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 (1), 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 (12, 13, 14, 15, 16, 17, 18, 19, 20), pursuing experiments in accordance with Yamagiwa's view (2), 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 continue its action, carcinoma may be the outcome, even though no specific agent has been interpolated.

1 The authors have not read the proof of this article.
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 (table 1) of irritating the epithelium and subcutis, and with each of them have produced, as Fischer (9), Haga (10), Jores (3) and others have done, an atypical growth of the epithelium (12); 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 investigations of Vollmann (4), Liebe (4), Tillmanns (5), and Schuchardt (6); and while coal-tar carcinoma has not yet been experimentally produced, it is nevertheless conceivable that one

| TABLE 1 |
|---|---|---|---|---|
| **Methods, and number of experiments** |
| **Experiment** | I | II | III | IV |
| Coal-tar painting of inner surface of ear every two or three days | | | | |
| Coal-tar painting of outer surface of ear every two or three days | | | | |
| Coal-tar painting of incised wounds on margin of ear every two or three days | | | | |
| Coal-tar painting on outer surface of ear every two or three days after repeated intradermal injection of scarlet oil | | | | |
| Number of rabbit ears employed | 50 | 26 | 31 | 30 | 137 |
might succeed if suitable methods of applying this irritant could be discovered.

Only Cazin (7) and Bayon (8) 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.

**TABLE 2**

A. *The result with regard to the rabbits in four earlier experiments which have survived over 70 days*

<table>
<thead>
<tr>
<th>NUMBER OF RABBITS AND EARS IN EACH EXPERIMENT</th>
<th>NUMBER OF EARS BEARING FOLLICULOEPITHELIOMA AMONG THE SURVIVING RABBITS</th>
<th>PERCENTAGE RESULT BETWEEN 2 AND 3</th>
</tr>
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<tbody>
<tr>
<td>Rabbits</td>
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<td>15</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
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</tr>
<tr>
<td>10</td>
<td>10</td>
<td>1</td>
</tr>
</tbody>
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B. *The result with regard to rabbits which have survived over 100 days*

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>13</td>
<td>13</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
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<td>7</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>1</td>
<td>17</td>
</tr>
</tbody>
</table>

The results of our own investigations with coal-tar have gradually approached our expectations; *follliculoepithelioma* have arisen in nearly all ears after a period longer than 100 days (table 2) from the beginning of the application, numbering in one instance twenty 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 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

1 We employ the term *follliculoepithelioma* for papillomatous new growths which reach the size of a rice grain; a full explanation of this term will be given in a succeeding paragraph.
earliest stage, sixteen 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: Atypical 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 fibroepitheliomata or fibromata. The sessile tumors measured over 1 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 ulcer. Microscopic examination disclosed an early stage of carcinoma. Certain of these growths continued to increase in dimension until they imitated even macroscopically the carcinomata 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 macro-and microscopically, the most typical carcinomata 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 carcinomata originated in folliculoepitheliomata, passing step by step through various stages until they 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, sixth, 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 (fig. 12).

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 adenitis, 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 2560 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 273d 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 273d 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 post mortem 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.
Sixth case of carcinoma

In this case, also, the occurrence of metastasis was proved. The tumor arose on the inner surface of the right ear of a white female rabbit about two years old, and weighing 2750 grams when the experiment was begun on July 2, 1915.

On the 261st day we observed a small elevation on the irritated surface; on the 292d day this nodule, which now measured 0.7 cm. by 0.5 cm., ulcerated. On the 302d day its diameter was 1 cm., and on the 312th day two lymph nodes at the root of the ear began to enlarge, the tumor having, in the meantime, attained a diameter of 1.3 cm.

On the 322d day (May 20, 1916) the animal began to emaciate; on this date the coal-tar was discontinued. On the 332d day the two swollen lymph nodes had reached about the size of a peanut, and the ulcerated tumor continued to enlarge, even though the applications of coal-tar had been stopped. On the 337th day the new growth measured 1.0 cm. by 1.5 cm., and the enlarged nodes had attained almost the size of a nut. The lymph nodes were extirpated and small fragments inoculated subcutaneously into 10 rabbits, but the result was negative. At the operation these nodes were found adherent to the surrounding tissues, a condition which the microscope proved to be due to the infiltrative growth of carcinomatous metastases into them. The cut surface of the node exhibited a cystic nodule about the size of a pea, with contents similar to those of the one just described. The cyst was lined by many layers of cancer cells and other parts of the node contained accumulations of these elements, but epithelial pearls could not be demonstrated. The node, therefore, undoubtedly contained metastases.

On the 338th day the rabbit died, extremely emaciated (1750 grams). The ulcerated folliculoepithelioma resembled human carcinoma in its histological aspects. The rest of the post-mortem findings were negative; in particular, neither macroscopic nor microscopic evidence of metastases in remote regions could be discovered.
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 folliculoepitheliomata had arisen, and on the 305th day many new sessile folliculoepitheliomata 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 folliculoepithelioma, 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 543d 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 folliculoepithelioma 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 car-
cinoma 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 four 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 and sixth cases.

