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INVESTIGATIONS ON THE SPIROPTERA CANCER V

ON THE GROWTH OF SMALL CARCINOMATA AND ON
PREDISPOSITION TO SPIROPTERA CANCER IN RATS
AND MICE

BY

JOHANNES FIBIGER



KØBENHAVN

HOVEDKOMMISSIONÆR: ANDR. FRED. HØST & SØN, KGL. HOF-BOGHANDEL

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In previous papers¹ report has been given on the results of my investigations on the occurrence and frequency of cancer in the stomach and in the tongue of black and white rats of different origin, and of bastards of the Norway rat (*M. decumanus*) and black and white rats, all infected with the *Spiroptera neoplastica* (*Gongylonema neoplasticum*).

The outcome of these experiments is not, however, limited to the chief results reported, but may also be useful in illustrating some questions which are still open to inquiry in the debate on the pathogenesis of cancer. In the following pages, therefore, as a contribution to this discussion account will be given of various details which supplement previous statements.

To the 19 cases of Spiroptera cancer in the fundus of the stomach, produced in my earliest experiments, altogether 65 new cases were added in the course of the last investigations. A considerable number of these new cases were only of very slight microscopic dimensions but still all so advanced that, in spite of their smallness, they revealed a structure, the carcinomatous nature of which admitted of no doubt whatever. For the study of the state of the epithelial cells, before the carcinomatous process has acquired its fully typical morphological character, these small cancers, therefore — as mentioned previously — cannot be used, whereas they will prove excel-

¹ Det Kgl. Danske Videnskabernes Selskab. Biologiske Meddelelser I, og I₁₀ 1918.

lent illustrations of the initial stages and phenomena of growth of the already developed carcinoma.

Furthermore, the last investigations have confirmed that the development of the Spiroptera cancer frequently takes place pluricentrically, viz: from multiple foci, as already demonstrated in my investigations of 1914, — and regarding the carcinoma of the tongue in rats infected with the Spiroptera they have shown that these carcinomata as well as the carcinoma of the tongue of the cat formerly described by BASHFORD¹) and the carcinomata of the tongue in man are sharply marked off.

Corresponding observations were now made regarding the carcinomata of the fundus of the stomach. Also here the very smallest cancerous foci proved to be sharply marked off, diffuse transition from atypical carcinomatous epithelium to neighbouring normal or hyperplastical epithelium not being traceable; and the sharp demarcation of the carcinoma remained unchanged also during its further development, as it became evident from the examination of larger carcinomata. In these too, no gradual transition could be traced; as a rule, it caused no difficulty to draw a line between the carcinomatous atypical growth and the adjacent neighbouring epithelium which had often undergone no other changes than more or less collateral hyperplasia. In some cases it was only very slightly thickened or nearly normal, in other cases displaced, curved or compressed, often undermined by the carcinoma which then lifted it up while penetrating into the connective tissue of the mucous membrane.

In other words, these investigations revealed phenomena quite identical with those formerly recorded by RIBBERT²,

¹ The Growth of Cancer under natural and experimental conditions. 1905 Scientific Report of the Imperial Cancer Research Fund. No. 2. Part III.

² Lehrbuch der allgemeinen Pathologie 1901. Geschwulstlehre 1904. Das Karzinom des Menschen. Bonn. 1911, and previous papers.

BORRMANN¹, BASHFORD², a. o. in their description of the conditions of growth of the squamous celled carcinoma and of other carcinomata. Thus, these observations on the Spiroptera carcinoma must be quoted in support of the well-known interpretations, which were first given by RIBBERT, of the said phenomena.

Also the extension of the Spiroptera carcinoma must be regarded as an expansive and invasive independent growth from its own resources only without any appositional increase in size by a successive seizing upon the immediately adjacent neighbouring epithelial cells on the part of the carcinomatous transformation.

My investigations hitherto indicate that the Spiroptera carcinoma, as other carcinomata previously examined, at any rate must take its origin from small very localized areas of cells although it cannot be precluded that only a single or a few cells might in reality prove to be the place of origin. Where very great carcinomatous areas are found, the considerable extension of the process may be due to the circumstance that several originally small and mutually separated and independent carcinomatous complexes of cells may have increased in size and conflued.

A proportional relation between the development of plain heterotopical proliferation of the epithelium and carcinomatous invasive downgrowth could not be traced. In a great number of cases the fundus of the stomach was found to be the seat of violent downgrowth of numerous epithelial projections and columns which invaded the muscularis mucosae and penetrated deep into submucosa, while in other cases large heterotopical retort-shaped epithelial crypts were besides found, real carcinomatous growth being observed in none

¹ Die Entstehung und das Wachstum des Hautcarcinoms. Zeitschrift. f. Krebsforschung. Bd. II. 1904.

² loc. cit.

of these cases. And several of these rats had survived the transmission of the Spiroptera for a considerable space of time, so that, without doubt, the fundus of their stomach had been the seat of these changes for a long period.

