sig narre til at søge dem i tørre og præparerede Stolper, der vel aldrig regulært huser Insekter eller deres Larver. Og som Rugeplads har Collett heller ikke hørt Hullerne omtalt i Norge.

Collett kommer til den Slutning, at Spætterne bearbejder Stolperne uden anden paaviselig Hensigt end som Tidsfordriv; og kun undtagelsesvis gøres Hullerne saa store, at de kan benyttes som Hvilested eller som Tilflugtssted i Uvejr.

En ganske tilsvarende Fremstilling er langt senere givet i Collett's Storværk om Norges Fugle[1].

Her i Landet henledte i 1913 Overklitfoged Dahlerup Opmærksomheden paa[2], at Flagspætterne til Tider ødelæg-

[1] R. Collett: Norges Fugle, ved Ørjan Olsen, 2det Bind, 1921, p. 28—30.

[2] C. F. Dahlerup: Nyanlæg af Skov i Klit. Tidsskrift for Skovvæsen, 25. Bd., Række B, 1913, p. 104.

ger Telefonstænger i Plantagerne; som et grelt Eksempel anfører han, at Telefonforbindelsen med Opsynsmanden i den vestlige Del af Skagens Plantage engang maatte afbrydes en hel Dag, fordi Telegrafvæsenet fandt det fornødent at flytte en Strækning af Linien til et for Spætteangreb mindre udsat Strøg. Desværre meddeles der intet om Angrebets Art.

I sin Forstzoologi henviser Boas til denne Meddelelse af Dahlerup og oplyser samtidig, at der i Landbohøjskolens Samling findes et Stykke af en jydsk Telegrafpæl, indsendt fra Statstelegrafen; den er ganske ødelagt, med store Huller, det ene saa stort, at Spætten har kunnet sidde i det. Det er her i Landet den Store Flagspætte, der angriber Stængerne. Iøvrigt henviser Boas til Collett's Meddelelse, der refereres ret udførligt. — Man tør formode, at Boas har haft sine Tvivl om Rigtigheden af den af Collett fremsatte Mening, at Spætterne skulde bearbejde Pælene alene for Tidsfordriv, siden han sætter Udtrykket »for Tidsfordriv« i Gaaseøjne.[1]

Disse kort gengivne Meddelelser om Spætter og Telegrafpæle har været mig bekendt længe; jeg har altid følt dem utilfredsstillende, men har ikke haft Lejlighed til selv at anstille Iagttagelser paa dette Felt, før jeg for kort Tid siden gjorde nogle smaa Iagttagelser, som paany bragte mig dette Problem i Erindring.

Paa en afsides Villavej i Holte fik jeg for kort Tid siden Øje paa en Mængde Rødgrankogler, som laa paa Havehækken og Fortovet under en Telefonpæl. Disse Kogler viste sig ved nærmere Eftersyn at være behandlet af Spætter paa den for disse Fugle karakteristiske Maade: Kogleskællene var ved Hug med det i Spidsen skarpe, mejsel-

[1] J. E. V. Boas: Dansk Forstzoologi, 2. Udg., 1923, p. 148—149.

formede Næb kløvet efter Længden, saa at Spætten har kunnet faa sin lange Tunge ind til Frøene; disse bliver hængende paa Tungens yderste klæbrige og tornede Spids. Højt oppe paa Telefonpælen saas en aflang Fordybning, Vidnesbyrd om, at her havde Spætten kilet Grankoglerne ind, een efter een, og bearbejdet dem. Det er vel kendt, at Spætten ikke bearbejder Grankoglerne paa selve Træet, da dens Fødder ikke egner sig til at sidde paa Grene med. Derfor bider den Koglerne af og flyver med dem i Næbbet hen til et Træ, i hvilket den i Forvejen har hugget et Hul, saa langt og dybt, at Koglen kan presses fast deri. Fuglen hager sig nu med Kløerne fast paa Stammen under Koglen og presser sin stive Hale ind mod Træet til Støtte; i denne Stilling kan Spætten bearbejde Koglen paa den beskrevne Maade. I Haven indenfor Hækken stod nogle koglebærende Graner, hvorfra Spætten har hentet Koglerne. Ved et senere Besøg paa Stedet saa jeg en Grankogle siddende indkilet i Hullet paa Telefonpælen. I en Lund ikke langt borte fandtes Spættens Redehul i et Elmetræ.[1]

Vi har altsaa her et haandgribeligt Eksempel paa, at Spætter kan benytte Telefonpæle paa samme Maade som Stammer i Skoven, og at denne deres Virksomhed efterlader tydelige Spor i Pælen i Form af anselige Huller. I vore Skove finder man hyppigt Stammer, der har Mærker efter denne Form for Spættevirksomhed — paa et og samme Træ kan der endog være flere Huller.

Hvis man vilde spørge: Hvorfor satte den paagældende Spætte ikke Koglerne fast i selve Granerne, hvorfra den tog Koglerne, men foretrak Telefonpælen, kan jeg dertil svare følgende: Jeg har gennem Aarene set Hundreder af Træer, Eg, Bøg, Birk, Røn, Pil, Skov- og Bjergfyr o. fl.,

[1] Om Spætten paa denne Lokalitet se iøvrigt Bemærkningen S. 10—11.

baade levende og udgaaede, der benyttedes af Spætter til Anbringelse af Kogler, men mellem dem ikke en eneste levende Rødgran; Grunden hertil er utvivlsomt den, at der selv fra de mindste Saar i Granens Bark i længere Tid flyder sejg, klæbende Harpiks ud, saa at Spættens Næb og Fjerklædning til stor Gene for Fuglen vilde blive indsmurt deri, hvis den gav sig til at hugge Hul i Granens Bark, satte Koglen fast deri og anbragte sig selv nedenunder for at bearbejde Koglen.

Jeg skal derefter omtale den anden Iagttagelse, jeg har gjort, idet jeg forudskikker den Oplysning, at jeg nylig er flyttet til en Villavej lige op ad Geel Skov. En Dag i Midten af Februar hørte jeg, da jeg aabnede min Gadedør, en Stor Flagspætte »tromme« og saa, at den sad oppe i Toppen af en Telefonpæl udenfor Haven. Med overordentlig hurtige, vibrerende Bevægelser med Næbbet mod Pælen frembragte den en Lyd, der nærmest kan sammenlignes med en kort Trommehvirvel, naar en Tromme røres med stor Hurtighed. Jeg nævnede denne Iagttagelse for Ornitologen, Konservator HARRY MADSEN, og han fortalte mig da, at han i de sidste 3 Aar havde set og hørt det samme paa en Villavej overfor Sorgenfri Park. Det lykkedes mig ikke at faa nogen Spætte at se paa de Telefonpæle, han havde udpeget, men derimod nok paa nogle andre Pæle paa samme Villavej. Det var ogsaa her den Store Flagspætte (*Dryobates major*), der trommede. En Gang iagttog jeg en Spætte en halv Time i Kikkerten; i smaa Sæt, forlænds og baglænds, rykkede den snart helt op til, snart lidt ned fra Stolpens Top; efter en kort Trommen saa den sig om ligesom lyttende, og efter en Pause trommede den paany; dette gentog sig flere Gange; da ingen anden Spætte svarede, fløj den omsider bort. Undertiden var det,

som om Spætten ikke var helt tilfreds med den frembragte Lyd, saa klatrede den helt til Tops og bankede med Næbbet paa den Hætte af Blik, som dækker Pælen foroven, hvorved Lyden fik en metallisk Klang.

Spætternes »Trommen« er et vel kendt Fænomen; i Almindelighed finder den Sted i Skoven, og en tør Gren tjener Fuglen til at tromme paa. Lyden er saa kraftig, at det gjalder i Skoven, og den kan høres langt bort, er derved let at kende fra den mere sindige og dæmpede Lyd, der fremkommer, naar Spætten hakker i Bark og Ved for at naa ind til Insekter og Larver. Det er paa Slutningen af Vinteren og hen paa Foraaret, man hører Spætterne »tromme«. Der kan næppe være Tvivl om, at det er et Meddelelsesmiddel særlig i Parringstiden; naar man er heldig og befinder sig et Sted mellem to i betydelig Afstand fra hinanden trommende Spætter, kan man høre, hvorledes de korresponderer og skiftes til at »røre Trommen«; og det er ikke alene Hannen, men ogsaa Hunnen, der kan tromme.

Efter disse smaa Iagttagelser gav jeg mig til at se efter i Litteraturen, om der skulde foreligge yderligere i Sagens Interesse. Jeg fandt da i det af Naumann med Bistand af talrige Medarbejdere udgivne mægtige Værk om Mellemeuropas Fugle, i hvis 4de Bind Spætterne er behandlet[1], en udførlig Redegørelse for Spætternes Forhold til Telegrafpæle i Tyskland; ogsaa der kender man til, at den Store Flagspætte, Grønspætten og Sortspætten beskadiger Telegrafstænger; og som Følge deraf har i nogle Distrikter en Ombytning af et stort Antal Stænger vist sig nødvendig. Maaden, hvorpaa Spætterne i Tyskland behandler Stængerne, ligner meget den af Collett beskrevne.

