STUDIES ON IMMUNITY IN CANCERS OF THE WHITE RAT.¹

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The most important biological phenomenon observed in the experimental study of the transplantable tumors of the white rats and mice consists in the so-called immunity or resistance of certain animals to the growth of these implantations. This resistance apparently depends upon a number of various complex conditions, most of which as yet present no tangible explanation. It consists in every case of two components, one of which is the power of the implanted cancer cell to unlimited proliferation, and the other, the reactivity of the host. When a spontaneous tumor is inoculated, it grows successfully only in a small number of the animals used, frequently in less than 10 per cent. In subsequent inoculations with material taken from the implanted tumors, the success of the takes increases until it may reach fully 100 per cent., which indicates that the implanted cancer cell increases its proliferating power — its virulence. The fact, on the other hand, that during all these transplantations a certain number of animals retain their immunity to the growth of the tumor, clearly shows the influence of the reactivity of the host. This latter quality is considered by the majority of the investigators to be a natural characteristic of this animal, a natural immunity.

Immunity may also be induced artificially by various methods. Ehrlich (1), who first succeeded in rendering an animal resistant by artificial means, proceeded in the following manner. He inoculated a certain number of animals with a spontaneous hemorrhagic tumor, the cells of which were known to be of very low virulence and to absorb in nearly every animal without producing a growth. He

¹ Conducted at the expense of the George Crocker Special Research Fund. Read before the meeting of the American Association for Cancer Research, May 2, 1910. Received for publication, May 24, 1910.
then inoculated the same animals with a very virulent tumor with
the result that a greater number of the treated animals were resist-
ant to the implantation of the virulent strain than the control ani-
mals, and this resistance held good against the subsequent inoculation
of nearly every other transplantable tumor, whether carcinoma or
sarcoma. This phenomenon, which Ehrlich calls *pan-immunity*,
appears to have a very wide application, and as is shown by the
investigations of Bashford (2), Schoene (3), Borrel (4) and
Bridré (5), may be induced by previous treatment, not only with
tumor tissue, but also with different normal organ tissue of the
same species of animals. Thus the immunity, or resistance to
growth of tumor, does not appear to have a clearly specific character.

Ehrlich maintains further that animals on whom a successful
inoculation of another tumor was done previously, *i.e.*, animals that
had a tumor growth, appeared to be immune against a subsequent
inoculation either of the same or another tumor. This observation
was one of the main factors which served Ehrlich in the construc-
tion of his "atreptic" hypothesis of the immunity to tumor growth.
Every implanted cancer cell, according to his idea, requires for the
success of its further proliferation a specific food which it finds in
the organisms of the host. When the first tumor is implanted into
the animal, it takes up all the specific food available in the organism
and consequently the cancer cell subsequently inoculated does not
find in the host any of this specific food, and, therefore, no tumor
growth takes place. This hypothesis of Ehrlich's will be consid-
ered with more detail later, but it must be stated here that both this
theory and the phenomenon itself did not seem to find any corrobo-
ration among the subsequent investigators.

Hertwig and Poll (6), Gierke (7), and Borrel, on repeating
these experiments, found opposite results. Borrel as well as Gierke
explain this discrepancy by the difference in the technique employed
in their investigations. They inoculated small pieces of the tumor
under the skin, while Ehrlich used an emulsion. They believe that
when an emulsion is used, only a small number of the cells pro-
liferate, while the rest is absorbed and consequently produces an
immunity against subsequent inoculation. When a small piece is
inoculated, on the other hand, all the cells participate in the pro-
liferation, no artificial immunity is produced, and the subsequent inoculation is successful.

Nor does Borrel agree with Ehrlich's explanation of his results, that the immunity to the growth of cancer depends upon certain peculiarities in the distribution of food, but believes that it is analogous to the bacterial immunity and is caused by certain cytolytic anti-bodies circulating in the blood. He maintains that the first tumor grew in the animal because the anti-bodies did not have the time to develop, and when they do develop, they cannot interfere with the continued growth of the first tumor, but they may interfere successfully with the grafting of the second tumor. Ehrlich, on the other hand, explains the lack of corroboration of his finding by the fact that he always uses in his experiments a very virulent tumor for the first inoculation, a tumor which according to his ideas is best able to obtain all the specific food from the host, while the other investigators use tumors of less virulence, and there consequently remains in the organism a sufficient amount of specific food for the successful growth of the subsequent inoculation. This explanation of Ehrlich's seemed to find proof in the experiments of Apolant (8) with mixtures of emulsions of the two different tumors, sarcoma and carcinoma, for instance. At first he produced on such inoculation a tumor with the character both of carcinoma and sarcoma, but if one of the tumors used was more virulent than the other, then its character prevailed in the implanted tumor and the cells of the other tumor were suppressed. When all these facts are analyzed, the weight of evidence seems to tend to the possibility that the experiments of Ehrlich, Gierke, and Borrel may all be correct and the differences are not due to faulty methods, but to the fact that the conditions are different in these various experiments.

