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FROM THE ANATOMICAL-PATHOLOGICAL INSTITUTE AND THE ZOOLOGICAL MUSEUM
OF THE UNIVERSITY OF COPENHAGEN

CONTRIBUTIONS TO THE BIOLOGY AND MORPHOLOGY OF *SPIROPTERA (GONGYLONEMA) NEOPLASTICA N. SP.*

BY

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THE nematode described in the present paper was first discovered by J. FIBIGER in the cardiac portion of the stomach of the rat. By a series of experiments he succeeded in proving that this nematode during its evolution uses cockroaches as a host and in the stomach of the rat is able to produce papillomatous — and in certain cases carcinomatous growths.

Further investigation on the morphology of the nematode was made by HJ. DITLEVSEN, who defined it as a hitherto not described species of the genus *Spiroptera*.

Before proceeding to a description of the morphological and biological features of the nematode a summary of the main points of the papers[1]) previously published by J. FIBIGER will be given here:

The nematode was originally found in 3 wild rats (m. decumanus) belonging to the stock of rats of the anatomical-pathological Institute of the University of Copenhagen and probably imported to Denmark from Dorpat.

In these 3 rats, isolated in the same cage for a long time, the cardiac portion of the stomach presented enormous papillomes, which in some respects were found similar to malignant epithelial tumours.

In these stomachs the nematode was originally found by microscopical examination of sections from the papillomatous ventral wall. Among the upper coats of the squamous-celled epithelium sharply outlined bodies of a complicated structure were observed, containing in several instances eggs with embryos.

In order to define these bodies as veritable sections of a nematode, a great erseries of sections was reconstructed and the length of the nematode thus determined as nearly 1,6 cm. the diameter being nearly 0,25 mm. Later on a few nematodes were prepared perfectly out of the stomachs after fixation in formaline.

Now, however, the papillomes of the cardiac portion of the stomach can with great probability be referred to the nematode. Both zoological and medical literature

[1]) Recherches sur un nématode et sur sa faculté de provoquer des néoformations papillomateuses et carcinomateuses dans l'estomac du rat. Acad. royale de sciences et de lettres de Danemark. Extrait du bulletin de l'année 1913 No. 1, — Untersuchungen über eine Nematode (Spiroptera sp. n.) und deren Fähigkeit, papillomatöse und carcinomatöse Geschwulstbildungen im Magen der Ratte hervorzurufen. Zeitschrift für Krebsforschung, Bd. XIII, 1913.

contains cases in which the occurrence of tumour-like growth or true tumours in the organs of animal or man, has been found coincident with the presence of helminthes (especially trematodes or nematodes) and a sort of causal relationship might probably exist here, in analogy with the now elucidated power of the Bilharzia worm to produce cancer in the urinary bladder of man. BORREL, moreover, has put forward the hypothesis that the development of cancer in mice and rats is due to an unknown virus, transmitted by various parasites, among which nematodes; and according to HAALAND there is evidence that the presence of nematodes in the subcutaneous tissue of the mammary gland in mice might be made responsible — at least to a large extent — for the frequency of inflammatory processes, associated with pathological changes of adenomatous and cystical character, from which a true cancer can develop. In order now to make out, whether a causal relationship might exist between the nematode found in the 3 rats from Dorpat and the pathological changes of their stomachs, a series of examinations were carried out. The results of these investigations having been published in full extent in "Zeitschrift für Krebsforschung"[1]), only a shorter summary will be given here.

Subcutaneous and intraperitoneal transplantation of tumour-cells from 2 of the 3 Dorpat rats into normal rats of various species gave negative results. In experiments with rats fed on tumour-cells no papillomatous growth was noted, and in rats, isolated for a longer time (until one year) in the same (uncleaned) cage, in which had been kept the 3 rats from Dorpat neither pathological changes of the cardiac portion of the stomach nor nematodes were observed.

In fact, no tumour-like growth of the stomach was found in the rats of the Institute except in the 3 rats already mentioned, which harboured the nematode and most likely were imported to Denmark from Dorpat,

Numerous examinations were made of wild rats from various localities in Copenhagen or from the neighbourhood and of white and black and white rats from different laboratories, but neither papillomatous growth nor nematodes similar to those which had initiated the investigation were observed.

Thus, pathological changes as the described no more than the nematode seemed to be known here in Denmark and the fact that the feeding experiments on tumour material, containing nematodes, had not been successful, might perhaps be due to an insusceptibility of Danish rats.

Another possibility — however — appeared to be still more obvious: Instead of being directly infective the nematode might be wanting a host, in which the embryos of the eggs could attain a higher stage of development, which would make them infectious to the rat. As according to a report made by GALEB in "Comptes rendus des séances de l'Académie des Sciences", Tome 87, 1878, it was possible, that cockroaches *(Periplaneta orientalis)* were able to serve as a host, investigations were made on several wild rats from a locality in Copenhagen where great numbers of *P. orientalis* were at hand.

Neither nematodes nor tumour growth, however, was found in any rat, and feeding experiments on normal rats with cockroaches from this locality also turned out negative.

A better result was given by some examinations made in another locality, a sugar refinery, which harboured lots of rats and cockroaches, although these were not *P. orientalis* but *P. americana.*

[1]) l. c.

Out of 61 rats *(M. decumanus)* caught in this locality and kept isolated in the institute — most of them until death, 40 rats presented the nematode in the epithelium of the cardiac portion of the stomach. Pathological changes were found in 18 among these 40 rats, in 9 cases as marked papillomatous tumours of the same type as described above, while in the rest of the cases the process must be considered a preliminary stage of true new growth. It was further determined that the nematode could not only be found in the cardiac portion of the stomach but also in the epithelium of the oesophagus, occasionally also in the epithelium of the tongue and in the mucous membrane of the mouth.

Observations made on rats fed on cockroaches *(P. americana)* caught in the refinery, gave the following result:

For these experiments 4 wild brown rats from the neighbourhood of Copenhagen and 53 black and white rats born and bred at the new anatomical-pathological Institute of the University were used. The modern, and hitherto unused stables here did not at all harbour cockroaches. The rats were fed on cockroaches for one or 2 periods, every period being of 3—70 days duration. The number of cockroaches used as feeding-material varied, 2 rats were given c. 100, the rest less. The rats with a few exceptions were not killed unless getting into agony. On the whole, the experiments gave the following results: In 3 rats the stomach presented no pathological changes and especially no nematodes. On the other hand parasites were found in different numbers in the remaining 54 rats, 37 of which presented pathological changes simultaneously. In 11 cases the process appeared to be but slightly marked, in 27 cases a higher stage of development was reached, which in 7 cases consisted in enormous tumour-growths of the described type.

The nematode and eggs found in these rats corresponded to those observed in the rats from Dorpat and in the rats caught in the sugar refinery. Most unfortunately — however — the sugar refinery was destroyed by fire in February 1912, and experiments consequently could not be carried on, as at this point of time no other locality in Copenhagen or elsewhere seemed to harbour the *P. americana.*

The results of all series of experiments are seen from the table:

Sort of rats	Number of rats examined	The worm found in	The worm found coincident with path. changes in	True newgrowth found in
Wild rats:				
m. decumanus	844	0	0	0
m. rattus	21	0	0	0
m. alexandrinus	2	0	0	0
Black and white laboratory rats	277	0	0	0
Total...	1144	0	0	0
m. decumanus from sugar refinery	61	40	18	9
Black and white laboratory rats fed on *P. americana* from sugar refinery	57	54	37	7
Total...	118	94	55	16

The results thus obtained admitted no doubt whatever as to the responsibility of the nematodes for the development of growths, nor to the fact that transmission of the nematode into the rat must take place by the *P. americana*. Further conclusions as to the part played by the cockroach during the transmission could not be drawn from these experiments. It might be possible that the cockroach transmitted the nematode into the rat, without serving as a host, in which the nematode went through a further development. Numerous examinations of rats, the stomach of which contained nematodes, now showed that eggs containing embryos were embedded in the squamous-celled epithelium of the cardiac portion of the stomach. The eggs are liberated by desquamation of the epithelium and pass with the excrements of the infected rats without further development. The excrements nearly always contain eggs, but never free embryos. It further appeared from the experiments that the eggs, when left in the laboratory for half a year in moist excrements of the rat did not change at all, neither did the excrements then contain free embryos.

As already mentioned the first experiments in which the rats were fed on tumour material from two of the Dorpat rats had given negative results. In later experiments eggs with embryos were used for feeding, but no transmission of the nematode into normal rats was reached.

On the whole investigations had now shown 1) that in excrements evacuated from infected rats no free embryos were found but only eggs containing embryos, 2) that the eggs directly transmitted into the stomach of rats did not develop into nematodes, 3) that the eggs when left in the excrements did not go through any further development — at any rate not during half a year. The nematode, however, actually being transmitted by the cockroach, these observations had left it beyond all discussion that the cockroach really served as a host in the proper sense of the word, and the parasite thus was sure to be found in their body.

Examination of the fatty bodies of the *P. americana* from the sugar refinery, however, gave negative results, as well as examination of the digestive tube, which in numerous cases contained neither eggs nor free embryos. On examination of protorax and the limbs, however, nematodes, which after their size and shape most likely must be considered further stages of the embryos, were found in the muscles, coiled up like trichines. As a matter of fact it might be quite another nematode as well. In order to disprove this, a series of experiments was made to transmit the nematode into cockroaches, in the muscles of which the absence of nematodes before the transmission could be absolutely taken for granted. In these experiments *P. orientalis* were used, fed partly on eggs extracted from nematodes, partly with rats' excrements containing eggs. The cockroaches were examined 42—60 days after discontinuance of the feeding.

The experiments are seen from the following table:

	Number of cockroaches examined	Nematodes found in the muscles of
Cockroaches fed on fæces....	18	17
— — eggs.....	9	9
Non-fed control cockroaches..	101	0

As will be seen from the table it did not cause any difficulty to produce deposits of nematodes in the muscles of the cockroaches. In no respects did these worms differ from the nematode found in *P. americana* and also here they were coiled up trichina-like in spirals, often surrounded by a fine capsule. On feeding rats on cockroaches *(P. orientalis)* infected in this way the nematode was further transmitted to rats, as seen from the following table which gives a view of the first experiments of this sort.

