Experimental Study of the Pathogenesis of Carcinoma

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The problem of the etiology of tumors, and in particular of carcinoma, has been investigated for a great many years, but has not yet been solved. Many suggestions have been offered, among which Virchow's and Cohnheim's are especially prominent; both of these, however, were the result of clinical experience rather than of experimental study; hence, the adherents of these hypotheses have tried subsequently to establish them by means of experiments, the object of which has been artificially to produce carcinoma. But all attempts have proved negative, until Fibiger, of Copenhagen, finally succeeded in producing papilloma and carcinoma in the stomach and the esophagus of rats by infecting them with spiroptera; the origin of these new growths he referred to irritation of the mucous membrane of the stomach and the esophagus by the parasite. Thus the soundness of Virchow's irritation hypothesis has been demonstrated experimentally for the first time.

To this problem we also have devoted ourselves for four years, pursuing experiments in accordance with Yamagiwa's view, which he has expressed as follows: The repetition or continuation of chronic irritation may cause a precancerous alteration in epithelium previously normal. If the irritant continues its action, carcinoma may be the outcome, even though no specific agent has been interpolated.

For our experiments, we have employed mainly the ear of the domestic rabbit, an organ in which no spontaneous new growth has ever been reported. Among the methods of mechanical or chemical irritation chosen, the painting of coal-tar upon the inner surface of the ear has been the most efficacious in producing carcinoma.

The investigations are being continued, but we wish to report here a summary of the results up to June 30, 1916.

We have tried various methods of irritating the epithelium and subcutis, and with each of them have produced, as Fischer, Haga, Jores and others have done, an atypical growth of the epithelium; but of all the methods, painting with coal-tar was most effective, as has been said, and it had the additional advantage, furthermore, of being a simple procedure.

It was hoped that epithelium which had once begun a career of atypical growth would proliferate more and more atypically as the applications of coal-tar were continued, until at last it acquired a true malignant character; for the occurrence of carcinoma in coal-tar workers has become well known since the inves-
tigations of Vollmann and Liebe,15 Tillmanns,16 and Schuchardt;17 and while coal-tar carcinoma has not yet been experimentally produced, it is nevertheless conceivable that one might succeed if suitable methods of applying this irritant could be discovered.

Only Cazin18 and Bayon19 appear to have employed coal-tar for an experimental investigation of the pathogenesis of carcinoma. The former brushed this material upon the inner surface of a dog’s ear, while the latter injected hypodermically a mixture of watery extract of coal-tar and lanolin; neither, however, produced more than a temporary atypical growth of the epithelium.

The results of our own investigations with coal-tar have gradually approached our expectations; folliculoepitheliomata have arisen in nearly all ears after a period longer than 100 days from the beginning of the application, numbering in one instance 20 on a single ear. The size of the lesions varied from that of a grain of rice to that of a sparrow’s egg. Some lesions which developed later showed a more malignant character and it could be proved, in fact, both macroscopically and microscopically, that we had produced from them eight cases of carcinoma in its earliest stage, 16 cases of carcinoma in an early stage, and seven cases of still more fully developed carcinoma. Furthermore, we were able to demonstrate the presence of metastases in the lymph nodes at the root of the ear and in the submaxillary region in two cases among the last seven of carcinoma.

Clinical Course of the Lesions

We have divided the course of events into four periods: a first period, characterized by atypical growth of the epithelium (before the appearance of folliculoepithelioma); a second, marked by the appearance of folliculoepithelioma benignum; a third, in which folliculoepithelioma malignum (carcinomatous alteration in a folliculoepithelioma) arises; and a fourth period, that of metastasis.

First period: A typical growth of the epithelium. After applications of coal-tar had been begun, the rabbits appeared uneasy and the irritated ear soon became swollen. One week later, the epithelium, especially that of the hair follicles, appeared hyperkeratotic; cystic dilatation of the follicles set in, and keratin was retained in them. At the same time, also, the hair began to come out. These changes became gradually more distinct during the period of 30 to 360 days.