[1] Naumann: Naturgeschichte der Vögel Mitteleuropas, neu bearbeitet. Herausgegeben von Dr. Carl R. Hennicke. IV. Band, 1901, p. 321—22.

Ligesom COLLETT afviser Forfatteren en Teori, der gaar ud paa, at Spætterne skulde hidlokkes af den syngende Tone, som Telegraftraadene ofte frembringer, naar de sættes i Svingninger af Vinden, idet Spætterne skulde antage denne Lyd for Insekters Summen; og i den Tro, at Insekterne var inde i Pælen, skulde Spætten give sig til at hakke i den for at naa ind til dem; men, siger Forf., Spætterne er nok for kloge til, at de ikke skulde kunne adskille Telegrafstængernes Summen fra Insekters.

Der er imidlertid den væsentlige Afvigelse fra COLLETT's Fremstilling, at efter den tyske er det netop Søgen efter Næring, der faar Spætterne til at angribe Telegrafstængerne. Thi der opholder sig ofte et stort Antal Insekter i Telegrafstængernes af Solheden frembragte lange Sprækker, samt hvor der er faldet Grene ud, eller hvor der er Huller efter Skruer. Paa saadanne Steder gaar Spætterne paa Insektjagt og udvider de forhaandenværende Huller og Revner. Endvidere lokkes Spætterne til af Flyvehuller efter Træhvepse (*Sirex*) i den Forventning, at der ogsaa er Larver af dem at finde i Pælene; da Larvernes Udvikling tager flere Aar, hænder det jo, at der findes Larver i Veddet endnu lang Tid efter, at Pælen er taget i Brug. Da det har vist sig, at ingen af de brugelige Imprægneringsmidler beskytter mod Spætteangreb, er det i Tyskland paabudt af Telegrafvæsenet, at alle Huller skal lukkes forsvarligt, inden Pælene tages i Brug.

Da jeg ikke andetsteds har set omtalt, at Insekter opholder sig paa nævnte Steder, har jeg i Skovegne undersøgt et Antal Telefonstolper og kan for saa vidt bekræfte Angivelsens Rigtighed, som ogsaa jeg har kunnet paavise Insekter og tillige andre Leddyr i dem. I Sprækker og Huller var der hyppig Myrer, desuden Edderkopper (bl. a.

Steatoda bipunctata, *Amaurobius fenestralis* og Springedderkopperne *Marptusa mucosa* og *Epiblema cingulatum*) og deres Ægspind med Unger[1]. Dernæst fandtes et ikke ringe Antal Pupper af Sækspindere (*Palaeporia tubulosa* og *Fumea casta*) i deres Sække samt hyppigt og stedvis i stort Antal Larver af flere Arter Sækmøl (*Coleophora*). Endvidere Gravehvepse (*Crabro*), Guldhvepse (*Chrysis*), Snyltehvepse (2 Arter Braconider), Pupper af en Dagsommerfugl (*Thecla*), Stankelben (*Tipula*) og Mejere (*Phalangium*). Ørentviste (*Forficula*) forekom hyppig, undertiden samlede i betydeligt Antal, baade Voksne og Unger. Et Sted fandtes et enormt Antal af den 2 mm lange Springhale *Allacma fusca*, ikke alene uden paa Pælen, men ogsaa i Revner og Sprækker.

I Skovegne slaar iøvrigt mange flere forskellige Insekter og Larver sig ned udenpaa Pælene. At mange Insekter og Larver derfra søger ind i Revner og Sprækker, fremgaar yderligere af, at Rester af Hudskelettet, baade af Biller, Aarevinger, Tovinger o. s. fr. findes i Mængde i de derinde anbragte Edderkoppespind. Da Revnerne tit strækker sig dybt ind i Pælene og har en betydelig Længde, er der god Plads for Edderkopper til at spinde deres Spind derinde, og de benytter sig i udstrakt Grad af Lejligheden; undertiden kan Revnerne tillige være ret brede paa Midten, saa at man kan stikke en Lillefinger derind.

Som nævnt siges det direkte hos Naumann, at Spætterne udvider Pælenes Sprækker for at faa fat i de deri skjulte Insekter. Selv har jeg ikke truffet Spætten ved dette Arbejde, men nok set Revner, som øjensynlig var udvidet ved Hakning af Spættenæb. Det var saaledes Tilfældet med

[1] Det er næppe almindeligt kendt, at den Store Flagspætte tager Edderkopper; Whiterby skriver dog i sin: »Practical Handbook of British Birds« (Vol. II, 1924, p. 42) om dens Føde: »spiders also freely taken«.

den Stolpe, som den S. 5—6 omtalte Spætte havde benyttet til Indkiling af Grankogler, og med nogle andre Stolper nær ved; disse Stolpers Revner var stedvis udvidet som ved Hug med Spættenæb, medens andre, fjernere staaende Stolper ikke viste noget tilsvarende. Jævnfør ogsaa Bemærkningen S. 13.

Endelig skal jeg omtale, at Prof. LÖNNBERG i Stockholm saa sent som i 1936 har publiceret den overraskende Nyhed, at Sortspætten i Sverige kan lave Reder i Pæle, der bærer Højspændingsledninger[1]. Spætter udmejsler som bekendt selv deres Rede i en Træstamme eller en Gren; fra et rundt Indgangshul fører en kort Gang ind til et Redekammer af betydelig Dybde. Til Rede kan Sortspætten altsaa iflg. LÖNNBERG vælge Stærkstrømspæle; disse Pæle har en betydelig Tykkelse og kan afgive Plads for et rummeligt Kammer. Som Eksempel kan nævnes: I en Pæl med en Diameter af 30 cm var Indgangshullet 15 cm højt og 10 cm bredt, Kammerets Dybde var 38 cm og dets Diameter 15 cm. I en anden Pæl strakte Kammeret sig 46 cm under Indgangshullets nedre Rand. Spætten er overmaade standhaftig ved dette Arbejde; hvis den har begyndt at udmejsle et Hul, og man dækker det med et Stykke Træ, hugger Spætten igennem dette eller hugger et nyt Hul nær det første. Hvis en Jernplade nagles over et paabegyndt Hul, laver Spætten et nyt i den samme Pæl paa Siden af det første eller i kort Afstand derfra. I eet Tilfælde havde Spætten 5 Gange begyndt at hugge Huller, og lige saa mange Gange blev de beslaaet med Jernplader, og først med det sjette Hul lykkedes det Spætten at fuldføre Kammeret.

[1] EINAR LÖNNBERG: Some examples of anomalous behaviour of Woodpeckers. Orgaan der Club van Nederlandsche Vogelkundigen, Jaarg. 9, No. 1, Juni 1936, p. 27—31.

Det er klart, skriver Lönnberg, at slige Udhulinger af Pælene betyder en ikke ringe Fare for, at disse kan bryde sammen i Storm og derved foraarsage svære Forstyrrelser paa Linien. Det er uheldigvis ikke noget lokalt Fænomen, men udstrakt over en betydelig Del af Sverige, omfattende 8 Provinser imellem 56° og 62° N. B. Og helt sjældent kan det heller ikke siges at være, siden et Eftersyn i Foraaret 1935 viste, at blandt 6000 Pæle var 360, altsaa 6 %, angrebet af Sortspætten. Ingen Imprægnering yder heller her tilstrækkelig Beskyttelse mod Sortspættens Angreb. Man har ogsaa forsøgt at skræmme Sortspætterne væk, men de fortsætter haardnakket deres Arbejde. Det har derfor vist sig nødvendigt at tillade at skyde saadanne Individer, der blev truffet i Færd med at angribe Pæle, der bærer Højspændingsledninger, uagtet Sortspætten, ligesom alle dens Slægtninge, er strengt fredet ved Lov.

Jeg havde ikke gjort mig nogen Forventning om, at man her i Danmark kunde faa at se noget lignende som det, Lönnberg har beskrevet. Stor var derfor min Overraskelse, da jeg d. 9. Juni i Aar (1937) i en Skov i Nordsjælland (Tokkekøb Hegn) pludselig stod over for det samme Fænomen. Langs en Sti inde i Skoven findes en Række Pæle, der tidligere har baaret Traade for Telefon eller Lysledning; men nu var Traadene fjernet. En af Pælene var, som det ofte er Tilfældet, støttet af en anden, lidt skraat stillet Pæl, og heri var Spættereden bygget. Pælens Omkreds var 66 cm, dens Diameter følgelig 21 cm. Indgangshullet befandt sig i en Højde af kun 1,7 m over Jorden, dets Tværmaal var 5 cm. Kammerets Dybde fra Loft til Bund var 30 cm, dets største Diameter fra Væg til Væg 11,5 cm, men 17 cm, hvis man maalte fra Indgangshullets Yderrand til Kammerets Bagvæg; Kammeret

var følgelig paa den modsatte Side af Indgangshullet kun begrænset af en 4 cm tyk Væg.