Number of rats	Each rat fed on cockroaches *(P. orientalis)*	Nematodes found in the stomach of rats	Nematodes and pathological changes found in the stomach of rats	New-growth found in rats
	Number of *P. or.*	Number of rats:	Number of rats:	Number of rats:
e 6	1/2	2	1	0
- 6	2 1/2—5	1	0	0
- 2	12	0	0	0
f 8	1—3	3	1	0
- 16	15—103	16	13	7
Total: 38		22	15	7
Control experiments: Rats fed on non-infected cockroaches *(P. orientalis)*				
43	11—50	0	0	0

Thus by feeding rats on cockroaches *(P. orientalis)* infected with fæces, (*f*) or eggs of the nematode, (*e*) the worm was transmitted into 22 out of 38 rats, while in 43 rats used as control animals and fed on non-infected cockroaches from the same locality, the nematode was not found at all.

After this the transmission of the nematode by *P. orientalis* does not cause any difficulty and for more than a year in a long series of experiments, has now[1]) been carried out quite regularly at the Institute. When for the feeding experiments is used a not too small amount of cockroaches, infected during a longer period by rat's excrements containing eggs, the infection of the rats will as a rule turn out successful. Out of the 16 rats noted above, fed each of them on 15—103 cockroaches, the nematode was observed in all of them without exception; in 13 rats anatomical changes were found simultaneously, and in 7 cases the changes were tumour growths of the same type as those originally observed.

The life-cycle of the nematode now, according to the observations already published and here only sketched, must be as follows: The nematode lives in the pavement epithelium of the stomach and of the oesophagus of the rat — occasionally also in the epithelium of the tongue and mouth. It here reaches sexual maturity and evacuates ripe eggs, containing embryos, which are liberated by desquamation of the epithelium and passed with the excrements.

[1]) December 1913.

On being consumed by the cockroaches (either *P. americana* or *P. orientalis*) the embryos are liberated and wander into the striped muscles of the protorax and the limbs. In these localities they are found after six weeks or more as larvæ coiled up trichina-like in spirals and surrounded by a capsule.

When at this point of time the rats eat the cockroaches the larvæ are freed from their capsules and wander into the squamous-celled epithelium of the fundus of the rat's stomach (sometimes also into the epithelium of the oesophagus, tongue and mouth) where after c. 2 months the females begin to evacuate eggs containing embryos.

As mentioned above, morphological investigations on the nematode were made by Hj. Ditlevsen, who has examined worms from the Dorpat-rats, from rats from the sugar refinery, and from black and white rats from various experiments; and finally has defined them as belonging to the very same, hitherto undetermined species of the genus *Spiroptera* as will be described in the following pages.

Thus, examination had shown that a causal relationship must exist between the nematode and the tumour-growths as in all cases the tumours were found coincident with the parasite, and growths of the same type had been developed by transmitting the worm into normal rats.

Out of all in all 118 rats (61 wild and 57 laboratory rats fed on *P. americana*) 94 harboured the spiroptera in their stomach. In 39 cases the nematode was the only abnormality observed; in 53 rats anatomical changes were found simultaneously. On the whole, these changes, with only a few exceptions, represented the very same type as those which had initiated the investigations and to a macroscopical view they only showed quantitatively mutual differences.

Out of 34 laboratory rats fed on *P. americana* 22 harboured the nematode not only in their stomach but also in their gullet; in 16 of these rats the mucous membrane of the gullet presented anatomical changes although of a less definite character than in the fundus of the stomach. In a few cases (4—5) the nematode was found also in the epithelium of the tongue, and in 1 case in the epithelial covering of the mouth.

In no rat was the gullet found affected without the stomach being affected too.

The nematode was never observed in the pyloric portion of the stomach, in the rest of the alimentary canal, nor in other organs. The nematode in question thus only lives in the upper portion of the rat's alimentary canal, paved with a squamous-celled epithelium. Only once was a *Spiroptera* found in a vein of the ventral wall; in all other cases the worm and free eggs were only found in the epithelial covering, where it is placed between stratum corneum and granulosum, producing irregular canals among the cells. Most frequently it is quite covered by the epithelium, but sometimes it may be seen protruding into the cavity of the fundus of the stomach. Very seldom it reaches further down touching the lower coats of the epithelium.

The anatomical changes of the stomach (figs. 10—16) were found only in the fundus, the pyloric portion being always quite normal.

In order to obtain a general view, the pathological changes in the rats fed on *P. americana* may be divided into: 1) slight changes, (16 rats) 2) definite changes (23 rats) 3) violent changes and tumour growths (16 rats). These groups, however, very smoothly pass into each other without sharp lines of demarcation. Out of the 16 rats fed on great quantities of arti-

ficially infected cockroaches *(P. orientalis)* 7 rats presented tumour growths, 6 definite hypertrophy of the epithelium, while 3 rats did not show any pathological changes — besides nematodes.

In the slightly definite cases (16 rats) the cardiac portion of the stomach was only changed very moderately, the hypertrophy of the mucous membrane being either circumscribed or diffuse. Corresponding to this, the microscopical examination showed a proliferation of the squamous-celled epithelium of the mucous membrane, combined with a definite hypertrophy and desquamation of the stratum corneum. As a rule, the mucous membrane and submucosa contained slight inflammatory processes consisting mainly in colonies of leucocytes, lymphocytes and plasma-cells.

In cases where the pathological changes must be characterized as definite (29 rats) the condition of the mucous membrane was diffusely hypertrophic, irregularly folded and wrinkled, often beset with small papillomatous prominences. In microscopical examination the mucous membrane, and the connective tissue of submucosa appeared to be proliferated, the latter forming the stock of small papillomes which protruded on the surface of the mucous membrane and were covered with hyperplastic epithelium; in some cases the inflammation had even reached the muscularis and the serosa.

The proliferation of the epithelium was very definite, not only was the stratum corneum hypertrophically changed but the lower coats as well; and often long tongues of epithelium were seen going deep down, displacing, and even perforating the muscularis mucosæ, so that submucosa contained spurs of epithelial islets.

In cases where the pathological changes showed themselves as violent or as tumour growths (23 rats in all) the stomach was very much enlarged, the wall of the cardiac portion very thick, and the mucous membrane set with petiolate irregularly branched papillomes or wall-like prominences, which in all cases made the ventral wall measure nearly 1—1½ mm. in sections; in several cases the cavity of the fundus was absolutely diminished by the papillomes, which in other cases not only caused an obliteration of the cavity but even protruded into the pyloric portion. The outside of the stomach was irregularly rugged, with globular or flat prominences; in some cases even petiolate protuberances were observed.

Microscopical examination showed that these changes were due to a further development of the tumours already described.

The connective tissue of the submucosa appeared to be a seat of violent inflammatory processes and new growth, so that branched coral- or crater-shaped prominences of connective tissue covered with coats of hyperplastic epithelium, projected into the stomach as enormous papillomes, 1—1½ cm. high.

By cooperation of these various processes:

Proliferation of the heterotopic epithelium, and inflammation and new growth of the connective tissue of submucosa, enormous pathological changes were produced. In fact, the proliferation of the heterotopic epithelium in several cases destroyed the papillomatous wall of the fundus to such an extent that it consisted of nothing but great masses of epithelium covered with a thin serosa. In such cases the bundles of muscularis were atrophic, dismembered or quite destroyed. In all cases a definite eosinophily was observed in mucosa and submucosa of the ventral wall.

Moreover, it must be pointed out that in 5 laboratory rats fed on cockroaches (in 4 cases

P. americana, in 1 case *P. orientalis*) microscopical examination of the cardiac portion of the stomach showed processes which differed from those found in the other rats. The changes, in fact, here appeared to be true cancerous growths, the mucous membrane and submucosa having changed into a tumour tissue of quite the same type as is generally found in true squamous-celled carcinoma in man.

In 2 of these rats carcinomatous metastases were observed in a lymph gland and in a lung respectively; in a third case the urinary bladder contained papillomatous growth which was probably a metastase too. Notwithstanding a very careful examination of serial sections neither nematodes nor eggs were found in any of these metastases, being thus metastases in the most strict sense of the term.

By these investigations carcinomatous tumours giving rise to metastases were produced experimentally for the first time, and the hypothesis put forward by Borrel and Haaland was thus verified, as it has now been proved, that nematodes play a causal part in the development of cancer in rats [1]).

Further particulars as to description and pictures of the growths and pathological changes described above will be found in the papers previously published by J. Fibiger.

As only in very few cases bacteria were found outside the most superficial coats of the epithelium of the ventral wall, it seems in advance to be unreasonable to make such microbes responsible for the development of tumour growth. It rather must be referred — as well as the local eosinophily — to some poisonous secretion of the worm, and the more so, as in the most rats, a rather constant and plain proportion could be stated between the degree of the pathological changes, the number of the parasites and the length of time in which the spiroptera had lived in the fundus of the stomach.

Owing to the ability of the nematode to produce new growths the name Spiroptera neoplastica is proposed.

As a supplement to this summary of the papers previously published, some later investigations concerning the biology of the worm, carried out by J. Fibiger in the anatomical-pathological Institute of the University of Copenhagen will be recorded here.

As already mentioned it had not been possible — notwithstanding thorough examination — to find the *Spiroptera* in any other wild Danish rat, than in those caught in the sugar-refinery, where *P. americana* were found in great numbers.

On continued examination this result was further confirmed, as the nematode was observed only in one of 3 rats (*Mus decumanus*) caught in a sugar-refinery connected with the one mentioned above.

[1]) Later on, moreover, carcinoma has been produced in the same way in several rats. In one of these rats metastases also were observed. (December 1913).

This locality too harboured *P. americana* in great numbers and it was not difficult to prove, that the muscles of these cockroaches also contained the nematode coiled up and placed in the same way as in those originally examined. Controlling examination of rats from other localities has constantly turned out negative, so that until now the nematode has not been found in 1025 rats belonging to the species *M. decumanus*, in 21 rats of the species *M. rattus*, and in 2 rats of the species *M. alexandrinus* caught in various localities in Copenhagen and the neighbourhood, nor in 277 black and white or white rats from different laboratories.

