STUDIES ON IMMUNITY IN CANCERS OF THE WHITE RAT.

THE SIGNIFICANCE OF ATHREPSIA.*

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In a previous communication, one of the present writers (1) showed that the result of tumor implantation is determined by two factors: first, whether or not the implanted tumor becomes ingrafted, and second, the rate of its growth if ingrafted.

The mechanism of ingrafting is common to tumor and normal tissue. Thus, Ribbert (2) has successfully implanted human skin into the ear of the rabbit. On the other hand, the ultimate fate and character of the graft depends on the rate of growth of the implanted tissue.

If the growth of the latter proceeds at approximately the same rate as that of the surrounding tissue, the implanted tissue develops into a small nodule which is ultimately absorbed. On the other hand, if the intensity of the cell proliferation in the implanted tumor exceeds that of the surrounding tissue, there will develop a large malignant tumor. The rate of growth of an implanted tumor and with it the ultimate character of the tumor depend upon an interaction between the power of proliferation of the tumor cell and the resistance of the host. The mere grafting of the tumor is independent of the phenomenon of cancer immunity.

A great deal of the confusion and controversy that exists in the attempted analysis of the results of the work on cancer immunity is caused by this lack of appreciation of the coexistence of the two factors determining the outcome of the tumor implantation. It seemed, therefore, desirable to undertake a revision of the work on cancer immunity.

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The most popular and most generally accepted theory of cancer immunity is the one advanced by Ehrlich and is commonly designated the athrepsia hypothesis. It is based on the following considerations:—

Immunity or resistance of the host to the growth of an inoculable cancer is not specific in its character, and an animal immune against carcinoma will be also immune against sarcoma. The methods used to induce an artificial immunization are likewise not specific. When a cancer of low virulence is inoculated into and absorbed by the host, the latter is immune in the majority of cases against subsequent inoculation of any of the most virulent cancers. The same immunization may also be accomplished by an inoculation of normal tissue cells of the same species of animals or even of cells of a phylogenetically closely related species of animals, as, for instance, with mouse cells in immunization of the rat, and *vice versa*. This non-specific character of cancer immunity Ehrlich designates by the name of panimmunity.

According to Ehrlich, as well as to most of the investigators on the subject, an artificial immunity may be induced only by treatment with living cells. Growth of inoculated cancers is due, of course, to cell activity. Ehrlich contends that the growth of a cancer indicates that the tumor cells possess a great avidity for a certain specific food within the organism of the host and consequently obtain it from the normal tissue cells of the organism. When cancer fails to grow, then either the organism of the host does not possess the necessary specific food or else the avidity of the cancer for this food is not strong enough to deprive the normal tissue cells of it. When the organism of the host is immunized by treatment with a cell emulsion, these cells bind the specific food and consequently the cancer cells inoculated subsequently do not find the necessary nourishment and die. Immunity, then, to cancer growth consists in the lack of food athrepsia.

The experimental evidence adduced by Ehrlich in support of his theory consists in the so-called zigzag transplantation, the second inoculation on a tumor-bearing animal and the inoculation of an emulsion consisting of a mixture of two tumors (carcinoma and sarcoma).

In the present investigation, a separate detailed study was made of each of the experimental proofs mentioned above, and in the following pages each of them will be considered separately. The studies on immunity in inoculable cancer, of which this investigation presents one phase, were conducted on white rats only, as these animals are better adapted for experimentation. For the present investigation, however, both rats and mice were used, and in all five different tumors served for the experiment. Two of these were rat tumors; an extremely virulent Ehrlich sarcoma, and the Flexner-Jobling carcinoma, which is not as virulent as the former. The three mouse tumors used were Ehrlich's carcinoma of the mouse (No. 33), Bashford's carcinoma of the mouse (No. 27), and Ehrlich's sarcoma of the mouse. The last named was the most virulent of the mouse tumors.

PANIMMUNITY.

Ehrlich did not consider the phenomenon of panimmunity a direct proof of athrepsia, but there must undoubtedly be a certain relation between the general non-specific character of the former and athrepsia, which is merely a phase in intracellular nutrition. Indeed, if athrepsia is to have a general application it must be capable of explaining the conditions of panimmunity against the different tumors used in this research, and it may be stated that in a general way the fact of panimmunity was found to be correct for the majority of cases. But there were factors observed in the course of these experiments which can not be explained on the basis of the theory of athrepsia. Of such experiments the following instances may be cited.

