JOHANNES FIBIGER

Investigations on *Spiroptera carcinoma* and the experimental induction of cancer

Nobel Lecture, December 12, 1927

Ladies and Gentlemen. Now that it is my honour to address the Swedish Society of Medicine, I should like to begin by expressing the debt of gratitude I owe to this Society.

In 1913, some six months after I had published my first work on *Spiroptera carcinoma,* the Swedish Society of Medicine did me the honour of making me its member. This was the first recognition of this kind which came to me after the appearance of my work, and I would like to mention again today, here in the home of the Society, the great pleasure which I felt then and which I still recall with sincere gratitude.

Before I go on to relate the principal results of my investigations on *Spiroptera carcinoma* and the experimental induction of cancer, I would like to be allowed to offer the Nobel Foundation and the Staff of Professors of the Caroline Medio-Surgical Institute my most sincere and humble thanks for the great honour they have done me by finding my experiments worthy of the Nobel Prize in Physiology or Medicine.

It is possible to trace back attempts at supplementing clinical and anatomical studies of cancer by means of experimental work on the disease's origins, development and dissemination in the organism over a great number of years; not only the first, primitive experiments made over 150 years ago, but many more recent ones met with only negative results up to a short time ago.

Reports by Hanau and Morau, published during the years 1889-1894, contained the earliest description of successful transplantation of cancer from rats and mice to animals of the same species, but it was only at the dawn of this century that work done by Loeb, Jensen, Borrel, Bashford, Ehrlich, Haaland, Murray, and others (especially the classic experiments carried out by C. O. Jensen) ushered in the first experimental phase in cancer research, the era of transplantation experiments. From this time on the study of the cancer diseases could justifiably be said to belong to the field of experimental pathology.

Although a great deal of new and useful experimental work on transplantation has been done and is still being done, it must be admitted that on the whole it has not come up to early expectations. The development of cancerous tumours in one animal to which particles of a cancer which has occurred spontaneously in another have been transferred, is no more than the continued development of diseased tissue already in being. In fact, the study of transplanted neoplasms really only provides us with a possibility of closer investigation of the development, and conditions for development, of existing cancer tissue; it does not enable us to undertake any of the most important tasks in cancer research - an explanation of the original causes and the conditions for the onset of cancer, and of the processes surrounding the earliest beginnings of the disease. Moreover, the significance of transplantation experiments has decreased since the successful results of tests on immunization against transplanted cancer have shown themselves to be invalid for spontaneously developed neoplasms.

It follows that experimental studies must, for our purposes, necessarily be carried out on cancer growths which are not transplanted fully established from one animal to another, but which are induced experimentally and then develop in normal tissue in previously healthy animals.

In other words the problem of inducing cancer would have to be solved before the disease could be made the subject of the kind of experimental work which has provided important results in the study of the pathology of other diseases.

Recent attempts at solving this problem, which has occupied numerous workers in vain for a great number of years, have made use of methods based on the three famous theories of the origins of cancer: Virchow's irritant theory, Cohnheim's embryo theory, and the theory which ascribes cancer to *parasites.*

But up to recent times neither the constant application of chemical or physical irritants, nor the introduction of embryonic tissue, nor the implantation of various kinds of microbes into healthy animals produced any result at all; it was only around 1910 that there were reports of experiments in which the development of cancer was observed in isolated animals. Experiments carried out by Clunet, Marie and Raulot Lapointe, for example, produced sarcoma in two rats which had been exposed to X-radiation; Askanazy had, in isolated cases, observed the development of cancer in rats inoculated with embryonic tissue; and finally we must mention the sarcomatous fowl tumours first described by Fujinami and Inamoto, and by Peyton Rous,

which could be produced by filtrates from tumour tissue, and which have generally come to be regarded as due to an invisible virus. There was doubt, however, as to whether these neoplasms could be considered as true cancers, and were really identical to normal sarcomatous tumours - and this doubt has still not been finally removed.

The first method which succeeded experimentally and systematically in bringing about a true epithelial carcinoma in healthy animals was the introduction of a Nematode, *Spiroptera neoplastica* or *Gongylonema neoplasticum,* into piebald rats, and it is as I mentioned the principal results of these experiments which I now have the honour of describing to, you.

The starting-point for these studies was my finding in 1907, in the stomach fundus of three wild rats captured originally in Dorpat, of extensive papillomatous tumours which virtually completely filled the stomach and emerged from the cardiac region which was lined with pavement epithelium (Fig. 1; *cf.* normal rat's stomach, Fig. 2). Under the microscope the epithelium showed peculiar formations which were reminiscent of a section through egg-containing parasites, particularly Nematodes (Fig. 3), and after reconstruction and serial section it became possible to prove that the epithelium did in fact contain such parasites. This was later confirmed definitely by the separation

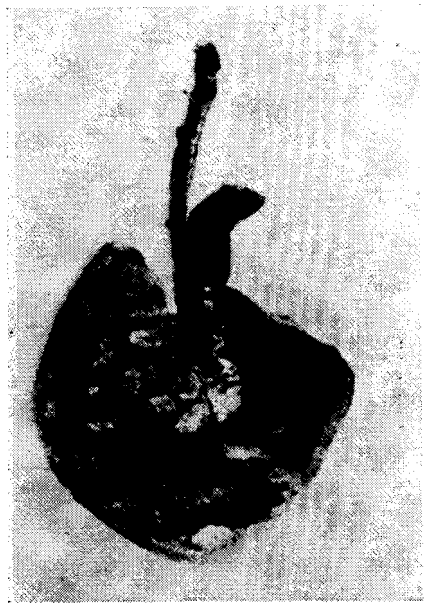

Fig. 1. Massive papillomata caused by *Spiroptera neoplastica* in the stomach fundus of a wild rat Gem Dorpat (natural size).

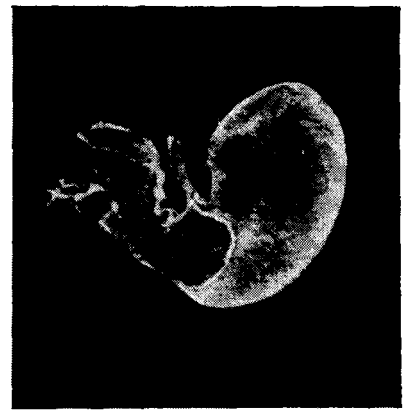

Fig. 2. Normal rat's stomach (natural size).

of isolated worms. My thoughts naturally turned to the possibility of these parasites having been the cause of the neoplasm developing - a supposition which was all the more justified since Borrel had already asserted in 1906, and subsequently, basing himself upon the presence of Helminthes and other types of animal parasites found in tumours in rats and mice, that such macro-parasites should be considered as having considerable significance in the genesis of the tumours. Haaland, too, had reported observations which could be used in support of this assumption; but the strongest argument for the pathological role of Helminthes in the development of cancer lay in the frequent appearance of cancerous neoplasms during schistosomiasis in the

Fig. 3. Spiroptera embedded in the fundus epithelium of a brindled rat.

human bladder. There appeared to be proof that in the case of these tumours the presence of worms in the cancerous tissue was neither coincidental nor ascribable to a secondary invasion. In order to investigate whether the tumours in the stomach fundus of my three rats were actually due to a Nematode, and to contribute to a discussion whether the transmission of such parasites could result in the experimental inception of the development of a cancer, I set up a series of experiments of which I am only able here to give you the main features.