Add to this several rats examined in the Institute the exact number of which can not be accounted for, the nematode was not observed in altogether nearly 1100 wild rats and nearly 300 laboratory rats.

Neither has the nematode been found in other wild cockroaches than in those (*P. americana*) living in the two refineries (see further below).

After all there is great evidence that in this country the nematode is bound to such localities (sugar-refineries) as contain the *P. americana*. These localities having in former days received raw sugar from West India where *P. americana* is very common, it seemed an obvious conclusion that the nematode must be found in the Danish West Indies. A series of investigations has been established and owing to kind assistance by the veterinary surgeon of the government SELMER, 6 dead rats (*M. rattus*) and c. 90 live cockroaches (*P. americana*) were sent to Denmark from St. Croix. Examinations in the anatomical-pathological Institute now showed that the cardiac portion of 2 of these rats contained specimens of the nematode; and in the muscles of 2 out of 6 cockroaches examined, the nematode was also observed. The rest of the cockroaches (84) were used — the $^{27}/_{7}$ 1913 — as feeding material on black and white normal laboratory rats, 6 out of which died resp. 18, 30, 44, 79, 85 and 141 days after the feeding.

In the epithelium of the cardiac portion of the stomach typical *Spiropteræ* were found in all the rats, in one, moreover, typical papillomatous growth of the same type as described above. One rat is still alive and infected, typical eggs having been found in his fæces. Thus, it must be considered very probable, that the *Spiroptera* in question originally was imported to Denmark with *P. americana* in the raw sugar from the Danish West Indies.

A series of investigations has further been carried out in order to transmit the nematode to other rodents. As already mentioned in a former paper it did not cause any difficulty to transmit the nematode to mice. Up till now[1]) the experiments completed comprise 61 mice, all of which were infected on feeding with cockroaches containing the *Spiroptera*. In 31 mice out of the same stock which had not been

[2]) December 1913.

fed on cockroaches, the nematode was not observed. The worm lives in the mouse quite in the same way as in the rat; it produces inflammation, proliferation and growth, occasionally associated with papillomes in the pavement epithelium of the fundus of the stomach and in the gullet. As far as can be seen from the experiments till now, the anatomical changes in mice are perhaps in some respects slightly different to the changes found in the rat, e. g. the nematode in mice seems more often to invade the deeper coats of the epithelium, sometimes also the deepest layers of the ventral wall. Development of real cancer has not yet been observed. Also in mice the nematode reaches sexual maturity and typical ripe eggs have been found in all the cases without exception — in the excrements of the mice, c. 2 months after the first feeding on cockroaches.

Also transmission of the nematode to rabbits has given positive results. From the $^{30}/_{4}$ till the $^{3}/_{5}$ 1913 8 rabbits were fed on 25 *P. americana* each, infected with rat's fæces containing eggs.

Rabbit	1	died	$^{23}/_{6}$	1913	in	the	gullet	7 *Spiroptera* (5 ♂, 2 ♀)
—	2	—	$^{8}/_{10}$	-	—	—	2	— (2 ♂)
—	3	—	$^{10}/_{10}$	-	—	—	5	— (5 ♀, no eggs)
—	4	—	-	-	—	—	1	— (♀)
—	5	—	-	-	—	—	5	— (1 ♂, 4 ♀ containing eggs)
—	6	—	-	-	—	—	2	— (2 ♂)
—	7	—	-	-	—	—	4	— (1 ♂, 3 ♀)
—	8	—	-	-	—	—	1	— (1 ♀)

No pathological changes were found in the gullet or mouth or tongue. The stomach was normal and contained no *Spiroptera;* eggs were not observed in the fæces.[1])

Experiments on guinea-pigs gave a similar result. From the $^{30}/_{4}$ till the $^{4}/_{5}$ 1913, 8 guinea-pigs were fed on 20 specimens of the *P. orientalis* each, infected with *Spiroptera* on eating rat's fæces containing eggs. In 6 guinea-pigs, which died spontaneously or were killed the $^{13}/_{5}$, $^{1}/_{7}$ and $^{11}/_{10}$ 1913 respectively, no nematodes were found. In 2 guinea-pigs killed the $^{11}/_{10}$ 1913 one male worm was found in the gullet of one of the animals, one female in the gullet of the other; apart from that the gullet, tongue, mouth and stomach were normal. Nematodes were not found elsewhere, in the fæces no eggs could be found.

The *Spiroptera* in question, thus, is not only able to live in wild rats (*M. decumanus*, *M. rattus*) and in white and black and white laboratory rats, but is very easily transmitted to white laboratory mice, rabbits and guinea-pigs.

[1]) In later experiments eggs have been observed (addition during the proof reading).

In all these rodents the nematode was only observed in the upper portion of the alimentary canal covered with squamous-celled epithelium, in rabbits and guinea-pigs only in the gullet, in rats and mice moreover, in the fundus of the stomach, which in these animals represents the continuation of the gullet, and occasionally in the epithelium of the tongue and mouth; whereas no other part of the digestive tube contained nematodes.

In order to ascertain whether any other species of cockroaches than *P. americana* and *P. orientalis* might be able to serve as a host, experiments were made on *Blatta germanica.*

For about 2 months (some of them from $^{13}/_{1}$—$^{14}/_{3}$, some from $^{27}/_{1}$—$^{5}/_{4}$, and others from $^{11}/_{4}$—$^{10}/_{6}$ 1913) a great number of *Blatta germanica* were fed on rat's excrements containing eggs of the *Spiroptera.* These cockroaches, in the muscles of which in a few cases after the feeding typical coiled-up nematodes were found, were consumed by black and white laboratory rats and white mice.

Black and white rats Number	Each rat fed on infected *Blatta germanica* Number of *Bl. germ.*	The rat died or was killed	Number of Spiroptera found in the fundus of the stomach
1	25	47 days after the feeding	4 (3 ♀ 1 ♂)
1	25	58 — —	4 (2 ♀ 2 ♂)
1	15	16 — —	0
1	15	41 — —	3 (♀)
Control experiments: Rats fed on non-infected cockroaches (*Blatta germanica*)			
6	125	93—114 — —	0
Mice.			
2	10	41 — —	0
2	18	52— 57 — —	In both of them Spiroptera and epithelial hyperplasia were found
Control experiments: Mice fed on non-infected cockroaches (*Blatta germanica*)			
4	13	14— 92 — —	0

Experiments have thus shown that *Blatta germanica* as well is able to serve as a host to the nematode.

In order to ascertain whether the nematode might be able to use the *Tenebrio molitor* (weal-worm) as a host in the same way as the *Spiroptera obtusa*, the following experiments were made:

A great number of larvæ and fully developed specimens of *Tenebrio molitor* from the $^{21}/_{6}$—$^{1}/_{7}$ 1911 were fed daily on fæces containing eggs, originating from wild brown infected rats (from the sugar-refinery). Likewise another number of *Tenebrio molitor* were fed daily from the $^{29}/_{6}$—$^{14}/_{8}$ 1912.

On examination of some *Tenebrio molitor* about $2^{1}/_{2}$ months after the beginning of the feeding no Spiroptera could be found in the muscles.

Black and white rats number	Each rat fed on	Spiroptera found in
12	c. 112 fully developed beetles, pupæ and larvæ	3 rats (in 2 one ♀, in 1 one ♂)
3	c. 107 fully developed beetles, pupæ and larvæ	1 — (one ♀)
Total 15		4 rats

The rats were killed 19—136 days after the feeding

Control-experiments: Rats fed on non-infected weal-worms from the same locality

5	numerous beetles, pupæ and larvæ	0
6	300 beetles, pupæ and larvæ	0
6	525 — — —	0
Total 17		0

The rats were killed 13—42 days after the feeding

Experiments, thus, have shown that not only the cockroaches (*P. americana*, *P. orientalis* and *Blatta germanica*) but also the *Tenebrio molitor* is able to serve as a host to the *Spiroptera* in question.

As the nematode here dealt with has not been described before we propose to give it the name *Spiroptera neoplastica*. The morphological examination of this species made in the zoological museum of the University of Copenhagen by H. DITLEVSEN has given the following results.

The shape of the Nematode, in question, is very divergent from that of the *Spiroptera obtusa* (SCHNEIDER) found frequently in the stomach of *Mus decumanus* and *Mus musculus*. The latter — especially the female — is short and clumsy, almost spindle-formed, whereas the *Spiroptera neoplastica* is thread-formed as a filaria.

When seen by reflected light, the colour is milky white, in a way that less transparent organs as intestine, ovaries become whiter, whereas the rest of the animal appears less distinctly, the chance support on which it is placed shining through it. When seen under the microscope in transmitted light it presents itself very pellucid.

As to dimensions of the two sexes a great difference is found, the female reaches a length of 6—8 ctm. sometimes even more, whereas the length of the male will very seldom be more than $1^1/_2$—2 ctm. The body has almost the same width throughout its whole length, being as far as the male is concerned between 110—130 μ. Young females at the beginning of their maturity have a width of 170—200 μ. In a a female the length of which measured c. 6 ctm., the width was found to be 326 μ.

Almost opposite the porus excretorius the body tapers very evenly and quickly towards the front part, which terminates bluntly in a cone. The hind part of the body keeps its width until the anus, narrowing then very suddently; the tail has the shape of a short cone the basal diameter of which is about one third of the height. This, however, is only the case with the females, whereas in the male the hind part and the tail have developed into a prominent bursa, the structure of which will be recorded later on.

The cuticula presents very distinct and fine transverse striæ which makes the body look almost annulated on its concave sides, according to the movements of the worm. The thickness of the cuticula almost at the middle of the animal measured 12,6 μ in a female of average size (c. 6 ctm.) 9,4 μ in a female of the length of 3 ctm. and 6,3 μ in male of average size.

