

STUDIES ON THE RELATION BETWEEN TUMOR SUS-  
CEPTIBILITY AND HEREDITY.

II. THE INCIDENCE OF TAR TUMORS IN STRAINS OF MICE HAVING A  
DIFFERING INCIDENCE OF SPONTANEOUS GROWTHS.

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(Received for publication, July 18, 1925.)

The ability to produce malignant new growths at will in laboratory animals has provided a new and valuable instrument for the investigation of cancer. Fibiger's<sup>1</sup> success in causing neoplasms in the stomach of rats infected with Spiroptera was followed shortly by Yamagiwa and Ichikawa's work<sup>2</sup> with coal tar upon rabbits; and the application of the coal tar method (Tsutsui<sup>3</sup>) to mice, the species in which transplanted and spontaneous tumors are best known, has opened the way to one of the most popular fields of present day medical research. The progress of the growths, the histological findings, the conditions of occurrence and so forth, have been treated in many papers. In all the published accounts it may be noted that there were wide variations in the response given by the animals subjected to treatment.

It is difficult to compare results from different laboratories. Nevertheless the reports indicate that the same tissue in different species shows different degrees of reaction to the treatment designed to elicit neoplasms. The skin of rabbits and mice responds rather readily to irritation with tar, while that of rats and guinea pigs appears to be highly resistant. Not only are species differences indicated but among individuals of the same species a wide range of variability is seen. Apparently no tests have been made heretofore to determine whether

<sup>1</sup> Fibiger, J., *Berl. klin. Woch.*, 1913, 1, 289; *Compt. rend. Soc. biol.*, 1920, lxxxiii, 1160.

<sup>2</sup> Yamagiwa, K., and Ichikawa, K., *Mitt. med. Fakult. k. Univ. Tokyo*, 1915-16, xv, 295; 1917-18, xix, 483; *J. Cancer Research*, 1918, iii, 1.

<sup>3</sup> Tsutsui, H., *Gann, Japan Z. Krebsforsch.*, 1918-19, xii, 17.

the individuals manifesting distinct differences may not come from families which have peculiar and characteristic grades of susceptibility.

For some years there have been reported strains of mice which show differences, apparently hereditary, in the rate of incidence of spontaneous tumors, notably those of the mammary gland. Two such strains bred at The Rockefeller Institute seem to afford suitable material for tests as to whether there are hereditary differences affecting the response to treatment with coal tar.

#### EXPERIMENTAL.

The stocks chosen were a strain showing a high tumor incidence, No. 1194 from the Lathrop mice, and a strain exhibiting a lesser incidence, which was originally obtained from Dr. Bagg at the Memorial Hospital. Strain 1194 comprises strong healthy mice, rather large and of the wild, or agouti, color. Of the bred females reaching the age of 6 months or more, 65 per cent develop mammary gland tumors while those not bred develop them in 20 per cent. The Bagg mice are albino but are also a vigorous stock, in which the animals producing young have a 28 per cent tumor rate and those not bred only 4 per cent. The average tumor age of Strain 1194 is 12 months and of the Bagg mice 18 months.

