THE EFFECT OF A GROWTH-RETARDING FACTOR FROM NORMAL TISSUES ON SPONTANEOUS CANCER OF MICE*

By JAMES B. MURPHY, M.D., and ERNEST STURM

(From the Laboratories of The Rockefeller Institute for Medical Research)

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Extracts of placenta and embryo skin have a definite inhibiting action on the growth of transplantable cancers of mice (1). The absence of any effect of these materials on sarcomas, and the finding that the inhibiting factor isolated from certain fowl tumors acts on sarcomas and not on carcinomas, suggested a degree of tissue specificity for these factors. The attempt to establish a principle applying to cancer in general on evidence derived from the transplantable tumors only leaves something to be desired, for these tumors always represent cells foreign to the host that maintains them. Therefore, we have deemed it necessary to test the above mentioned factor on the spontaneous or natural cancers made up of malignant cells derived from the animal's own tissues (2).

The mammary tumors of mice have been studied because of the number available as by-products of the genetic studies, and because of the extensive data available on the natural history of these neoplasms. There is a surprising uniformity in the figures published by different investigators, using different strains of mice, on such points as the growth of autografts, local recurrence following surgical removal, spontaneous retrogression, and multiple foci of malignant change. In the present communication a report will be made on both the local and general effect upon these natural cancers of inhibiting materials that are known to act on transplanted tumors.

Materials

The tumor mice utilized came from three principal sources, and were from several strains. The largest number were from the general breeding room of

* This investigation was carried out under the Rutherford Donation.

The Rockefeller Institute, where the stock of Swiss origin is inbred though not a pure line strain. Figures for the tumor rate, while known to be high, cannot be accurately determined by the available records. The second source was our genetic breeding room, where the mammary tumor strains are descendents of the original Abbe Lathrop stock. The third source, and the second largest, was the Roscoe Jackson Memorial Laboratory, and the animals came from several strains of high tumor incidence. The size of the tumors at the time that the mice were sent into the laboratory was rarely under 1.0 x 0.9 cm. and rarely over 1.8 x 1.5 cm. About 29 per cent of the animals had more than one primary tumor.

The extracts of mouse placenta and embryo skin were prepared in the manner described in earlier publications. The material, collected during the latter part of pregnancy, was minced, spread in a thin layer, and placed in a vacuum jar over sulfuric acid. After evacuation it was kept in the freezing box until desiccation was complete. The finely powdered material was thoroughly extracted with water (0.1 gm. to 1 cc.) and centrifuged. The supernatant fluid was used for the tests.

Effect of the Extracts on Local Postoperative Recurrence

The early invasion of the skin or deeper tissues by mammary cancer in mice is unusual. Probably owing to the looseness of the tissues, the tumors grow as more or less discrete masses in the subcutaneous tissue and can be easily separated. In spite of the apparent discreteness of the masses and the removal of all immediately adjacent normal tissue, local recurrence is not uncommon. The first tests were on the effect of the inhibiting extract on this manifestation.

Experiment.—The group was composed of 58 tumors removed and the wound bathed with embryo skin extract and 100 tumors removed and the operative field similarly treated with placenta extract. The operation consisted of a radical removal from the etherized animal of the tumor and adjacent tissue, under aseptic precautions. After the wound was sutured, from 0.05 to 0.2 cc. of the tumor extract, depending on the size of the wound, was injected into the area and then it was sealed with collodion to prevent leakage. In the controls collodion was used in the same way. There was rarely any evidence of infection and healing took place promptly. No further treatments were given and the animals were kept under close observation until their death. Diagnosis was made by sections of the original tumors and any nodules found at autopsy were removed and also sectioned. No animals were included as negative for local recurrence unless they survived over 5 weeks. The average length of life after operation was approximately 3 months for the group.

The results are shown in Table I, which for comparison includes our control series in which the tumors were removed without other

treatment, two earlier series from this laboratory, and two groups reported by other investigators.

The recurrence rate following operative removal of mouse cancers reported by different investigators with different strains of mice is remarkably constant, the maximum variation between the groups being from 46.2 per cent to 54.0 per cent. The constancy of these figures gives even greater significance to the evidence of suppression

TABLE I

Local Recurrence Following Operation

	No. operated	No. recurred	Percentage recurrence
Controls			
Murray*	48	23	47.9
Haaland†	174	96	54.0
Murphy and Morton‡	39	18	46.2
Nakahara§	50	26	52.0
Present series	144	69	47.9
Total controls	455	232	50.9
Placenta treated	100	6	6.0
Embryo skin treated	59	15	25.9

* Murray, J. A., *3rd Scient. Rep. Inv. Imp. Cancer Research Fund*, London, 1908, 69.

