THE EFFECT OF THE SHWARTZMAN REACTION WITH BACTERIAL FILTRATE ON TRANSPLANTABLE TUMORS IN ANIMALS

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In 1928 Shwartzman (1) described a phenomenon of local skin reactivity to bacterial filtrates which became known as the Shwartzman phenomenon. He observed that the injection of 0.25 c.c. of bacterial filtrate of B. typhosus into the abdominal skin of a normal rabbit produced no reaction except perhaps a mild erythema. If, however, after a period of twenty-four hours as little as 0.01 c.c. of the toxic substance per kilogram of body weight was injected intravenously, a blue discoloration appeared at the site of the first injection within a period of two hours. This discoloration increased rapidly so that at the end of four hours it was extremely pronounced, presenting a dark blue central area with a deep red zone at the periphery. Histologic sections of such an area showed a severe hemorrhagic and necrotic reaction characterized by edema of the skin, ruptured blood vessels, extensive subcutaneous hemorrhages, widespread leukocytic infiltration, and pronounced necrobiosis of fixed and wandering cells both within and outside the blood vessels. This phenomenon has been reproduced in the liver, kidneys (2), testes, intestines, lymphatic glands, lung, thymus (3) stomach (4) and knee-joints (5). It has also been elicited with a great variety of micro-organisms (6) and with vaccine virus. The reaction has been observed in rabbits, horses, goats, and guinea-pigs (3).

Gratia and Linz (3) obtained the reaction with a colon bacillus filtrate in liposarcomata in four guinea-pigs. Shwartzman and Michailovsky (7) elicited similar suggestive results with a meningococcus filtrate in mice bearing sarcoma 180. In neither instance was a sensitizing injection used. The present paper deals with a larger series of tumor-bearing animals treated according to the original technic of Shwartzman (1).

Method

Mouse sarcoma 180, Flexner-Jobling rat carcinoma, Walker rat carcinosarcoma 256, and the Rous chicken sarcoma 1 were used. These were selected because of their high growth energy and degree of malignancy. The behavior of these transplantable tumors in the hosts has been reported elsewhere (8).

Young healthy animals were used. The tumor material, selected from solid non-ulcerated growths, was inoculated subcutaneously by means of a trocar. The animals were given the usual mixed laboratory diet (9) with an unlimited supply of fresh water. The tumor-bearing animals were divided into groups, some to be treated with bacterial filtrates and others to be used as controls. The progress in the growth of the tumors was ascertained by measuring them at stated intervals by means of calipers.
As a source of bacterial toxin, the filtrate of a six-day-old broth culture of *B. typhosus* was used. It contained 1500 reacting units per c.c. In a preliminary trial it was found that the filtrate was extremely toxic, so that it became necessary to dilute it with six parts of normal saline. The optimal dose of the diluted filtrate was found to be 0.1 c.c. intratumorally and 0.2 c.c. intraperitoneally. These amounts are equivalent to 0.0145 c.c. and 0.029 c.c. of undiluted filtrate, respectively.

**Results**

*Xenopus Sarcoma 180*

**Control Group:** Ten mice bearing seven-day-old tumors constituted the control group. All these animals developed large tumors which showed no spontaneous retrogression at any time. The average length of life was twenty-five days. The dimensions of the tumors at the beginning of the experiment ranged from $1.1 \times 0.8$ mm. to $1.2 \times 1.4$ mm., and at death most of them were approximately 2.4 cm. in diameter. No metastases were observed.