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 in the preceding paragraphs, divided the course of our experiments into four periods, according to the macroscopical findings, so we wish to discuss in the following pages the microscopic 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 into 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 form 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.

Eight cases of carcinoma in the earliest stage were discovered among seven irritated ears (see tables 3, 4, and 5).

(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 folli-
cles 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 (dis-

<table>
<thead>
<tr>
<th>CASES</th>
<th>EXPERIMENTAL SERIES</th>
<th>SEX</th>
<th>SIDE</th>
<th>DAYS классифицирован Микроскопические диагностированные COULD BE</th>
<th>DAYS TO DEATH OF RABBIT</th>
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<tr>
<td>Carcinoma</td>
<td>1st case</td>
<td>III</td>
<td>Right</td>
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<td>222</td>
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<tr>
<td></td>
<td>2d case</td>
<td>I</td>
<td>Right</td>
<td>103</td>
<td>103*</td>
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<td>3d case</td>
<td>I</td>
<td>Right</td>
<td>151</td>
<td>180</td>
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<tr>
<td>Carcinoma in its early stage</td>
<td>1st case</td>
<td>I</td>
<td>Right</td>
<td>103</td>
<td>103*</td>
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<td>I</td>
<td>Left</td>
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<td>103*</td>
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<td>103*</td>
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<td>Left</td>
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<td>103*</td>
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<td>stage</td>
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<td>156</td>
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<tr>
<td></td>
<td>3d case</td>
<td>II</td>
<td>Left</td>
<td>194</td>
<td>255</td>
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</table>

*The second case of complete carcinoma, the first, second, and third cases of
carcinoma in its early stage, and the first case of carcinoma in its earliest stage,
were all seen in one rabbit.

sociation) and individual cells leave the main group (emancipa-
tion); 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 find-
Carcinoma... This rabbit bore the fourth case of carcinoma in its earliest stage. These cases produced in the same rabbit.

Carcinoma in its early stage... This rabbit bore the fifth case of carcinoma. These cases produced in the same rabbit.

Carcinoma in its earliest stage... This rabbit bore the sixth case of carcinoma. These cases produced in the same animal.

So far we have produced sixteen cases of carcinoma in its early stage among thirteen ears in ten rabbits (see tables 3, 4, and 5).

(3) Fully developed carcinoma. Our seven cases of carci-
noma, 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 car-

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<th>CASE</th>
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<th>DAYS TO DEATH OF RABBIT</th>
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<td>565</td>
<td>589 (still living)</td>
</tr>
<tr>
<td>10th case of carcinoma in its early stage</td>
<td>♀</td>
<td>Left</td>
<td>565</td>
<td>589 (still living)</td>
</tr>
</tbody>
</table>

These tumors developed from cutaneous horns in the second case of cutaneous horns in the first experiment (see Table 6).

These seven cases of carcinoma occurred on the inner surface of seven ears in seven rabbits.

*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 (20).
APPENDIX

Experiments on the artificial production of cutaneous horns

Three rabbits remaining from the first experiment were used for this investigation, the purpose of which was to observe the fate of the experimentally produced new growths after painting with coal-tar had been discontinued. The experiment began on June 12, 1915. To our surprise, the great majority of the

Table 6
First experiment

<table>
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<th>Cases</th>
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<th>Days After Treatment Was Started</th>
<th>Days from Beginning of Experiment</th>
<th>Number of Cutaneous Horns Developing from Folliculoepitheliomata</th>
<th>Number of Folliculoepitheliomata Unchanged or Decreasing in Size</th>
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folliculoepitheliomata developed into cutaneous horns (see table 6). This outcome was so interesting that one of us (Ichi-kawa) continued this investigation into the pathogenesis of cutaneous horns (see table 7), and found that 34 among 45 sessile folliculoepitheliomata developed into horns (75%) and 10 among 19 pedunculated folliculoepitheliomata (53%). The folliculoepitheliomata which did not develop into horns gradually decreased in size and finally vanished, though a few of them first underwent a secondary fibromatous alteration. Most of the cutaneous horns increased in size after the appli-
cations of tar had been discontinued, and, furthermore, we observed horns developing from the neighbouring epithelium. Thus there was required for the production of cutaneous horns the application of coal-tar for a certain length of time, followed by a period of freedom from irritation. In the further course of the experiment, one of these horns gradually took on the character of an early carcinoma and another that of a carcinoma in a somewhat more advanced stage (see table 5). The details have already been discussed in the preceding paragraphs.

**SUMMARY**

The following conclusions may be drawn:

1. Papillomatous new growths (which we term folliculo-epitheliomata) may be produced on the rabbit's ear by the ap-
plication of coal-tar for 30 to 100 days (text figure 1). The proportion of folliculoepitheliomata to the total number of ears treated gradually rises.

Text Fig. 1. Gradual rise in percentage of folliculoepitheliomata with the passage of time.
2. By the repeated application of coal-tar, eight cases of carcinoma in its earliest stage, sixteen in an early stage, and seven complete carcinomata were produced. The carcinomatous 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 neighbouring epithelium, as is the case in man.