Experiment 1.—Twenty-nine rats which had been previously immunized against an inoculation of a rat carcinoma (Flexner-Jobling) were inoculated four weeks later with a rat sarcoma (Ehrlich). Of these rats, twenty-three remained immune and only six, or 20 per cent., of the inoculated animals grew the tumor, while of twenty control rats, 17, or 85 per cent., took the tumor.

Of these two tumors, the Flexner-Jobling carcinoma grows much more slowly than the Ehrlich rat sarcoma and does not reach as large a size. Consequently the former tumor has less avidity for the specific food than the sarcoma, and does not, according to the Ehrlich theory, use up all this food of the host. Therefore, a suffi-

cient amount of specific food should be left for the subsequently inoculated sarcoma cells, and it should be possible to inoculate the latter successfully in all the treated animals.

The following experiment shows again that all the facts of panimmunity can not be explained on the basis of athrepsia.

Experiment 2.—Twenty rats were inoculated with a mouse sarcoma, and four weeks later, when the grafts were completely absorbed, they were inoculated with a rat sarcoma. Of the fifteen rats which survived at the last examination, the tumor grew only in two. This shows 13 per cent. of takes, while in the twenty control animals, the tumor took in 90 per cent.

It will be shown later that according to the theory of athrepsia the cells of a mouse tumor are unable to anchor the specific food of the rat. Consequently the specific food needed for the success of the inoculation of a rat sarcoma should exist in the same quantity in the rats treated with mouse sarcoma as in the control animals. Still the rats treated with mouse tissue were immune to the inoculation of rat tumor. The fact that it is possible to immunize a rat with mouse tissue, and *vice versa,* was previously observed by Carl Lewin and by one of the present writers.

Another factor observed in the course of these experiments on panimmunity, which is of great importance in the consideration of the significance of athrepsia, is the following: whenever a tumor succeeds in growing in one of the previously treated animals, it attains the same size and virulence as in the control animals.

This phenomenon indicates that panimmunity with its non-specific character is in reality an immunity to tissue grafting. On the other hand, when the cancer cells are ingrafted, such preliminary treatment of the animals with living tissue, as is used in experiments on panimmunity, does not exert any influence whatever on the subsequent growth or virulence of the developing tumor. The truth of this assertion is also evident from the fact that no immunization with living tissue is successful if it is done subsequently to the inoculation of the tumor.

The experiments of Rous (3) with inoculation of embryonic tissue show that by the same method an animal may be immunized against the grafting of embryonic cells. Here again is apparent the non-specificity of immunity in cancer. The panimmunity is in

reality an immunity against implantation of tissue. But since in all experimental cancer work with the inoculable tumors the first step always consists in the inoculation of a piece of the tumor, such tissue immunity obscures the actual immunity to the growth and development of the cancer.

ZIGZAG TRANSPLANTATIONS.

This phenomenon, which served as the main experimental basis for the formation of the theory of athrepsia, consists in the following: When a mouse sarcoma is inoculated into a rat, it grows there normally for about eight or ten days, then ceases to grow and is absorbed. But if before absorption takes place the nodule is removed from the rat and transferred to a mouse, it continues to grow normally and may be subsequently transferred to another rat, where it will grow for about eight days, and so on.

Ehrlich explains the result of these experiments by the supposition that tumor cells in order to proliferate must obtain a certain specific food x, which they can find only in an animal of the same species as that from which the tumor cells have come. Cells of a mouse tumor inoculated into a rat proliferate for a few days, as long as the food x, which they brought over from the mouse, lasts. When this is used up, they do not find this specific food in the rat and cease to grow, but grow again when transferred to a mouse, where they find again this specific food. On the basis of this supposition, Ehrlich created the general theory that whenever cancer cells fail to proliferate, it means that they fail to obtain the food x, either because the normal body cells have greater avidity for this food than the cancer cells, or else the cells with which the animals were immunized anchored all the specific food and the cancer cells inoculated subsequently could not obtain it.

The experiments of zigzag transplantation were undertaken by the writers on all the tumors used in this investigation. In the course of these experiments certain facts were observed which indicate that athrepsia can not account for all the phenomena observed with zigzag transplantations.

The inoculation of the rat sarcoma and rat carcinoma into mice and of the Ehrlich mouse carcinoma into rats uniformly produced

small nodules in the host of different species. When these nodules were retransferred into animals of the species from which the original tumors were derived, for instance, when a rat sarcoma was retransplanted from a mouse into a rat, not a single good tumor growth could be produced by any of the three tumors mentioned above.