Two factors have to be accounted for in the consideration of the powers of resistance of the host to tumor growth. When a tumor is inoculated, the cells are grafted on the host, and whether the graft will be successful or not depends upon the ability of the host to supply a connective tissue stroma or "scaffolding," as Bashford calls it. It also depends upon the amount of the vascularization of this stroma. Russell (9) has shown that the difference in the morphological appearance between a successful and unsuccessful
tumor graft consists just in such a difference of the formation of a stroma. Indeed the investigations of the writer (10) seem to make it plausible that the fact of the success of the implantation of the cancers of the white rat and mouse, as compared with other laboratory animals, may be explained by such a great cellular reactivity of these animals. It seems feasible that during this stage of grafting or cohesion of the implanted cells to the host the result will be identical whether this cell possesses great power for proliferation, or simply a normal embryonic cell, as in the experiments of Askanazy (11). On the other hand, after the implanted cancer cell becomes ingrafted and is consequently able to develop its proliferating power, it again depends upon an interaction of a different nature between the implanted cell and the resistance of the host, whether there will take place unlimited proliferation and growth of cancer, or whether the cancer cell will succeed only in proliferating for a while, will form a small nodule and then become absorbed, and consequently will be just as innocuous to the organism of the host as an implantation of normal tissue. Every worker in cancer inoculation knows by experience that when pieces of a tumor are implanted in a set of animals, there will take place a tumor growth in a certain number of animals, in others the graft will disappear completely, while in others again there will appear a small nodule, which will later disappear. The two latter sets of animals are both ultimately immune to the inoculation of the tumor.

It stands to reason to suppose that resistance to the grafting of the implanted cell and resistance to its subsequent unlimited growth may be produced in a different manner. Such a difference is very well illustrated in the fact that, as was shown by Haaland (12), a tumor inoculated succeeds but poorly on a gravid animal, while, on the other hand, Herzog (13) has shown that when a tumor 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. It is possible then that a tumor may find conditions favorable for its own growth and at the same time produce within the host an immunity to subsequent tumor implantation, as was the case in Ehrlich's experiment, while tumors used by the other investigators may not possess that property.
It is clear then that an artificial production of immunity against tumor growth depends upon a great variety of conditions, nor is there a unanimity of opinion as regards the *modus operandi* of this phenomenon. Borrel inclines to the view that the organism of an immune animal contains some anti-bodies of a peculiar nature, which restrain the growth, or rather exert a toxic influence upon the implanted cells, while Ehrlich and Bashford maintain that resistance to growth of implanted tumor is a purely cellular activity and that an artificial immunity can be induced only by inoculation of living cells.

But an analysis of the investigations on immunity does not seem to bear out fully the latter opinion. When an animal is immunized by a previous inoculation of normal tissue, all this tissue is completely absorbed by the time the tumor is inoculated, ten days later. Very instructive in this connection is the work of Woglom (14). He found that the spleen extirpated and then inoculated subcutaneously into the same animal induces resistance against growth of tumor. Extirpation of the spleen alone does not induce any resistance. If the resistance is caused in this case by the live functions of the cells of the spleen, they could act most effectively when the spleen was *in situ*, and the mice ought to have been naturally resistant. It is true that blood serum deprived of cells and tissue heated or crushed and frozen, do not appear to induce immunity, but this fact may be just as easily explained by the supposition that such a treatment of tissue is too severe and changes the chemical composition of those constituent parts of the injected cells, which induce the organism of the host to form anti-bodies. The weight of evidence seems to be against the idea that the cells used for immunization remain alive indefinitely in the new host and induce the resistance by their life functions. Ehrlich's atreptic theory resembles closely the exhaustion theory elaborated by Pasteur in explanation of bacterial immunity, but while it very ingeniously explains certain phenomena in the immunity to growth of tumor, it does not seem to be sufficient for the complete elucidation of all the data. It is also true that so far not a single experimental fact was adduced to prove the existence of anti-bodies in the blood serum of immune animals, nor was it possible to show the existence of
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passive immunity (the experiments of Clowes and Baeslack (15) received no confirmation), but such negative results can hardly be of great value, since immunity to growth of cancer, while possibly similar, cannot by the nature of the process be identical with bacterial immunity.

