A FACTOR FROM NORMAL TISSUES INHIBITING TUMOR GROWTH*

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

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

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A factor has been demonstrated in certain tumors of the fowl which has a definite inhibiting or neutralizing action on the transmitting agents of these tumors (1). It has proved to be non-specific in its effect, as shown by the fact that it will slow down or actually prevent the growth of a mouse sarcoma (2). It has been suggested that this factor may be related to the control or balancing mechanism of normal cells. The present paper is a report of an investigation designed to test the possible presence of a growth-inhibiting factor in active normal tissues (3).

It might be expected that tissues with the greatest growth energy would require a greater concentration of the hypothetical balancing factor to insure controlled and regulated growth. Therefore, the embryonic tissues have been utilized as the main source of material investigated.

Material and Method

Several strains of standard transplantable mouse tumors have served as the medium on which the growth-inhibiting factors were tested, the principal ones being the Bashford Adenocarcinoma No. 63, Carcinoma 48, and Sarcoma 180.

Following the method which had given the best yield of the inhibiting substance from the fowl tumors, desiccates of the tissue were employed. The tissues or organs to be studied were removed, minced finely, spread in a thin layer, and placed immediately in a vacuum jar over sulfuric acid. After being evacuated down to about 1 mm. mercury the container was placed in a freezing box and allowed to remain until desiccation was complete. The dry flakes were ground to a fine powder and stored in sealed tubes at ice box temperature until used. The test solutions were prepared by thoroughly extracting 0.1 gm. of the desiccate with 2 cc. of water, by pumping back and forth in a syringe through a coarse needle.

* This investigation was carried out under the Rutherford Donation.

The larger particles were centrifuged out and generally the supernatant fluid was heated at 48°C. for 30 minutes. The Bashford tumor was cut into generous sized grafts and these were nicked in several places to give a greater area of exposed surface. Half of these were placed in the test solution and half in normal salt solution. The time of contact was only that required for loading the grafts into trocars. A certain amount of the extract was carried along with the inoculated material. With No. 48 and the sarcoma, cell suspensions were generally used for inoculation. These were prepared by pressing the tumor through a fine wire mesh, and half of the *Brei* was suspended in the tissue extract and the other half in normal

TABLE I

Effect of Extracts of Mouse Placenta and Embryo Skin on Mouse Tumor Bashford 63

Material inoculated with Bashford tumor graft	No. of experiments	No. of mice inoculated	No. of tumors	Average size of tumors	No. negative	Per cent negative
				cm.		
Extract of dry mouse placenta	19	225	91	1.02 x 0.82	134	59.6
Salt solution (grafts inoculated in same animals as above)		211	163	1.43 x 1.10	48	22.7
Salt solution (grafts inoculated in other animals)		110	109	1.73 x 1.36	18	16.4
Extract of dry embryo skin	10	144	64	1.09 x 0.97	80	55.5
Salt solution (grafts inoculated in same animals as above)		84	66	1.38 x 1.08	18	21.4
Salt solution (grafts inoculated in other animals)		90	69	1.66 x 1.36	25	27.7
Extract of fresh placenta or embryo skin	8	79	56		23	29.1
Salt solution		70	40		30	40.2

salt solution in a ratio of 1:1 between cells and suspending fluid. From 0.05 to 0.1 cc. of these suspensions were injected. In order to secure a more complete check each animal inoculated with the tumor and tissue extract in one groin received the control inoculation of the tumor in salt solution in the other groin.[1] Besides these controls additional animals were frequently inoculated in both groins with the same tumor material in salt solution.

[1] It was perhaps not sufficiently emphasized in our earlier papers on the inhibitors in the fowl tumors that the test and the control materials were both inoculated in each fowl, using the intradermal site where accurate measurements may be obtained. By having each test controlled in the same animal the natural variation in degree of susceptibility is eliminated in judging the results.

The Effect of Homologous Tissue Extracts on the Growth of Transplantable Tumors

The first investigation was carried out with various homologous tissues, but only two strains of tumors were used as indicators.

