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ON THE TRANSMISSION OF SPIROPTERA NEOPLASTICA (GONGYLONEMA N.) TO THE RAT AS A METHOD OF PRODUCING CANCER EXPERIMENTALLY

BY

JOHANNES FIBIGER

WITH ONE PLATE

KØBENHAVN
HOVEDKOMMISSIONÆR: ANDR. FRED. HØST & SØN, KGL. HOF-BOGHANDEL
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(From the Anatomo-Pathological Institute of the University of Copenhagen).

That our knowledge of the etiology and pathogenesis of cancer is still but very limited, may essentially be referred to the fact that any attempt to draw this chapter of tumor's pathology within the reach of experimental investigation has hitherto failed, whereas experiments have been employed with perfect success on other oncological domains.

As generally known it was through the investigations of BORREL, LOEB and C. O. JENSEN — above all the epoch-making communications from the pen of JENSEN (1902—1903) — that the modern era of experimental cancer research was initiated and numerous pathologists (APOLANT, BASHFORD, EHRLICH, HAALAND, LEWIN, MURRAY, TYZZER, a. o.) impelled to enter upon transplantations of spontaneous tumors in mice and rats.

The doubt soon afterwards put forward whether or not a comparison of tumors in these animals with those in man be lawful, has been silenced long ago. It is now generally agreed that malignant growths in mice and rats harmonize so perfectly with those in man that conclusions of analogy are quite permissible within wide ranges.

The enormous work devoted to the study of transplantable tumors in recent years has given results of great and lasting value. The biological deviations of the tumor cells from those of the normal tissue have been thoroughly studied; their power of infinitely continued propagation through — so to speak — innumerous generations of cells has been evidenced by means of transplantations carried out for years on extensive series of experimental animals. Important information hitherto not approachable has been acquired, e. g. regarding the vitality of the tumor cells and their resistance to chemical and physical agents, the genesis of the stroma, the changes and growth of the tumor tissue, the metabolism of the tumor-bearing animals, and so on.

The utmost interest further attaches to the fact that differences have been proved in the ability — not only of the species and the races, but also of the individual animals — of serving as a favourable soil for the growth of transplanted cells, — and that this predisposition of the organism can be made subject to changes by experiments.

Finally, these investigations, and the transplantations on the whole, have created a base for therapeutic experiments which although not yet coming up to the expectations originally formed of them have revealed unknown possibilities as to the treatment of malignant growth.

Thus new territory has been gained on various domains of oncology by means of transplantations, — but the fundamental question of the etiology and pathogenesis of cancer has not yet essentially approached solution.

Let it be remembered that transplantation — as often emphasized — only permits the study of the continued propagation of a fully developed spontaneous tumor. Transplantation of tumor tissue has justly been termed an artificial metastasis formation, differing from the natural in so far only that it does not occur in the spontaneously attacked individual which bears the primary tumor, but in another animal, to which portions of the mother tumor have been transferred experimentally. By transplantations fully developed tumor tissue is inoculated, but development of tumor cells from normal tissue is not provoked.

Thus, strictly speaking, the results of the transplantations can only be utilized by the study of the continued growth and the metastasis formation of the fully developed tumor tissue. To make them apply — without further circumstances — to the spontaneously developed real primary tumor would a priori scarcely be reasonable. More likely might diverging conditions be expected. The existence of such differences and their great importance already appears from the fact, that in the laboratories of the Imperial Cancer Research Fund it has proved impossible (BASHFORD 1913)[1] to prevent development of spontaneous growths by methods immunizing against transplanted tumors — even in the same animals.

Thus, not only must the inquiry of the morphological and causal origin of cancer, but also the study of other problems, be based upon investigations which cannot be executed along sufficiently rational lines until the producing of primary cancer at will has come within the reach of our endeavours. To gain this long sought-for goal is thus the chief object of experimental cancer research, as it has been pointed out by several authors in the discussion of the value of transplantations, at first and most stringently perhaps by ORTH.[2]

As a contribution to the solution of this problem the ensuing investigations will be recorded.

In previous papers[3] I have described the development of inflammatory processes and papillomatous growth — combined in some cases with real carcinomatous transformation and metastasis formation — in black and white laboratory rats infected with a round worm which lives in the pavement epithelium of the fundus of the stomach. The develop-

[1] Berliner klin. Wochenschrift. 1913. No. 2.

[2] Berliner klin. Wochenschrift No. 11 and 12. 1905. Sitzungsberichte der Kgl. preussischen Akademie der Wissenschaften. 1909.

[3] Académie Royale des Sciences et des Lettres de Danemark. Extrait du Bulletin de l'année 1913 No. 1. Hospitalstidende 1913 and 1914. Zeitschrift f. Krebsforschung 1913 and 1914.

ing of the parasite from egg to larva takes place in hosts, viz. cockroaches (*Periplaneta americana, Periplaneta orientalis, Phyllodromia germanica*). This nematode had been defined by mag. scient. DITLEVSEN as a new species belonging to the group of the Spiropterae, and in an ensuing detailed zoological description[1] the name of *Spiroptera neoplastica* or *Gongylonema neoplasticum* was proposed. Later on the American zoologist RANSOM[2] has asserted that, no doubt, the parasite in question must be referred to the genus of *Gongylonema*, the name of *Gongylonema neoplasticum* being, thus, preferable, a proposition to which mag. scient. DITLEVSEN is inclined to subscribe.[3]

As mentioned in previous papers I have succeeded in finding the nematode firstly in wild Norway rats (*M. decumanus*) and in cockroaches captured in some sugar refineries in Copenhagen, and secondly in wild house rats (*M. rattus*) and in cockroaches (*P. americana*) caught in St. Croix. In rats originating from other localities it has not been possible to find the nematode although my investigations carried out for that special purpose altogether comprise more than 1800 rats (about 1300 Danish wild rats (*M. decumanus* and *M. rattus*), nearly 500 black and white rats from Danish laboratories and from dealers in England, Holland and Germany, and, furthermore, some specimens of *M. rattus alexandrinus*. Nor did I ever succeed in finding the nematode in other wild living cockroaches in this country than in the *P. americana* captured in the said sugar refineries. These refineries having formerly received raw products from the Danish West Indies where — according to my investigations — the nematode seems to be of frequent occurrence, I have set forth the assumption that the nematode has originally

[1] Mindeskrift for Japetus Steenstrup. 1914.

[2] The Life History of Gongylonema scutatum. Journal of Parasitology. Decbr. 1915.

[3] Ueber *Gongylonema neoplasticum* (*Spiroptera* [*Gongylonema*] *neoplastica*) Fibiger Ditlevsen 1914. Centralbl. f. Bakt. 81. Bd. 1918.

been imported to Denmark from the Danish West Indies, and, thus, is actually a tropical species.