Kammerets Dybdemaal kunde imidlertid først tages, efter at det var tømt for et anseligt Indhold bestaaende hovedsagelig af Mos, iblandet med Fuglefjer og Haar af Raadyr. Reden var altsaa oprindelig bygget af en Spætte — og der kan vel næppe være Tvivl om, at det har været den Store Flagspætte — er forladt af denne og derefter taget i Besiddelse af en anden Fugl, efter Redestoffet og Smaastykker af Æggeskaller at dømme en Musvit (*Parus major*), der har fyldt Kammerets Bund med Redemateriale.

Paa samme Pæl saas adskillige andre Mærker efter Spættehakning, nogle af dem ret overfladiske, andre noget dybere, kegleformet tilspidset indefter med en Dybde af indtil 4 cm, formodentlig Steder for Prøvehakning, inden Spætten har bestemt sig for det Sted paa Pælen, hvor den har fuldført det beskrevne Redekammer; nogle, af en langstrakt Form og ret dybe, saa ud til oprindelig at have været Sprækker, som Spætten havde bearbejdet og udvidet.

Hermed er denne lille Redegørelse afsluttet, og Resultatet kan sammenfattes saaledes:

Alle de Funktioner, hvortil Spætterne normalt benytter Skovens Stammer og Grene, nemlig til:

Søgen efter Insekter
Fastkiling af Kogler
Trommen i Parringstiden
Redebygning

kan Spætten henlægge til Pæle, der bærer Telefon-, Telegraf- og Stærkstrømsledninger. Der er ingen Plads for den Teori, at Spætterne skulde bearbejde Pælene for Tidsfordriv.

I Danmark er den Store Flagspætte den eneste Art Spætte, der kan siges at være ret almindelig, og selv den

kan næppe antages at gøre større Skade paa nævnte Anlæg, da den kun optræder ret faatallig her i Landet og derfor som Regel i Skovene kan finde tilstrækkeligt af Træer og Grene, der egner sig til at tilfredsstille dens Behov i de forskellige Retninger.

I samme Afhandling omtaler Collett, at de større Spættearter tilfredsstiller deres Trang til Virksomhed ogsaa derved, at de især i afsides Egne ikke sjældent angriber Tømmervægge paa ældre Huse eller Lader og ogsaa herved kan anrette virkelig Skade. Saaledes iagttoges ved en Gaard paa Hvaløerne en Udlade, hvori der fandtes 22 Huller, og paa en anden nærliggende Gaard 8 Huller, alle hakkede af Sortspætten. Enkelte af Hullerne var saa store, at Fuglen kunde faa Plads indenfor, og den iagttoges ofte siddende her og titte ud. Den samme Fremstilling findes gentaget i Collett's Værk om Norges Fugle (2. Bd., 1921, p. 29—30).

Ogsaa denne Skildring er gaaet over i Boas' Forstzoologi (p. 149), med den Tilføjelse, at her fra Landet har man intet erfaret i den Retning — ikke saa mærkeligt, da Sortspætten er en sjælden Fugl hos os.

Denne Form for Sortspættens »Trang til Virksomhed« finder imidlertid sin naturlige Forklaring gennem en Meddelelse af den udmærkede Naturiagttager Gösta Kihlén, som er offentliggjort i Jägerskiöld og Kolthoff's Værk om Nordens Fugle.[1] I al sin Korthed giver Kihlén's Beretning saa anskueligt og klart et Billede af Spættens Arbejde og Hensigten dermed, at jeg vil citere den ordret:

»Ej sällan skaffar sig spillkråkan[2] en vinterbostad i

[1] L. A. Jägerskiöld och Gustaf Kolthoff: Nordens Fåglar (p. 122—23). Andra Upplagen. Stockholm 1926.

[2] Det svenske Navn for Sortspætten.

torpstugor och mindre hus, som ligga i skogen, hon hackar då ett hål på väggen och tillbringar natten på vinden. Om ett sådant hål spikas igen, hugger hon ett nytt bredvid. I Värmland ser man ofta skogsstugor, vilkas gavlar visa hål vid hål, de flesta täckta av påspikade brädlappor. Får spillkråkan behålla sitt första hål i fred, så hackar hon intet nytt. Hon lämnar sin tillflykt i ljusningen och kommer regelbundent åter i skymningen.«

Sædvanligvis tilbringer Spætterne Natten i et Hul i et Træ eller i en Hule, som de selv udmejsler i en Træstamme. Men under særlige Forhold skaffer Spætterne sig altsaa »Tag over Hovedet« paa Lofter, i Udhuse og lignende Steder, naar de kan skaffe sig Adgang dertil ved at hugge Hul i en Trævæg. Der er heller ikke i dette Forhold nogen som helst Anledning til at se et Udslag af »Trang til Virksomhed«.

I Bindet om Fuglene i det nye zoologiske Samleværk skriver Prof. STRESEMANN om den Store Flagspætte: »Der grosse Buntspecht (*Dryobates major*) frisst keine Ameisen«.[1]

Da jeg læste dette, kom jeg til at tænke paa en Oplevelse, jeg havde d. 16. Juni 1935 i Gribskov i Nordsjælland: Paa ret nært Hold saa jeg den Store Flagspætte arbejde ivrigt i en Myretue, rodende og hakkende og øjensynlig gørende sig til Gode med Beboerne. Da Spætten nogen Tid efter, rimeligvis foruroliget ved min Nærhed, forlod Tuen, saa jeg, at der var rømmet slemt op i den; Myrerne løb forvildede hid og did, og der laa Masser af Larver strøet om i Ruinerne af Tuen. En hjembragt Prøve viste, at det var den gule Myre (*Lasius flavus*).

[1] E. STRESEMANN: Aves, i: Handbuch der Zoologie, VII[2], 1934, p. 457.

Stresemann er ikke den eneste, der bestrider, at den Store Flagspætte tager Myrer. Saaledes skriver Yarrell: »It is rarely seen on the ground, for it does not make ants its prey«.[1] Og hos Naumann læser man følgende: (l. c. p. 286): »Er lebt von allerlei Insekten deren Eiern, Larven und Puppen . . ., aber nicht von Ameisen und Ameisenpuppen.« I en Anmærkning under Teksten bemærkes dog, at en Iagttagelse af Werner og Leverkühn strider herimod, idet de i en i Juni Maaned ved Kiel nedlagt Stor Flagspætte fandt Hals og Svælg propfuld af Myrer. Ogsaa Collett (l. c. 1921, p. 11) meddeler, at flere af ham undersøgte Store Flagspætter havde Myrer i Tarmkanalen.

De nævnte Analyser af Fordøjelseskanalens Indhold hos den Store Flagspætte og min direkte Iagttagelse af dens Plyndring af en Myretue maa være tilstrækkelige Beviser for, at Paastanden om, at denne Spætte ikke efterstræber Myrer, er uholdbar.

[1] W. Yarrell: A History of British Birds, Vol. II, 1876—82, p. 471.

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BY

G. HEVESY, J. J. HOLST AND A. KROGH

KØBENHAVN
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Anatomical Introduction.

The hard part of a tooth is composed of three distinct substances viz. the dental substance proper, dentine, the enamel, and the cement. The dentine constitutes by far the largest portion; the enamel is found in a comparatively thin layer partly covering the dentine; and the cement covers the surface of the root in a thin layer. In the case of the canines of cats we found the weight of the enamel ash to be 11,2 % of that of the dentine ash, the weight of the enamel before ashing being equivalent to about 9,7 % of that of the dentine.

The dentine is penetrated throughout by fine tubes (dentinal tubes) starting from that side of the dentine which faces the pulpa cavity; they have an initial diameter of 2 to 8 μ and do not much diminish in size at first as they approach the surface; the distance between adjacent tubules is about two or three times their width. From the tubules numerous immeasurably fine branches are given off and penetrate the hard intertubular substance. Near the periphery of the dentine, the tubules, which by division and subdivision have become very fine, terminate imperceptibly in free ends. It is reported that tubules have been observed passing into the enamel in the teeth of marsupial animals, and to a less marked degree in human teeth. In this case they pass, not into the enamel prisms, but into the inter-prismatic substance. The enamel is made

up of microscopic columns, very hard and dense, arranged close side by side, and fixed at one extremity on to the subjacent surface of the dentine. The enamel columns have the form of six-sided prisms. Their diameter is about 0.005 mm. They are united by a small amount of substance which appears to be similar to the intercellular substance of an epithelium. The small amount (about 1 %)[1] of organic matter in the enamel is probably found to a large extent in the above mentioned connective substance. In marsupials and some rodents there are regular canaliculi in the interprismatic substance.

The central cavity of a tooth is occupied by a soft and very vascular dental pulp, containing cells, blood-vessels, nerves, and fine connective-tissue fibres. The cells are partly disseminated in the matrix and partly form a stratum at the surface of the pulp. These superficial cells, the odontoblasts, send out elongations into the tubules in the dentine. It is through the intermediary of the pulp that constituents of the blood get into the hard tissues of the teeth.

Chemical composition of the teeth.

a) Dentine.

