THE EFFICACY OF "COLEY'S TOXIN" IN THE TREATMENT OF SARCOMA *

AN EXPERIMENTAL STUDY

ALEXANDER BRUNSCHWIG, M.D.

CHICAGO, ILL.

FROM THE DEPARTMENT OF SURGERY AND DIVISION OF ROENTGENOLOGY OF THE DEPARTMENT OF MEDICINE, UNIVERSITY OF CHICAGO, CHICAGO, ILL.

SINCE the observations many years ago, that patients with sarcoma who accidentally contracted erysipelas sometimes exhibited regression of the neoplasm, attempts have been made to treat sarcomata by injection of bacterial products, particularly those of *Streptococcus erysipelatis* combined with *Bacillus prodigiosus*. Interest in laboratory research in this question has developed in the last few years and a number of publications have appeared indicating that certain bacterial products, especially those of meningococcus and *B. coli*, were capable of inducing partial or complete liquefaction sometimes with complete regression of transplantable neoplasms in mice (and other animals). For a detailed review of the literature the reader is referred to the report of M. J. Shear[3] (1935).

There is some question as to the mechanism of such phenomena. According to some it is essentially an immunologic reaction of the nature of a Schartzman phenomenon. On the other hand, Shear and Andervont[4] have extracted from *B. coli* filtrates a "hemorrhage producing fraction" whose action would appear to be a direct and specific one upon the capillaries of the tumor. Furthermore, Andervont[1] has indicated that the reactivity of a neoplasm to bacterial products depends to some extent upon the hereditary factors of the animal.

Among clinicians the late W. B. Coley[2] was the most enthusiastic proponent of the treatment of sarcoma, especially of bone, by bacterial products in man. For many years he advocated the use of killed suspensions of *Streptococcus erysipelatis* combined with *B. prodigiosus* as an adjuvant to surgical and irradiation therapy. This mixture has become popularly known as "Coley's toxins." While the evaluation of the efficacy of the toxins alone under such conditions is difficult, a review of the accumulated case reports in the literature leaves an impression that one cannot conclude, at present at least, that the use of toxins was totally ineffective in all cases.

The purpose of the experiments recorded below was to study the effect of "Coley's toxins" (Parke Davis & Co.) and other bacterial products upon sarcomata in rats induced from the animals' own tissues as a result of subcutaneous injection in the interscapular region of a suspension of benzpyrene

* This work was facilitated by a grant from the Cancer Research Institute of the Chicago Woman's Club. Submitted for publication March 15, 1938.

or methylcholanthrene (2 to 3 mg. per animal) in lard. Sarcomata began to appear after four and one-half months following injection. The animals employed were of unknown genetic history; the colony was derived from the Wistar strain coupled with those obtained from a local dealer.

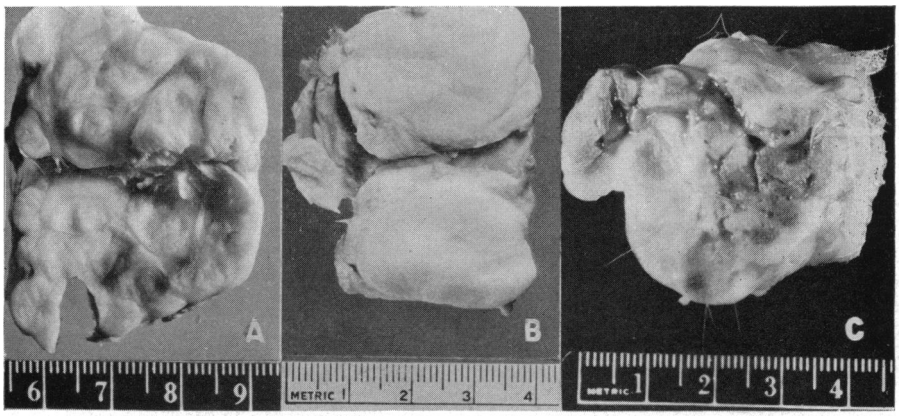

FIG. 1.—Compare with Fig. 2. (A) Sarcoma from a rat receiving intraperitoneal injections of Coley's toxins but exhibiting no reaction; there are a few small areas of spontaneous hemorrhage and necrosis. (B) Sarcoma from an animal injected as in (A) showing firm, dense whitish tissue, also no reaction. (C) Sarcoma from a control rat, that received no injections. Partially collapsed central cavity was filled by orange-red fluid; there is also spontaneous necrosis of the central portion of the tumor.