In the front part of the body the cuticula presents most peculiar changes: towards the proximal half of the oesophagus the annulation loses its regular build, and disappears successively until it is finally replaced by some large vesicular prominences more or less globular, egg-shaped or sausage-shaped, like those found in *Gongylonema* MOLIN (Pl. III fig. 17).

These prominences are of unequal size not only according to the different individuals but within a single worm too. Relatively and absolutely they are most conspicuous in the bigger females. On contraction of the front part, performed even by this species to an extraordinary degree, the vesiculæ become more prominent, whereas on extension they present themselves less distinctly, and almost disappear

when the worm has reached its most extended state. They are not found in the larval stages.

The significance of these vesiculæ probably is to facilitate the movements and invasion of the worm into the mucuous membrane of the stomach of the rat. Its way of moving the front part by extending it as much as possible and afterwards contracting it violently, might support this theory, at any rate there is a great resemblance between this way of moving and the one known from the earth worm.

The front part of the nematode is devoid of real lips. The mouth has the shape of an equilateral triangle the edges of which are very easily moved forwards and backwards, and used incessantly during the moving of the worm. This fact may be observed most distinctly in the larva, escaped from its capsule in the muscles of the cockroach. At this stage of development the front part in all essentials has the same aspect as that of the fully developed nematode. When freed from its capsule the larva appears exceedingly agile, moving constantly onwards in search of spots suitable for intrusion.

The worm being placed into a solution of physiological common salt the movements of its front end may very easily be studied under the microscope: it bends itself into all directions protruding and retracting incessantly the lip-shaped edges of its mouth.

It has not been possible to decide with certainty whether papillæ may be found in this species or not; some have been met with, but they were rather inconspicuous, and even under high magnifying power (Zeiss Apochr. 2 m. m.) they were not constantly observed in the different individuals. Just under the ridge due to the edges of the mouth a laterally placed very small warty papilla was observed; it is also to be seen in fig. 18; but as before said it has not been found in all individuals. The cervical papillæ, characteristic of Gongylonema are entirely absent.

In the same fig. a little lower down and sublaterally placed, some jagged lines are found, passing obliquely upward and inward. They are very unequal as far as their distinctness is concerned. They seem to be very low, list-shaped ridges, most prominent in such spots as in the fig. present the triangular point. It may be that the latter presents a sublateral papilla, the shape of which then differs from that of the laterally placed papilla, mentioned above.

The lateral bands are easily perceptible passing throughout the length of the animal. In the male they reach the width of c. 60 μ; in the female, however, a width of about twice as much. They are sharply marked against the musculature and divided into 2 side halves, through the middle of which is seen a single row of very small but distinct nuclei (see fig. 22). These are placed very regu-

larly in a straight line, the distance between them being quite alike. The protoplasm of the sideband throughout its whole length is finely granulated, but each nucleus is surrounded by a group of bigger granules and a double row of dark spots is thus seen throughout the lateral band.

An observation similar to this was made by Looss with regard to the Filaria loa. The lateral band can be traced right up to the front part of the worm, decreasing here somewhat in width. Towards the tail it disappears in the anal part, keeping, however, its width throughout nearly its whole length. In the hindmost part the nuclei do not seem so regularly placed as described above (see fig. 19), it is possible however, that shrinking during conservation might have caused this irregularity.

Throughout the middle of the lateral band the excretory canal can be observed as a somewhat sinuous or at times nearly straight line (fig. 19 a, 22).

The irregular course of this canal may correspond to a more or less pronounced state of contraction in the animal. However it deserves to be noticed that in the specimen, represented in the fig. 19, the sinuosity of the intestine seems to imply a high degree of contraction, whereas the canal of excretion does not present any condition to match.

According to the figures of Looss the excretory canal of the Anchylostomum has a much more sinuous course than is the case with the spiroptera in question. In Anchylostomum it is seen rather frequently that the bendings of the canal form parallel lines (Looss, l. c. Pl. I, fig. 4, Pl. IV, fig. 38, and in other places); the canal of this species can never be straight even when the worm is extended to its utmost ability, and most of the nematodes will present conditions similar to this.

The porus excretorius is situated on the middle of the ventral side half way from the nerve-ring to the limit between the two halves of the oesophagus, Pl. III, fig. 17 ex. p. Just in front of the porus excretorius the canal penetrates a bigger cell (Looss: "carrying cell") which most likely contains a cavity (vesicula of excretion). The course of the canal, however, can not be traced here on account of the lack of chitin-intima. On the whole, conditions here seem to be somewhat similar to those known from Ankylostoma and Filaria loa, as described by Looss.

The front part of the digestive canal consists of a short pharynx clothed with a rather thin chitin-intima. The length of the pharyngeal cavity measures c. 53 μ in a female of 6 ctm., the diameter being 20 μ in optical section. In fact, the pharynx — especially in the larvæ from the muscles of the cockroach — has some resemblance to that of the *Rhabditidæ*, the shape being probably likewise triangular prismatic.

In the pharynx of the larvæ the chitin-intima is relatively thicker and more prominent than is the case in a fully-developed nematode, a fact which makes their pharynx still more like that of the Rhabditidæ.

The oesophagus is divided into two distinct parts. The cephalic part is rather short and slender and suddently passes into the other half which is conspicuously thicker and longer with fairly uniform width throughout the length, and only increasing inconspicuously in the hindmost part. The proportion of the length in the two halves is about 1 to 6. In an oesophagus, the whole length of which measured 2,7 mm. the diameter was found to be 26,4 μ in the slender part whereas in the thicker half a little anterior to its passing into the chylus-intestine the diameter was found to be 52,8 μ or exactly twice the size of the front part.

The length of the oesophagus differs conspicuously as far as the two sexes are concerned. In a female specimen the length of which was 6 ctm. the oesophagus measured 7 mm, coming thus to $^1/_9$ of the whole length of the worm. In a male of $1^1/_2$ ctm. the oesophagus measured 2,8 mm. or about $^1/_4$ of the length.

Round the middle of the front part of the oesophagus the nerve-ring is very distinct both in the fully developed worm and in the larva (see Pl. III fig. 17, and Pl. IV fig. 28). In the male specimen of $1^1/_2$ ctm. mentioned above, the diameter of the nerve-ring measured 45 μ. In optical sections the central part of the nerve-ring is seen to consist of a granulated substance surrounded by an apparently homogenous coat, which presents itself exceedingly thin on the side facing the oesophagus. Similar spurs of sarcoplasma as described by Looss in the *Filaria loa* are found reaching from the muscular layer towards the nerve-ring (fig. 18).

The oesophagus is as usual marked off from the chylus-intestine by a constriction, the chitin-intima, however, being traceable somewhat further down.

As far down as this chitin-intima reaches the intestine presents a peculiar differentiation with faint transversal striæ, probably consisting of muscular tissue. Caudal to this part some conspicuous intestinal valves are observed, sometimes arranged in two rows as may be seen in the fig. 23.

On the whole, the chylus-intestine is but slightly developed, its width being almost corresponding to that of the thin front part of the oesophagus. Most frequently it appears as a fine line throughout the worm; only its hind part is sometimes rather sinuous and the lumen here somewhat more dilated than is the case in the rest of the intestinal canal. Rectum decreases in width (fig. 19). The anal musculature is well developed. The intestine in the female opens at a distance of 210 μ from the tip of the tail, the shape of which is regularly conical with rounded point. The tail, as well as the front part of the worm, shows a great power of contraction. On the specimen, pictured on the fig. 19, the tail has fairly its natural shape and length; when contracted it will only present half the length and become thick and folded, often ventrally bent. In the male, which has a large bursa and only one aperture for the intestinal and genital duct, the anus is situated at a distance

of 165 μ from the tip of the tail, — which — considering the small size of the male — will be relatively a great deal more than in the female.

The tail of the male has the shape of a cork-screw as is generally the case in all the species belonging to the genus in question. The windings are distinct although not so marked as may sometimes be observed. The bursa has a rather considerable size. In a male of average size (c. 1 1/2 ctm.) the bursa has a length of 462 μ and a maximal width of 170 μ, measured from one edge to the other. Its shape is asymetrical which is no uncommon fact in these nematodes; this, however, not only applies to the exterior shape of the bursa, it also may affect the placing and relative size of the papillæ on both sides, as well as the mutual size and shape of the spicules. Even SCHNEIDER describes this. [1])

"Bursa und Papillen sind bei einer grossen Zahl von Species asymmetrisch gebildet und gestellt. Die eine Seite der Bursa ist länger und breiter, zugleich rücken die auf derselben stehenden Papillen weiter nach vorne und aus einander. Die Spicula sind immer ungleich und bei den verschiedenen Species von sehr verschiedener Gestalt." —

In the species concerned one side of the bursa very distinctly appears to be nearly 1/3 shorter than the other side, the measure mentioned (462 μ) thus applies to the longer one. The bursa, on the whole, has a longish oval shape with a distinct constriction on the middle, somewhat more pronounced on the longer side and placed more caudally on the shorter side. The lower convexity is somewhat more distinct on the longer side. Towards the tip of the tail the bursa narrows very considerably, the side edges approaching each other. Almost in front of the hindmost set of papillæ a small dilatation is observed after which the lips converge to a small rounded lingulate tip, reaching c. 14 μ outside the tip of the tail.

Besides the 4 preanal papillæ constantly present in the genus of Spiroptera 4 postanal papillæ are found on each side. They are all equally shaped, most slender on their point of fixation to the bursa, and evenly increasing in width; the outermost part forming a headshaped or rather discoidal dilatation. The 4 preanal papillæ almost have the same size; they are bigger than the postanal papillæ and measure c. 20 μ in length. Among the postanal papillæ the set next to the anogenital duct is the largest, after which they gradually decrease in size, rendering those proximate to the tip of the tail only c. 10 μ in length.

As already mentioned a distinct asymmetry is to be noted in regard to their placing; all the row of papillæ on the longer side of the bursa has been drawn somewhat more cephalic than those of the shorter side. It is, however, the question only

[1]) ANTON SCHNEIDER: Monographie der Nematoden p. 84.

of an insignificant deviation in distance on both sides, not even 10 μ, I suppose. The exact proportion can not easily be given in fixed numbers, the torsion of the tail preventing its being placed in one plane.

