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(*STERNA PARADISAEA* PONTOPPIDAN)
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Synopsis

The Arctic Tern (*Sterna paradisaea* Pontoppidan) breeds in the polar regions and winters in the antarctic zone, performing the most extensive migratory movements known in any bird. The crossing of the Antarctic Ocean, a flight of more than 3000 km, forms the most critical part of the long migration. It is demonstrated in the present paper that this oceanic passage follows the meridional cyclone trajectories, in which way the terns are aided in their flight by obtaining a considerable increase to their ground speed. The peculiar moulting system in the Arctic Tern is discussed, and it is shown that it must be explained as an adaptation to the extremely strenuous flight across the Antarctic Ocean. The winter range in the pack-ice zone and the flight routes during the autumn and spring migration across the Antarctic Ocean are outlined. Differences in the timing of the wing moult between adult and immature birds are pointed out, and it is demonstrated that a number of terns, particularly immature birds, are drifted eastwards by the strong winds. In this way the birds may perform a complete circumnavigation of the Antarctic Continent before they return to South Africa for the spring migration towards the north. Many one-year old birds spend the summer in the Humboldt Current, off South America, and do not continue to South Africa until the following spring. In such cases the circumnavigation takes two years. The regular drifting of young birds around the Antarctic Continent is a result of the special situation in the young birds, which are more liable than the adult birds to be caught by the wind. The circumnavigation is compared with similar movements in other antarctic sea birds. Evidently, it must be regarded as an advantageous adaptation of considerable survival value to the birds. An attempt is made to map the complicated migration pattern of the Arctic Tern within the antarctic zone (fig. 8).

The Arctic Tern has the longest migratory flight of any bird. It literally moves from pole to pole. It has its main breeding range in the Arctic region, where it is known to breed as far north as dry land extends, and has recently been found to breed at Cape Morris Jessup on 83°40′N, the northern point of Greenland (Røen 1965, p. 128). On the other hand, it winters in the Antarctic region, where it has been recorded as far south as 74°S.

The migration route, leading from the Arctic to the Antarctic region has been described a number of times, most recently by Kullenberg (1946, p. 58), Fisher and Lockley (1954, p. 140) and Dorst (1962, p. 175). The route is not known in detail and an exact analysis of the complete migration complex in this species has never been attempted. The ringing records, which have piled up in recent years, have never been treated in their entirety, but there are a number of special studies, like that of Radford (1961, p. 174) on recoveries of British specimens.

I do not intend to describe or comment on the migration between the breeding places and the southern subtropical zone, to which I cannot add anything new, but it is necessary to outline briefly this long movement, because it is fundamental to the understanding of the subsequent winter migration in the south polar region.

The great flight takes place along two, and only two, migratory routes, the first one along the continental shelf of Western Europe and southwards along West Africa, and the second one along western North and South America. The first route is the main one, while the latter is of lesser importance and is very little known. Somewhere in eastern Siberia and in northern Alaska a migratory divide must have developed, separating populations which migrate to the west from those migrating to the east. The

populations of the Bering Sea, Bering Straits, easternmost Siberia
and westernmost Alaska move southwards along the west coast
of America and part of them are said to winter in the Humboldt
Current off Chile. The evidence for this, summarized by MURPHY
(1936, vol. 2, p. 1100–1101), is very meagre, however. Virtually
all observations refer to October-November records of migrating
birds (the last date being 3. December) or to June-July records
of summering one-year old non-breeders. It is safe to conclude
that the migrants using this western American route usually
continue across Drake Strait to the Antarctic zone.

The Arctic Terns breeding in Canada, Greenland, north-
eastern United States, Northern Europe and the coasts of
Northern Siberia (except the eastern parts) use the south-west
European – west African migratory route. In order to reach
S.W. European waters the Siberian populations must carry out
an extensive westward migration along the coasts of the Polar Sea
and south-west along the coasts of Norway and the British Isles.
The populations of the nearctic region must carry out a trans-
atlantic crossing in the zone between $60°N$ and $50°N$. This long
oceanic voyage takes place in a direction which is almost due
east, but probably has a slight southern component, as the birds
reach the European coastal waters in the area from Scotland to
Western France. This has been demonstrated by a number of
autumn (September-October) recoveries of birds ringed in
Western Greenland, Labrador, and New Brunswick.

During the transatlantic flight the terns do not feed and do
not attempt to rest by alighting on the surface of the sea. Before
the flight the birds have accumulated ample fat resources (cf.
SALOMONSEN 1951, p. 347), but nevertheless it is necessary to
accomplish the oceanic crossing as fast as possible. In this period
of the year the wing- and tail-feathers are comparatively worn,
being more than half a year old. It is important, therefore, that
the terns during the transatlantic flight are aided by the westerly
winds which prevail over the boreal areas of the seas in the
northern hemisphere (fig. 1) and which give them an almost
constant tail wind and considerably add to their ground speed.

A similar utilization of the westerly winds in the northern
seas takes place in some other sea birds, primarily in the large
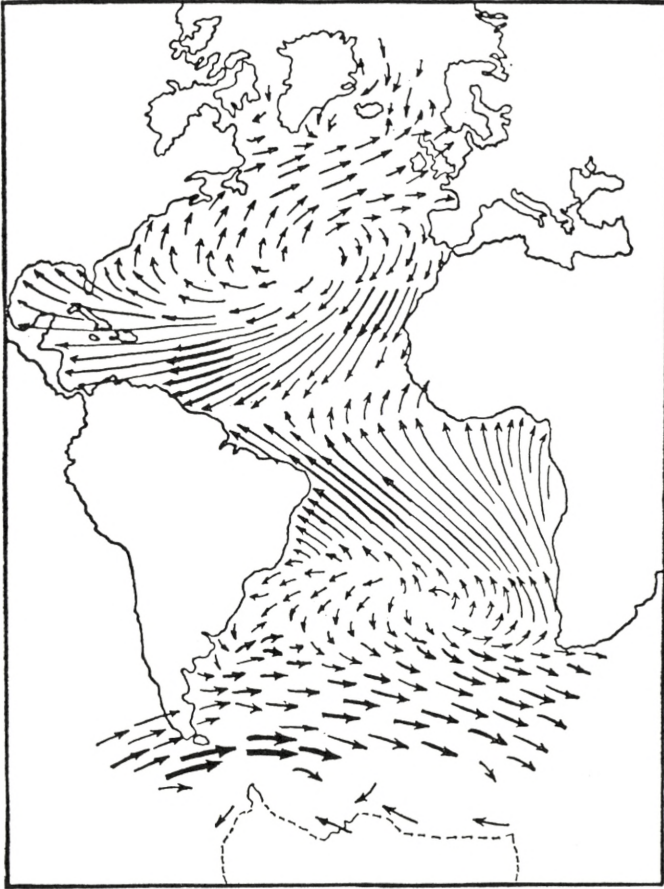shear-waters, such as *Puffinus gravis, P. griseus* and *P. tenuiro-*

Fig. 1.   Prevailing winds in the Atlantic Ocean in August. (After DORST 1962).

stris. Altogether, the use of wind as an aid during oceanic migrations is a wide-spread phenomenon among sea birds; cf. the excellent summary by DORST (1962, p. 181–199).

When on their return flight in the spring (latter half of April and May) the Arctic Terns are forced to make the transatlantic crossing against the westerly winds the force of the winds is as a rule much slighter. In addition, their wing- and tail-feathers are new and strong, their growth having been completed only one or two months previously.

Arrival in South Africa usually takes place in October or November, sometimes in September, but there is very little exact

information. One-year old birds summering in South African waters obscure the picture, making it difficult to ascertain when the migrants arrive in the autumn. Just as in Chile Arctic Terns may winter in South Africa, but by no means appear to be common as winter visitors. The species is "mainly a passage migrant" (CLANCEY 1965, p. 313), contrary to the Common Tern (*Sterna hirundo*), which is a "general visitor to all coasts" (CLANCEY, *loc. cit.*), where it is "fairly abundant" (SCLATER 1906, p. 441). This remarkable difference between the two species is borne out by the recoveries of ringed birds. The South African records of Arctic Terns include altogether nine birds, namely four British (Nov., Dec., two Jan.), one Swedish (Dec.), one Greenland (Oct.) and two Canadian birds (both Nov.), besides a one year old Faeroe bird summering there (Aug.). The European populations of the Common Tern are allohiemic, the British and West European populations wintering in tropical W. African waters and never reaching as far south as S. Africa (RADFORD 1961, p. 176), while the Scandinavian birds have their main winter quarters in S. Africa. There are altogether 20 recoveries of Scandinavian birds in S. Africa, namely one Danish, four Norwegian, eight Swedish and seven Finnish birds, from the period September —April, including a summering one-year old bird from May and June, respectively. These figures, which I have compiled from various lists of ringing recoveries (cf. SALOMONSEN 1967, p. 265), indicate that the Common Tern is the main contributor to the wintering tern population in S. Africa, while the Arctic Tern is a much more uncommon winter visitor. The situation of the Arctic Tern in S. Africa resembles somewhat that in Chile.

According to KULLENBERG (1946, p. 16) some of the Arctic Terns moving southwards during the autumn migration somewhere off West Africa branch off in a westerly direction, crossing the Atlantic in the zone of the doldrums and the southeastern trade winds (cf. fig. 1), reaching the coastal waters of S. America somewhere in southern Brazil. However, evidence for the existence of this migratory route is only indirect. There are, so far I know, no actual observations of terns migrating along this route. Records of Arctic Terns moving southward in October off Mar del Plata in Argentina (MURPHY 1936, vol. 2, p. 1101) tend to show, however, that an east-west crossing of the Atlantic does take place,

but this is probably carried out by only a small number of birds. During their Atlantic crossing the birds may benefit not only from the favourable wind conditions, but also from the large quantities of macroplankton in the border zones of the equatorial countercurrent, particularly at its northern boundary, brought about by the extensive upwelling of water from deeper layers in this area. It is a wellknown fact, of course, that upwelled water induces large amounts of nutrients (phosphates, nitrates, etc.) to the surface water layers, giving rise to a high organic production (cf. REDFIELD, KETCHUM and RICHARDS 1963, p. 65; WOOSTER and REID 1963, p. 276; LA FOND 1966, p. 957). It is doubtful, however, whether the Arctic Tern feeds at all during such a long oceanic crossing. A few terns, having finished the crossing and moved south along the eastern coast of Argentina, probably spend the winter in the waters off the Falkland Islands and southeastern Argentina, but the few records known are unsatisfactory and not quite certain.

KULLENBERG in his paper 1946 summarized the current ideas about the movements of the Arctic Tern in a map, shown in this paper as fig. 2. This map has formed the basis for most other maps on the subject in recent publications. It shows the migration
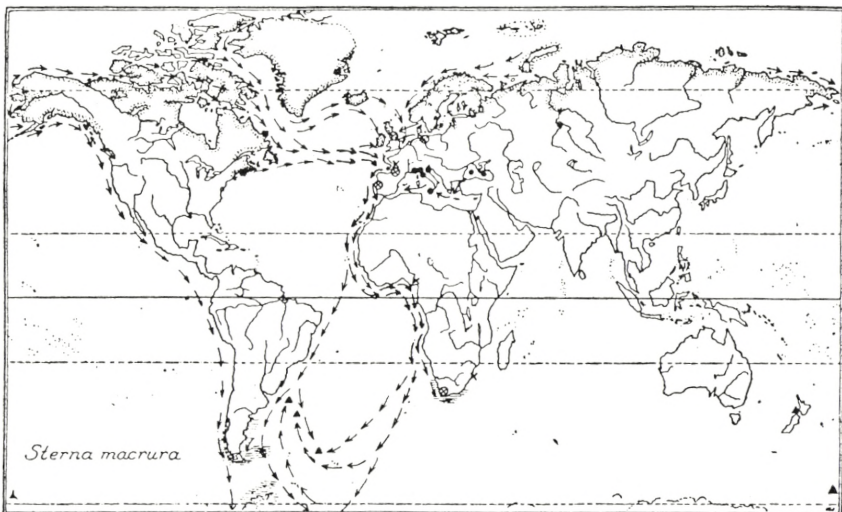

Fig. 2. The generally accepted view-points concerning breeding range, winter quarters and migratory routes of the Arctic Tern. Breeding range is dotted, winter quarter hatched, migratory routes shown by arrows. (After KULLENBERG 1946).

route of the Arctic Tern to form a loop, the birds heading south-
west from S. Africa to the antarctic waters and then, in spring,
northwest to the waters east of Argentina. This view is purely
speculative, and quite apart from there not being a single obser-
vation to support it, it is evident at once that the theory cannot
be correct. When leaving S. Africa for the south the terns are
subject to the heavy storms and gales of "the Roaring Forties and
the Shrieking Fifties", which constantly blow west to east (cf.
fig. 1). It is quite out of the question that the terns should be able
to fight these powerful forces, especially as the birds in this
period of the year have extremely weak and worn wings. I had
seen no better attempt to explain the movements of the Arctic
Tern in winter until the map published by STORR (1958, p. 60),
rendered here as fig. 6, which is much nearer the truth.

It can now be concluded, however, that the Arctic Terns
wintering in the waters around southern S. America and off
S. Africa constitute only a small fraction of the whole population
of this species. The main wintering areas are situated in the
Antarctic pack-ice zone, where hundreds of thousands of indi-
viduals spend the winter. Recent expeditions and collectings have
shown this to be the case, but it is noteworthy that the existence
of this winter quarter was completely unknown until quite
recently. Although specimens were collected in the Antarctic zone
by several expeditions in the 19th century and some of them
identified by the expert on *Laridae* HOWARD SAUNDERS (1896,
p. 66), the occurrences of the species in the Antarctic were
regarded as mere exceptions by most students, even by MURPHY
as late as 1936 (MURPHY 1936, vol. 2, p. 1102). In the sub-
sequent year FALLA (1937) published the results of the Austral-
asian Antarctic Expedition 1911–14 and the B.A.N.Z. Antarctic
Research Expedition 1929–31, and demonstrated with a wealth
of facts that the Arctic Tern was numerous in the Antarctic zone
in winter. Mainly because of FALLA's results MURPHY (1938, p. 7)
changed his mind, and since then almost every Antarctic expedi-
tion has added to our knowledge about the south-polar winter
quarters of the Arctic Tern.

When leaving S. African waters for the final stage of the
autumn migration towards the Antarctic pack-ice zone the terns
face a gloomy situation. Not only have they exhausted their
resources and their strength during the long journey already

carried out, but their efficiency of flight is considerably reduced owing to the weak and worn-out condition of their wing-feathers. Before them is a stretch of more than 3000 km of open ocean which they are forced to traverse before they can start moulting the flight-feathers. The possibilities for resting (on islands or icebergs) under the long and strenuous ocean crossing are extremely slight, and it is necessary, therefore, to get through the flight as fast as possible. All evidence tends to show that the terns are in a great hurry, and even if they should happen to pass an island they do not remain there for any length of time (cf. Downes 1952, p. 307). The same must hold good of encounters with icebergs; these are as huge as islands, but very scattered. They occur in the Atlantic northwards to about 40°S (cf. fig. 7). During the overseas voyage the terns are exposed to incessant westerly storms, against which they cannot possibly fight, owing to their present debility. It is unavoidable, therefore, that they are carried more or less to the east by the wind before they reach the border zone of the pack-ice, which constitutes their winter quarters. The birds which, for some reason, lag behind on their southward journey along West Africa will arrive so late in South Africa that they will not have time enough for the journey to the Antarctic before the moult commences. Consequently, they must remain in the South African waters. The majority of the Arctic Terns wintering there probably belong to such belated rear parties. Recoveries of ringed birds have demonstrated that odd birds may winter as far north as the Ivory Coast (December) and Ghana (February) in tropical West Africa.

It is appropriate here to insert some remarks on the moult of the flight-feathers, which has not been properly understood until quite recently. STRESEMANN (1966, p. 254) in his excellent monograph on the avian moult has demonstrated that the moult of the flight-feathers in the *adult* individuals of the Arctic Tern begins in the last days of December and is finished before the end of February, *i. e.* in a period of about 60 days. The flight feathers are only moulted once a year in this species.

The moulting processes of the Arctic Tern differ considerably from those of its allies (*Sterna hirundo, S. albifrons, S. sandvicensis, Chlidonias leucoptera, C. nigra*), which move from Europe to the coasts (or fresh-waters) of West and South Africa, but do not continue across the southern ocean to the Antarctic region. In

these species the migration takes place in a more leisurely way and is not impeded by the very slow moult, which takes place at the same time and which continues without interruption from July–August to April–May, *i. e.* the entire non-breeding period. These species can afford to moult the flight-feathers twice a year, and some of the innermost primaries even three times a year, in the lengthy moulting period they have at their disposal (STRESEMANN 1966, p. 247–268).

The moulting conditions in the five species of terns mentioned above demonstrate that a performance of a flight from Europe to South Africa is compatible with a coinciding wing-moult. The Arctic Tern is the only species which postpones the wing-moult until the arrival in the winter-quarters, and at the same time is the only species which continues the migration from South Africa across the Antarctic Ocean. The aberrant moulting condition in the Arctic Tern must be explained, therefore, as an adaptation to the extremely strenuous and demanding flight across the stormy seas from South Africa to the Antarctic, which can be carried out succesfully only under special conditions. Selection will soon eliminate individuals unable to stand the iron test. As already said, the birds are in a rather poor physical state when they start the south-polar journey. The wings are very worn and the conditions for building up new fat resources during the stay in South African waters are not too good. Nevertheless, the strength of the wings is very heavily taxed during the south-polar flight, and it must be an absolute condition that all remiges and rectrices are functioning perfectly and not weakened by growth or shedding processes. This requirement holds good both for the autumn and spring passage. Consequently, the moult has to be restricted to the comparatively short resting period in the winter quarters. This implies that the moult must be speeded up at a considerable rate when compared with conditions in all other species of terns. All observers agree that the flight of the Arctic Tern in the winter quarters is considerably hampered by the poor condition of the heavily moulting birds, which causes a lack of endurance against strong winds. The weak and awkward flight and the reluctance to rise from the safe ground has been noticed even by early observers, but was for a long time held to be a character of the Antarctic Tern (*Sterna vittata*) (*e. g.* by MURPHY (*loc. cit.*) and

KULLENBERG (*loc. cit.*)), until FALLA (1937, p. 252) rightly
ascribed it to the Arctic Tern. FALLA adds that in February the
terns are more frequently on the wing than earlier, by then
showing signs of recovery from the extreme conditions of moult.
This agrees with the examination of skins made by STRESEMANN
(1966, p. 257), who found that in five adult February birds three
had completed the wing moult and two had almost finished it,
only the outermost primary still showing growth. A single speci-
men from 25. February examined by FRIEDMANN (1945, p. 312)
likewise had only the outermost primary still in growth. The
body moult continues until about the middle of March, when the
nuptial plumage is completely established. Shortly before this the
adult birds start on the northward spring migration towards the
breeding places.

A parallel development has taken place in the Great Shear-
water (*Puffinus gravis*), which crosses the equator during its
migration from the southern hemisphere and spends the southern
winter in the North Atlantic. Just like the Arctic Tern it postpones
the moult of the flight-feathers (but not of the body-feathers)
until arrival in the winter quarters. By far the majority of the
population moult the flight-feathers off the coast of S. W. Green-
land, usually in August, while gathered in large flocks. During
this wing-moult the Great Shearwater "although not completely
unable to fly, can only rise with difficulty from the surface in
calm weather" (SALOMONSEN 1951, p. 43). The largest flock of
moulting birds I ever saw in Greenland was met with on the
22. August 1954 about one mile off the west-coast at 61°N.
I estimated the flock to comprise about 30,000 individuals. The
birds, which dotted the surface of the sea for about 9 miles, were
very reluctant to rise when I passed the area onboard a steamer.
MARSHALL and SERVENTY (1956, p. 943) found the same post-
ponement of the wing-moult in the related species *Puffinus
griseus* and *P. tenuirostris*, which migrate to the oceans of the
northern hemisphere, just like *P. gravis*. STORR (1958, p. 61) has
already drawn attention to the parallel development in wing-
moult in the Arctic Tern and in the said shearwaters.

The moult of the *young* Arctic Terns differs in some important
points from that of the adult birds, but strangely enough this has
escaped the notice of STRESEMANN. In *Sterna hirundo* and other

related species the young birds in their first winter do not start the moult of the juvenal remiges until January, *i. e.* about half a year later than the adult birds. The moult is not finished until August, but the next wing moult starts already in September and continues to May, when the now two year old birds arrive in the areas aorund the breeding places, although usually not yet breeding (STRESEMANN 1966, p. 250). The young Arctic Terns have developed a similar postponement of the wing moult, but the delay compared with the situation in the adult birds is of about two month's duration only. STRESEMANN (*loc. cit.*, p. 256) has examined a young bird from 1. March with the two outer primaries still in growth, and another specimen from 16. March still in possession of two old primaries, not yet shed. These stages correspond to those in two adult individuals captured 15. January and 22. January, respectively (STRESEMANN, *loc. cit.*, p. 257), *i. e.* about two months previously. On the other hand, three young birds from 10., 12., and 19. January, respectively, had not yet started the wing-moult (STRESEMANN, *loc. cit.*, p. 255). FALLA (1937, p. 251) has examined 10 specimens captured in the period from 17. January to 6. February. Among these were seven adult birds, all of which were in wing-moult, while the remaining three specimens were young birds, all obtained on the 25. January, which had not yet started the wing moult, but were still in possession of the old remiges "much faded and worn". This tends to show that the young birds do not usually start the moult of flight feathers until the end of January and that they are still in full moult in the middle of March, when the adult birds have had new flight feathers for about a fortnight.

It is noteworthy that the ornithologists of the Willem Barendsz Expedition 1946–47 and 1947–48, who daily encountered Arctic Terns in the Weddell Sea area from 25. December, did not observe moulting birds (with moulting holes in the wings) until 31. January and then continued to see moulting birds until 27. March (BIERMAN and VOOUS 1950, p. 112). I strongly suspect that the majority of these birds must have been individuals in their first or second winter.

It is not possible to state when the wing moult of the young birds is completed. Probably it happens sometimes in April, but the moult may occasionally draw out until June. MURPHY (1936,

vol. 2, p. 1101) examined a one year old specimen, captured
26. June, which was spending its second summer in Chilean
waters, with "new quills appearing in wings and tail". All other
specimens from May–June examined by MURPHY had finished
the moult. The main point is, however, that the period in which
the wings are weak and the flight impeded is considerably
protracted in the young birds.

The departure from South Africa for the south-polar flight
takes place from the latter part of October to the first part of
January, the adult birds probably leaving earlier than the young
ones. The latter start the moult later and are, therefore, not so
pressed for time. Evidently the greater part of the migrants start
from the leeward areas east of the Cape, but they are soon exposed
to the full force of the western storms.

The shortest distance to the pack-ice zone is found in a due
south direction, but the westerly winds will carry the birds more
or less to the east, and the resulting flight direction will be E.S.E.
or S.E. I do not think that this direction is achieved by a passive
drifting to the east of birds which aim at a southern direction.
It is more reasonable to suppose that the flight direction is genet-
ically fixed, and that selection has favoured this direction because
of its superior survival value for the migrants. By following this
direction the birds are aided by the wind without being led too far
away from their destination.