In the study of the phenomenon of immunity to cancer growth, all the experience gained in the study of bacterial immunity should be closely followed, but new methods will have to be devised before the actual explanation will be reached. The fact that an animal organism creates anti-bodies against enzymes or any proteid matter introduced into the circulation is a certain indication of the fact that the existence of anti-bodies in the immune animal cannot be denied a priori. The aim of the present investigation consisted in the search for a substance which would not contain living cells and would still be able to immunize an animal against the growth of an implanted tumor. The work was done with Ehrlich’s sarcoma of a white rat, for a transplant of which the writer is indebted to Dr. Simon Flexner. This tumor is of a very malignant type, takes in from 100 per cent. to 80 per cent. and grows to a very large size, frequently measuring two inches by one inch in about three weeks after inoculation.

A description of the different methods of treatment of animals employed in order to create a condition of immunity to cancer growth without the assistance of living cells will now be given.

Treatment with Arsalazin.—In view of the great inhibitory influence of the modern arsenical preparations (atoxyl and arsalazin) on trypanosomes and other animal parasites, and in view further of the frequent reports of the influence of arsenicals on human cancer, it is interesting to learn whether these drugs have any influence upon the growth of a transplantable sarcoma of a rat.

Sticker (16) reported that a combination of blood-serum and atoxyl retards growth of his transplantable sarcoma of a dog and occasionally even arrests completely its development. On the other hand, Uhlenhuth and Weidanz (17) state that atoxyl not only does not retard growth of carcinoma of a mouse, but even seems to enhance it.

Since the growth of sarcoma is more readily influenced than
carcinoma—and this fact may have explained the discrepancy between the results of Sticker and Uhlenhuth and Weidanz, the experiments were repeated on the sarcoma of the rat. Arsazetin was used instead of atoxyl, since it was found by Ehrlich (18) to be the more efficient of the two.

In our experiments, forty rats were treated for six weeks by hypodermic injections of one cubic centimeter of 4 per cent. solution of arsazetin, first every two days and then every four days. Three weeks after the beginning of the treatment the tumor was inoculated.

| TABLE I.  
| Arsazetin. |
|----------------|----------------|
| Treated animals | Controls |
| Number of rats inoculated with tumor | 40 | 20 |
| Number of rats surviving at final examination | 32 | 20 |
| Number of rats with tumors | 28 | 19 |
| Percentage of takes | 87 | 95 |

As Table I shows, the arsazetin had no influence upon the growth of the tumor, though the quantity of the drug used was sufficient to impair considerably the general health of the animals. In nearly every animal a condition was created similar to the state of a waltzing mouse.

Treatment with Sodium Oleate.—It was stated above, that while no protective bodies were found in the blood of animals immune to the growth of cancer by the ordinary methods used in bacteriology, the possibility of the existence in the blood serum of some peculiar kind of anti-bodies cannot be excluded a priori. The investigations of Kyes (19) and Noguchi (20) have shown that a certain kind of cytolytic immune bodies become activated on the addition of lecithin and other lipoids, and Noguchi has further proven that sodium oleate seems to possess the strongest cytolytic action of all the lipid substances. For this reason a certain number of animals were treated with an injection of a solution of sodium oleate previous to the inoculation of the tumor.

While the difference between the treated animals and the controls is not sufficient to indicate any actual influence of the lipid used, the result is suggestive, and in view of the theoretical importance
of such a method of investigation, this part of the research is still being continued with different variations.

**TABLE II.**

<table>
<thead>
<tr>
<th>Sodium Oleate</th>
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<table>
<thead>
<tr>
<th></th>
<th>Treated animals</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats inoculated with tumor</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Number of rats surviving at final examination</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Number of rats with tumors</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Percentage of takes</td>
<td>77 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>

_Treatment with Autolyzed Tissue._—The investigations of Salkowskii (21), M. Jacoby (22), P. A. Levene (23) and others, have shown that a great many of the so-called vital functions of the cell are due to the activities of the endocellular enzymes. These enzymes, while constituting an integral component part of the cell, may remain under certain conditions uninjured after the death of the cell. Furthermore, the investigations of Petry (24), Blumen-thal and Wolf (25), C. Neuberg (26), Yoshimoto (27) and others have shown that the endocellular enzymes of the cancer cells seem to act differently both qualitatively and quantitatively from the enzymes of normal cells. In view of the further fact that the investigations of Müller (28), Opie (29), Blum (30), Conradi (31) and others have shown that these endocellular ferments play an important rôle in a number of pathological processes, and may be the means to which the organism resorts in order to elaborate protective substances—Conradi and Blum have shown that autolyzed tissue may act as an antitoxin—it seems feasible _a priori_ that the resistance induced by normal mouse or rat tissue inoculated subcutaneously may also be due not to the function of a live cell, but to some peculiar type of an endocellular ferment. This assumption seems the more plausible since the best method of liberating these endocellular enzymes consists in the autolysis of tissues, and the tissue introduced under the skin in order to induce immunity is put under conditions most favorable for subsequent autolysis.