Second period: Appearance of folliculoepithelioma. About 50 days after irritation had been begun, we noticed certain circumscribed regions in which the epithelium of one or more hair follicles was hyperkeratotic to a high degree. These areas became gradually more and more elevated, and eventually developed into papillomatous new growths which were very variable in size and shape, though roughly divisible into two groups: pedunculated and sessile. The largest of the former attained approximately the size of a nut, some, however, being larger. In some cases, the interstitial connective tissue increased in amount, the growths then appearing like fibroepithelioma or fibroma. The sessile tumors measured over one cm. in diameter across the base. On the cut surface of all these growths there were visible a number of dilated hair follicles, in which keratin had accumulated. Many of the tumors continued to grow after the irritant had been withdrawn (provided it had been applied for a sufficiently long period), more and more of the horny substance accumulated in the dilated follicles, and finally these neoplasms came to resemble the cutaneous horns found in the human subject. If broken off at the base, new horns grew again at the same site or from the neighbouring epithelium.

Third period: Production of carcinoma.

In the course of continued application of
coal-tar to these new growths, the cut surface of some of them became so irregular that we could not differentiate macroscopically one hair follicle from another. The microscope showed a high degree of atypical proliferation of the epithelium (carcinoma in its earliest stage). The surface of these new growths then ulcerated, the tumors increased in size, and the walls of the ulcers gradually thickened, so that they came to resemble rodent ulcers. Microscopic examination disclosed an early stage of carcinoma. Certain of these growths continued to increase in dimension until they imitated even macroscopically the carcinoma of man. Thus our first and fifth cases presented elevated nodules which had, under the microscope, the appearance of a carcinoma more advanced than those described as being in an early stage. The second, fourth, sixth and seventh cases of carcinoma arose from sessile folliculoepitheliomata, while the third developed from a pedunculated tumor. The second, third, fourth, sixth and seventh cases of carcinoma were, both macroscopically and microscopically, the most typical carcinomatous in our series.

Fourth period: Metastasis. The third, fourth, sixth and seventh cases, among these seven examples of experimentally produced carcinoma, exhibited a swelling of the regional lymph nodes, and in the fourth and the sixth cases these nodes were proved by the microscope to contain metastatic deposits from the primary tumor. The nodes of the third case were the seat of a suppurative adenitis, yet it is conceivable that they may have contained carcinomatous metastases before the infection developed; those of the seventh have not yet been extirpated, but it may be supposed that they contain metastatic deposits, inasmuch as the primary tumor was more advanced than either of the two in which metastasis was definitely proved.

Microscopical observation demonstrated that the carcinomatous originated in folliculoepitheliomata, passing step by step through various stages until it reached that of completely developed carcinoma. Since the sequence of events was similar in all, we shall briefly describe here only the representative cases of carcinoma (third, fourth and seventh cases).

Third Case of Carcinoma
This growth arose on the inner surface of the right ear in a black female rabbit, about two years old, in which applications of coal-tar had been begun on December 15, 1914, and repeated every two or three days. Seventy-five days after the first painting, a pedunculated papilloma was found in the irritated area; this tumor grew slowly until, 111 days later it had become more and more hyperkeratotic, had assumed the sessile form, and had ulcerated. It spread along the surface and its edges thickened gradually, so that it came to resemble a rodent ulcer; but it seemed to invade the deeper tissues also, an area on the outer surface of the ear opposite to it becoming gradually elevated and finally ulcerating. After 130 days, the ear was perforated by an opening that soon became large enough to admit the little finger, and finally extended to the free border.

On the 151st day small pieces from the thickened wall of this perforating ulcer were removed for microscopical examination. The microscope showed that the new growth had already become malignant, resembling a carcinoma in its epithelial pearls and columns of epithelium; furthermore, there could be demonstrated the formation of thrombi in the veins, and a remarkable infiltration of the surrounding tissues.

The lymph nodes at the root of the affected ear began to swell after perforation had occurred, and reached the size of a large nut before the animal died. As they were the seat of suppurative aden-
itis, it is impossible to say whether or not they contained metastases.

The tumor in this rabbit continued to increase in size until the animal died from emaciation on the 180th day.

Fourth Case of Carcinoma

This is a case in which the occurrence of metastasis was proved.