In contrast to this, an extensive and typical carcinoma would have developed in other rats, the stomach of which presented only scattered slight heterotopical downgrowth of a hyperplastic, but, for the rest, apparently healthy epithelium. And, finally, it would be seen that the cells of heterotopically growing epithelial columns disclosed no special tendency to carcinomatous transformation; on the contrary, the carcinomatous growth must often be considered to originate from normally localized epithelium, so that already from the very outset invasive growth of atypical cells had taken place in the upper layers of the connective tissue of mucosa. Thus, heterotopical downgrowth of hyperplastic, but, for the rest, unaltered, epithelium in the fundus of the stomach of rats infected with the Spiroptera can be explained neither as an obligatory initial stage nor as an absolute portent of carcinomatous growth, any more than development of cancer represents the terminal stage of these epithelial proliferations.

The observations here reported thus yield a supplement to the data already in hand as regards the right of maintaining a sharp distinction between plain hyperplastic and heterotopical epithelial proliferation — and real carcinomatous processes. The observations speak in disfavour of the current opinion that development of carcinoma must be regarded as the last stage of a process which, initiated by an atypical epithelial proliferation, gradually passes through series of transitory stages into a carcinoma, that represents the culmination, — an explanation which has been applied in recent days also to the development of the carcinomata, produced experimentally by painting coal tar upon the ears of rabbits

(YAMAGIWA and ICHIKAWA)¹. As distinct from this the phenomena observed in the Spiroptera infection will show that development of carcinoma must be regarded as a specific process, which under certain conditions joins the hyperplastic, heterotopical proliferation of the epithelium, and accompanies the latter as an independent complication. That the heterotopical proliferation and the development of cancer must be looked upon as phenomena of different nature, will be further discussed in the following pages, in which the influence of the Spiroptera infection upon the fundus of the stomach in mice, as well as predisposition to development of cancer will be dealt with (see pag. 24—25).

In my previous investigations I observed that, as a rule, several or numerous Spiropterae were discernible in stomachs which exhibited stronger or pronounced changes, while stomachs with slight or doubtful changes would harbour only a single or a few parasites; and that a fairly proportional relation would generally exist between the degree of the changes, the number of the Spiropterae, and the length of the time in which these had lived in the fundus of the stomach. At the same time, however, I laid emphasis upon exceptions from this rule, as in certain cases the stomach will contain but a few nematodes in spite of pronounced and carcinomatous changes, — a fact which I ascribed to the original considerable number of the Spiropterae having been diminished by a later emigration or dying off, analogous to the emigration of other species of nematodes, e. g. ascarides.

That Spiropterae which have lived for a long time in the epithelium of the stomach, in certain cases are apt to emigrate has been demonstrated in previous papers. Minor importance

¹ Experimentelle Studien über die Pathogenese der Epithelialgeschwülste. *Mitteil. der med. Fakultät der Universität zu Tokyo*. 1915, and later papers. Recently such carcinomata have been produced also on the skin of mice (TSUTSUI, *Gann* XII, July 1918).

must, however, be assigned to a dying off of the nematodes, as both in rats and mice they have appeared to be able to live and to produce eggs in the fundus of the stomach for a space of time which may exceed one year. Greater importance, on the other hand, must be attached to the emigration, which, no doubt, plays a far more prominent part than I supposed at first.

In most cases of these experiments I was prevented from controlling the frequently unreliable and impracticable macroscopical estimation of the number of nematodes by an exact enumeration of these after having prepared them out of the epithelium of the stomach, as I wanted to keep this organ undamaged for subsequent histological examination, and I have therefore been obliged to estimate, as a control, the number of nematodes in the serial sections, a procedure, by which of course no exact information is obtainable. The Spiropterae being, however, relatively very large (the males may attain a length of $\frac{1}{2}$ —1 cm., the females a length of 4—5 cm. or more) the number of parasites must of course be very small in cases where serial sections of all the fundus of the stomach contain but a few parts of them and only in a single area or here and there, while no parasites are traceable in any other portion of the organ (and it is absolutely certain that Spiropterae have not fallen out of the sections). Only in unquestionable cases of this kind have I designated the number of Spiropterae as "one or two" or "a few". The expressions "a greater number" or "many" were employed in cases where a considerable number of the nematode was visible at the macroscopical examination or at the inspection with the lens, or where numerous parts of Spiropterae were found in all or in most of the serial sections on microscopical examination.

The following table gives a view of the number of nematodes met with in the fundus of the stomach of rats belonging to experiments communicated in a previous paper¹:

Days passed from the transmission (or the first transmission) of the Spiroptera to death of the rat	All rats	A greater number or many Spiropterae	Carcinomatous rats	one or two or a few Spiropterae	Carcinomatous rats
45—89 days.....	52	48	29	4	0
90—298 —	50	19	9	31	16
Total...	102	67	38	35	16

¹ Det Kgl. Danske Videnskabernes Selskab. Biologiske Meddelelser I, 1918 (s. p. 18—20).

From the table it will be seen that out of 52 rats which survived the transmission (or the first transmission) of Spiroptera for from about 6 weeks to 3 months, 48 contained in the fundus of their stomach a greater number or many parasites (and in 29 cases carcinomatous growth) while the fundus of the stomach in the other 4 harboured but a few parasites. In these 4 rats the cardiac portion of the stomach exhibited only slight non-cancerous changes.

Among 50 rats which survived the transmission for a longer time, only 19 contained many nematodes in the fundus of their stomach (in 9 cases carcinomatous growth), while in no less than 31 rats the number of nematodes, found in the cardiac portion of the stomach, was to be designated as 1—2 or «a few». And out of these 31 stomachs 16 proved to be carcinomatous.