This supposition has gained corroboration by extensive investigations carried out by W. F. WASSINK[1] in the Cancer Research Institute of the Netherlands (ANTONI VAN LEEUWENHOEK HUIS) in Amsterdam.

On examining more than 1000 wild rats (virtually all *M. decumanus*) from Amsterdam he did not succeeed in finding the *Spiroptera neoplastica* in any of them. Among 625 rats (*M. rattus*) caught in ships, 5 were found to harbour the parasite in their stomach, and these 5 rats came from ships in South American service. Finally, the nematode was found in 16 out of 40 rats (*M. decumanus* and *rattus*), captured in Surinam. In 2 of these rats the fundus of the stomach, moreover, contained large papillomes.

On examining great numbers of cockroaches (*P. americana*, *P. orientalis*, *Phyllodromia germanica*) from Amsterdam, WASSINK only found the parasite in individuals (*P. americana* and *P. orientalis*) originating from a sugar refinery which had formerly received sugar from The West Indies. In this refinery which housed no rats, the parasite appeared to live in mice.[2] Furthermore the nematode has been found in cockroaches (*P. americana*) caught in Paramaribo. On the other hand, rats and cockroaches from ships in East Indian service were not infected.

Thus, the results of these investigations are in perfect keeping with those of my inquiries, and it hardly admits of doubt now, that *Spiroptera neoplastica* (*Gongylonema neoplasticum*) is indigenous to the tropical countries (the West Indies, South America) from which, living as parasites in the cockroaches (*P. americana*) and rats, they are imported

[1] Nederl. Tijdsschrift voor Geneeskunde 1916 No. 13.

[2] Through the courtesy of Prof. DE VRIES, director of the Cancer Institute of the Netherlands, and of Dr. WASSINK I have had the opportunity of examining preparations of the Spiropteræ observed.

to Europe and will here be found in such localities (sugar refineries) as offer to the host animals favourable conditions of life.

In my earliest experiments,[1] published in 1913, carcinoma had only been produced in 4 rats, but an ensuing paper[2] (1914) brought the report of 15 new observations of carcinomatous growth of the same type, produced in the same way also in black and white laboratory rats. Thus, Spiroptera cancer had altogether been produced experimentally in 19 cases. In my experiments of 1914 comprising 91 rats, pronounced inflammatory changes and papillary transformation of the fundus of the stomach were observed in 33 animals, in 12 of these associated with carcinoma.

Thus, the number of observations was great enough to permit the assumption that development of cancer in rats infected with the Spiroptera, far from being a rare phenomenon, on the contrary, was of so frequent occurrence that the transmission of the Spiroptera might perhaps turn out to be used as a generally suitable method of producing cancer experimentally. This assumption was still further confirmed in continued experiments. In 1916 Spiroptera cancer had thus been produced in 42 rats altogether.[3]

As a matter of course, a practical method of producing cancer must needs give positive results in a great number of the experimental animals and within a reasonable space of time, otherwise it can be of no use in experimental studies of the setting in, the development, conditions of growth, etc. of the carcinoma. If by the procedure employed, cancer develops but now and then, and only after the lapse of a considerable space of time, the suitability of the method will be strongly limited or precluded.

[1] loc. cit.
[2] loc. cit.
[3] 16. Skandinaviske Naturforskermøte i Kristiania 10.—14. Juli 1916.

In order to examine the suitability of the infection with the Spiroptera it was thus necessary to put the method to a thorough test in a series of experiments. It would thus be possible to obtain a reliable estimation of its effective power and perhaps a standard too.

It was my hope to be able to base such a test upon examinations carried out in foreign institutes, to which cockroaches, infected with the Spiroptera, had been sent, but greater series of experiments, as far as I know, have not been executed abroad, a fact which may most naturally be ascribed to the war.

In the Anatomo-Pathological Institute of the University of Copenhagen a considerable number of new experiments have been performed since the publication of my first papers. In the first investigations various circumstances — the non-occurrence of carcinomatous growth in several Norway rats and white mice, infected with the nematode, had been suggestive of the fact that the transmission of Spiropteræ might appear to cause carcinomatous growth with different frequency in the different species and races of rats or related rodents. In my last experiments, therefore, not only black and white laboratory rats of different origin, but also Norway rats (*Mus decumanus*) and wild house rats (*Mus rattus*), bastards of Norway rats and black and white rats, and a few Alexandrine rats (*M. alexandrinus*) have been employed, as well as white laboratory mice, wild house mice (*Mus musculus*), forest mice (*M. sylvaticus*) aud finally a few specimens of *Hypudæus glareola* and Japanese waltzing mice.

Spiroptera neoplastica has now been bred in this Institute for about 7 years. Not only the large light brown cockroach (*Periplaneta americana*), but also the more common black brown species (*P. orientalis*) has been used as host to the parasite, as at that time *P. americana* was too difficult to procure, and at present may perhaps be quite extinct in this country since our sugar refineries have been forced to reduce or even to suspend their work during the war. For the last eighteen months we have succeeded in establishing a regular breeding of *P. americana* without difficulty (s. p. 27—28) and in the last experiments, therefore, only this species has been used. The Spiroptera is transmitted very easily to all rodents above mentioned, and in all of them lives in the fundus of the stomach, in the gullet, and occasionally also in the epithelium of the tongue and of the mouth.

Furthermore the Spiroptera can be transmitted to rabbits, guineapigs, squirrels (*Scirrhus vulgaris*), and hedgehogs (*Erinaceus europæus*), living in these animals in the upper parts of the alimentary canal covered with squamous celled epithelium.

But the Spiroptera is not only able to live in the alimentary canal. I have succeeded in transmitting it to the mucous membrane of vagina in rats, where it seems to live just as well, in so far as it reaches full development and sexual maturity also in this locality. The mucous membrane of vagina becomes the seat of desquamatous inflammatory processes with pronounced epithelial hyperplasia. Papillomes or carcinomatous growth, however, has not been observed, but only a few experiments have hitherto been performed.

According to preliminary investigations the power of the Spiroptera to influence the stomach in rats seems to be the same, no matter whether the rats are infected with nematodes which have lived as parasites in rats or in mice. In 3 black and white rats infected with cockroaches which were merely fed on excrements of spiroptera-infected mice (white laboratory mice, forest mice (*M. sylvaticus*), development of cancer has also been produced in the fundus of the stomach.

More than 900 experimental animals have been used for the entire series of experiments, but only a minor part of this great material — the investigations on the black and white laboratory rats and white laboratory mice — has as yet so far undergone final revision that a view of the chief results may be obtained.