An attempt to demonstrate papillomata or worms of the type sought in

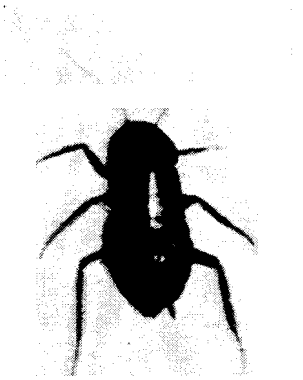

Fig. 4. *Periplaneta orientalis.*

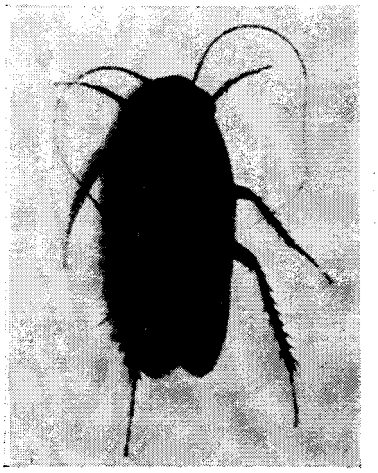

Fig. 5. *Periplaneta americana.*

other rats came to naught, since investigation of the fundus in nearly 1,200 wild rats and laboratory animals gave only negative results. Negative results were also obtained when I tried to induce neoplasms by means of papilloma tissue introduced into healthy rats. Not even feeding the rats on tumour tissue produced positive results, and no tumours developed in rats kept for protracted periods (up to one year) in uncleaned cages previously occupied by diseased animals. There seemed, therefore, to be hardly any question of the direct transmission of Nematodes. This left the possibility of the Nematodes being transmitted through an intermediate host, in which the embryo-containing egg could undergo further development.

The literature was now found to countain a reference in 1878 to Galeb having found a Nematode, *Filaria rhytipfeuritis,* in the stomach of rats. This had previously been discovered as a parasite in the fatty bodies of the common cockroach, *Periplaneta orientalis* (Fig. 4), and after three rats had been fed on cockroaches infected with this Nematode, Galeb was able to find worms in their stomachs, although no pathological change was evident.

Accordingly, I started on experiments in which rats were fed on cockroaches of the same species as those used by Galeb; these, too, were unsuccessful, the stomachs of rats captured in the same locality as the cockroaches containing neither worms nor papillomata. Results were only obtained when I changed over to experiments on rats which had lived in a sugar refinery where there were large numbers of *Periplaneta americana,* a species cockroach very seldom found in Europe (Fig. 5). Nematodes of the type under consideration were found in the stomachs of no less than 40 out of 61 wild brown rats from this refinery, and in 18 of these there were signs of pathological changes in the stomach fundus. In nine cases these were in the form of very extensive papillomatous neoplasms.

I began on fresh feeding experiments, now using cockroaches of the Peri*planeta americana* type from the sugar refinery, and these gave positive results. Nematodes of the type I was seeking appeared in 54 out of 57 rats fed on this species of cockroach, and of these 37 had stomachs exhibiting epithelial proliferation and papillomatous changes. Seven of the rats had large, papillomatous tumours.

I had thus been successful in tracing Nematodes and papillomatous tumours of the type under consideration in rats, and this had been achieved by feeding the animals on cockroaches from a given locality so as to infect healthy rats with Nematodes and to bring about the formation of neoplasms in their stomachs.

Fig. 6. Mature eggs of *Spiroptera neoplastica* (x 280 approx.).

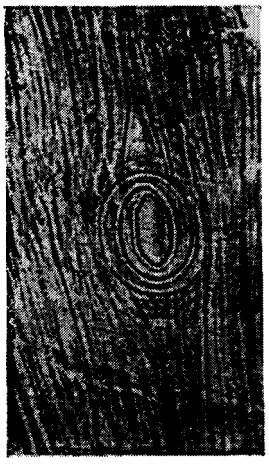

Fig. 7. Coiled Spiroptera larvae in cockroach muscles.

Further research now showed that the larvae of the Nematodes were not, like the Filaria of Galeb, contained in the fatty bodies of the cockroaches but in the cross-striated muscle, and that their development was on the following pattern: after the infected cockroaches (or only their muscles) are

eaten by the rats, the larvae are liberated and these invade the upper parts of the rat's alimentary canal, which is lined with pavement epithelium - the mouth lining, tongue, oesophagus and stomach fundus. Here they develop further, reach maturity and after about 45 days start to lay eggs, which are surrounded by a double membrane and contain embryos (Fig. 6). The eggs are evacuated with the rat's faeces. When a cockroach feeds on rat faeces containing these eggs, or on the Nematode eggs by themselves, it liberates the embryos which then penetrate into the cockroach's muscles and continue to develop. After 5-6 weeks they appear as Trichina-like, spirally-coiled larvae encysted in a thin capsule (Fig. 7). This completes the Nematode's development in the host and intermediate host.

Research work carried out by Hjalmar Ditlevsen, a member of the Staff of the University Zoological Museum, indicated that these Nematodes did not match the Galeb worm, but had rather to be added to the Spiroptera as a new species which had not so far been described. It was given the name *Spiroptera neoplustica,* which has since been changed to *Gongylonema neoplasticum.* In its fully developed form the male is ½-1 cm long, with a diameter of 0.1-0.16 mm (Fig. 8). The female is 4-5 cm long with a diameter of 0.2-0.25 mm (Fig. 9).

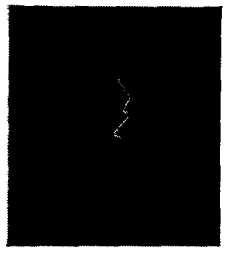

Fig. 8. *Spiroptera neoplastica*- male (natural size).

Fig. 9. *Spiroptera neoplastica*- female (natural size).

The presence of the Nematode in cockroaches and rats from the sugar refinery which I have mentioned, which had earlier received consignments of raw material from the West Indies, suggested that it must be a species coming originally from the tropics; when tests carried out on rats and cockroaches gathered in what used to be the Danish West Indies succeeded in showing the Nematodes, and when research done by others had the same result, it strengthened me in my belief that their proper habitat is in tropical countries from which they are brought into Europe with rats or cockroaches.

A fully developed papilloma in the stomach fundus was not only observed, as I have described, in wild rats from the sugar refinery, but also in piebald laboratory animals which were fed with Spiroptera-containing cockroaches in order to induce tumours experimentally. Five of these rats developed, in addition to papillomata, quite typical invasive squamous-celled carcinoma (Figs. 10, 11 and 12) which in two of them was accompanied by metastases; in one animal this affected the lung (Fig. 13), while in the other a lymph gland was involved (Fig. 14).