Owing to the fact that a number of the animals had been fed on cockroaches in which the number of larvæ was not exactly known, the possibility (according to previous statements¹) was now at hand that the small number of Spiroptera in these rats might not alone be due to a later reduction but also to the circumstance that originally only a small number of larvæ had been transmitted to these rats, and that a further reduction might possibly have taken place by emigration of the parasites immediately after the transmission.

As a control, therefore, the following table is set up, comprising all rats fed on Spiroptera larvæ in a number of 300—800².

Days passed from the transmission (or the first transmission) of the Spiroptera to death of the rat	All rats	A greater number or many Spiroptera	Carcinomatous rats	One or two or a few Spiroptera	Carcinomatous rats
45—89 days	22	22	12	0	0
90—298 —	31	15	9	16	5
Total...	53	37	21	16	5

¹ l. c. p. 8.

² As a matter of course the figures 300, 800 etc. do not in all cases mean exactly 300, 800, etc., but only an approximate number, as a few larvæ may be destroyed during the preparation (see my previous paper (Det Kgl. Danske Vidensk. Selsk. Biol. Medd. I, 1918 pag. 30).

A reduction of the number of Spiropterae from several hundreds to a single or a few immediately or shortly after the transmission was never observed¹, and when, in spite of such great doses of worms, the number can be reduced so considerably, as must have been the case in 16 of the above rats which survived the transmission of 300—800 Spiropterae for 3 months or a longer period, this is only to be explained by the fact that the nematodes, originally invaded, have decreased in number not only by an early, but also, and especially, by a later reduction, whether this be due to a loosening of the Spiropterae coincident with the desquamating epithelium, the former being carried into the cavity of the stomach and from there into the intestines, — or to the Spiropterae's actively leaving the epithelial cells.

Thus, it is beyond doubt that in rats infected with Spiropterae, a considerable emigration of worms from the fundus of the stomach will take place in numerous cases, when a period of 3 months or more has passed after the transmission. The contents of Spiropterae in the fundus of the stomach — already as early as 3 months after the transmission — may be so reduced that the total epithelial covering contains but one or two, or a few parasites.

This observation now becomes worthy of special interest by the fact that the frequency of the carcinoma of the fundus of the stomach among the longest lived rats proved to be about the same, no matter whether the stomach contained a great or a small number or only a few worms.

And entirely corresponding observations are met with in the Spiroptera infection of the tongue, as mentioned in the preceding report.² Among 5 rats, in the tongue of which cancer was produced, only 3 harboured the Spiroptera in the epithelium of their tongue, whereas Spiropterae were not traceable in 2 of these rats. And in the former 3 cases the rats had survived the transmission of Spiropterae for at most 2 or 3½ months, in the latter 2 cases, on the other hand, for from 5¾ to 6¾ months.

These observations evidence that Spiroptera cancer both in the fundus of the stomach and in the tongue, being once pro-

¹ Det Kgl. Danske Vidensk. Selskab. Biol. Medd. I, 1918 p. 13.

² ibidem. I₁₀ 1918.

duced, is able to subsist and continue developing even if the influence — the Spiropteraæ — which originally impelled its forthcoming, partly or completely disappears. Also an observation of cancer in the stomach of a mouse, mentioned below (p. 22) indicates such a disappearance.

This, per se, cannot in so far surprise, as evidence has already been brought forward, that the cells of the Spiroptera carcinoma are able to continue their growth independently, without co-operation of the Spiropteraæ, viz: that the worms are absent from the metastatic deposits, a fact formerly¹ proved and now confirmed in later experiments.

Nor will it be difficult to quote examples of analogous facts in cases where known etiological momenta are at hand as the causative factors of the development of cancer. I shall only draw attention to the continued growth of the X-ray carcinoma after cessation of exposure to the rays, and to the coal tar carcinoma, produced experimentally these last years by YAMAGIWA and ICHIKAWA and TSUTSUI² on the skin of rabbits and mice —, and being able to grow on after the discontinuance of the tar paintings.

Some investigations recently published by C. O. JENSEN³ deserve special interest in this connection, having confirmed that certain tumor-like formations in beets and other plants, according to the view of ERWIN SMITH⁴ may be parallelized with real malignant tumor growth in higher animals,

¹ Académie Royale des Sciences et des Lettres de Danemark. Extr. du Bull. de l'année 1913.

Hospitalstidende and Zeitschrift f. Krebsforschung 1913 and 1914.

² loc. cit. pag. 7.

³ Undersøgelser vedrørende nogle svulstlignende Dannelser hos Planter. Investigations upon certain tumor-like formations in plants. Den Kongelige Veterinær- og Landbohøjskole. Aarsskrift 1918.

⁴ E. F. SMITH, NELLIE A. BROWN and C. O. TOWNSEND: Crown Gall of Plants, its cause and remedy. U. S. Department of Agriculture. Bur. of. Plant Industry. Bull. 213. Washington 1911. ERWIN SMITH: Pflanzenkrebs versus Menschenkrebs. Centralblatt f. Bakteriologie. 1912.