On analysing a great number of dry human dentine samples Bowes and Murray[2] found a loss in weight of the fresh tissue on ignition amounting to 29—29.7 %. The losses on ignition found in some of our experiments can be seen in table 1, in which we have also included for the sake of comparison the values found for the tibia and jaw.

[1] J. H. Bowes and M. M. Murray, Biochem. J. **29**, 12, 2721, 1935.
[2] J. H. Bowes and M. M. Murray, Biochem. J. **30**, 977, 1936.

Table 1.

Albino rat 200 g.

Organ		Loss on ignition in % of fresh weight
Incisors	Proximal end	33.6
	Distal end	25.0
	Average	26.4
Molars		27.0
Tibia	Head	79.1
	Average	63.2

Cat 4 kg.

Incisors	32.0
Canine	35.0
Molar	38.0
Jaw	50.4
Tibia epiphysis	66.8
Tibia diaphysis	36.7

The average values found for the chief constituents of the dentine by BOWES and MURRAY are seen in table 2.

Table 2.

Analyses of dentine of human teeth (% in dry dentine).

	Slight hypoplasia	Severe hypoplasia
Ash	71.09	70.28
Ca	27.79	26.96
P	13.81	13.5
CO_2	3.18	3.10
Mg	0.835	0.728
Cl	—	0.023

BOWES and MURRAY give the following average figures for the composition of the enamel:

Table 3.

Analyses of enamel of human teeth.

	Slight hypoplasia	Severe hypoplasia
Ash	95.38	94.67
Ca	37.07	35.81
P	17.22	17.72

	Slight hypoplasia	Severe hypoplasia
CO_2	1.952	2.434
Mg	0.464	0.477
Cl	0.3	0.19
Fe[1]	0.25	—

As is seen from the above figures phosphorus is the second most abundant mineral constituent of the teeth, its share in the dentine amounting to 13.5—13.8 % and in the enamel to 17.2—17.7 % while in the dentine ash 18.2—18.4 % in that of the enamel 18.4—19.4 % were found. In the ash of the incisors of rats an even higher phosphorus content of 20 % was found. Bone ash contains an only slightly lower amount of phosphorus than tooth ash, the values found varying between 17.9 and 18.5 %.

In distinction to the chief constituents of the teeth the minor constituents vary within wide limits. The composition of the mineral constituents of the teeth corresponds approximately to a mixed crystal of the minerals hydroxide-apatite and carbonate apatite, the former predominating strongly. As in apatite minerals the OH^- ions of the tooth apatite can be replaced to a certain extent by F^- ions for example. The degree of replacement of OH^- by F^-, will depend primarily on the fluorine content of the blood during the development period of the tooth and also on that which circulates in the fully calcified tooth. The fluorine content of the blood will depend on the fluorine content of the food and water taken up. It is thus easy to explain why the fluorine content of the teeth varies within wide limits (see table 4). The high fluorine content of the teeth

[1] G. Montelius, J. F. McIntosh and Y. C. Ma J. dent. Res. **13**, 73, 1933. The amount of copper present in teeth is 10^{-4}—10^{-5} gm. per gm. E. Tiede and H. Chomse, Ber. d. dt. chem. Ges. **67**, 1988, 1934.

of human beings living at Colorado Springs is due to the high fluorine content of the water which amounts to up to 2 mgm. per liter. The high fluorine content of the teeth of some North African sheep is to be explained by the high fluorine content, above 0.02 %, of the soil on which they graze. On such soil plants of high fluorine content grow, are eaten by the sheep, and lead to an abnormally high fluorine content of the blood plasma, which in turn leads to an abnormally high replacement of OH^- by F^- in the teeth.

Table 4.

Fluorine content of teeth ash.

		%
Man[1]	teeth	0.03
Marine animals	teeth	0.69 —0.74
Rats[2]	teeth	0.006—0.03
Man[3] New York	dentine	0.065
Man New York	enamel	0
Man[3] Colorado Springs	dentine	0.112
Man Colorado Springs	enamel	0.065
Man[4]	dentine	0.030
Man[4]	enamel	0.005
Calves[4]	dentine	0.022
Calves[4]	enamel	0.0057
Sharks[4]	teeth	0.89
Sheep, young from neighbourhood of Norwegian aluminium factory where fluorides are utilised[4]	incisors	0.45—0.49
Sheep[5] North Africa	teeth	0.04
Sheep[5] North Africa, attacked by fluorine disease	teeth	0.32—0.46

The much higher fluorine content of animals living in sea water is also due to the comparatively high fluorine con-

[1] R. Klement, Naturw. **21**, 662, 1933.

[2] G. R. Sharpless and E. V. McCollum, J. Nutrit. **6**, 163, 1933. J. H. Bowes and M. M. Murray, Biochem. J. l. c.

[3] H. Boissevain and W. F. Drea, J. Dent. Res. **13**, 495, 1933.

[4] K. Roholm, Fluorine Intoxication, Copenhagen 1937 p. 260.

[5] M. Gand, A. Chavnot and M. Langlais, Bull. Inst. Hyg. Maroc. Nos. I—II, 1934, comp. also Roholm loc. cit. p. 42.

tent of the latter. In the same way that F^- replaces OH^- in the apatite lattice, magnesium for example replaces calcium. Human dentine ash has a magnesium content[1] of 1.18—1.39 % whereas human enamel ash has only 0.42 %. While the calcification of the tooth tissue is presumably the result of specific cell activity, it is quite possible that later on a replacement of calcium by for example magnesium takes place, governed chiefly by solubility (chemical affinity) conditions, and it is quite conceivable that in the course of time more and more calcium is replaced by magnesium if the magnesium: calcium ratio is in favour of such an exchange. The enamel being characterised by a decidedly poorer lymph circulation than the dentine, the much lower magnesium content of the former can easily be accounted for by a reference to the above considerations. The 0.42 % magnesium found in the human enamel possibly got into the latter wholly or to a large extent during the formation of the enamel tissue. The presence of as much as 4 % of magnesium in elephant dentine is possibly due to a high magnesium content of its food or to a high magnesium retention in its blood. It is also of interest to remark that the magnesium content of the teeth found in prehistoric skeletons is only one third of that found in teeth of recent generations, furthermore that carious teeth[2] show a greatly increased magnesium content. Besides the elements discussed above, spectroscopic investigation[3] revealed the presence of traces of Na, Ag, Sr, Ba, Cr, Sn, Zn, Mn, Ti, Ni, V, Al, Si, B and Cu in dental tissue.

[1] M. M. Murray, Biochem. J. **30**, 1568, 1936.

[2] T. Francia, Ann. Clin. Odoniat. **8**, 685, 1931; M. M. Murray and J. H. Bowes, Brit. Dent. J. **61**, 473, 1936.

[3] E. Lowater and M. M. Murray, Biochem. J. **31**, 837, 1937.

That the concentration of the minor constituents of the teeth does not fluctuate between still wider limits is due to the narrow limits within which the concentration of most elements in the blood plasma is restricted. This is caused partly by a prevention of the resorption of excessive quantities of the elements, conspiciously shown in the case of calcium, and partly by prompt removal chiefly through the kidneys of excessive amounts of the mineral constituents present in the plasma. But even in spite of this levelling mechanism of the blood plasma some of the mineral constitutents are deposited to a noxious extent in the tooth tissue, as is seen above in the case of fluorine, and it is quite possible that even an excessive replacement of for example the calcium by magnesium, sodium, or potassium might lower the resistance of teeth to disease.

While the conclusions given above are based partly on hypothetical assumptions, in the case of lead, which also replaces calcium in the crystal lattice, the accumulation in the teeth with time can clearly be shown. While small children have only negligible amounts of lead in their teeth, the lead content increases with age[1], the increase being markedly greater in the case of carnivorous than herbivorous animals, presumably on account of a greater lead intake in their normal nourishment. In the case of lead poisoning the lead content of teeth is greatly increased. All these observations support the hypothesis, that even in fully formed teeth an exchange of mineral constituents is regularly taking place. To test this hypothesis we have studied the exchange of phosphorus by means of labelled phosphorus atoms.

[1] F. Pfrieme, Arch. f. Hyg. **111**, 232, 1934.

Phosphorus exchange in teeth.

We investigated the movement of the phosphorus atoms both in the teeth of fully grown and growing animals by using labelled phosphorus atoms as an indicator. By adding radioactive phosphorus, prepared from sulphur by the action of neutrons, to food administered to animals at a known date, it is possible to distinguish the phosphorus atoms which were present in the food sample and which have been retained and deposited in the organism, from those already present in the body and the teeth at the start of the experiment. We can thus follow the movement of the phosphorus atoms taken in for example a glass of milk and investigate if and to what extent these particular atoms get into the teeth and how they are distributed there.