The way was now open for further research into the possibility of the planned, systematic inducement and close study of neoplasm and the development of cancer, and experiments along these lines were begun in the fol-

Fig. 10. *Spiroptera* carcinoma in stomach fundus of brindled rat fed on *Periplaneta americana* (natural size).

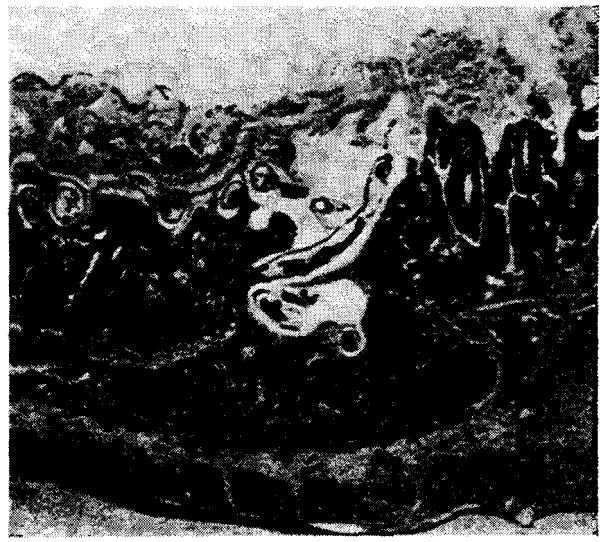

Fig. 2. Part of stomach fundus wall of brindled rat fed on Spiroptera, showing Spiroptera and heterotopic and incipient invasive epithelial growth (highly magnified).

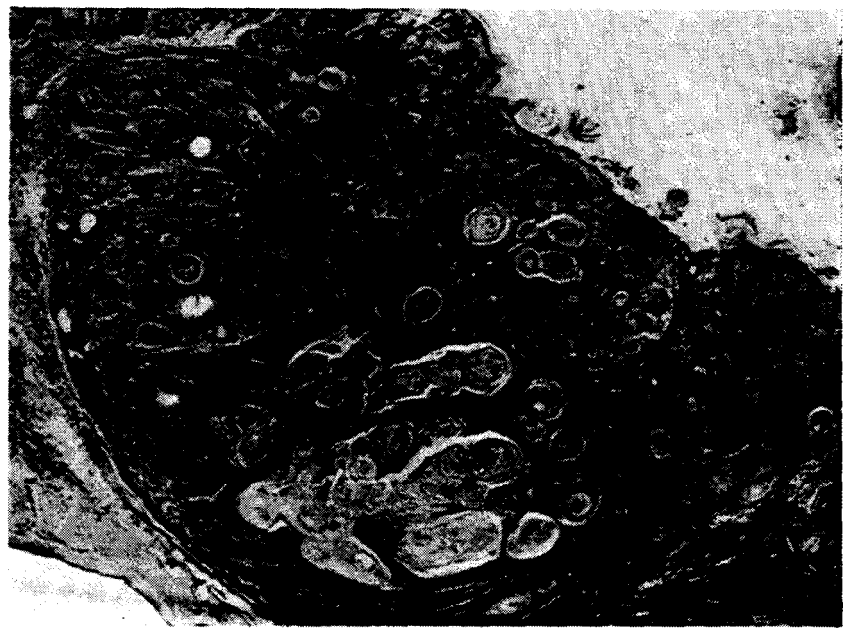

Fig. 12. *Spiropters carcinoma* in fundus of brindled rat fed on *P. americana (highly* magnified).

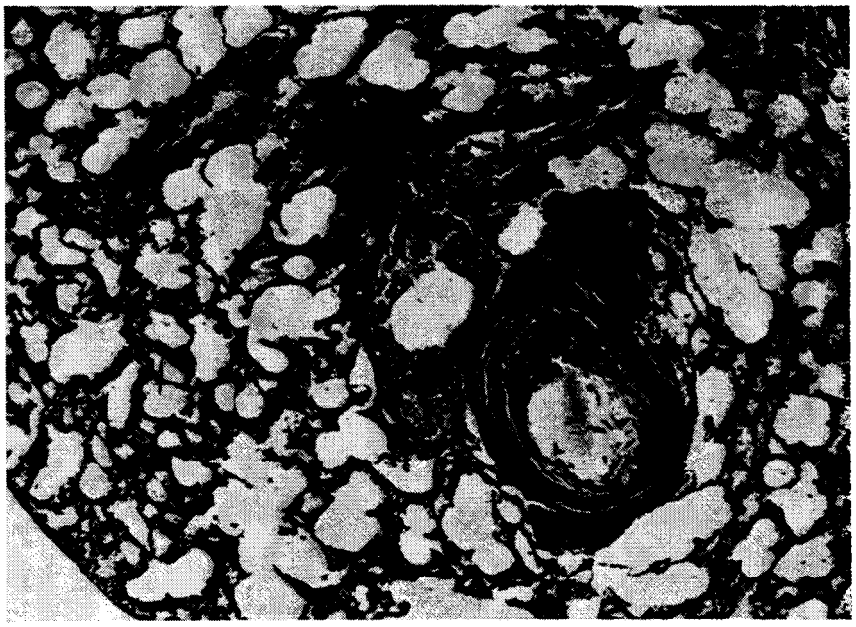

Fig. 13. Lung metastasis from *Spiroptera carcinoma* of stomach in brindled rat (highly magnified).

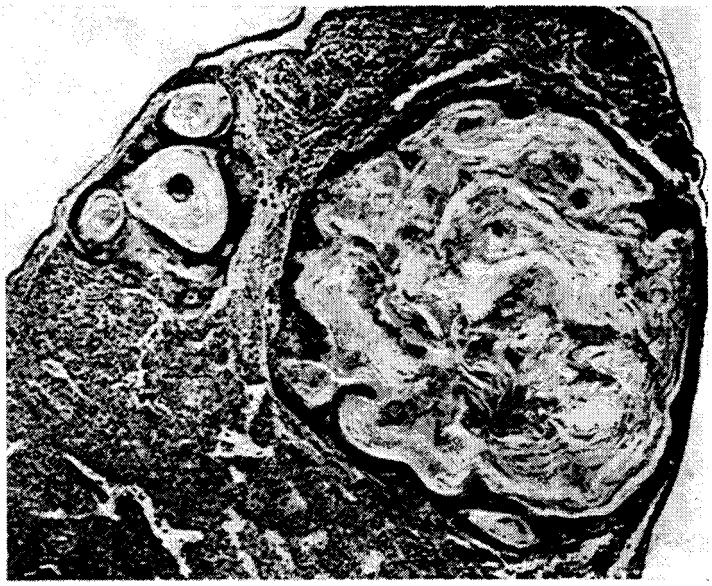

Fig. 14. Metastasis in retroperitoneal lymph gland from *Spiroptera carcinoma* of stomach in brindled rat (highly magnified).

lowing year. Various species of rats and mice were used, most of them being piebald laboratory animals. The Gongylonema were transmitted partly by feeding the rats on cockroaches which had been infected by feeding on rat faeces containing the eggs, partly by feeding them only cockroach muscle containing larvae, and finally by means of direct injection into the rat's stomach of Gongylonema larvae prepared from muscle.