Control Observations.—Prior to evaluation of the effects of the bacterial products, control observations were made in a series of benzpyrene sarcomata to determine their natural course, especially as to the incidence and magnitude

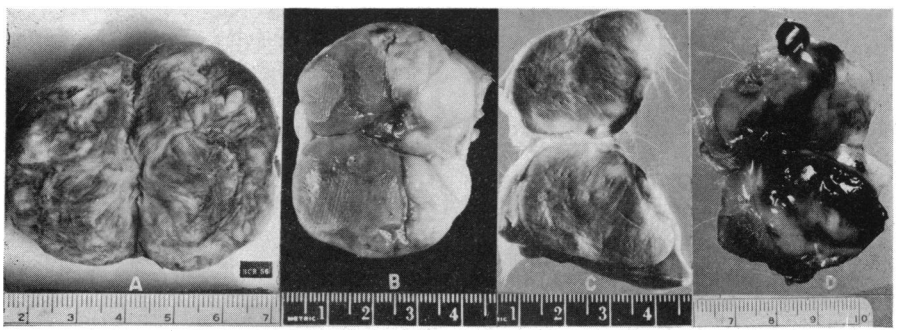

FIG. 2.—(A) Relatively mild diffuse hyperemia in a sarcoma from an animal injected with 2 cc. of Coley's toxins 24 hrs. previously. Animal died spontaneously. (B) Moderately severe hyperemic reaction in a sarcoma from an animal injected 2 days previously with 2 cc. of killed 7-day dextrose broth cultures of *Streptococcus erysipelatis*. Note absence of reaction throughout a large portion of the tumor. (C) Moderately severe hyperemic reaction throughout a tumor from an animal injected with 6.5 cc. of Coley's toxins during 21 days, and killed 22 days after the first injection. There was no apparent growth restraint. (D) Intense hemorrhagic reaction in the tumor from an animal injected 24 hrs. previously with 1 cc. of Coley's toxins. Animal died spontaneously.

of spontaneous hemorrhages and necrosis. In 15 animals, specifically set aside for these observations from the larger group employed in the following experiments, it was found that the consistency of the tumors themselves varied from very hard, even suggesting the presence of bone, to a soft cystic con-

sistency denoting spontaneous liquefaction. The animals were killed at varying periods up to 60 days following daily palpation of the tumor, once it had become 1 cm. or larger in diameter. In 12 cases the surfaces of the tumors appeared grayish and semitranslucent. On cut surface, *small* scattered areas of recent or old hemorrhage were often seen and in some tumors large, central yellowish areas of necrosis were present. In two instances large blebs of reddish-orange fluid were present, once within the tumor and once beneath the "capsule." These blebs composed a large part of the gross bulk of the neoplasms and gave it a cystic quality on palpation *in vivo*. Adjacent to, or surrounding the blebs, the tumor tissue was reddish-yellow, necrotic and amorphous. In another instance the entire tumor, measuring 5 by 3 cm., became cystic while under observation, and at necropsy was found to be composed of semisolid, reddish-orange, amorphous material resembling a clot in consistency but not in color.

In the series reviewed above, and in a number of other rats bearing similar tumors and employed in other experiments, it was observed that the natural rate of growth in these neoplasms varied widely. Some grew only several millimeters in diameter over a period of three to four weeks while others grew to 10 cm. in diameter over a similar period.

In none of the animals observed in the appended experiments was there evidence that intraperitoneal injections of the bacterial products resulted in growth restraint of the sarcomata; on the other hand, some effects were noted.

When the animals died or were killed and the tumors excised and bisected the following criteria were employed to record results:

(1) Grayish or yellowish-gray tumors were regarded as exhibiting no effects due to bacterial injections.

(2) Large yellowish areas of central necrosis were regarded as spontaneous changes.

(3) Areas of liquefaction, where the fluid and surrounding tumor tissue exhibited an *orange-red color*, were regarded as spontaneous changes since they had been observed in the control series described above.

(4) Diffuse hyperemia (red or dark red color) with or without areas of softening, the latter when present resembling a blood clot and distinctly different from the areas of liquefaction observed in (3) were regarded as effects due to injection of bacterial products since in no instance in the above control or in other experiments were such changes observed to occur spontaneously.

Experiment 1.—Intraperitoneal injection of Coley's toxins in rats bearing benzpyrene sarcomata. This product proved to be quite toxic for the animals in the doses administered, since of a total of 22 animals, 10 died within 48 hours after receiving one injection of 1 to 2 cc.

Of the latter, four tumors showed positive effects, six were negative.

Of the remaining 12, Coley's toxins in doses of 1 to 2 cc. were administered every 24 to 48 hours to total doses of 6 to 14 cc. over periods varying up to 25 days. The animals were killed at intervals of 4 to 30 days following the first injection. Of this series three showed positive effects, and nine were negative.

Experiment 2.—Intraperitoneal injection of killed (heating to 56° C. for 12 hours) 7-day dextrose broth cultures of *Streptococcus erysipelatis* obtained from the stock of the Department of Bacteriology of the University of Chicago. In 15 animals exhibiting benzpyrene sarcomata, doses of 3 to 5 cc. were injected at intervals of one to two days. This preparation was not as toxic as the product used in Experiment 1. The animals were killed 1½ to 78 days following total doses of 3 to 16 cc. Of the 15 tumors two exhibited positive reactions.

Experiment 3.—Intraperitoneal injections of killed 7-day dextrose broth cultures of a hemo-

lytic strain of *B. coli*. In eight tumor bearing animals 5 cc. doses were injected every one to two days. Two animals died after one injection; in one of them the tumor exhibited a positive reaction. Six animals were permitted to survive 14 to 27 days receiving total doses of 14 to 26 cc. In two of the latter series the tumor exhibited a positive reaction, and four were negative.