The dentine contains 14 % and the enamel 17.5 % of phosphorus in the form of phosphate (PO_4). It is the movement of these phosphate radicles which we actually investigate. For the sake of brevity we shall often use the word phosphorus in discussing the behaviour of the phosphate[1] radicle. We may recall that the phosphorus taken with food, amounting in the case of an adult to somewhat more than 1 gm. per day, is to a large extent (in most cases up to about 80 %) absorbed from the gut and gets into the blood stream. Adult human blood contains 44—50 mgm. % of phosphorus of which only 2—5 mgm. % are present as inorganic P. Very different views have been put forward on the formation of the bone and tooth tissue, but they all consider the blood plasma as saturated or nearly saturated with calcium phosphate and the precipit-

[1] The expression radioactive phosphate is ambiguous, since in such a radicle either the phosphorus or the oxygen atoms, possibly even both, may be radioactive.

ation of the latter from the plasma as being of paramount importance for the ossification process. The solubility of calcium phosphate in the plasma is very strongly affected by the presence of proteins, carbonate and bicarbonate ions, and possibly also other constituents. It is also dependent on the acidity of the blood, slight changes in which may be sufficient to produce precipitation. It seems very probable that it is not simple calcium phosphate but a complex salt of the apatite type, a solid solution of hydroxide apatite and carbonate apatite, that precipitates.

In addition to the inorganic phosphate, blood contains a phosphoric ester at a comparatively high concentration which is mainly found in the corpuscles; as it cannot yield phosphate ions by dissociation, this ester does not affect the saturation of the blood with respect to calcium phosphate. However, as ROBISON[1] discovered, the cartilage and osteid contain an enzyme, phosphatase, which hydrolyses this ester, thus setting free inorganic phosphate, whereby the concentration of the phosphate ions increases and a supersaturation occurs, followed by a precipitation of the calcium phosphate in the matrix of the tissue. With the discovery of the bone phosphatase a second agency (in addition to the acidity change) of great importance was found, regulating the calcium phosphate precipitation leading to ossification. ROBISON found that the enzyme had the greatest activity in ossifying cartilage, bones, and teeth of very young animals, the activity per unit weight of tissue decreasing with age. Although the plasma contains on an average only 0.5 mgm. of phosphorus present as phosphoric ester per 100 ccs. this is completely hydro-

[1] Comp. R. ROBISON, The significance of phosphorus esters in metabolism. New York 1912.

lysable by the bone phosphatase and thus supplies phosphate ion amounting to about $^1/_6$ of the inorganic phosphorus present in the plasma, an amount amply sufficient to bring about a supersaturation and a subsequent precipitation of calcium phosphate, or more correctly of the apatite-like bone substance, from the already nearly or fully saturated plasma. The conclusions arrived at in this paper are independent of the special mechanism assumed for the ossification process.

Distribution of labelled phosphorus in the incisors of rats.

The rapidly growing incisors of rats are very suitable for studying the distribution of phosphorus. According to

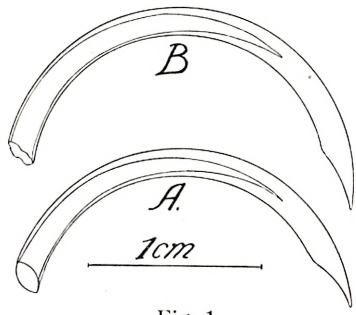

Fig. 1.

Fridericia and Gudjonsson[1] the average extrusive incisor growth per week is 2.7 mm. in the case of adults and 3.4 mm. for young rats. As seen in Fig. 1 A the cross section of the pulpa is very large at the proximal end and gets narrower toward the distal end, the last millimetres

[1] L. S. Fridericia and S. V. Gudjonsson, Kgl. Danske Vid. Selsk. Biol. Medd. XIII, 12, 1936; comp. also W. G. Downs jun., Proc. Soc. exp. Biol. and Med. **28**, 813, 1931, who finds an average growh per week of 2.78 mm. for 6 months old animals.

of the teeth being free of pulpa. The problem we have to investigate is how the distribution of newly formed calcium phosphate in the incisor takes place. Two extreme cases must be envisaged:

a) the labelled phosphate is deposited in close proximity to the pulp from which it is derived, while the tissue formed at an earlier date is pushed along in the direction of growth;

b) the labelled phosphate is equally distributed throughout the incisor.

Cutting incisors transversally into pieces and analysing these separately revealed the fact that the largest part of the labelled phosphate is found in those regions of the incisor where the pulpa is strongly developed, but that some of the labelled phosphate is found all through the incisoral tissue (Tables 5 and 6).

Table 5.

Distribution of labelled phosphorus, contained in the normal diet, found in the incisor after 2 days. Weight of the rat 210 gm. + denotes upper ÷ lower teeth.

Part of the incisor	Weight of ash in mgm.	% of labelled P taken found	% of the labelled P per mgm. ash
Proximal I +	38.2	0.42	0.011
Proximal II +	40.8	0.47	0.012
Proximal I ÷	29.2	0.37	0.013
Proximal II ÷	27.2	0.38	0.014
Middle	92.6	0.125	0.00135
Distal I +	115.2	0.072	0.00063
Distal II ÷	36.4	0.008	0.00022

Percentage of labelled P found in the total incisors = 1.85. Average per 1 mgm. ash = 0.005. Biggest ratio between proximal and distal end = 60.

Table 6.

Distribution of labelled phosphorus, administered in the normal diet, found in the incisor after 7 days. Weight of the rat 240 gms.

Part of the incisor	Weight of ash in mgm.	% of labelled P taken found	% of the labelled P per mgm. ash
Proximal I +	25.0	0.28	0.011
Proximal II +	23.6	0.31	0.013
Proximal I ÷	21.8	0.29	0 013
Proximal II ÷	31.6	0.32	0.010
Middle +	81.6	0.204	0.0032
Middle ÷	68.0	0.206	0.0031
Distal + and ÷	29.3	0.020	0.00074

Percentage of labelled P found in the total incisors = 1.69. Average percentage per 1 mgm. ash = 0.006. Biggest ratio between proximal and distal part = 18.

In the experiments now to be described the distal part of the incisor was removed by operation one day before labelling the phosphorus present in the blood. In these experiments the radioactive P was not added to the food but given in the form of subcutaneous injections. 2 days, 5 days and 8 days after the administration of the labelled phosphorus the end part of the freshly grown incisor was again removed by operation and its radioactivity ascertained. The distal parts removed were all outside the range of the pulp. The figures obtained are seen in Table 7 and those from a similar experiment in Table 8.

Table 7.

Days after intake of labelled P	Weight of the tissue in mgm.	% of the labelled P found in 1 mgm. fresh tissue
2	42.8	0.00089
5	16.4	0.00030
8	20.4	0.00066
13 (rat killed)	26.6	0.00090

Percentage of the labelled P found in 1 mgm. of average incisor tissue = 0.0076. The removed distal ends contained 8 to 25 times less labelled P than the average tissue.

Table 8.

Days after intake of labelled P	Weight of the tissue in mgm.	% of the labelled P found in 1 mgm. fresh tissue
5	14.4	0.00040
8	11.0	0.00044
13 (rat killed)	21.3	0.00062

Percentage of the labelled P found in 1 mgm. of average incisor tissue = 0.0062. The removed distal ends contained 10 to 16 times less labelled P than the average tissue.

Though the figures in the tables above clearly show that the deposition of labelled phosphorus is not restricted to the regions in the vicinity of the pulp, but that the labelled phosphorus is to be found even in the most remote part of the incisors, we attempted to obtain incisors with an appreciably larger pulp-free part. As is well known, rats, being rodents, grind their teeth and thus continually remove parts of the pulp-free end of the growing incisors. By eliminating the upper incisors the animal was prevented from gnawing and incisors were thus obtained in which the distal pulp-free end had a length of 10.5 mm. as shown in fig. 1 B. The result of this experiment is seen in Table 9 and the diagram fig. 2.

Table 9.

Distribution in the incisor after 3 days of labelled phosphorus, injected subcutaneously.
Weight of the rat about 200 gms.

Part of the incisor (comp. Fig. 2)	Weight of the tissue in mgm.	Weight of the ash in mgm.	% labelled P found per mgm. tissue	% labelled P found per mgm. ash
I (Proximal end)	13.1	9.2	0.0103	0.0151
II	14.5	11.0	0.0079	0.0116
III	24.0	17.1	0.0026	0.0040
IV	15.3	11.0	0.00021	0.00030
V (Distal end)	12.0	9.0	0.000033	0.000044

The content of labelled P varies between 0.01 % at the proximal end and 0.000033 % at the pulp-free distal end, thus diminishing by a factor of 1/300. On comparing the activity of the ash obtained by igniting the incisor the figures work out to be 0.015 % and 0.000044 % respectively, corresponding to a factor of 1/340. The average content of labelled P in 1 mgm. tissue was found to be 0.0041 %, in 1 mgm. ash 0.0059 %. Fig. 2 shows both the location of

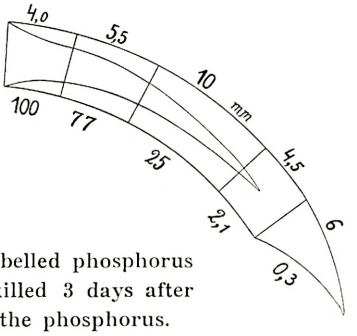

Fig. 2. Distribution of labelled phosphorus in the incisor of a rat killed 3 days after the administration of the phosphorus.