With Ehrlich's mouse sarcoma and Bashford's mouse carcinoma, the results of the experiments appeared to be more successful, i. e., when a nodule of the mouse sarcoma or carcinoma, after a sojourn of eight to ten days in a rat, was removed and inoculated into a mouse, it grew, in a certain number of cases, into a good sized tumor. The number of such successful inoculations, however, was much smaller than the number of takes in control animals, i. e., in animals that were inoculated with tumor material which was taken directly from a mouse. The following series of experiments illustrates this point.

Bashford's Mouse Carcinoma Inoculated into 20 Rats; 2, 4, 6, and 8 Days Later Nodules Were Removed from the Rats and Inoculated into Mice.

	2 days later.	4 days later	6 days later.	8 days later.	Total.	Control.
No. of mice inoculated	10	10	15	10	45	20
No. of mice surviving at final examination	8	9	14	9	40	20
No. of mice with tumors	4	1	4	1	10	18
Per cent. of takes					25	90

Ehrlich's Mouse Carcinoma Inoculated into 20 Rats; 2, 4, 6, and 8 Days Later Nodules Were Removed from the Rats and Inoculated into Mice.

	2 days later.	4 days later.	6 days later.	8 days later.	Total.	Control.
No. of mice inoculated	10	10	10	7	37	20
No. of mice surviving at final examination	9	6	10	1	26	18
No. of mice with tumors	1	2	0	1	4	14
Per cent. of takes					15.4	77.8

An analysis of these experiments on zigzag transplantations shows that all tumors, or at least all the tumors with which this investigation was conducted, can be successfully grafted on the phylogenetically closely related animals, i. e., from rat to mouse and *vice versa*. The same holds true, according to Rous, for em-

bryonic tissue. But the ingrafted cancer cells do not proliferate indefinitely and become malignant in the host of a foreign species. The explanation of this phenomenon given by Ehrlich does not seem to be adequate to explain all the factors in connection with it. If a mouse tumor fails to grow in the rat only for lack of specific food, then it should retain its normal virulence when transferred into a host of its own species. But experiments show that the virulence of a mouse or rat tumor is so diminished by its eight to ten days sojourn in a host of a different species that it either fails to take at all when reinoculated into a host of its own species, or it takes in a very small percentage. It is evident then that the organism of the temporary host has a decidedly noxious effect upon the proliferating activities of the tumor cells, and, moreover, while the capacity for ingrafting in an animal of a foreign species is common to all the tumors studied and is non-specific, the amount of injury sustained through the sojourn in the foreign host is different with the different tumors.

SECOND INOCULATION ON A TUMOR-BEARING ANIMAL.

Ehrlich maintains that animals on which a successful inoculation of another tumor was previously made appeared to be immune against a subsequent inoculation, either of the same or another tumor. The opposite results found by Hertwig and Poll (4), Gierke (5), and Borrel (6), he explains by the fact that in their experiments they used for the primary inoculation tumors of low virulence and slow growth, while in his experiments the animals received for the first inoculation an extremely virulent tumor. Only a very virulent, rapidly growing tumor anchors all the specific food x and consequently makes the successful take of the subsequently inoculated tumor impossible. A slowly growing tumor leaves a sufficient amount of the specific food for the successful take of a subsequent inoculation.

Experiments with a double inoculation were conducted with the Flexner-Jobling rat carcinoma and with Ehrlich's rat sarcoma. The latter is an extremely virulent and rapidly growing tumor, and still a subsequent inoculation of the carcinoma was just as successful on an animal bearing a large sarcoma as the inoculation of

the sarcoma on an animal with a growth of carcinoma. The most plausible explanation of the discrepancies in similar experiments of different investigators consists in the different behavior of the different tumor cells after a successful grafting. This latter factor is most evident in the series of experiments on the inoculation of an emulsion consisting of two tumors.

INOCULATION OF AN EMULSION CONSISTING OF TWO TUMORS.

Apolant (7) inoculated an emulsion of a carcinoma and sarcoma into an animal and developed a mixed tumor showing the structure both of the carcinoma and sarcoma. If one of the tumors was originally more virulent than the other, or if its virulence was increased artificially, then the resulting growth showed the structure of the more virulent tumor only. Ehrlich adduces the results of this experiment as another proof of the fact that the more virulent tumor cells obtain all the specific food of the host, causing the cells of the weaker tumor to die of starvation.