Studies on the Crown Gall of Plants. Its relation to Human Cancer. The Journal of Cancer Research. Vol. I. 1916.

and that these formations are due to infection with the *Bacterium tumefaciens*, described by ERWIN SMITH. Furthermore, it has been evidenced in these investigations that in elder tumors of this kind the bacteria die off, and C. O. JENSEN calls attention precisely to the correspondence between this fact and the independent growth of the metastases of the *Spiroptera* carcinoma.

Thus, the analogy must be extended so far as to concern also the primary *Spiroptera* carcinoma, as this too continues its growth after disappearance of the causative factor. That the *Spiroptera* in the *Spiroptera* cancer like the *Bacterium tumefaciens* in plant cancer may vanish, is of the greatest significance also in another respect, as it makes room for the possibility that more tumors than those hitherto known may be actually of parasitary origin which is not traceable, because the parasites have disappeared before the tumor is fully developed, and examination takes place.

As to the *Spiroptera*'s mode of action I am not able to give further particulars, the examinations concerned comprising up to now but a smaller number of introductory experiments, and having been delayed for want of black and white rats which, owing to the war, could not be imported from abroad. My first assumption that the *Spiroptera*, on analogy with other worms, acts by some poisonous secretion, has been widely accepted (SALOMONSEN¹, AFOLANT², VERSÉ³, BOVERI⁴, YAMAGIWA and ICHIKAWA⁵, WASSINK⁶, a. o.), and still seems to me more acceptable than the assump-

¹ Smaa-Arbejder. Kjøbenhavn. 1917.

² Die experimentelle Erforschung der Geschwülste. KOLLE-WASERMANN's Handbuch. 1913.

³ Das Problem der Geschwulstmalignität. Jena. 1914.

⁴ Zur Frage der Entstehung maligner Tumoren. Jena. 1914.

⁵ loc. cit.

⁶ Over Gezwollen bij dem Fazant, veroorzaakt door een Worm. Amsterdam. 1916.

tion that the Spiropterae, in harmony with BORREL'S well-known hypothesis, are the carriers of a specific cancer virus. The supposed poisonous secretion as well as the supposed virus might cause the development of cancer either by influencing the epithelial cells directly, or indirectly especially by producing an inflammation which again favours or gives rise to the carcinomatous proliferation of the cells. Although RIBBERT'S renowned theory of the part played by the inflammatory process in the earliest genesis of cancer, has met with opposition from several investigators, the influence of the inflammation in general upon the development of cancer is still so commonly recognized that, without doubt, also carcinomata, arising after invasion of parasites, are generally considered due to the inflammation which is a very frequent phenomenon in such carcinomata. Thus, the Bilharzia carcinoma in the urinary bladder is generally looked upon as an effect of the chronic Bilharzia cystitis. ASKANAZY¹ and MARCHAND² regard the liver carcinoma in the presence of infection with *Opistorchis felineus* as a formation developed after the chronic cholangitis due to this trematode, HAALAND³ has suggested that development of carcinomata of mamma in mice might be referred to a mastitis, caused by nematodes, and precisely the Spiroptera carcinoma is, according to BORST⁴ and HERXHEIMER⁵ a. o. to be regarded as a result of the Spiroptera inflammation in the fundus of the rat's stomach.

¹ Parasiten als Krankheitserreger. Aschoff's Handbuch der patholog. Anatomie 1913, and other papers.

² Die tierischen Parasiten des Menschen. MARCHAND UND KREHL: Handbuch der allg. Pathologie. I. 1908.

³ Proceedings of the Royal Society. 1911. Fourth Scientific Report of the Imperial Cancer Research Fund. 1911.

⁴ Echte Geschwülste. Aschoff's Handbuch der pathol. Anatomie. 1913.

⁵ SCHMAUS-HERXHEIMER: Grundriss der pathologischen Anatomie. 1915.

Perhaps this assumption is correct, but a definitive solution of the question is not yet at hand.

That inflammation is an extraordinarily frequent and almost constant phenomenon in stomachs infected with the Spiroptera, is beyond doubt. But an approximately proportional relation between the development of the inflammation and the carcinoma is not traceable. There is, thus, absolutely no possibility of having by macroscopical examination of the fundus of the stomach any opinion whatever, as to whether or not carcinoma has developed in consequence of the stronger or slighter degree of the inflammatory changes. Carcinoma may sometimes be lacking in stomachs, the walls of which are strongly thickened and the seat of a violent inflammation and enormous papillary excrecences, and conversely, carcinomatous growth may occur in stomachs which are but slightly altered, small carcinomata being sometimes traceable in stomachs, the wall of which is but doubtfully affected and exhibiting thickenings that are due simply to a very slight epithelial hyperplasia or to phenomena of contraction.

That the carcinoma is bound by law to take its rise from papillary excrecences must thus be precluded. The papillomatous formations are no necessary stage preceding cancer, and carcinomata may as well develop in stomachs where such formations are completely wanting.