Experiment.—Fresh tissues were utilized for the earlier experiments and they included fresh mouse placenta, embryo skin, and skinless embryos, with the extracts both heated and unheated. These extracts tested by 222 inoculations of the Bashford 63 failed to show any detectable effect on either the number of takes or the growth rates of the resulting tumors. Likewise, extracts of desiccated skinless embryos and mouse blood, tested by 102 inoculations of the same tumor, showed no influence on the resulting tumors as compared with the controls.

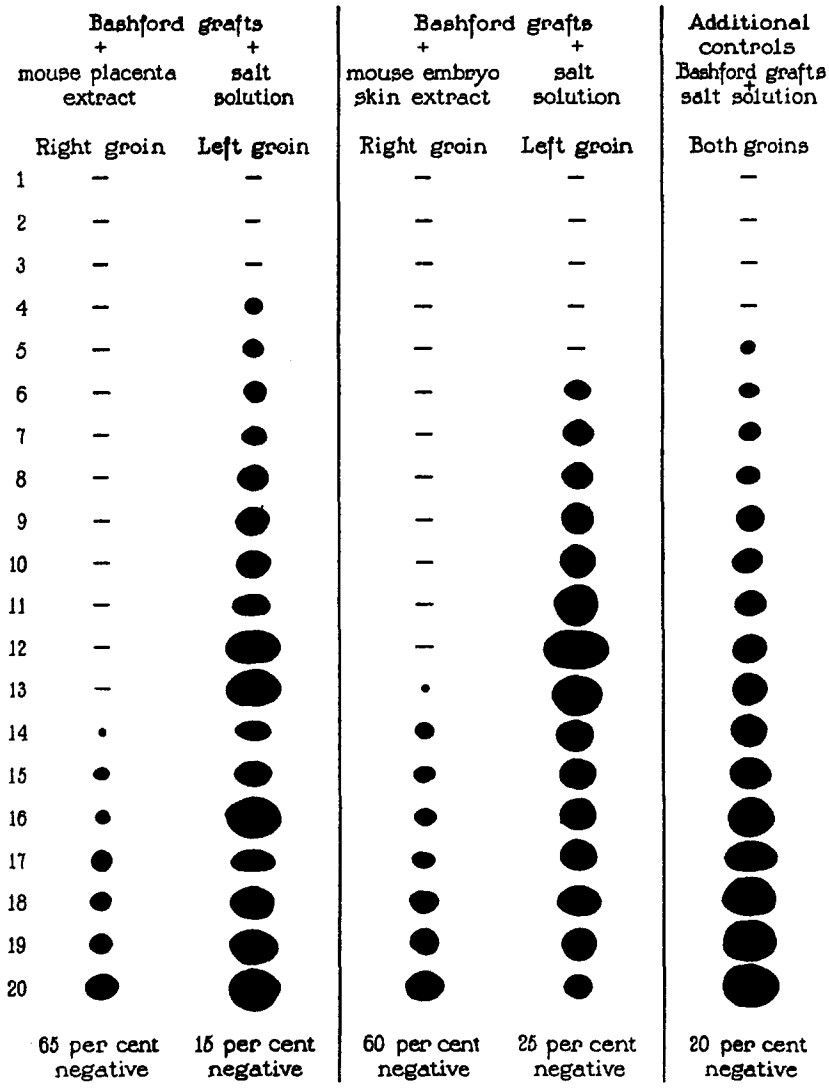
The major experiments were directed towards testing extracts of desiccated mouse placenta and embryo skin, as preliminary tests had indicated an effect on tumors by extracts of these two tissues.

The methods used were those described above. The length of contact between the tumor graft and the solutions was at the maximum 20 minutes. As the heating of the extract at 48°C. for 30 minutes had no material effect on the result all of the experimental results are grouped together. In the majority of instances the control material of tumor immersed in salt solution for a corresponding time was inoculated into the same animal, as well as in other animals. The results, including the average size of the tumor, are given in Table I.

