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Phenomenon of Local Skin Reactivity to Bacterial Filtrates in the Treatment of Mouse Sarcoma 180.

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The phenomenon of local skin reactivity to bacterial filtrates described by one of us (Shwartzman[1]) was later also reproduced in the liver, kidney (Shwartzman[2]), testis, intestines, lymphatic glands, lungs, thymus, guinea pig liposarcoma (Gratia and Linz[3]), stomach (Karsner, Ecker and Jackson[4]), and knee joints (Moritz and Morley[5]). It was elicited with a great variety of microorganisms (Shwartzman[6]) and also with vaccine virus as the preparatory factor (Gratia and Linz[3]). The animals in which the phenomenon was observed were rabbits (Shwartzman[1]), horses, goats (Shwartzman[8]), and guinea pigs (Gratia and Linz[3]). It could not be reproduced in mice and rats (Shwartzman[7]). Assuming that malignant tumors may be of parasitic etiology, Gratia and Linz[3] thought that the hypothetical virus should then be capable of inducing a state of reactivity in the tumor tissue and thus render it susceptible to reacting factors in the blood stream. Five guinea pigs bearing liposarcoma were injected intravenously with *B. coli* culture filtrate. Two guinea pigs which died 24 hours later and 2 killed 48 hours later showed at autopsy hemorrhagic lesions in the tumor tissue and no lesions in other organs. The fifth guinea pig was left alive for

[1] Shwartzman, G., *J. Exp. Med.*, 1928, **48**, 247; *J. Inf. Dis.*, 1931, **48**, 339.

[2] Shwartzman, G., *J. Exp. Med.*, 1930, **51**, 571.

[3] Gratia and Linz, *Comp. Rend. Soc. Biol.*, 1931, Oct. 23.

[4] Karsner, Ecker and Jackson, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 319.

[5] Moritz and Morley, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 321.

[6] Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 207.

[7] Shwartzman, G., unpublished observations.

[8] Shwartzman, G., *J. Exp. Med.*, in press.

further observations. They selected guinea pigs because of their susceptibility to the phenomenon of local reactivity to bacterial filtrates.

Since it was deemed important to determine whether this phenomenon could be reproduced in transplantable tumors in mice, the effect of bacterial filtrates upon a mouse sarcoma 180 (Crocker Institute) was studied by the present authors. This strain of sarcoma was selected on account of its high growth energy and malignancy. The bacterial filtrate employed was of high phenomenon-producing potency, as previously determined in rabbits (Shwartzman[1]), namely "agar washings" filtrate of meningococcus 44D group I (*i. e.*, filtrate No. 1700 containing 1350 reacting units per cc.). The results can be summarized as follows:

Group I. Nine mice bearing 18-day-old tumors were each injected intravenously with 0.5 cc. of filtrate No. 1700. The injection killed 2 mice within 24 hours. At autopsy there was found extensive hemorrhage in the entire tumor mass and no evidence of it in any other tissue or organ. Twenty-four hours after the intravenous injection the surviving mice showed also extensive hemorrhage in the tumor mass including previously healthy borders. The necrotic mass hardened and separated, leaving a bed of granulation tissue. However, at the borders growth reappeared. Further treatment consisted of intravenous and intraperitoneal injections of 0.5 cc. of the same filtrate 10 and 12 days after the first injection, respectively. Four more mice died from the injections. The surviving mice showed again hemorrhage in the areas of new growth. In one mouse the tumor regressed similarly but began to grow again. In the remaining 2 mice the necrotic mass separated, the granulation tissue filled the bed and complete healing resulted. The mice showed no growth 2 months after tumor inoculation. Their general condition was excellent. One mouse was killed at this time. No growth was discovered in any organ.

Group II a. (4 mice). Mice I and IV received each intravenous injections of 0.25 cc. of filtrate No. 1700 on the 18th, 20th, and 23rd days of tumor growth. Mice II and III received these injections on the 30th and 34th days, in addition. Extensive hemorrhagic necrosis was evident in the tumor masses of all the treated mice within 24 hours after the first injection. The tumor of Mouse I gradually reduced in size until on the 35th day after tumor inoculation there was no tumor left. The healing proceeded uneventfully. Mouse II lost the tumor but died on the 35th day of tumor inoculation. Autopsy showed grossly no tumor growth and no

metastasis. The tumor of Mouse III regressed after the first 3 injections but later increased in size. It died on the 31st day of tumor growth. The tumor of Mouse IV completely disappeared. The mouse appeared completely free of growth on the 35th day of tumor inoculation.

Group II b. (4 mice). Mice V-VIII received each intraperitoneal injections of 0.25 cc. of filtrate No. 1700 on the 18th day of tumor growth. Mice VII and VIII died in 14 and 48 hours after the injection, respectively. The autopsy showed extensive hemorrhagic necrosis of the tumors, and no lesions in other organs. Mice V and VI which showed a similar picture 24 hours after the injection later developed large tumors.