The two spicules are exceedingly different in shape and size, one being only 93 μ in length, whereas the other measures 528 μ, or nearly 6 times the length of the first one. The shorter spicule is sword-shaped with rounded point. In the proximal end it reaches its maximal width, the diameter being about 9 μ; after which it is tapering evenly towards the tip, being at the same time slightly curved. It seems to be hollow throughout the length, presenting along each edge a double contour with distinct transverse striæ. Thus, in optical section it has the aspect of two converging rods, meeting in the end and transversally striated (P. IV fig. 31). The above description applies to the spicula when seen in profile. In a preparation dorso-ventrally compressed, it is squeezed together and presents quite another appearance. It then apparently will have its maximal width at the middle, showing a longitudinal thickening which in fact will be nothing but a fold due to the pressure (Pl. IV fig. 24). The ring-shaped profile of the proximal end of the spicule as seen on the fig. distinctly shows the interior cavity, mentioned above. The longer spicule nearly has a uniform width throughout its length, but for a small dilatation in its proximal end. It contains a cavity similar to that of the thicker spicula. In optical sections, however, the slender spicula appears as two parallel lines, turning off each other at some distance before the tip and meeting together in the very end of the spicula, — whereas the thicker spicula were seen as two converging rods meeting in the distal end. In other words: The point of the longer spicule most of all resembles a strongly twisted piece of wire (Pl. IV fig. 30). When seen under high magnifying power the point of the long spicule looks as if surrounded by a fine membrane, in optical sections resembling a ferrule of a sword (Pl. IV fig. 30), or it might be considered somewhat similar to the so-called "cutter" in certain Polychæte setæ. When the spicules are at rest and in their natural position in the worm, the tips are almost converging or at any rate placed quite near to one another, whereas for the rest the spicules are diverging and form a distinct angle. In the fig. 24 the shorter spicule is retracted unnaturally far. Most frequently the longer spicule will be seen protruded, stretching out of the anogenital duct with almost half its length. It will then be strongly bent, the protruded distal part at a right angle to the proximal part. The curving takes place somewhat within the anus. The shorter spicule was never found protruded in any case.

Under high magnifying power both spicules appear to be surrounded by a most peculiar apparatus which shall now be explained, without laying claim to any complete correctness. Especially it is possible that the thing found extends further back or

more caudal in the animal than shown in the figure. However, it has proved impossible to state exactly the most posterior limit. Each of the spicules is surrounded by a sheath, the surface of which seems to consist of a chitin-membrane; on examination with immersion (apochr. 2 mm.) it presents a most peculiar spotted surface, each spot being due to a diminutive circle and most likely representing holes or grooves. Somewhat more caudally situated, not far from the distal end of the thicker spicula, another piece of sheath is found, the object of which possibly might be to make the two spicules keep their distance and to support the whole sheath-apparatus. The latter piece seems bigger and more thick-walled than the sheaths which compass each spicule. A fact which renders it difficult to accomplish an examination of this part of the worm, is the highly developed muscular system which is found here.

From the neighbourhood of the anus, broad muscular bands arise, running partly ventrodorsally on each side of Rectum and Ductus ejaculatorius, partly obliquely forwards, partly obliquely backwards or flabelliform towards both sides.

The interior genital organs are found corresponding to the usual type. The testis being single presents quite a straight line with no curvings, and almost reaches as far as to the proximal end of the oesophagus, where it turns, running back in caudal direction, parallel to itself, and terminating in a small retort-shaped dilatation. The returning branch is always quite short (Pl. IV fig. 29). In exceptional cases the course may be somewhat different, f. instance when after returning the testis turns once more, and for a little while takes a cephalic direction, after which it terminates in the usual way, bent caudal. In no specimen was the testis found surpassing the limit between the oesophagus and the intestine. Usually the point of turning will be at a distance of c. $^1/_2$ mm. from the said limit. The testis does not vary in width, the minimal diameter being near to the turning where it measures 24 μ. In the middle of its length it is about 60 μ and its maximal width is found to be 72 μ. The hind part of the genital duct presents a differentiation; posterior to testis it narrows to a narrow duct, which evidently serves as nothing but a conduct-pipe, and therefore may be pointed out as Vas deferens. It is c. 70 μ in length, although no exact measure can be given as seen from flg. 27 Pl. IV; the distal end of the testis is tapering evenly into a conical part, which passes into the vas deferens without any distinct line of demarcation. The vas deferens then again increases evenly in width and passes into a dilated part reaching towards the anus, nearly in front of which it narrows into a short Ductus ejaculatorius. The dilated part which is to be marked out as vesicula seminalis has a length of a little more than one mm. and often is brimful of spermatozoa. When placing the worm alive under the cover-slip the spermatozoa often by the pressure may be forced out through the anogenital opening. They then appear as small regular globular-cells.

In the female the vulva is situated rather near the tail. In a female of $2^1/_2$ ctm. having reached its maturity, the vulva is placed 2,5 mm. from the tip of the tail; in a female of about 6 ctm. the distance from the vulva to the end of the tail is 7 mm. In a female of 3,6 ctm. the vulva is situated almost 4 mm. from the tip of the tail. It is very difficult to obtain absolutely exact measurements; in the first place preparations of females tolerably extended are found but very seldom; as a rule they will be considerably curved, bending from one side to the other and — which is still worse — in several cases twisted. Secondly, even exact measurements may not be in keeping with the facts on account of the great power of contraction met with in this species, as the measurements will vary according to the momentary state of contraction. Generally, however, the distance from the vulva to the tail may vary between $^1/_8$ and $^1/_{10}$ of the whole length of the animal.

It is no easy task to fix the place of the vulva; the two uteri are extending far forwards and backwards in the body and often — especially in bigger specimens — are filled with eggs to such a degree that the whole cavity of the worm may be taken up by these organs, the intestinal canal and the ovaries being forced aside; as the vulva is but little prominent itself it often may take a long time to find it.

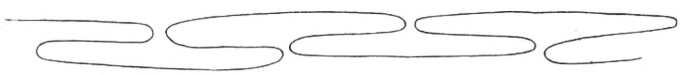

Fig. 1.

The interior female organs as already hinted, are double. The vagina extends from the vulva a little way in the direction of the head, after which the two uteri are branching off, one of them in the cephalic the other in the caudal direction.

Near the limit between oesophagus and the Chylus-intestine, and somewhat posterior to the vulva the two uteri pass into resp. the anterior and posterior Receptaculum seminis which by a short curved oviduct communicate with the corresponding ovary. It seems to be the rule that the end of the front ovary is pointing towards the tail, the end of the hindmost ovary towards the head. The ovaries are strongly bent and apparently rather gratuitously; on further examination the windings appear to be rather typical; deviations may be found as was the case with the testes in the male — but they are not the rule. The windings always correspond to the longitudinal axis of the animal, nearly constantly ventro-dorsally bent or vice versa. The course of the ovary is either cephalic or caudalic. After a little while it suddenly turns, running now parallel to its first course but taking a converse direction. After some time it turns again and now keeps this course at a distance twice the length of the first one; it then turns again and runs towards the first point of curving where once more it turns. The course of the ovary thus describes a line corresponding to the longitudinal axis of the animal and presents itself as follows: (Text fig. 1).

A part of the mucous membrane from the ventricle of a rat enclosing the Spiroptera, when seen under the microscope first of all presents the winding passage in which the worm is placed. Scattered round the worm eggs will be found in the epithelium. It probably may be beyond doubt that the pairing takes place in this passage in the mucous membrane, as the worm does not leave its place here in order to seek refuge in the lumen of the stomach. To be sure, it may sometimes be observed projecting into the lumen with a shorter or longer part of its body, but no specimen has been found free in the stomach. Sometimes two specimens are found in the same passage, a male and a female. They are then placed quite near to each other, the front part being turned in the same direction. However, a pairing never is observed although a case as the one just described may probably be explained as the introduction; or possibly the pairing itself may have taken place.

In the mature female eggs are constantly evacuated through the vulva; the vagina as a rule contains eggs in the act of leaving the worm. Round the female the epithelium of the stomach of the host is seen to contain eggs. When by desquamation of the epithelium, afterwards passed into the lumen of the stomach, the eggs are mixed with the contents of the latter and finally evacuated with the excrements, in which on microscopical examination they are easily found.

The egg is very regularly egg-shaped, thickest in the middle and evenly tapering towards the poles; its maximal diameter is about 60 μ, the minimal being about 40 μ. In optical sections the shell is found somewhat thickened at the poles, in this respect the egg is quite similar to that of *Spiroptera obtusa* as figured by LEUCKART. The shape too resembles that of the latter. See LEUCKART: Die menschlichen Parasiten II fig. 85 p. 113, and present paper Pl. I fig. 8.

Under high magnifying power the egg presents a distinct line of demarcation between the thinner middle part of the shell and the thicker field at the poles; which seems to be of great consequence when the young ones are about to leave the egg. In the uterus, however, all stages of development are present; in ripe eggs the larva is found coiled up; at this stage it is thickest in its front end, decreasing evenly in width towards the tail.

Fig. 2.

The very day after the feeding of the cockroach on ripe eggs the intestinal canal of the latter contains free larvæ and empty eggs simultaneously. An extraordinary number of eggs apparently, however, is uninfluenced by the new conditions. This especially is the case when the cockroaches are fed on eggs originating directly from mature females, instead of being fed on rat's excrements; and the reason is, probably, that in fact several of the eggs are not ripe for hatching, although by way of microscopical examinations it is not possible to prove this. Most likely some changes

are taking place within the egg during its stay in the mucous membrane of the rat's stomach and its passing through the intestine canal; in this way, to be sure, it has reached a stage of development, which eggs extracted from the uterus have not yet obtained. The not fully developed eggs thus are not hatched in the intestine of the cockroach but passed with the excrements without change.