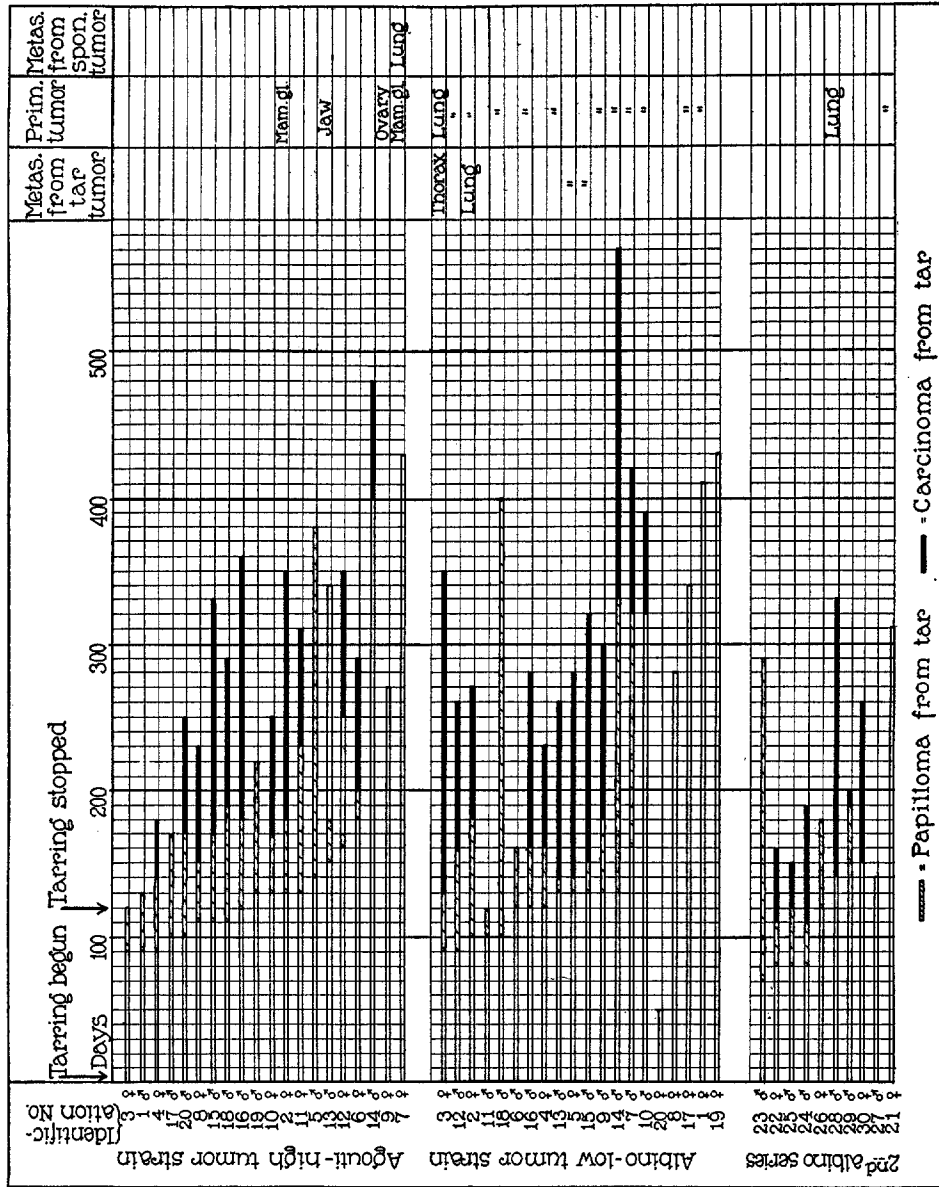
The technique used was to paint the mice three times weekly for 4 months on a spot between the shoulders, applying approximately 1 drop from an applicator dipped in a solution made from coal tar residue. Depilation was found unnecessary but the hair was clipped before the first painting.

The tar solution used was a benzene extract free from acids, bases, and phenols made from the residue from a coke oven in which the tar had been distilled at a temperature of approximately 377°C. The residue was obtained from the Barrett Company who have very kindly cooperated with us a number of times by providing both residue and extract. We wish to extend our thanks to them.<sup>4</sup>

For the experiment 20 mice from each strain were selected. Approximately half were males and half were females that had not been bred. Tar was applied for 4 months, from the middle of November to the middle of March. The first painting was usually followed by a loss of hair from the treated area. Desquamation could be observed sometimes after a few paintings but the first papillomatous elevation was not noted until after the 90th day. The elevations were strongly keratinizing, often producing horns of remarkable size similar to those pictured by other investigators. When carcinomatous changes occurred they became evident about the 140th day or later, either as an induration at the base of the horn or, if the latter had not persisted, at its former site. Occasionally they appeared without the formation of a papilloma. Experience has shown that the induration