**Group 1:** Twenty mice bearing seven-day-old tumors were sensitized intratumorally with 0.1 c.c. of the diluted *B. typhosus* filtrate, and twenty-four hours later were injected intraperitoneally with 0.2 c.c. of the diluted filtrate. In 14 of the mice, the tumors showed pronounced and violent hemorrhagic and necrotic reactions on the following day. In some instances these were evident externally through the skin. The hemorrhagic and necrotic masses soon hardened, desiccated, and sloughed off, leaving a bed of granulation tissue, which finally healed completely. The remaining 6 mice showed a less severe reaction. In them recurrences of the tumor growth took place at the borders of the sloughed areas after ten (3 mice), twelve (2 mice) and thirteen (1 mouse) days. These were treated repeatedly until complete sloughing and healing resulted, the average number of repetitions being four or five. Following each successive treatment, shrinkage and inhibition of tumor growth were observed within five days of the intraperitoneal injection. The mice appeared in good condition throughout the experimental period, and when killed five months after therapy they showed no tumor growth.

**Group 2:** Ten mice bearing seven-day-old tumors were treated with normal saline, instead of bacterial filtrate, both intratumorally and intraperitoneally. These injections had no demonstrable effect on tumor growth. The dimensions of the tumors at the beginning of the experiment ranged from $1 \times 0.9$ mm. to $1.4 \times 0.9$ mm., and at death they averaged about 2 cm. in diameter. The average duration of life was twenty-one days.

In order to rule out the possibility that the tumor changes were due to either localized focal necroses induced by the injected material or were the end-results of a non-specific foreign protein reaction, two additional control groups were used. **Group 3** received the filtrate only intratumorally and **group 4** only intraperitoneally.

**Group 3 (Control):** Five mice bearing seven-day-old tumors were each given an intratumoral injection of 0.1 c.c. of the diluted filtrate of *B. typhosus*. These revealed only a marked primary erythema with a local traumatic induration in the vicinity of the needle track. Histologically the tissues showed
a moderate leukocytic infiltration, but no necrosis or hemorrhage. The initial dimensions of the tumors ranged from $0.9 \times 7$ mm. to 1.1 mm., and at death they averaged 2.2 cm. in diameter. The mice ran a progressive and downward course and died of nutritional failure and of sepsis on the twentieth day following tumor inoculation. Autopsy failed to disclose any gross or histologic retrogressive changes in the tumor tissue.

**Group 4 (Control):** Five mice bearing seven-day-old tumors were each given an intraperitoneal injection of 0.2 c.c. diluted *B. typhosus* filtrate. One mouse died within twenty-four hours following the injection and autopsy failed to disclose any gross or histologic changes in the tumor tissue. One mouse showed slight surface reddening of the tumor on the day following the intraperitoneal injection. This, however, disappeared in a few hours, leaving the tumor unchanged in appearance or consistency. The other mice showed no gross structural changes in the tumor nor any growth inhibitory phenomena.

Four days following the original intraperitoneal injection, 3 mice (including the one that had exhibited slight tumor reddening) were each given a second intraperitoneal injection of 0.2 c.c. of the diluted *B. typhosus* filtrate. The tumors showed no gross changes nor evidence of retrogression. On the contrary, they continued to grow and the mice died on the twelfth, thirteenth and twentieth days after the tumor inoculations. The remaining mouse, which had received but one injection, died ten days after tumor inoculation. Autopsies on all four mice failed to reveal any gross or histologic inflammatory, necrobiotic, or regressive changes in the tumor tissues which might be attributed to the intraperitoneal injection of the bacterial filtrate.

*Flexner-Jobling Rat Carcinoma*

**Control Group:** Ten adult albino rats bearing twelve-day-old tumors, $2 \times 1$ cm. average size, were allowed to remain uninoculated. All died within thirty-two days of tumor transplantation. Autopsy revealed only necrosis of the central portions of the tumors, and histologic examination failed to show any retrogressive changes in the growing portion of the neoplasm.