The S.E. direction of the flight over the Antarctic Ocean
exactly corresponds with the course of the cyclone trajectories
leading across the sea to the Antarctic continent, and I do not
hesitate to conclude that the migration of the terns is intimately
connected with these air movements. This is an important point.
The terns need all support for the crossing, which forms the most
dangerous part of their long journey, and following the cyclones
downwind they can achieve an increase to their ground speed of
no less than 20–60 km/hr, with a mean of 34 km/hr. (ASTA-
PENKO 1964, table 7, p. 69). It is doubtful whether the birds could
accomplish the ocean crossing with their worn wings and unfit
physical condition without the support of these air movements.
Altogether, the whole migration system in this species, the routes
chosen (along southwestern South America and South Africa),
the absence of the species in the Pacific, the situation of the winter

quarters as outlined below, appear to be one large scale con-
sequence of the drastic dependence on the cyclones during the
last and crucial part of the migration. The terns can depend on
the presence of these cyclones, for in the migration time (October–
December) they occur virtually every day. Nevertheless, it must
happen rather frequently that the terns are overcome by the
hardships of the oversea flight, judging from the comparatively
great number of cases in which exhausted and dying birds have
been caught onboard ships or washed ashore during migration.
Elsewhere such phenomena occur very rarely among such good
flyers as terns, and bearing in mind the small number of obser-
vations made on tern migration in the antarctic and subantarctic
zone, the number of records of exhausted birds is remarkable.
There are two records from the "Valdivia" Expedition (cf. p. 23),
one described by Mörzer Bruyns and Voous (cf. p. 19), and a
number of records from Australia, during autumn migration
(p. 20) as well as spring migration (p. 34).

It is possible now to give a satisfactory description of the said
air movements owing to the considerable advances in Antarctic
meteorology in recent years. Athmospheric disturbances contin-
uously develop in the region of the strong temperature gradient
of the subtropical convergence zone, fluctuating around 45°S and
called the polar front by meteorologists. These disturbances give
rise to cyclonic systems which move with the westerly winds in
a zonal (annular) pattern and may even cross from one ocean to
the other. Owing to circulation obstructions, which are usually
blocking-anticyclones of south-polar origin, or more rarely oro-
graphic barriers, polar front depressions are also deflected to the
south, forming meridional cyclones. On reaching the antarctic
front they divide into an eastern and a western branch. The
trajectories of these meridional cyclones in the antarctic summer
are shown in fig. 3. The whole system is fairly staple and is fun-
damental for the elucidation of the tern migration.

When leaving S. Africa the majority of the Arctic Terns,
moving in a southeasterly direction, assumably will reach the
area of the Kerguelen trajectory and arrive at the pack-ice belt
at localities situated from about 50°E to 110°E. A minority of
birds, leaving South Africa at more western longitudes, follow the
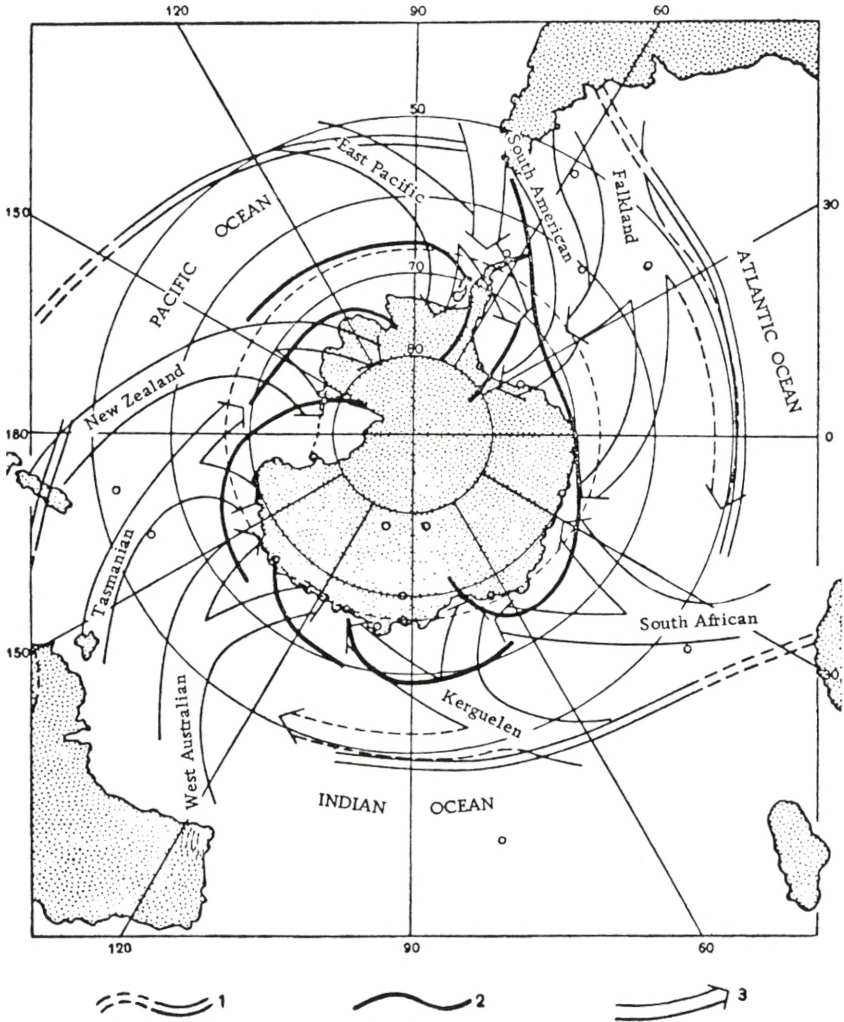South African trajectory. Another minority, following the east-

Fig. 3. Cyclone trajectories over the Antarctic Ocean and position of major fronts in the southern summer. 1. Polar front, 2. Antarctic front (tentative), 3. Meridional cyclone trajectories. (After Astapenko 1964).

ward zonal branch of the Kerguelen system (cf. fig. 3) will reach the West Australian trajectory and follow it to the south. Altogether the entire tern population starting from S. Africa will reach the pack-ice in the area from about 30°E to 150°E, with the greatest concentration in the central parts.

Trajectories situated further east (Tasmanian, New Zealand)

are only utilized by the terns on rare exceptions. It is noteworthy that no meridional cyclones develop in the greater part of the Pacific, which probably explains why the Arctic Tern does not migrate south in the eastern and central Pacific.

The Arctic Terns leaving South America will follow the South American and the Falkland trajectories and end up in the eastern Weddell Sea. A smaller number will go further east, until about 30° E, where they meet the more eastern birds, of South African origin. The trajectory shown in fig. 3 to move almost due south west of Cape Horn leading towards the west coast of Palmer Peninsula is probably used by a few birds moving south far out to sea, *i. e.* several hundred miles off the Chilean coast, but it cannot possibly be a much frequented route. The westerly winds in northern Drake Strait are extremely strong and will usually cause the birds to drift farther east, through the strait. In November, the main migration month, only 5 per cent of 888 observations showed quiet or almost calm weather, while no less than 40 per cent showed storms or gales from N.W., W. or S.W. with a force of 7–12 on the Beaufort Scale, *i. e.* a velocity higher than 28 knots (Oceanographic Atlas 1957, fig. 50, p. 42).

The migration routes outlined above are purely theoretical, but the few observations of terns during their ocean crossing agree very well with the theory. On 26. October 1929 one individual was observed on 40° S, 28° E and the following day another individual on 41° S, 31° E, *i. e.* southeast of S. Africa (Falla 1937, p. 253).

Very interesting observations have been made on Heard Island (53° S, 73°30′ E) by Downes (1952, p. 306); cf. fig. 4 for this and other geographical localities. This island is situated in the Kerguelen cyclone trajectory. In November–December Arctic Terns were seen daily in small bands, the first birds observed as early as 22. October, the last in the first week of January. The passage of terns past the island was fairly continuous, and apparently the individual terns did not remain more than one day throughout the time they were seen. It was easy to distinguish the Arctic Tern from the Antarctic Tern in the month of December, because by this time the Antarctic Tern had acquired its breeding plumage with black cap and red bill, whereas the Arctic Tern was in winter plumage with white forehead and blackish bill.
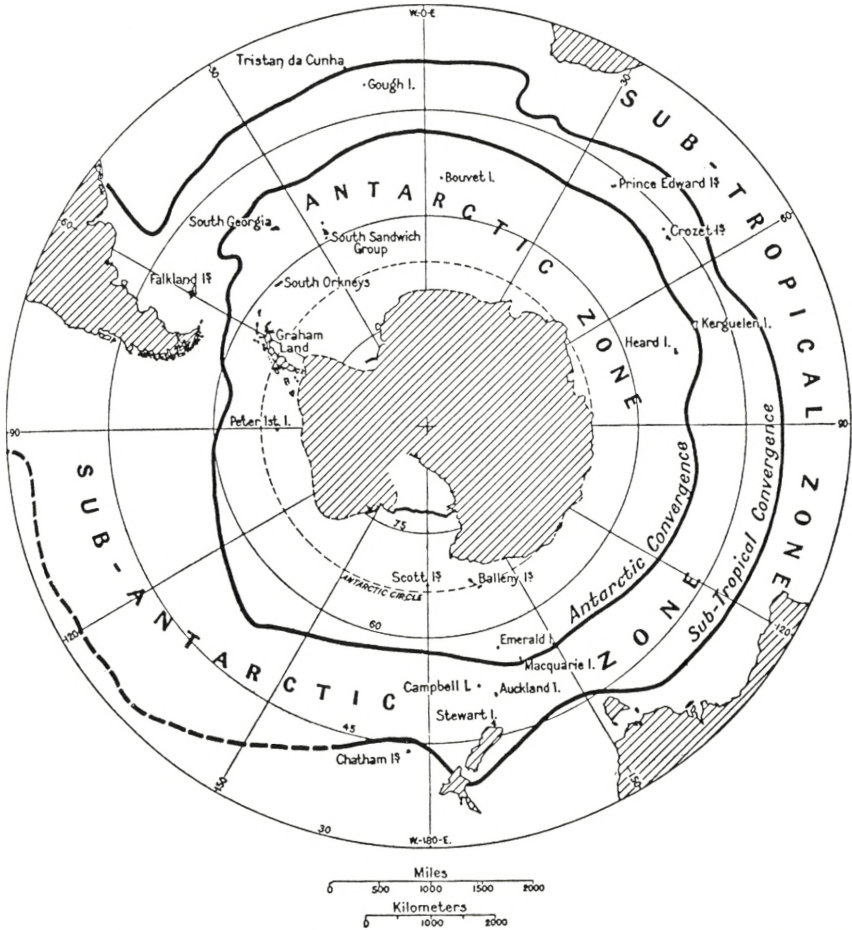
Fig. 4. The Antarctic Continent and the surrounding ocean, in south-polar pro-
jection, showing the zones of surface water and their convergences.
(After Murphy 1936).

The Antarctic Tern does not breed on Heard Island until the
Arctic Terns have left in early January. This may be due to inter-
specific competition for food between the two species. Further
north, in Kerguelen Island, where the Arctic Tern does not occur,
the endemic Kerguelen Tern (*Sterna virgata*) breeds in November
and the Antarctic Tern in January–February (Falla 1937, p. 255
and p. 259; Paulian 1953, p. 213 and p. 215), which tends to
show that the interspecific competition between these closely
related birds must be an important factor. Still further north, on

Amsterdam Island, where neither the Arctic Tern nor the Kergue-
len Tern occurs, the Antarctic Tern breeds in October–November
(PAULIAN, *loc. cit.*, p. 215).

Heard Island, situated rather far north in the Antarctic zone,
forms the northern boundary of the migratory area of the Arctic
Tern. On Kerguelen Island, which is situated almost on the
Antarctic convergence, the Arctic Tern has never been met with.
PAULIAN (*loc. cit.*, p. 218) who spent November–December on the
island expressly states that he did not see it, adding, however,
that other work prevented him from concentrating on sea birds
in this period.

On the other hand, it is possible that the Arctic Tern appears
on Macquarie Island (54°30′S, 159°E, situated on the Tasmanian
cyclone trajectory; cf. fig. 3–4) during autumn migration. FALLA
(1937, p. 261) noticed "white-capped terns" at this island in the
beginning of December, holding them to be Antarctic Terns, but
since this species has usually acquired its black-capped breeding
plumage by this time DOWNES (1952, p. 309) is inclined to think
that the birds observed by FALLA actually were Arctic Terns.
If this is correct, the Arctic Terns passing Macquarie Island must
belong to populations wintering farther east than hitherto known.
Heading towards the pack-ice belt along the Tasmanian cyclone
trajectory the terns could not reach their destination until east of
150°E, and in these regions the Arctic Tern appears to be a very
rare winter visitor. I do not think, therefore, that Macquarie
Island is situated on the regular autumn migration route of the
Arctic Tern.

A minority of the Arctic Terns leaving South African waters
are carried away by strong westerly gales and drifting across the
southern Indian Ocean in due eastern direction in the subtropical
zone between 30°S and 40°S and in extreme cases reaching the
western and southern coasts of Australia and Tasmania and even
New Zealand. The prevalence of the western winds is as pro-
nounced at these latitudes as further south in the "roaring forties",
which distinctly appears from a comparison between the wind
directions on Amsterdam Island and Kerguelen Island (fig. 5).
The long eastward drift of the terns is undoubtedly due to strong
zonal cyclones along the polar front; cf. fig. 3. It must be re-
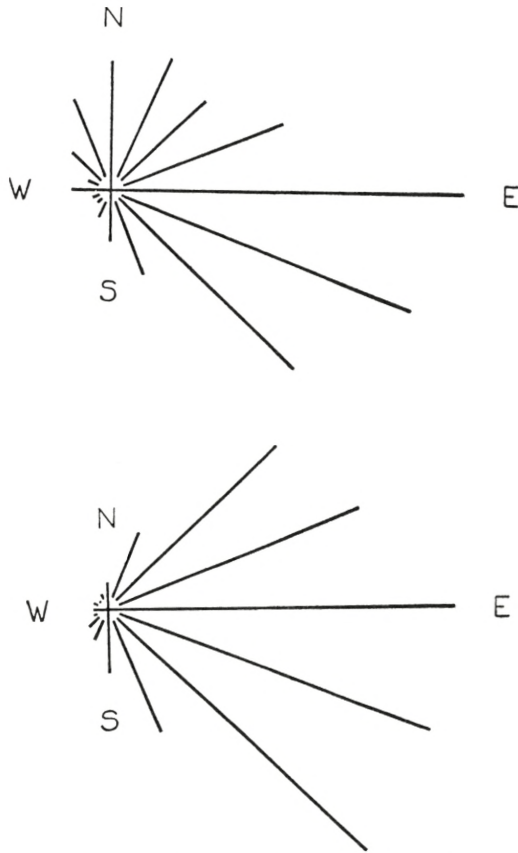marked that the polar front is situated considerably higher to the

Fig. 5. Frequency of wind directions at Amsterdam Island (above) and Kerguelen Island (below), based on observations in 1952. (After PAULIAN 1954).

north in the migration time (October–November) than shown on the map fig. 3, which gives the condition in summer (January–February).

As there is a considerable amount of shipping in the northern parts of the Antarctic Ocean these areas are better known from an ornithological point of view than the areas further south. The following observations of Arctic Terns in these areas have been published (enumerated in a west-to-east direction):

33°41′S, 40°38′E, 21. November 1961, one "found on deck of the ship in a very exhausted state", in first winter plumage, feathers very worn, wing moult not started (Mörzer Bruyns and Voous 1964, p. 117).

2*

37°S, 57°18′E, 23. October, ♂ "vix ad.", collected during the voyage of
    the yacht "Venus" (Saunders 1896, p. 66).
30°S, 60°E, 3. October 1929, five observed flying south (Falla 1930,
    p. 178).
Amsterdam Island (39°S, 78°E), 29. November 1951, one collected,
    "probablement pas adulte" (Paulian 1953, p. 217).
From 33°30′S, 41°E to 37°30′S, 79°30′E, 21.–26. November 1961, one
    or two observed each day (except 25. November) (Mörzer Bruyns
    and Voous 1964, p. 117).
Australia: Six specimens collected, all found dead, washed ashore, or in
    a weak condition, with worn plumage and old faded wing feathers;
    three records are from S.W. Australia, two from the south coast
    and one from Tasmania, two of the specimens collected in October
    (one as early as 10. October), three in November and one in
    December (Storr 1958, p. 59; Hindwood 1958, p. 259).
New Zealand: One specimen collected in December and a few others
    without date (Fleming 1953, p. 51).

This list of records, although short, shows that the oceanic
observations soon thin out when moving from west to east. The
majority was made between 40°E and 60°E, a few between 60°E
and 80°E, but none further east. This shows that the birds blown
eastward by the wind steadily attempt to break through to the
south, penetrating the wind barrier in order to reach the Antarctic
zone; cf. the observation on 30°S, 60°E of five birds "flying
south". Sooner or later most of them succeed in doing so,
although many of them probably succumb. At any rate, east of
80°E the Arctic Tern must be extremely rare on these northern
latitudes, but odd specimens, nevertheless, are blown as far east
as Australia and New Zealand.

Storr (1958, p. 60) has given a correct picture of the migra-
tion route of the Arctic Terns which are drifted eastwards to
Australia. His illustration is given here as fig. 6. It must be kept
in mind, however, that this route represents the exception, not
the rule.

The Arctic Tern spends the winter in the border zone of the
pack-ice. The water temperature here is rarely above 0°C, which
corresponds to the conditions in summer in the high-arctic region
in the northern hemisphere. The terns are mostly seen resting on
ice-floes or fishing in leads among the pack. In the period of
strong wing moult (late December and all of January in adult
birds) they keep to the resting places, even clinging to powdered
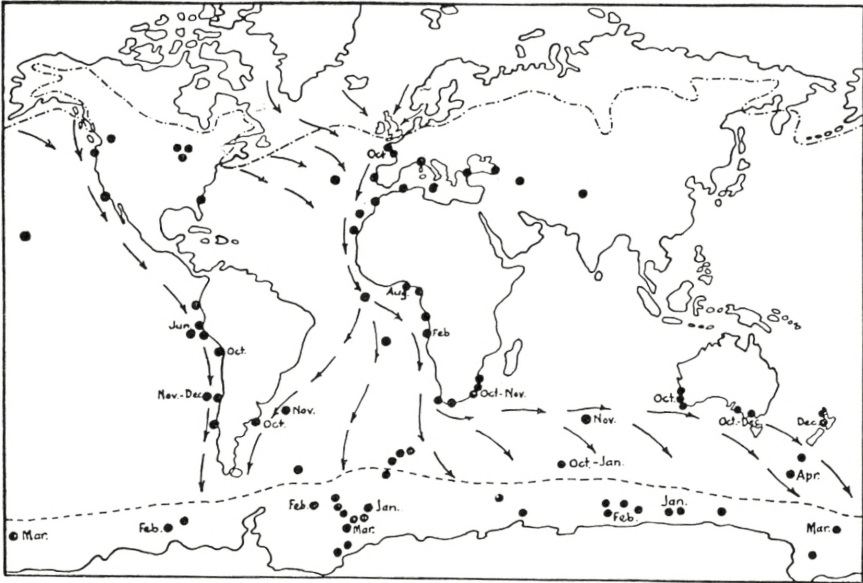
Fig. 6. Autumn migration and eastward drift towards Australia and New Zealand of the Arctic Tern, indicated by arrows. Solid circles show records of the species outside of the breeding ground (not complete); —·—·— southern limit of breeding range; – – – northern limit of Antarctic pack-ice belt. (After Storr 1958).

brash rather than taking to the wing, but in between they do some fishing in the narrow creeks between the floes, or occasionally undertake a short communal flight. They occur in flocks of varying size, from a dozen or less up to several thousand. Particularly large congregations appear when strong gales have pressed large areas of ice floes together into a congested mass. The birds then concentrate in enormous flocks which at a distance may appear as dark patches on the ice. The terns are virtually absent from the areas of open water, they appear at most a few miles away from the ice-edge, but never at considerable distances from the pack (Falla 1937, p. 253; Routh 1949, p. 590; Bierman and Voous 1950, p. 109; v. Oordt and Kruijt 1954, p. 278; Holgersen 1957, p. 68). The food conditions at the ice-edge are particularly favourable for the terns. Macroplankton, the so-called "krill", appears here in large masses, as a consequence of the immense upwelling of deep sea water along the south-polar continent (cf. above, p. 7).

Keeping to the ice-edge and its immediate surroundings the

Arctic Tern in its winter range is restricted to a rather narrow zone, usually only a few miles wide. Generally, the ice-edge forms a solid line, with minor indentations only, and ships attempting to force their way through are soon barred from further progress. Sometimes there may be a transition from isolated stream of ice to close pack, but this is not a usual condition. Open water may of course lie to the south of the ice-edge, but generally there is much more ice than open water between the ice-edge and the shores of the Antarctic continent (MACKINTOSH and HERDMAN 1940, p. 290). Patches of open water within the ice-fields as well as the open land water may also be frequented by the Arctic Tern, but apparently it does not occur in particularly large numbers here (FALLA 1937, p. 252).

The geographical distribution of the Arctic Tern in the south-polar zone roughly coincides with the East Antarctic. This was to be expected on the basis of the migration routes outlined above. The postulated arrival area of the migrating terns was said above (p. 15) to extend from the eastern Weddell Sea to about 150°E, and the terns apparently do not move much outside this hypothetical range. The eastern limit of the winter quarters runs about 170°E, *i. e.* at the west coast of Ross Sea, but the terns are irregular and scarce east of 150°E. As early as 11. December (in 1930) FALLA (1937, p. 253) while making observations at the ice-edge at 63°S, 166°E saw a single bird flying S.E., *i. e.* heading towards Ross Sea.

The greatest concentrations of terns, totalling hundred of thousands of birds, are found between 150°E and westwards to at least Enderby Land (about 55°E), according to FALLA's careful investigations. The only south-polar recovery of a ringed Arctic Tern has been made in the central parts of these areas, namely an adult bird ringed on the nest on Saltholm Island, Denmark, 22. May 1958, recovered in the pack-ice off Wilkes Land at 65°08'S, 111°15'E on 4. February 1959 (JOHANSEN 1959, p. 40). In the pack-ice belt at about 90°E Arctic Terns were daily observed "singly or in small flocks" between 18. February and 28. March 1903 by the German South-polar Expedition (VAN-HÖFFEN 1905, p. 507). In the same area, from about 85°E to 100°E, Arctic Terns were frequently met with by ROUTH (1949, p. 590) during the "Balaena" Expedition 1946–47. West of

Enderby Land the population is probably thinning out, but the sector between Enderby Land and Weddell Sea is very poorly known from an ornithological point of view. However, as far west as Coats Land, at the east coast of Weddell Sea, Arctic Terns were seen in thousands at 72°–74°S, 22°W in early March 1904 by the Scotia Expedition (CLARKE 1913, p. 268). The German "Valdivia" Expedition 1898–99 observed (and captured) only a single bird in the pack-ice on 64°14′S, 53°30′E; it flew onboard the vessel in an exhausted state. Further west (until 8°E) no terns were observed in the pack-ice in late November, but another exhausted individual was found on board the ship on 2. December (VANHÖFFEN 1901, p. 312). It should be noted, however, that there may have been more birds, VANHÖFFEN's observations not always being very accurate.

The Willem Barendsz Expedition when in rather heavy pack-ice on 63°–64°S, 22°–23°W, i. e. southeast of the South Sandwich Islands on 13.–24. March 1947 recorded almost daily flocks of several hundreds of individuals (BIERMAN and VOOUS 1950, p. 114). As mentioned above (p. 12) there are reasons to believe that the majority of these birds were immature individuals.

The majority of birds wintering in the eastern Weddell Sea are undoubtedly of South American origin, having followed the South American or Falkland cyclone trajectories.