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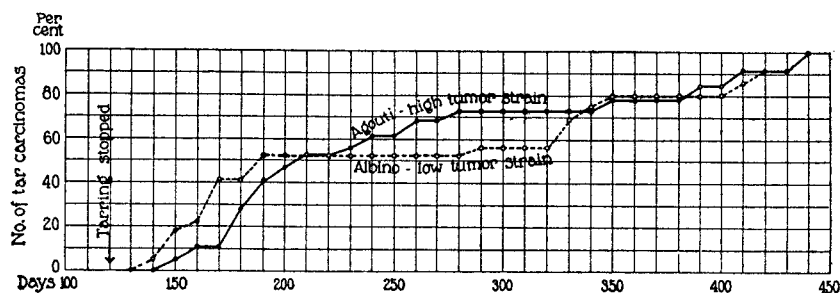
<sup>4</sup> For a more detailed account of the preparation of the tar product see Murphy, Jas. B., and Landsteiner, K., *J. Exp. Med.*, 1925, xli, 807.



TEXT-FIG. 1.

referred to is indicative of carcinomatous transformation of the cells but the diagnosis was confirmed by sections taken at the time of death.

In general the results of the experiment show that no significant difference in the response to tar painting was given by the two strains of mice with differing rates of incidence of spontaneous tumors. Of the 20 mice from the strain with high incidence, all of which survived the period of painting (120 days) 12 eventually developed carcinomas of the skin (60 per cent); from the low tumor strain, the albinos, 19 individuals lived 120 days and again 12 produced carcinomas, approximately the same percentage. Since the mice lived differing lengths of time, it is important to analyze the results more closely. The course of the experiments may be followed in the graphs. The percentages



TEXT-FIG. 2.

from which the graphs were made, are based upon small numbers of individuals and therefore cannot be significant in detail but they are of value in indicating the general progress of events.

The histories of the individuals presented in the first two sections of Text-fig. 1 are condensed and compared in Text-fig. 2. It will be seen that the carcinomatous transformations occurred slightly earlier in the albinos or low tumor strain than in the agoutis. By the 140th day 1 of the 18 mice living at that period showed distinct induration while none of the high tumor strain did. For a time the albinos continued to yield a larger number of individuals with carcinoma, showing at the 170th day 7 out of 17, or 41 per cent, as against 2 out of 18 or 11 per cent among the agoutis; but by the 210th day the percentages were even (9 out of 17 or 53 per cent in each case). From the 190th