† Haaland, M., *4th Scient. Rep. Inv. Imp. Cancer Research Fund*, London, 1911, 1.

‡ Murphy, Jas. B., and Morton, J. J., *J. Exp. Med.*, 1915, **22**, 800.

§ Nakahara, W., *J. Exp. Med.*, 1925, **41**, 347.

of local recurrence by the two tissue extracts, particularly by placenta extract. While the figures for skin extract show a significant reduction over the expected recurrence rate it is doubtful if this represents the full potency of the material. The larger number of recurrences in this group took place in a lot of animals treated with a single preparation which was probably inactive; but this was not evident until too late to make further tests to establish the point. There seems no doubt that there is a definite reduction in local recurrence due to the treatment of the operative field with the tissue extracts.

The Influence of the Tissue Extracts on the Fate of Autografts from Spontaneous Tumors

The autograft of a spontaneous tumor may be considered as an artificial metastasis and as such might be expected to grow in practically every instance. As a matter of fact, the percentage of takes is so high that it is probable that the failures are due to accidental loss of the graft through extrusion or destruction by local infection.

Experiments.—The experimental group for this test consisted of 111 mice with spontaneous mammary cancer. The primary tumors were removed surgically, and from the outer surface two large sized grafts were cut out. One of these was immersed for a few moments in one of the test fluids and the other in Ringer's solution for the same length of time. The two grafts were then reinoculated into opposite sides of the host of origin. The operation wound was also treated with the tissue extract. No further treatment was given to these animals and they were kept under observation for their duration of life. The diagnosis of the original tumors and the fates of the autografts were confirmed by microscopic sections.

The results are presented in two ways for better comparison. In Table II the figures are given for takes of the treated and untreated autografts in the same animals. Table III shows the figures for the untreated autografts compared with a larger number of controls in which autografts had been returned to animals without treatment of the animal or graft.

The figures for the growth of autografts collected in this laboratory and those reported by others are remarkably uniform, with the greatest variation between groups of 1.9 per cent. The failure of takes in 10.9 per cent and 18.5 per cent of the untreated grafts inoculated into animals which had received a treated graft and whose wound had been flushed with one of the tissue extracts, suggested that there is a general effect from the local injections. Here, as with the first experiments, it is doubtful if the figures for the effect of embryo skin extract give the correct idea of the full strength of this factor. Most of the positive grafts occurred in a lot of mice treated with an extract which we have reason to believe was inactive as stated in the foregoing experiment. The results cannot be explained on a possible interference between the double grafts. An analysis of an earlier un-

published study, in which 52 double autografts were made, showed that in 86.5 per cent both grew, and in only 7 per cent one grew and the

TABLE II

Effect of Embryo Skin and Placenta Extracts on Autografts of Tumors

	No. which grew	Per cent which grew	Per cent doubtful or no growth
46 tumors removed			
Autografts treated with embryo skin extract.	29	63.1	36.9
Autografts not treated	41	89.1	10.9
65 tumors removed			
Autografts treated with placenta extract	21	32.3	67.7
Autografts not treated	53	81.5	18.5
64 tumors removed			
Autografts, no treatment of graft or wound	62	96.9	3.1

	No.	Per cent
Autografts treated with embryo skin, negative or definitely smaller than control	33	71.7
Autografts treated with placenta, negative or definitely smaller than control	63	96.9

TABLE III

Growth of Autografts

	No. of autografts	No. which grew	Per cent which grew
Controls			
Haaland*	62	60	96.9
Murphy and Morton*	29	28	96.6
Nakahara*	25	24	96.0
Additional Rockefeller Institute series	202	192	95.0
Total controls	318	304	95.6
Placenta treated	65	21	32.3
Embryo skin treated	46	28	60.9

* For references see Table I.

other did not. The retardation of growth of tumors resulting from the treated graft, where inhibition was not complete, was very evident.

The Distal Effect of Inhibiting Factors on Tumors

The history of the established spontaneous cancers of mice, particularly the mammary tumors, has been studied by a number of investigators. The most complete data are those collected by Haaland (3) based on 353 tumors and those by Woglom (4) based on 2000 tumors. Haaland found five, or 1.4 per cent which retrogressed more or less completely, while Woglom had only 0.8 per cent which did not show a progressive course. Our figures would be close to Woglom's. We have not seen a well established tumor retrogress among the large number which have been followed in this laboratory. There was a suggestion of an effect on the control graft when the inhibiting extracts were injected into another part of the body, in the test just described. In the next experiments we have considered its possible action on an established primary tumor when injected at a distance.