In the remaining part of the Antarctic zone, i. e. the region between the western Weddell Sea and westwards to Ross Sea the Arctic Tern is extremely scarce, and in most areas virtually absent. Most records are from the end of February or from March, i. e. during the latter part of the terns' residence in the Antarctic when they have regained their vigour after the wing moult.

In the western Weddell Sea an Arctic Tern was collected by the Scotia Expedition on 25. February 1904 at 64°38′S, 35°13′W. It was in winter plumage and "in the midst of quill moult" (MURPHY 1936, vol. 2., p. 1102), i. e. certainly a younger bird. Another specimen was obtained by the same expedition on 23. March 1904 at 68°32′S, 12°49′W, being in summer plumage and having finished the moult, probably being a fully adult bird. I do know of no other records from these parts of the Antarctic, and it is noteworthy that the many expeditions to Graham Land

and the island groups in the Scotia Sea have never met with the Arctic Tern.

In the Bellingshausen Sea there is a single record of a specimen collected by the Norwegian Antarctic Expedition at Peter I Island 5. February 1929, being a female "moulting into summer plumage" (HOLGERSEN 1945, p. 69), but apparently some other specimens were observed. In the Amundsen Sea the U.S. Antarctic Service Expedition collected a female in the pack-ice at 70°30′S, 106°W on the 25. February 1940 (FRIEDMANN 1945, p. 312). The expedition did not obtain this species during its visit to Palmer Land, where altogether 13 specimens of the Antarctic Tern were collected.

In the westernmost part of the West Antarctic two specimens were collected by the Antarctic Expedition under JAMES CLARKE Ross on 12. March 1841 at 66°S, 157°W (SAUNDERS 1896, p. 66), and two individuals were observed on 16. February 1952 on about 70°S, 178°W by v. OORDT and KRUIJT (1954, p. 278).

In addition to these very scattered records from the West Antarctic, careful notes on the occurrence of terns in this part of the south-polar regions have been made by HOLGERSEN (1957, p. 68–71) on the "Brategg" Expedition 1947–48. In the middle of February terns were seen daily at the ice-edge in the sector between 80°W and 100°W, but never more than a few dozen a day. HOLGERSEN gives good reasons for the assumption that these birds were Arctic and not Antarctic Terns. Further west one individual was seen on 31. January at 68°13′S, 120°16′W, but this was the only record during the cruise from middle December to the end of January between 100°W and 175°W, and "we should have seen them if there had been any as we spent days cruising along the ice edge" (HOLGERSEN, loc. cit., p. 68).

We may conclude that the Arctic Tern is very scarce in the West Antarctic in February–March and apparently completely absent in October–January, going by the negative evidence that no specimens have been collected and no terns have been observed in this period.

The antarctic continent can be divided into three parts on the basis of tern distribution in summer: (1) The winter quarter of the Arctic Tern, from 150°E westwards to 30°W. (2) The breeding area of the Antarctic Tern on the islands in the Scotia Sea and

Weddell Sea and on Palmer Peninsula, between 30°W and 80°W.
(3) The remaining sector, between 80°W and 150°E, which is
virtually devoid of terns. The two species of terns have com-
pletely separate ranges, the Arctic Tern keeping to the ice-edge,
and the Antarctic Tern requiring icefree sea or areas with only a
very slight coverage of ice near its breeding places on firm land.
The only places on the continent where this condition is present
are the northern coast and the west coast of Palmer Peninsula
(Oceanographic Atlas 1957, fig. 32–33). Smaller parts of the coast
of Wilkes Land and along the eastern coast of Ross Sea may
temporarily be icefree, at least in March, and Antarctic Terns have
been recorded from the latter area, although some doubt is
attached to this record (FRIEDMANN 1945, p. 313).

After the arrival to the edge of the pack-ice belt the Arctic
Terns are still under the influence of the wind conditions.

Owing to the constant anticyclonic situation in the highlands
of the eastern Antarctic continent, downslope (katabatic) winds
blow regularly towards the marginal regions, where they turn
eastwards owing to the earth's rotation. Out at sea these east
winds are usually rather weak and do not penetrate further north
than about 65°–66°S.

In October–December when the Arctic Terns arrive at their
winter quarters the outer zone of the pack-ice is situated far out
in the region with westerly winds (cf. fig. 7), and the terns
wintering there are exposed to the full force of the storms. During
heavy gales they may easily be carried passively away in an
eastern direction, particularly when, during the wing moult, they
have a reduced capability of flight. During the following months
the pack-ice belt narrows and shrinks considerably, as seen in
fig. 7, but parts of it are still within the reach of the westerly
storms. Furthermore, the ice conditions differ widely from one
year to the next. In summer the situation of the ice-edge at the
entrance to Ross Sea has been found to vary as much as 400 miles.
In 1913 the ice-edge at long. 123°E was situated at 54°S as late
as 18. February, and several flocks of terns were observed there
by HUNTER, while in 1929 the ice-edge at long. 79°E was situated
as far south as 64°S as early as 11. December (FALLA 1937,
p. 252).

The situation of the pack-ice in relation to the prevailing winds
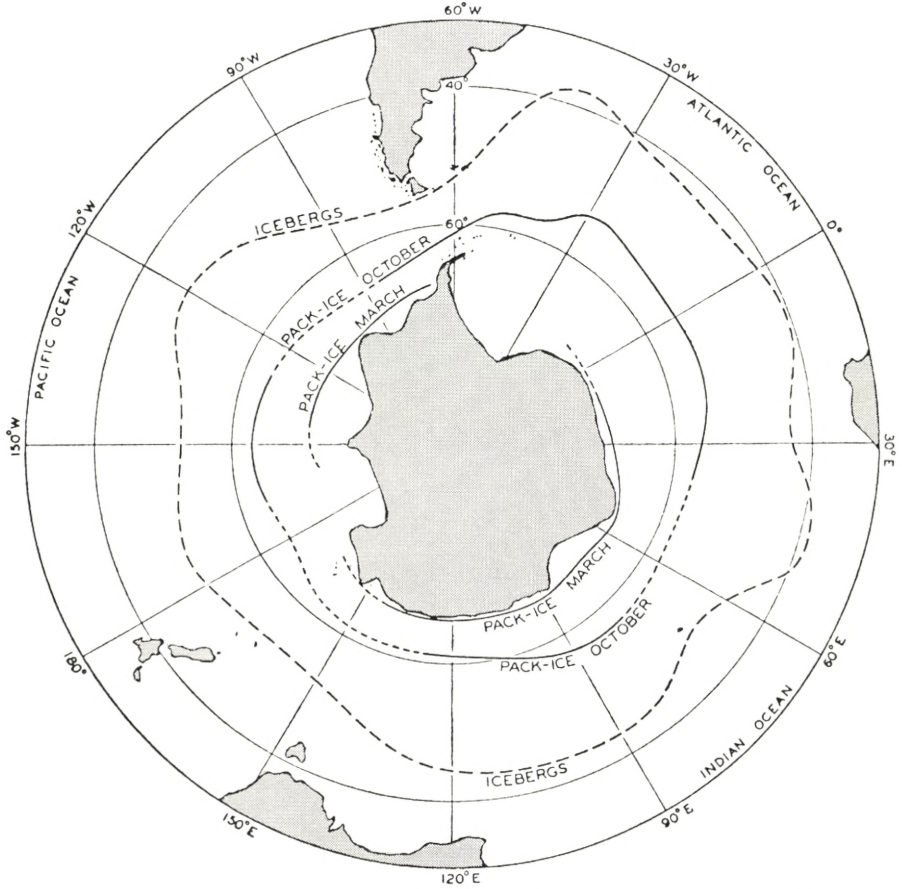
Fig. 7. Average northern limit of the Antarctic pack-ice belt in March and October, and average northern limit of icebergs. (After SVERDRUP, JOHNSON and FLEMING 1946).

makes it evident that the risk of the terns being drifted eastwards while staying in their winter quarters cannot be ruled out. The younger birds are more exposed to this danger than the adults. They roam about more, even out to sea, are unexperienced, and have not yet developed any fixed adherence to a definite wintering place. In addition, their wings are weaker, and the wing moult is prolonged well into March or even April. I am convinced that many—perhaps the majority—of the stray birds recorded in late summer (February–March) east of the Ross Sea and in the Amundsen and Bellingshausen Seas have been drifted to the

east from the ordinary wintering area in the East Antarctic, and
that the greater part are young birds. It is possible that some of
the birds observed by Holgersen in Bellingshausen Sea in
February have come directly from western Chilean waters, having
crossed Drake Strait (cf. discussion above, p. 16). A few of these
birds may during winter even penetrate westwards in the direc-
tion of Ross Sea, utilizing the east wind which blows at high
latitudes. Such a movement must be exceptional, however, par-
ticularly since the east winds in this sector of the Antarctic are
irregular and erratic, as will be shown below. It is very unlikely,
at any rate, that the Arctic Terns occurring in West Antarctic
originate from the Weddell Sea regions, *i. e.* that they should have
forced their way against the very strong westerly winds in the
Drake Strait. Some few individuals may possibly filter down the
west coast of Palmer Peninsula, along which the wind is weaker
and even may blow to the east, but these areas are within the
premises of the Antarctic Terns, and the wintering Arctic Terns,
therefore, would probably be inferior as competitors.

One more fact supports the assumption that the majority of
the Arctic Terns occurring in the Amundsen and Bellingshausen
Seas have a western origin. Birds which have entered the Ross
Sea area are exposed primarily to westerly winds which blow
right to the coast, because the cyclonic trajectories continue south-
wards far into the continent itself. As said above the anticyclone
over the high plateau of East Antarctic is very staple and only
rarely disrupted by disturbances. The lower plateau of West
Antarctic, on the other hand, is frequently traversed by pertur-
bations. Cyclonic systems moving into the eastern Ross Sea, along
one of the major paths of entry, find relatively easy access across
West Antarctic (Sabbagh 1962, p. 103). This phenomenon, which
appears distinctly in fig. 3, makes it still more doubtful that
Arctic Terns should move in an anticlockwise direction along
West Antarctic.

Direct evidence of the eastward movement of birds into the
West Antarctic is practically lacking. I can refer, however, to the
observation quoted above (p. 22) of a single bird flying south-
east at 63°S, 166°E. Indirect evidence is furnished by the fact
that young Arctic Terns in great number spend their first summer,
when they are about one year old, in the off-shore zone of the

Humboldt Current. According to MURPHY (1936, vol. 2, p. 1100), BECK found Arctic Terns to be numerous in June 1913 off the coasts of central Peru, where they occurred up to 300 km out to sea. BECK observed them daily, passing singly, in groups of six or seven or even being "common", and he collected a number of specimens. It is out of the question that all these birds should have entered the Humboldt Current *via* Drake Strait, *i. e.* originating from the Weddell Sea, because this would imply a strenuous fight against the westerly storms over a long distance. Consequently, it must be assumed that the majority of the birds originated from further west, *i. e.* from the Bellingshausen and Amundsen Seas. Having already emphasized the improbability of a Weddell Sea or Chilean origin of any great number of the Arctic Terns wintering in the West Antarctic seas, it is fair to conclude that an essential part of the West Antarctic population must have derived from the East Antarctic, west of Ross Sea.

It is possible that hydrological factors may give rise to certain dislocations of the Arctic Terns wintering in the pack-ice, but these do not appear to be of any special importance. The pack-ice steadily drifts with the wind, but the direction of the drift deviates about 45° from the wind direction, *i. e.* it has a northern component, which, however, is much less pronounced than the eastern one. The reason for this deviation is the fact that the surface water layers are continuously transported to the north, owing to the upwelling of deep sea water along the coasts of the antarctic continent. When these northgoing water masses reach the Antarctic Convergence they sink abruptly down below the subantarctic water, which results in a sharp rise of temperature at the surface. When resting on the ice floes the terns are passively drifted in an easterly direction, but the speed is slight – from the view point of the birds – amounting to about one knot, only in Drake Strait reaching two knots; nevertheless, this may sum up.

The West Wind Drift is replaced by the East Wind Drift close to the coast, but this is of still less significance to the terns. The East Wind Drift is narrow, only about 2° wide, running approximately parallel to the shore line, and is of a weak order, because the east winds blowing over the coastal waters are weak, as said already. The northern limit of the East Wind Drift lies on about 66° S, along the so-called Antarctic Divergence, which separates the East and West Wind Drift and which is characterized by much upwelling (DEACON 1937, p. 10; SVERDRUP, JOHNSON and FLEMING 1946, p. 623; ASTAPENKO 1964, p. 15). The two drifts are circumpolar, but the East Wind Drift is interrupted at Graham Land and the adjoining South Shetland Islands. These landmasses force the current towards the north where it is directly

diverted into the West Wind Drift. On the west coast of Palmer Penin-
sula the East Wind Drift is narrow and very weak, but in the Bellings-
hausen Sea it soon regenerates.

This system of currents is extremely important for the organic
productivity in the sea and for the life conditions of the marine fauna,
but probably does not exert any appreciable influence on the localization
of the Arctic Terns.

In early March, when the moult of the flight-feathers is com-
pleted and the wings have regained their vigour, at least in the
adult birds, the spring migration in the Arctic Tern is initiated.
It is very unlikely that the terns follow the same route as during
the autumn migration from South Africa. All evidence tends to
show that the terns move westwards from their winter quarter in
the East Antarctic. The pack-ice is now (in March) reduced to
its minimum size, and the ice edge is generally situated within
the zone of eastern winds. The terns undoubtedly utilize these
winds in their westward journey, but after the long period of rest
on the wintering grounds the birds are vigorous enough to move
against the westerly winds if necessary, at least for some distance
and provided the wind is not too strong. The terns continue
westwards to the Weddell Sea area, where they make a turn,
moving northwards or northeastwards towards South Africa, in
this way having the advantage of side-wind or even tail-wind.
Cf. the routes given in fig. 8.

A number of observations support the view points set forth
above. V. OORDT and KRUIJT (1954, p. 278) followed the first
part of the migration in the East Antarctic in early March 1952.
On 1. March they observed altogether 15 birds, all flying in a
westward direction at 63°31′S, 106°53′E, moving against a strong
westerly wind (force 8–9). Other individuals were observed flying
westwards on 5. March at 64°S, 84°E against a westerly wind of
force 3–6. In the next days (6.–7. March), when the expedition
had left the pack-ice belt, further individuals were noticed, all
flying westwards against westerly winds of varying force, the last
observation being made at 64°09′S, 74°17′E. No further terns
were encountered until 11. March, when at 60°S, 39°E five
scattered birds were observed flying southwestwards, against a
S.W.-wind with force 4–5. These latter birds must have been on
their way to Weddell Sea. During the remaining voyage, which
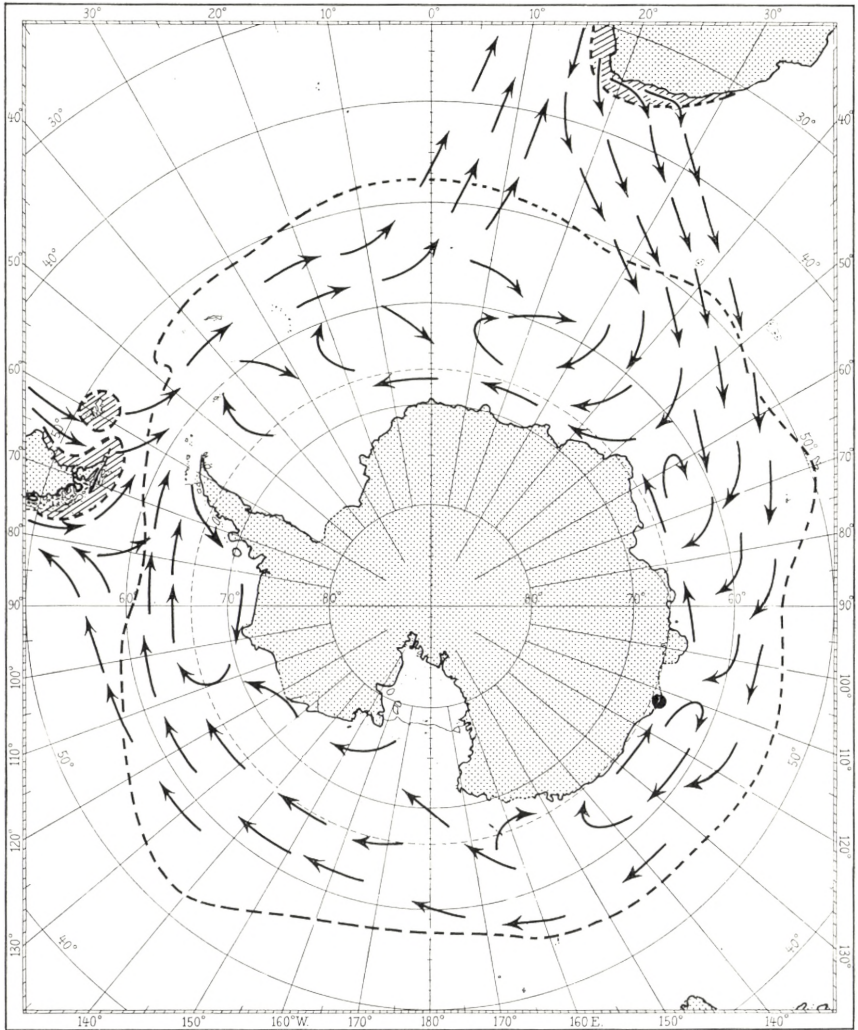
Fig. 8. Migratory system, indicated by arrows, of the Arctic Tern in the Ant-
arctic Ocean, according to the view-points accepted in the present paper, some-
what schematical. The winter quarters off South America and South Africa are
hatched, the position of the Antarctic recovery of a ringed Arctic Tern is shown
by the solid circle, the Antarctic Convergence by the dashed line.

followed a straight northwestern direction to Cape Town (within
the zone in which the autumn migration of the Arctic Terns took
place), v. OORDT and KRUIJT did not see any terns at all. This
tends to show that the Arctic Tern does not migrate towards the
north in this area. After the arrival at Cape Town on 19. March

hundreds of terns were seen by the said observers in the sea west of Cape Peninsula and in Table Bay (but some of them might have been Common Terns).

Interesting observations were made also during the "Willem Barendsz Expedition". On 12.–15. April 1947 small flocks of Arctic Terns were observed steadily moving in northward direction in the area 55°–47°S, 0°–8°E (BIERMAN and VOOUS 1950, p. 114), *i. e.* in an area which was predicted above to be on the migration route when the terns had turned to the north from the Weddell Sea region.

This route, leading towards N.–N.E. from the Weddell Sea region, is undoubtedly the main path of the Arctic Tern on its way to the north. It is used not only by the main population wintering in East Antarctic, but also by the birds wintering in the Weddell Sea. It is even used by the majority of the terns wintering in the Bellingshausen Sea, which move with the westerly winds through Drake Strait and then join the other populations on their way northeastwards to South Africa. Part of the populations of the region further west, *i. e.* from the Amundsen Sea and westwards to about Ross Sea, probably go northeastwards to the waters off the Chilean west coast.

The northeastward route of the Weddell Sea winter birds is purely theoretical, but it is the only possible one. It is unrealistic to believe that the terns would move in a northwestern direction through the Drake Strait in order to reach Chilean waters. Such a crossing could be carried out during one of the rare periods of fairly calm weather, and some few individuals may probably succeed in making such a crossing, but generally the terns would run into one continuous succession of westerly storms, which would tax the strength of these light birds too heavily. An insistence by a population to follow this migratory route would challenge the selective forces beyond the limit of probability. This poses an interesting problem, however. It was argued above (p. 16) that the majority of birds wintering in the Weddell Sea originated from Chilean waters but now I advocate the view that these birds migrate north to South Africa and do not return to Chile. This discrepancy must imply that very few adult birds are involved in this migration, bearing in mind that adult terns generally return to their former breeding places. As a consequence

of this view it may be concluded that the migratory route along the west coast of the Americas must be used by a minority of birds only.

The recognition of this route is based mainly on the presence of large numbers of one year old birds observed and collected in the Humboldt Current in June–July in their second summer, and on the autumn migration recorded in the waters off western Chile in November–December. There are very few records of migrating Arctic Terns along the western coast of North America, Central America and northern South America, and the observations may in many cases refer to one-year old birds which have spent their first summer in the northern hemisphere and now are on their way south during their second autumn migration. It must be borne in mind that during their first summer the young Arctic Terns may wander as far north as the Arctic region. This has been proved by the recovery of ringed specimens, the northern-most record being a bird ringed as nestling in western Greenland 22. July 1947, captured in James Bay, Ontario, in arctic Canada, 4. July 1948. It was shot in a breeding colony of Arctic Terns, but was not itself in breeding condition (SALOMONSEN 1949, p. 251 and 255). There are reasons to believe, therefore, that only very few adult birds participate in the migration along the western coasts of America.

The birds passing the Chilean waters on their way south can be divided into four groups, according to age, origin and destination:

Individuals originating from the breeding area, including juvenile birds and adult ones, migrating

> A-1: southwards to the Bellingshausen Sea.
> A-2: southeastwards to the Weddell Sea.

One-year old individuals (occasionally two-year old birds) summering in the Humboldt Current, more rarely further north off the west coast of the Americas, migrating

> B-1: southwards to the Bellingshausen Sea.
> B-2: southwards to the Weddell Sea.

Groups A-1 and B-1 comprise only a small number of birds, as has been demonstrated already in this paper. A few of these birds may move westwards as far as Ross Sea, but during their

spring migration return to the east and go northeastwards to Chilean waters. The adult birds as well as those which are now in their third summer continue northwards along the Pacific coast of America towards the breeding places. The juvenile birds, now in their second summer, remain generally in Chilean waters until they go south during the subsequent autumn migration.

Group A-2 comprises only very few adult birds, because these run a grave risk of being wiped out by selection, as shown above. The juvenile birds may probably move northeastwards to South Africa during spring-migration and survive.

In my opinion group B-2 comprises the main bulk of the birds migrating from Chilean waters to the Antarctic. The majority are of Atlantic, not of Pacific, origin, and comprise individuals which during their first winter have drifted eastwards from the East Antarctic. In this way they finally reach the Amundsen and the Bellingshausen Seas and during their spring migration continue northeastwards to the Humboldt Current. During their second winter the majority of these birds migrate southeastwards to Weddell Sea, and next spring northeastwards to South Africa, from where they started for the Antarctic about one and a half years previously. They have in this way performed a circumnavigation of the Antarctic Continent. It is very probable that some individuals carry out this circumnavigation in one season, continuing the migration directly to South Africa via Drake Strait during their first spring.

The regular drifting of a number of young birds from East Antarctic in a clockwise direction towards the Amundsen and Bellingshausen Seas must be viewed in the light of the special situation of the young birds, not least their moulting condition. When the adult birds in early March gradually start the spring migration westwards towards Weddell Sea the greater number of young birds are not in a very good condition to follow, owing to the belated moult of the flight feathers. At the same time, their urge of migration is much weaker and their sense of direction less acute than in the adult birds. Moreover, owing to their lack of experience they sometimes roam far out to sea. They are, therefore, much more liable than the adult birds to be caught by the wind. Evidently, the birds have been adapted to this situation and utilize the wind power to their own advantage. The

circumnavigation, therefore, must undoubtedly be of a considerable survival value to the birds.