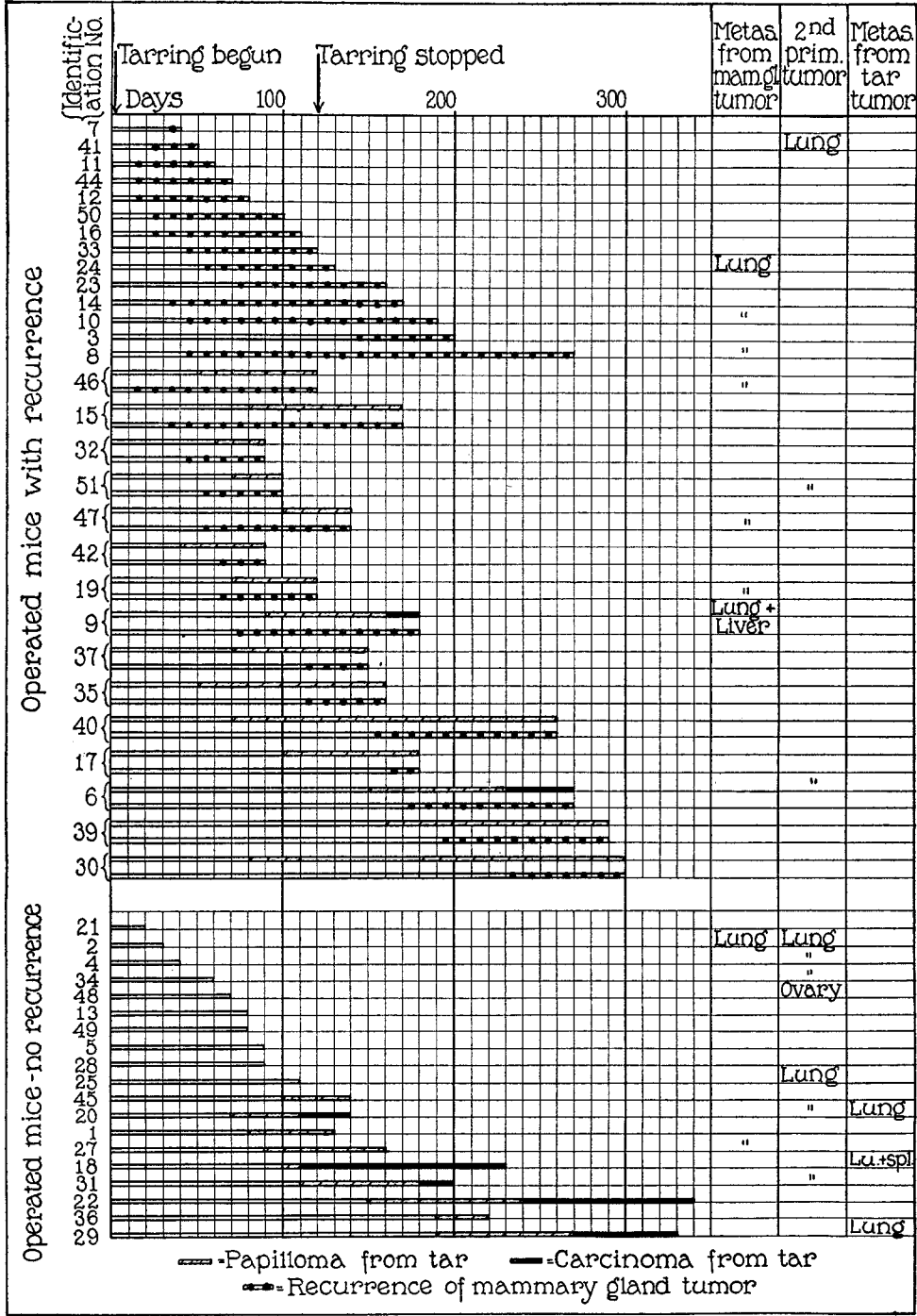
day the percentages are figured from the total number of carcinomas which had appeared in the group and the total number of animals living, or already dead, as a result of the neoplasm. From the 230th to 330th day the tumor rates of the agoutis exceeded that of the albinos but from the latter time to the end of the observations the rates were practically parallel. Three mice of the high tumor strain and five of the low exhibited a high degree of resistance for a considerable period but by the 440th day the sole survivor in each group had a cancer, but of very recent origin in one case.

We see then, that there was a gradual increase in the incidence of tar cancer in both strains, beginning with about the 150th day. The slight variations shown are not regarded as significant. It is to be noted in relation to the period of tarring that papillomas sometimes occur during the 4th month although they may not appear until after the applications have ceased. In the experiment now under consideration the carcinomatous changes took place only after the tarring was discontinued.

The series described were tarred during the winter months. In April an additional group of 10 albinos from the low tumor strain was subjected to the treatment. A few of these animals developed papillomas earlier (80th day) than either the agoutis or the first albinos and induration also was detected sooner in certain cases—in 2 of them before the end of the tarring (Text-fig. 1). Derom<sup>5</sup> has conducted experiments from which he concluded that the temperature of the tar and heating the tissues play a rôle in susceptibility to tarpainting. The observations here reported are too meagre to be the basis of definite conclusions in regard to seasonal variations but they are not contradictory to Derom's point of view. Certainly they show the variability which may occur within a given strain and the inadvisability of using so called standardized controls.