As a chief argument in favour of the importance of the inflammation, the constant occurrence of subepithelial inflammation in the earliest stage of carcinoma has been emphasized, but on examining microscopically the fundus of stomachs, such inflammations will by no means always prove to be pronounced in cases where beginning or small Spiroptera carcinomata are developing. In some cases only very slight or doubtful inflammatory changes are found, in a single case these may even seem to be wanting, as also it has some-

times been seen in heterotopical non-cancerous epithelial proliferation in these stomachs. Nor will greater Spiroptera carcinomata necessarily be associated with pronounced inflammation, whereas in cases of very extensive carcinomatous growth inflammation may often be easily traceable and violent. But the far advanced inflammatory process in these extensive carcinomata will without doubt often be due to a secondary invasion of bacteria and fungus threads from the stagnant content of the stomach. In my investigations now finished I have not unfrequently succeeded in tracing out such microbes in the necrosed tissue of such carcinomata and in its surroundings.

Thus, altogether great disharmony exists between the occurrence of the inflammation and that of the Spiroptera carcinoma in the fundus of the stomach, and that a pronounced glossitis needs not necessarily be found together with the Spiroptera carcinoma in the tongue, may be seen from a preceding paper.¹

An essential or even unquestionable influence on the growth of the Spiroptera carcinoma once developed cannot, thus, be assigned to the inflammation. On the other hand, it cannot be precluded that the slighter or stronger acute subepithelial inflammatory changes which, associated with the epithelial proliferation, are generally the immediate effect of the invasion of the Spiroptera, might be co-operative in the establishment of conditions for the earliest carcinomatous proliferation. But these inflammatory processes then must be able to disappear quickly, leaving sometimes no distinct trace behind them.

As emphasized in the discussion of the genesis of cancer, the inflammatory changes need not be in causal relationship to the carcinomatous growth at all. Also in the development of the Spiroptera cancer small masses of lym-

¹ l. c. pag. 10.

phocytes and slight proliferation of the connective tissue might possibly be an effect of the carcinomatous growth or these processes altogether be parallel, mutually independent processes, results of the same pathogenetical power. And the possibility of the carcinomatous growth being an independent process can so much the less be left out of consideration, as there are reasons for assuming that epithelial proliferation and tumor growth due to the invasion of other nematodes, may arise without the co-operation of inflammatory processes being distinctly traceable. This is the case with the epithelial hyperplasia and papillomatosis due to the *Trichodes crassicauda*, observed by LÖWENSTEIN¹; WASIELEWSKI² and the present writer³ in the urinary bladder of rats. Nor has WASSINK⁴ succeeded in tracing out inflammatory processes in such fibromata (fibro-sarcomata) as occur in the intestines of pheasants and are due to a worm of the genus *Heterachis*.

A definite determination of the influence of the inflammation upon the early genesis of the Spiroptera carcinoma must, therefore, be reserved for future investigations.

In whatever manner the mode of action of the Spiroptera infection or the mutual relation between the carcinoma and the inflammation be comprehended, it remains at any rate an unsolved enigma, why cancer may develop in some rats which have survived the transmission of Spiropterae only for a shorter time, and whose stomach is but slightly affected,

¹ Epithelwucherungen und Papillomenbildungen verursacht durch ein Trichosoma. Brunn's Beiträge zur klin. Chirurgie. 1910. Bd. 69. *Trichodes crassicauda*, eine causa directa in der Aetiologie der Tumoren, ibidem. 1911. Bd. 76. Berlin. klin. Wochenschr. 1913. No. 16.

² Zum Nachweis tierischer Parasiten in Gewebswucherungen. Bericht über die 6. Tagung der freien Vereinigung f. Mikrobiologie in Berlin. Centralbl. f. Bakteriologie und Parasitenkunde 1912.

³ Berlin. klin. Wochenschrift. 1913. No. 16. (Polemic remarks to LÖWENSTEIN).

⁴ loc. cit.

but will not arise in other rats which have survived the transmission for a longer period and whose stomach is the seat of pronounced epithelial hyperplasia, violent chronic inflammation, and considerable papillary transformation.

As a matter of course, the cause of the occurrence or non-occurrence of the carcinoma may be sought in variations of the qualities of the Spiroptera; but even if significance must be ascribed to such variations, this explanation will not prove satisfactory in all cases, and especially not when rats, belonging to the same stock and apparently exhibiting no differences, are influenced in highly different degrees within the same space of time, although they have been infected repeatedly with such great quantities of Spiroptera that the possibility of some animals having been infected only with slightly "toxigenic" and other animals merely with strongly "toxigenic" parasites, seems precluded.

Most naturally by this circumstance we shall be led to think of the possibility of a different susceptibility of the rats to the cancer producing power of the Spiroptera infection, in other words: of a different individual predisposition to the development of Spiroptera cancer.

Among the constitutional causative factors of predisposition to cancer, generally emphasized, the sex will hardly be of any consequence, carcinoma being found with about the same relative frequency in males as in females. Nor is the age of the animals likely to have been of any great importance. As previously mentioned¹, it proved impossible to realize an exact estimation of the age; but a greater frequency of the carcinoma amongst unquestionably elder than amongst unquestionably younger animals could not be ascertained, and Spiroptera cancer occurred in rats which both on the transmission of the Spiroptera and on death were absolutely young. Furthermore, the way of living and feeding was quite uniform in all

¹ l. c. p. 8.

experiments and, thus, can hardly be taken into consideration. That debilitation due to intercurrent accidental diseases which had in numerous cases conveyed death, might have been a predisposing factor, is not probable, carcinoma being found just as frequently in rats which were killed on arbitrarily fixed dates, and whose only or main affection was the Spiroptera infection in the fundus of their stomach, — as in rats which died spontaneously from complicating diseases.