Some young birds, being victims of a misleading sense of direction, will attempt to move northwards in too eastern longitudes, to the effect that they sooner or later drift further eastwards. Under these circumstances they may eventually reach Australia. Two such cases are known, and, strangely enough, both individuals had been ringed. The first one was caught alive off Fremantle in Western Australia on 16. May 1956, having been ringed as nestling near Murmansk at the White Sea 5. July 1955 (HINDWOOD 1958, p. 261). The second bird was found dead near Perth in Western Australia on 10. June 1963, having been ringed as nestling near Stockholm in Sweden on 27. June 1962 (SERVENTY 1963, p. 22). The occurrence at Macquarie Island on 20. April 1950 of two Arctic Terns, which were collected there by N. J. FAVALORO (DOWNES 1952, p. 309) is another instance of this eastward drift during spring migration. Naturally, also adult individuals may participate in this eastward drifting, but the majority must definitely be younger birds.

The circumnavigation of the Antarctic Continent by a varying number of young Arctic Terns is not so peculiar as it may seem at first glance. Recent ringing of the Giant Petrel (*Macronectes giganteus* (Gmelin)) on the breeding places on Palmer Peninsula, South Orkney Islands and Heard and Macquarie Island[1] (STONEHOUSE 1958, p. 204; HITCHCOCK & CARRICK 1958, p. 54) has demonstrated that the juvenile individuals of this species perform enormous migrations which follow the westerly winds in the subantarctic zone, while the adult birds are more or less stationary. Recoveries of juvenile birds have shown that a circumnavigation can be easily carried out in less than two months. Evidently, many other species of petrels as well as albatrosses perform such clockwise movements around the Antarctic continent (cf. DORST 1962, p. 196), but this has not yet been proved by ringing.

STONEHOUSE (*loc. cit.*, p. 206) emphasizes the importance of the westerly winds in aiding the Giant Petrels in their movement from west to east, but impeding their flight to an extraordinary

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[1] The specimens ringed on Macquarie Island may belong to the related species *Macronectes halli* Mathews (BOURNE and WARHAM 1966, p. 45).

degree if they attempt to fly against it. These birds "fly easily
with the wind, but only with the greatest difficulty against it".
This, of course, holds good to a still greater degree for the Arctic
Tern. Being a much smaller and lighter bird, it is much less apt
to resist strong winds. Transportability by wind (*i. e.* possibility
for drifting) increases geometrically with decrease in weight, and
"the Arctic Tern is so light that in a breeze it cannot keep a
straight course" (WYNNE-EDWARDS 1935, p. 326). On the other
side, by means of its highly developed manoeuvrability and flight
control the Arctic Tern is extremely well suited to utilize the wind
power to its advantage.

On the map fig. 8 I have attempted to illustrate the complete
migratory system of the Arctic Tern within the Antarctic zone,
in its close dependence on the winds. The representation is
somewhat schematical, it is true, and it has not been possible to
separate all details in the complicated pattern. Needless to say,
many particulars have not been proved, but I hope that the map
may be of some use as a basis for future studies.

Summary

The stupendous flight of the Arctic Tern (*Sterna paradisaea*
Pontoppidan) from the breeding places in the polar region to the
winter quarters in the antarctic zone has been described a num-
ber of times, but the migratory routes are not known in detail,
and virtually nothing has been published on the migratory move-
ments in the huge area south of Africa and South America.
Two migratory routes are recognized generally, as shown in fig. 2.
It is shown in the present paper that the Pacific flyway is of little
importance. A comparatively small number of Arctic Terns spend
the winter in the coastal waters of South Africa and southern
South America, but by far the majority continue the autumn
migration further south, crossing the Antarctic Ocean and
wintering in the border zone of the pack-ice belt.

The crossing of the Antarctic Ocean, a flight of more than
3000 km, forms the most dangerous and difficult part of the long
migration. When leaving South African waters for the south,
the terns have exhausted their resources during the extensive

journey already carried out, and their efficiency of flight is considerably reduced owing to the weak condition of their wing-feathers, which are almost one year old, having not been renewed (in adult birds) since the previous stay in the winter quarters. During the oversea voyage across the Antarctic Ocean the terns are exposed to incessant westerly storms (cf. fig. 1), against which they cannot possibly fight, owing to their present debility. It is inavoidable, therefore, that they are drifted more or less to the east by the wind.

The renewal of the flight feathers in the Arctic Tern does not take place until the crossing of the Antarctic Ocean has been accomplished and the winter quarters have been reached. In all other palearctic species of terns the wing-moult takes place without interruption during the entire non-breeding period, *i. e.* about 300 days. The Arctic Tern is the only species which continues the migration from South Africa across the Antarctic Ocean. The aberrant moulting condition in the Arctic Tern must be explained, therefore, as an adaptation to the extremely strenuous and demanding flight across the stormy seas from South Africa to the Antarctic, which can be carried out successfully only under special conditions, *i. e.* the moult must be restricted to the comparatively short resting period in the winter quarters. Consequently, the moult must be considerably speeded up when comparing with conditions in all other species of terns.

The wing-moult of the young Arctic Terns in their first winter is delayed up to two months in comparison with that in the adult birds. It never starts until the end of January and is probably not over until April, but moulting specimens have been recorded from as late as June.

The shortest distance from South Africa to the pack-ice belt is found in a due south direction, but when leaving South Africa on the migration across the southern ocean the Arctic Terns are drifted eastwards by the westerly storms and gales, and the resulting flight direction will be E.S.E. or S.E. This direction, which undoubtedly is genetically fixed, exactly corresponds with the course of the meridional cyclone trajectories leading across the sea to the Antarctic Continent (cf. fig. 3). All evidence tends to show that the migration of the terns is intimately connected with these air movements.

When leaving South Africa the majority of Arctic Terns assumably reach the area of the Kerguelen trajectory (cf. fig. 3), while smaller numbers will follow the South African or the West Australian trajectory. The Arctic Terns leaving South America will follow the South American and the Falkland trajectories. According to these migration routes the Arctic Terns will reach the pack-ice in the area from about 150° E westwards to the eastern Weddell Sea. Observations during recent expeditions have demonstrated that the winter range of the Arctic Terns covers the area delimited above on the basis of the theoretical migratory routes. Even the few observations of terns during the actual ocean crossing support the theoretical considerations. Special emphasis has been laid on the careful observations by Downes on Heard Island; cf. fig. 4 for this and other geographical localities.

It is probable that some individuals migrating southwards well off the Chilean coast use the trajectory leading almost due south, west of Cape Horn (cf. fig. 3). It cannot possibly be a much frequented route, because the westerly winds in northern Drake Strait are extremely strong and will usually drift the birds farther east, through the strait.

A minority of individuals leaving South African waters are carried away by strong westerly gales and drift across the southern Indian Ocean in due eastern direction in the subtropical zone between 30° S and 40° S, in extreme cases reaching western and southern Australia and even New Zealand. The prevalence of western winds is as pronounced at these latitudes as further south, as shown in fig. 5. The long eastward drift of the terns is probably due to strong zonal cyclones along the polar front. All known cases of this drift migration have been enumerated on p. 19–20, and the probable migration route has been outlined on the map fig. 6.

The geographical distribution of the Arctic Tern in the south-polar zone extends from about 150° E westwards to eastern Weddell Sea (about 30°), as outlined above on the basis of observations and collections made on various expeditions. In the remaining part of the antarctic zone, roughly the area from Weddell Sea westwards to Ross Sea, the Arctic Tern is extremely scarce, and in most localities completely absent.

During the first part of their sojourn in the outer zone of the

pack-ice belt the terns are still subject to the influence of the westerly winds and may easily, during heavy gales, be carried away in an eastern direction, particularly when during wing-moult they have a reduced capability of flight. Later in the season the pack-ice belt narrows considerably (cf. fig. 7), and the ice-edge gradually moves within the reach of the continental eastern winds. It is evident, however, that the risk of the terns being drifted eastwards during their stay in the winter quarters cannot be ruled out. The young birds are more exposed to this danger than the adults. It is most probable to assume that the majority of the stray birds recorded in late summer (February–March) east of the Ross Sea area and in the Amundsen and Bellings-hausen Seas have been drifted to the east from the ordinary wintering area in the East Antarctic, and that the greater part are young birds.

The return journey to the breeding places in March and early April leads from the East Antarctic in a westerly direction towards the Weddell Sea region, and during this flight the terns undoubtedly utilize the continental east winds. Off the Weddell Sea the migratory birds turn to the north or to northeast towards South Africa, in this way having the advantage of side-wind or even tail-wind; cf. the routes given in fig. 8. A number of observ-ations support these view-points.

The route leading N.–N.E. from the Weddell Sea region is undoubtedly the main path of the Arctic Tern on its way to the north. It is used not only by the main population wintering in East Antarctic, but also by the birds wintering in the Weddell Sea. It is even used by many of the birds wintering in the Bellingshausen Sea, which move with the westerly winds through Drake Strait and then join the other populations on their way to South Africa. The birds originating from Bellingshausen Sea (or from further west in the West Antarctic) have been drifted to these regions from the East Antarctic, as described above. They are mostly young birds, and in returning to South Africa in spring they have performed a complete circumnavigation of the Antarctic Continent during their first winter. Not all young birds of the West Antarctic population take this route, but many, perhaps the majority, continue from the Amundsen and Bellings-hausen Seas to the N.E. to the Humboldt Current, where they

spend their first summer. The subsequent autumn they move southeastwards to Weddell Sea, following the South American cyclone trajectory, and the following spring go northeastwards to South Africa. In this way the antarctic circumnavigation takes two years.

The regular drifting of a number of young birds around the Antarctic Continent in a clockwise direction must be viewed in the light of the special situation of the young birds, *i. e.* their moulting conditions, their lack of adherence to a certain circumscribed winter quarter, their lack of experience and their less acute sense of direction. They are, therefore, much more liable than the adult birds to be caught by the wind. Evidently, the birds have been adapted to this situation and utilize the wind power to their own advantage.

The antarctic circumnavigation of the young Arctic Terns has a parallel in similar movements in the subantarctic zone performed by the immature Giant Petrels (*Macronectes giganteus* (Gmelin)), and undoubtedly also by many other sea birds.

In fig. 8 an attempt is made in a schematical way to illustrate the complete migratory system of the Arctic Tern within the antarctic zone.

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FOLKE RØNNIKE

THE PLANT HORMONE EFFECT OF SERUM FROM HUMANS

AND

SOME INDIVIDUAL SPECIES OF ANIMALS

With Special Consideration of the Effect of Serum from
Women during Pregnancy and in Early Puerperium
and also from Children

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With Special Consideration of the Effect of Serum from
Women during Pregnancy and in Early Puerperium
and also from Children

Synopsis

1) Serum from human individuals, cattle, hens and pigs carries out an inhibitory effect on the growth of roots of wheat, *Triticum savitum Lam.*

The effect of serum from various species can be mutually different. The same is valid for the effect of serum from different breeds within the same species.

2) Serum from children in the 1/2–3 year-old age group acts less inhibitory than serum from adult subjects. This is confirmation of the Czech investigation with *Lupinus albus L.* in 1961.

Serum from younger subjects in cattle and hens has a less inhibitory effect than serum from adult individuals; on the other hand the opposite condition has been shown in pigs.

Thus it is not a general biological phenomenon that serum from non-adult subjects acts in a less inhibitory manner than serum from adult subjects.

3) In adult human individuals serum from early puerperal women has a less inhibitory effect than serum from non-pregnant women.

Individual sera of pregnant women acts less inhibitory than serum from non-pregnant women.

CONTENTS

1. Introduction

a. On the plant hormone effect in human individuals in general.
It is known that in human serum and urine there are to be found
substances of a chemical structure that is partly identical with,
and partly strongly similar to, genuine plant hormones.

However, the substances in the human and animal organism
that have an influence on the metabolism of plants have been the
subject of very infrequent investigations. A summary of these
was given in the author's doctoral thesis (Rønnike 1961). In this
work it was shown that the effect of human serum was due to
substances that could be characterized as phytohormones or plant
hormones. From this will be seen such organic substances, which
at low concentrations promote, inhibit or qualitatively influence
the growth of plants so that the effect is not dependent on the
energy content of the substances or on their content of essential
elements (Larsen 1955). Out of several hundred subjects the
author found very few whose serum showed special strong phyto-
hormone effect. This renders probable that these persons were
physiologically identical with one of the described subjects of
Kögl, Haagen Smit & Erxleben (1933) with specially strong
urine-auxine-effect.

*b. The plant hormone effect in children and individual species
of animals.* Čížková, Ulrychová & Růžička (1964) investigated
the effect of serum from children (older than 9 months) and
adolescents and found that serum from these subjects was less
inhibitory on the growth of plant roots of *Lupinus albus L.* than
serum from adult individuals. A similar difference in the effect
of serum from children and adults has been confirmed in the
present work (section 4). In addition a comparison has been
made of the effect of the serum from non-adult and adult cows,

hens and pigs. In cows and hens the same difference between the two ages as in humans was found. On the other hand in pigs it was found that there was a more inhibitory effect with the non-adult subjects than with the adult.

c. *The plant hormone effect in pregnant women.* As early as 1,500 B.C. the Egyptians had described a plant hormone effect of the urine of pregnant women. By comparison of the Carlsberg VIII papyrus in Copenhagen with similar prescriptions found in the medical papyrus in Berlin, IVERSEN (1939) was able to give a summary of a translation in which the following sentence appears: "You shall put wheat and barley into purses of cloth, the woman shall pass her water on it every day . . . if both sprout she will give birth . . . if they do not sprout, she will not give birth . . ."

In his treatise IVERSEN discusses the channels through which these prescriptions were introduced into European popular medicine where they have remained well known until this very day. BAYON (1939) wrote a monograph on the existence of the phenomenon in European popular medicine. In Danish popular medicine the phenomenon was described by MØLLER (1940).

A number of authors have been occupied with the phytohormone effect of urine from pregnant women: to name a few, KÖGL et al. 1933, MANGER 1933, HOFFMANN 1934, BAK 1936, SCHWIND 1938, WILBERG 1944, TOBIAS 1950 and v. BRESKA 1951. Most of them thought that they were able to confirm that the urine of pregnant women had a different effect on the growth of plants than the urine of non-pregnant women. KÖGL et al., who, from a quantitative point of view, employed the best plant test (coleoptile curvature test) of the above investigators, found by an investigation of the urine of ten 8 to 9-month-pregnant women a plant hormone excretion of about three times that of non-pregnant.

In this present work (section 5) a comparison is undertaken of the effect of serum from non-pregnant, pregnant and early puerperal women. An inhibitory effect could be shown with certainty in the early-puerperal that could quantitatively be characterized as being similar to the effect in children. By investigation of separate sera a less inhibitory effect of serum from the pregnant subjects than from the non-pregnant could also be shown.

In the present series it was, however. with pooled sera from these two human categories not possible to show any difference.

The statistic symbols that are employed in this treatise are in accordance with SNEDECOR 1956.

2. Technique

a. Methods of measuring the plant root inhibitory activity in blood and urine, and blood and urine fractions respectively. In plant physiology a large number of standard methods are employed for the quantitative estimation of plant biological activity, LARSEN (1955). The methods used in this investigation are described in the present section. There are two preliminary growth methods, I and II, and then the procedure proper with the two growth-in-solution methods, A and B. The two preliminary growth methods, I and II, and the two growth-in-solution methods, A and B, can be used interchangeably, I with II and A with B.

The principle of the quantitative methods described here is fundamentally the very simple one of measuring the length increments from roots of *Triticum sativum Lam.*, variety "Starke".

b. Preliminary growth, method I. Figure 1 shows the uniform sowing of wheat grains on wet filter paper, guided by holes in an acryl sheet. In Figure 2 the filter sheet after sowing is seen being wound round a plastic cylinder measuring 26×7 cm. Figure 3 shows preliminary growth of wheat at 48 hours at $22°C$ between sheets of wet filter paper rolled on plastic cylinders standing in a container of water. All the cylinders of one experiment have been placed in the same polythene bag.

c. Preliminary growth, method II. Another method of preliminary growth of wheat plants appears in Figure 4, which shows wheat grains sown on specially-constructed $21 \times 30 \times 0.6$ cm acryl sheets in which 90 depressions in the shape of spherical segments have been made. The diameter measured in the plane of the sheet is 1.5 cm. The depth of the depressions is 3–4 mm. Two acryl sheets are used together, one serving as the base and one as the cover, separated by two sheets of filter paper. A glass ball (8 mm in diameter) is placed in each corner depression to prevent the sheets from being pressed together too hard. The acryl sheets are held together by two rubber bands. The setting
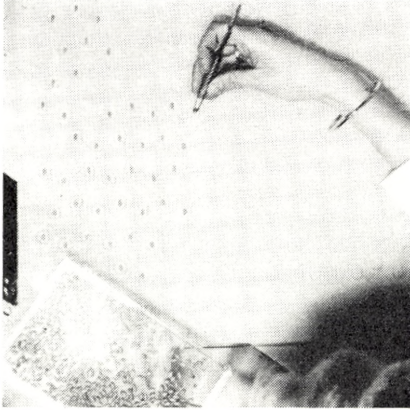
Fig. 1. Sowing of wheat grains.
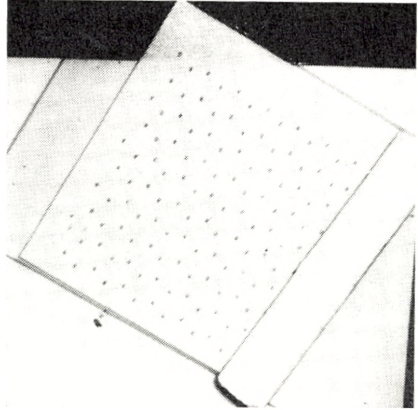
Fig. 2. Rolling a filter-paper sown with wheat round a plastic cylinder.

Fig. 3. Preliminary growth of wheat at 22°C (Figures 1–3: Method I of preliminary growth).
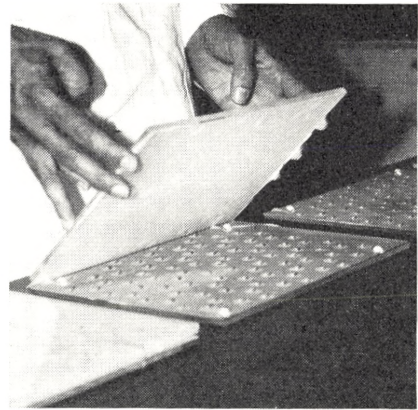
Fig. 4. Grains sown between acryl sheets (Method II of preliminary growth).

up of 15 pairs of sheets with about 1,300 sown grains, of which 35–50 per cent will prove usable for testing, takes $1^1/_2$ hours.

Figure 5 shows an air-cooling incubator used for the experiments. In the door of the incubator there is a built-in tube system that ensures forced circulation of air at constant temperature throughout the experiments, the cold air being sucked in from the refrigerating-plate compartment at the top of the incubator. A constancy of $\pm 0.2°C$ could be obtained. In the bottom compartment of the incubator a plastic container $(31 \times 32 \times 25$ cm)
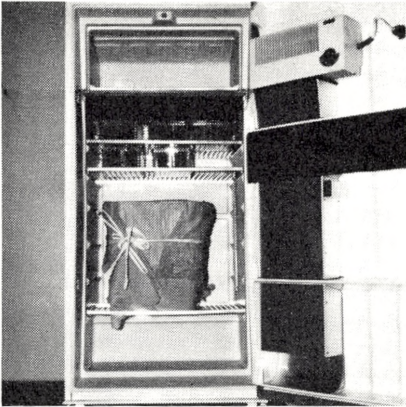
Fig. 5. Air cooling incubator for preliminary growth and growth in solution.

Fig. 6. Selection and measurement of plants before growth in solution.
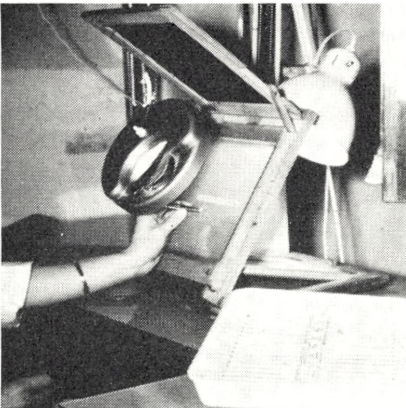
Fig. 7. A measuring setup.
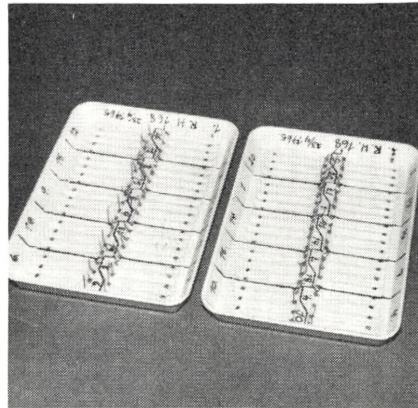
Fig. 8. Growth in polystyrol trays.

can be seen covered by a polythene sheet. The container holds up to 15 of the preliminary-growth sheet pairs. The water level is 2–3 cm above the bottom of the container. Only ion-exchanged + distilled water has been used. After 48 hours of preliminary growth at $22°C$ the plants have a length of 20 ± 10 mm.

   d. *The preparation and measurement of plants prior to, and after, growth in solutions.* Figure 6 illustrates the selection and measurement of plants prior to growth in the solutions. During the handling of the plants it is advantageous to wear special magnifying glasses. A millimeter scale fitted on an angled knife
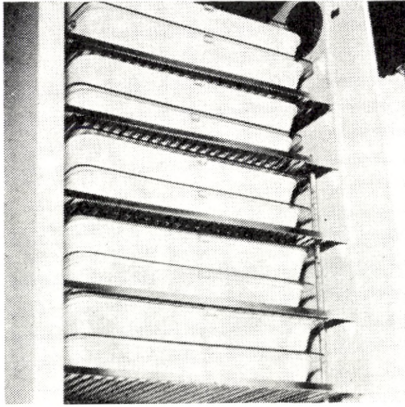
Fig. 9. A complete experiment of 20 so-
lutions, each with 20 seedlings (Figures
8–9: Growth in solution, method A).

Fig. 10. Arrangement of 40 cups, each
containing 10 plants (Growth in solu-
tion, method B).

is used for the measuring. Only the central positive geotropic of
the seminal roots are used for the experiments, the lateral seminal
plagiogeotropic roots are, in the main—see later—cut off. In the
standard method only plants with a central root length of 20–22
(± 5) mm are used. A $17 \times 22 \times 0.9$ cm tray, divided into 40 sec-
tions of $4 \times 2 \times 0.9$ cm, filled with re-distilled water may be used
for preliminary floating of the plants before these are moved to
the trays of method A (Figure 8) or the cups of method B (Figure
10)—see later.

Time will be saved if a dictating machine with a foot control
switch is available for recording the measurements.

The final measurement of plants after growth in the solutions
(after 24 hours in the standard method) is best done by means
of a setup as shown in Figure 7: a millimeter scale behind a
large lens.

e. *Growth in solution, method A.* On the right of Figure 7
can be seen one of the special polystyrol trays (obtainable from
"Dansk Formstof", 7 Lergravsvej, Copenhagen S) of method A
of the growth-in-solution methods, with the solutions to be tested.
Five pairs of these trays cover a complete experiment. Figure 8
shows a pair of trays, i.e. a whole experiment in miniature, re-
peated five times in the aggregate experiment, where each pair
of trays has its own random succession of the twenty different
solutions of the experiment, i.e. a block experiment arrangement.

f. Growth in solution, method B. Figure 10 shows a setup consisting of 40 cylinder-shaped 25 ml cups measuring 5×2.5 cm (obtainable from the firm of "Nunc", Algade, Roskilde, Denmark). The growth-cups setup occupies far less space than the polystyrol-tray setup. The cups are discarded after use. In the plastic lids 10 holes of 3.6 mm diameter have been cut. The holes are peripheraly arranged so that the upper inner edge of the cups partially closes the holes, thus allowing access for the roots only and preventing the whole plants from falling into the liquid. Another type of lid (obtainable from "Helge Buchs Eftflg.", 55 Kapelvej, Copenhagen N.) is made of acryl plastic and has an inner diameter of 29 mm and a thickness of 4.5 mm, with 10 part-cylindrical, part-conical holes, 5×2.6 mm. This gives extremely good support for the seedlings and renders unnessary the time-consuming cutting off of the lateral seminal roots of the plants.