In the ensuing pages a report of these results will be given. It is my hope, later on, to add the statement of the results of the entire series of my experiments.

For the investigations in question only white and black or white (not albinotical) laboratory rats were employed, belonging to a stock which for years had been bred in this institute. The exact age of each rat cannot be stated. Old animals, however, were only exceptionally used; as a rule, the experimental rats were adult younger individuals, whose age on transmission of the nematode must be estimated at about 4—6 months or somewhat more. The weight of the rats at the transmission was in most cases about 90—150 gr., some few rats weighing less, a considerable number more (150—200 gr.); in a minor number of elder animals the weight amounted to 210—270 gr. In some of the investigations the Spiropteræ, as in earlier experiments, were transmitted to the rat by simply feeding the latter on cockroaches infected with the nematodes. In most of my experiments, however, another manner of proceeding has been practised, the rats being fed on larvæ which under the microscope were liberated from the muscles of the prothorax and the limbs of the cockroaches; the larvæ were deposited in a 0,9 % normal saline solution and transferred to the rats either on a piece of white bread, or placed directly on the tongue, or finally, as was most frequently the case, injected into the stomach, a caoutchouc catheter being conveniently used as a stomach pump. As a rule, 100 larvæ[1] were transmitted at a time in 2,3 or several injections; the greatest number of injected larvæ was 600—800,[1] but this only took place in a small number of experiments.

Injection of free larvæ was originally employed for two reasons: Firstly, in order to secure that approximately the same number of nematodes was transmitted to the rats within each series of experiments, more homogeneous conditions being thus obtained. Secondly, in order to prevent the inconvenience of some rats being only infected with a minimal number of larvæ or not infected at all. To ascribe slight or missing infection to the feeding on cockroaches used in earlier experiments appeared to be the more reasonable as the contents of larvæ in the muscles of the cockroaches turned out to vary exceedingly, and sometimes to be very small even in individuals which for a considerable and equally long time have been fed on rat's excrements containing Spiroptera eggs.

Out of a greater series of experiments some examples may be briefly noted: in 4 cockroaches, equally fed for 85 days, the muscles of prothorax and femora contained respectively 24, 58, 63 and 107 larvæ, while at the same time 4 cockroaches of another experiment of the same duration were found to contain resp. 195, 241, 306 and 324 larvæ; furthermore, in the muscles of the limbs of 5 cockroaches in one experiment resp. 3, 11, 43, 49 and 131 larvæ were found, and in another after feeding for the same space of time 7, 15, 25, 239 and 506 larvæ. If as feeding material cockroaches are employed whose muscles contain approximately as great a number of larvæ as was the case in some of the experiments just quoted, the infection of the rat's stomach with a very considerable number of larvæ might, a priori, seem to be unquestionable. It must, however, be remembered that the larvæ coiled-up in the muscles of the cockroaches — even if presenting no conspicuous divergences in morphological respect, can by no means all of them be considered fully developed and capable of invading the stomach of the rat. This, of course, is due to the fact that they have entered the muscles of the cockroaches not coincidently but gradually during the feeding of the latter on rat's excrements containing eggs. The capacity of all the larvæ to invade the epithelium of the rat's alimentary canal cannot be taken for absolutely granted unless the cockroaches are used as feeding material only after so long periods, that all larvæ incorporated in their muscles have reached the stage of full development and are encysted. This, however, is difficult to carry through as cockroaches, in order to be strongly infected with Spiropteræ, must be fed on rat's

[1] As a matter of fact, the figures 100—800 do not in all cases mean exactly the cited figures, but only an approximate number of larvæ, as a few larvæ may be destroyed during the preparation (see pag. 30).

excrements for so long a period that a great number of them will die, especially when *P. orientalis* is used. This species has proved more liable to succumb to a strong infection with Spiropteræ than *P. americana.* In the experiments concerned, the cockroaches, as a rule, were used 85—90 days after the beginning of the feeding on rat's excrements.

The uncertainty of the results obtained by feeding the rats on cockroaches is further increased by the fact that several of the larvæ inclosed in the muscles will be injured or even killed when the cockroaches are bitten to pieces and chewed by the rats.

Finally, the fact that not all larvæ which have entered the fundus of the stomach remain and undergo further development there, increases the uncertainty. If cockroaches infected with Spiropteræ are used as feeding material, only a very small number of the nematodes will often be found in the fundus some little time after the act of feeding, and, after feeding on fixed numbers of liberated fully developed larvæ, it can be directly ascertained that the fundus only contains a varying larger or smaller fractional part of the transmitted number. Also the gullet, pharynx, tongue and parts of the mouth may contain Spiropteræ, the majority, however, cannot be traced, having only for a shorter space of time, if at all, visited the epithelium of the fundus. This disadvantage may occur no matter whether larvæ be used, placed on a piece of white bread, fixed directly on the tongue or injected into the stomach through a catheter.

As examples be it noted that in rats having obtained free Spiroptera larvæ by injection into the stomach or by application in the mouth, the fundus was found to contain:

In 1 rat	after 1 day	8	out of	c. 100	transmitted	larvæ.
- 4 rats	— 2 days	resp. 9, 11, 11 and 14	- -	—	—	—
- 1 rat	— 3 —	21	- -	—	—	—
- 1 rat	— 5 —	9	- -	—	—	—
- 1 rat	— 12 —	21	- -	c. 200	—	—
- 1 rat	— 13 —	36	- -	c. 750	—	—
- 1 rat	— 14 —	40	- -	c. 650	—	—
- 1 rat	— 15 —	c. 70	- -	c. 500	—	—

The examples quoted also make evident that by transmission of greater numbers of larvæ a considerable but by no means proportionally larger number will remain in the fundus.

That only a minority of the larvæ transmitted may be found in the fundus, is, no doubt, due to various factors. Invasion of a relatively small number might perhaps be referred to the fact that the fundus by more or less pronounced contractions or otherwise reacts differently on the invasion of the parasites. Another possibility cannot be precluded, that the ability of the larvæ to invade the epithelium may vary even if only encysted and apparently fully developed specimens are chosen at the preparation.

