A STUDY OF CANCER IMMUNITY BY THE METHOD OF CULTIVATING TISSUES OUTSIDE THE BODY.*

BY ROBERT A. LAMBERT, M.D., AND FREDERIC M. HANES, M.D.
(From the Department of Pathology of the College of Physicians and Surgeons, Columbia University, New York.)

The phenomena of resistance and susceptibility to the transplantable tumors of rats and mice have been exhaustively studied. Extended observations have been made on the behavior of carcinoma grafts in immune mice. All of the recognized methods for the demonstration of specific antibodies have been employed in the study of the body fluids of immune animals. It may be stated summarily that immunity to tumor growth does not correspond to any of the types of immunity produced by the reaction of the body to injections of alien cells or to infective organisms and their products. Moreover, Russell (1) has shown that in immune mice inoculated cancer tissue fails to grow because of the absence of a specific stroma reaction on the part of the host, and not because of the presence of cytotoxic or cytolytic substances acting on the implanted grafts. Or, stated differently, in immune animals implanted cancer cells lose their power of calling forth a stroma reaction. He observed that, notwithstanding the absence of a stroma, the tumor cells remained alive and proliferated for eight to ten days.

The method of cultivating tissues outside the body, devised by Harrison (2) for the study of the developing nerve fiber in frog embryos, and modified by Burrows (3) in its application to warm-blooded animals, affords another means for studying the reaction of immunity to cancer, one, too, in which any reactive phenomena may be observed in all stages in a single specimen. Tumor cells growing in blood plasma find in the fibrin network an artificial stroma, so that the influence of the plasma alone may be held accountable if cancer cells which grow very readily in plasma from

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normal animals fail to grow or are modified in their growth in plasma from immune animals. In the present investigation we have applied this method to the study of tumor immunity, using a virulent rat sarcoma from Ehrlich's laboratory. It may be well, before describing our experiments, to review very briefly the subject of cancer immunity.

Two kinds of immunity to the transplantable rat and mouse tumors are recognizable, natural and artificial. The percentage of naturally immune animals (nullers) varies with age and with different varieties and strains. Old animals are, as a rule, less susceptible than young. Animals in which tumors have grown for a time and then undergone retrogression and absorption are highly resistant to further inoculations. This refractory condition may be regarded as a natural resistance artificially increased through the absorption of tumor tissue. Animals may be artificially immunized in various ways. Ehrlich (4) showed that mice inoculated with large doses of a hemorrhagic mouse carcinoma of very low virulence were rendered immune to more virulent carcinomata, and to a less extent to sarcoma. Loeb (5) was able to immunize rats in a similar manner, that is, by the inoculation of tumor tissue of artificially diminished resistance (heat). Others have shown that different degrees of resistance may be induced by the injection of suitable quantities of various homologous tissues, as, embryo (Schöne (7)), blood (Bashford (6)), liver (Bridré (8)), etc. Woglon (9) found that the subcutaneous inoculation of a mouse's own spleen gave results comparable to those obtained when spleen tissue from other mice was used. More recently, successful immunization through the use of tissues from alien species has been reported (C. Lewin (10), I. Levin (11)). Furthermore, I. Levin (11) has found that the autolyzed products of normal organs are quite as efficient as the fresh intact cells in the production of immunity. Ehrlich's athreptic immunity, to any wide application of which many objections have been advanced, constitutes another kind of immunity, but since it has no direct bearing on our experiments, a discussion of it will be omitted.

In the present study we have used six types of immune animals: (1) nullers from a strain of white rats in which sarcoma inocula-
tions gave 80 to 90 per cent. of takes; (2) nullers from a hooded variety of rats in which the percentage of takes was only 20 to 30 (racial immunity); (3) immune old animals whose immunity was probably attributable to their age; (4) animals in which both sarcoma and carcinoma inoculations had been negative (pan-immunity); (5) animals whose tumors (sarcoma) had undergone retrogression and absorption; (6) artificially immunized rats from a series of animals that had received two cubic centimeters of rat's blood, and had resisted subsequent sarcoma inoculations. The percentage of resistant rats in the series was 40.

In all, eighteen immune rats were used, and from each sufficient plasma was obtained for fifteen to twenty culture preparations. We found that sarcoma grew readily in the plasma from every animal used. The character of the growth did not differ essentially from that observed in control specimens in which plasma from normal and tumor-bearing animals was employed.