After use the lids are disinfected with a strong hydrochloric acid solution.

In the standard method 20 plants are grown in each solution, 10 in each of two cups. In many experiments, however, 30 or 40 plants have been used in each solution.

The cups are coded at the beginning of each experiment, and the succession of the cups during the experiment is a random one.

The centre compartment of the incubator of Figure 5 contains 5 plastic boxes of cups with plants of the growth-in-solution method B.

3. Variability in the Inhibitory Effect of Human Serum on Root Growth

a. The preparation of the serum and salt samples. The blood samples were usually taken in the morning. The samples were left for a few hours and then centrifuged, the serum was removed with a pipette. On many occasions the blood serum was kept undiluted for many days at $-25°C$. Such storage, as demonstrated below, had no effect on the obtained values. The serum samples were diluted mostly to 0.5 or 1 per cent with a salt solution of half the concentration used by SHIVE (1915), i.e. 2.5 mM $Ca(NO_3)_2$, 7.5 mM $MgSO_4$ and 9.0 mM KH_2PO_4, denoted in the
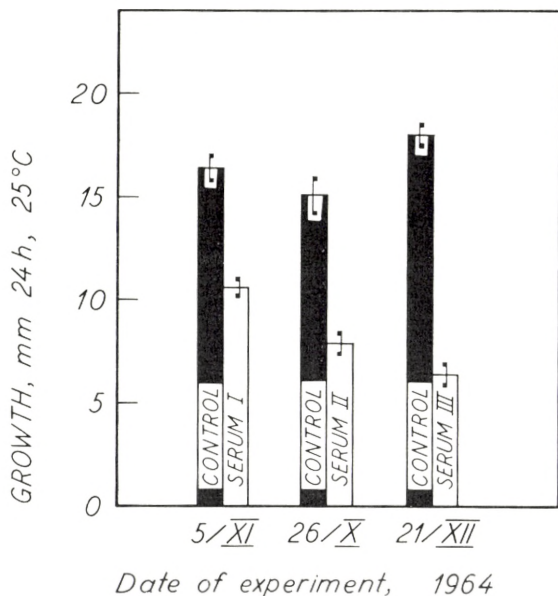
Fig. 11. Variability in the effect of three types of serum. Serum I, serum with a low inhibitory effect. Serum II, serum with an intermediate inhibitory effect. Serum III, serum with a high inhibitory effect. Growth in control (Shive's solution) and in serum diluted to 1 per cent with Shive's solution. Shown at the head of each column is the S.D.

following as "Shive". Sometimes, however, the concentrations used by Shive were used (the experiments in Figure 14). In some experiments a phosphate buffer solution 1/150 M at pH 6.0 with addition of 5 mM $Ca(NO_3)_2$ has been used. The serum dilutions were prepared immediately prior to use. Growth in salt solution without serum served as a control.

At first *Lupinus albus L.* was used by the author, but was abandoned in favour of *Triticum sativum Lam.* as relatively large quantities of serum—0.1 ml—were required for each Lupinus plant compared with about 12.5 μl for each Triticum plant.

b. The variability in the effect of one fresh serum sample within a single experiment. In experiments without addition of serum, standardized as described above, the S.D. of a single determination was 1–2 mm and with addition of serum to 1 per cent, 0.5–1.0 mm.

Figure 11 illustrates the variability in the effect of fresh serum samples in three different experiments. The experiments de-
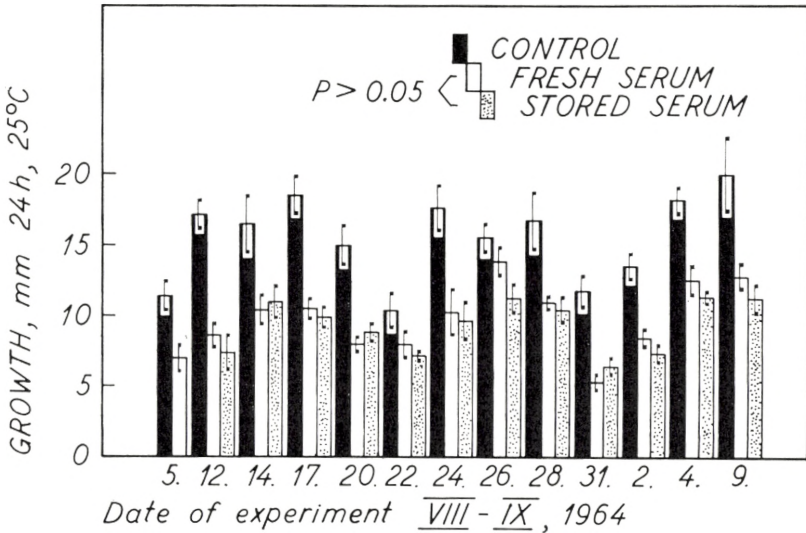
Fig. 12. Effect of storage of serum taken on a single day of one normal person. Comparison with the effect of non-stored serum in 13 experiments. Serum diluted to 1 per cent with Shive's solution. – First column in each experiment: Control growth, i.e. growth in Shive's solution. – Second column in each experiment: Growth in dilution of non-stored serum. – Third column in each experiment: Growth in dilution of serum stored from 5/VIII 1964. – Shown at the top of each column is $t_{.05}s_{\bar{x}}$. – Conclusion: No difference in inhibitory effect of stored and non-stored serum (P >0.5).

monstrate the effect of sera with, respectively, a low, a medium and a high inhibitory effect. Each column value in Figure 11 is the mean of growth of 200 roots. The S.D. is shown at the head of each column.

c. *The variability in the effect of non-stored serum compared with stored serum.* The influence of serum is also demonstrated by the thirteen experiments of Figure 12. Serum from a single, normal person was used in each of the 13 experiments carried out on different days. On the first day of the experiments a large serum sample was prepared and pipetted off in 2 ml portions, these were kept at $-25°C$ until use. In each experiment the effect of one stored portion was compared with freshly prepared serum. Figure 12 shows the variability of the mean growth in: (1) Control solution (Shive's solution), (2) 1 per cent diluted serum, prepared from samples of freshly drawn blood, and (3) 1 per cent diluted serum, prepared from serum stored at $-25°C$.
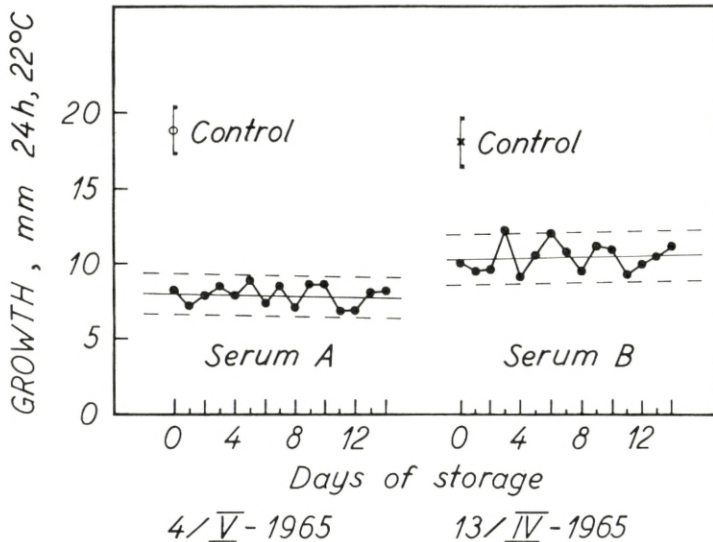
Fig. 13. Effect of storage of serum taken on many days from two persons, A and B. Two experiments: 4/V 1965, experiment with serum of person A and 13/IV 1965, experiment with serum of person B. Serum diluted to 1 per cent with Shive's solution. – Solid straight lines: Lines of the estimating equations for the two sets of data. Dashed lines: Zones of $\pm t_{.05} s_{Y \cdot X}$. – Conclusion: No significant regression ($P > 0.5$) in the two experiments, i.e. storage had no effect on the inhibitory effect of serum.

It is seen that although variations occur in the total growth, no significant difference could be demonstrated between the freshly prepared serum and the stored serum, ($P > 0.05$). Thus only small variations may have occurred in the inhibitory effect of the blood from the subject during the period of the sampling.

That storage at $-25°C$ does not alter the inhibitory effect is also demonstrated by the two experiments from two normal persons A and B shown in Figure 13. The serum samples from each person were each examined in a single experiment in such a way that all the determinations of the effect of all the serum samples of each person were carried out simultaneously. Serum prepared from a blood sample taken daily on 15 consecutive days and stored undiluted at $-25°C$ for up to 15 days is without influence on the inhibitory effect of serum from the two persons.

These experiments also demonstrate that only slight variations in the inhibitory effect of serum seem to occur in normal persons.

d. *Influence of serum concentration, growth time, growth tem-perature and pooling of serum.* The influence of variation of serum concentration, growth time, growth temperature and pool-ing of serum is described in section 6.

4. The Inhibitory Effect of Serum from Children, Chickens, Calves and Young Pigs compared with the Effect of Serum from Adult Individuals

a. The effect of serum from different species and breeds. The effect of serum from different species can show large variations. Thus in table 1 it can be seen how the effect of serum from pigs differs strongly from the effect of serum from the human individual and cattle.

The effect of serum from different breeds within the same species of animal can also vary, table 2. For instance, in hens it is possible to show a difference between White Leghorns and Brown Leghorns and between Brown Leghorns and White Ply-mouth Rocks, and in cattle between Red Danish Milk Breed and Black and White Danish Milk Breed.

b. The effect of serum from children and adults. Čížková, Ulrychová & Růžička (1964) have shown that serum from children has a less inhibitory effect than serum from adults. Čížková et al. used *Lupinus albus L.* In order to investigate whether it would be possible to demonstrate this difference with

TABLE 1. The Effect of Serum from Different Species.

SPECIES	No. of individuals	Growth, 24 hrs., 22°C $\pm t._{05}s_{\bar{x}}$ mm
Man (*Homo sapiens*)	7	7.3 ± 0.4
Cattle (*Bos taurus L.*), Red Danish Milk Breed	6	8.3 ± 1.7
Pig (*Sus scrofa L.*), Danish Landrace	7	13.4 ± 1.3

Growth in serum diluted to 1 per cent with Shive's solution. One experiment: 18/I 1966.

TABLE 2. The Effect of Serum from Different Breeds.

BREEDS Date of experiment	No. of individuals	Growth, 24 hrs., 22°C $\pm t._{05}s_{\bar{x}}$ mm	Probality of no difference
Hens (*Gallus domesticus L.*) White Leghorn . . . . Brown Leghorn 6/X 1965	10 10	13.5 ± 0.4 12.1 ± 0.8	0.01 < P < 0.05
Hens (*Gallus domesticus L.*) Brown Leghorn . . . . White Plymouth Rocks . . . 25/IX 1965	10 10	12.9 ± 1.2 10.8 ± 0.9	0.005 < P < 0.01
Cattle (*Bos taurus L.*) Red Danish Milk Breed . . . Black and White Danish Milk Breed 26/XI 1965	10 10	12.3 ± 1.2 14.1 ± 1.2	0.025 < P < 0.05

Growth in serum diluted to 1 per cent with Shive's solution. Three experiments.

other plant species the experiments were carried out with *Triticum sativum Lam.*

The results are shown in Figure 14; in each of the 15 experiments an equal number of children and adults took part, in all 72 children and 72 adults.

Figure 14 shows that the plant roots grew longest in the control solution, next longest in the serum from children and least in the serum from adults (P < 0.001). Thus serum from young children inhibits the growth of the cells in the meristems near the tips of the plant roots less than serum from adult individuals, and so the experiments confirm the results of Čížková et al.

It should be pointed out that the experiments in Figure 14 were carried out with a technique that varies slightly from that described in section 2 as the experiments were carried out in a period prior to the complete standardization of the technique.

Čížková et al. also found that the inhibitory effect increased with age, so that the effect at 15–18 years of age corresponded to that of adults.
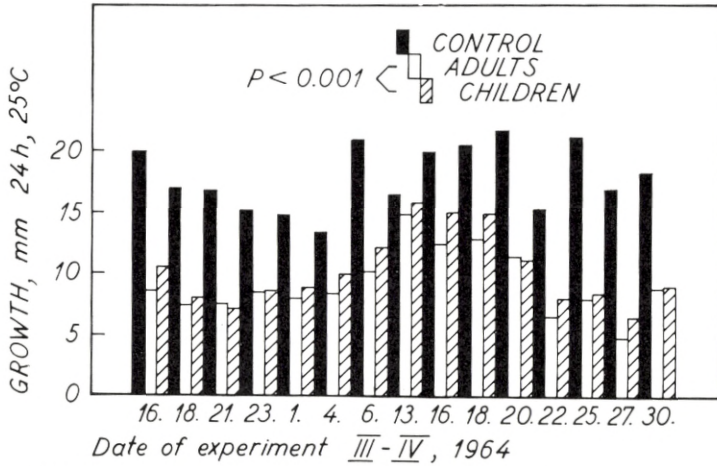
Fig. 14. Effect of serum from children and adults. 15 experiments with serum from 72 different normal children $^1/_2$–3 years old and 72 different normal adults. – First column in each experiment: Growth in control (Shive's solution). – Second column in each experiment: Average growth in 1 per cent dilutions of serum from 5 (4–5) different normal adults. – Third column in each experiment: Average growth in 1 per cent dilutions of serum from 5 (4–5) different children. – Conclusion: Lesser degree of inhibition of serum from children compared with serum of adults (P <0.001).

Comparative experiments with serum from children of various ages and adults are illustrated in Figure 15. It can be seen how the difference in the effect of serum from children and serum from adults is clearly marked in the two experiments with children under the age of 2. On the other hand a significant difference could only be demonstrated in one of the two experiments with children in the 6–8 age group, and no difference could be shown in either of the two experiments with children aged about 15. The results of these experiments are in agreement with those of Čížková et al.

c. *The effect of serum from non-adult and adult cattle and hens.* In order to see whether a difference comparable with that that exists between children and adults could also be shown in animals, determinations of serum from hens, cattle and pigs were carried out.

The effect of serum from hens of the White Plymouth Rocks breed can be seen in Figure 16. As a control in each experiment a number of about-one-year-old hens were used. In the experiments the effect of serum from the hens was compared with
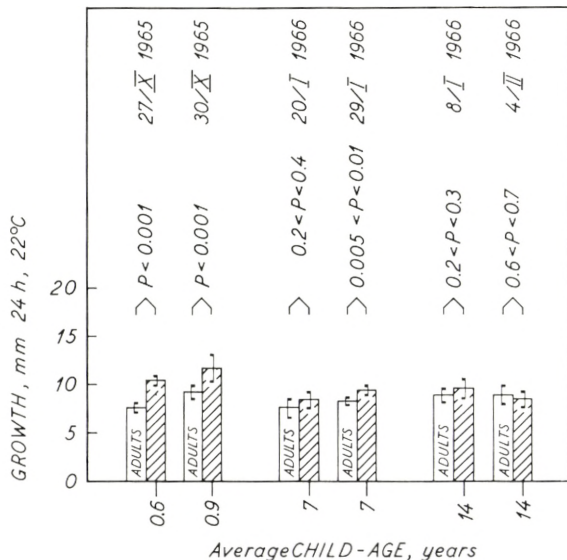
Fig. 15. Effect of serum from children at different ages. 6 experiments with serum of 50 different normal children at different ages and serum of 46 different normal adults. Serum diluted to 1 per cent with Shive's solution. – First column in each experiment: Average growth in dilutions of serum from about 10 adults. – Second column in each experiment: Average growth in dilutions of serum from about 10 children. Shown at the head of each column is $t_{.05}s_{\bar{x}}$. – Conclusion: Only at the two youngest ages did all the children have inhibitory serum effects different from adults.

serum from 17-, 69-, and 125-day-old chickens. The blood from the 17-day-old chickens was obtained by decapitation, whilst the blood from the others was taken from a wing vein.

A clear difference could be shown in all three experiments ($P < 0.001$, $P < 0.001$ and $0.01 < P < 0.025$).

The inhibitory effect of serum from cattle is illustrated by the last three experiments in Figure 16. A comparison of the effect of serum from 3-week-old calves and 24-week-old heifers was made with the effect of serum from adult cattle. In both cases a difference could be shown between the younger animal and the adult ($P < 0.001$). On the other hand it was not possible to show a difference between the effect from calves and the effect from heifers—the last experiment in Figure 16.

In respect to hens and cattle the same relation as in man could thus be shown: a less inhibitory effect in the non-adult subjects compared with the effect from adult.
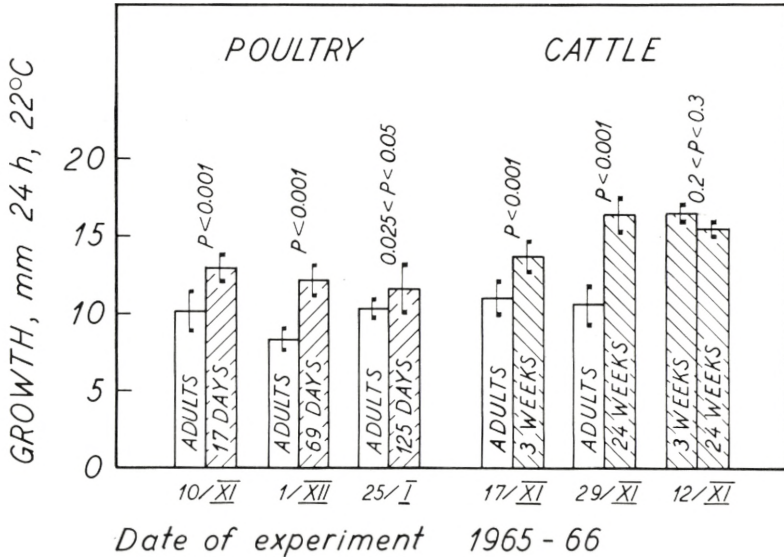
Fig. 16. Effect of serum from hens (White Plymouth Rocks) and cattle (Red Danish Milk Breed) at different ages. 6 experiments showing effect of dilutions to 1 per cent of serum from 118 different animals. – First column in each experiment: Average growth in serum dilutions of about 10 adult animals (except experiment 12/XI 1965). – Second column in each experiment: Average growth in serum dilutions of about 10 non-adult animals. – At the head of each column is shown $t_{.05}s_{\bar{x}}$. – Conclusion: Lesser inhibitory effect of serum from non-adult animals of hens and cattle compared with the effect of serum from adult individuals.

d. *The effect of serum from non-adult and adult pigs.* In table 3 the inhibitory effect of serum from adult pigs is reproduced compared with the effect of serum from five-week-old and six-

TABLE 3. The Effect of Serum from Pigs at Different Ages.

AVERAGE AGE Pigs (*Sus* scrofa *L.*) Danish Landrace Date of experiment	No. of individuals	Growth, 24 hrs., 22°C $\pm t_{.05}s_{\bar{x}}$ mm	Probability of no difference
3–5 weeks	9	12.7 ± 1.5	
3 years	10	15.5 ± 0.3	$0.001 < P < 0.005$
3/XII 1965			
6 month................	10	14.9 ± 1.2	
3 years	10	17.2 ± 0.9	$0.005 < P < 0.01$
9/XII 1965			

Growth in serum diluted to 1 per cent with Shive's solution. Two experiments.

2*
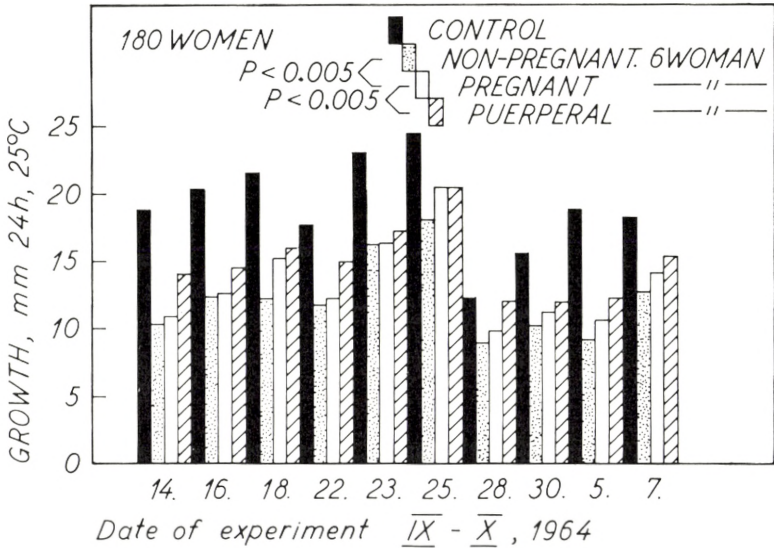
Fig. 17. Effect of serum from non-pregnant, pregnant and puerperal women. 10 experiments with growth of about 4000 plants in dilutions to 1 per cent of serum from 180 women. – First column in each experiment: Growth in control solution (Shive's solution). – Second column in each experiment: Average growth in dilutions of serum from 6 different, non-pregnant, normal women. – Third column in each experiment: Average growth in dilutions of serum from 6 different women, normal pregnant in the third trimester. – Fourth column in each experiment: Average growth in dilutions of serum from 6 different women in normal, early puerperium. – Conclusion: Differences between effect of serum of the three categories of women (P < 0.005).

month-old pigs. It can be seen that in pigs a stronger inhibitory effect of the serum from younger animals could be demonstrated compared with the effect from adult animals: that is to say, the inverse relation to that which obtains in man and the two other investigated species of animal.

Thus it appears not to be a general phenomenon that the inhibitory effect of the serum from adult subjects is greater than the effect from non-adult subjects.