Experiment 4.—Intraperitoneal injection of killed 7-day broth cultures of *B. prodigiosus*. The latter strain was obtained from the stock cultures of the Department of Bacteriology. Seven tumor bearing rats received daily doses of 2, 3, 4 and 4 cc. respectively for four days. One died six days after the first injection. The tumor exhibited a positive reaction. The remaining six animals were observed for 28 days and when killed all the tumors were negative. At no time during the period of observation did any of the tumors exhibit softening.

Experiment 5.—Control experiments were conducted upon eight tumor bearing rats to show that doses of previously heated (56° C.), sterile dextrose broth equivalent in volume to the bacterial suspensions used above, would not induce hemorrhagic reactions in the tumor.

Experiment 6.—Foci of acute chemical inflammation were induced by the subcutaneous injections of 0.2 cc. of croton oil in 12 rats. Forty-eight hours later, 2 cc. of Coley's toxins were injected intraperitoneally in six animals. All animals were killed three to five days later. The acute inflammatory reactions were approximately of equal intensity in the control and injected rats.

Histologic Study.—A histologic study of the tumors which grossly exhibited hyperemia and hemorrhagic necrosis as a result of injection of bacterial products showed essentially the same picture exhibited in the small foci of spontaneous necrosis or spontaneous hemorrhage in control tumors or such areas in tumors not affected by these products, *i.e.*, widely dilated capillaries; interstitial exudate of red blood cells; local polymorphonuclear infiltration; shrunken appearance of the tumor cells, *etc.* The tumors produced in the above animals exhibited varying histologic pictures and consisted of large spindle cell, small spindle cell or "pleomorphic cell" sarcomata. No correlation was possible between the histologic type of the tumor and its positive or negative response to intraperitoneal injections of bacterial suspensions. In none of the animals was there macroscopic evidence of lesions in other organs or tissues that might have been ascribed to the injected materials.

Discussion.—In the foregoing observations no evidence was obtained to indicate that Coley's toxins, or the other bacterial suspensions, inhibited the growth of sarcomata induced from the animals' own tissues by carcinogenic hydrocarbons. The acute hyperemia and hemorrhagic exudations that did occur, presumably due to such injections, did not result in appreciable growth restraint. As far as can be determined, the only other observations of this type reported upon sarcomata induced from the animals' own tissues by hydrocarbons are those of Andervont. This investigator found that *B. coli* filtrates "produced hemorrhage with regularity in primary 1:2:5:6 dibenzanthracene tumors," but that "thus far, complete recession of primary dibenzanthracene sarcomata has not occurred."

The mechanism of the "hemorrhagic reaction" observed in some of the cases remains obscure. The assumption that it is an "immunologic phenomenon" also necessitates the assumption that the tumor tissue was primarily hypersensitive to certain bacterial products, a fact not yet demonstrated. The work of Shear and Andervont in obtaining a fraction from *B. coli* filtrates which exhibited a high potency as regards hemorrhage production in transplantable tumors would suggest that bacteria elaborate, in some form or another, a substance highly specific for the vascular system of tumors. Experiment VI was performed to observe whether in an acute inflammatory focus the capillaries are in a hypersensitive state in regard to bacterial products. It was felt that a tumor presenting in a substantial part of its mass inflammatory reaction due to spontaneous necroses might thus prove to be hypersensitive to

bacterial products. The results of this experiment, however, were negative. Furthermore, in the observations made above it would appear that the hyperemic and hemorrhagic reaction when once inducted are not ephemeral phenomena but persist for some time.

SUMMARY

(1) The effects of intraperitoneal injections of "Coley's toxins," of killed 7-day dextrose broth cultures of *Streptococcus erysipelatis,* of killed 7-day dextrose broth cultures *B. prodigiosus,* and of killed 7-day broth cultures of *B. coli* were studied upon sarcomata of the subcutaneous tissues of rats, induced from the animals' own tissues by carcinogenic hydrocarbons. Transplanted neoplasms were not employed.

(2) No evidence of inhibition of growth of the tumors was obtained.

(3) In a significant number of instances the tumors of injected animals exhibited a marked hyperemic and hemorrhagic reaction. It is assumed that this was a reaction to the injection of bacterial products since such changes were not observed in control animals, and were definitely of a different type, macroscopically, than the spontaneous degenerative changes sometimes observed in such neoplasms. These reactions, as indicated, did not appear to markedly restrain the growth of the neoplasm.

REFERENCES

[1] Andervont, H. B.: Reaction of Mice and of Various Mouse Tumors to the Injection of Bacterial Products. Am. Jour. Cancer, **27**, 77, 1936.

[2] Coley, W. B.: The Treatment of Sarcoma of the Long Bones. ANNALS OF SURGERY, **97**, 434, 1933.

[3] Shear, M. J.: Studies on the Chemical Treatment of Tumors. II. Am. Jour. Cancer, **25**, 66, 1935.

[4] Shear, M. J., and Andervont, H. B.: The Chemical Treatment of Tumors. III and IV. Proc. Soc. Exper. Biol. and Med., **34**, 323 and 325, 1936.