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.

F. DURAN-REYNALS. (Introduced by J. B. Murphy.)

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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.

As a corollary to this principle it was found on further analysis that the degree of pre-existing necrosis is, generally speaking, a good index of the degree of tumor susceptibility. Necrosis is the expression of a special state of vascular fragility which renders the rapidly growing tumor vulnerable to the injected toxin, even if the latter is practically harmless for the normal animal. Apitz[3] had already noticed a definite relation existing between the microscopic areas of necrosis and the incidence of hemorrhages.

The tumors most susceptible to the *B. coli* toxin were the fast-growing malignant transplantable tumors of rats and mice. As a result of the phenomenon a good many tumors regressed partially or totally. Regression was also observed after an apparently negative reaction in the tumor. On the other hand, the slower growing malignant spontaneous carcinomas of mice were found practically non-susceptible and their further course was not modified at all.

It is obvious that the phenomenon is conditioned by 2 sets of factors: the intrinsic factor depending on the sensitivity of the tumor itself, and the extrinsic one, depending on the activity, quantity, and route of inoculation of the toxin. Additional studies showed that both sets of factors could vicariously substitute for each other to a rather large extent. The very susceptible tumors required smaller doses of toxin to produce the same effect, and the same result was also obtained with filtrates of low toxicity as with much more active filtrates. Also, the route of inoculation did not seem to be a matter of much importance. This suggested that perhaps one could obtain with the refractory spontaneous tumors results similar to those obtained in transplantable tumors, but by using filtrates of higher toxicity.

Accordingly a series of tests was carried out on 52 mice bearing mammary carcinomas using a very toxic "agar washing" of human typhoid bacillus (supplied by Dr. Shwartzman), and filtrates of 6-day-old broth cultures of Mouse Typhoid I (*B. enteritidis*), Mouse Typhoid II (probably *B. paratyphosus* B.), and Mouse Typhoid III. The filtrates of Strain II of mouse typhoid were also very toxic; the filtrates of the other two strains were less so. The malignant character of the tumors was histologically confirmed in every case, either from biopsy or at the post-mortem examination.

In the first experiment 3 mice were injected intraperitoneally with 0.5 cc. of filtrate of the "agar washing" of the human typhoid. The 3 animals died in less than 6 hours without any alteration in their tumors.

In the second experiment 13 mice were first injected with 0.1 cc.

TABLE I.

Effect of Injection of Toxin of Mouse Typhoid Bacillus on Spontaneous Carcinomas of the Mouse.

	No. of mice	Size of tumors		Intensity of phenomenon in tumor after 1st injection			Dead within 48 hours	Evolution of tumor in survivors			Partial or total inhibition
		Large*	Small†	Strong	Mild or doubtful	Negative		Steady growth	Partial inhibition	Total regression	
1st series											%
1st injection .25-.5 cc. intraperiton. followed by 3-9 similar weekly injections .1-.2 cc.	22	16 (72%)	6 (27%)	11 (50%)	5 (22%)	6 (27%)	11 (50%)	5	4	2	27
2nd series											
1st injections of 0.1 cc. intraperiton. or sub. cutan. followed by 3-9 similar weekly injections	14	2 (14%)	12 (85%)	4 (28%)	2 (14%)	8 (57%)	1‡ (7%)	5	4	4	57
Total	36										38

* Tumors of a size from 2.5 x 3.0 cm. to 3.5 x 4.0 cm.

† Tumors of a size from 0.4 x 0.6 cm. to 1.5 x 2.0 cm. The few tumors of an intermediate size have been classed with one group or another according to their special growth characteristics.

‡ Mouse already sick before injection.

of toxin from Mouse Typhoid II intraperitoneally, and this was repeated at weekly intervals. The following results were obtained. Nine mice died within the first 6 days, 4 of which showed hemorrhages in their tumors. Of the 4 remaining 2 showed a weak phenomenon and the growth of the tumors was not modified. One mouse showed no reaction, but there was a temporary retrogression of the tumor. The last mouse had a medium sized papillary adenocarcinoma which retrogressed to a small nodule, proving on histological examination to be made up only of connective tissue.

In the third and most extensive experiment 36 mice were treated with a mixture of equal parts of toxin and of the 3 strains of mouse typhoid. Mice have been classified in 2 groups according to the amount of toxin given in the first injection. Simply by chance it so happened that the animals treated with the higher doses were also those bearing larger tumors, so that these 2 factors must be considered together. The details and results of the test are also given in Table I.

The group of mice having larger tumors and receiving the larger dose of toxin had a higher incidence, greater intensity of local reaction, a higher early mortality, and a lower percent of partial, or total inhibition than did the group with the preponderance of small tumors, receiving the smaller doses of toxin.

These observations duplicate similar findings on transplantable rapidly growing malignant tumors.

Moreover, Table I shows that inhibition is better observed when small or medium sized tumors are treated with small doses of toxin. The mortality under such conditions is very low. It is noteworthy that inhibition is observed without any apparent reaction taking place in the tumors and, consequently without any danger to the life of the animal. Thus, out of 14 cases of partial or total inhibition 3 gave a strong tumor reaction, 4 gave mild or doubtful reactions, and 7 negative reactions. The average length of life from the beginning of treatment in mice showing partial inhibition was 80 days, while the average for mice whose tumors were not inhibited was 50 days. The average length of life in the 6 mice showing total inhibition was 171 days. In 3 of them no tumor could be found at autopsy. In the other 3 cases the tumor recurred after the mice had remained free from disease for 46, 60, and 180 days respectively.

The facts brought to light in transplanted as well as in spontaneous tumors establish the principle that the newly formed vessels of malignant growth, either as a consequence of an excessive per-

meability or through some other cause, are extremely sensitive to injections of blood-carried bacterial toxins and it is this fact which creates a very special state, the *tumor vulnerability* which is responsible for the regression of malignant growths.

8160 C

Inhibiting Action of Placenta Extract on a Transplantable Malignant Epithelioma of the Rabbit.

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Previous work has been reported showing the existence in mouse placenta of a factor markedly inhibiting the growth of both transplantable and spontaneous carcinoma of the mouse.[1] Placenta from rats and rabbits were later found to be equally effective in retarding the growth of transplantable mouse carcinoma.[2] The present study is an extension of this observation to include a tumor of a different species.

The epithelioma of Brown-Pearce was selected for this work. The tumor is extremely malignant and invasive when grafted into the testicle but if inoculated into the skin it eventually regresses after a period of active growth.

The mouse placenta was prepared according to the technique described in the above-mentioned publications. Late term placenta was finely minced, dried *in vacuo* and kept in the cold room. The desiccated material was ground, extracted with 10 volumes of Ringer's solution or plain water and then centrifuged; the supernatant fluid was used as such or diluted with 10 volumes of Ringer's solution. In some cases the extract was heated to 48°C., a procedure which did not seem to modify in either direction the activity of the extract.

The tumor cells from healthy testicle growths were passed through a masher and suspended in a few cc. of Ringer's solution. This suspension was squeezed through bolting cloth and divided into 2 parts. These were mixed with an equal volume of placenta extract

*Fellow of the C.R.B. Educational Foundation.

1 Murphy, Jas. B., and Sturm, E., *Science*, 1933, **77**, 631.

2 Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1934, **60**, 293.