5. The Inhibitory Effect of Individual Serum Samples from Normal Pregnant and Puerperal Women

a. The effect of serum from non-pregnant, pregnant and puerperal women. The effect of serum from 60 non-pregnant, 60 pregnant and 60 early-puerperal normal women was investigated in 10 mutually independent experiments (Figure 17). In each
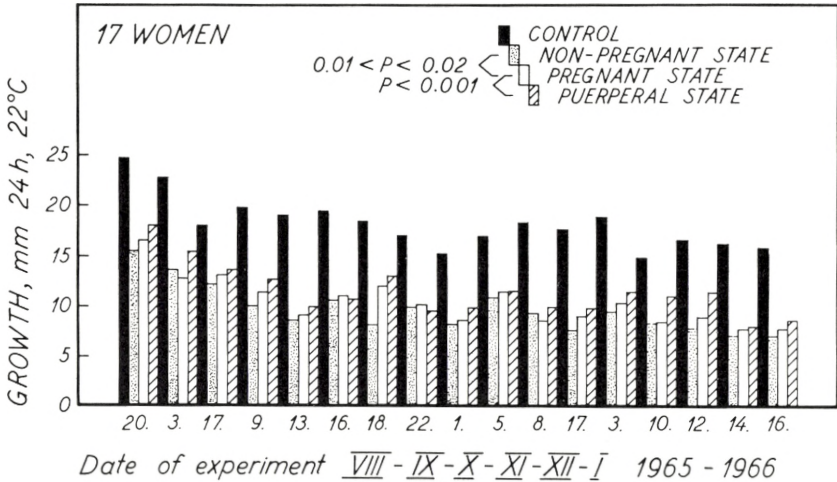
Fig. 18. Effect of serum from 17 different women during and after normal preg-
nancy. 17 experiments with growth of about 6000 plants. Each experiment shows
the effect of serum from a single women in the pregnant and early puerperal state
and 1 month or later after delivery. – First column in each experiment: Growth in
control solution (Shive's solution). – Second column in each experiment: Average
growth in dilutions to 1 per cent of serum from 6 (5–7) different days 1 month
and/or later after delivery. – Third column in each experiment: Average growth
in dilutions to 1 per cent of serum from 6 (5–7) different days during late pregnancy.
– Fourth column in each experiment: Average growth in dilutions to 1 per cent
of serum from 6 (5–7) different days in early puerperium. – Conclusion: Differences
between effect of serum of the three categories of physiological states (0.01 <P <0.02
and P <0.001).

experiment an average of separate determinations on 6 non-
pregnant, 6 pregnant (in the third trimester) and 6 early puerperal
women was carried out. It can be seen from Figure 17 that the
largest inhibitory effect could be shown from the non-pregnant,
the least from the early puerperal and an intermediary effect
from the serum from pregnant women. By calculations that in-
clude the separate experiments only it will often not be possible
to show any difference, on the other hand by calculations that
include all mean values of the three categories of women a
significant difference can be shown with the significant figure as
quoted on the figure.

    b. *The effect of serum from 17 women during and after preg-
nancy.* The effect of serum during pregnancy, in early puerperium
and one month or more after delivery was investigated in 17
normal women (Figure 18). Separate determinations were made
of 18 serum samples from each woman: these were 6 samples
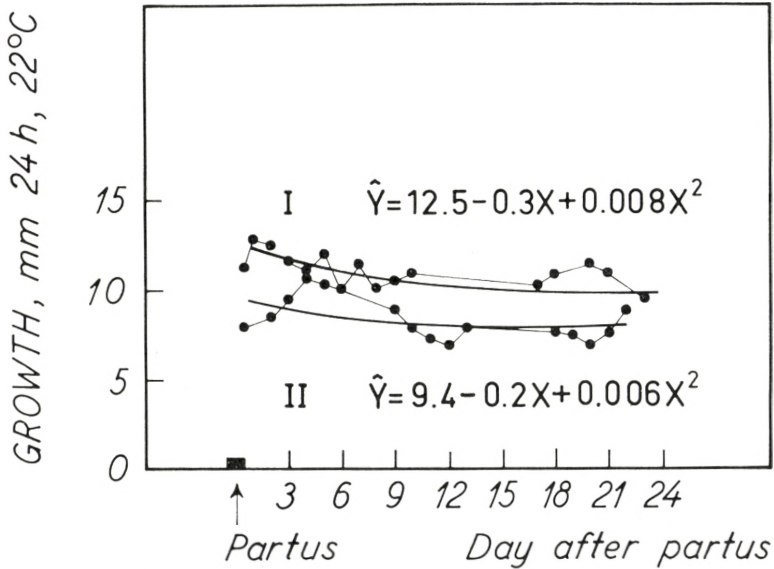
Fig. 19. Effect of serum of two women I and II in three normal puerperal weeks after delivery. Solid curved lines: Estimated parabolas of two sets of data. – Conclusion: Puerperal effect of less inhibition disappeared three weeks after delivery.

taken on 6 different days during the later pregnancy, 6 samples taken on 6 days in the early puerperium and 6 samples from 6 days at least one month after delivery. In this series of experiments it also could be shown that on an average the strongest inhibitory effect occurs in the non-pregnant, an intermediary effect in the pregnant and the weakest inhibitory effect in the early puerperium.

  c. *The effect of serum from two puerperal women in the three weeks after delivery.* The effect of serum in the first three weeks after delivery from two women, *I* and *II*, is shown in Figure 19. Two second degree polynomials are shown in the figure: the graphical representations of these take into account the experimental data so that the sum of the square of the distances from the parabolas to the experimentally-found points is a minimum. The possibility that these curves are curvilinear is over 99 per cent and as constant level (where $dy/dx = 0$) is obtained after three weeks there is thus evidence that a lesser inhibition exists in the period just after delivery, and also evidence that this reduction disappears after three weeks. This experiment is thus in agreement with the average results of the previously described experiments.

6. The Inhibitory Effect of Pooled Serum

a. Pooled serum samples compared with individual serum samples. Individual determinations with serum from different individuals often show a large variation caused partly by analysis error and partly by the individual variation. This complicates a comparison of the effect of serum from different subject categories. In a comparison of the effect of serum from non-pregnant, pregnant and puerperal women it is thus necessary either to investigate serum from a very large number of subjects or, if fewer subjects are available, to investigate each subject repeatedly.

In order to investigate if the number of analyses could be reduced by employing pooled serum, a comparison was made of the growth in 1 per cent dilutions of individual sera and 1 per cent dilutions of a pooled serum produced from the same sera. The result of this comparison appears in Figure 20 where in two experiments a determination was made of the effect of serum from 20 women: that is 10 non-pregnant women in the experiment on 10/II 1966 and 10 early puerperal women in the experiment on 29/I 1966.

It can be seen that it has not been possible to demonstrate a difference between the average effect of the individual sera and that of the pooled serum. As expected it can also be seen that the standard deviation is less for the pooled serum than for the individual sera.

The experiments thus appear to show that pooled serum ought to be preferred for a comparison in groups of several subjects based on the greater precision with which it is possible to determine the average values and because fewer of the time-consuming plant-experiments need be made.

b. The effect of pooled serum from non-pregnant, pregnant and puerperal women. In a series of experiments a comparison was made of pooled sera from 60 non-pregnant, 60 pregnant and 60 early-puerperal normal women. Here a difference could be shown between the serum of the early-puerperal women compared with the serum of the other two categories: on the other hand no difference could be demonstrated in the present series between the effect of the serum from the pregnant women and the non-pregnant women.

Reproduced in table 4 are the results from one of the experiments in this series. Serum from the puerperal women had the
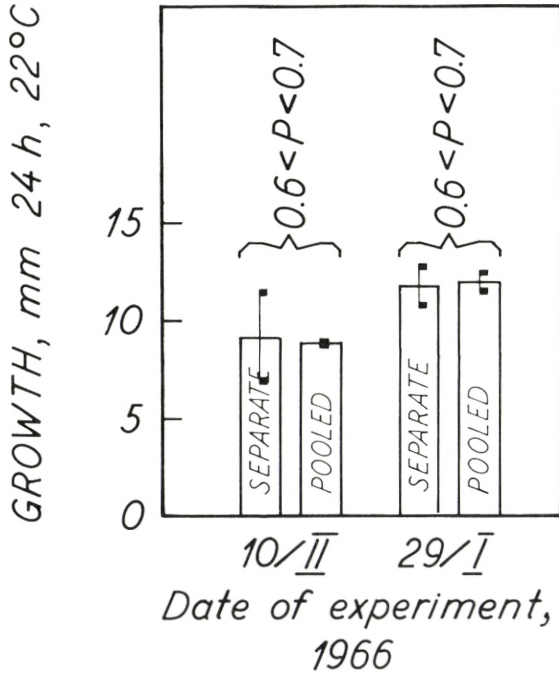
Fig. 20. Pooled serum compared with samples of individual serum. Two experiments: 10/II 1966: Serum from ten normal, non-pregnant women and 29/I 1966: Serum from ten normal, puerperal women. Serum diluted to 1 per cent with Shive's solution. At the head of each column is shown ±S.D. – Conclusion: No difference of average growth in two types of serum dilutions, lesser S.D. in pooled serum dilutions.

least inhibitory effect, 11.5 ± 0.4 mm, compared with 8.9 ± 0.6 mm in the pooled serum of the pregnant women and 8.7 ± 0.3 mm in the pooled serum of the non-pregnant women.

 c. *The effect of variation of concentration of pooled serum from puerperal and non-pregnant women.* The effect of different concentrations of the pooled serum from 0.25 to 5.0 per cent from puerperal and non-pregnant women is shown in Figure 21. Also shown in this figure is the effect of 5 mM Ca^{++} and 10^{-6} M of the antiauxin para-chloro-isophenoxy-butyric acid (PCIB) at the same serum concentrations. The serum is diluted with a phosphate buffer 1/150 M at pH 6.0. After dilution adjustment is made with 0.1 N HCl to pH 6.0. It can be seen that an increase in the concentration of the serum alone does not cause any large increase in the inhibition. The difference between the two types

TABLE 4. The Effect of Pooled Serum from Puerperal, Pregnant and non-Pregnant, Normal Women.

TYPE OF POOLED SERUM (No. of determinations)	Average growth of 20 plants 24 hrs., 22°C $\pm$ t. $_{05}s_{\bar{x}}$ mm	Average growth of 120–140 plants $\pm$ t. $_{05}s_{\bar{x}}$ mm
Pooled serum of 60 puerperal women (6)	11.9 ± 1.1 11.9 ± 0.9 11.6 ± 1.4 11.4 ± 0.8 11.4 ± 1.3 10.8 ± 1.0	11.5 ± 0.4
Pooled serum of 60 pregnant women (6)	9.4 ± 1.5 9.4 ± 0.9 9.2 ± 1.0 9.1 ± 1.0 8.5 ± 0.9 8.0 ± 0.8	8.9 ± 0.6
Pooled serum of 60 non-pregnant women (7)	9.2 ± 0.8 9.1 ± 0.6 9.0 ± 0.8 8.7 ± 0.7 8.5 ± 0.9 8.4 ± 0.7 8.3 ± 1.0	8.7 ± 0.3

Pooled serum diluted to 1 per cent with Shive's solution. One experiment: 24/X 1966.

of serum is also small. The addition of 5 mM Ca^{++} markedly changes the effect of the two sera so that a distinct correlation between the concentration of the serum and its inhibitory effect is demonstrable. This becomes more distinct on the addition of both 5 mM Ca^{++} and 10^{-6} M PCIB. Here the growth increases strongly without the addition of serum. At the highest serum concentration a stronger inhibitory effect is obtained by the addition of Ca^{++} and PCIB than without the addition of Ca^{++}. For all the curves in Figure 21 it may be said that the pooled serum from puerperal women has shown less inhibitory effect than pooled serum from non-pregnant.

Reproduced in Figure 22 is the effect of a variation of two
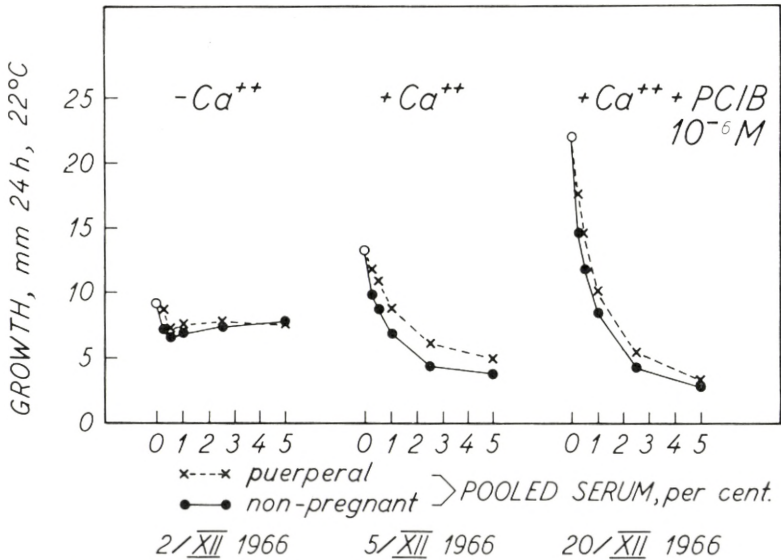
Fig. 21. Effect of different concentrations of serum without and with addition of 5 mM $Ca(NO_3)_2$ and 10^{-6} M of the antiauxin PCIB. Dilution of pooled serum with 1/150 M phosphate buffer at pH 6.0 to 0.25, 0.5, 1.0, 2.5 and 5.0 per cent. Three independent experiments. – Conclusion: Lesser puerperal inhibitory effect present in all cases of changed growth of plants.

PCIB concentrations for two different concentrations of pooled serum from puerperal and non-pregnant women: 0.5 and 5.0 per cent. The serum is diluted with Sørensen's phosphate buffer 1/150 M + 5 mM $Ca(NO_3)_2$ at pH 6.0. It can be seen that an increase of the concentration of PCIB to 10^{-5} causes a further growth of the roots of the plants used in the experiments; on the other hand an increase of the concentration to 10^{-4} results in normal growth. The effect of the serum is seen to be reduced in that no inhibitory effect of the serum is obtained on dilution to 0.5 per cent and the inhibition by dilution to 5.0 per cent is reduced. The serum from the puerperal women in relation to that from the non-pregnant is seen in all three curves to have the less inhibitory effect.

To sum up it may be said that despite the growth variation on addition of Ca^{++} and PCIB pooled serum from the puerperal shows a less inhibitory effect compared with serum from the non-pregnant women.

d. *The effect of variation of pH-value.* The effect of a variation of pH in pooled puerperal and pooled non-pregnant serum
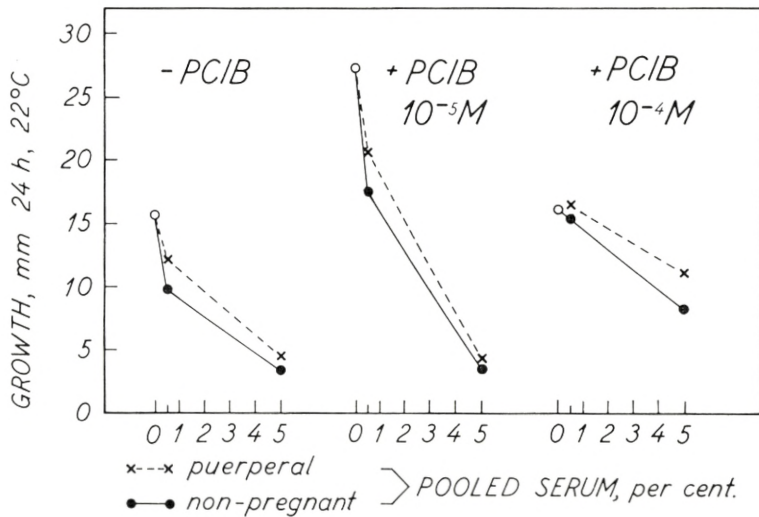
Fig. 22. Effect of two concentrations of serum at two concentrations (10^{-5} and 10^{-4} M) of the antiauxin PCIB. A single, factorial experiment. Dilution of pooled serum with 1/150 M phosphate buffer at pH 6.0 and 5 mM $Ca(NO_3)_2$ to 0.5 and 5.0 per cent. – Conclusion: Lesser puerperal inhibitory effect present in all cases of changed growth of plants.
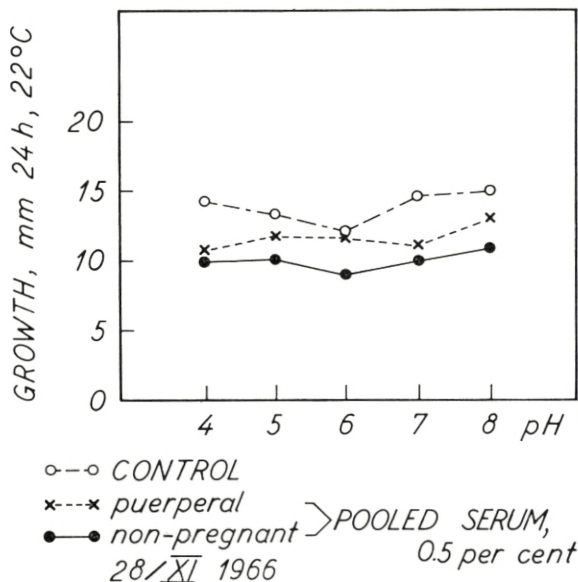
Fig. 23. Effect of different pH-values. Growth in pooled serum diluted to 0.5 per cent with 1/150 M phosphate buffer and 5 mM $Ca(NO_3)_2$. – Conclusion: Lesser puerperal inhibitory effect present at all pH-values.
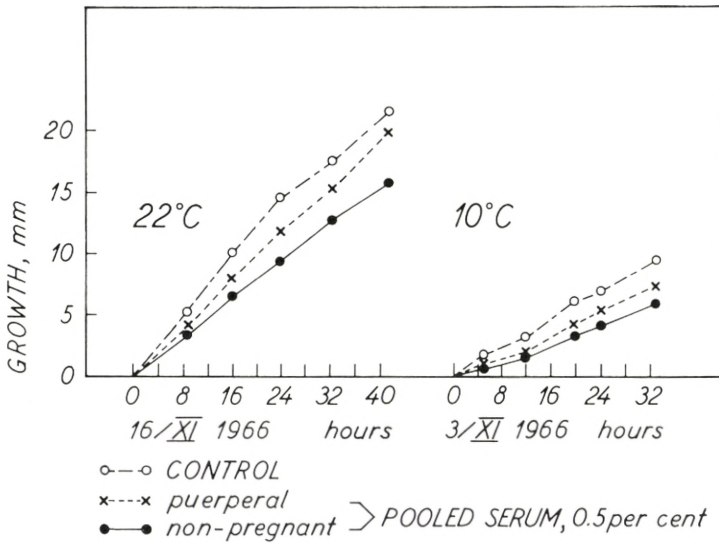
Fig. 24. Effect of different durations of growth at two temperatures, 10°C and 22°C. Serum diluted to 0.5 per cent with 1/150 M phosphate buffer at pH 6.0 and 5 mM $Ca(NO_3)_2$. – Conclusion: Lesser inhibitory puerperal effect present at both temperatures and at all times of growth.

diluted to 0.5 per cent is seen in Figure 23. Serum from the two categories of women is diluted with 5 mM $Ca(NO_3)_2$ in a phosphate buffer, 1/150 M at pH 6.0, as an outlet thinner. After this the adjustment of the pH values to pH 4.0, 5.0, 6.0, 7.0 and 8.0 is made by the addition of 0.1 N HCl or NaOH. Figure 23 also shows the larger growth in the dilutions with pooled puerperal serum than with pooled non-pregnant serum for the investigated pH values.

e. *The effect of growth time and growth temperature.* Figure 24 shows two experiments with growth in 0.5 per cent pooled serum from puerperal and non-pregnant women. The growth of the plants took place at respectively 10° and 22°C for up to 43 hours. On the whole proportionality between the growth and the length of the experiment could be demonstrated. Thus, in the serum solutions, there is in fact an actual inhibition of growth and not, for example, a delayed commencement of growth. As expected the quicker growth took place at 22°C.

In these experiments as well the serum from puerperal women had the less inhibitory effect.

7. Discussion

The discovered less inhibitory effect of serum from children in relation to adults is confirmation of the results of the Czech investigations made in 1961 (Čížková, Ulrychová & Růžička). *Lupinus albus L.* was used in these Czech investigations, whilst in the present investigations *Triticum sativum Lam.* was used. Thus, that it has been possible to show the same phenomenon with two different species of plants must be considered as additional confirmation of the existence of the phenomenon.

A similar relation as that in human individuals has, as mentioned, also now been shown in cattle and hens. On the other hand serum from non-adult pigs had a larger inhibitory effect than serum from adult pigs. In human individuals it has also been possible to show a less inhibitory effect in puerperal women in the first days after delivery compared with other normal adult persons. On the other hand it must be considered as uncertain if there is a difference in the inhibitory effect of serum from pregnant women compared with non-pregnant women.

The root inhibitory effect of human serum is determined by substances that must be characterized as phytohormones of predominently auxin type. These substances occur partly in the free state and partly bound to the serum proteins (Rønnike 1961). A dominating part of the inhibitory effect of urine and serum is due in all probability to indole-3-acetic acid, but it is also conceivable that other substances are playing a role as an antagonistic effect between various substances can also appear. An antagonistic effect can thus appear partly in the form of greater or lesser inactivation by binding, partly thereby that perhaps substances with a growth promoting effect may be found.

The difference in the effect of individual and pooled sera from pregnant women where the inhibition of the individual sera showed disparity in effect opposite the pooled sera, can thus possibly find an explanation in that the individual sera with the most strong inhibition contains either a deficit of growth promoting substances or a deficit of inactivating substances or both.

The experiments available up to now do not permit however a definite decision on whether there exists a qualitative difference as an explanation of the various serum effects and until further

it is quite natural to assume that the variation in the concentration of the same substances in various subjects is the most important factor. An experimental support of this is found in the experiments in section 6 with Ca^{++} and the antiauxin para-chlorophenoxybutyric acid (PCIB). Sufficiently thorough analyses of the results of the experiments with serum, Ca^{++} and PCIB are difficult to carry aut, but it should be stated that the antagonism between Ca^{++} and PCIB opposite pooled puerperal and pooled non-pregnant serum was fairly equally pronounced opposite both the two concerned types of serum at all the investigated concentrations of serum (Figures 21 and 22). In other experiments, too, it has not been possible to find a combination of serum, Ca^{++} and PCIB concentrations where the difference between the two serum types was diminished or augmented.

Thus, these relationships make it probable that the difference in the effect of serum from puerperal and non-pregnant women is more of a quantitative type than a qualitative one.

The less inhibitory effect in young subjects has as well as in human individuals also been shown in cattle and hens. No investigations exist that can show if the inhibitory effect in these species of animals in a qualitative respect differs from that in the human individual. It does not seem however to be a general biological phenomenon that serum from non-adult subjects works less inhibitory that serum from adult subjects, which the determinations on non-adult pigs seem to show as the effect here, as mentioned, was found to be more inhibitory than the effect in adult pigs.

It is uncertain as to the reason for this discrepancy between the species. Many considerations of a similar nature to those above about the interplay between inhibitory and promoting substances can be mentioned, to which also can be added considerations of different selectivity in the excretory activities in the various species of animals.

8. Acknowledgments

9. Summary

(1) Serum from human individuals, cattle, hens and pigs carries out an inhibitory effect on the growth of roots of wheat, *Triticum sativum Lam.*

The effect of serum from various species can be mutually different. The same is valid for the effect of serum from different breeds within the same species.

(2) Serum from children in the $1/2$–3 year-old age group acts less inhibitory than serum from adult subjects. This is confirmation of the Czech investigation with *Lupinus albus L.* in 1961.

It has now also been possible to show that serum from younger subjects has a less inhibitory effect than from adult in cattle and hens, on the other hand the opposite condition has been shown in pigs.

Thus it is not a general biological phenomenon that serum from non-adult subjects acts in a less inhibitory manner than serum from adult subjects.

(3) In human individuals it has now also been shown that serum from early puerperal women has a less inhibitory effect than serum from non-pregnant and pregnant women. On the other hand it is uncertain if the serum from pregnant women acts less inhibitory than serum from non-pregnant women as determinations on individual sera showed this difference while in the present series this was not the case with determinations on pooled sera.

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FOLKE RØNNIKE

THE INHIBITION OF EHRLICH ASCITES TUMOUR CELL GROWTH BY HUMAN SERUM ESPECIALLY SERUM FROM WOMEN IN PREGNANCY AND THE EARLY PUERPERIUM

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CONTENTS

1. Introduction

Addition of serum to synthetic cell culture media promotes cell growth, and is, in fact, essential in the great majority of human and animal cell culture methods of to-day.

In general it is not clear which type of serum—autologous, homologous or heterologous—is most effective, nor is the mechanism of action fully understood. It is probable that high molecular weight serum proteins with their physico-chemical properties are effective detoxicants, but low molecular weight substances also give some unknown, but necessary, character to the culture medium (FOLEY & EPSTEIN 1960, LEVINTOW & EAGLE 1961).