The content of Spiropteræ of the fundus, however, is further diminished in some rats, owing to the fact that a smaller or greater number will leave the epithelium a shorter or longer time after invasion. The evidence of such an after-emigration has been pointed out in previous papers, and the part performed by it will be dealt with in an ensuing communication

Thus, the employment of liberated larvæ for the infection of the rats, has not quite come up to expectations. As seen from the table, this procedure, to be sure, renders it possible to infect the rats with numerous Spiropteræ; a uniform infection of all the animals of an experiment is, however, not approachable. Whether this method be preferable to the less circumstantial feeding on strongly infected cockroaches, seems to me the more doubtful as both methods easily give rise to severe Spiroptera inflammation in the fundus, and it will appear from the following pages that the new experiments in regard to development of cancer have given about the same result, no matter how the transmission took place. My chief reason for using to such an extent the feeding on larvæ in these experiments was, however, that this procedure more easily permits the invasion of numerous Spiropteræ into the epithelium of the tongue, which among other things has just been my object in a number of the experiments.

When, as a rule, no single transmission of numerous larvæ but several transmissions with intervals of some days or 1—1½ weeks were employed, it was partly in order to furnish greater security with regard to a considerable invasion, partly because the rats too often were found to succumb to a sudden transmission of great numbers of larvæ which might give rise to severe inflammatory, sometimes hemorrhagical, processes in the fundus of the stomach and often bring about death.

The rats infected with the Spiroptera were kept isolated and fed entirely on white bread. In order to grant the carcinomatous growth as long as possible to develop, the rats, as a rule, were not killed before getting into agony. A number of rats, however, exhibiting no pronounced symptoms of disease was put to death.

The macroscopical changes of the fundus of the stomach did not differ essentially from those previously described,[1] the chief phenomenon being thickening of the stomach wall, hyperplasia and desquamation of the epithelium.

[1] l. c. p. 5.

Furthermore, papillary wallshaped, and tuberous tumor-like excrescences were observed in numerous cases, often diminishing or even obliterating the cavity of the stomach. Also the gullet was the seat of thickening of the wall and epithelial hyperplasia, and in a considerable number of rats, infected mostly by transmission of free Spiroptera larvæ, corresponding changes were noticed in the mucous membrane and epithelium of the tongue and pharynx. These changes being thoroughly dealt with in an ensuing paper, will be subjected to no particular inquiry here.

The microscopical examination was carried out in serial sections. Having previously experienced that Spiroptera cancer in the fundus of the stomach may often develop in areas of too slight an extension to permit macroscopical identification, I had no alternative but to examine the fundus in toto, viz: to cut it completely in serial sections. In no other way would it be possible to trace out all, even the very smallest, tumors. To extend this procedure to the whole amount of rats would, however, be a work hardly practicable and, in fact, of no real use, as a considerable number of the animals died so soon after the transmission that the chance of their being carcinomatous was but very small.

Where to draw the line correctly, could not, of course, be decided in advance. In previous experiments carcinoma had developed 66 days[1] after transmission of the Spiroptera. In the course of these experiments carcinomatous growth was observed in some rats which died already 45—50 days after the transmission. The possibility of cancer developing still quicker could not, however, be precluded. I have, therefore, preferred to examine in toto the fundus of the stomach in all rats — a small number excepted — which lived 30 days or more after the transmission of the Spiroptera, or

[1] Hospitalstidende and Zeitschrift f. Krebsforschung 1914.

— if more than one transmission was used — after the first of these. Besides all portions of the fundus, the immediately adjacent parts of the gullet and of the glandular pars pylorica were cut in serial sections (at 10 μ), every sixth of which — in several cases every fifth or third — were subjected to microscopical examination. In a number of cases every second or every section of some parts was examined. Also the tongue, the pillars of fauces, and parts of the mucous membrane of the mouth were examined in serial sections.

The histological changes too, in all essentials, appeared to be identical with those originally described,[1] the chief phenomenon being inflammatory processes, hyperplasia, down growth of the epithelium and development of cancer. The degree of the changes varied most conspicuously, ranging — as far as the inflammation is concerned — from superficial, slight or even doubtful to strongly pronounced, deep, sometimes ulcerous acute or chronical processes, — and for the part of the epithelial proliferation — from slight thickening and hyperkeratosis to pronounced, often enormous hyperplasia with desquamation and heterotopical deep downgrowth into the connective tissue of mucosa and submucosa. In 2—3 cases besides typical keratinizing carcinoma of the common type an enormous development of papillary excrescences was noticed, containing numerous closely set poches and crypts lined with slightly horny or not horny squamous epithelium, and exhibiting a structure similar to that of the papillomes met with in the urinary bladder of man. Heterotopical infiltrative growth of this tissue was never observed.

Thus, I have only felt entitled to fix the diagnosis of carcinoma in such cases as exhibited — through a series of sections — the following changes:

1) heterotopical downgrowth of epithelial cells belonging not only to the normal type of the basal epithelial layers

[1] l. c. p. 5.

but mixed up with atypical and keratinized cells in abundance partly arranged as spherical masses and horny globes.

2) infiltrative growth of these heterotopical and atypical epithelial cells into deeper layers, splitting up invasively the elements of the connective tissue of mucosa and the muscle cells of muscularis mucosæ, forming isles and spurs in the latter or — as most frequently seen — penetrating through this membrane into the superficial or deeper layers of submucosa.

Heterotopical, non-invasive downgrowth of solid epithelial projections and columns without atypical structures was in no case diagnosticated as carcinoma.

In other words, the demands I have made on the diagnosis of carcinoma have been rather severe, more severe perhaps than has sometimes been the case in investigations on the early stages of carcinomatous growth. This reservation, of course, has permitted only such changes to be diagnosticated as carcinoma, as disclose the absolutely characteristical carcinomatous structure. I have not tried to trace out the preceeding, very earliest stages of development, in which the morphological character has not yet come out strongly. To characterize these stages and to prove whether they have any pronounced morphological distinctive feature at all, is a problem worthy of further special examination.

The experimental material originally comprised 214 rats, out of which 11 died immediately after the transmission of the Spiroptera or were not infected at all. 69 did not survive the transmission for more than at most 29 days. Out of the remaining 134, 18 were subjected to no sufficient microscopical examination or to none at all, the fundus of their stomach exhibiting but slight or practically no macroscopical changes. At this stage of the experiments, I had no clear understanding that carcinomatous growth —

as later investigations have shown — may also occur in stomachs with extremely slight or doubtful macroscopical changes.

Thus, in the table below will be found the remaining 116 rats, whose fundus was subjected to a thorough microscopical examination in serial sections, comprising all the fundus of the stomach in 107 rats. In 8 carcinomatous rats only a few greater areas — in which extensive development of cancer was found — were examined, in one rat only one half of the fundus, the other half being used for a feeding experiment; carcinomatous growth was not met with in this rat.

18 out of the 116 rats have been splenectomized[1] anterior to the transmission of the Spiroptera, and survived this for at least 45 days. Carcinomatous growth developing only in 5 out of these 18 rats, whereas, on the other hand, 13 out of 19 controls infected simultaneously and with the same Spiropteræ were found to be carcinomatous, I have admitted these splenectomized rats to the table, acknowledging that the result of these experiments did not suggest that extirpation of the spleen has favoured development of cancer.