The new growth arose on the inner surface of the ear of a two-year-old, black, male rabbit, weighing 2,560 grams at the beginning of the experiment (June 16, 1915). One hundred and thirty-three days later, we observed that a small papillomatous growth (the early phase of folliculoepithelioma) the size of a grain of rice, had arisen from a whitish spot on the irritated surface. On the 165th day its diameter measured 2.3 cm and its surface had become ulcerated. The ulcerated surface was elevated above the level of the surrounding epithelium, and the wall of the ulcer gradually thickened, so that the growth came to resemble macroscopically a rodent ulcer. Two-thirds of the tumor was removed, together with the ear cartilage and the epithelium of the opposite side (outer surface), and the growth was inoculated into the subcutaneous tissues of the back and the ear in 20 rabbits. Microscopical investigation of the extirpated sample proved that the neoplasm had already assumed the characteristics of carcinoma, the nodule exhibiting those features of "carcinoma in its early stage" which we shall describe in a following paragraph.

The remainder of the tumor, left in situ, showed an inclination to diminish in size for a while after the operation, but it gradually regained its dimensions and on the 273rd day (76 days after the operation, March 15, 1916) its diameter had reached 2.8 cm. At that time we observed a nodule on the outer surface of the ear in a situation corresponding to that of this growth on the inner surface; the new nodule, like the primary tumor, finally ulcerated. The irritant was then discontinued to see whether the tumors would continue to grow in its absence. This proved to be the case, for the nodule grew slowly, spreading along the surface, and the lymph nodes at the root of the ear began to swell; furthermore, the animal commenced to lose weight. On the 294th day both ulcers had increased still more in size, the regional lymph nodes had enlarged to about the size of a peanut, and emaciation had become even more noticeable.

On the 350th day the primary ulcer on the inner surface of the ear measured 5.2 cm. by 5.3 cm., and the secondary one on the outer surface 3.5 cm. by 4.5 cm. One of the lymph nodes at the root of the ear had attained nearly the size of a sparrow's egg, and the others the size of a peanut. On the 351st day these nodes were extirpated, and inoculated, in small fragments, into the subcutaneous tissue of the back in 10 rabbits. That portion reserved for histological examination contained no metastatic deposits, and the result of the transplantation was negative. On the 366th day (June 16, 1916) the animal died, extremely emaciated, its weight having been reduced almost to half the body weight at the time of the first coal-tar application. The tumor had continued to grow, and when the rabbit died the neoplasm was about twice as large as when the irritant was discontinued (on the 273rd day); its size was now 5.5 cm. by 6.5 cm., while the secondary nodule was 4.5 cm. by 5.2 cm.

At the postmortem examination, solid and cystic metastatic nodules varying from miliary size to the dimensions of a soja peanut were discovered in the two lymph nodes at the root of the ear and in one of the submaxillary nodes; the former were found, upon microscopic examination, to consist of accumulations of cancer cells while the latter contained a mucous fluid in which yellowish flocculi, composed of degenerated...
epithelial cells and leucocytes, were suspended. The walls of such cysts were covered with a many-layered epithelium which, in several places, had formed epithelial pearls and was infiltrating the lymph sinus and the surrounding tissues. One of the enlarged submaxillary nodes yielded similar findings. In no other organ, however, were we able to find either gross or microscopical evidence of metastasis.

Seventh Case of Carcinoma
This tumor developed from a cutaneous horn. It arose on the inner surface of the right ear of a two-year-old, brown, female rabbit after long continued irritation with coal-tar (November 19, 1914 to June 12, 1916); after the 205th day the irritant was discontinued, in order that the behavior of the lesion in the absence of its exciting cause might be observed.

By the 205th day two sessile and two pedunculated folliculoeipitheliomata had arisen, and on the 305th day many new sessile folliculoeipitheliomata were observed; nearly all these tumors continued to enlarge and developed into cutaneous horns, one of which gave origin to a carcinoma (seventh case); it is this one in particular which we now wish to discuss.

This, the seventh case of carcinoma, arose on the 286th day from a diffusely elevated spot on the irritated surface, which elevated and enlarged gradually until it had become a sessile folliculoeipithelioma and, by the 508th day, a cutaneous horn; it grew both in length and width, measuring on the 513th day 1.5 cm. by 1 cm. It was conical in shape; the free end was hard and dry, and the base elastic and soft. On the 518th day a small area at the base ulcerated, and on the 520th day the free end of the horn fell off; the portion now remaining consisted of an ulcer with edges elevated 0.5 cm. from the surrounding epithelium and an even, finely granulated surface. On the 543rd day (May 15, 1916) the diameter of this ulcer had reached 1.5 cm., and a small diffuse elevation was noticed on the opposite (outer) surface of the ear, which became gradually larger and at last ulcerated like the primary folliculoeipithelioma on the inner surface. There was considerable venous engorgement. On the 565th day the primary ulcer was 2.3 cm. by 1.5 cm., and the lymph nodes at the root of the ear began to swell, one of them being about the size of a soja peanut; both ulcer and nodes continued to increase in size. A fragment removed for microscopical examination had all the characteristics of a rodent ulcer; the epithelium had infiltrated into the lymph vessels and the veins and formed a thrombosis of carcinoma cells in them; furthermore, it had penetrated the ear cartilage and had reached the subcutaneous tissue on the opposite side, where it produced on the outer surface the ulcer just mentioned. This case is still under observation, the animal being still well, and the lymph nodes will be transplanted as soon as they are large enough.

