THE EFFECT OF EXTRACTS OF DIFFERENT ORGANS AND TISSUES UPON THE VIABILITY OF TRANSPLANTABLE TUMORS

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It is a well established clinical fact that organs vary greatly in their susceptibilities to neoplastic growth. Laboratory studies have also indicated that different organs are not equally susceptible to tumor implantation and to the development of spontaneous tumors. Such differences in tumor development may well be accounted for by the presence in the normal tissues of tumor-growth-inhibiting substances. The occurrence of such substances is indicated by observations recorded by Woglom (1), Sugiura and Benedict (2), de Gaëtani and Blothner (3), Furth et al (4).

The investigation here recorded has to do with the action of aqueous extracts of organs and tissues on the viability of tumor transplants.

Materials and Methods

The organs and tissues to be studied were removed from freshly killed, healthy, young adult albino rats in the morning before feeding. After the removal of all extraneous tissue and fat, the organ or tissue was washed with Locke-Ringer solution and dried on filter paper. The tissue was then weighed, ground thoroughly with sea-sand in a mortar, and taken up with ten times its own weight of Locke-Ringer solution at pH 7.4. After standing twenty-four hours at 7° C., the mixture was centrifuged at high speed (2,300 r.p.m.) for fifteen minutes. Two cubic centimeter portions of the supernatant fluid were put into small Jena glass weighing bottles having ground glass stoppers, and allowed to come to the temperature of the refrigerator, 4–5° C. Immediately small pieces of fresh tumor tissue (generally eleven pieces to each bottle), each weighing about 6 mg., were placed in these solutions and left for definite periods of time at 4–5° C., the bottle being shaken gently twice in twenty-four hours. The tumor fragments were then inoculated into animals by the usual trocar method, each animal usually receiving a single graft in the right or left axilla. Each set of experiments included as a control animals inoculated with untreated tumor tissue shortly after removal from the tumor-bearing animal. Aseptic precautions were taken throughout.

The tumors used were mouse sarcoma 180 and Flexner-Jobling rat carcinoma. Our previous studies (5, 6) indicated that these tumors show marked differences in their reaction to different hydrogen-ion concentrations. Because of possible changes in the reaction of the extract on standing we determined the hydrogen-ion concentration of the tissue extracts, by the colorimetric method, before and after immersion of tumor tissues.

Effect of Heterologous Tissue Extracts on the Growth of Mouse Sarcoma 180

Tissue extracts were prepared with fresh rat heart, lung, liver, kidney, leg muscle, testis, brain, placenta, and embryo and pieces of mouse sarcoma 180 were placed in these as described above. After varying periods of time, the fragments were inoculated into mice, subcutaneously in the region of the axilla. The results are given in Table I.

In the course of the investigation determinations were made of the influence of protein-free Locke-Ringer solution (pH 7.4) upon the proliferating capacity of mouse sarcoma 180 at 4–5° C., the procedure being the same as already described. It was found that immersion of fragments of mouse sarcoma 180 for from 24 to 120 hours, at 4–5° C., was without effect, the tumors subsequently growing normally when implanted in animals. Similar treatment for 168 hours produced 20 per cent inhibition. Immersion of tumor fragments for 288 hours resulted in complete failure of growth upon implantation.

Of the tissue extracts, that of spleen was found to exert the most destructive action upon the growth capacity of mouse sarcoma 180, while lung and placenta extracts showed the least effect. Embryo, kidney, heart, liver, leg muscle, brain and testis extracts had an intermediate effect. Thus immersion of tumor fragments for 120 hours in spleen extract at 4–5° C. produced complete inhibition of growth. On the other hand, fragments of tumor remained 100 per cent viable after immersion for the same time in lung or placenta extract. Fragments immersed in extracts of kidney, heart, liver, leg muscle, brain and testis for 120 hours yielded from 80 to 90 per cent takes, but only 60 per cent of takes were obtained with tissue treated with embryo extract.

It is interesting to note that fragments of mouse sarcoma 180 immersed in rat blood serum or Locke-Ringer solution at pH 7.4 for 120 hours at 4–5° C. remained viable, the takes being the same as in untreated controls, as shown in Table II.

The inhibitory action of spleen tissue upon tumor growth was recognized as early as 1912, when Oser and Pribram (7) found that transplantable rat sarcoma grew more rapidly in splenectomized animals, and that retrogression or cessation of tumor growth could be induced in sarcoma-bearing rats by the subcutaneous injection of spleen pulp. Since then numerous papers have appeared upon the effect of spleen on the growth of animal tumors (8–44).

It appears from our study that the aqueous extract of fresh spleen tissue contains a greater amount of substance which is antagonistic to tumor growth than extracts of other organs and tissues mentioned in this paper. Some questions arise in connection with these results. Is the different toxicity of the tissue extracts due to variation in the degree of absorption by the tumor tissue? Or to the chemical composition? Or to some other factor?