ANIMAL INOCULATIONS.

To test the viability of sarcomatous tissue cultivated in vitro, animal inoculations were made. Chart I shows graphically the tumors resulting from the implantation of pieces of sarcoma that had been incubated for three days at 37° C., and were growing satisfactorily. In this series both the original pieces and the outgrowths embedded in plasma were used for inoculation. We have, however, obtained tumors when only the outgrowth was inoculated. Chart I is also designed to present a comparison of the tumors resulting from the inoculation of pieces of sarcoma cultivated in plasma from normal animals and of tumors from pieces grown in immune plasma. It is seen that the percentage of takes is as high, and the rate of growth as rapid, in the one as in the other. If, however, we compare the entire series of inoculations with sarcoma cultivated in vitro with a series of inoculations with sarcoma taken directly from the animal bearing the tumor, as is done in routine laboratory transplantations, we find two differences: first, the percentage of takes is 50 as compared with 80 to 90 in the inoculations with fresh tissue; and secondly, the number of late regressing tumors is about twice as great in the series from cultivated tissues.
The lowered percentage of successful inoculations is not, in this instance, necessarily an evidence of diminished virulence, since the pieces used were at least ten times smaller than those ordinarily

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**Chart 1.** The tumors on the left resulted from the inoculation of pieces of rat sarcoma cultivated three days in plasma from *immune* animals. The tumors on the right occurred in animals inoculated with sarcoma cultivated three days in plasma from *normal* and *tumor-bearing* rats. In each series there were fifteen rats; those not developing tumors were not charted. Six retrogressing tumors are noted, three in each group. The first row of figures indicates the number of days after inoculation.
implanted. Furthermore, the mass of plasma about the pieces of cultivated sarcoma was possibly an obstacle to early vascularization of the grafts. In order to test this possibility, animals were inoculated with bits of fresh sarcoma embedded in plasma. The yield of tumors was slightly greater (60 per cent.) than was obtained from the pieces of cultivated sarcoma. The relatively large number of regressing tumors, however, can hardly be interpreted other than as an evidence of lowered virulence.

Inoculations of mouse sarcoma cultivated for two days in rat plasma showed also a smaller percentage of takes than was observed in the inoculations with fresh tissue, but no regressing tumors have been noted.

Small pieces of sarcoma placed in hanging drops of blood serum and incubated at 37° C. were inoculated after twenty-four hours (twelve animals), thirty-six hours (eight animals), forty-eight hours (eight animals), and ninety-six hours (eight animals). Three tumors resulted from the twenty-four hour specimens, and two tumors from the thirty-six hour specimens. The inoculations from the forty-eight hour and ninety-six hour preparations were negative. Sarcoma cells incubated in blood serum, then, live at least thirty-six hours. A larger number of inoculations, however, would be necessary in order to determine definitely the duration of life of sarcoma cells under these conditions. We have obtained tumors from the inoculation of pieces of sarcoma after nine days incubation in plasma.

SUBCULTURES.

We have made subcultures of specimens of sarcoma growing in plasma by transferring to new medium the original piece of tissue or a portion of the outgrowth. Strictly speaking, transplantation of the original piece to new plasma does not constitute a subculture. The majority of the specimens cultivated in a single medium do not grow longer than five or six days, but, if before cell death takes place they are transferred to fresh plasma, active growth is renewed. We have in this way carried pieces through five drops of plasma, making the transfer at intervals of three to five days. In these subcultures, plasma from both normal and immune animals was used, and growth was as vigorous in the one as in the other.
CONCLUSIONS.

1. Sarcoma cultivated in plasma from immune animals grows quite as vigorously as in plasma from normal and tumor-bearing animals, thus affording further proof of the absence of specific cytolytic substances in the body fluids of animals immune to transplantable cancer.

2. Animals may be successfully inoculated with sarcoma cultivated in vitro, although, in the case of rat sarcoma, there is evidence of diminished virulence.

3. Subcultures of sarcoma cultivated in vitro may be made by transferring to fresh plasma the original piece of tissue, or a portion of the outgrowth. The duration of life of sarcoma cells under these conditions seems dependent only on a renewal of the medium.

BIBLIOGRAPHY.