Group II c. (4 mice). Mice IX-XII received each intravenous injections of 0.5 cc. of filtrate No. 1700 on the 18th, 20th, and 23rd days of tumor growth. Mice IX, X, and XII received in addition intravenous injections of the same dose on the 30th and 34th days of tumor inoculation. Here again, the first injection elicited severe hemorrhage in 24 hours. Finally, Mouse IX showed complete regression which was followed by scab formation. There was, however, a doubtful growth observed at the borders on the 37th day of tumor inoculation. The tumor of Mouse X showed a gradual regression to about 1/10 of the growth present before the first injection. After the 4th injection the growth reappeared. Mice XI and XII lost their tumors completely on the 4th week of tumor inoculation and no growth was evident later.

Group II d. (4 mice). Mice XIII-XVI received intraperitoneal injections of 0.5 cc. of filtrate No. 1700 on the 18th day of tumor growth. Mouse XIV died 24 hours later. It showed extensive hemorrhage in the tumor mass. Mice XIII and XV received in addition similar injections on the 20th and 23rd days and Mouse XVI on the 20th, 23rd, and 30th days of tumor growth. The tumor of Mouse XIII regressed to about 1/8 of the size before the first injection. The third injection killed it. The autopsy showed a large necrotic mass with doubtfully active growth. Mouse XV, dead on the 37th day of tumor inoculation, was found at autopsy free of growth. The tumor of Mouse XVI regressed to 1/6 of the size before the first injection and reappeared later.

Group III. In this group 15 mice bearing 17-day-old tumors were each injected intravenously with 0.5 cc. of filtrate No. 1700. Three mice died 24 hours later. They showed hemorrhagic necrosis of the tumors. Within the following 2 weeks there was observed complete regression of the tumor growth in 10 mice. Three of these

mice appeared completely free of tumors on the third week, while in 7 of these mice the growth began again. In 2 mice the growth was uninfluenced at any time after injection.

Control Groups. Nineteen mice in all were set aside as controls, a few for each experimental group. They all developed large tumors, which showed no spontaneous regression at any time. Two of these mice were injected intravenously each with 0.5 cc. of 2% glucose broth containing 0.4% phenol. There was no effect on the development of the tumor observed.

The number of mice employed is too small as yet to allow any conclusions as to the percentage of temporary or complete regressions obtained. However, inasmuch as the tumor strain employed is of high growth energy and malignancy and only very rarely regresses spontaneously, the following can be concluded:

It is possible to elicit prompt hemorrhage in the tumor tissue of mouse sarcoma No. 180 via the blood stream by means of bacterial "agar washings" filtrates (*i. e.,* meningococcus) of high potency in the phenomenon of local skin reactivity to bacterial filtrates.

The first appearance of the effect described resembles very closely the latter phenomenon. The hemorrhage leads to progressive damage of the tumor, which may be followed either by its complete elimination and healing, or by striking regression with further slow reappearance of tumor growth. The individual variations are probably due to spontaneous and active acquired immunity to the reacting factors observed in the phenomenon under discussion (Shwartzman[8]). The effect on tumors appears to be selective, inasmuch as the intravenous and intraperitoneal injections of the filtrates produce no hemorrhagic lesions in other organs of the mouse.

The injections are toxic and kill a large number of mice. Once recovered from the immediate effect, they remain in a good general condition. The repeated intravenous injections of 0.5 cc. of the filtrates employed were most destructive for the tumors; next in effectiveness were repeated intravenous injections of 0.25 cc. and repeated intraperitoneal injections of 0.5 cc. of the filtrates. Repeated intraperitoneal injections of 0.25 cc. remained without definite effect.

This report is of a preliminary nature because of insufficient number of mice and lack of histological studies.

Although there appears to be a close resemblance between the reaction described and the phenomenon of local skin reactivity to bacterial filtrates, it does not necessarily mean that a virus must be responsible for the state of reactivity of the tumor cells to bacterial

filtrates introduced via the blood stream. Studies on other possible explanations are under way.

Torrey and Kahn[9] found that filtrates of certain gram positive anaerobes remained without effect upon transplantable tumors of mice and rats. Inasmuch as their purpose was to employ preparations of high proteolytic activities but the phenomenon-producing potency was not determined, the negative results reported by them are not considered contradictory.

The observations reported in this paper are considered of interest because there appears to be a remarkable selective destruction of a tumor of high malignancy and of rapid growth; and also because being obtained in mice they offer an opportunity for further thorough studies on the relation of the "phenomenon of local skin reactivity to bacterial filtrates" to problems of tumor growth.

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B. coli Bacteriophage in the Treatment of B. coli Peritonitis in Mice.

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The bacteriophage which was used for this study was found to produce a complete lysis of 2 virulent strains of *B. coli* which we obtained from patients with peritonitis. One one hundred billionth of a cc. of this bacteriophage caused lysis of approximately one billion bacteria of strain "T" in 10 cc. of broth. The minimal lethal dose of the "T" strain of *B. coli* for mice at the time of the experiments was 1-5 million organisms which killed within 5 to 12 hours after injection of an actively growing, 3-hour, 2% dextrose cooked meat medium culture. Fifty million bacteria which constituted 10-50 M.L.D.'s were suspended in 0.5 cc. of broth and inoculated intraperitoneally into a series of mice. 0.5 cc. of bacteriophage was injected simultaneously into 2 of these mice, and into 2 more mice at varying intervals up to 4½ hours after the bacterial inoculation. This approached closely the lethal period for control animals. In the control series plain broth injections were given at the same intervals as phage. The table shows

[9] Torrey and Kahn, *J. Cancer Research*, 1929.