Greater importance must perhaps be assigned to inbreeding, no matter whether the influence (BAUR)¹ must be sought in cumulation of recessive qualities, in frequent occurrence of cellular mutation, or in constitutional debility of unknown origin. As it is well-known, the theories of the influence of heredity on the development of cancer have been supported by modern investigation, which indicates that spontaneous cancer will develop more frequently in mice of cancerous ancestry than in mice devoid of a cancerous pedigree. (TYZZER,² MURRAY³, BASHFORD⁴, SLYE⁵).

It cannot thus be precluded that the origin of the different individual susceptibility which, according to my experiments now finished, must be supposed to bear a part in the development of the Spiroptera cancer in black and white rats, must be sought in inherited peculiarities, and that the frequent occurrence of such peculiarities may have been due to in-

¹ Indavlsproblemet. Den Kgl. Veterinær- og Landbohøjskoles Aarskrift 1918.

² A Series of Spontaneous Tumors in Mice. Journ. of med. Research. 1907. 5th Report of Cancer commission of Harvard University. 1909.

³ Cancerous Ancestry and the incidence of Cancer in Mice. 4th Report of the Imperial Cancer Research Fund. 1911.

⁴ Das Krebsproblem. Berl. klin. Wochenschrift. 1913. No. 1.

Proceedings of the New York Path. Society. Middleton Goldsmith Lecture. 1912.

⁵ The inheritability of spontaneous tumors of specific organs, and of specific types in mice. 5th Report. Journ. of Cancer Research I. 1916, and other papers.

breeding which is sure to have taken place to a great extent among the experimental animals.

This, too, is a problem of great importance, the solution of which must be reserved for future investigations aiming at this special purpose.

As expressed by BASHFORD a. o., comparative studies on the occurrence of cancer in man and animals have made it almost certain that irritants will not be equally effective in developing cancer in all species of animals. It is as an experimental proof of this assumption, and of the right of speaking at all of a different predisposition to development of cancer, that I shall state the results of my provisionally finished investigations on the influence of the Spiroptera infection on white laboratory mice.

As mentioned in previous papers¹, it must a priori be expected that carcinoma in the fundus of the stomach would be of frequent occurrence in white mice infected with the Spiroptera, these animals being generally regarded as more disposed to development of cancer than rats. Furthermore, observations made by MURRAY² already in 1908 had proved the incidence of cancer in the fundus of the stomach in mice, and besides, my introductory examinations had shown that the Spiroptera are able to live in the same way in the fundus of the mice's stomach as in that of the rats. At the outset, the action of the parasites must be expected to be more powerful in mice than in rats, on account of the far smaller size and weight of the former, and the small dimensions of their stomach. Finally, the fundus of the stomach in mice infected with the Spiroptera, on preliminary investigation, proved to be the seat of epithelial hyperplasia and inflammation of the same kind as those generally met with in the rats.

¹ Hospitalstidende and Zeitschrift f. Krebsforschung. 1914.

² The zoological distribution of cancer.

3rd Report of the Imperial Cancer Research Fund. 1908.

These observations were fully confirmed in the first greater series of experiments accomplished. It could be ascertained that the Spiroptera live and produce their eggs for at least just as long a time or longer in the white mice as in the black and white laboratory rats, and that the fundus of the stomach in mice is subjected to just as intense affections and undergoes essentially the same changes. Pronounced epithelial hyperplasia, considerable, often heavy and heterotopical downgrowth, compact or retort-shaped downgrowing epithelial projections, violent inflammation and papillary transformation of the mucous membrane, on the whole, was found with the same frequency in the mice as in the rats.

It was then all the more striking that carcinoma was not traceable in a single mouse, although these experiments, the results of which was stated at the Meeting of Scandinavian Naturalists in Kristiania 1916¹, comprise altogether 207 mice, among which also inbreeding will have taken place to a great extent. 56 mice had survived the transmission of Spiroptera for 45—597 days, viz: just as long or much longer than is necessary for cancer to develop in the fundus of the stomach of black and white laboratory rats (17 mice survived the transmission of the Spiroptera for 6 weeks — 3 months, 8 for 3—5¹/₂ months, 18 for 6—12 months, 13 for about 1—1²/₃ year. In 35 mice very considerable heterotopical proliferation of the epithelium was found, often penetrating deep into submucosa.)

It is further important to emphasize that mice — as commonly stated — at the most, but very rarely live as long as the rats, viz: for 3 years (BASHFORD², BORREL³, HAALAND⁴

¹ Forhandlingerne ved 16. Skandinaviske Naturforsker møde i Kristiania 1916.

² Second scientific report of the Imperial Cancer Research Fund. 1905. S. 9.

³ Le probleme étiologique du cancer. Ann. de l'Institut Pasteur. 1908.

⁴ Les tumeurs de la souris. Ann. de l'Institut Pasteur. 1905 and

C. O. JENSEN¹, MURRAY)², and it will thus be seen that the Spiroptera in a great number of mice had influenced the fundus of the stomach for a very considerable time or for a longer time than black and white laboratory rats will generally be able to sustain the influence, and finally, that a great number of mice, being on transmission of the Spiroptera at least 4 months old, on death belonged to age-groups, in which carcinoma in other organs most frequently manifests itself in these animals. (MURRAY).

