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Reaction of Transplantable and Spontaneous Tumors to Blood-carried Bacterial Toxins in Animals Unsusceptible to the Shwartzman Phenomenon.

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Gratia and Linz[1] described a phenomenon characterized by a violent hemorrhagic and necrotic reaction in the tumor in guinea pigs bearing large transplantable lipo-sarcomas, shortly after intravenous injection of a filtrate of *B. coli* broth culture. The authors identified this phenomenon with that of "Sanarelli-Shwartzman", and also with similar focal hemorrhagic reactions resulting from intravenous injection of bacterial filtrates in rabbits injected by a number of bacteria and filterable viruses.* Shwartzman and Michalowsky[3] described the same phenomenon in a rather extensive series of mice bearing Sarcoma 180. Guinea pigs as well as mice may die within 24 hours following the injection.[1, 3] Some of the mouse tumors may temporarily regress or completely disappear.

Besides their intrinsic value, 2 considerations make these findings particularly interesting: (1) mice and rats are unsusceptible to the ordinary Shwartzman phenomenon, and it would seem that special conditions existing in the tumor render its vessels, supplied by the host, apt to react with the blood-carried bacterial toxins. (2) This state of reactivity of the tumor in either susceptible or non-susceptible animals is a permanent one, without the need of any previous local injection of bacterial filtrate as is the case with the ordinary Shwartzman phenomenon in rabbits and guinea pigs.

We have studied the phenomenon described by Gratia and Linz on a variety of neoplastic processes in animals unsusceptible to the Shwartzman phenomenon, namely, rats and mice. As a source of bacterial toxin we used filtrates of 6-day-old broth cultures of *B. coli* and injected them into the peritoneum or vein in the amount of 0.5 cc. for mice and 1 cc. for rats. We have divided our results into 2

[1] Gratia, A., and Linz, R., *Compt. rend. Soc. biol.*, 1931, **108**, 427; *Ann. Inst. Pasteur*, 1932, **49**, 131.

* As early as 1907 S. P. Beebe and M. Tracy[2] described the regression of several cases of dog lymphosarcoma under the influence of the "Coley toxin" from *Streptococcus, B. coli,* and *B. prodigiosus.*

[2] Beebe, S. P., and Tracy, M., *J. Am. Med. Assn.*, 1907, **49**, 1493.

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

groups, according to whether the phenomenon was positive or negative.

1st group (positively reacting tumors). This group includes 203 mice or rats bearing rapidly growing malignant transplantable tumors which give a high percentage of takes and rarely retrogress. The tumors differed widely in age, size, and degree of necrosis at the time of the toxin injection. The results are summarized in Table I.

TABLE I.

No. of animals	Tumor	% showing positive phenomenon	% of regressed tumors	% death within 24 hours following injection
61 mice	S/37 sarcoma	67	20	0
68 "	180 "	60	10	10
21 "	M/63 adeno-carcinoma	57	0	47
19 "	Twort " "	70	0	0
34 rats	Walker sarcoma	88	0	41

In the tumors included in Table I the intensity of the phenomenon bears a direct relationship with the age and size of the tumors. While very young, perfectly healthy tumors often give a negative phenomenon, large tumors, averaging 1x1 cm. for mice and 2x1 cm. for rats, give practically 100% positive results. The strongly positive phenomenon is characterized by an extensive hemorrhagic condition, which in most cases may be observed externally through the skin. A more moderate phenomenon requires the opening of the growth to be detected. The very necrotic parts, where vessels are obliterated, are naturally free from hemorrhage. In the more prominent cases the phenomenon can be detected in the tumor as early as one hour after the peritoneal injection of bacterial filtrate.

2nd group (negatively reacting tumors). This group includes 69 mice and rats bearing: (1) comparatively slowly growing spontaneous or transplantable malignant tumors which rarely or never regress, (2) rapidly growing malignant tumors which eventually regress (heterologous graft), and (3) benign embryomas or granulomas, rapidly developing, which eventually regress. The detailed account is as follows:

19 mice bearing spontaneous malignant mammary adeno-carcinomas.

6 mice bearing the transplantable malignant Harding and Passey melanotic sarcoma.