These experiments were repeated with two emulsions. One, consisting of the cells of the Flexner-Jobling carcinoma and of Ehrlich's sarcoma of the rat, was injected into rats, and another, consisting of Bashford's carcinoma and Ehrlich's mouse sarcoma, was injected into mice. In both instances the sarcoma was a great deal more virulent than the carcinoma. The relation in virulence between the Flexner-Jobling carcinoma and the Ehrlich rat sarcoma, on the one hand, and the Bashford carcinoma and the Ehrlich mouse sarcoma, on the other, is quite identical, but a most surprising difference was observed in the results of the two experiments. While in the rats, on the injection of the emulsion, there developed a mixed tumor showing the structure both of carcinoma and sarcoma, the tumor in the mice appeared to be pure sarcoma. Thus it seems that different tumors behave in a different manner when introduced simultaneously into an organism.

Of all the experiments stated above, the zigzag transplantations undoubtedly offer the best field for the analysis of the different phenomena of immunity in cancer. The apparent success of an inoculation of a mouse tumor into a rat may mean only the success of the initial grafting, since the rat possesses a congenital racial im-

munity against the growth of a mouse cancer. Indeed, the nodule which develops in the rat upon an inoculation of a mouse tumor never develops into a large malignant growth. There is consequently a condition here in which the dual character of the immunity to an inoculable tumor is perfectly apparent. The rat is susceptible to the grafting of a mouse sarcoma, but immune against its malignant development. Another proof of these two conditions in cancer immunity is shown in the influence of pregnancy on inoculations of a tumor. Haaland (8) found that an inoculable tumor does not take well on a gravid animal, and Ehrlich explains this fact on the supposition that during pregnancy all the available food is used up by the body cells. Herzog (9) has shown, on the other hand, that when a tumor-bearing animal becomes pregnant the tumor appears to grow faster. In other words, pregnancy produces a resistance against the grafting of tumor cells, while it enhances the proliferation of the tumor cells present in the organism.

The success of the grafting of the inoculated cells undoubtedly depends upon conditions of nutrition. Thus, athrepsia may serve to explain the conditions of resistance and susceptibility to the grafting of tumor as well as tissue cells. The formation of a highly vascularized stroma around the successfully ingrafted tissue and the lack of stroma formation in an unsuccessful graft (Russell (10)) also shows the importance of nutrition during this phase of the inoculation of a tumor.

On the other hand, the same experiments on zigzag transplantations clearly indicate that differences in nutrition can not explain the reason why in one successful tumor graft a small nodule will form which is subsequently absorbed, while in another the nodule will grow into a large tumor and kill the animal.

As stated above, this investigation has shown that a lack of specific food alone can not explain the failure of a mouse carcinoma or sarcoma to develop into a malignant tumor in the rat. The organism of the rat has a direct influence upon the mouse tumor and its virulence is greatly diminished after a sojourn of a few days in this animal. The organism of the rat has consequently a noxious effect upon the proliferating activities of the cells of the mouse tumor. It is of great interest to note in this connection that

upon an inoculation of these weakened cells into another generation of mice, the normal virulence of the former returns. The following series of experiments illustrates this point.

Experiment 3.—Twenty mice were inoculated with pieces of the Ehrlich mouse sarcoma taken from rats. Nineteen mice survived at the last examination, and of these, one took the tumor, which represents 5 per cent. of takes. The tumor from this mouse was inoculated in twenty other mice and the tumor took in fourteen, or 70 per cent.

It appears from these experiments that a mouse tumor through a sojourn in the rat reacquires the characteristics of a spontaneous tumor; namely, it takes in a small percentage of inoculated animals, and this percentage increases in subsequent generations. But the diminution of the virulence of the tumor which takes place in the organism of the rat is due to congenital racial immunity of the latter. Furthermore, the immunity of the rat against mouse tumor is not due to conditions of nutrition, but is accompanied by an active noxious influence of the organism of the rat on the mouse tumor. Thus indirect evidence is brought forward through this investigation to show that immunity to cancer growth, but not to tumor grafting, is caused not by differences in intracellular nutrition, but by an active inhibitory influence of the organism of the host on the cancer cells. The search for direct evidence and the true nature of this inhibitory influence is the aim of further studies on immunity in cancer of the white rat.

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