4. The seventh and sixteenth 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 and sixth cases 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 (2) has been confirmed: The repetition or continuation of chronic irritation may cause a precancerous alteration in epithelium previously normal. If the irritant continue its action, carcinoma may be the outcome, even though no specific agent has been interpolated.

In conclusion, we desire to acknowledge our indebtedness to the Japanese Cancer Research Fund for its support in this work, to Dr. Mann and Dr. Horiuchi for their courtesy in correcting our English, and to Dr. Kikuta for the photographs and photomicrographs.

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EXPLANATION OF ABBREVIATIONS

Ab.c., Cystic abscess
C.d., Carcinomatous change in a folliculoepithelioma
Ch., Cartilaginous layer of rabbit's ear
D.ep., Dissociated epithelial cells
E.n., Network of carcinomatous epithelium
F., Fibromatous portion
F.c., Folliculoepithelioma carcinomatous
F.n., Fibromatous nodule
I.ep., Infiltrative growth by carcinomatous epithelium
L., Lymphatic follicle
M.c.a., Metastatic accumulation of carcinoma cells
M.c.w., Metastatic carcinoma cell layer on wall of cystic abscess
M.i.g., Metastatic carcinoma cells exhibiting infiltrative growth
M.l., Metastasis of carcinoma cells into lymph sinus in a lymph node
N.ep., Relatively normal epithelium
P.h., Perforation
Th.l., Thrombosis of lymph vessels caused by infiltrative growth of carcinomatous epithelium
Th.v., Thrombosis of vein caused by infiltrative growth of carcinomatous epithelium
R., Thickened wall of rodent ulcer

PLATE 1

Fig. 1. Multiple hyperkeratotic sessile folliculoepitheliomata on inner surface of ear on 204th day. Natural size.
Fig. 2. First case of cutaneous horn, on the 390th day after first application of coal-tar, and the 217th day after its withdrawal. Natural size.
Fig. 3. Second case of cutaneous horn, on the 422d day after first application of coal-tar, and the 217th day after its withdrawal. Natural size.
Fig. 4. Fourth case of carcinoma in its early stage; inner surface of ear, 165th day. Two-thirds natural size.
Fig. 5. Fourth case of carcinoma, on the 273d day after first application of coal-tar, and the 77th day after its withdrawal. Natural size.
Fig. 6. Secondary ulcer and central nodule on outer surface of ear; fourth case of carcinoma. This picture was taken on same day as figure 5. Natural size.
Fig. 7. Fourth case of carcinoma in its earliest stage, 118th day. Hematoxylin-eosin. Zeiss microplanar 35 mm.
Fig. 8. Fourteenth case of carcinoma in its early stage, 90th day. Hematoxylin-eosin. Zeiss microplanar 35 mm.
Fig. 9. Thrombosis of vein by infiltrative growth of carcinomatous epithelium; thirteenth case of carcinoma in its early stage, 90th day. Hematoxylin-eosin. Zeiss projectionocular 2, obj. aa.

Fig. 10. Carcinomatous portion in fourth case of carcinoma in its early stage, 197th day. Notice dissociation of epithelial cells (D. ep.). Hematoxylin-eosin. Zeiss projectionocular 4, obj. aa.

Fig. 11. Second case of carcinoma (rodent ulcer), 103d day. Hematoxylin-eosin. Zeiss microplanar 35 mm.

Fig. 12. Third case of carcinoma, 151st day. Hematoxylin-eosin. Zeiss microplanar 50 mm.
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PLATE 2
PLATE 3

Fig. 13. Fourth case of carcinoma in its early stage, 197th day. Hematoxylin-eosin. Zeiss, microplanar 50 mm.

Fig. 14. Fifth case of carcinoma, 120th day. Hematoxylin-eosin. Zeiss, projectionocular 2, obj. aa.

Fig. 15. Sixth case of carcinoma. Invasion of lymph channels at periphery of tumor. Hematoxylin-eosin. Zeiss, project. oc. 2, obj. aa.

Fig. 16. Seventh case of carcinoma, 505th day. Thrombi of cancer cells in lymph vessels. Hematoxylin-eosin. Zeiss, project. oc. 2, obj. aa.
PLATE 4

Fig. 17. Metastasis in regional lymph nodes (fourth case). Hematoxylin-eosin. Zeiss project. oc. 4, obj. aa.

Fig. 18. Metastasis in regional lymph node (sixth case); cancer cells in lymph sinus. Hematoxylin-eosin. Zeiss project. oc. 2, obj. aa.

Fig. 19. Metastasis in regional lymph node (sixth case); stratified cancer cells cover the wall of a cystic abscess, which contains mucous fluid, degenerated epithelial cells, eosinophiles, and lymphocytes. Hematoxylin-eosin. Zeiss project. oc. 4, obj. aa.

Fig. 20. Metastasis in regional lymph node (fourth case). The metastatic cancer cells invaded the parotid gland, causing adhesion to the surrounding tissues. Hematoxylin-eosin. Zeiss microplanar 50 mm.