In an attempt to answer these questions, we investigated first the hydrogen-ion concentration of the tissue extracts. As pointed out in a previous paper (6), when the pH of the medium (a Clark's buffer mixture solution or a Locke-Ringer solution) is 4.0 or 10.0, the growth capacity of mouse sarcoma 180 is completely destroyed in twenty-four hours at 4–5° C. At pH 5.0, 69 per cent inhibition, and at pH 9.0, 61 per cent inhibition occurred. On the other hand,

TABLE I: *Results of Transplanting Mouse Sarcoma 180 after Immersion in Various Tissue Extracts at 4–5° C.*

Exp. no.	Number of tumor transplants	Nature of tissue extract	Duration of exposure (hours)	Growth of transplants (per cent)	Remarks
1	10	Untreated	—	100	Rapid growth
	10	Spleen	24	100	Rapid growth
	10	Spleen	48	80	2 tumors did not grow; 6 grew slowly; others grew rapidly
	10	Kidney	24	100	Rapid growth
	10	Kidney	48	100	8 tumors grew slowly; others grew rapidly
	10	Liver	24	100	2 tumors grew slowly; others grew rapidly
	10	Liver	48	100	6 tumors grew slowly; others grew rapidly
2	10	Untreated	—	100	Rapid growth
	10	Heart	24	100	Rapid growth
	10	Heart	48	100	1 tumor grew very slowly; 4 grew slowly; others grew rapidly
	10	Muscle	24	100	Rapid growth
	10	Muscle	48	100	3 tumors grew slowly; others grew rapidly
	10	Lung	24	100	Rapid growth
	10	Lung	48	100	5 tumors grew slowly; others grew rapidly
3	10	Untreated	—	100	Rapid growth
	10	Spleen	72	90	1 tumor did not grow; 7 showed no growth in first week, normal thereafter; 2 grew normally
	10	Spleen	120	10	9 tumors did not grow; 1 grew slowly
	10	Brain	24	100	4 tumors grew slowly; others grew rapidly
	10	Brain	48	100	4 tumors grew slowly; others grew rapidly
	10	Testis	24	100	3 tumors grew slowly; others grew rapidly
	10	Testis	48	100	4 tumors grew slowly; others grew rapidly
4	16	Untreated	—	100	Rapid growth
	10	Kidney	72	100	Rapid growth
	10	Kidney	120	80	2 tumors did not grow; 7 grew very slowly; 1 grew slowly
	10	Liver	72	100	3 tumors grew slowly; others grew rapidly
	10	Liver	120	90	1 tumor did not grow; 8 grew very slowly; one grew rapidly
	10	Lung	72	100	Rapid growth
	10	Lung	120	100	7 tumors grew very slowly; one grew slowly; others grew rapidly
5	10	Untreated	—	100	Rapid growth
	10	Spleen	96	0	No growth
	10	Spleen	120	0	No growth
	10	Embryo	96	100	1 tumor grew slowly; others grew rapidly
	10	Embryo	120	60	4 tumors did not grow; 5 grew very slowly; one grew rapidly

TABLE I—*Continued*

Exp. no.	Number of tumor transplants	Nature of tissue extract	Duration of exposure (hours)	Growth of transplants (per cent)	Remarks
5	10	Placenta	96	100	1 tumor grew slowly; others grew rapidly
	10	Placenta	120	100	5 tumors grew very slowly; 3 grew slowly; others grew rapidly
6	10	Untreated	—	100	1 tumor grew slowly; others grew rapidly
	10	Spleen	120	0	No growth
	10	Kidney	120	80	2 tumors did not grow; 7 grew slowly; one grew rapidly
	10	Brain	72	100	Rapid growth
	10	Brain	120	90	1 tumor did not grow; 4 grew slowly; others grew rapidly
	10	Testis	72	100	5 tumors grew slowly; others grew rapidly
	10	Testis	120	90	1 tumor did not grow; others grew slowly
7	10	Untreated	—	100	Rapid growth
	10	Spleen	96	10	9 tumors did not grow; one grew slowly
	10	Spleen	120	0	No growth
	10	Embryo	96	100	3 tumors grew slowly; others grew rapidly
	10	Embryo	120	60	4 tumors did not grow; 4 grew slowly; others grew rapidly
	10	Placenta	96	100	2 tumors grew slowly; others grew rapidly
	10	Placenta	120	100	6 tumors grew very slowly; 3 grew slowly; one grew rapidly
8	10	Untreated	—	100	Rapid growth
	10	Heart	96	100	5 tumors grew slowly; others grew rapidly
	10	Heart	120	80	2 tumors did not grow; 5 grew very slowly; others grew slowly
	10	Muscle	96	100	Rapid growth
	10	Muscle	120	90	1 tumor did not grow; 7 grew very slowly; others grew rapidly
	10	Lung	96	100	Rapid growth
	10	Lung	120	100	6 tumors grew very slowly; 2 grew slowly; others grew rapidly