The opening of the ripe egg takes place in the following way:

The thicker part of the shell at the poles actually consists of two layers. When passed into the intestine of the cockroach these two layers probably influenced by the secretion (Text fig. 2) begin to diverge, forming a cavity between them. The connection with the rest of the shell is then loosened, the outer lamelle falls off first, after which the inner one is broken, perhaps influenced by some pressure from the foetus. In the fig. the two lamellæ at one of the poles are observed to diverge from each other, at the other pole the outer lamella is gone, the inner one is broken, the hind part of the young one forcing its way through the hole thus made. At the same time the inner lamella has loosened itself from the rest of the egg, remaining only as a sort of collar which will soon fall off. The emptied egg now appears as a ring, consisting of the middle part of the shell, devoid of the thickened polar fields. To a cursory view these empty eggs will now be found somewhat similar to the eggs of Trichocephalus, flattened at the poles, on a more thorough examination, however, no mistake is possible.

The larva when freed from the egg has the shape of a long comma; presenting the greatest width in the front part and tapering evenly towards the hind part; the tip of the tail is rounded. On fresh preparations the larva shows no interior differentiation; it is covered with a rather thin cuticula, somewhat thicker in front. Its length is about 250 μ, its maximal width 13 μ.

Apparently the period in which the larva may be found in the chylus-intestine of the cockroach is but of very short duration; even two days after the feeding they are gone, as well as the eggs and the empty shells. It has not yet been discovered where the larva goes to, on leaving the chylus-intestine; most probably it will make its way into the intestinal wall, although it has not been found there on fresh preparations. A rather long time is passed after the feeding until it can be found in the musculature (about 20 days), for the present it must be unexplained where it resides till then.

After having entered the muscles of the cockroach the larva encysts itself, being then coiled-up trichina-like. The capsule, however, in the Spiroptera-larva generally is but little prominent, — as distinct from those known from the trichina. Most frequently no capsule at all will be observed at first sight; only the larva will be seen among the muscular fibres, coiled-up in a most graceful and regular spiral. As a rule the tail is placed in the centre, the head at the outside.

The shape of the capsule is somewhat differing according to the various individuals; most probably this is proportionate partly with the space which is left to the disposal of the worm during the act of encysting, partly with the state of contraction of the surrounding muscles. The circumference generally is nearly circular and somewhat flattened, the capsule being thus lenticular and thickest in the middle. Often, however, it has a more or less longish shape almost as a somewhat flattened egg.

As a rule the worm if not disturbed will keep rather quiet within its capsule, small sliding movements being observed as the spiral is contracted or relaxed; greater movements no more than changing of place will occur.

When isolated from the surrounding muscular tissue and placed under a coverslip in a solution of physiological common salt the larva within its capsule will show itself most extraordinarily animated, owing to the unusual conditions. It moves about inside the capsule in a most energetic way, describing with its body now a spiral, now a figure of eight, the head sometimes in the centre of the capsule, sometimes in the periphery.

Under the microscope the isolated capsule presents itself very thick-walled, composed of a thick granular mass the consistence of which is similar to that of a thick protoplasm. A membrane of connective tissue will generally not be found. The capsule probably consists of degenerated muscular tissue from the surrounding muscles. After a while the movements of the larva grow more animated; it begins trying to break through its capsule, now and then putting its head into the wall of the capsule which very easily yields to the pressure. As the worm draws back into the cavity a canal is left which will contract but very slowly. By and by several bored passages may be found in the wall, reaching more or less deep into it and often wound according to the direction of the worm. Finally it may happen that the worm gets quite through the wall and escapes. This fact has been dwelt on at some length as it seems to throw light upon the peculiar consistency of the capsule. Most likely the unusual toughness, which is evidently characteristic of it, will be of great consequence to the larva, sheltering it from the pressure of the surrounding muscles during their contraction. The text-figure 3 shows such a capsule and its cavity which has harboured the larva, as well as the bored canal through which it has escaped; also some other canals half contracted appear distinctly.

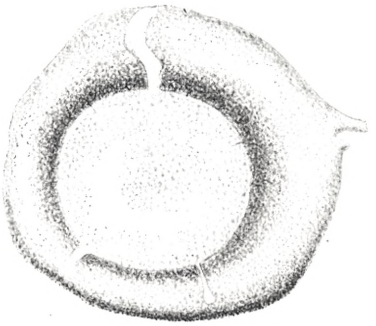

Fig. 3.

The encysted larva measures about 1 mm. in length, most frequently somewhat less, varying conspicuously in size; the smallest one found had a length of 792 μ,

the biggest one a length of 1215 μ, whereas a larva from a stomach of a rat taken one day after the feeding measured 1248 μ, having thus evidently been still longer, as in one day it will hardly be able to grow as long as that in the mucous membrane of the stomach of the rat. On the whole, it is growing but very slowly during the first days after the transmission to the alimentary canal of the rat, as will be referred to later on.

The shape is slender (fig. 32) tapering but slightly towards the front end and keeping its width until the anal region where rather quickly it narrows into a conical tail. The front end is similar to that of the fully developed worm as far as shape and appearance is concerned (fig. 26). The tail often terminates in two pointed papillæ-shaped projections the shape of which is varying much according to the different individuals. Sometimes three projections will be found of unequal size, while in other cases it proves very difficult to make out whether two or three are present. A most peculiar wing-shaped prominence may also be found in some cases, the edge of which is serrated or fringed.

The pharynx is relatively longer than that of the fully developed worm and the Chitin-intima is comparatively thicker, a fact which will sometimes recall the Rhabditidæ. As to the shape of the diameter I have not yet been able to make out whether it is prismatic or not. Oesophagus at this stage of development has about the same length as the intestine; frequently, however, it is somewhat shorter; during the following period of development the oesophagus, however, grows more quickly than the intestine, as the front half of the animal increases considerably in length, being at a certain point of time longer than the intestine. When later on the hind part of the worm shows the greatest rate of growth the length of oesophagus comparatively will decrease by and by. Even in the encysted larva the oesophagus has the shape of the fully developed worm, the front part being slender, passing with a rather sudden dilatation into the thicker proximal half. The nerve-ring shows itself very distinctly and half way from the latter to the limit beetwen the thinner and thicker part of the oesophagus the porus excretorius is situated (figs. 28 and 32). The genital rudiment appears as a small egg-shaped body far back in the animal, nearly on the spot where the vulva will be found later on. Even at this stage of development it seems to consist of a number of cells, or at any rate of a syncytium with several nuclei.

When feeding the rat on cockroaches containing encysted larvæ these will be found to have entered into the mucous membrane of the stomach already the next day. As above mentioned they may not only be observed here; not seldom they are found in the mucous membrane of the oesophagus or of the tongue, so that it may be supposed that the larvæ already are freed from the capsules while in the mouth of the rat, owing perhaps to a purely mechanical influence during the chewing.

When liberated, the larva will immediately seek a spot on the mucous membrane fit for invasion. There is nothing to show that after this it changes its place; fully developed worms may thus be found in the mucous membrane of the oesophagus as well as in the root of the tongue; most of the larvæ, however, are swallowed and passed with the food into the stomach where they are to be found in the sqamous-celled epithelium of the cardiac portion.

During the first period the larvæ seem to grow but very slowly, having reached after 10 days only double the length of that which they had on the transmission. Then, however, the growth is quickened very considerably; a few weeks after the feeding they measure about ten times the length of the encysted larva.

Experiments as to the growth and development of the larvæ were carried out in the following way: On some fixed day a number of rats were fed on infected cock-roaches; the next day one of the rats was killed and its stomach and oesophagus was examined, and so on. The larvæ from the various rats were examined and measured, and thus an inventory was procured which gave a view of the case concerned. Besides the fact already described, these experiments, however, permit no further conclusions as to the growth of the larva, the comparative length of oesophagus and the development of the genital-apparatus, — the material being too limited.

In order to get hold of the larvæ the mucous membrane was scraped with a scalpel but this method only gave very few larvæ sometimes but one or two. Greater series of measurements were thus precluded. If measurements were undertaken on a larger scale an inordinate number of rats would be required.

The following table gives a view of measurements of the length of the larvæ, carried out on live specimens from the stomach or from oesophagus of the rat.

Larva	.....	1 day	after	the	feeding	1247 μ	stomach
—	.....	2 days	—	—		1056 μ	oesophagus
—	.....	3 —	—	—		1220 μ	stomach
—	.....	4 —	—	—		891 μ	—
—	.....	6 —	—	—		1255 μ	—
—	.....	8 —	—	—		1750 μ	—
—	.....	10 —	—	—		2268 μ	—
—	(female)	16 —	—	—		11,7 mm.	—

After having been in the stomach of the rat for about 8 days the tail of the larva presents itself as that of the fully developed worm. A larva in this stage of development has been observed in the moult; the tip of the tail was still trifid, but after the ensuing moult the tail would turn out single; the hind part of the worm had drawn somewhat back from the old cuticula, which was here already loosened, and the new shape of the tail appeared within. In another larva the tail 10 days after the transmission to the stomach of the rat still presented the bifid stage.

As may be seen from the above mentioned the spiroptera in question most likely has its home in America. The larvæ are found encysted in the muscles of the *Periplaneta americana.* In his descriptions of the *Filaria rhytipleuritis* Seurat[1]) refers to an observation made by the Brasilian helminthologist Macalhães who in the Periplaneta americana has found a larva which he defines as that of the Filaria rhytipleuritis. Seurat now shows that owing to various incongruities in their characteristic features this larva can not be that of the Filaria rhytipleuritis. Most unfortunately I have not been able to get hold of the paper by Magalhães, in my opinion, however, it appears from Seurat's report that the larva observed by Magalhães probably must be identified with that of the spiroptera which has been dealt with in the present paper.

Finally a few words shall be added about the systematic position of the Spiroptera in question. It is beyond doubt that it is closely related to the group established by Molin under the name of *Gongylonema; Myzomimus*, Stiles, 1892[2]). Like the Spiroptera s. str. the species in question has four pairs of preanal papillæ and lacks the cervical papillæ characteristic to the group of Gongylonema[3]) to which it approaches on account of the above mentioned prominences on the front part of the body.