The outcome of a typical experiment is shown in Text-fig. 1.

A similar test was carried out on the effect of the embryo skin and placenta extracts on Mouse Sarcoma 180. The results of these are given in Table II.

The extracts of desiccated mouse placenta and mouse embryo skin give evidence of inhibiting the growth of the Bashford Mouse Tumor 63. The test involved 369 inoculations controlled by 495 inoculations of the same tumor and the effect is shown not only by the material reduction in the number of takes of the treated grafts but by the slow rate of growth of such tumors as were not completely suppressed. In fact there were very few instances in which the tumor arising from the treated graft was not definitely smaller than that from the control graft inoculated into the same animal. There was an absolute failure of extracts prepared from fresh placenta or embryo skin to produce any effect on the growth of this tumor. The Mouse Sarcoma 180 which was definitely retarded by the inhibitor from chicken tumors (2) was unaffected by extracts either from fresh or desiccated mouse tissues.



TEXT-FIG. 1. Effect of homologous tissue extracts on mouse tumor.

Attempts to Induce General Resistance

While there is nothing in the above described experiments to suggest that the results are in any way connected with the well known induced

resistance to transplantable tumors, it seemed advisable to test this point. The method by which this resistant state is brought about is

TABLE II

Effect of Homologous Tissue Extracts on Mouse Sarcoma 180

Material inoculated with Mouse Tumor 180	No. of experiments	No. of mice inoculated	No. of tumors	Average size of tumors	No. negative	Per cent negative
				cm.		
Extract of dry mouse placenta	10	86	85	2.05 x 1.75	1	1.2
Salt solution		69	69	2.20 x 1.90	0	0.0
Extract of dry embryo skin	5	49	49	1.70 x 1.30	0	0.0
Salt solution		50	50	1.70 x 1.20	0	0.0
Extract of fresh placenta	2	12	12	1.80 x 1.55	0	0.0
Salt solution		12	12	2.10 x 1.70	0	0.0
Extract of dry skinless embryo	7	59	59	2.35 x 1.85	0	0.0
Salt solution		47	47	2.55 x 2.15	1	2.0
Extract of fresh skinless embryo	2	12	12	2.20 x 1.00	0	0.0
Salt solution		12	12	2.10 x 1.70	1	0.0

TABLE III

Effect of Previous Injection of Tissue Extracts on the Growth of Tumors

	No. of experiments	No. of mice inoculated	No. negative	Per cent negative
0.2 cc. placenta or embryo skin extract injected 7 days prior to inoculation of Bashford tumor	4	44	14	31.8
Bashford tumor alone (control)		34	8	23.5
0.2 cc. placenta of embryo skin extract injected 7 days prior to inoculation of Sarcoma 180	2	12	1	8.3
Sarcoma 180 alone (control)		18	0	0.0

the injection of homologous living normal cells into the animal 7 to 10 days prior to the inoculation of the tumor.

Experiment.—Based on four experiments, 44 mice were given subcutaneously 0.2 cc. of either an extract of dry placenta or dry embryo skin, and 7 days later

these mice were inoculated with grafts of the Bashford tumor. At the same time 34 controls were inoculated with the same tumor. A similar experiment was carried out on mice subsequently inoculated with Sarcoma 180. The results of these tests are given in Table III.

There is no evidence from these experiments that extracts of desiccated placenta or embryo skin induce a subsequent general resistance to inoculated tumor.