The second albino group just mentioned was studied as an additional control for another series of mice. Those previously dealt with had been individuals from strains which produced tumors but which had not yet grown them themselves. It seemed pertinent to enquire

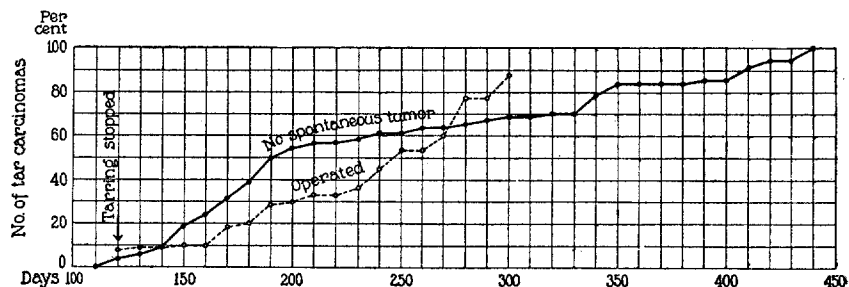
<sup>5</sup> Derom, E., *Bull. Assn. franç. étude cancer*, 1924, xiii, 422.



TEXT-FIG. 3.

whether individuals which already had developed tumors would behave differently in response to tar.

Since mice with spontaneous tumors of the mammary gland rarely live more than 2 or 3 months, most of them could not be expected to survive the period of tar painting (4 months) unless the tumors were removed. It is difficult to obtain a large number of operable tumors at any one time, and so the collection of mice with suitable tumors was begun in the fall and continued several months. 48 of them were at length acquired, from a number of different strains. More than half were agouti; and most of the remainder were albino, though a few were chocolate, cinnamon, silver, or spotted. The tumor ablations were performed under ether by Dr. James B. Murphy and Mr. Ernest Sturm, to whom acknowledgement is here made. The growths were taken out soon after the tumor had been discovered. When the wound was healed and the mice in good condition, painting 3 times a week with coal tar extract was begun and continued for 120

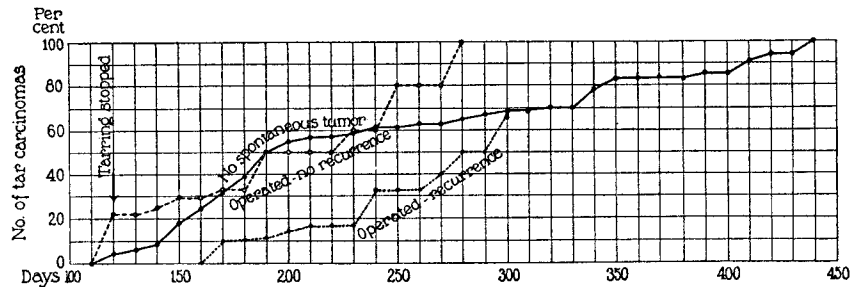


TEXT-FIG. 4.

days as in the experiment already described. With the mice first operated upon, treatment was begun in December, and the others were subjected to it at intervals, the last of them in April. This unavoidable spreading of the experiment over several months necessitated a second group of albinos as controls, tarred synchronously with the mice last operated upon, in addition to the groups with high and low tumor incidence, on which treatment had been started in November.