The figures below give the relative amounts of labelled phosphorus present in 1 mgm. of fresh tissue in the section in question. The figures above give the length of the section in mm.

the pulp and the distribution of the labelled phosphorus. It is seen clearly that the bulk of the labelled phosphorus atoms are to be found in the vicinity of the pulp but an amount which is far from being negligible reaches even the remotest part of the incisor. In Fig. 2 we have inserted the relative abundance figures of the labelled phosphorus present in the different parts of the incisor. A part of the first sector amounting to 1.4 mm. grew during the time which elapsed between the injection of the labelled P and the killing of the animal; the other parts were present before. Out of 204 parts of labelled phosphorus only 100 were found in the first sector and consequently not more

than 30 in the part actually grown, the remaining 174 or more being at least partly located in the parts present before injecting the phosphorus.

In seeking an explanation of the presence of labelled phosphorus at a very considerable distance from the pulp we must remember that even the most remote incisal part of the tooth contains organic constituents. The constituents of the blood plasma penetrate through the latter and exchange of phosphate radicles and possibly also some ossification occurs in situ, though only to a modest extent on account of the poor circulation in comparison with that in the vicinity of the pulp. For part V (fig. 2) we found a loss of weight on ignition amounting to 25 % of the weight of the tissue dried in a vacuum dessicator; part I lost 29.8 %; and the average loss on ignition was found to be 27.4 %. That bones rich in organic constituents, i. e. such in which a comparatively effective circulation takes place, take up more radioactive phosphorus than the diaphysic bones poor in organic constituents had already been found previously in our investigations and is also to be seen in an example discussed on page 23.

Phosphorus exchange in growing rats.

It is tempting to explain the parallelism between the abundance of organic substance present in the tissue investigated and the percentage of labelled phosphorus present by assuming that the latter is chiefly present in the organic substance and not in the calcium phosphate of the bone or teeth. This possibility must however be discarded because blood weighing as much as an incisor contains after the lapse of few days less than 0.01 % of the labelled

phosphorus taken, while that found in the incisors exceeds 1 %. In view of the importance of this point we tested the effect of the removal of the pulp on the exchange data. The experiment was carried out on a young rat which increased in weight from 87 to 110 gm. in the course of the 5 days which elapsed between the subcutaneous administration of the labelled phosphorus and the killing of the animal. Before the analysis the pulp was removed from

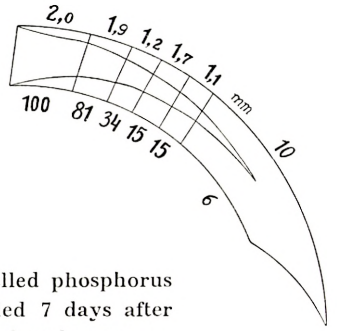

Fig. 3. Distribution of labelled phosphorus in the incisor of a rat killed 7 days after administration of the phosphorus.

The figures below give the relative amounts of labelled phosphorus present in 1 mgm. of fresh tissue in the section in question. The figures above give the length of the section in mm. 2.0 should read 2.8.

the two upper incisors and the activity of these incisors compared with that of the two lower incisors containing the pulp: we also measured the activity of the extracted pulp. The results are seen in Table 10.

Table 10.

	Fresh weight	Ash weight	% of the labelled P found per mgm. ash	Loss of weight on ignition
Lower Incisors + pulp	58.2	37.7	0.0192	35.2 %
Upper Incisors — pulp	33.2	22.9	0.0185	31.0 %
Tibia	800.1	123.2	0.0234	84.6 %

The activity of the extracted pulp was very weak and only amounted to about 3 % of that of the upper incisors. Should the exchange of phosphorus in the calcium phosphate of the teeth be very small, it is however quite possible that the amount of labelled phosphorus present in the pulp would no longer be negligible (comp. p. 26).

While in the experiments described above emphasis was laid on the investigation of the remote incisal end, in the following experiment we cut the proximal end of the incisor into small pieces and compared their activity with that of the distal end. The results are seen in Table 11, I denoting the united parts nearest to the jaw of all four incisors (comp. Fig. 3).

Table 11.

Distribution of labelled phosphorus, injected subcutaneously, in the incisor after 7 days. Weight of the rat about 200 gm.

Part of the incisor (comp. Fig. 3)	Length in mm.	Weight of the fresh tissue in mgm.	% of the labelled P found per mgm. tissue
I	2.8	7.3	0.0156
II	1.9	16.8	0.0127
III	1.2	21.3	0.0054
IV	1.7	25.1	0.0025
V	1.1	28.2	0.0025
VI	10.0	163.7	0.00096

Average % of labelled P per mgm. tissue = 0.0028, per mgm. ash 0.0038.

The investigation of the labelled P content of the head (A), the central (B) and lower part (C) of the tibia gave the following figures.

While in the proximal end of the incisor the phosphorus exchange is much greater than in any part of the tibia, the exchange of the average phosphorus atoms in

Table 12.

	Weight of the fresh tissue in mgm.	Weight of ash in mgm.	% of the labelled P found per mgm. ash
A	1131.1	144.5	0.0080
B	312.4	130.9	0.0026
C	243.8	63.5	0.0027

Average % of labelled P per mgm. tissue = 0.00098; per mgm. ash 0.0049.

the tibia is about 29 % greater than that of the average P atoms of the incisor; this is due to the fact that contrary to the tibia a large part of the incisor exchanges phosphorus atoms in the course of 7 days only to a very small extent. The figures in Table 12 cannot be compared directly with those obtained from fully grown animals for the following reason: the growing animal being much smaller the percentage of labelled P obtained for the same weight of the organ becomes larger; furthermore growth much facilitates the uptake of phosphorus. We can, however, compare the ratio of the labelled P content of the incisor and of other organs; the value of this ratio for the tibia, for example, is found to be not appreciably different in the cases discussed above. As to the labelled phosphorus content of the blood, this amounted after the animal was killed to only 0.04 % per gram of blood; assuming a total amount of 10 ccs. of blood all but 0.4 % of the labelled phosphorus administered left the blood of the animal in the course of 5 days.

The exchange of labelled phosphorus in molars.

In contrast to the incisors, molars of adult rats do not grow, so the labelled phosphorus found in the latter is due solely to exchange processes; the blood stream circulating through the molar carries labelled phosphate ions

which enter into exchange processes with the calcium phosphate of the molar tissue. Such exchange processes also take place in the incisors simultaneously with the formation of new ossification products. In the molars of adult animals, however, we encounter chiefly the former process; but though growth can be excluded we cannot discard the possibility of dissolution of tooth tissue at one place and a corresponding precipitation of calcium phosphate at another along the boundary between the circulating fluid and the tooth tissue. Small fluctuations in the acidity or parathormone concentration of the blood are sufficient to cause such a process. The molars of the rat decribed on p. 15 showed a content of labelled phosphorus amounting to 0.0013 % per mgm. of tissue and 0.0018 % per mgm. of ash, which is less than in the average incisor. The loss on ignition was found to be 26.9 %. We thought furthermore that it would be of interest to compare the labelled P content of the incisors, molars and skeleton, choosing the tibia as representative of the latter. The figures obtained are seen in Table 13. The

Table 13.

Organ	Labelled P found in 1 mgm. fresh tissue as a percentage of the amount given	Labelled P found in 1 mgm. ash as a percentage of the amount given	Loss in weight on ignition
Incisor	0.0033	0.0044	26.4 %
Molar	0.0013	0.0018	26.9 %
Tibia	0.0024	0.0064	63.2 %

fresh tissue of the incisors contains more labelled P than equal weights of either the molars or the tibia, but comparing the ashes the tibia has a content three times as large as the incisors and eight times as large as the molars.

A comparison of the labelled P content of the ash is in general preferable to that of the fresh tissue, the former comparison giving information about the percentage of phosphorus atoms replaced by labelled ones. The total P content of the ash of the incisors varies between 19.6 and 20.0 %, and that of the molars and the tibia is only slightly smaller, about 18 %. A closer analysis of the tibia revealed the parallelism already mentioned between the content of organic tissue and labelled phosphorus; that is shown in Table 14. The rat was killed 3 days after the administration of the labelled P.

Table 14.

Part of the tibia, see fig. 4	Weight of the fresh tissue in mgm.	% of the labelled P found in 1 mgm. tissue	% of the labelled P found in 1 mgm. ash	Loss on ignition
a_1	215.4	0.0028	0.0134	79.1
b_1	64.4	0.0033	0.0064	48.3
c	78.8	0.0019	0.0033	42.0
b_2	33.1	0.0015	0.0028	47.5
a_2	81.5	0.0014	0.0034	58.7

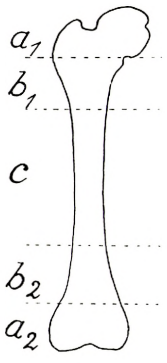

Fig. 4.

As seen from the figures of Tables 13 and 14 the head of the tibia has exchanged a part of its phosphorus content eight times as large as that exchanged by the molars.