TABLE IV

Effect of Heterologous Tissue Extracts on the Bashford Tumor

Material inoculated with Bashford 63	No. of experiments	No. of mice inoculated	No. of tumors	Average size of tumors	No. negative	Per cent negative
				cm.		
Extract of desiccated rabbit placenta	6	68	23	1.52 x 1.30	45	66.2
Salt solution		68	47	1.97 x 1.40	21	30.9
Extract of desiccated rat placenta	5	59	17	1.10 x 0.90	42	71.1
Salt solution		59	43	1.60 x 1.20	16	27.1
Extract of desiccated rat embryo skin	4	40	18	0.90 x 0.70	22	55.0
Salt solution		40	26	1.40 x 1.00	12	30.0
Extract of fresh rabbit placenta	2	17	14	1.32 x 0.95	3	17.6
Salt solution		17	13	1.30 x 1.05	4	23.5

Inhibiting Action of Heterologous Tissue Extracts

As noted above, the inhibitors from chicken sarcomas are not species limited in their action, as shown by their effect on mouse sarcoma. In the next group of experiments we tested heterologous tissue extracts on transplantable mouse tumors.

Experiment.—In a series of 293 inoculations, controlled by 283 inoculations, the following heterologous tissue extracts showed no very definite effect on either the Bashford Tumor 63 or Carcinoma 48; desiccated and fresh calf thymus, desiccated kidney and spleen of the rat and rabbit, early and term human placenta, and early cow, rabbit, and hog placenta.

The main experiments were tests of rat and rabbit placenta and rat embryo

skin. The methods employed were those described above. The materials were collected during the later stages of pregnancy. The data from tests on Bashford tumor grafts are given in Table IV, and for cell suspensions of Tumor 48 in Table V. The results of a typical experiment are shown in Text-fig. 2.

The inhibiting action of rat placenta and embryo skin and of rabbit placenta is quite definite. As in the case of the homologous tissues the action is manifest not only by the complete suppression of growth in a high percentage, but where the suppression was not complete the growth rate of the resulting tumors was almost invariably distinctly slower than that shown by the control grafts in the same

TABLE V

Effect of Heterologous Tissue Extracts on Mouse Carcinoma 48

Material inoculated with cell suspension of Mouse Tumor 180	No. of experiments	No. of mice inoculated	No. of tumors	Average size of tumors	No. negative	Per cent negative
				cm.		
Extract of desiccated rabbit placenta	28	275	114	1.19 x 0.92	161	58.5
Salt solution		275	220	1.58 x 1.14	55	20.0
Extract of desiccated rat placenta	2	20	7	1.40 x 1.00	13	65.0
Salt solution		20	14	1.50 x 1.10	6	30.0

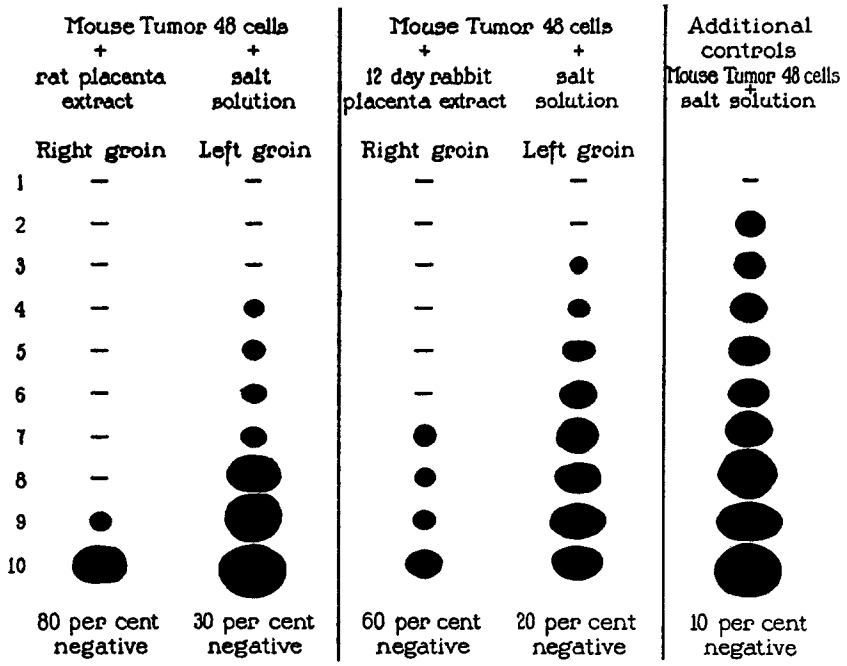
animals. These points are well shown by Text-fig. 2. The failure of the extracts of fresh tissue are just as evident with heterologous as with homologous tissues. There was no definite effect from extracts of desiccated early cow, hog, or human placentas or from human placentas at term.