It was observed that the Gongylonema could not only live parasitically in rats and mice, but could also be transferred to the pavement epithelium-lined section of the upper alimentary canal in guinea pigs, rabbits, hedgehogs, and squirrels. The common cockroach and the flour-mite, *Tenebrio molitor,* may serve as intermediate host as well as *P. americana.* My tests on the Gongylonema were carried out mainly on their natural host, *P. americana,* which were bred at the Institute for this purpose.

The experiments showed that in the stomach of a rat infected with a single Gongylonema, or with a small number, only epithelial hyperplasia or a slight inflammation occurred. If, on the other hand, the infection involved a large number of Gongylonema, then there was pronounced proliferation of the epithelium and of the connective tissue of the mucous membranes, accompanied by heterotopic growth at depth and the formation of papillomata. In the most pronounced cases there were deep-seated epithelial crypts, which might affect the entire stomach wall, and massive papillomata which could completely block the stomach lumen. In a number of animals squamous-celled carcinoma developed as well, typified partly by pronounced cornification with atypical cell formation and bulbous epithelial formations, partly (and particularly) by invasive growth penetrating the mucous membranes and muscularis mucosae and reaching down into - or through - the submucosa.

Desquamative inflammation and epithelial hypcrplasia occurred in the mouth, ocsophagus, and on the tongue. Characteristic carcinoma may be found on the tongue (Fig. 15, normal tongue; Figs. 16-18, *Spiroptera carcinoma* of the tongue).

The *Spiroptera carcinoma* is accompanied by metastases, localized mainly in the lungs; here they are, however, frequently only to be found by means of microscopic examination of serial sections. Metastases were discovered in six out of 33 brindled rats whose lungs were examined in this manner; in only one instance have I observed a gland metastasis. The structure of the metastases resembles exactly that of the primary growth, they never contain either Spiroptera or eggs, and it is only the proliferation of the cells without

any contribution by the parasite which indicates that metastases (in the usual sense of the term) are involved.

By and large, the stage of development of the carcinoma is proportional to the length of life of the rats after the transmission of the Spiroptera. Among piebald laboratory rats the earliest observation of carcinoma was 45 days later.

5. Normal rat's ton

Fig.15. Normal rat's tongue.

roptera carcinoma in r

Fig. 16. *Spiroptera carcinoma* in rat's tongue.

Fig. 17. *Spiroptera carcinoma* in rat's tongue (x 24).

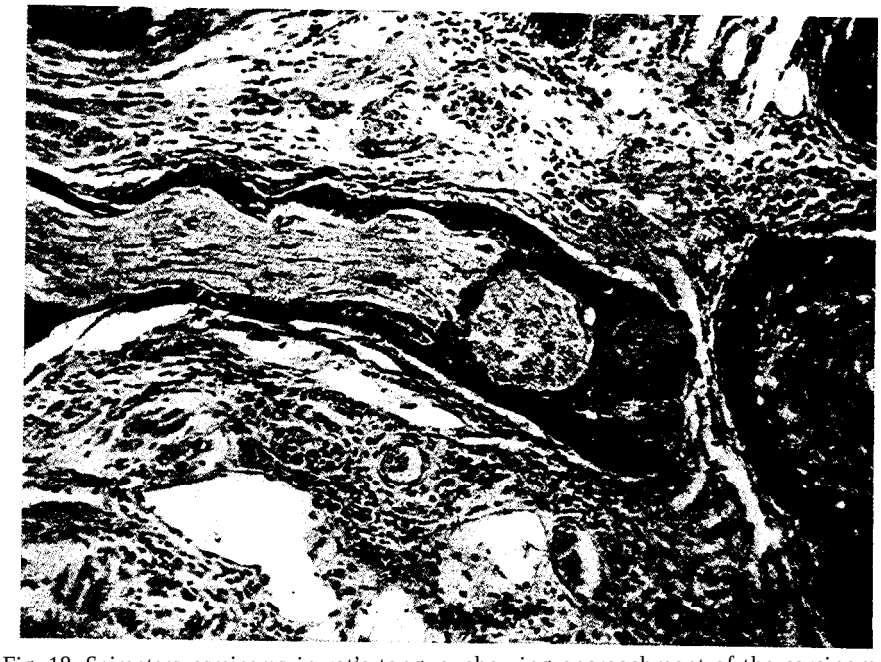

Fig. 18. *Spiroptera carcinoma* in rat's tongue, showing encroachment of the carcinoma into the lymphatic space surrounding a nerve (x 130).

Fig. 19. Primary *Spiroptera carcinoma* in stomach fundus of white mouse (x 50).

The transfer of Gongylonema also produced changes in wild rats *(M. decumanes),* black rats *(M. rattus),* white mice, grey mice *(M. musculus),* and field mice *(M. sylvaticus),* and these were in most instances of the same type. In white mice I found instances where the carcinoma had completely penetrated the stomach wall (Fig. 19) and in one case had led to its perforation. In one mouse the lungs contained a metastasis the size of an orange pip, while in another enormous metastases were found in the peritoneum and in the abdominal glands (Fig. 20).

I should finally like to emphasize that *Spiroptera carcinoma* is transplantable, this having been done during the study of the disease in a mouse (Figs.21 and 22). It was possible to transplant the tumour through four generations in the course of a year.

We may thus summarize the results of the experiments I have described in the following terms: the carcinoma produced by *Gongylonema neoplasticum* is, in its structure, a typical epithelioma, or squamous-celled carcinoma; it is invasive and destructive in its growth, produces metastases and may be transplanted. Carcinoma of the stomach was produced experimentally in brindled rats in more than 100 cases, and carcinoma of the tongue in seven cases. As an illustration of the incidence of carcinoma of the stomach in the piebald rats, I might mention that out of 102 of these animals which survived the transmission of Spiroptera by 45 days or longer, more or lesspro-

nounced stomach carcinoma developed in 54 - that is to say, rather more than half.

I shall return to the appearance of carcinoma in other rats and in mice at a later stage.