The cases of carcinoma just described exhibited the following macroscopic and microscopic signs of spontaneous carcinoma: (1) Exhaustion and emaciation, resembling the cachexia of cancer patients. The animal in which the seventh case of carcinoma arose is not yet emaciated, it is true, but this may be because the carcinoma is still fairly recent. (2) The tumors infiltrated the subcutaneous tissue, the lymph channels and the blood vessels, and exhibited the histological features characteristic of rodent ulcer. Again, these carcinomata grew not only in the presence of the irritant, but also after it had been discontinued; thus in the fourth case of carcinoma the tumor doubled in size in the 93 days following the withdrawal of the coal-tar applications. (3) The presence of metastases was proved in the regional lymph nodes in the fourth.
Such facts make it evident that these experimentally produced carcinomata closely resemble carcinoma in man, and especially that type found among workers in coal-tar.

**Histology of the Lesions**

As we have divided the course of our experiments according to the macroscopical findings, so we wish to discuss in the following pages the microscopical appearances characteristic of these four periods.

**First period: Atypical growth of the epithelium.** The epithelium, and especially that at the periphery of the hair follicles, gradually undergoes hyperplasia: (1) each layer increases considerably in thickness; (2) many symmetrical mitoses are found in its basal layer; (3) the hair follicles become cystic; (4) the basal layer grows irregular in outline, owing to the projection of processes which ramify in the surrounding subcutaneous tissues. These are the earliest stages in the development of folliculoepithelioma benignum and malignum. Furthermore, the blood vessels, especially the veins and capillaries, soon dilate, while eosinophiles and lymphocytes escape in the neighboring connective tissues. The duration of this period is very variable; according to the individual animal it may occupy from 30 to 350 days, the average being about 100 days.

**Second period: Appearance of folliculoepithelioma.** The circumscribed papillomatous elevations on the irritated surface, which we have already described, consist of one or more hair follicles; in them, hyperplasia and hyperkeratosis of the epithelium are present in high degree, the process resembling that seen in the first period already mentioned, except that it is more advanced. From these elevations arose new growths, which we divide into two general types, pedunculated folliculoepithelioma and sessile folliculoepithelioma, though they are very variable both in size and shape.

(1) Pedunculated folliculoepithelioma. This arises when hair follicles in the central or the peripheral part of the papillomatous epithelial elevation are lifted upward by the pressure of epithelial growth, and continue to proliferate. As these new growths arise primarily from the epithelium of the hair follicle, we call them folliculoepithelioma rather than papilloma. In their subsequent growth, some of these pedunculated folliculoepitheliomata underwent an increase in their connective tissue, thus becoming fibromatous; others came to resemble the sessile type; and still others (10 in all) developed into cutaneous horns.

(2) Sessile folliculoepithelioma. This type arose from one or more hair follicles when the papillomatous epithelium showed a higher degree of hyperplasia and hyperkeratosis. The cystic hair follicles are arranged almost directly on the ear cartilage, and the epithelium and corium extending upward between them for septa or papillary processes, the spaces between which are filled with collections of concentric keratinized epithelial cells. Hence these new growths have wide bases, and are elevated but little from the surface of the skin; occasionally, however, one or more hair follicles may push upward, as in the pedunculated variety. Thirty-four of these sessile folliculoepitheliomata developed into cutaneous horns when we stopped the coal-tar.

Some of these cutaneous horns diminished gradually in size and at last disappeared, but an equal number continued to grow for 365 days after the coal-tar had been discontinued.

The blood vessels, especially the veins and capillaries, become greatly dilated, and eosinophiles and lymphocytes are found in the interstitial connective tissue. Many symmetrical bipolar mitotic figures occur in the basal layer of the epithelium. Mucous degeneration of the
interstitial tissue was not found in these folliculoepitheliomata.