Human and animal sera also contain low molecular weight substances which modify growth of cells from higher plants (RØNNIKE 1961, 1967, 1969, 1970), but, again, the reasons for this are unknown. Generally, however, plant cell cultures differ from animal cell cultures, in that they can be maintained on wholly synthetic media, without addition of serum proteins or other high molecular weight substances (LARSEN 1962).

The author has demonstrated that serum from pregnant women, women in the puerperium, and children, has effects on plant cell cultures different from other human serum (RØNNIKE 1969). The effect of serum from pregnant and non-pregnant women on human and animal cell cultures has not been much studied. MEYER, PENTTINEN & SAXÉN (1964) found ". . . that it is obvious that pregnancy has an effect on the growth-controlling capacity of the serum. The changes in the serum which occur during pregnancy and are responsible for the differences in the growth behaviour of cells in cell culture are unknown. The more frequent clumping of cells at the end of pregnancy could be connected with the increase in e.g. beta-lipoproteins at that time.

1*

The obvious disappearance of the clumping phenomenon after the first weeks of pregnancy remains unsolved . . .".

REJNEK, BEDNARIK, RERÁBKOVÁ & DOLEZAL (1963) observed some differences in HeLa cell culture growth in the presence of, respectively, pregnant and non-pregnant serum, but no author has made further investigation of this interesting phenomenon.

This author, inspired by his basic plant physiology studies, undertook examination of the effect of fresh human serum on the growth of Ehrlich ascites tumor cells in a culture already optimal as regards its serum component (i.e. 10 % embryonic bovine serum was added to the basic medium, and the Ehrlich cells were adapted to this milieu over a considerable time before the studies).

The categories of serum studied were obtained from: 1) healthy young men, 2) healthy young women, 3) healthy pregnant women, and 4) healthy women early in the puerperium. An inhibitory effect on the Ehrlich cell growth was observed, increasing from category 1) to category 4) serum; i.e. women's serum is more toxic to the Enrlich cells than men's and still more so when the women are pregnant or in the early puerperium.

The following report documents these observations.

2. Methods

a. *Cell type and culture medium.* Throughout this study, the common standard culture technique with cells in suspension was used.

The Institute of Pharmacology of the University of Bonn provided the Ehrlich ascites tumor cell suspension (KARZEL 1965, KARZEL & BREULL 1968, KARZEL & SCHMID 1968) from a continous culture.

The standard culture medium, to which sera were added to obtain test preparations, was "FIB 41 B" (BRIAND 1969), to which was added 10 % embryonic bovine serum (Flow Laboratory, Ltd., Irvine, Scotland/Dansk Mikrobiologisk, Skoletoften 16, Grundfør pr. 8332 Hinnerup Danmark). Control tubes contained this standard medium only.

b. *Cell suspensions.* Fresh culture medium was added daily after disposal of between 50 % and 80 % of the culture. From one

to four times monthly, cells were centrifuged down (300 G for 5 mins.) and the culture medium completely replaced.

    c. *Preparation and treatment of human serum.* Following venepuncture, the blood was allowed to stand for at least one hour. Coagulated blood was thereafter freed from the test tube sides with a sterile metal probe, and the whole centrifuged at 3,000 G for 10 minutes. The serum was drawn off with a sterile disposible syringe. Serum dilutions were always made on the day of vene-puncture, and if the preparation was not used the same day, it was stored under sterile conditions at ≤ 20 °C.

    d. *Experiment technique.* In preparation of the cell suspension before addition of serum (or the control solution), a greater or lesser number of cells were centrifuged down (300 G for 5 min.), and the cell concentration adjusted by the addition of a greater or lesser quantity of the basic culture medium. While the cell suspension was being added to the serum preparation, or the control solutions, even cell distribution was ensured by magnetic stirring. In general, 1 ml cell suspension, containing approximately 60×10^4 cells, was added to 1 ml of the serum preparation, or control solution.

    The experimental cultures themselves were conducted in radiation sterilized reagent tubes, 100×13 mm with lids ("Nunclon"®, Algade 8, Roskilde, Danmark). Standard sterile cell culture technique with sterile disposible syringes and needles was used. All procedures involving cell nutrition and transfer were conducted in locked containers with front walls of glass. Glass ware, stoppers etc. were cleaned in "7 X" fluid (Gateway International 107, N. Virgil Ave. Los Angeles, California 90004/Bjellekjær Madsen, Vesterbrog. 69, København, Danmark). Utensils were autoclave, dry air, or radiation sterilized. (Radiation sterilization by Radest, Formervangen 16, 2600 Glostrup, Danmark).

    Cell growth took place at 37 °C in a thermostat with forced circulation. All changes of nutritional medium, and cell transfers, were conducted at room temperature.

    Generally, each *Experiment* comprised 5 test tubes for each of some 20–25 serum admixtures in addition to 10 control tubes. The arrangement of these tubes in the thermostat was randomized.

    Whenever the word "*Experiment*" is written with a capital *E*, such an experimental run is meant.

e. *Cell counts.* Bürger-Türck counting chambers were used. The cells were counted without staining or after addition of a few drops of Lissamine Green to each 2 ml. sample. The counted numbers of cells for each tube is denoted N, which lay between 0 and 200 (approximately). The total number of cells in each tube (2 ml suspension) is obtained by multiplying by 10^4.

(From subsequent studies (HILDEN, BIRGER JENSEN & RØNNIKE) it seems probable that approximately 1/10 of the variation between the reagent tubes with identical serum admixture (replicates) can be explained by count variation, except for very low N (less than 10), where counting variability is the major source of variation. This will affect the standard errors of the means (SEM) recorded in Figures 1, 2, 3, 4, 6, 8 and 9. This counting variation, taking into account other fluctuations inherent in the investigation, is of an acceptable order).

3. Results

a. *Control cultures.* The variation in numbers of cells in the control cultures after 24 hours growth is shown in Figs. 1 & 10. It is apparent that the initial number of cells was a significant factor (Fig. 10: positive regression between the initial number of cells and the number after 24 hours growth), but attempts to hold the control rates of growth constant by regulating the initial number of cells were not successful (Fig. 1). The marked variations depend, therefore, on factors which cannot be adequately controlled even by the present, highly standardized technique.

b. *Human serum concentration.* After addition of human serum to the cultures (which already contained embryonic bovine serum $10\ ^0/_0$ v/v), cell growth was invariably inhibited after a period of 24 hrs. (Fig. 2). It is seen that both a pooled serum preparation from 10 healthy donors and a serum preparation from one donor had marked inhibitory effect at $15–20\ ^0/_0$ serum dilution, while growth in a $10\ ^0/_0$ dilution was between $30–40\ ^0/_0$ of control culture growth. At higher dilutions the serum inhibitory action decreased rapidly. Slight differences in inhibitory effects could accordingly be best identified at about $10\ ^0/_0$ dilution. This level of dilution was therefore chosen as the standard for tests comparing the various serum categories. (In plant physiology studies of comparable principle, the optimal dilution was $1\ ^0/_0$ v/v).
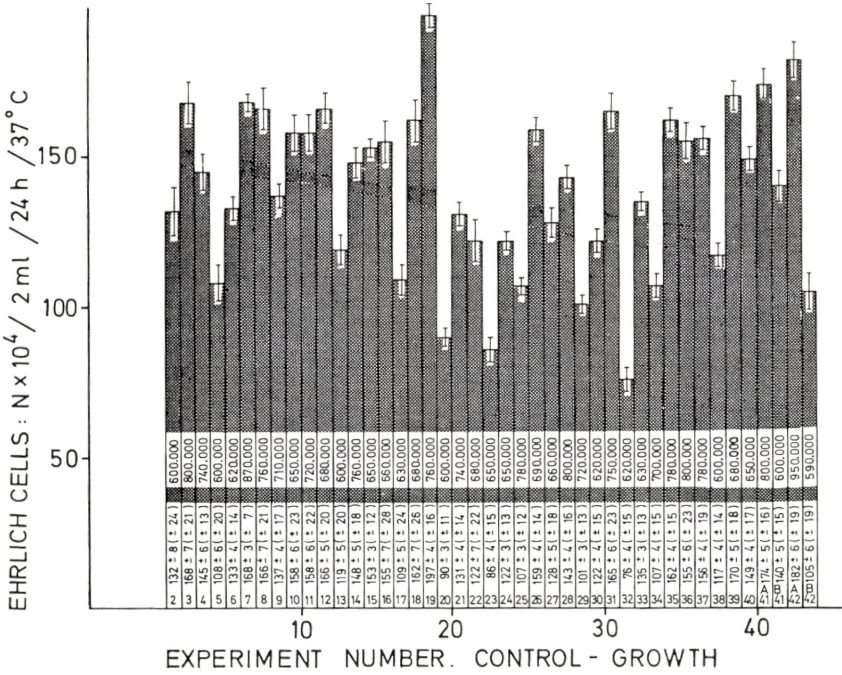
Fig. 1.

Control culture growth. March-August 1970.

Consecutive values of optimal growth in control cultures ("Fibiger 41 B" + foetal bovine serum 10 % v/v). At the column peaks SEM is shown.

Under the columns are shown: 1) Experiment numbers. 2) Number of cells in colonies $\pm$ SEM ($\pm$ SD between test tubes) $\times$ 10^4 after 24 hours ("Time 24"). 3) Number of cells at the beginning of the experiment "Time 0").

Ordinate: No. of Ehrlich cells/2 ml after 24 hrs. culture at 37 °C.

Conclusion: Wide variation of growth in control cultures during Experiment period.

c. *Serum from men compared with serum from women in the early puerperium.* It became early apparent that there was considerable overlapping variation in the inhibitory action of the 10 % serum dilutions from the different donor categories, as also observed in plant cell studies. Thus, to identify any differences in effect between serum from the different donor groups, it was necessary to study many members within the same group. Because of the limited number of hours in a working day p. p., several Experiments were often necessary to secure this. In order to combine figures from several Experiments the following formula was applied:
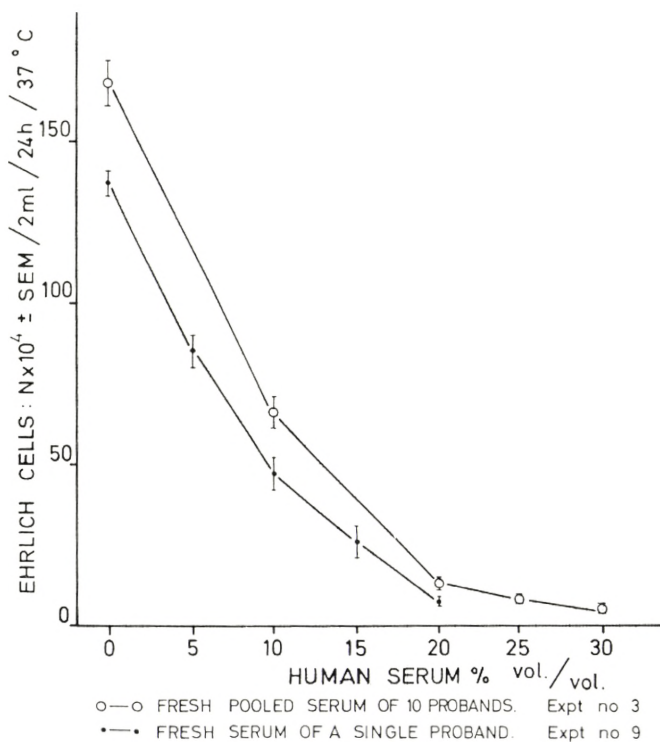
Fig. 2.

Growth in dilutions of human serum.

Abscissa: Concentration of human serum in the control solution ("Fibiger 41 B" + foetal bovine serum $10^0/_0$ v/v).

Ordinate: No. of Ehrlich cells/2 ml after 24 hrs. incubation at 37 °C. The vertical lines denote SEM.

Conclusion: $30–40^0/_0$ growth inhibition by $10^0/_0$ dilution of human serum.

$$t = \frac{\left[\left(\dfrac{(\Sigma r)_1}{M+1}\right)_\text{I} + \left(\dfrac{(\Sigma r)_1}{M+1}\right)_\text{II} + \cdots\right] - \left[\left(\dfrac{m_1}{2}\right)_\text{I} + \left(\dfrac{m_1}{2}\right)_\text{II} + \cdots\right]}{\sqrt{\dfrac{1}{12}\left[\left(\dfrac{m_1 \cdot m_2}{M+1}\right)_\text{I} + \left(\dfrac{m_1 \cdot m_2}{M+1}\right)_\text{II} + \cdots\right]}}$$

[D.F.: ∞]

in which Roman numerals identify the quantities relating to each individual Experiment. Within each individual Experiment, $(\Sigma r)_1$ denotes the Wilcoxon rank sum pertaining to the first proband group, while $m_1(m_2)$ is the number of probands in the first (the second) group; finally, $M = m_1 + m_2$.
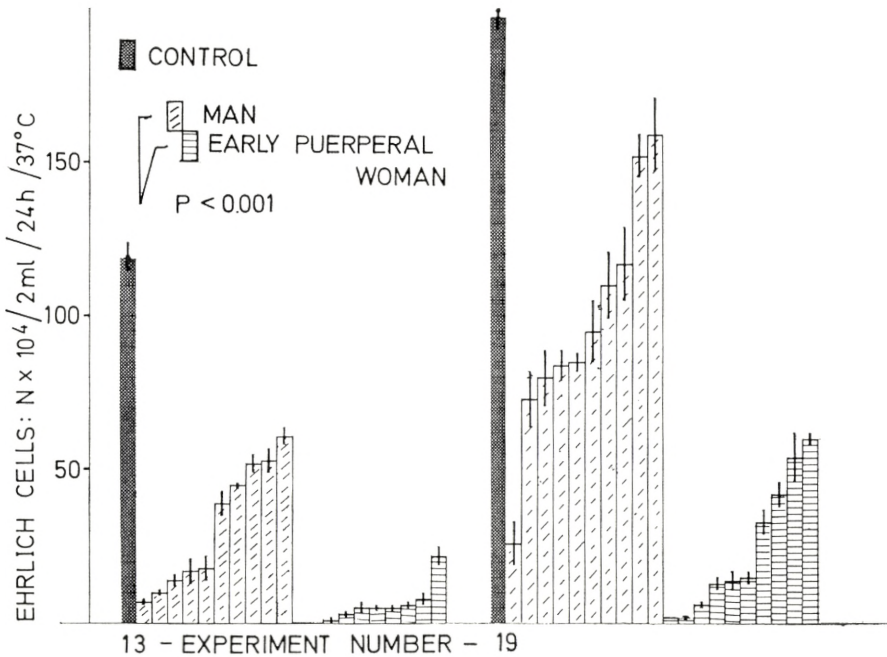
Fig. 3.

Cell growth inhibition by serum – from men, and from early puerperal women.

Human serum dilution: $10^0/_0$ v/v in the control medium ("FIB 41 B" + $10^0/_0$ foetal bovine serum).

Independent Experiments Nos. 13–19. For each is given: 1) Control growth ± SEM. 2) Growth in $10^0/_0$ dilution of human serum from each of 20 donors (10 men and 10 early puerperal women) ± SEM.

The probability of significant difference between the two categories (Experiment 13 and 19 combined) is shown on the graph.

Conclusion: A: Early puerperal serum markedly more inhibitory than male serum.

B: High growth in control cultures apparently paralleled by high growth in human serum cultures (Experiment 19 growth higher than Experiment 13 growth – Compare Fig. 4).

Fig. 3 shows that the difference in inhibitory effect between serum from men and serum from women in the early puerperium is established at the 0.1 $^0/_0$ significance level. Comparison of values found in Experiments 13 & 19 further shows that higher initial cell counts are correlated with greater growth in sera, whether this be slightly inhibiting male serum or strongly inhibiting early puerperal serum.

d. *Control growth – serum growth* (1). That higher control values were associated with higher growth in serum seems also to be apparent from the test described in section f (Fig. 6). Results
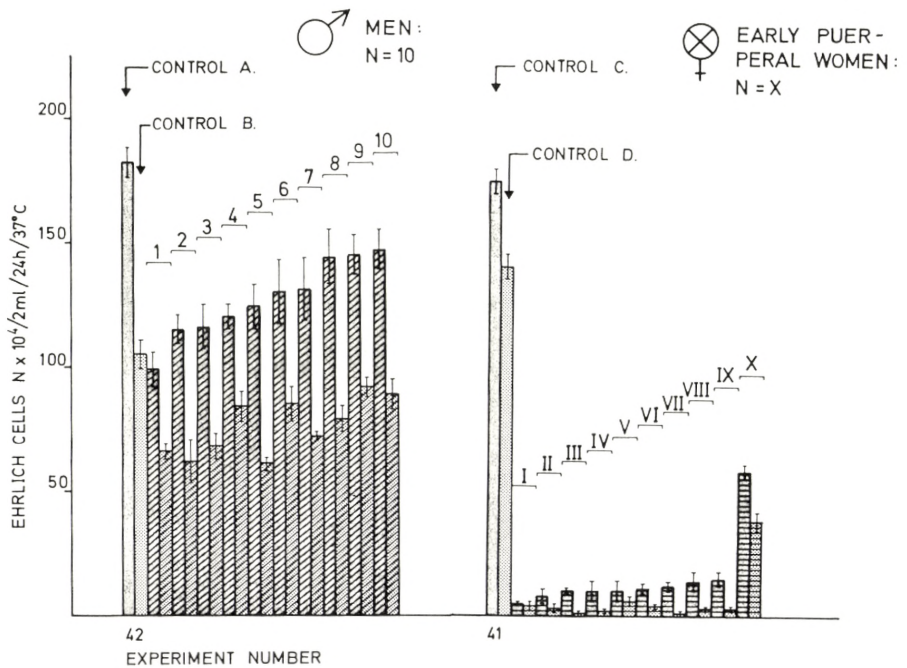
Fig. 4.

Relationship between control and human serum growth.

Duplicate tests of serum from 10 men (1-10) (Experiment 42) and serum from 10 puerperal women (I-X) (Experiment 41),– first test initial number of cells "large", second test initial number of cells "few".

When the initial number of cells was large, growth in both control and serum dilutions was higher than when the initial number of cells was few.

A + C: initial number of cells in control solutions: "large".

B + D: initial number of cells in control solutions: "few".

Conclusion: High control growth parallelled by higher growth in human serum.

of Experiments to confirm these observations appear in Fig. 4. Cells were added to serum preparations obtained from each of 10 male donors, and from each of 10 female donors in the early puerperium. A control test was also run using the control preparation. Growth after the addition of both many and few cells was studied. In all tests growth was higher after 24 hrs. when the initial number of cells added was large. This was particularly marked in the puerperal serum trial, where the increase was more than proportional to the control trial increase.

e. *Control growth – serum growth* (2). In the previous section d, the relationship between control and serum growth was analysed, and it was found that high control growth was associated
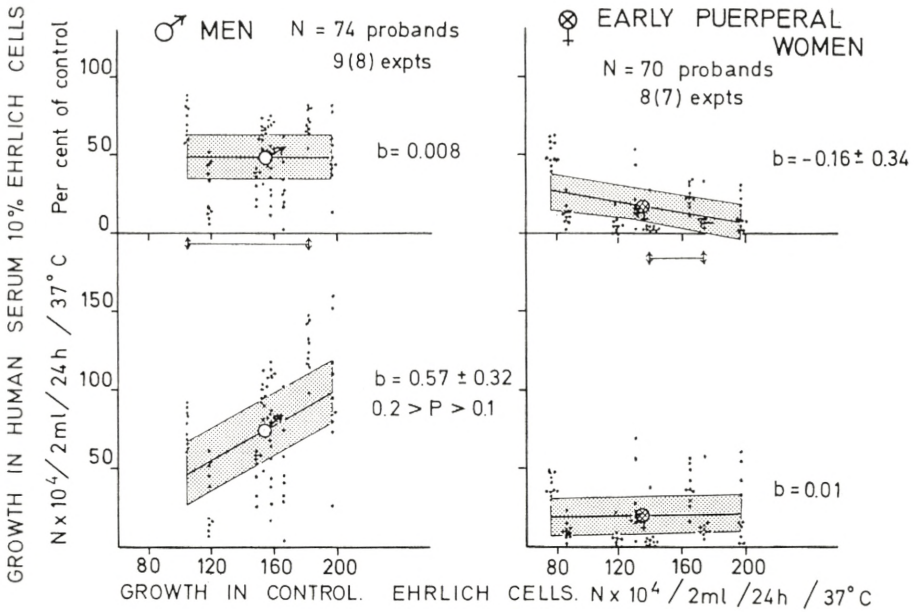
Fig. 5.

Relationship between growth in control medium and growth in male serum and early puerperal serum.

Two sets of male probands and two sets of early puerperal probands (marked "↕") are identical in each of two Experiments. Their Experiment results are also included in Fig. 4. None of the regression coefficients varies from 0 – i. e. no correlation is apparent between control and human serum growth (see Fig. 4, the analysis of variance in Table 1, and text).

Conclusion: The interexperimental variability of this figure is so large that the positive correlation between control and human serum growth demonstrated in Fig. 4 is not apparent.

with high serum growth. It may be noted that this finding depends on an experimental procedure which avoids interexperimental variability. Otherwise, even though the results of various tests are in fact consistent, this can be obscured. Fig. 5 records the results of cell growth: in serum from 74 men (9 tests): in serum from 70 early puerperal women (8 tests); and in the control preparation. In no instance was regression certainly positive. Accordingly, the analysis of variance (Table 1) indicates that the variation between Experiments around the regression line is too great to be attributed to the variability of the means of each individual Experiment ($P < 0.001$). Considerably more Experiments than recorded in Figure 5 (9 and 8 Experiments, resp.) would be necessary to display clearly the connection which is evidenced by the above-

TABLE 1. Analysis of variance. Growth in serum from men. Actual values from Fig. 5.

Type of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Variation explained by linear regression....................	1	21 539.6	21 539.6
Deviations of the nine experiments from their common regression line	7	540 473.3	72 210.5***
Between subjects, within experiments.................	75	46 663.6	622.2

$F_{(7,75)} = 116.1 \quad P < 0.001$
$F_{(1,7)} = 0.298 \quad P > 0.1$

explained experimental set-up (Fig 4), in which the trend is not obscured by inter-experimental variation.

It is this large interexperimental variability that necessitates the experimental procedure employed in the studies described in sections *c*, *f*, *g*, and *h*.

f. *Cell growth in human serum:* α) *Men's serum, women's serum – non-pregnant women, pregnant women, early puerperal women.* β) *Additional indicated studies. The influence of oral contraceptives. Serum from children under 2 yrs., and from post-menopausal women.*

α) Fig. 6 shows findings from comparison of successively more inhibitory serum categories. Thus the least inhibitory men's serum is compared with more active non-pregnant women's serum (Experiments 11 + 15, $P < 0.001$ by the formula of section c); non-pregnant women's serum is compared with early and late pregnant women's serum (Experiments 16, 26, 27, 28 combined: $P < 0.001$), and finally late pregnant women's serum is compared with early puerperal women's serum (Experiments 31 + 32: $P < 0.001$), which was the most actively inhibitory.