Table I.

Time of survival after the transmission of the Spiropterae	Number of rats		Carcinomatous rats	
30— 44 days...............	14		0	
45— 89 —	37 (11)	102 (32)	20 (6)	54 (17)
90—119 —	15 (6)		9 (3)	
120—179 —	26 (8)		14 (3)	
180—298 —	24 (7)		11 (5)	
Total...	116		54	

(The figures in parenthesis refer to the number of rats infected by feeding on cockroaches and not on free larvæ).

[1] These rats belong to a series of experiments still unfinished, concerning the question whether or not the spleen acts as a protective organ against cancer.

The Table shows that in 54 out of 116 rats carcinoma of the fundus of the stomach was found (46,5 %).

All rats have been arranged in the table in groups according to the length of the period in which they survived the transmission of the Spiropterae or if more than one transmission was used — the first of them.

Most naturally it cannot a priori be taken for granted that the Spiropteræ — when more than one transmission was used — have entered the fundus at the first of them and not at the ensuing ones. The manner of grouping employed, thus, need not represent the exact points of time of the parasite's invading these rats, but as far as the shortest-lived rats are concerned, essential errors will scarcely be found.

According to experience a period of 45—50 days fairly corresponds to the time which must at least pass after the transmission of the nematode before the females can reach sexual maturity and begin evacuating fully ripe eggs containing embryos, a fact which has served me as a control in my examination of the stomach of the rats infected not only at the earliest 45 days before death but also a shorter time before death, that the nematodes may actually have invaded the stomach at the first and not alone at the later transmissions. This criterion, of course, is inapplicable in cases where rats have lived so long that not only nematodes which have entered the stomach after the first transmission, but also those invaded later on, have reached the said stage of development. The intervals between the transmissions being, however, of rather short duration, the incorrectness can be of no greater importance at a grouping within such wide limits as have been used here.

In order to control the right of estimating the relative frequency of carcinomatous growth within the different groups according to the figures quoted, the following tables are given, in which errors like those mentioned cannot occur.

Table II.

Time of survival after the transmission of the Spiropteræ	Number of rats	Carcinomatous rats
45— 89 days	19	11
90—119 —	6	3
120—179 —	13	7
180—298 —	18	10
Total...	56	31

Table III.

Time of survival after the transmission of the Spiropteræ	Number of rats	Carcinomatous rats
45— 89 days	11	6
90—119 —	5	2
120—179 —	9	4
180—298 —	9	5
Total...	34	17

Table II comprises the rats whose time of survival after the invasion of the Spiropteræ — no matter after which transmission it may have taken place, — can be fixed with certainty within the figures limiting each group.

Table III, in corresponding groups, contains all rats to which Spiropteræ have not been transmitted but once, and whose survival after transmission can thus be stated with absolute certainty.

These tables present the same results as table I in as far as the deviations are no greater than may be explained by the smallness of the figures.

From Table I it is seen that in the rats (14) having survived the transmission (or the first transmission) of Spiropteræ only for 1—1 1/2 month (30—44 days) carcinomatous growth was not met with, while among 102 rats surviving the transmission for 1 1/2 month (45 days) or some longer period (298 days being maximum) carcinoma was observed in 54 (about 53 %). In 3 out of these rats not only the fundus of the stomach but also the tongue appeared to be the seat of a carcinoma which will be described in an ensuing paper.

The grouping used in the tables according to time passed from transmission of the Spiroptera to death of the rats, makes it evident that the relative frequency in the different groups varies within so narrow limits that — as far as the small figures permit — it must be judged to be about the same or little different, no matter how much time, exceeding 44 days, may have passed from the transmission to the death of the rats.

The fact that neither in rats surviving the transmission for 3—4 months, nor in rats having survived transmission for 4—10 months the frequency of carcinomatous growth was found to be superior to that met with in rats which had only survived transmission for about six weeks—3 months, might possibly suggest that all carcinomata or the great majority may have developed early and even during the first $1^1/_2$—3 months after transmission.

If this be actually the case, it must be expected that the carcinomata would generally appear to be of slighter dimensions in the short-lived rats than in the long-lived rats, provided that the rate of growth be approximately the same. Exact judgment based upon volumetric measuring of reconstructions after serial sections, of course, being unattainable, I have tried to control the estimation of the dimensions of the carcinomata by measuring in serial sections their greatest extent along the mucous membrane, and by counting the numbers of sections (at 10 μ) in which each carcinoma was traceable. In these investigations as in those previously published, multiple (pluricentric) development of cancer (viz. 2 or more (to 5) mutually distant and apparently independent sharply demarcated carcinomatous foci) was found in numerous cases, in each of which the greatest carcinomatous focus was used at the measurement.

It now appeared that in the 20 carcinomatous rats which had survived the transmission of the Spiroptera only for $1^1/_2$—3 months even the largest foci were of very small dimensions (see the Plate fig. 1). No less than 17 out of these carcinomata took up areas of the mucous membrane the extent of which in serial sections did not exceed 1 mm. any more than the total thickness of all the sections. Also the dimensions of the remaining 3 carcinomata of this group were found to be rather insignificant. The invasive downgrowth was relatively slightly pronounced and only exceptionally entered the deeper layers of submucosa.

Among the carcinomata found in the 23 rats which survived the transmission of the nematode for 3—6 months, only 5, on the other hand, were of slight dimensions and 8 somewhat larger. The remaining 10 were far more developed, taking up areas of mucosa of 2,5—5 mm and traceable through numerous serial sections, often penetrating submucosa and reaching down into muscularis (see the Plate fig. 2). And finally, out of the carcinomata in the 11 rats which survived the transmission for more than 6 months, only 3 small, 1 somewhat larger and 7 very large and invasive ones were found. Even though a grouping established according to the principles here quoted must necessarily be encumbered with errors, it is beyond doubt that in long-lived rats carcinomatous growth of greater dimensions than in short-lived rats will generally be met with. The probability of the majority of all carcinomata having developed during the first six weeks—3 months after the transmission is, thus, further increased.

But to ascribe early development to all the carcinomata is not permissible, carcinomata of smaller extension being also in some cases met with in long-lived rats. And add to this the fact that multiple development of cancer occurred more frequently in rats which survived the transmission of Spiropteræ for a longer period than in rats of shorter time of survival, — as may be seen from the table below. This table contains the 46 carcinomatous rats, the fundus of whose stomach was subjected to a complete examination in serial sections.