Furthermore we compared the labelled P content of the incisors, the molars, and the tibia in the case of a rat weighing 220 gms. killed 1 hour after the labelled phosphorus had been administered by subcutaneous injection. The results are seen in Table 15.

Table 15.

Organ	Labelled P found in 1 mgm. fresh tissue as a percentage of the amount given	Labelled P found in 1 mgm. ash as a percentage of the amount given	Loss in weight on ignition
Incisor	0.00046	0.00062	26.0
Molar	0.00025	0.00034	27.4
Jaw	0.00125	0.0020	36.3
Tibia head	0.0024	0.0077	68.7
Tibia residue	0.00068	0.0014	52.7

The incisor was cut in 5 pieces the labelled P content of which is seen below, I denoting the proximal end.

Table 16.

	Weight of the fresh tissue in mgm.	Labelled P found in 1 mgm. fresh tissue as a percentage of the amount given
I	6.1	0.0062
II	14.4	0.0027
III	236.2	0.00040
IV	33.0	0
V	19.6	0

One cc. of plasma contained 0.5 % of the activity injected; assuming a plasma content of 10 cc., only 5 % of the labelled phosphorus injected was present in the blood after the lapse of 1 hour. Only 1 hour after administering the labelled phosphorus the tibia phosphorus was found to be 1000 times less active than the blood phosphorus, while for the molars the corresponding ratio was found to be 5000 and for the incisors (inclusive of growth) 2700. We also determined the activity of the acid soluble phosphorus extracted from the muscles of the rat and found 1 mgm. to contain 0.042 % of the labelled P given. From this figure and those found for the activity of the tooth and tibia phosphorus to be seen in Table 16 it follows that a comparatively fast phosphorus exchange is taking place

in the muscle compared with that ascertained in the bones and the teeth.

Exchange of phosphorus in the teeth of cats.

For the teeth of young cats[1] killed a few hours after the subcutaneous injection of the labelled phosphorus the results seen in Table 17 and 18 were obtained.

Table 17.

Cat weighing 2 kg. killed after $3^1/_2$ hours.

Tooth	Weight of ash in mgm.	% of injected labelled P present in the tooth	% of the labelled P per mgm. ash of the tooth
Upper molar	123	0.016	0.00013
Lower molar	110	0.014	0.00013
Upper canine	108	0.044	0.00041
Lower canine	91	0.040	0.00044

Table 18.

Cat weighing 2.5 kgm., killed after $1^1/_2$ hours.

Tooth	Weight of ash mgm.	% of injected labelled P present in the tooth	% of the labelled P per mgm. ash of the tooth
10 Incisors	94.8	0.0032	0.000034
Canine	148	0.019	0.00013
Jaw	126.3	—	0.00037

We also investigated fully grown cats. A cat weighing about 4.5 kgm. and killed three days after administration of the labelled P gave the figures seen in Table 19. In this experiment the labelled P injected was not of negligible weight but amounted to 15 mgm. (corresponding to about 75 mgm.

[1] The heads of the cats were kindly given to us by Professor Lundsgaard; they were obtained in the course of an investigation on the distribution of labelled phosphorus carried out by him and one of the present writers. In the first mentioned case 1 mgm. plasma P was found to contain after $3^1/_2$ hours 1.6 % of the activity injected, i. e. about 2300 times as much as that present in 1 mg. of the upper molar P.

sodium phosphate). The labelled phosphorus used in this experiment was kindly presented to us by Prof. LAWRENCE and was prepared by the action of high speed deuterium ions on phosphorus and accordingly contained a comparatively large amount of normal phosphorus. The injection of 15 mgm. P into a cat contained in its blood only about 10 mgm. inorganic P leads to an accelerated excretion and the figures are thus not entirely comparable with those of the last described experiment, which was furthermore carried out on a growing cat.

Table 19.

Cat weighing 4.5 kgm., killed after 3 days.

	Weight of ash in mgm.	% of injected labelled P present in the teeth	% of the labelled P per 100 mgm. ash of the tooth
Molars	690.5	0.0080	0.0012
Upper canines	768.4	0.0076	0.0010
Lower canines	635.2	0.0068	0.0013

The corresponding enamels weighed 29.3, 34.3 and 55 mgm. The canine enamel was found to contain less than $^1/_{80}$ of the labelled P content of the corresponding dentine.

In another experiment a strong preparation was administered in three portions, 5 days, 2 days and 1 day before killing the animal, each portion containing 40 mgm. P. The results are seen in Table 20.

Table 20.

Cat weighing 4 kgm., killed after 5 days.

	Weight of teeth in mgm.	Weight of ash in mgm.	% of injected labelled P present in the teeth	% of the labelled P per 100 mgm. ash of the tooth
Molar	323.3	186.0	0.0027	0.0015
Canine	422.2	274.3	0.0038	0.0014
8 Incisors	172.5	117.5	0.0021	0.0018

The enamel obtained is discussed on page 28. In investigating the incisors of rats we found the activity to be due almost exclusively to the phosphate of the mineral constituents, the pulp being only slightly active. In the earlier experiments conditions were however very different from those obtaining in the above mentioned case. The uptake of labelled P in the teeth of a cat is much smaller than in the incisors of a rat and correspondingly the ratio of labelled P in the plasma to labelled P in the teeth is much larger in the case of the cat. Now a high blood activity will lead to a comparatively high pulp activity and we must expect a greater share of the pulp[1] in the total activity of the tooth in the case of cat teeth. To test this point we removed the pulp of some of the canine teeth and compared the activity of the dissected and the total canine. We found an activity ratio of 3 : 4, showing that a quarter of the activity of the canines of a fully grown cat is due to the pulp.

A comparison of the figures of Tables 17 and 18 with those of 19 and 20 shows that the uptake of labelled P in young animals is greater than in fully grown ones and also that while in the former case the canines take up 3 to 4 times as much labelled P (per mgm. ash) as the molars, in the latter case no such difference is found. As has already been mentioned above the figures for the two sets of experiments are not entirely comparable, but no objection can be raised against a comparison of the ratio of the canine and molar uptake, which differs very markedly in the case of growing rats from the ratio for fully

[1] Human tooth pulp was found by H. C. Hodge (Proc. Soc. Exp. Biol. Med. **35**, 53, 1936) to contain 0.70 % phospholipins besides other phosphorus compounds.

grown animals. The following is a possible explanation of this difference: the labelled P uptake in the teeth of young rats is due partly to a growth of the teeth and not to an exchange process; since in the cat the canines grow faster than the molars the uptake is greater in the former case. One would be inclined to object to this explanation on the the short duration of the experiment, the growth in ground of the course of few hours being considered entirely negligible. This objection is however unwarranted. The molars of the growing cat weighed 116 mgm. and those of the fully grown animal 691 mgm. It does not take longer than a few years for the growing cat to become fully grown so the yearly growth of a molar will be above 100 mgm. Let us now calculate the amount of tooth ash formed on the assumption that the labelled phosphorus found in the tooth is due to growth. A molar of the growing cat took up 0.016 % labelled P during 3.5 hours. The labelled P which we injected into growing cats had in most cases a negligible weight originally, but very soon after the injection it mixed with the inorganic phosphate of the plasma (corresponding to about 5 mgm. P) and from that moment we must consider the labelled P as having a weight of about 5 mgm. 0.016 % of the labelled P will therefore correspond to 0.0008 mgm. P. The next step is that a large part of the labelled phosphorus leaves the plasma and is replaced by other phosphorus atoms coming from different bodily organs and also from the blood corpuscles. The result is that 0.016 % of the activity given no longer represents 0.0008 mgm. P but a greater weight, our scale of indication becoming less and less sensitive. From the experiences of Prof. LUNDSGAARD and one of us on the exchange of phosphorus present in the plasma we can estimate roughly that the amount

of P which corresponds after the lapse of 3.5 hours to 0.016 % of activity is about 0.008 mgm. in the case discussed. To transform from phosphorus weight to ash weight we have to multiply by six. The weight of the tooth thus increases by 0.04 mgm. in 3.5 hours and about 100 mgm. in a year. The order of magnitude of the growth observed and that calculated on the assumption that the uptake of labelled P is due to growth and to exchange is thus the same.

A very simple but instructive calculation can be carried out in the case of a fully grown cat into which as much as 120 mgm. labelled P was injected. We can calculate how many milligrams of these 120 mgm. are to be found after the lapse of 5 days in a single tooth. Making use of the figures quoted in table 20 we find that a canine takes up 0.005 mgm. and a molar 0.003 mgm.

The behaviour of the enamel.

The difference in the mechanical properties of dentine and enamel is very pronounced. The hardness of anterior enamel is nearly half as great as that of hardened tool-steel, while dentine compares closely with brass[1]. The hardness is taken as the pressure in kilograms necessary to push a steel ball into the test piece.

The above mentioned difference is not due to a pronounced difference in the relative abundance of the mineral constituents of dentine and enamel, as discussed on p. 5, but to the following conditions. The amount of organic constituents + water found in dentine is about six times as large as the amount present in enamel, the calcification of the enamel tissue being thus carried through much more effectively than that of the dentine tissue. Bowes and Mur-

[1] H. C. Hodge, J. Dental Res. 15, 251 (1936).