β) Many of the young non-pregnant women took oral contraceptives (50 %), but this was not apparently a factor in serum inhibitory activity (Fig. 6, Experiments 33 + 34). Serum from children under 2 yrs., and from post-menopausal women was also studied in a single pilot Experiment and was found to have
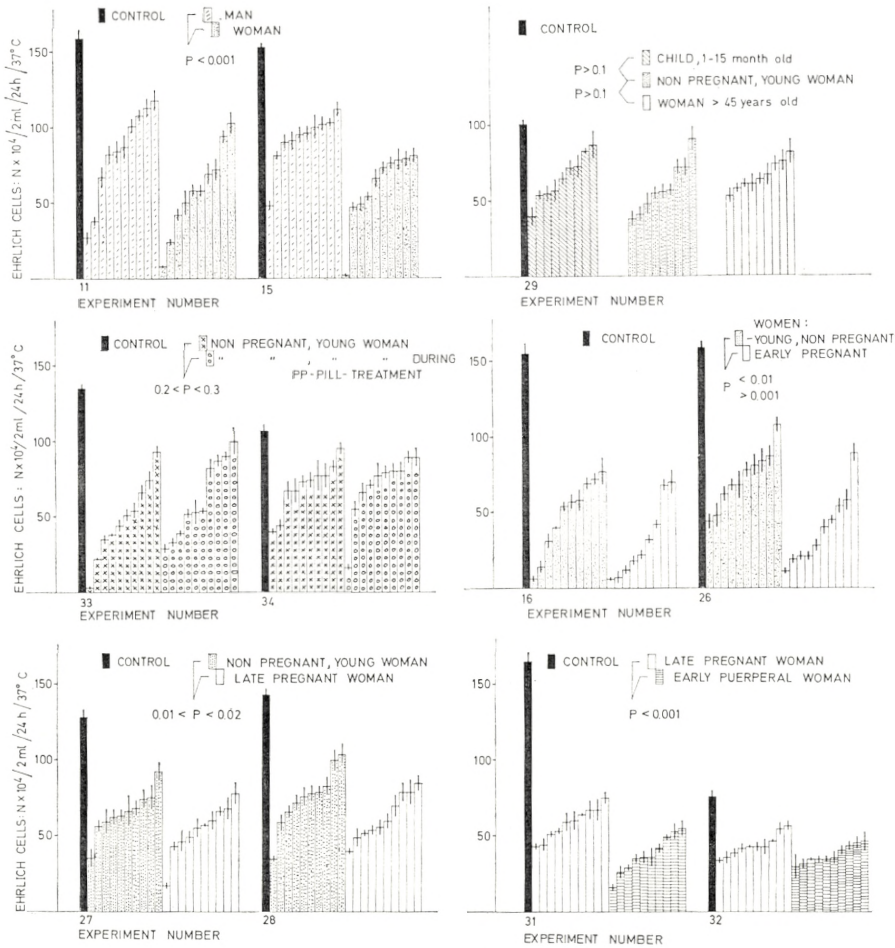
Fig. 6.

Cell growth in human serum of different categories.

Basic categories: 1) Men (Experiment 11 and 15), 2) healthy non-pregnant women (Experiments 11, 15, 16, 26, 27 and 28), 3) women – early (Experiments 16 and 26), and late (Experiments 27, 28, 31 and 32) pregnant, 4) early puerperal women Experiments 31 and 32).

Additional categories: 1) Serum from women taking oral contraceptives (Experiment 33 and 34), 2) Serum from children under 2 yrs. old and from post-menopausal women – compared with serum from non-pregnant young women (Experiment 29).

Serum from, in all, 226 individuals tested. Each column represents cell growth in freshly prepared $10^0/_0$ v/v human serum. Control cultures were run for each test. $\pm$ SEM are shown at the column peaks. Level of significance is shown over the columns. The diagrams are presented in order of increasing inhibitory activity of the serum categories.

Conclusion: From category 1 (men) to category 4 (puerperal women) there is progressively increasing serum inhibitory activity.

Oral contraceptives are without significance. Serum from children under 2 yrs. and from post-menopausal women, has activity similar to serum from young non-pregnant women (category 2).
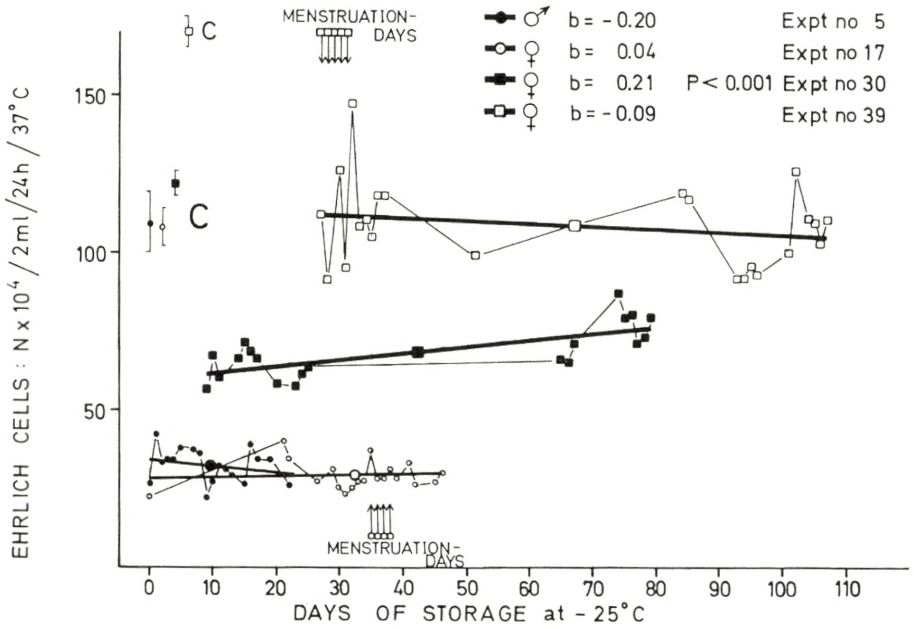
Fig. 7.

Storage of serum.

The influence of storage up to 108 days on serum from three women and one man. Four independent Experiments. The regression line for each proband is shown.

Conclusion: No relevant influence of storage at ≤ 20 °C on activity of serum from 4 probands. Two of the women menstruated during the periods of blood-sampling. No change in serum activity was evident.

inhibitory activity similar to that of the young non-pregnant womans category (Experiment 29).

g. *Storage of serum.* Fig. 7 gives the results of 4 experiments with sera from 4 different individuals, obtained by repeated blood-sampling and stored for up to 108 days. Only in one case was there difference in inhibitory capacity—Experiment no. 30, $b = 0.21 \times 10^4$ cells/day, $P < 0.001$, mild fall off of inhibition. The experimental design does not allow differentiation between an effect of storage, and an "in vivo" change in the properties of the individual's serum but the absence of a similar trend in the other 3 subjects speak in favour of an "in vivo" change.

Variation in results of Experiments with serum with weak inhibitory properties (Experiment 39) was greater than in serum with strong inhibitory properties (Experiments 5 + 17), but this difference in variation could be predicted from the curve in Fig. 2.
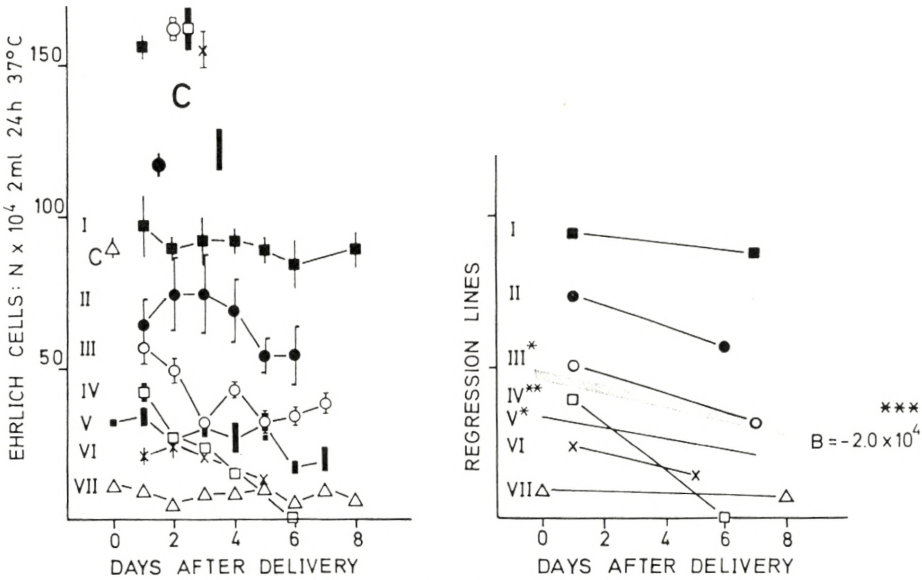
Fig. 8.

Inhibition of cell growth by serum from women in the first eight days of the puerperium.

Left of the Figure: Individual findings in each of 7 probands (I–VII) ± SEM with respective control values ± SEM.

Right of the Figure: Regression lines corresponding with graphs on left of Figure, and the combined regression line.

Conclusion: Inhibitory activity of serum increases in the first 8 post partum days.

Two donors had menstruation as marked in Fig. 7. No difference in serum activity was detected.

h. *Growth in early and late puerperal serum.* As recorded in Fig. 6 the serum with the greatest inhibitory effect was from women in the early puerperium, followed by serum from pregnant women. Figs. 8 & 9 demonstrate the increasing of the inhibitory activity from delivery over the first 8 days, and also the falling off of this activity during the first 3–4 post partum weeks. The graph on the left of Fig. 8 shows the individual growth values ± SEM for each of the seven women (I–VII) from delivery to the eighth day of the puerperium. Results of the controls run concurrently with each specific serum test are shown uppermost on the graph. On the right of Fig. 8 the regression lines corresponding to the curves on the left side are appended. Only three of the curves slope significantly (*: $P < 0.05$, **: $P < 0.01$) while the total regression line "summing" the 7 regression lines (the broad line
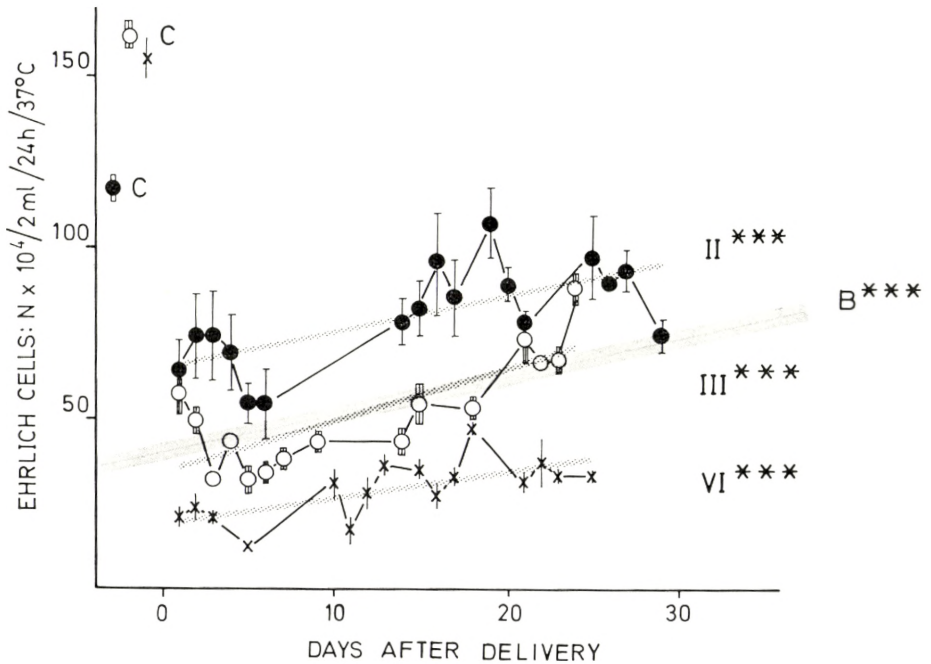
Fig. 9.

Inhibition of cell growth by serum taken during the first 4 weeks post partum.

Conclusion: Serum inhibitory activity, having increased during the first 8 post partum days (Fig. 8), reduced markedly 24–28 days post partum.

Three probands studied. Regression lines for each, and the combined regression line are shown.

on the Figure) has value $B = -2.0 \times 10^4$ cells/day ($P < 0.001$) indicating that serum's inhibitory activity increases during the first 8 days post partum in these 7 women. This statement can be extended to puerperal women in general ($P \leq 1/64$).

Figure 9 shows cell growth in serum from the first 29 post partum days. Results of three tests together with regression line for each and the shared regression line are recorded. Despite the fact that the data obtained from study of serum during the first 8 days is included, where fall in growth was noted, all lines demonstrate increase in growth (reduced inhibition) $P < 0.0005$, $B = 1.27 \times 10^4$ cells/day. Falling off of inhibitory serum activity is, therefore, marked from the 2nd to 4th post partum weeks in these 3 women, and fairly certainly in all women.

It is not possible to judge from the curves in Fig. 9 how long after pregnancy the activity of serum falls to the level found in the non-pregnant group.
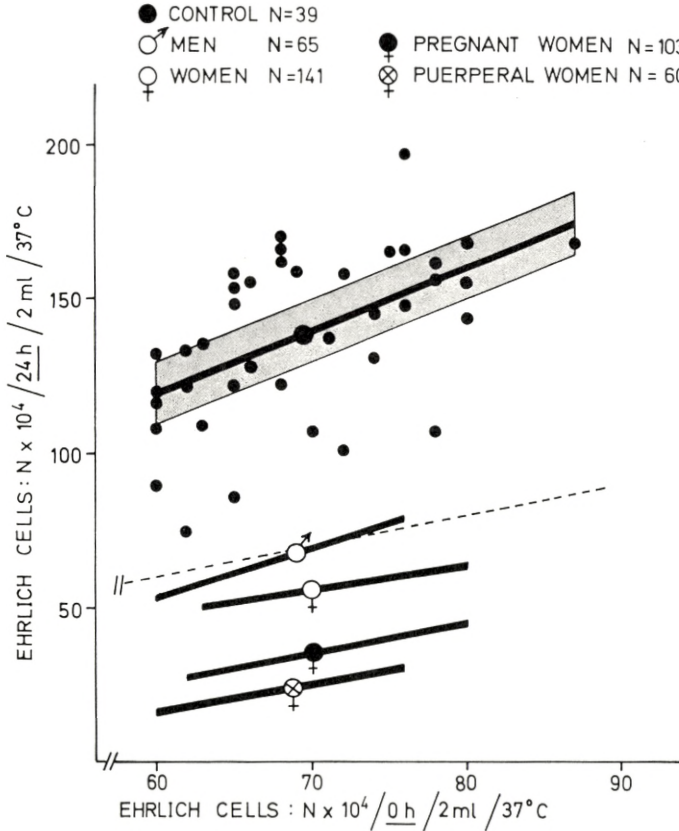
Fig. 10.

Cell counts at the beginning and completion of experiments.

Abscissa: Initial cell counts.

Ordinate: Cell counts after 24 hours growth in: 1) Control culture medium. 2) $10^0/_0$ v/v serum dilution from 65 men. 3) $10^0/_0$ v/v serum dilution from 141 non-pregnant women. 4) $10^0/_0$ v/v serum dilution from 103 pregnant women. 5) $10^0/_0$ v/v serum dilution from 60 puerperal women. 6) The dotted line in the middle of the graph denotes no net growth.

In the control study, individual Experiment values, the grand average, the regression line and the confidence interval for this line are shown. For the human serum Experiments the grand average and regression lines are given.

Conclusion: Number of cells in control colonies doubled after 24 hrs. growth. No net growth of cells in mens' serum dilution.

Net cell death in womens' serum dilution – most marked when serum was from women in the early puerperium.

i. *Cell counts – initial and final. α) Grand averages and regressions for all control and serum category studies.* Fig. 10 gives the initial (time 0), and final (time 24), cell counts in the control and category studies (the categories comprised, respectively, 65 healthy

men; 141 healthy women; 103 healthy pregnant women, and 60 healthy women in the early puerperium). The control values were also discussed in section a. The grand average of the 39 control values $\bar{y} = 139 \pm 4$ $(\pm 28) \times 10^4$, $s_{Y.X} = 22.2 \times 10^4$. The initial number of cells $\bar{x} = 70 \times 10^4$ (the factor 10^4 is omitted in the following equations).

For the 65 men: $\bar{y}_{total} = 68 \pm 4$ (± 35), $s_{Y.X} = 33.4$; for the 141 women $y_{total} = 56 \pm 2$ (± 25), $s_{Y.X} = 24.3$ $(P_{men-women}:$ $< 0.005, > 0.001)$; for the 103 pregnant women $\bar{y}_{total} = 37 \pm 2$ (± 24), $s_{Y.X} = 23.5$, and for the 60 early puerperal women $\bar{y}_{total} = 24 \pm 2$ (± 18), $s_{Y.X} = 17.1$. It can be seen that the large spread gives rise to considerable overlap between the different serum categories.

The regression line for the control values increases significantly, but none of the lines for the serum category values slopes significantly. As discussed in section e, the large interexperimental variation is again an important factor. Nevertheless the difference between the average in all 4 categories is clear. The graduated increase in serum's inhibitory effect from male to early puerperal women is again apparent from comparison of the grand averages for each serum category.

β) *Interpretation in terms of growth acceleration, growth inhibition and cell destruction.* The stippled line in the middle of the graph in Fig. 10 gives a base line of no growth after 24 hrs. Generally speaking, as previously mentioned, growth in the controls doubled the initial number of cells in the colony. The male serum line comes near to the stippled line suggesting total growth inhibition, whereas the lines of the other serum categories fall progressively more below the stippled line, suggesting not only inhibited growth but a net cell mortality.

4. Discussion

Earlier studies on the effect of serum from pregnant women on animal and human cell growth have been few.

MEYER, PENTTINEN & SAXÉN (1963) in Finland, and REJNEK, BEDNARIK, RERABKOVA & DOLEZAL (1963) in Czechoslovakia, found that fresh serum from pregnant women modified growth

of HeLa cell culture growth. The difference in technique make it difficult to relate the above authors' findings with those of this study. MEYER et al. found differences in activities of serum from early and late pregnancy, and also that "delivery" serum, 6 days post partum serum and complicated pregnancy serum, relatively often gave rise to a "clumping" phenomenon. Both the Finnish and Czechoslovakian studies lack probability calculations to support the presented data and conclusions. REJNEK et al. ascribe the effects of pregnancy serum to an abnormal alpha-lipoprotein.

In this study here presented, it is demonstrated that freshly prepared serum contains one or more substances which inhibit cell growth in cultures which are otherwise optimal. The serum categories tested—male, female, pregnant women's, early puerperal women's—evidenced increasing inhibition capacity in the foregoing order.

In the author's comparable plant cell studies (men's serum not tested), an opposite phenomenon was observed—i.e. that length of root cell growth was most inhibited by non-pregnant women's serum.

If plant cells from the above-earth part of a plant—e.g. koleoptile cells—are considered, observed effects are comparable with those in this Ehrlich cell study.

In further contrast, the effect of serum from young children (2/12—1 yr.) in plant studies was comparable with the effects of pregnancy and early puerperal serum, but in the Ehrlich cell study comparable with only non-pregnant women's serum (Fig. 6, Experiment 29).

This may well indicate that the active serum component(s) are not the same in the two studies.

Fig. 10 shows that men's serum holds cell cultures static (i.e. no net growth), whereas in increasing degree, there was a net mortality when women's, pregnant women's and puerperal women's sera were tested.

It could be that men's serum has a purely inhibitory effect, while women's is to degrees lytic, but the individual records rule out this possibility. On occasion, there was good cell growth in men's serum, but there was also net cell mortality in some of the individual Experiments (e.g. no. 13, Fig. 3), with the exception of one individual proband where there was no net growth. Basic

difference between men's and women's serum may therefore be quantitative and not qualitative.

Comparisons of repeated blood-samplings from the same proband over a period of time indicate that storage does not affect serum activity. Fig. 7 records the results of a study of 3 individuals in which no difference in serum inhibitory activity could be demonstrated after storage. In a fourth individual a slight falling off of serum inhibition was noted, but it would seem that storage of serum at < 20 °C has no effects on serum's inhibition of Ehrlich cell growth (see: PENTTINEN & SAXÉN 1957). Combined significance was not tested because the degree of inhibition, and times of storage were very different in each case. It is noted that in plant cell studies, the effects of serum storage were also negligible (RØNNIKE 1967).

Seven early puerperal probands were individually studied daily for 7 (8) days after delivery (Fig. 8). All the regression lines, shown on the right of the Figure, slope down. The combined regression coefficient $B = -2.0 \times 10^4$ cells/day is certainly negative.

One of the seven (no. IV, Fig. 8) was qualitatively completely different from the others. There was total cell lysis in her serum after 6 days. Unfortunately, it was not possible to continue the studies with her.

The falling off of serum's inhibitory effect from the 6–8th day to the 25–30th day of the puerperium (Fig. 9 – 3 studies) was apparent in the linear regression but there was no clear curving. At what point effects return to a non-pregnant niveau is, therefore, obscure. Fig. 10 shows the average difference between early puerperal and non-pregnancy serum to be about 30 ordinate units. Probably, therefore, the low early puerperal values, as represented by the regression coefficients, return to "normal" over 3–4 weeks i.e. (56 – 24): 1.27 days. In plant studies the comparable period was 3 weeks after delivery (RØNNIKE 1967).

In plant studies, early pregnancy and late pregnancy sera were found to have equivalent effects on root cell growth (RØNNIKE, not published). This was also apparent in the Ehrlich cell study. If there is, in fact, a difference, the tests used are not sufficiently sensitive to demonstrate it. Extended further studies were not made. Nevertheless, MEYER et al., as mentioned, record difference in the "clumping" activity of serum from early and late pregnancy. The

inhibitory activity of serum during menstruation was studied in only two probands (Fig. 9): one serum had constant marked inhibitory capacity, while the other was only mildly inhibitory. Menstruation produced no observable changes. If this proves to be a constant finding, it may be reasonable to assume that the steroid hormones are not involved in serum inhibition of Ehrlich cell growth.

While no definite conclusions can be drawn at present, current studies indicate that the active serum factor(s) is probably a protein. It is thermolabile, inactivated at 56 °C, and is not dialysable. Sephadex G-200 fractionation has shown that the biologically active proteins are among the first eluates, and therefore the molecular weights must be high.

This characteristic is the opposite of that found for serum and urine substances active in plant cell growth.

The puerperal serum factor inhibiting Ehrlich cell growth can be inactivated by repeated absorption with washed concentrated cell suspensions of the serum dilution, which may indicate that the inhibition is due to an antigen/antibody reaction.

Observation of comparable cell cultures in male serum, and in puerperal serum, has thown that proportionately more cells in the latter cultures take up Trypane Blue stain after one hours incubation at 37°C. This indicates a higher proportion of dead cells.

Biological testing of the serum protein fractions after separation on Sephadex and Sepharose columns are in progress.

Pilot experiments indicate, that some coagulation factors in the blood may be significant.

Acknowledgments

6. Summary

A. Freshly prepared human serum inhibits growth of Ehrlich ascites tumour cells in otherwise favorably nutrient suspension cultures.

B. Serum from men is less active than serum from women. Serum from non-pregnant women is less active than serum from pregnant women, which is, in turn, less active than serum from women in the early puerperium.

C. Three weeks into puerperium inhibitory activity of serum is declined.

D. Storage of serum at $\leqq 20\ ^{\circ}\text{C}$ does not affect inhibitory properties.

E. Influence of pregnancy serum on Ehrlich cell growth and plant cell growth, is compared.

F. Earlier studies of possible effects of pregnancy serum on cell growth (MEYER, PENTTINEN & SAXÉN, 1963; REJNEK, BEDNARIK, RERÁBKOVÁ & DOLEZAL, 1963) are compared with this study.

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