Time of survival after the transmission of the Spiropterae	Number of rats	Multiple development of cancer occurred in
$1^1/_2$—3 months	20	5
3—6 months	18	8
6 months and longer	8	7

The dimensions of the multiple foci in one and the same fundus were only exceptionally about the same. Most frequently a larger focus was accompanied by several smaller foci of approximately equal size.

On the whole, it may be assumed that although the majority of all the carcinomata have developed early and, in fact, about 6 weeks—3 months after the transmission of the Spiroptera, a minor part of the tumors may have developed later on, and, besides, that all foci in cases of multiple development of cancer have not come into existence coincidently but successively. But as mentioned above, it cannot be precluded that differences in size of the carcinomata might be due to a different rate of growth within the same space of time, all of them having thus developed at a very early point of time.[1]

[1] The slight development of the carcinomata met with in some cases can hardly be ascribed to the fact that the rats in question were especially young and thus might be regarded as less disposed to rapid development of cancer, e. g. be it only mentioned that in 2 rats weighing on the transmission of the Spiroptera 200 gr. each, and

From the experiments, however, it follows with certainty that carcinomatous growth in the stomach of rats infected with Spiropteræ may develop rapidly. The estimation of the rate of growth being, however, only appropriate when regarded in proportion to the span of life of the rats, the rapidity is only absolute, not relative. DONALDSON[1] who assumes that, dating from birth, the average span of life of the albino rat (descending from the Norway rat (*M. decumanus*) is 3 years, asserts that all data regarding duration of the life processes in these rats must be multiplied by 30 before being comparable to data of the corresponding processes in man. In other words: days must be turned into months. If this assumption be transferable to the tumor growth in the rats concerned, the rapid development of some of these (45 days) will correspond to $3^3/_4$ years in man. May be that this period has been calculated too high, but even if reduced considerably, nay even to the half of it — evidence is at hand, that the development of cancer in the fundus of the stomach of rats, infected with the Spiroptera can take place at a rate which, compared with the span of life of the rat, need not actually differ essentially from observations met with in the development of cancer in man.

In rats which survived the transmission of the nematode for more than 44 days, development of carcinoma, as mentioned above, occurred with a frequency corresponding to 53 %. It cannot, however, be precluded that the frequency might have proved still greater, if a number of the animals, which died early, had lived longer, the possibility being as

having at this point of time reached full development, only small carcinomata were found although the rats did not die till resp. ca. 4 and ca. 8 months later.

1) The Rat. Memoirs of the Wistar Institute of Anatomy and Biology. Philadelphia 1915.

mentioned at hand that the development of the carcinoma in some cases sets in late.

A frequency of 53 %, however, is very considerable and much greater than the frequency stated in my previous experiments, a fact which may probably in the first place be due to the way of proceeding in the experiments here reported, where a complete examination in serial sections has not only been carried out of such stomachs as presented pronounced pathological or particularly papillary changes — as was the case in previous experiments — but also of stomachs which were apparently healthy or only slightly changed.

The above results have been attained by investigations carried out on black and white rats belonging to a stock which has for years been bred in the Institute. That inbreeding has taken place in this stock admits of no doubt whatever, and it cannot be precluded, that this circumstance may possibly have augmented the frequency of cancer in the stock, as it will be mentioned in an ensuing paper. It may however be pointed out, that infection with Spiropteræ does not, however, gives rise to development of cancer merely in these rats specially experimented on. Also in black and white rats from other Danish medical Institutes Spiroptera cancer has occurred, as well as in black and white rats, bought in England (in 5 out of 8), in Holland and in bastards of black and white rats and Norway rats (*M. decumanus*).

Biological distinction between *M. decumanus* and *M. rattus* being possible neither by investigations on precipitin or on complement fixation, nor by anaphylactic tests (GRAETZ),[1] only the morphological peculiarities and amongst these especially the shape of the skull, can be used as the distinctive mark between these species.[2]

[1] Zeitschrift f. Immunitätsforschung VI. 1910.

[2] DIEUDONNÉ und OTTO: Pest. Handbuch der pathogenen Mikroorganismen, herausgegeb. von Kolle u. Wassermann. Bd. IV. 1912.

All black and white rats examined were now, according to the shape of their skull, — judged to be descendents of the *M. decumanus*. But also in the fundus of the stomach of a house rat (*M. rattus*) I have recently been able to produce development of Spiroptera cancer. In my experiments provisionally finished and rather small in number — carried out on wild Norway rats (*M. decumanus*) I have not yet succeeded in producing carcinomatous growth by infection with the Spiroptera.

Spiroptera cancer in the fundus of the stomach has up till now been produced in 84 rats — previous experiments included.

That in some rats whose stomach was the seat of carcinomatous growth, Spiroptera cancer has developed also in the tongue, will be further discussed in an ensuing paper.

Spiroptera cancer has further been produced in the tongue of a rat whose cardiac portion of the stomach appeared to be non-cancerous, and in the fundus of the stomach in 3 white mice. In 2 of these very large metastases were found. These observations will also be reported later.

In the fundus of the stomach the Spiroptera cancer only very exceptionally attains the stage of development distinguishable by macroscopical examination. In accordance to metastatic deposits of the common carcinomata found in rats and mice, metastasis formation also here appeared to be most frequently met with in the lungs. After microscopical examination of practically all organs, including examination in serial sections of the lungs, metastases were found in my earlier experiments in 5 out of 18 rats (located in 3 cases in the lungs, in 1 case in a lymphatic gland, in 1 (somewhat doubtful) case in the urinary bladder). In the experiments here reported the lungs of 15 rats were cut completely in serial sections, metastases being found in 3 animals. Altogether, in special examinations on metastasis formation, metastases have, thus, till now been found in 8 out of 33 rats and in 2 of 3 white mice mentioned above in whose stomach Spiroptera cancer had developed. The metastases were only in 4 cases of larger dimensions.

From the above observations it follows that Spiroptera cancer has been produced in black and white rats of different origin, and in rats belonging to the stock specially examined develops rapidly and with a frequency which among individuals which survive the transmission of the nematode for about six weeks or more, exceeds 50 %. The possible influence of in-breeding mentioned above permits, a priori, no general application of this figure to all black and white rats or any stock whatever. Also differences of races or other factors may perhaps increase or diminish the frequency of the Spiroptera carcinoma, but at any rate it may be pointed out, that in the transmission of the *Spiroptera neoplastica* (*Gongylonema neoplasticum*) to black and white rats, a manner of proceeding has been attained which can be used as an applicable method for experimental studies of the way of developing and the conditions of growth of the carcinoma, as well as of other problems the intimate nature of which have till now been inapproachable to experimental investigation.