tumor fragments immersed in solutions at pH 6.0, 7.0 and 8.0 for twenty-four hours at 4–5° C. grew normally when implanted into mice. For this reason the approximate pH of the tissue extracts was determined colorimetrically befor and after immersion of tumor tissues. It was found that before immersion the tissue extracts had pH values of 6.8 to 7.4. After twenty-four hours the mean pH values were between 6.5 and 7.0, which indicates that the inactivation of the growth capacity of mouse sarcoma 180 at 4–5° C. is not due to a change in the reaction of the liquid medium.

It was thought that a possible factor in the death of tumor tissues might be the hemoglobin and protein concentration of the medium. The 10 per cent

TABLE II: *Results of Transplanting Mouse Sarcoma 180 After Immersion in Serum or Salt Solution at 4–5° C.*

Duration of exposure (hours)	Growth of transplants (per cent)	
	Rat blood serum	Locke-Ringer solution
24	100	100
48	100	100
72	100	100
96	100	100
120	100	100
144	100	55
168	70	20
192	50	10
240	30	5
288	10	0
312	0	0

organ and tissue extracts prepared aseptically with a Locke-Ringer solution at pH 7.4, as described above, showed different color intensities and turbidities. The color intensity of the extracts, in decreasing order, were as follows: liver, deep red; spleen, deep red; placenta, red; lung, red; kidney, reddish brown; heart, pink; embryo, pink; testis, faint pink; leg muscle, whitish; brain, whitish. The extracts of the brain and testis were decidedly turbid, followed in order by the liver, placenta, kidney, heart, and leg muscle extracts. The extracts of embryo, spleen, and lung were only slightly turbid.

The intensity of color and the density of colloidal particles can be more accurately determined by means of a photoelectric photometer (45). Since the absorption of light is a function of the color content, the size, and the number of the particles, measurements taken by this method give values for a sum total of color and turbidity.

In the present case, 14 c.c. of each tissue extract in a Pyrex test tube, 18 mm. diameter, was placed in the apparatus and the light allowed to pass horizontally through the sides of the tube, the current output of the photoelectric cell was read, and the value so obtained compared with that given by the pure solvent, in this case 14 c.c. of Locke-Ringer solution (350 micro-amperes). The average values from 3 to 5 separate samples of each tissue, arranged according to the increasing reading of the micro-ammeter, are as follows: spleen 20, kidney 28, liver 57, lung 61, placenta 74, heart 84, leg muscle 104, testis 126, brain 144, embryo 214 micro-amperes. Mouse sarcoma 180 and Flexner-Jobling rat carcinoma gave 81 and 195 micro-amperes, respectively. These tumor tissue extracts were prepared in the same manner as normal tissue extracts, from non-necrotic areas of the rat and mouse tumors.

The splenic extract showed the greatest absorption of light and the embryo extract the least. In order to obtain a truer comparison of light absorption by various tissue extracts, these figures are expressed in logarithmic relationship, using the formula of log B — log U, where B is the reading of the pure Locke-Ringer solution, and U the reading with a tissue extract. If the greatest light absorption—in this case that of the spleen extract—is represented by

100 and the light absorption of the remaining tissue extracts is calculated in terms of this, the relative light absorption by the various extracts may be brought out more clearly. The percentages are as follows: spleen 100, kidney 88, liver 63, lung 61, placenta 54, mouse sarcoma 51, heart 50, leg muscle 42, testis 36, brain 31, Flexner-Jobling rat carcinoma 20, and embryo 17 per cent.

It will be seen, therefore, that there is no relationship between the hemoglobin content of various tissue extracts and their deleterious action, for although the spleen extract exerted the greatest inhibitory action on the growth capacity of mouse sarcoma 180 and incidentally had the greatest light absorption power, the extracts of the lung and placenta, which had about 60 per cent light absorption power in respect to spleen, showed the least toxic effect upon sarcoma growth.

Further proof of the specific inhibitory action of spleen extract upon the growth of tumor grafts is seen from the following experiments.

Extracts of spleen were prepared as before, and portions were diluted with an equal volume of Locke-Ringer solution. The color intensity and turbidity of the diluted extracts were determined by the photoelectric photometer, which gave an average reading value of 72 micro-amperes, equal to 55 per cent of the original undiluted spleen extract. As before, fragments of fresh tumor tissue were placed in undiluted and diluted spleen extracts, both adjusted colorimetrically to pH 7.4. After standing for definite periods of time at 4–5° C. the tumor fragments were implanted into mice in the usual way. The inhibitory action of the diluted spleen extract was not diminished, the number of takes being practically the same as with undiluted spleen extract. The inhibition of tumor growth is, therefore, not due to hemoglobin. This is in accord with our results on the effect of an anemia-producing diet on tumors, in which we came to the conclusion that no relationship exists between nutritional anemia and tumor growth (46). It is generally known, however, that presence of hemoglobin in cultures of tumor tissues interferes with the outgrowth of tumor cells.