Experiment.—In this group there were 142 primary tumors, 75 of which were treated by intraperitoneal injection of placenta extract and 67 with embryo skin extract. The injections of 1 cc. were made at weekly intervals, from six to fifteen injections, depending on the response of the tumor. A small piece was removed for diagnosis before the injections were started, in half the animals, and in the other half after the tumor had become stationary, or at autopsy. Otherwise the tumors were not interfered with. As there was no difference in the outcome of the treatments these animals were all grouped together. Weekly measurements were made and complete autopsies were performed at death. No tumors were included in the series unless the animal lived more than 5 weeks, except those dying as the result of growth of the tumors. The general health of the mice seemed unaffected by the treatment.

The results are shown in Table IV.

Among the two groups there were 45 tumors which continued to grow but apparently at a slower rate than in an untreated series. The average progress of 43 of these treated tumors in animals which lived for 4 weeks, compared with 41 of an untreated group is shown in Text-fig. 1. A comparison of this control group with a large number from earlier studies on growth rate shows this to be a fair average.

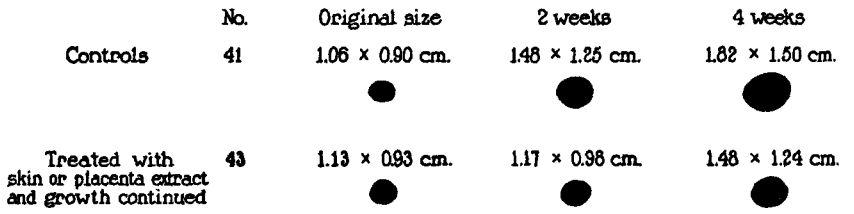
The growth-inhibiting action of the two tissue extracts is evidenced by the fact that 67 per cent and 69 per cent of the two groups treated showed no further growth of the tumors after the injections had been begun, and may be considered to have lived out their normal span of

life, a number of the animals having survived over 6 months and two over a year. The tumors not completely stopped progressed at what appeared to be a slower rate than is usually shown by tumors of this type. The difference in effectiveness between the skin and placenta extracts is not marked here, for the use of an inactive preparation in one of a series of injections would not be so noticeable as in the first two experiments in which a single treatment was given.

TABLE IV

Effect of Embryo Skin and Placenta Extracts on Spontaneous Tumors

	No. of tumors	Continued growth	Stationary or slight retrogression	Marked retrogression	Complete absorption	Total showing inhibition
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
Treated with skin extract.....	67	32.8	28.4	14.9	23.9	67.2
Treated with placenta extract.....	75	30.7	21.3	26.7	21.3	69.3



TEXT-FIG. 1. The average growth rate of 43 spontaneous tumors in animals treated with placenta or embryo skin extracts, which continued growth after treatment, compared with the average growth rate of 41 untreated spontaneous tumors.

The number of complete regressions, 32 out of 142 tumors making up the series (a percentage of 22.5) is striking when compared with the fate of tumors in untreated mice in which spontaneous absorption occurs in less than 1 per cent. Tumors showing temporary retrogression followed by subsequent growth are not included among those classed as affected by the treatment. With untreated mice the rule is progressive growth until the death of the animal, but with considerable variation in the growth rate of individual tumors. Cessation of

growth for any great length of time is unusual,[1] and retrogression to any marked degree is rarely seen. This experience is in agreement with Woglom's report, in which among 2000 mice with spontaneous tumors thirteen retrogressed and three fluctuated in growth rate, or remained stationary in size. Thus 99.2 per cent of 2000 untreated spontaneous cancers of mice pursued "their usual progressive course."

The Occurrence of New Primary Tumors in the Treated Groups

It is well known that mice not uncommonly have multiple primary foci of malignant change in the mammary tissue, the reported figures on this condition yielding an average of about 18 per cent. Haaland (5) collected the first systematic laboratory data and found that after the animal was sent into the laboratory with at least one established primary tumor, some 35 per cent developed additional new tumors before death. It is of interest to analyze the figures for the groups of mice in the above described experiments subjected to treatment with placenta or embryo skin extracts from the point of view of the new tumors developed.

Experiment.—All of the animals in the foregoing experiments were followed closely for their duration of life, and the appearance of new tumors or the finding of them at autopsy was recorded. They may be considered in two groups; one in which the tumors were removed, the operative field treated with one of the extracts, and in which each animal received an autograft which had been immersed in one of the extracts; in the second group the tumors were not removed and the mice received weekly intraperitoneal injections of one of the extracts, the total number varying from six to fifteen. For controls we have two earlier series followed in this laboratory, a recent series the direct control for the present experimental groups, and the figures published by Haaland. The collected data are given in Table V.