4 mice bearing the transplantable malignant Walker rat tumor.

10 rats bearing the transplantable malignant S/37 or 180 mouse sarcoma.

20 mice bearing benign embryomas.

10 mice bearing kieselguhr granulomas.

None of these tumors or granulomas showed any definite hemorrhagic phenomenon. Only 2 spontaneous tumors and 2 mouse tumors growing in rats showed a doubtful mild phenomenon. Three spontaneous tumors were tested a second time 24 hours after the tumor had been "prepared" by injection of 0.2 cc. of filtrate into the mass. Again no modification in the tumor could be noticed. It is worth pointing out that some of the mouse tumors growing in rats had at the time of injection attained a mass of 2.2x0.9 cm. of perfectly healthy tissue with actively dividing cells and apparently perfect blood supply, as shown by histological examination. Such growths were the result of grafting large pieces of tumors under the skin of the heterologous host.

From the results obtained with the tumors so far studied, it appears that only those growths showing at the same time *malignancy* and *rapidity of growth* show the phenomenon of Gratia and Linz.

The mechanism of death as a result of the reaction in the tumor is not understood, and no significant general lesions have been found at the autopsies. Numerous control tests have shown that normal mice and rats stand the injection of the bacterial filtrate without marked alterations in their health. Additional experiments have shown that the general resistance of the animals to the ordinary Shwartzman phenomenon is not changed by the existence of the tumor. Also small tumors may be found unsusceptible despite their growing in an animal bearing large susceptible tumors. The frequency of death seems to be in a direct relationship with the size and age of the tumor, but besides this there is another intrinsic factor depending on the strain of tumor itself. The Bashford adenocarcinoma seems, from this point of view, to be the most "toxic" of all the strains so far studied.

Animals in which, as a consequence of the phenomenon, the tumors regressed were found resistant to regrafting. The intimate cause of this tumor regression is now being investigated.

In view of the fact that transplantable tumors are often infected by various bacteria, we have secured sterile tumors, and found that they are as sensitive to the phenomenon as the infected ones. Moreover, filtrates of tumors showing a strong phenomenon, injected into the skin of rabbits, have failed to sensitize this organ to intravenous injection of a potent bacterial filtrate made 24 hours later.

Work is underway extending these studies to more true tumors and granulomas occurring in animals both susceptible and resistant

to the ordinary Shwartzman phenomenon in order to obtain more information about the relationship existing between malignancy and rapidity of growth on the one hand, and ability to react to bacterial toxins on the other hand.

Summary. Whereas rapidly growing transplantable malignant tumors in rats and mice are very susceptible to blood-carried *B. coli* toxin, slow-growing malignant spontaneous or transplantable tumors, malignant tumors rapidly growing in heterologous hosts, embryomas, and granulomas are practically non-susceptible.

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Contribution to the Etiology of Encephalitis. Differentiation of Encephalitis by Protection Tests.

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Muckenfuss, Armstrong, and McCordock[1, 2] and Webster and Fite[3] have reported that the encephalitis epidemic in St. Louis during the summer and autumn of 1933 is communicable, by inoculation, to monkeys and mice. In addition, Webster and Fite[3] reported that the encephalitis prevailing in Kansas City at the same time is likewise communicable to mice; that the infectious agent from the St. Louis and Kansas City cases is filterable, is readily transmissible to mice, is highly virulent when instilled into the nasal passages of mice, and is neutralized by the serum of encephalitis convalescents from the 1933 epidemic.

We have continued our studies of the effect on the encephalitis virus of various sera derived from cases of encephalitis and from immunized monkeys, and will report the result of these tests in the present paper.

Monkeys injected with the virus develop in their sera protective properties similar to those in the sera of convalescent St. Louis and Kansas City encephalitis cases. Again, serum from a monkey injected with one strain of virus from a St. Louis case protects not only against that strain of virus but against 2 other strains from St.

[1] Leake, J. P., *J. Am. Med. Assn.*, 1933, **101**, 928.

[2] Muckenfuss, R. S., Armstrong, C., and McCordock, H. A., *Pub. Health Rep.*, 1933, **48**, 1341.

[3] Webster, Leslie T., and Fite, George L., *Science*, **1933, 78, 463.**