[1]) Seurat: Sur l'habitat et les migrations du Spirura talpæ Gmel. (= Spiroptera strumosa Rud.) Comptes rendus de la Soc. de Biol. Année 1911. T. II p. 606.

[2]) Ch. W. Stiles: Notes on parasites: Journ. of Comp. Med. and Vet. Arch. Wash. D. C. 1892.

[3]) Michele Stossich: Filarie e Spiroptere, lavoro monografico. Trieste 1897. p. 130.

EXPLANATION OF THE PLATES

Plates I—II.

Fig. 1. Female Spiroptera. Natural size.
— 2. Male Spiroptera. Natural size.
— 3. Part of the in nersurface of a stomach. The epithelium of the fundus containing two spiroptera, no pathological changes.
— 4. Spiroptera imbedded in the epithelium of the fundus of the stomach.
— 5. Transverse section of a gullet containing spiroptera.
— 6. Spiroptera in the epithelium of the fundus of the stomach.
— 7. — — — — — — —
— 8. Ripe eggs of the Spiroptera. $\times {}^{280}/_{1}$.
— 9. Larva of a Spiroptera in its capsule, imbedded in the muscles of a cockroach.
— 10. Tuberous tumour in the stomach of a rat, infected with Spiroptera.
— 11. Enormous papillomatous tumour in the stomach of a wild rat from sugar-refinery. Natural size.
— 12. Transverse section of the same stomach. $\times {}^{2}/_{1}$.
— 13. Carcinomatous tumour in the fundus of the stomach. Black and white rat fed on cockroaches *(P. americana)*. Natural size.
— 14. Carcinomatous tumour in the ventral wall of the fundus of a black and white rat, fed on cockroaches *(P. orientalis)*. High power.
— 15. Carcinomatous tumour in the ventral wall of the fundns of a black and white rat, fed on cockroaches *(P. americana)*. High power.
— 16. Metastases in a retroperitoneal lymphatic gland of a black and white rat, fed on cockroaches *(P. ame-ricana)* $\times {}^{6\text{-}7}/_{1}$.

(The figures 2—16 have been published before in „Zeitschrift für Krebsforschung“ 1913, the figures 11, 13, 15 og 16 in „Berliner klinische Wochenschrift“ 1913.)

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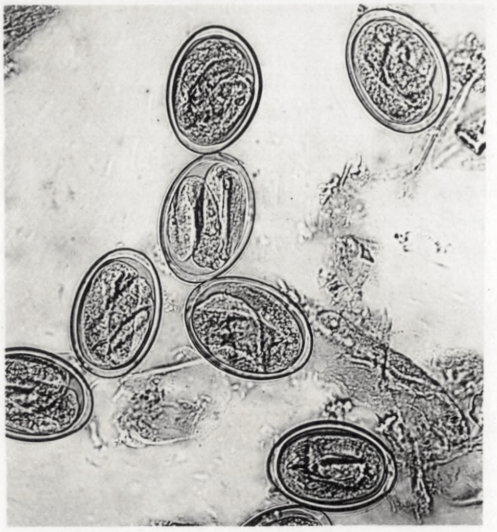

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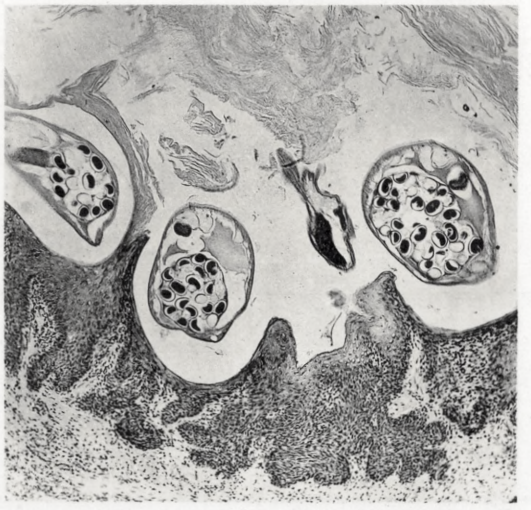

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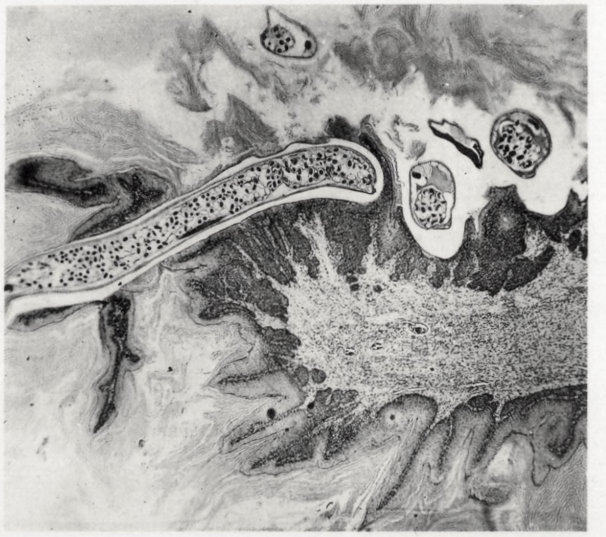

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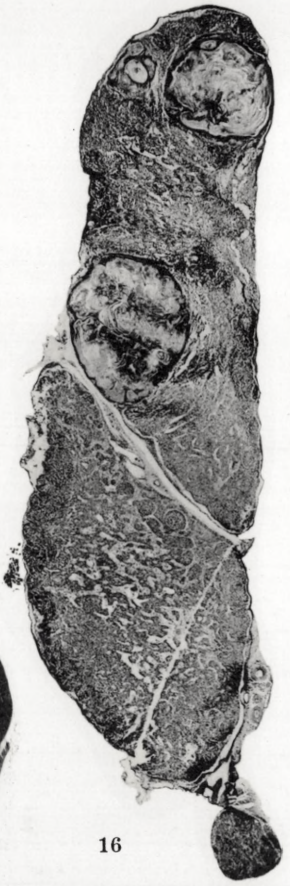

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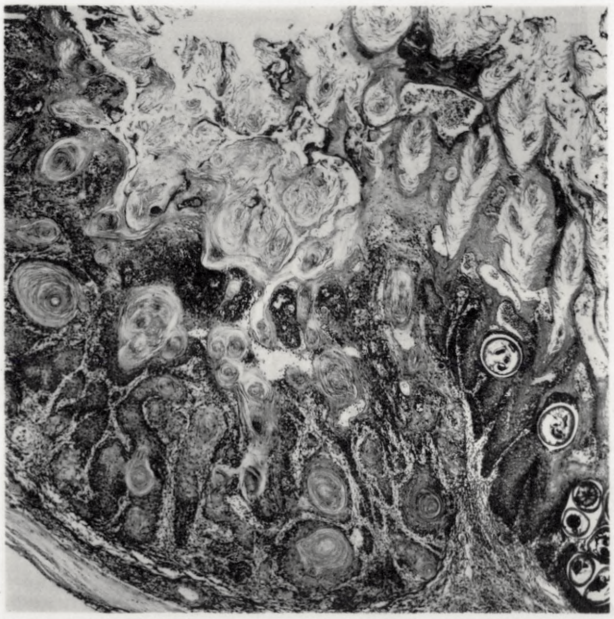

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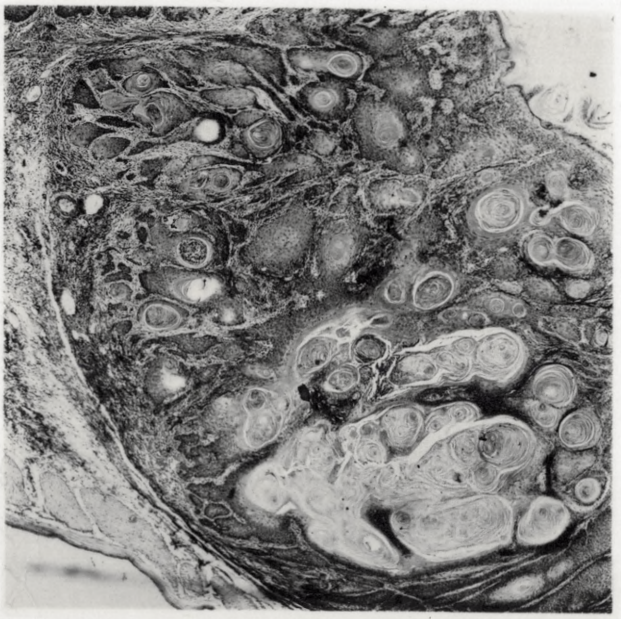

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Pacht & Crone phototyp.

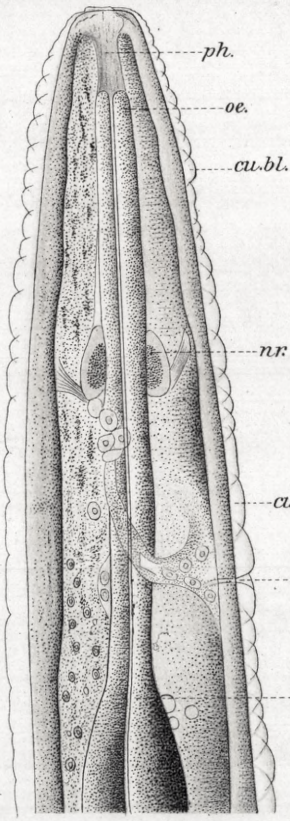
Reference letters to Plate III and IV.

t. Testis
vd. Vas deferens
i. Intestine
vs. Vesicula seminalis
l. sp. Long spicule
k. sp. Short spicule
sh. Sheath apparatus for the spicules
oe. Oesophagus
m. Musculature
cu. Cuticula
k. Nucleus
exc. Excretory canal
lat. Lateral band
med. Median band
g. Genital-rudiment
pr. p. Preanal papillæ
po. p. Postanal papillæ
o. Egg
rs. Receptaculum seminis
ovd. Oviduct
am. Annal musculature
vu. Vulva
vg. Vagina
ut. Uterus

Plate III.