The Effect of Age of Placenta on Inhibiting Action

The absence of any very definite inhibiting action of the extracts of early hog, cow, and human placentas and of human placentas at term opened the question as to whether this was due to the gap between the species or to the age of the placentas. We had some preliminary evidence that early stages of rabbit placenta were not active, while the later stages were active. On the basis of this evidence

of a possible bearing of the age of the placenta on its inhibiting action, a systematic investigation of the point was taken up.

Experiments.—Rabbit placentas were collected at the 12th, 15th, 19th, 22nd, 26th, and 28th day of pregnancy (30 day term) and prepared in the way described above. The extracts of the desiccates were tested on Mouse Tumor 48 in the usual way, with control inoculations of the tumor suspension in salt solution into

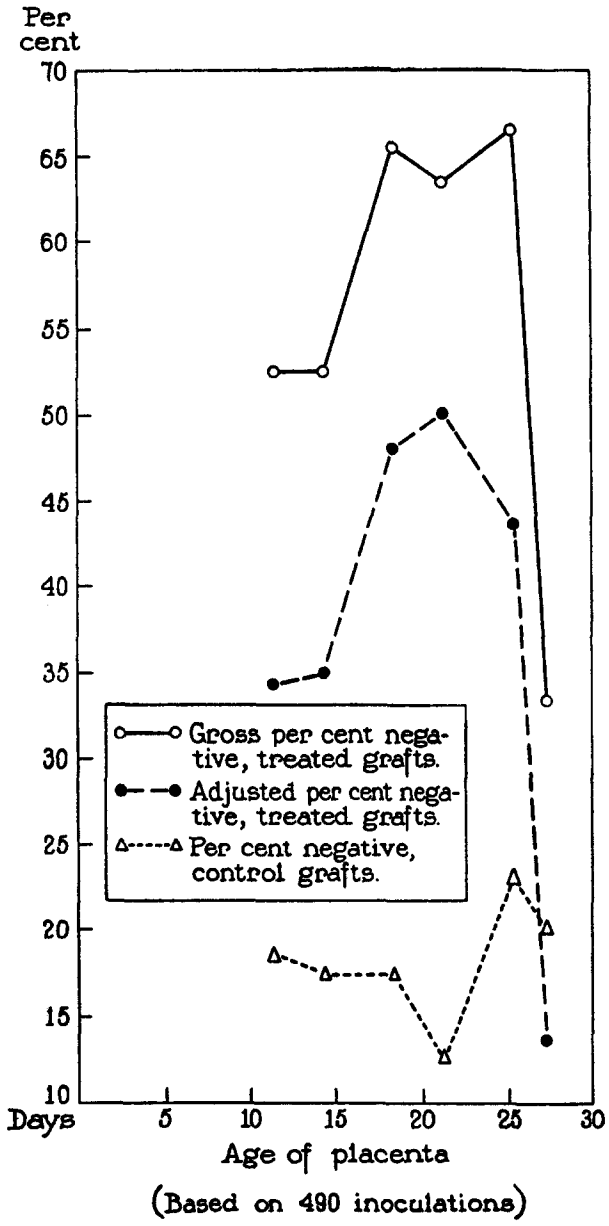


TEXT-FIG. 2. Effect of heterologous placenta extract on mouse carcinoma cells.

the same animals. The results of this test, based on 490 inoculations, are shown in Text-fig. 3. The data for the early stage placentas which were inactive are not included.

In the light of the above described results it would appear that the lack of inhibiting property encountered in the early cow, hog, and human placenta and term human placenta was probably due to the age of the organ rather than to the difference in species.[2] No doubt the

[2] Tests on cow placentas between the 7th and 8th month of pregnancy are in progress. The present indications are that appreciable amounts of the inhibiting factor are present.