The Effect of Homologous Tissue Extracts on the Growth of Flexner-Jobling Rat Carcinoma

Since Flexner-Jobling rat carcinoma had been found to be more sensitive to various chemical and physical agents than mouse sarcoma 180, duplicate experiments were carried out with the former tumor. As before, fragments of fresh tumor tissue were placed in extracts of organs and tissues of normal young adult rats, and after standing for various lengths of time at 4–5° C. were implanted into rats. The results of these experiments are shown in Table III.

Table III shows clearly that the growth capacity of the Flexner-Jobling rat carcinoma was either completely or partially inhibited when tumor fragments were immersed in various tissue extracts for 48 hours at a temperature of 4–5° C. The inhibitory action of the spleen extract was greatest. Extracts of liver, heart, lung, leg muscle and kidney were less toxic to the transplanted tumor. The extracts of various tissues, however, showed a much greater inhibitory effect upon the proliferating capacity of the Flexner-Jobling

TABLE III: *Results of Transplanting Flexner-Jobling Rat Carcinoma after Immersion in Various Tissue Extracts at 4–5° C.*

Exp. no.	Number of tumor transplants	Nature of tissue extract	Duration of exposure (hours)	Growth of transplants (per cent)	Remarks
1	10	Untreated	—	80	2 tumors did not grow; 4 grew slowly; others grew rapidly
	10	Spleen	24	10	9 tumors did not grow; one grew slowly
	10	Spleen	48	0	No growth
	10	Liver	24	20	8 tumors did not grow; 2 grew slowly
	10	Liver	48	0	No growth
	10	Muscle	24	30	7 tumors did not grow; others grew rapidly
	10	Muscle	48	0	No growth
2	10	Untreated	—	70	3 tumors did not grow; others grew rapidly
	10	Spleen	24	30	7 tumors did not grow; others grew rapidly
	10	Spleen	48	0	No growth
	10	Liver	24	80	2 tumors did not grow; others grew rapidly
	10	Liver	48	50	5 tumors did not grow; others grew normally
	10	Kidney	24	50	5 tumors did not grow; others grew rapidly
	10	Kidney	48	30	7 tumors die not grow; others grew normally
3	14	Untreated	—	93	1 tumor did not grow; others grew rapidly
	10	Spleen	24	40	6 tumors did not grow; 3 grew slowly; one grew rapidly
	10	Spleen	48	10	9 tumors did not grow; one grew slowly
	10	Heart	24	60	4 tumors did not grow; others grew normally
	10	Heart	48	30	7 tumors did not grow; others grew normally
	10	Lung	24	80	2 tumors did not grow; others grew normally
	10	Lung	48	50	5 tumors did not grow; others grew normally

rat carcinoma than did the sera of mouse, rat, guinea-pig, rabbit, and man (47). Tumor fragments immersed in sera for 24 hours at 4–5° C. remained viable, the growth being the same as in the untreated controls. Total inhibition of growth was brought about only when tumor fragments had remained in blood serum for 96 hours.

In the course of the investigation determinations were made of the influence of aqueous extracts of the spleen of immune and tumor-bearing rats upon the proliferating capacity of Flexner-Jobling rat carcinoma and mouse sarcoma 180 at 4–5° C. The spleens taken from animals bearing large Flexner-Jobling rat carcinomas were two to three times the size of those of normal animals. The inhibitory action of splenic extracts of immune and tumor-bearing rats did not differ appreciably from that of normal rats.

Summary

1. An investigation has been made of the effects of immersing fragments of mouse sarcoma 180 and of Flexner Jobling carcinoma in aqueous extracts of fresh rat heart, lung, liver, kidney, spleen, brain, leg muscle, testis, embryo and placenta, prior to transplantation.

2. The growth capacity of mouse sarcoma 180 and Flexner-Jobling rat carcinoma was markedly inhibited when fragments of these tumors were treated with splenic extract before implantation.

3. The inactivation of the growth capacity of the carcinoma and sarcoma cells by extracts of embryo, kidney, heart, liver, leg muscle, brain and testis was less than in splenic extract. The extracts of the lung and placenta were least toxic to these tumors, but were more toxic than the sera of mouse, rat, guinea-pig, rabbit and man.

4. No significant difference was found in the growth-inhibiting action of aqueous extracts of spleens from normal rats, from immune rats, and from rats bearing progressively growing Flexner-Jobling rat carcinoma.

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