Third period: Production of carcinoma. This period we have divided into three stages: (1) earliest stage of carcinoma; (2) early stage of carcinoma; (3) fully developed carcinoma.

(1) Earliest stage of carcinoma. It is difficult to differentiate these very young carcinomata from benign folliculoepithelioma. However, the following characteristics will help in the differential diagnosis. All or a part of the epithelium of these new growths assumes a fainter stain with hematoxylin than does normal epithelium or that of the benign folliculoepithelioma; the sprout-like processes developed by atypical proliferation of the basal epithelium of the hair follicle become more angular at their basal layer, and the processes grow very irregular in thickness; the interstitial connective tissue becomes loose or shows a slight mucous degeneration; lateral and downward penetration of the cancerous epithelium can be demonstrated.

We believe that such changes as these must be the initial step in the transformation to carcinoma.

(2) Early stage of carcinoma. By prolonging the irritation, we have produced many cases of folliculoepitheliomata exhibiting more advanced changes than are found in the earliest stage of carcinoma. Such tumors show the following characteristics. The interstitial connective tissue becomes loose and edematous, the sprout-like processes in the basal layer of the hair follicles are more angular than in the first stage, these sprouts grow even more irregular in thickness, lateral and downward invasion takes place to a higher degree than in the previous stage, and the invading processes anastomose with one another to form a network. The intercellular spaces become wider (dissociation) and individual cells leave the main group (emancipation); the epithelium takes a fainter stain with hematoxylin than does normal epithelium or that exhibiting atypical growth. Two among these cases showed invasion of the veins, and one into the lymphatic channels; the dilatation of the capillaries and the veins is generally more advanced than in the first period.

These are, in general, the characteristics of our carcinomata in their early stage, but the process varies in intensity, and some of the lesions resembled complete carcinoma. The findings are like those described by Ribbert and others in early carcinoma.

So far we have produced 16 cases of carcinoma in its early stage among 13 ears in 10 rabbits.

(3) Fully developed carcinoma. Our seven cases of carcinoma, which arose after further repetition of the coal-tar applications, resembled closely in their histological characteristics the spontaneous carcinomata of man. The features distinguishing the previous stage become more advanced, especially the infiltrative growth of the epithelium. This is now really striking, the carcinomatous cells invading the surrounding subcutaneous tissue both downward and laterally, growing into the veins and the lymphatic channels, penetrating the aural cartilage, and forming ulcerated new growths of a similar nature on the opposite surface of the ear. Mucous degeneration of the interstitial connective tissue was noticed in almost all cases of carcinoma.

Fourth period: Metastasis. This has been already discussed under other headings. Other problems concerning the histogenesis of experimentally produced carcinoma in the rabbit have been already suggested in previous reports, but will be taken up again in full. 10

Summary
The following conclusions may be drawn:

1. Papillomatous new growths (which we term folliculoepitheliomata) may be produced on the rabbit’s ear by the appli-
cation of coal-tar for 30 to 100 days. The proportion of folliculoepitheliomata to the total number of ears treated gradually rises.

2. By the repeated application of coal-tar, eight cases of carcinoma in its earliest stage, 16 in an early stage, and seven complete carcinomata were produced. The carcinomatus change was discovered between the 55th and the 360th day; in most of the cases it was found after the 150th day.

3. The hyperkeratotic pedunculated or sessile folliculoepitheliomata produced by irritation with coal-tar continued to grow after the irritant had been discontinued, and eventually developed into cutaneous horns. Some of these horns grew for a year after the withdrawal of the coal-tar, while others fell off spontaneously; in the great majority of the latter animals new cutaneous horns grew again from the same base, or from the neighboring epithelium, as is the case in man.

4. The seventh and 16th cases of carcinoma in its early stage developed from cutaneous horns about 300 days after the tar had been discontinued.

5. The presence of metastasis was microscopically proved in the regional lymph nodes in the fourth case of carcinoma.

6. The animals which bore folliculoepitheliomata did not begin to emaciate while the new growth maintained its benign character.

7. Yamagiwa’s hypothesis has been confirmed: The repetition or continuation of chronic irritation may cause a precancerous alteration in epithelium previously normal. If the irritant continues its action, carcinoma may be the outcome, even though no specific agent has been interpolated.

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