But other ways of proceeding may probably appear to be applicable too. YAMAGIWA and ICHIKAWA have recorded a series of investigations, dating from 1915—1917, in which by means of painting coal tar upon the ears of rabbits, they have succeeded in producing real carcinomatous growth. According to the last communication from these authors (1918)[1] development of real cancer has occurred in 12 out of about 200 rabbits (in 3 cases combined with metastasis

[1] Experimentelle Studien über die Pathogenese der Epithelialgeschwülste. III. Mitteilung. Mitteilungen der medizinischen Fakultät der Kaiserlichen Universität zu Tokyo. Bd. XIX. 4. Heft. 1918.

formation), and besides, early stages of cancer in a larger number of animals.

Furthermore be mentioned that K. SECHER in investigations[1] carried out in my institute on the influence of oats-feeding upon the tongue of the rat has observed development of cancer of the tongue in 1 rat. This and some similar observations made by myself may perhaps indicate that the oats-feeding may also turn out to be applicable as a method of producing cancer experimentally in rats.

Although it may appear from previous papers as from the above pages that the technique employed in producing Spiroptera cancer must be considered exceedingly simple, information as to some details in the manner of proceeding used in this Institute will be sure to facilitate investigations in other laboratories.

In working out greater series of experiments for that purpose the requirement will be to secure that cockroaches infected with the Spiroptera are at hand in abundance at all times. As mentioned above the parasite is able to fulfill its development from egg to larva not only in the American cockroaches (*P. americana*) but also in other species (*P. orientalis* and *Phyllodromia germanica*), and in the meal-beetle (*Tenebrio molitor*). In the latter the developing of the nematode, however, seems to take place with some difficulty. Neither is the use of *Phyllodromia germanica* advisable. This cockroach being rather small is not able to harbour more than a small number of larvæ in its muscles, and, moreover, is not easily kept and isolated in limited localities. The much larger common black brown cockroach (*P. orientalis*) is better fit for use and has thus exclusively been employed as a host for the nematode in my experiments dating from 1913—1914. But this species can bear no strong infection with Spiropteræ during longer periods, and a great number of the infected animals will therefore very frequently die before greater quantities of larvæ can be stored in their muscles.

By far to be preferred is the large light brown American species (*P. americana*) which, according to the above information, must be considered the proper host of the Spiroptera. The difficulty of procuring these cockroaches in Europe, indigenous to the tropical and subtropical parts of America, has been alluded to in preceding pages.

[1] Hospitalstidende 23/10 1918 no. 43.

Artificial breeding, however, does not cause any difficulties, provided that the requisite temperature is procured. In this institute an electrical thermostate (temperature 25—30° C.) is used in which the cockroaches, isolated in wooden boxes supplied with fine wire cowers, will get on most excellently. As soon as the females have evacuated their egg capsules, these are picked up and placed in glass reservoirs in the thermostate. According to observations hitherto made the larvæ under these conditions will leave the egg capsule after about 6 weeks. The time required for the larvæ to go through the whole process of moulting and to become fully developed animals with fully developed wings is, however, rather considerable and cannot yet be stated with certainty, but as far as I have been able to make out it may preliminarily be estimated at least 7—8 months.

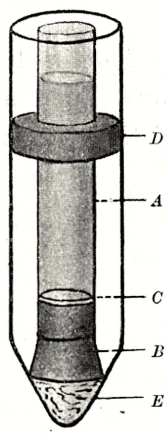
The larvæ are fed on white bread soaked in water; when fully developed, the cockroaches may at once begin to be fed on rat's excrements containing Spiroptera eggs. The best plan is to prepare an emulsion of the solid excrements in a normal saline solution, to centrifuge the emulsion and to feed the animals on the sediment. No other food is required.

That the excrements actually contain Spiroptera eggs can be controlled most efficiently by the method of Telemann (Deutsche medicinische Wochenschrift 1908).

As a matter of course the period in which the cockroaches must be fed on rat's excrements in order to be subjected to a violent invading of the Spiroptera larvæ into their muscles must vary according to the number of eggs contained in the excrements, and, thus, cannot be stated. Feeding for 6 weeks—2 months will frequently suffice, but if really a considerable invasion of fully developed and encysted larvæ is to be secured, a feeding of longer duration will suit the purpose better. How much the contents of larvæ in the muscles may vary will be seen from the preceding pages (12). In my experiments cockroaches are as a rule not used until 3 months have passed since the feeding was started.

The greatest number of larvæ is stored in the strongly developed muscles of the wings, of the breast rings (especially *prothorax*) and of the femora. When it has been ascertained by microscopical examination that these parts of the muscles contain fully developed encysted larvæ, the cockroaches are ready for use.

As mentioned above, transmission of the Spiroptera to the rat most conveniently takes place by feeding them on cockroaches which, especially after inanition for 36—48 hours, they will eat up greedily. The cockroaches are killed by cutting off the head, the hindpart of the body comprising the alimentary canal infected with microbes is removed, the wings and the outside joints of the limbs (*tibia* and *tarsus*) are cut off, so that only limbs and *prothorax* are used as feeding material. Exact particulars as to the quantities of muscles required cannot be given (see p. 12). If evidence be at hand that the muscles in question contain larvæ in abundance, the muscles of 3—5 cockroaches will be sufficient for each rat, 1—2 for each mouse. If too many cockroaches are used the animals will die at once or soon afterwards (see p. 14). If feeding on fixed numbers cf cockroaches is wanted, direct preparation under the microscope must be executed. *Prothorax* and *femora* are cut off, the chitin covering is split, the muscles cautiously removed by means of fine needles and placed on slides in a normal saline solution. By cautious use of the needles, parts of the muscles containing larvæ are now easily isolated. The larvæ are often expelled from their capsules by these manipulations, but still better by placing the muscle particles in a solution of pepsine and hydrochloric acid (50 centigram pepsine to 100 gram 1/20 normal hydrochloric acid). A complete digestion has not been aimed at, not being necessary as nearly all larvæ are free after having stayed but for a short time (c. 20 minutes) in the said solution. With some practice it is now easy work to liberate and to count a considerable number of larvæ, without removing more than a very small quantity of muscle tissue. In order to isolate the larvæ still more efficiently they are centrifuged in a common laboratory centrifuge (diameter 27 ctm). Apparently no harm whatever is done to them by the centrifuging, at any rate after a rotation for 15 minutes (2000 turns per minute) they appear just as movable as before and fully capable of invading. The sediment containing all larvæ and muscle particles is easily sucked up from the common conical centrifuge glass into a caoutchouk catheter (Charrière No. 9) and injected into the stomach of the rat. If application of the larvæ directly in the mouth cavity, or on small pieces of white bread is wanted, the following manner of proceeding has turned out to be appropriate, by working out of which the first laboratory attendant of the institute H. P. PEDERSEN, has lent excellent assistance.