Before I go on to deal with the results of these experiments in greater detail, it ought first to be mentioned that this research gave experimental proof of the correctness of Barrel's theory that worms, like certain other animal parasites, possess the ability to produce neoplasms. Research similar to mine later provided further proof of this. In 1921, for example, the work of Bullock and Curtis (whose experiments covered a very wide scope) showed that the sarcoma which Barrel and others had described as occurring in the liver of rats when it has been a site for the cysticercus of *Tænia crassicollis* may be induced experimentally by feeding rats with the eggs of this Taxia. It was these experiments which provided us with the possibility of a systematic, experimental inducement of *sarcoma.*

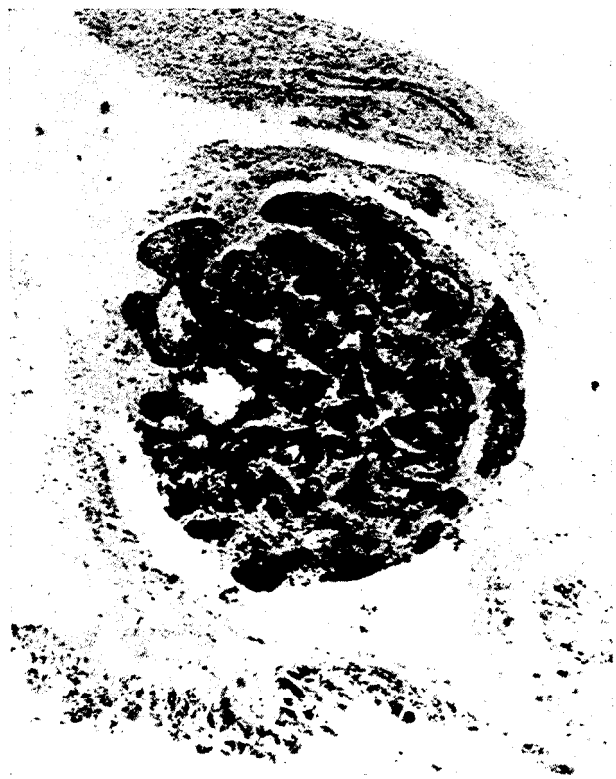

Fig. 20. Peritoneal lymph gland metastasis from the tumour in Fig. 19.

Fig. 21. Mouse with subcutaneously transplanted *Spiroptera carcinoma,* 84 days after transplantation - 1st generation.

In very recent times (1925) Yokogawa has published details of experiments in Formosa which have led to the discovery of a new type of Gongylonema known as G. *orientale,* which from the morphology viewpoint closely resembles G. *neoplasticum;* this, again, lives as a parasite in the stomach of rats, and makes use of cockroaches *(P. americana, P. australasiæ)* as an intermediate host during its development. Feeding rats on cockroaches whose muscles contained the larvae of the parasite showed that these Gongylonema, too, can bring about stomach carcinomas.

This therefore removes any shadow of a doubt that the Helminthes must be included among the causative agents of cancer, and the quite considerable number of studies and observations in which Nematodes and other Helminthes were shown to be present in various kinds of benign and malignant neoplasms must now be considered in a rather different light from formerly; although the possibility of fortuitous coincidence cannot, of course, be entirely ruled out, there are nevertheless grounds for suspecting that in such cases the parasites can have a pathogenetic significance. It would take too

long to go here into all the observations of this kind which have been made, and I shall therefore mention only the Helminthes which must be assumed to play a greater or lesser role in the development of tumours and cancers in human beings.

As we have already mentioned, *Schizortomum hæmatobium's* aetiological importance in the development of cancer of the bladder must be considered as proven. Nor can it be doubted that other Trematodes, such as *Opistorchis felineus*, *Schizostomum japonicum* and *Clonorchis sinensis* can, in certain cases, bring about primary carcinoma of the liver, and that *Schizostomum Mansoni* can be the cause of polyps and carcinoma in the colon.

In a number of cases, too, it has been found that the presence of *Echinococci* is accompanied by a primary carcinoma of the liver.

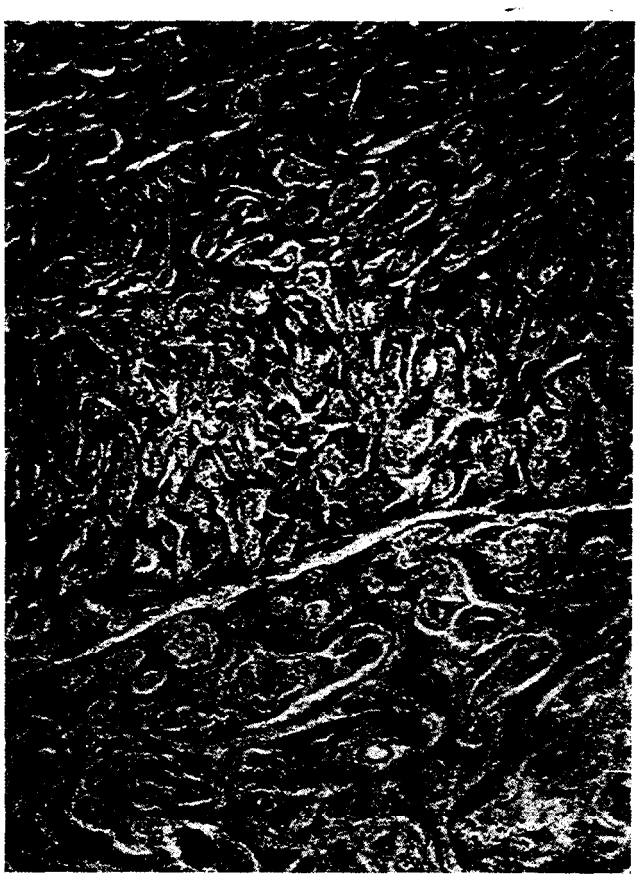

Fig. 22. Transplanted *Spiroptera carcinoma-2nd* generation (x 50).

In connection with the significance of the Nematodes, we must mention the statement by the American cancer research worker Ewing, that he has fairly frequently found Trichina in cases of carcinoma of the tongue; in Europe, too, there have been a whole series of reports of carcinoma in organs lying close to trichinous muscles in humans suffering from chronic trichinosis, in particular the breasts.

So far as can be ascertained, *Gongylonema neoplasticum* is never found in humans. On the other hand, four reports from America and one, or possibly two, from Italy indicate that another type of Gongylonema, probably G. *pulchrum,* can live in the mucous membrane of the human lips and tongue; this type is normally parasitic in the tongue and oesophagus of the pig and uses dung-beetles *(Aphodius* and others) as an intermediate host. In all the patients under observation, however, there were only very slight changes in the mucous membrane; there were no instances of carcinoma or similar processes, and there was likewise no cancer of the oesophagus in pigs infected with this Gongylonema.

This seems therefore to suggest that the Helminthes which must be assumed to result in the development of cancer are quite few in number.

If we wish to come to a conclusion on this question we must, however, keep in mind the fact that experiments on *Gongylonema neoplasticum* have (as I shall explain later) proved that this parasite may leave the cancer tissue after producing the carcinoma, which then still continues to grow. In cases where tumours have been brought about by Helminthes it consequently cannot always be assumed that these will be present; in such cases the original aetiological role of the Hehninthes will be masked. This poses the possibility of a greater number of malignant neoplasms being due to such parasites than can be proved to be the case, and the part they play in the onset of tumours may in fact be greater than appears from our observations.

Even bearing this possibility in mind, we are nevertheless not justified in ascribing to the Helminthes any extensive aetiological role in the pathology of cancer in Man.