Fig. 17. Male. Front end. Apochr. 8 mm. Comp. 8.
— 18. Male. Head. Apochr. 2 mm. Comp. 4.
— 19. Female. Caudal end. Obj. C. Oc. 2.
— 20. Female. Uterus and Vagina. Apochr. 8 mm. Comp. 4.
— 21. Female. Vagina in profil. Apochr. 8 mm. Comp. 8.
— 22. Male. Apochr. 8 mm. Comp. 8. After a preparation mounted in Canada-balsam, stained with Saffronine.
— 23. Male. Limit between Oesophagus and Intestine. Apochr. 3 mm. Comp. 4.

17.



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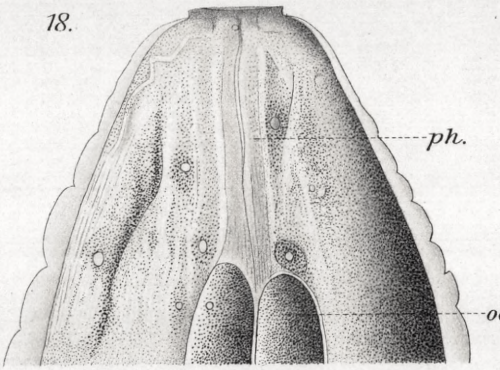

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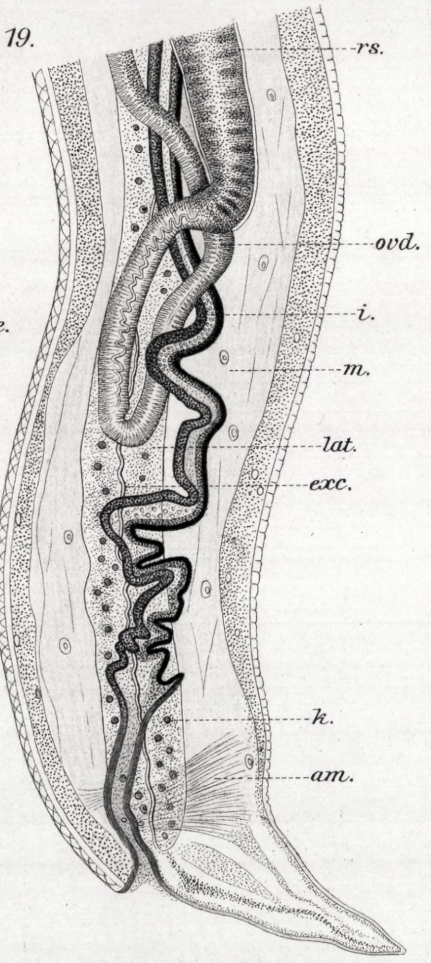

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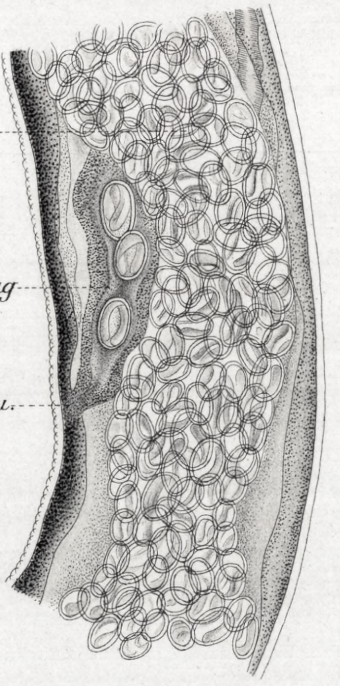

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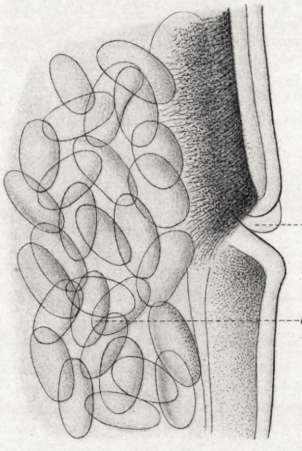

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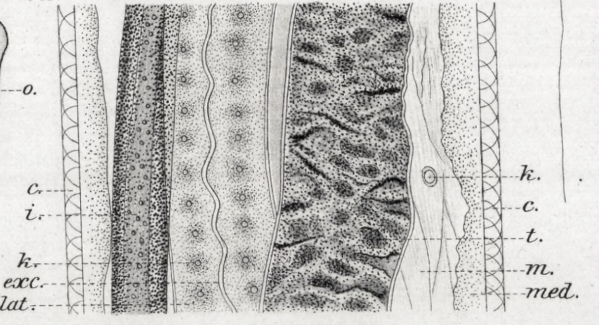

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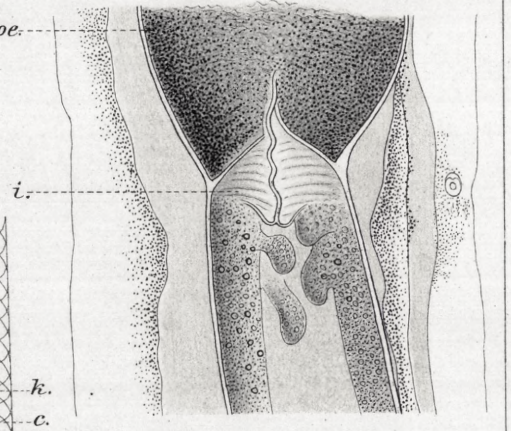
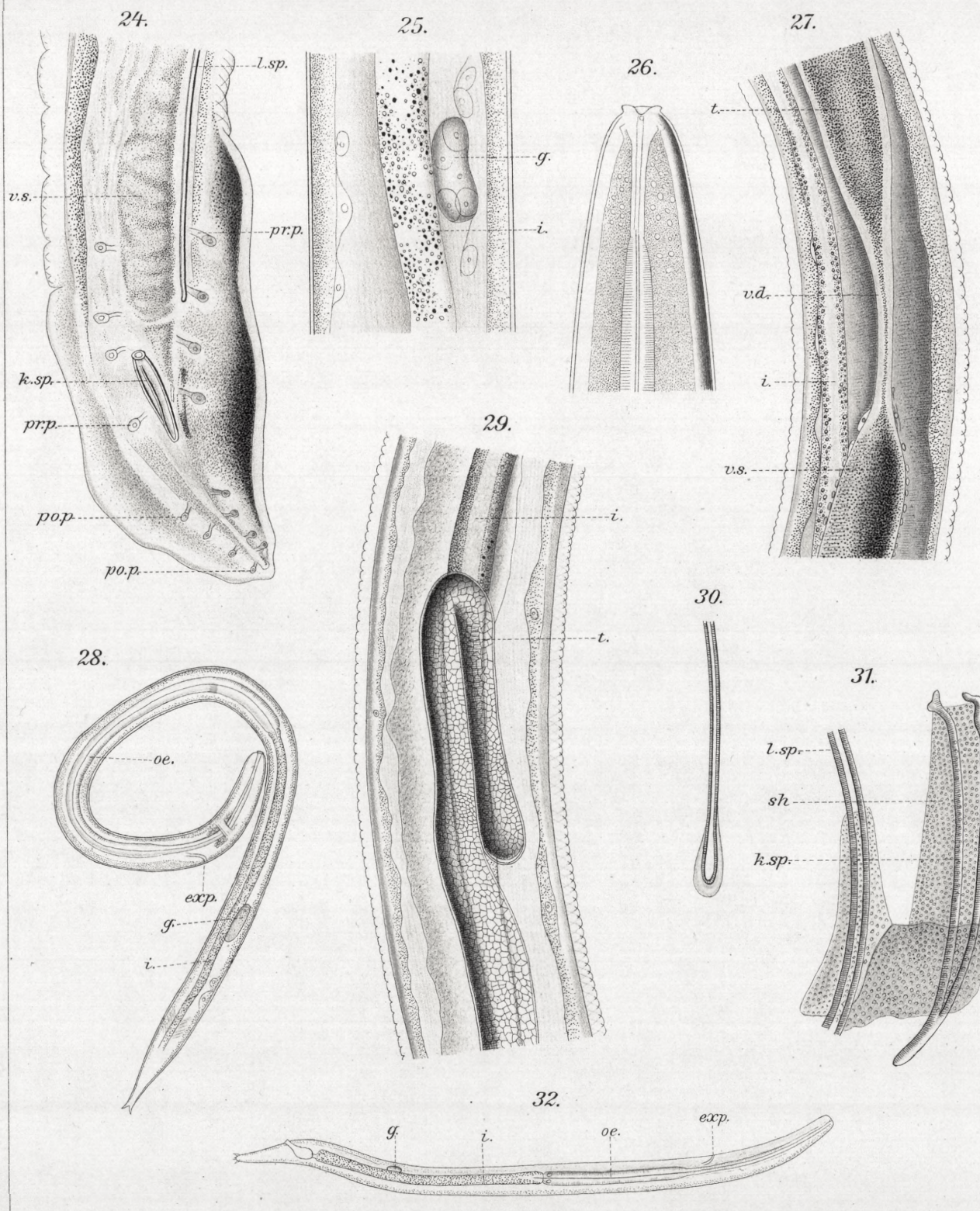


Plate IV.

Fig. 24. Male. Caudal end. Apochr. 8 mm. Comp. 4.
— 25. Larva from the Prothorax of Periplaneta americana Apochr. 2 mm. Comp. 4. After a living specimen.
— 26. Larva from the Prothorax of Periplaneta americana. Front end. Apochr. 2 mm. Comp. 4.
— 27. Male. Testes. Ves. sem. Apochr. 8 mm. Comp. 4.
— 28. Larva from the ventricle of a rat, four days after the feeding with 50 cockroaches. Apochr. 8 mm. Comp. 4. After a living specimen.
— 29. Male. The bent cephalic end of the Testis. Apochr. 8 mm. Comp. 8.
— 30. The tip of the long spicule. Apochr. 2 mm. Comp. 4.
— 31. The short spicule, a part of the long spicule and the chitinous sheath-apparatus. Apochr. 2 mm. Comp. 4.
— 32. Larva from the Prothorax of Periplaneta americana. Apochr. 8 mm. Comp. 4. After a living specimen.


24.
l.sp.
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pr.p.
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pr.p.
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