Per cent
70
65
60
55
50
45
40
35
30
25
20
15
10
Days
5
10
15
20
25
30
Gross per cent negative, treated grafts.
Adjusted per cent negative, treated grafts.
Per cent negative, control grafts.
Age of placenta
(Based on 490 inoculations)

TEXT-FIG. 3

curve as represented in Text-fig. 3 might be modified by an increased number of tests, but a sufficient number are available to establish the main points. There seems no immediate explanation in the sudden drop between the 26th and 28th days, 2 days before term.

DISCUSSION

This investigation was undertaken on the hypothesis that the balanced state and orderly growth of normal cells is maintained by the interaction of two forces, one which stimulates growth and the other which retards it. Under this hypothesis the break in the balance between the factors, either the loss of the retarding or the accumulation of the stimulating factor, would lead to uncontrolled growth. While the reported results demonstrate the presence of a retarding factor when the extracts of certain active tissues are tested against transplantable cancer—a fact which would seem to support the hypothesis—the question raised is far too complex to justify such a conclusion yet. Nor do we consider that there is sufficient evidence to show that the inhibitor from normal tissues is of the same order or acts in the same manner as the inhibitor isolated from certain fowl tumors. However, there are points of probable significance. The inhibitor from the fowl tumors could be demonstrated only in extracts from desiccated tumors, and the normal tissue factor is evident only in desiccates of placenta and embryo skin. With both materials their action is not species limited but appears to be tissue limited, the factor from chicken tumors acting only on sarcomas, and that from placenta and skin only on carcinomas. This specificity of action and the fact that the inhibitors are not demonstrable in extracts of fresh tissues are taken as evidence that they are not simply proteolytic enzymes or some other substance generally injurious to cells.

The explanation of the release of the inhibitor factor by desiccation of the tissues is not evident. It may be that in the tissues it is closely associated with the growth factor which neutralizes its action, and only becomes demonstrable when it is dissociated from the combination through desiccation.

Before the hypothesis suggested can be seriously entertained, the premise that normal cell balance is maintained by the interaction of two forces must be established. While there is a suggestion of some

such mechanism in the work of Spemann (4) and others the principle is still far from being established.

SUMMARY

Extracts of desiccated embryo skin and placenta have been found to exert a definite retarding action on the growth of two transplantable carcinomas of mice, but they were without effect on sarcomas. In tests involving some 828 inoculations of the tumor cells and the extracts, complete suppression of growth occurred in from 55 to 71 per cent of instances, as compared with 21 to 27 per cent in the controls, and where growth was not completely suppressed some retardation was found in practically every instance. To judge from findings in rabbits the inhibitor is not demonstrable in the placenta until the beginning of the second third of pregnancy, reaches its maximum by the last third, but disappears about 2 or 3 days before term. Extracts of fresh placenta are without effect, and no very definite inhibition was noted in extracts of a variety of other desiccated or fresh tissues. The conclusions here reported are based on the results of over 3800 inoculations.

BIBLIOGRAPHY

1. Murphy, Jas. B., Helmer, O. M., Claude, A., and Sturm, E., *Science*, 1931, **73,** 266. Murphy, Jas. B., *Tr. Assn. Am. Physn.*, 1931, **46,** 182. Sittenfield, M. J., Johnson, A. S., and Jobling, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1931, **28,** 517. Gye, W. E., and Purdy, W. J., The cause of cancer, London, Cassell and Co., 461. Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1932, **56,** 103.
2. Murphy, Jas. B., and Sturm, E., *Science*, 1931, **74,** 180; *J. Exp. Med.*, 1932, **56,** 483.
3. Murphy, Jas. B., and Sturm, E., *Science*, 1932, **75,** 540 (preliminary note).
4. Spemann, H., *Naturwissenschaften*, 1933, **27,** 505.