A glass tube *A* the length of which is about 6,5 cm. and the diameter 1 cm. is closed at one end by means of a caoutchouk stopple *B*. The end of this stopple, enclosed in the tube, is covered with a circular piece of filtering paper *C* the diameter of which is a little larger than the inside diameter of the tube. A caoutchouk ring *D* is put upon tbe tube, being of such dimensions that the tube and the encircling ring will fit without difficulty into a common centrifuge glass. The emulsion of larvæ and small muscular particles is poured into the tube *A* which is then placed in the centrifuge glass in such a way that the lower plane of the caoutchouk stopple reposes on the conical bottom of the glass which is covered beforehand with a firmly squeezed layer of cotton *E*. The caoutchouk ring prevents the tube from getting into a sloping position within the glass and protects it against heavy shaking. The glass containing the tube is now fixed in the centrifuge and centrifuged for 15 minutes after which the tube is cautiously removed and the liquid poured out. The larvæ, then, together with small particles of muscle tissue will be found on the filtering paper which can removed (if necessary by means of a fine glass pin) together with the caoutchouk stopple by cautiously turning the latter out of the tube. Microscopical examination will show that the poured out liquid as well as the sides of the tube contain but an exceedingly small number of larvæ, the majority (about 95 %) being stored on the paper.

The only way in which to control the success of the infection of the living rats is by ascertaining that the excrements about six weeks (45—50 days) after the transmission of the Spiroptera harbour the characteristical eggs of the nematode. Often, however, these are but scantily at hand at this point of time, and sometimes only traceable with intervals of 1—2 days (TELEMANN's method).

By autopsy of the rats their tongue, gullet and stomach must be removed in natural cohesion, and without being opened placed in a solution of formaldehyde alcohol, which at the same time is injected into the stomach through the gullet. After fixation for 24 hours the stomach is cut into two symmetrical halves, passing a shaving knife through major and minor curvature. In this way the best view of the changes of the fundus portion is obtained.

That a thorough microscopical examination in serial sections of the complete cardiac portion is absolutely needed to secure a reliable judgment as to the developing or non-developing of cancer, has been mentioned in the preceding pages.

I desire to acknowledge my indebtedness to the CARLSBERG Fund and the W. BENDIX Legacy for their support of these investigations.

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Færdig fra Trykkeriet d. 7. Decbr. 1918.

Explanation of the plate.

Fig. 1. Small carcinoma in the fundus of the stomach of a black and white rat, which died 45 days after the transmission of the Spiropterae. The carcinoma could be traced in 66 serial sections (at 10 μ). $\times \frac{50}{1}$.

- 2. Carcinoma in the fundus of the stomach of a black and white rat, which died 152 days after the transmission of the Spiropterae. The carcinoma could be traced in about 400 serial sections (at 10 μ). $\times \frac{24}{1}$.

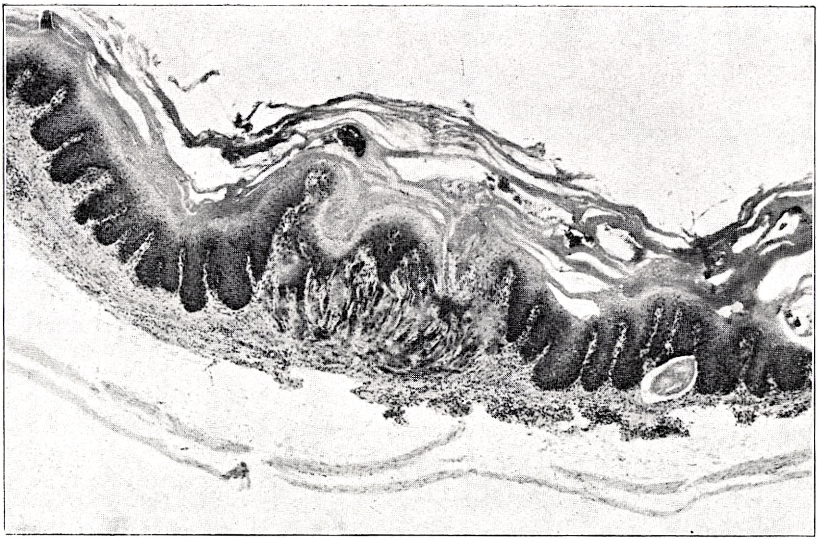

Fig. 1.

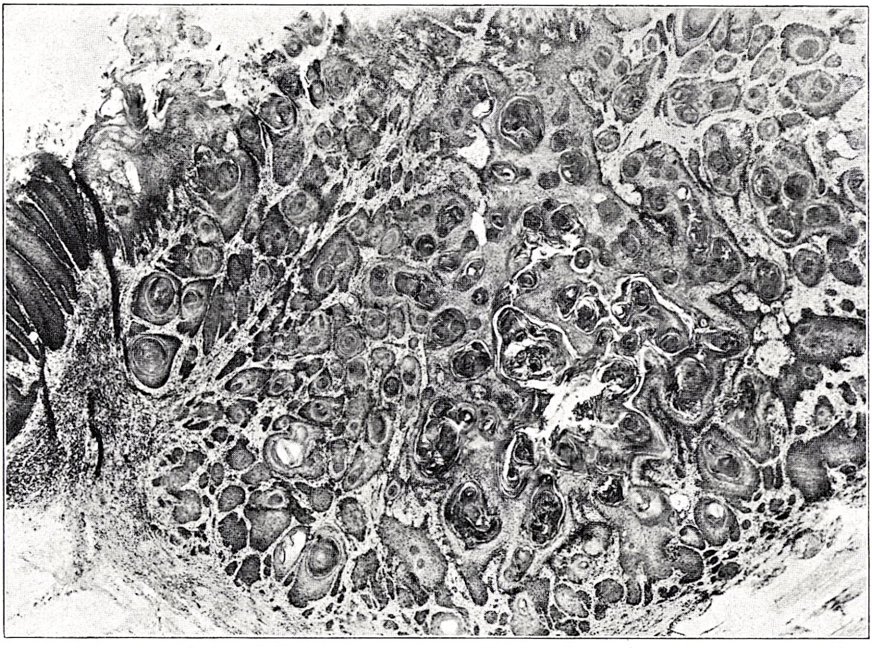

Fig. 2.

DET KGL. DANSKE VIDENSKABERNES SELSKABS SKRIFTER

8DE RÆKKE

NATURVIDENSKABELIG OG MATHEMATISK AFDELING

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