Here as with the other data collected from the studies of spontaneous cancer in mice, there is little variation between the figures collected by different investigators and from different strains. The incidence of new tumors in the untreated animals which had previously had one or more tumors, is 37.5 per cent, and the maximum spread between groups is from 32.0 to 43.0 per cent. Among the 210 treated mice the rate was 3.3 per cent, and in the 99 receiving several treatments only

[1] Slye believes that pregnancy retards the growth of spontaneous neoplasms in mice (Slye, M., *J. Cancer Research*, 1920, **5**, 25), but as none of the mice in our series were pregnant this point need not be considered.

one new tumor developed, or a rate of about 1 per cent. These figures on the prevention of new foci of malignant change are as definite as those for the other points investigated. The study is being extended to cover the possibility of preventing the origin of a first tumor in mice of families normally showing a high tumor rate. The observa-

TABLE V

Development of New Tumors

	No. of tumor animals	No. which developed new tumors	Percentage which developed new tumors
Controls			
Haaland*	209	73	35.0
Nakahara*	50	16	32.0
Early Rockefeller Institute series	138	53	38.5
Present series	125	54	43.2
Total controls	522	196	37.5
Tumors removed			
One treatment skin extract	46	3	6.3
One treatment placenta extract	65	3	4.6
Tumors not removed			
Several injections skin extract	42	1	2.4
Several injections placenta extract	57	0	0.0
Total treated	210	7	3.3

* For references see Table I.

tion will include internal tumors as well as those of the mammary gland.

Histological Study

The tumors composing the above described experimental material represent the usual types of mammary gland neoplasm. Histologically those grafts or unoperated tumors which had been affected by the tissue extracts showed no striking change in their structure. The only prominent feature was the reduction or complete absence of mitotic figures in the tumors after treatment, compared with the biopsy sections. There was a suggestion of more differentiation in the treated tumors and some showed an increase in the connective tissue surrounding and invading the mass, but these differences were

not sufficiently uniform to justify a conclusion as to their importance. No increase in cellular reaction was noted about the grafts, but a systematic investigation of the early changes was not made. A more complete study is in progress.

DISCUSSION

There are so many gaps in our evidence that it is untimely to discuss the bearing of these results on the hypothesis outlined in the foregoing paper (1), which has served as a basis for the investigation. There is no direct evidence as yet that the factor or substance in embryo skin and placenta extracts is related to the hypothetical control mechanism for normal cells. The lack of any reaction on the part of the host and the fact that the inhibitors are effective when the tumors are treated *in vitro* before reinoculation suggest that the action is a direct one on the cancer cells rather than a stimulation of the natural resistance mechanism of the body. Stimulation of the latter is the general interpretation accorded to the results obtained with generalized X-ray, dry heat, and fatty acids (6) which seem to represent an entirely different process from the action of the inhibitors. Our present impression is that the inhibitors affect directly the malignancy of the cell. However, any even tentative conclusion must await a more definite understanding of the control mechanism of normal cells.

The findings as concerns the various points investigated, prevention of postoperative recurrence, growth of autografts, the stoppage of further growth of well established cancer, followed in many instances by absorption of the tumor, and the prevention of new foci of malignancy, all indicate a very definite and pronounced effect of the extract of embryo skin and placenta on the growth and development of malignant cells. There were no direct controls for this series in which other tissue extracts were utilized, for such extracts had shown no inhibiting effect on transplanted cancer. It was judged more important to test the two materials which had proved effective with the transplantable tumors on the largest possible number of spontaneous cancers. We have records of unsuccessful attempts to influence the growth of spontaneous tumors by injection of homologous and heterologous serum, red blood cells, and a variety of other protein

materials which would indicate that the present results are not due to some general non-specific reaction.[2]

SUMMARY

Extracts of desiccated homologous embryo skin and placenta decrease markedly the rate of postoperative local recurrence after the surgical removal of spontaneous cancer of mice. Autografts after a short period of contact with these extracts either failed to grow, or, in the majority of instances, their subsequent growth was definitely retarded. Intraperitoneal injection of the extracts was followed by cessation of growth of established tumors in more than two-thirds of the animals treated, and among these many of the tumors regressed and over 20 per cent were completely absorbed. Tumor mice treated with either extract rarely developed new malignant foci, though this happened frequently in untreated mice.

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[2] This statement is based on an unpublished study of a large series of spontaneous tumors. No doubt other attempts of this sort have been made, and as negative results have not been reported.