More of the mice submitted to operation died before the end of the tarring than of those not so treated even when the spontaneous tumor did not recur. By the 120th day only 28 of the mice operated upon were living compared with 49 in the group without spontaneous tumors. This was probably a result of the treatment received. The tumor mice were somewhat older than those without spontaneous tumors but not sufficiently so for age to have been an important factor

in the fatalities. Several of the mice operated upon developed papillomas after tarring earlier than did any in the control groups (50th to 70th day as against the 80th or 90th) (Text-figs. 1 and 3), and papillomas continued to be more frequent up to the 150th day. On the other hand the percentage of carcinoma developing in the operated series lagged behind that of the mice without spontaneous tumors until the 280th day. These differences are apparent from a comparison of Text-figs. 1 and 3. Text-fig. 4 affords a comparison of the percentage of incidence of carcinomas found at successive periods in the two groups of mice. The percentages are calculated as in the preceding graph. Although the series operated upon lagged behind the control the difference is not very marked. It will be seen that in general they behaved in a very similar way. If, however, we



TEXT-FIG. 5.

distinguish in the series operated upon between those mice which did and those which did not have recurrences of the spontaneous mammary gland tumor, and compare the developments with those in the control mice, we find (Text-figs. 1 and 3) that it is the animals with recurrences which develop papillomas earlier, whereas malignant transformation rarely occurred in them. Of the 16 mice with recurrences which lived more than 120 days only 2 ever yielded carcinomas while 5 of the 9 which showed no recurrence eventually developed such growths. Text-fig. 5 shows the percentage of carcinoma at successive periods occurring among the three groups of mice: (1) those which had no recurrence, (2) those from which a mammary gland tumor had been removed and which did have a recurrence, and (3) those which had not developed a mammary gland tumor. The total number of



individuals in each group is very small so that the results are not conclusive but a difference in the response given by the mice with recurrences seems to be indicated. The first carcinomas in the series operated upon which showed no recurrences appeared at the 120th day when 2 of the 9 survivors (22 per cent) showed induration. In the non-operated group 2 carcinomas had appeared at this time (4 per cent) among the 49 surviving animals. None had occurred in the 19 mice operated upon which developed a recurrence then or later. Indeed, in the series last mentioned no carcinoma was found until the 170th day when 1 occurred in the group of 11 individuals as contrasted with 14 out of 44 (32 per cent) and 2 out of 6 (33 per cent) in the other 2 series. Unfortunately the mortality from the tarring was rather high among the mice from which the spontaneous tumors had been removed, so that even fewer individuals are represented in the later figures. However, the persistent absence of tar carcinomas in the mice with recurrences would seem important. At 230 days only 1 had died of such a carcinoma and 5 were still free from it (though 4 had papillomas), giving a group incidence of 17 per cent, whereas among those without recurrence 3 out of 5,—a total which includes both the living animals and those already dead from the neoplasm,—or 60 per cent, had developed a malignant growth. The control mice which had not been operated upon also showed a high incidence of malignancy, the record showing that 24 out of 41 of them (58 per cent) had tar tumors. At the 260th day there continued to be fewer tumors among the mice that had recurrences (2 out of 6) than among the mice without recurrences (4 out of 5) and also among the control animals (26 out of 41). By the 310th day all the mice with recurrences had died. The 2 mice without recurrences which were still living both had developed tar tumors.

The 2 oldest survivors among the animals with recurrences died after 290 and 300 days respectively without having developed more than a papilloma. Among the control animals 4 lived from 400 to 430 days from the beginning of the tarring without developing a neoplasm; but about the 410th day 1 of them developed a carcinoma where the tar had been applied, thus showing that a delay of as much as 290 days may elapse before there is a neoplastic response.