**Treated Group:** Twenty adult albino rats bearing twelve-day-old tumors, 2 cm. in diameter, were each given an intratumoral injection of 1 c.c. of the diluted *B. typhosus* filtrate. The injections were made by inserting the needle at the periphery of the tumor and carrying it into the center, where the fluid was injected. This procedure produced no visible reaction at the site of the needle prick. Twenty-four hours later an intraperitoneal injection of 1 c.c. of diluted filtrate was administered. The following morning the tumors had become softened, spongy, fluctuant, and deeply reddened. In 8 rats the tumors disintegrated, ulcerated, and sloughed out at the end of eight days, in 6 in ten days, in 5 in eleven days, and in 1 in twelve days. The ulcerated areas were replaced by granulation tissue. No recurrences appeared. The rats were active and well nourished when killed three months after the first filtrate inoculation. At autopsy no gross or histologic evidence of tumor tissue was found. The site of the tumor was occupied by dense scar tissue.
Walker Rat Carcinosarcoma 256

Control Group: Ten adult albino rats bearing fourteen-day-old tumors, $2 \times 2.3$ cm. in diameter, were allowed to remain uninoculated. All died within forty-three days of tumor transplantation. Autopsy revealed necrosis only of the central two-thirds of the tumors. Along the edges there was widespread extension. Histologic examination failed to show any retrogressive signs in the growing portion of the tumor.

Treated Group: Thirty adult albino rats bearing fourteen-day-old tumors, 2 cm. in diameter, were treated in the same manner as were the rats bearing the Flexner-Jobling tumor. The resulting softening and reddening of the tumors were identical with the observations in the former group. In 7 rats the tumor had completely sloughed out in nine days, in 12 in eleven days, in 6 in thirteen days, in 3 in fifteen days, and in 1 in sixteen days. One rat showed two small recurrences on the eighteenth day, which completely sloughed upon being treated as before, on the twenty-sixth day after the first filtrate injection. The ulcerated area in every animal was completely replaced by scar tissue; no recurrences, except in the one rat referred to, were observed. The rats remained active and well nourished. They were killed one hundred and ten days after the first filtrate inoculation. At autopsy no gross or histologic evidence of tumor tissue was found. The site of the tumor was occupied by dense scar tissue.

Rous Chicken Sarcoma

Five healthy adult Plymouth Rock chickens, bearing seven-day-old tumors in the right pectoral muscles, were each given 1 c.c. of the undiluted B. typhosus filtrate intratumorally, preliminary studies having revealed that the filtrate was not toxic to chickens and could be used in large doses. Twenty-four hours after the intratumoral injection, 1 c.c. of the undiluted filtrate was administered into the wing vein. The following morning an extremely dark blue discoloration of the sarcomatous mass was observed. This later became softened, lost its outline, and finally disappeared. Three chickens, after being observed for a period of two months without revealing signs or symptoms of recurrence, died of intercurrent infections. Autopsy failed to disclose any neoplasia at the site of inoculation or in remote regions. The other two chickens were killed four months after the tumor inoculation, and these too failed to show evidence of primary or metastatic new growths. The average duration of life of non-treated tumor-bearing chickens is twenty-one to twenty-five days.

Summary and Conclusions

(1) The Shwartzman phenomenon was elicited in tumor tissue of mouse sarcoma 180, Flexner-Jobling rat carcinoma, Walker rat carcinosarcoma 256, and Rous chicken sarcoma.

(2) The tumor tissue was first "prepared" or sensitized by bacterial filtrate and thus rendered susceptible to an intraperitoneal or intravenous injection of the same filtrate, as, for example, B. typhosus filtrate.
(3) The phenomenon was characterized, in tumor tissue, by a violent hemorrhagic and necrotic reaction, followed either by a complete sloughing and healing or by slow recurrence of the tumor growth, which again responded to filtrate injections.

(4) In control experiments in which saline was substituted for the filtrate, or the filtrate injected only intratumorally or only intraperitoneally, the Shwartzman phenomenon was not obtained.

Note: Since these experiments were reported, we have attempted to duplicate these animal findings in a patient with an inoperable recurrence of a carcinoma of the breast. Such a recurrence in the axillary skin was sensitized by the injection of \( B. typhosus \) filtrate into it. Twenty-four hours later the patient received similar filtrate intravenously. Upon her death, four days later, the tumor nodule was completely necrotic and virtually replaced by a hemorrhagic effusion; the vessels of the area were necrotic.

Bibliography