As noted in the report from the Meeting of Naturalists these experiments did not, however, give absolute evidence that carcinoma of the fundus of the stomach may not at all develop in white mice infected with the Spiroptera. It might be possible that cancer would occur if mice could live infected with the Spiroptera for a still longer time.

Furthermore, if mice were employed which were very old in the first stages of the infection, different results might perhaps be obtained, and cancer of the fundus of the stomach might finally be of so unfrequent occurrence in mice that my experiments were not sufficiently extensive. After I have completed the experiments, some new cases of carcinoma of unknown origin in the fundus of the stomach in mice have been observed (SLYE³, LITTLE and TYZZER⁴, ITAMI⁵), especially amongst a strain of mice, whose term of life is considerably longer than usual (SLYE).

Spontaneous tumors in mice. 4th scientific report of the Imperial Cancer Research Fund. 1911.

¹ Der Krebs der Tiere. VIII. internat. tierärztl. Kongress. Budapest. 1905.

² The heredity of cancer in mice. XVIIth internat. Congress of Med. London. 1913.

³ Comparative Pathology of Cancer of the stomach. Journ. of Cancer Research. 1917.

⁴ Journal of med. Research. 1916. quoted by SLYE.

⁵ Proceedings of the New York Pathological Society. 1916. quoted by SLYE.

I have, therefore, commenced a new series of experiments, comprising about 100 white mice among which a considerable number were rather old or absolutely old. Also among these mice in-breeding will have taken place. These experiments are far from finished, and only a few animals, the fundus of whose stomach exhibited specially violent changes, have hitherto been subjected to a complete examination. A full view of the entire result of the experiments, thus, cannot yet be had, but evidence has been obtained that Spiroptera cancer may actually develop in the fundus of the stomach in white mice.

Altogether 3 cases have been observed.

In the first case a small, slightly developed carcinoma was found in the fundus of the stomach, combined with gastritis and adenomatous transformation of the glands¹ of the pyloric portion, in a mouse which had survived the transmission of the Spiroptera for 355 days, and on death was 18—20 months old.

In the second case extremely violent carcinomatous growth was observed in the fundus of the stomach, as well as numerous metastases in cavum peritonei, in a mouse which died 482 days after the transmission of the Spiroptera, being then about 2 years old. Post mortem examination of this mouse showed no Spiroptera, but, that the fundus of the stomach must actually have harboured the parasites for a longer time, was evidenced by the fact that the excrements of the living mouse contained Spiroptera eggs 322 days after the transmission. It therefore admits of no doubt that the carcinoma in this case, on analogy with Spiroptera carcinomata of the tongue in rats previously described², has continued its growth and obtained its considerable size independent of the disappearance of the Spiroptera prior to the death of the mouse. There is no possibility of stating exactly the length of time in which the Spiropterae have lived in the fundus of

¹ The adenomatous transformation of the glands was very pronounced. Furthermore, heterotopical, non-infiltrative downgrowth of the epithelium was found, penetrating into submucosa as strongly demarcated cysts. Neither in this case any more than in a few similar cases previously observed, may the changes be considered as cancer of the pyloric portion, but only as such heterotopical proliferations of the epithelium as may be parallelized to analogous changes in the stomach and intestines of man.

² Det Kgl. Danske Videnskabernes Selskab. Biologiske Meddelelser I₁₀ 1918.

the stomach, but according to examination of the excrements, this period must at least have been 11 months. Transplantation of a metastasis gave positive result, and tumor has hitherto been maintained through 4 generations.

In both these cases the mice, which did not belong to the stock of the institute previously employed, but to another, were absolutely old on death, and these cases may in so far perhaps suggest that Spiroptera cancer in the fundus of the stomach like other carcinomata in mice will attain a higher development in old than in young animals. But the observations yield no security to the view that advanced age should favour development of cancer, upon the whole. At any rate the carcinoma had attained such enormous dimensions in the second mouse that its very earliest development must be assumed to have commenced long before the death of the animal, and in fact, at a time when the mouse could not yet be considered old.

Special interest in this respect attaches to the third mouse the fundus of whose stomach had undergone carcinomatous transformation.

In this animal, which belonged to the proper stock of the institute, and died 410 days after the transmission of the Spiroptera, an acute purulent peritonitis had arisen in consequence of a perforation of the stomach wall, the enormous thickening of which was due to an extensive carcinoma which penetrated infiltratively through all the wall. The carcinomatous fundus of the stomach and the gullet contained Spiropterae. The pyloric portion was healthy. In the right lung a metastasis, scarcely as large as a hemp seed and in pronounced necrosis, was noticed.

In this case the mouse on transmission of the Spiroptera was only 6 weeks old (its weight being 6 gr.), and on death about 15 months old (weight 15 gr.). That the earliest incipient development of cancer has taken its rise while the mouse was still young, hardly admits of any doubt.

There is thus a possibility, that, for the development of Spiroptera cancer in the fundus of the stomach in mice,

not only an individual predisposition is required, but furthermore, that the mice notwithstanding their age on transmission of the Spiroptera must live for so long a time that the influence of the parasite becomes of rather long duration.