Nor can the endemic appearance of *Spiroptera carcinoma* among rats coupled to our knowledge of the presence and effects of the Gongylonema in human beings serve as a foundation for the theory, put forward in recent times, that the comparatively high incidence of cancer in the inhabitants of districts of buildings infested with rats and cockroaches may be caused by the transmission of a carcinogenic virus through *Gongylonema neoplasticum* or other Nematodes. Research on Spiroptera, again, does not give unqual-

ified support for the theories that cancer is in general ascribable to macro-parasites or microbes.

All things considered, the present state of our knowledge allows us to allot the Helminthes only a modest place among the causes of neoplasms among humans, a fact that I stressed in my first work back in 1913.

In the course of the research work on *Spiroptera carcinoma,* it became possible for the first time to induce typical, metastasizing carcinomas systematically and at will. This provided *experimental proof* that the start of a cancer can, in agreement with the theory of Virchow, be brought about by external, exogenic influences, and lent support to experiments on the effects of long-term irritants of other kinds.

Experiments of this nature were first undertaken by Yamagiwa and Ichikawa, who chose coal-tar as the irritant; the carcinogenic effects of this had been observed in clinical practice, but in previous experiments had yielded only negative results. Works by Yamagiwa and Ichikawa over the years 1915-1918 however, proved, as a principal result, that a monthly application of coal-tar to the ears of rabbits was able to produce a skin cancer. Tsutsui reported, in 1918, that painting tar on the skin of the mouse would produce the same effect, and these experiments were taken further by Fibiger and Bang who were able, in 1920, to corroborate and supplement Tsutsui's results. This laid the foundation for a method of inducing cancer at will which is now in use in laboratories all over the world.

As I continue my account of the information which the study of *Spiroptera carcinoma* has contributed to our knowledge of the pathology of cancer, I shall add and compare some of these to the results which came from research on experimental tar cancer.

Before doing so, however, I should mention that the effect of the Gongylonema is generally taken to be due to their constant production of toxic substances; I originally suspected this through the analogy with the well-known fact that a number of the Helminthes exude pathogenic secretions.

A continuous excretion of this sort is not, however, essential for a carcinoma, once it has developed, to continue to grow. As tests showed, the growth of the fully developed carcinoma continues irrespective of whether the Gongylonema leave the tumour tissue or not; this is exactly analogous both with the continued growth of experimental tar cancer after the cessation of painting-on the tar, and with clinical observations that the development of chimney-sweep's cancer and cancers caused by aniline and X-rays

does not come to a halt because the patients are removed from the influences which have given rise to the disease.

The fact that in a number of cases the *Spiroptera carcinoma* could be produced both on the tongue and in the stomach provided experimental proof that multiple carcinomas in the alimentary canal can be due to the formation of a primary cancer in different sites, stemming from the same cause but otherwise entirely independent. The experiments furthermore made it possible to confirm that in its earliest stages a carcinoma develops pluricentrically, starting from separate and well-defined small groups of cells or perhaps even from individual cells, and that its continued development is through *expansive, non-appositional* growth, in line with the view put forward earlier by Ribbert and others to explain the development of a carcinoma.

No proportional relationship was found between the appearance of carcinoma and the degree of development of epithelial hyperplasia and heterotopic growth at depth. In rats which had survived the transfer of Spiroptera by a long period the wall of the stomach could be the site of deeply penetrating cell masses or large retort-shaped epithelial cysts without a carcinoma having developed, and contrariwise carcinoma might develop in other animals starting from epithelium exhibiting only moderate and non-heterotopic hyperplasia. Again, there was no correlation to be found between the formation of a carcinoma, the development of inflammation and papillomata. Violent development of papillomata was not necessarily accompanied by carcinoma, and carcinoma could develop without papillomata.

Accordingly neither heterotopic benign epithelial proliferation nor papillomatosis need necessarily be preliminary stages of carcinoma; development of carcinoma follows as a *characteristic process* upon the hyperplastic growth of epithelium, irrespective of whether this is slight or pronounced, heterotopic or not, and whether at the same time as the epithelial hyperplasia there is substantial inflammation, proliferation of connective tissue and papillomatous changes, or whether there is no papilloma and only slight inflammation. This is not so say that there is never any relation between inflammation and carcinoma, but only that inflammation is not an essential factor in the genesis of the carcinoma and cannot unequivocally be regarded as a process which is absolutely essential for the onset of a cancer. If inflammation is present, it can most probably be seen as an accompanying phenomenon in the development of the cancer or as a secondary process which may even, particularly when it is far-developed, be regarded as having a defensive character and in its most pronounced forms (e.g. in mixed infec-

tion) can bring the cancer to decompose almost completely; this may be observed in cases of experimental tar cancer in the mouse. As you will know, Murphy and other workers even regard the lymphocyte reaction during cancer as being a significant part of the processes involved in general in the resistance to cancer. This is, however, a problem which can hardly be considered as finally resolved.

The disproportion between heterotopic epithelial hyperplasia, inflammation and papilloma formation on the one hand and the development of carcinoma on the other appeared especially clearly in the effects of the Gongylonema upon wild rats *(M. decumanus* and *M. rattus),* white mice, domestic *mice (M. musculus)* and field *mice (M. sylvaticus).* These can all survive the transfer of Spiroptera for as long a period as the brindled laboratory rats, and frequently even longer. The development and biological state of the Gongylonema, furthermore, seem to be entirely unaltered during their parasitic existence in these various rodents, in whose stomachs they give rise to substantial changes of exactly the same sort as those observed in brindled rats, and sometimes more extensive still.

Nevertheless *Spiroptera carcinoma* develops much less frequently in these animals than in the brindled rats, among whom - as I have said - stomach cancers developed in more than half of those who survived the transfer of Spiroptera by 45 days or longer. During experiments undertaken in collaboration with C. Krebs carcinoma was found in the stomach on only one out of 38 black rats *(M. rattus)* and only 11 out of 34 brown rats *(M. decumanus).* Among 56 white mice who, like all these rats, survived the infection with the parasites for at least 75 days and often very much longer, only three were found to have carcinomas. Despite numerous attempts, it was not possible to induce *Spiroptera carcinoma* in field mice and domestic mice.

Thus the development of a cancer following the same exogenic influence, and under the same conditions, does not always occur within the same period of time in all animals of the same species; what is more, it occurs with varying frequency among animals of different, though closely related, species.

This gave an experimental indication of a varying individual-and species-predisposition towards cancer, which was corroborated by later research on Cysticercus *sarcoma* and experimentally induced tar cancer. I will content myself here with mentioning that (so far as I know) it has only been possible to produce tar cancer on the skin of the rat in one single case, despite numerous attempts in various laboratories.