RAY[1] found organic matter in human enamel to an extent of only 1 %. As there is more organic matter[2] in enamel near the junction with the underlying tissue, the dentine, than in the part equidistant from the dentine and the surface of the teeth, the outer part of enamel must contain even less than 1 % organic matter. The latter appears to be[3] a protein containing tyrosin and resembling reticulin.

Another outstanding difference between dentine and enamel seems to be the size and degree of orientation of the crystallites present in these. As to the orientation it has been stated[4] that enamel of high quality gives X-ray diagrams of a high degree of orientation, while enamel of poor quality does not. On igniting dentine an X-ray diagram characteristic of β-$Ca_3(PO_4)_2$ is often but not always observed[5]; this is never shown by ignited enamel. As it was found[6] that β-$Ca_3(PO_4)_2$ is formed when an excess of PO_4-ion is present, it was concluded that the dentine apatite often adsorbs an excess of phosphate ion which promotes the formation of β-$Ca_3(PO_4)_2$ on ignition. In the case of enamel forming larger crystallites, no excess of PO_4-ions being present, no β-$Ca_3(PO_4)_2$ formation was observed on ignition. While important information may be obtained by the study of X-ray diagrams the interpretation of the latter must be made with care.

Phosphorus exchange in the enamel.

In view of the connection found between the content of organic matter and phosphorus exchange in the teeth it

[1] J. H. BOWES and M. M. MURRAY, Biochem. J. 29, 721 (1935).
[2] C. F. BODECKER, J. Dental Res. 6, 2, 117 (1923).
[3] P. PINCUS, Nature 138, 970 (1936).
[4] J. THEWLIS, Naturw. 25, 42 (1937).
[5] W. F. BALE, M. L. LEFEVRE and H. C. HODGE, Naturw. 24, 976 (1936).
[6] G. TRÖMMEL and H. MÖLLER, Z. anorgan. Chem. 206, 227 (1932).

did not appear very promising to look for a pronounced exchange in the enamel. The enamel investigated by us was in some cases removed mechanically while in others we succeeded in separating the enamel of cat teeth after igniting the tooth very carefully. The enamel, having a different expansion coefficient from the dentine, splits off during the ignition process and can thus be removed. The method of separation used recently by various workers[1], in which the tooth is pulverized and placed in an organic liquid of suitable density when the heavier enamel settles to the bottom of the tube, is not suitable for our purpose. The reason is that some dentine often sticks on the pulverized enamel; assuming that the dentine is strongly active and the enamel not, we see that the presence of traces of dentine in the enamel might falsify the analysis.

We made several experiments with the enamel of cat teeth but in most cases with negative results, the exchange in equal weights of enamel being at least 20 times as small as that found in the molars of cats. In one case we got a positive effect, the canine of a fully grown cat five days after injecting the labelled phosphorus showing a radioactivity of 26 relative units (kicks per minute), one enamel sample showing 0.6, and another 0.7 kicks. The first mentioned enamel was separated by grinding it off from the dentine, while the second one was obtained by the same method from the uppermost enamel layer. The ash weight of the canine was 277.3 and that of the enamel samples 33.1 and 19.1 mgm. We are however reluctant to accept this positive result. On account of its smaller weight and greater distance from the underlying dentine, the outermost layer should be less active than the second enamel

[1] comp. P. J. Brekhus and W. O. Armstrong, J. Dental Res. 15, 23, 1935.

layer unless the labelled phosphorus present in the saliva (which latter contains[1] 13.4 mgm. per 100 ccs.) can interact with the outer layer of the enamel; this is not very probable, the uppermost layers excepted. We intend to follow up the problem of the phosphorus exchange in enamel using phosphorus preparations of greater activity.

Exchange of phosphorus in human teeth.

Other things being equal the exchange of phosphorus in teeth will be determined by the efficiency of lymph circulation in the tooth. Exchange experiments can thus be carried out to obtain information on the latter point. It does not look improbable that the growth of caries will be facilitated by a poor circulation; to decide this point we compared the phosphorus exchange in two teeth of the same individual (16 years old) removed simultaneously, one on account of caries, the other, a healthy one, to space the patients teeth better; about a two hundred thousandth part of the labelled phosphorus was found in each of the teeth investigated, a quantity sufficient to be measured but not large enough to permit the exact comparison necessary to decide the point discussed above. The weights of the whole fresh teeth were 800 and 540 mgm. and of the ash obtained on ignition 465 and 330 mgm. respectively; this corresponds to a loss on ignition of 58 and 61 % respectively. The time which elapsed between the injection of the radioactive phosphorus and the extraction of the teeth was 7 days. Through the very great kindness of Professor LAWRENCE we were able to continue these experiments using a much stronger radioactive phosphorus sample prepared by him with the aid of his powerful cyclotron. 900

[1] M. KARSHAN, J. Dental Res. 15, 388, 1936.

mgm. labelled sodium phosphate per os were administred to a patient 25 years old. 4 days later 10 necrotic teeth and 5 days later still, three more, fairly well preserved, living teeth were extracted. Of the $2.5 \cdot 10^6$ relative radioactive units we can estimate that about $1.8 \cdot 10^6$ were absorbed. As is seen in table 21 6 relative units were found in a fairly well preserved tooth on an average, showing that about 1 : 300,000 part of the labelled phosphorus atoms enter a single tooth; in the case of a 16 years old boy about 1 : 200,000 was found. In the latter case an activity of only 0.5 units (kicks per minute) was shown by a single tooth, and the estimate was accordingly only a very rough one. From the above result it follows that about 1 : 300,000

Table 21.

Labelled phosphorus in the teeth of a 25 year old patient.

a) Necrotic Roots.

Nr.	Fresh weigh	Ash weight	Relative labelled P content In total root	Relative labelled P content In 100 mgm. root ash
1	223.7	138.1	2.7	1.96
2	284.1	180.1	4.9	2.72
3	199.4	127.1	2.5	1.97
4	230.5	143.0	1.4	0.98
5	124.8	76.5	3.9	5.13
6	435.2	268.5	5.9	2.19
7	205.7	127.1	4.9	3.86
8	169.5	109	1.6	1.47
9	172.5	106.6	3.8	3.57
10	183.5	115.0	2.1	1.83

b) Necrotic Crowns.

	Fresh weight	Ash weight	Relative labelled P content In total crown	Relative labelled P content In 100 mgm. crown ash
One single crown	65.8	39.1	3.7	9.4
Fragments of several crowns	241.7	149.8	12.3	8.2

c) Almost normal roots.

Nr.	Fresh weight	Ash weight	Relative labelled P content In total root	Relative labelled P content In 100 mgm. root ash
1	377.4	241.0	2.8	1.16
2	651.1	413.6	3.7	0.9
3	685.9	430.2	6.7	1.56

d) Almost normal crowns.

Nr.	Fresh weight	Ash weight	Relative labelled P content In total crown	Relative labelled P content In 100 mgm. crown ash
1	533.5	338.7	1.9	0.56
2	862.9	670.9	1.8	0.27
3	464.1	429.9	1.8	0.42

part of the phosphorus taken up with the food finds its way into each tooth of an adult. In the course of time these phosphorus atoms get replaced by others taken up with the food or originating from other bodily organs. The normal diet containing about 2 gm. of phosphorus per day and a human tooth containing about 150 mgm. P, the replacement of 1 % of tooth P by that taken up with the food will take 250 days. Simultaneously a further replacement of the phosphorus atoms takes place with phosphorus originating from other bodily organs.

Summary.

It has been shown that an exchange of phosphorus atoms present in the teeth with those present in the blood plasma takes place.

During the growth of the incisors of rats the newly deposited phosphorus atoms are to a large extent found in close vicinity of the dental pulp, but even in the most

remote part of the incisor the presence of newly substituted phosphorus atoms can be established. An exchange of phosphorus atoms thus takes place even in those parts of the incisors which are entirely outside the range of the pulp. The exchange in the molars was found to be less pronounced than that in the incisors, this being presumably due to the fact that these teeth do not grow.

In the teeth of young cats within few hours, besides an exchange of phosphorus atoms, an increase in the labelled phosphorus content due to the growth of the teeth could already be ascertained.

An exchange of phosphorus has also been proved for human teeth, 1 : 300,000 of the phosphorus administered being found in each tooth. The replacement of 1 % of the phosphorus content of a human tooth by phosphorus atoms taken up with the food takes about 250 days.

The labelled (radioactive) phosphorus applied was partly prepared by us from sulphur under the action of neutrons emitted by a radium-beryllium mixture, most kindly put at our disposal by Professor Niels Bohr, and partly formed a generous gift from Professor Lawrence of the University of California. Besides thanking Professor Bohr and Professor Lawrence, we would also like to express our best thanks to Miss Hilde Levi, Miss A. L. Lindberg and Mr. O. Rebbe for their assistance.

(From the Institute of Theoretical Physics, the Dentistry School and the Zoophysiological Laboratory, Copenhagen.)

Færdig fra Trykkeriet den 23. November 1957.

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