New York Section

8158 C

Effect of Walker Rat Sarcoma on Growth of Transplanted Cancer.

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A factor has been demonstrated in extracts of certain chicken tumors which if concentrated is capable of neutralizing the filtrable transmitting agents of chicken tumors and of retarding the growth of mammalian sarcomas.[1] A factor has also been obtained from certain active normal tissues, placenta, embryo skin,[2] and testicle[3] which has a definite inhibiting action on the growth of mammalian carcinoma. The present investigation was undertaken to determine whether an inhibiting factor could be demonstrated in mammalian tumors.

Preliminary tests made with several of the transplantable tumors indicated that the Walker tumor gave the best intimation of the presence of an inhibiting factor, so the experiments were confined to this tumor. It grows in nearly 100% of inoculated rats, seldom retrogresses, and causes death of the animal in from 6 to 7 weeks Tumors of this type from 3 to 4 weeks old were finely minced, spread in a thin layer and placed in a freezing box until desiccation was complete. After being finely powdered, 1 gm. was thoroughly extracted with 10 cc. of Ringer's solution and centrifuged. The supernatant fluid was used for the tests.

The effect of this material was tested on Mouse Sarcoma 180, Carcinoma 48, and the Brown-Pearce rabbit epithelioma.

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1 Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1932, **56**, 107; *Ibid.*, 1932, **56**, 483; *Science*, 1932, **75**, 540.

2 Murphy, Jas. B., and Sturm, E., *Science*, 1933, **77**, 631; *J. Exp. Med.*, 1934, **60**, 293.

3 Duran-Reynals, F., *J. Exp. Med.*, 1931, **54**, 493; Tanzer, R. C., *J. Exp. Med.*, 1932, **55**, 455.

Experiments with Mouse Sarcoma 180. In the first group of experiments the tumor material was prepared by first mincing, and then after the addition of a few drops of saline solution it was squeezed through bolting cloth. This gives a finely divided cell suspension. A portion of this was mixed with an equal volume of the Walker tumor extract and allowed to stand at room temperature for 60 minutes. As control the same amount of tumor cell suspension was diluted with an equal volume of Ringer's solution and kept under the same conditions as the test material. At the end of the period, mice were injected into one groin with 0.1 cc. of the test material and in the other with an equal amount of the control suspension. The results of 10 such experiments are given in Table I.

TABLE I.

Exp. No.	No. of mice	Tumors	
		Experiment, %	Control, %
1	6	0	33.3
2	11	18.2	81.8
3	9	0	44.4
4	7	0	42.8
5	8	12.5	62.5
6	9	0	44.4
7	10	70.0	70.0
25	6	0	66.6
31	11	0	81.8
27	8	0	50.0
Total	79	12.7	64.5

It is evident from Table I that the extract yields a factor or substance which prevents or retards the growth of Sarcoma 180.

In a second group of experiments with 59 mice the tumor suspension was prepared by passage through a wire mesh, which gave a suspension composed of fairly large cell clumps. This suspension was tested in the same manner as in the first group. No evidence of inhibition was observed.

Experiments on Mouse Carcinoma 48. Exactly the same procedure was next applied to a dilute cell suspension of Carcinoma 48. Results may be summarized as follows: In 19 mice injected tumors grew in 36.8% in the side injected with mixtures of cell suspension plus tumor extract, while they grew in 68.4% in the control side. The experiments were then repeated inoculating grafts which had been immersed for 1 hour in the tumor extract, instead of cell suspensions. No inhibition could be observed under such conditions.

Brown-Pearce epithelioma of the rabbit. The tumor material was prepared in the same manner as that described above for the first

group of experiments. The rabbits were shaved in both flanks, and one side was injected intradermally in several areas with the tumor cell suspension plus Walker tumor extract, and the other side with the cell suspension diluted with salt solution. The results are given in Table II.

TABLE II.

Rabbit No.	Experiment			Control		
	No. of inoculations	No. of tumors	% tumors	No. of inoculations	No. of tumors	% tumors
519	4	0	0	4	2	50
520	4	0	0	4	4	100
570	3	0	0	4	4	100
571	4	0	0	5	4	80
578	7	0	0	4	4	100
579	8	0	0	4	3	75
Total	30	0	0	25	21	84

It is evident from Table II that the extract of the Walker tumor had a marked inhibiting effect on the growth of the epithelioma.

The question arose whether the action of the extract is on the tumor cells or on the host. The problem may be a complex one, but that at least part of the inhibiting effect is due to a direct action on the tumor cells is suggested by the following experiment.

A bolting cloth cell suspension of Mouse Tumor 180 was divided into 3 equal parts. Two of them were allowed to remain in contact with the Walker tumor extract for 10 and 75 minutes respectively before inoculation. The 3rd portion was allowed to stand for 75 minutes and was mixed with the extract 10 minutes before injection. These tests were controlled by injections of similar cell suspensions diluted with saline solution. These were injected into the opposite side of the same mice which had received the test inoculations. Results are given in Table III.

TABLE III.

Material injected	No. of mice	Tumors	
		Experiment %	Control %
Tumor cells after contact with the extract for 75 minutes	10	0	81.8
Tumor cells after contact with the extract for 10 minutes	13	84.6	84.6
Tumor cells after standing 75 minutes and mixed 10 minutes with the extract	10	60.0	40.0

Table III shows a marked inhibiting effect when the cells had remained in contact for a long time with the extract, while no evidence of inhibition was found when the duration of contact was

short. On the other hand, treatment of mice bearing either the Sarcoma 180 or Carcinoma 48 with the Walker tumor extract injected intraperitoneally had no effect. A possible inhibiting action on cells of the 180 tumor of desiccated extracts of the Brown-Pearce epithelioma of the rabbit, Sarcoma 180, Carcinoma 48, and a mouse melanoma has also been investigated. Results were consistently negative.

Conclusions. An extract of desiccated Walker rat tumor under certain experimental conditions prevents or retards the growth of Mouse Sarcoma 180, Mouse Carcinoma 48, and the Brown-Pearce epithelioma of the rabbit implanted in the skin. The action seems to be at least partially due to a direct effect on the cells, and is not shared by extracts of other transplantable tumors studied.

8159 C

Reaction of Spontaneous Mouse Carcinomas to Blood-Carried Bacterial Toxins.

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We reported[1] a study on the vascular reactions of a series of growths ranging from benign embryomas and granulomas to very malignant transplantable carcinomas and sarcomas of mice and rats to a blood-carried *B. coli* toxin of low potency. The phenomenon of the tumor reactivity to bacterial toxin was first described by Gratia and Linz,[2] and confirmed by Shwartzman and Michalowsky.[3] The reaction shows the general characteristics of the Shwartzman phenomenon. Apitz[4] has further proved that still other factors capable of inducing the latter phenomenon in rabbits also induce the phenomenon of the tumor reactivity.

From our investigations we concluded that only those growths showing at the same time *malignancy* and *rapidity of growth* present the phenomenon of Gratia and Linz.

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1 Duran-Reynals, F., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 341.

2 Gratia, A., and Linz, R., *Compt. rend. Soc. Biol.*, 1931, **108**, 427; *Ann. Inst. Pasteur*, 1932, **49**, 131.

3 Shwartzman, G., and Michalowsky, N., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 737.

4 Apitz, K., *Z. Krebsforsch.*, 1933, **40**, 50.