Tests on this made by Paul Møller in my Institute yielded only negative results, although the tar used for these would produce cancer in all the mice surviving by one month a q-month course of painting. On the other hand, all the six most long-lived rats (who died only some 10-15 months after the course of painting with tar) developed a *primary carcinoma of the lung.*

This notion of a predisposition to cancer therefore not only applies to a differing individual-, race- or species-predisposition to develop the same form of cancer after the same carcinogenic effect; we must assume that it can vary within the same species as regards differing carcinogenic factors. Tar cancer develops quite easily among mice who will only contract *Spiroptera carcinoma* with the greatest difficulty, although both tar and Gongylonema produce a carcinoma of identical structure. The experiments of *Spiroptera carcinoma* also provided an indication of the existence of a varying predisposition among the organs, since, although I was never able to observe an incipient or extant carcinoma in the oesophagus of either rats or mice, the epithelium of this organ does not differ from that of the stomach fundus, and in the majority of the animals it contained numerous Gongylonema and was often the site of a pronounced and sometimes heterotopic epithelial hyperplasia. Yokogawa obtained exactly the same results with rats infected with *Gongylonema orientale.*

On the whole it seems, as Bashford had suspected as far back as 1912, that there may very likely be special predispositions, but no common general predisposition to all forms of cancer, to cancer in all organs or to all carcinogenic influences.

At the present time it is impossible either to make an overall, confident assessment of the influences to which these predispositions must be attributed, or to weigh up the importance of the special factors, the effects of which upon susceptibility towards cancer are discussed and studied in recent works: endocrine secretion, the significance of the spleen, the composition and salt, lipoid or vitamin content of the diet, etc., the effects of pregnancy, various diseases, the iso-haemagglutinin content of the blood, inherited and constitutional characteristics of various kinds, and so on.

I will discuss only one factor: old age, the considerable importance of which as a generally predisposing characteristic towards all forms of cancer has long been a universally accepted doctrine. This doctrine has not, however, found confirmation in a series of experimental works. Even my very early experiments indicated that *Spiroptera carcinoma* appeared with the same facility in both young and old rats, and later work done in my Institute by

Fridtjof Bang (as well as experiments carried out elsewhere) proved that in like conditions young mice contracted tar cancer just as frequently and just as rapidly as did old ones. It is possible to point to clinical experience, too, which bears out these experiments showing that it is the time at which the effect of the carcinogenic influence begins (and the nature, duration, and intensity of this effect) which determines the time of life at which cancer develops, rather than any particular age-predisposition.

As you will know, clinical experience of chimney-sweep's cancer and aniline cancer has made it clear that the appearance of a demonstrable and morphologically typical cancer need not be the immediate consequence of a carcinogenic influence - it may only appear after this has ceased to have any effect and after a certain period (which may be quite lengthy) has elapsed; conclusive proof of the existence of such a period of latency was first provided by research work on tar cancer, and in particular that of Fridtjof Bang and of A. Leitch.

I have attempted briefly to outline the most important results obtained from experiments on *Spiroptera carcinoma.*

Of the later methods for the experimental induction and study of cancer, that of painting mice with tar has so far shown itself to be the most suitable. We still do not know the specially active ingredient (or ingredients) in this highly complex substance, but we have incontrovertible proof that all types of coal-tar possess this property of inducing cancer in rabbits and mice. Tar has also shown itself, however, to be capable of causing malignant neoplasms in the lungs, breasts, testes, stomach, and other organs, and it will thus be possible to make use of the experimental induction of tar cancer in the pathological study of a number of forms of the disease.

In research on therapy, too, experimental work will provide an invaluable, and indeed indispensable, foundation. As an example of this I might mention a series of experiments carried out by Paul Møller and myself on the prevention of metastasis in cancer cases.

These were done on mice suffering from cancer of the skin, induced by tar painting. As an immunizing measure we employed subcutaneous injections of an emulsion made from sterile, living skin from a mouse foetus; earlier transplantation experiments had shown this to be effective in preventing the development of transplanted tumour tissue. The tests covered in all 293 mice, kept under identical conditions, who had a small area of the skin of the back treated every other day with the same coal-tar and in the

same manner over a period of four months. The skin emulsion was injected into 156 of these animals from 2-7 times, while the remaining 137 mice acted as a control. Skin cancer was contracted by 127 (81%) of the treated animals and 102 (74.4%) of the controls, but metastasis occurred in only 38 (i.e. approx. 30%) of the treated mice as against 59 (about 58%) of the control animals.

This immunization treatment had thus, like earlier results, shown itself to be ineffectual in preventing primary cancer formation, although reducing the formation of metastases by about 50%.

There is consequently no doubt that the formation of metastases can be inhibited by this method, and I might add that after proper statistical checking these results have proved quite indisputable. I need not emphasize, therefore, that only further experiments covering a wider scope can show what methods can be evolved on the basis of our results, to serve as a therapeutic measure to combat cancer in Man. These methods will have to be free of the technical difficulties which affected ours; these included the fact that the tissue had not only to be homologous and sterile but also living, in order to be certain of avoiding the danger of hypersensitivity to cancer development which it had been shown could result from the injection of dead tissue. A detailed explanation of the processes by which metastasis is inhibited has still not been possible.

Other influences than those I have mentioned here have formed the subject of research by other workers in recent times, and have proved capable of inducing cancer. A point of interest in the study of occupational cancers is that it has been proved that carcinoma of the skin in mice can be produced not only by tar, but also by other products of the distillation and combustion of coal-soot, pitch, the paraffin oils and similar substances. Long-standing observations of experimentally induced X-ray sarcoma have been supplemented by recent reports of carcinomas from the same cause. The effect of traumata has been proved by tests of various kinds; of special importance is research showing the development of cancer in the gall bladder of the guinea-pig following on the penetration of gall-stone fragments or other foreign bodies into this organ.

Of even greater interest is the experimental work done by Carrel. I can only mention here the most important results of this, which show that by means of the inoculation of embryonic fowl tissue into fowls accompanied by the injection of extremely dilute solutions of tar, arsenic or indole (benzopyrrole) it is possible to induce rapidly growing and highly malignant, me-

tastasizing and transplantable neoplasms identical to the Rous sarcoma; like the latter, these can be transmitted to healthy fowls by theinoculation of cell-free filtrates of the tumour tissue. Moreover, the filtrate is able, *in vitro,* to bring about the conversion of the blood macrophages into malignant tumour cells, and it has furthermore been shown by Fischer that a similar conversion can be brought about by the cultivation of spleen macrophages in a nutrient medium to which a small quantity of arsenic acid has been added.

Carrel's work has thrown new light on a number of problems. First and foremost, it is now difficult to uphold the theory that the Rous sarcoma must be attributed to an invisible virus, since sarcomas of exactly the same type were seen to be produced during these experiments by the effects of chemically unorganized compounds upon embryo tissue. The carcinogenic property of the tissue filtrate must therefore be assumed to depend upon the effects of a *cellular product,* possibly an enzyme.

These tests also provided support for further studies on the formation of malignant tumours following the inoculation of embryo tissue, and can also be seen as corroborating Cohnheim's theory. The conversion of the macrophages into tumour cells also suggests that neoplasms can develop from non-differentiated cells as well as embryo cells, and that foreign substances introduced into the organism can produce tumours in embryonic tissue; finally, it indicates that the formation of neoplasms does not depend solely upon local factors, but also on others originating from the organism generally.