As already stated microscopic examinations were made of the

growths found at autopsy save in a few cases in which postmortem changes or other causes made it impossible. The malignant tar tumors proved to be carcinomas except in one instance (No. 18 in the operated group) which was a carcinosarcoma. Metastasis was found in 7 cases, usually to the lung but in No. 3, an albino, the mediastinum, diaphragm, and thoracic wall were also involved; and in No. 18 a metastasis from the sarcomatous portion of the tar tumor was found in the spleen. A number of tumors developed in regions outside the painted area. The only 2 of them in the mammary gland appeared in the agouti strain which ordinarily has a high incidence of mammary gland tumors. One was found in Mouse 7 which had not had a tar epithelioma, whereas the other developed in Mouse 2 about 100 days after the tar tumor had been first recorded. It is evident that a tumor produced by tar does not suffice to protect the host absolutely against the development of a spontaneous tumor in another organ. Male 13 which had developed in response to the tarring a small papilloma that persisted for only a short time, was very much later seen to have an infiltrating epithelioma of the jaw. Since mice remove tar from a painted area by licking it off it is perhaps surprising that epithelioma on the jaw or tongue is not more frequently encountered. In the case mentioned the neoplasm could not certainly be regarded as a result of the tarring since jaw tumors occur spontaneously in the strain. In two other cases the ovary was the site of a carcinoma but the most frequent region of occurrence for tumors developing at a distance from the tarred region was the lung. It is interesting to note that of the 23 pulmonary growths found none was in the agouti or high tumor strain in which the incidence of spontaneous lung tumors is very low.

#### SUMMARY AND DISCUSSION.

1. Two strains of mice, one with a high, the other with a comparatively low incidence of spontaneous tumors of the mammary gland, when painted between the shoulders with coal tar extract developed tar tumors with about practically identical frequency.

Possibly this result was to have been expected. If tumors of certain organs or tissues are specific in heredity, a conception for which there is some evidence, then assuredly a high degree of incidence for one tissue, as for example the mammary gland, does not necessarily mean

a high incidence for another tissue, such as the skin, when subjected to tarring. To test the influence of heredity on the response of the skin to tar painting it would be best to employ strains of animals exhibiting differences in the incidence of spontaneous cancer of the skin. Unfortunately no such material was available. We can state on the basis of our experiments only that no hereditary differences in the response of the skin to tar painting were demonstrable in two strains of mice manifesting markedly different percentage incidences for spontaneous mammary growths. It is possible, of course, that the natural differences in the two strains may have been wiped out by the tar treatment. Such a view is suggested by the result of other work in this laboratory which has shown that tar painting increases markedly the incidence of tumors of the lung<sup>6</sup> and destroys the resistance to transplantable tumors (unpublished work).

2. Mice from which spontaneous mammary tumors had been removed were treated with tar. The percentage incidence of the resulting tar tumors was similar to that met with in the controls, except possibly in the case of such animals as showed a recurrence of the spontaneous growth. In them the development of tar tumors seemed to be delayed and possibly prevented. The numbers involved are too small to be conclusive.

Murray<sup>7</sup> has stated that "the induction of a fresh primary growth after a first has been definitely established, meets with a very intense resistance whether the first tumor be of the same parent tissue or another." His conclusions are based partly upon certain retarring experiments but more especially upon a series of mice from which spontaneous mammary gland tumors had been removed, which were then tarred. Apparently these latter did not have recurrences of the spontaneous growth. Only 1 of them developed a papilloma and none carcinoma. The tar was applied twice weekly in Murray's experiment instead of 3 times as in ours and for this as for many other reasons a direct comparison is not possible. It is furthermore not clear what the expectation for his group would have been. Our

<sup>6</sup> Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1925, xlii, 693.

<sup>7</sup> Murray, J. A., *8th Scient. Rep. Imperial Cancer Research Fund*, London, 1923, 75.

conclusions, however, seem only partly to confirm his for we find that if any protection exists against a tar cancer after the ablation of a spontaneous mammary growth, it is only when a recurrence has taken place. From certain additional tarring experiments Murray concluded<sup>8</sup> that if a second tarring is begun before cancer has developed from the first, susceptibility is increased. Truffi<sup>9</sup> also has found a diminished refractoriness after tarring. Among our mice in which the mammary gland recurrence appeared during or after the tar painting, no increase in susceptibility but rather the reverse was found. Future experimentation must determine to which among several variables this divergence of results is to be attributed.

<sup>8</sup> Murray, J. A., *Brit. Med. J.*, 1924, ii, 1004.

<sup>9</sup> Truffi, M., *Riforma med.*, 1924, xi, 985.