It would be useless to discuss further this and other possibilities before the investigations are quite finished, the material hitherto revised being too little extensive to serve as foundation for a reliable numeric estimation of the frequency of the Spiroptera cancer in white mice. The experiments hitherto performed suggest that the Spiroptera carcinoma in mice is of far smaller frequency, and only develops after much longer exposure to the influence of the parasites than it does in black and white rats, but the experiments also demonstrate that the carcinoma in mice may attain an extension and power of metastasing which exceeds all that has hitherto been observed in rats. It is, moreover, noteworthy that transplantation in one of the cases gave positive results.

Up to now, my investigations on the influence of the Spiroptera infection on house mice (*Mus musculus*), forest mice (*M. sylvaticus*), and wild Norway rats (*M. decumanus*) are not extensive enough either, to permit reliable numeric statistical statements, but, at any rate, they may suggest that the Spiroptera carcinoma also in these animals is of unfrequent occurrence, in as far as on examining preliminarily altogether about 100 animals most of them *M. musc.* and *M. sylv.*, I have not yet succeeded in ascertaining any case, although the Spiroptera infection in all the animals as in black and white rats and white mice, give rise to inflammatory processes, papillary transformation of the mucous membrane and a considerable or violent proliferation of the epithelium, associated with extensive, often heterotopical downgrowth.

I have previously mentioned the difference between plain

heterotopical hyperplasia of the epithelium and carcinomatous growth, a difference which by these last observations has become so much more conspicuous, that there is further ground for regarding these processes as differing in nature. To take the epithelial proliferation, combined with heterotopical downgrowth, as a precarcinomatous phenomenon, is only permissible in as far as such proliferations in a certain number of cases are associated with development of cancer.

But, furthermore, the proliferative processes cannot in all animals with equal right be characterized as pre-carcinomatous. Hyperplastical and heterotopical epithelial proliferation of the same structure and extension, indicating in one species (e. g. black and white rats) with no little probability the possibility of combination with cancer, in another and nearly allied species (e. g. white mice) can only with far greater reservation be taken as a portent of a coming development of cancer.

Hence indication is given that not only differences according to individuals but also differences according to species and — probably according to race too — exist in the susceptibility to the cancer producing power of the Spiroptera infection, but it cannot be precluded that different predisposition according to species and race may be due to other factors than is different individual predisposition.

It cannot of course be claimed that the investigations which have here been outlined, afford anything approaching to an elucidation of the enigmatical problem of the predisposition to cancer. In support of the well-known cellular theories, the developing of the Spiroptera carcinoma from small demarcated foci of cells or from single cells, may naturally be quoted as a hold to the theory that peculiarities of distinct cells condition the power of the Spiroptera infection to cause the carcinomatous proliferation of these cells, irrespective of the mode of action of the infection.

As previously emphasized in the discussion of the predisposition to cancer (BASHFORD a. o.) the fact will perhaps prove to be, that different species or individuals are not only differently susceptible to one and the same cancer producing factor, but also, that susceptibility to one factor needs not mean susceptibility to all or to several other factors, in other words: that the different organisms are not equally influenced or influenced at all by all agents which cause the development of malignant tumors, but only by some. And perhaps susceptibility to one and the same influence may prove to be not only constitutionally but also locally different, bound to distinct groups of cells in different organs of the different species, races, etc. In this connection I only wish to state that spontaneous metastasing periosteal sarcoma of the bones has been observed in my laboratory by K. SECHER¹ in a black and white rat infected with the Spiroptera, the fundus of whose stomach exhibited very violent pathological changes, but no carcinoma, and that I myself have observed spontaneous tumor growth in 3 Spiroptera infected mice, whose stomach was also strongly affected but not carcinomatous, — in two of these mice carcinoma mammae, in one a tumor mammae, diagnosed as a sarcoma, were observed.

Furthermore, the highly striking fact must be noted, that in spite of hundreds of examinations I have not yet succeeded in tracing carcinoma or cancer-like changes of the gullet in Spiroptera infected rats, although this organ shows morphologically no essential difference from the fundus of the stomach and exceedingly often is the seat of numerous Spiroptera, pronounced inflammation, and considerable epithelial hyperplasia, and although not only practically always quite the same changes, but very frequently also carcinoma has developed in the fundus of the stomach of the same animal.

¹ Kasuistische Beiträge zur Kenntnis der Geschwülste bei Tieren. Zeitschrift f. Krebsforschng. 16. Bd. 1917.

The possibility thus seems obvious, that the epithelium of the gullet is less susceptible to the cancer producing power of the Spiroptera invasion than is the epithelium of the stomach. Neither is it perhaps any accident that carcinoma of the tongue in only one of the cases observed¹ was found in the portion, in which the Spiroptera glossitis is generally most pronounced.

Of course, wide-ranging special investigations are required to prove the value of these hypothetical reflections which, like other reflections in this paper, illustrate, how further investigations on the development and the conditions of growth of the Spiroptera carcinoma may yield a contribution to the solution of numerous and great problems still unsolved in the study on the genesis of cancer.

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

¹ Det Kgl. Danske Videnskabernes Selskab. Biologiske Meddelelser I₁₀ 1918.

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