It is however difficult at present to know how much importance can be attributed as a whole to the results of Carrel's work on the aetiology and pathogenesis of tumours, partly because the neoplasms he induced developed only from embryo rather than adult tissue, and that exclusively in fowls, and partly because in all instances they belong to the much discussed Rous type of sarcoma; the classification of this type alongside the sarcomas affecting mammals and human beings is problematical, and it is not recognized by many pathologists.

Recent work done by Askanazy has shown that the effect of arsenic is also able in the rat to foster the development of teratoids from inoculated embryonic tissue, and that there can be a simultaneous formation of sarcomas. If further experiments on mammals, involving entirely typical sarcomas and carcinomas, make it possible to obtain results matching those reached by Carrel, then the experimental study of tumours will have entered a new and fruitful era.

I will now close. The limited time at my disposal has permitted me to discuss only a few of the significant, principal results of modern cancer research, without mentioning either the vast number of other experiments which have been carried out, or all of the scientists who are working in this field. But what I have been able to tell you will have made it clear that the experimental induction of tumours in modern time has made it possible to realize that the causes of cancer include animal parasites and physical and chemical influences of various kinds, and that endogenic as well as exogenic factors must be regarded as playing a part in causing the disease.

Although even recent works attribute importance to microparasites and invisible viruses as causes of cancer, there has been no convincing evidence offered for the microbial origin of cancer in Man, and all theories of this kind must be regarded as based upon insufficient grounds.

The term cancer covers diseases of widely differing origins and of many different kinds, sharing the common feature of an uncontrolled, apparently autonomous, atypical and invasive growth of the cancer cells, with an ability to find a fertile site for development after metastasis in widely varying tissues in the organism. The anatomical and biological changes undergone by the cells in the course of their conversion into tumour cells, which give mature cancer cells their characteristic stamp, are far from well enough known; but the substantial improvements which have been made in the past few years in the methods of cultivating tissue *in vitro* have opened up new possibilities for studies of this kind, and have - as will be evident from what I have been telling you here - already given promising results. Warburg's work on the metabolism of cancer tissue, too, has meant a useful increase in our knowledge; I would mention only the very great importance which his experiments have taught us to attribute to glycolysis in the metabolism of the cancer cell.

The study of the manifold problems presented by cancer has, in recent years, seemed to offer many more riddles than were previously thought to exist; but the history of medicine has never known a period in which problems could be attacked in so many different ways as those made accessible today by the working methods now at our command.

Publications on Spiroptera carcinoma by Johannes Fibiger

1. *Recherches sur un nématode et sur sa faculté de provoquer des néformations papillomateuses et carcinomateuses dans l'estomac du rat,* Académie Royale des Sciences et des Lettres de Danemark, 1913.
2. <<Über eine durch Nematoden *(Spiroptera* sp. n.) hervorgerufene papillomatöse und carcinomatöse Geschwulstbildung im Magen der Ratte>>, *Klin. Wochschr.,* (1913); <<Undersøgelser over en Nematode *(Spiroptera* sp. n.) og dens Evne til at fremkalde papillomatøse og carcinomatøse Svulster i Rottens Ventrike1>>, *Hospitalstidende,* (1913).
3. <<Untersuchungen über eine Nematode *(Spiroptera* sp. n.) und deren Fähigkeit, papillomatöse und carcinomatöse Geschwülste im Magen der Ratte hervorzurufen>>, *Z.Krebsforsch.,* 13 (1913).
4. <<Sur le développement de tumeurs papillomateuses et carcinomateuses dans l'estomac du rat sous l'action d'un ver nématode>>, *Troisième Conférence Internationale pour l'Étude du Cancer, Bruxelles,* 1913.
5. In collaboration with Hj . Ditlevsen: <<Contributions to the biology and morphology of *Spiroptera (Gongylonema) neoplastica* sp. n.>>, *Mindeskrift for Japetus Steenstrup,* (1914).
6. <<Weitere Untersuchungen über das Spiroptera carcinom der Ratte>>, Z. *Krebsforsch.,* 14 (1914) ; <<Fortsatte Undersøgelser over Spiroptera carcinomet hos Rotten>>, *Hospitalstidende,* (1914).
7. <<Über Disposition der Ratten und Mäuse für *die* Wirkung der *Spiroptera neoplastica>>, Zentr. Allgem. Pathol. & Pathol. Anat., 27* (1916).
8. <<Undersøgelser over *Spiroptera neoplastica's* Indvirkning paa Mus>>, *Foredrag paa Naturforskermødet i Kristiania,* 1916.
9. <<Investigations on the Spiroptera Cancer, III: On the transmission of *Spiroptera neoplastica (Gongylonema neoplasticum)* to the rat as a method of producing cancer experimentally,, *Kgl. Danske Videnskab. Sefskab, Biol. Medd.,* I, 9 (1918).
10. <<Investigations on the Spiroptera Cancer. IV: Spiroptera cancer of the tongue>>, *Kgl. Danske Videnskab. Selskab, Biol. Medd.,* I, 10 (1918).
11. <<Investigations on the Spiroptera Cancer. V : On the growth of small carcinomata and on predisposition to Spiroptera Cancer in rats and mice>>, *Kgl. Danske Videnskab. Selskab, Biol. Medd.,* I,11(1918).
12. <<Investigations on the Spiroptera Cancer. VI: A transplantable Spiroptera carcinoma of the mouse>>, *Kgl. Danske Videnskab. Selskab, Biol. Medd.,* I, 14 (1919).
    <<Sur la transmission aux rats de la Spiroptera neoplastica (Gongylonema neoplasticum)>>, *Compt. Rend. Soc. Biol.,* (1920).
    <<Carcinome spiroptérien de la langue du rat>>, *Compt. Rend. Soc. Biol.,* (1920).
    <<Sur l'evolution et la croissance du carcinome spiroptérien>>, *Compt. Rend. Soc. Biol.,* (1920).
    <<Recherches sur le carcinome spiropterien de la souris blanche et sur sa transplantabilité>>, *Compt. Rend. Soc. Biol.,* (1920).
13. <<On Spiroptera carcinomata and their relation to true malignant tumours; with some remarks on cancer age>>, *J. Cancer Research,* 4 (1919).

14. <<Untersuchungen über das Spiropterakarzinom der Ratte und der Maus>>, Z. *Krebsforsch.,* 17 (1919).
15. <<Recherches sur la production expérimentale du cancer chez le rat et la souris>>, *Bull. Assoc. Franc. Etude Cancer,* (1921).
16. <<Le cancer spiroptérien et les autres cancers à parasites animaux>>, *Bull. Assoc. Franc. Etude Cancer, (* 1923) .
17. <<Nieuwere onderzoekingen over den kanker>>, *Ned. Tijdschr. Geneesk.,* Eerste Helft, No. 14 (1923).