In view of all these considerations, a series of animals were treated with autolized tissue. The organ used for immunization
was the liver of "Nullers," that is, rats naturally resistant to tumor implantation. The tissue was kept under aseptic precautions at body temperature for two weeks, then the autolysed tissue was mixed with about double the quantity of normal salt solution, ground thoroughly with sand, filtered, and one cubic centimeter of the solution injected subcutaneously.

**TABLE III.**

*Treatment with Autolyzed Tissue.*

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats inoculated with tumor.</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>No. of rats surviving at the final examination, 25 days after inoculation.</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>No. of days before inoculation that autolysed liver was injected.</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>No. of days after inoculation that autolysed liver was injected.</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>No. of rats without growth or with small abortive nodules.</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>No. of rats with tumors.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Per cent. of takes.</td>
<td>60</td>
<td>39</td>
</tr>
</tbody>
</table>

The results compare quite favorably with those of the investigators who induced immunity with normal tissue. Thus it seems possible to produce in rats a certain amount of resistance to growth of tumor by treatment with tissue of which the cells are killed, but the endocellular ferments apparently remain active. It is interesting to note here that autolysed liver tissue seems to immunize equally well, whether used before or after the inoculation of the tumor, which fact may be of great importance in view of the possibility expressed above, that the resisting influence of the host may be of two kinds, one to the tumor implantation, the other to tumor growth.

*Ligation of the Blood Vessels of the Spleen.*—The spleen tissue gives the best results in immunization, as is shown by the work of Bridré, and it is also the most active tissue on autolysis, but the spleen of a rat is so small, that a sufficient amount of material for autolysis could not be collected. Another method was, therefore, tried. An abdominal incision was made on a rat and all the
vessels of the spleen were ligated and from four to twelve days after the operation the tumor was inoculated. In another experiment the operation was done nine days after the inoculation.

| Table IV.  
| Ligation of Vessels of the Spleen.  
<p>|</p>
<table>
<thead>
<tr>
<th>After the operation</th>
<th>Before the operation</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats inoculated with tumor</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Number of rats surviving</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Number of rats with tumors</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Percentage of takes</td>
<td>61</td>
<td>100</td>
</tr>
</tbody>
</table>

The table shows that the immunizing influence of such a ligated spleen is not as strong as the influence of autolysed liver injected subcutaneously, and also that this immunizing influence is exerted only on the grafting of the tumor and not on its continued growth. The action of the ligated spleen is most probably comparatively weak on account of the rapid absorption of the organ, which prevents the autolysis, and indeed no traces of such a spleen were found in the abdominal cavity two days after the operation.

Treatment with Alien Tissue.—The most important facts adduced by Ehrlich in support of his atreptic theory of tumor growth and immunity consist in his so-called zig-zag transplantations. He showed, namely, that when a tumor of a mouse is inoculated into a rat, it grows normally for eight or ten days and then ceases its growth and becomes absorbed, but if before the absorption takes place, the tumor is inoculated back into a mouse, it grows there to a large size. Ehrlich’s explanation of this phenomenon is that a mouse tumor needs for its continued growth a certain food stuff X, which it can only find in the mouse organism. When it is introduced into the rat, it carries along from the mouse the X food, and as long as this storage of specific food lasts, the tumor grows; when it is exhausted, the tumor cells die, but when they are returned to the mouse, they find again the necessary food and proliferate. In order to give this contention a further test, a series of experiments was undertaken which consisted in the subcutaneous inoculation into a rat, of the normal skin and spleen tissue of a mouse, followed in a few days by a subcutaneous inoculation of
the tumor. The aim of this treatment was to accustom the tumor cells to mouse tissue and then to observe whether such a rat tumor, which had the opportunity to obtain during its growth the food supplied by the inoculated normal mouse tissue, would not grow more readily when subsequently inoculated into a mouse.

The results of this investigation were negative, but the extremely interesting fact was observed that a certain number of the rats treated with mouse tissue appeared immune against growth of the rat sarcoma. The following table will illustrate this phenomenon.

**TABLE V.**

*Treatment with Alien Tissue.*

<table>
<thead>
<tr>
<th>Treated animals</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rats inoculated with tumor</td>
<td>40</td>
</tr>
<tr>
<td>Number of rats surviving at final examination</td>
<td>27</td>
</tr>
<tr>
<td>Number of rats with tumors</td>
<td>10</td>
</tr>
<tr>
<td>Percentage of takes</td>
<td>37</td>
</tr>
</tbody>
</table>

Similar positive results were obtained recently by C. Lewin (32), who succeeded in immunizing rats with mouse tumors and *vice versa*. This possibility to immunize an animal against growth of tumor by treatment with alien tissue seems to indicate that Ehrlich's atreptic theory, while possibly of value in the explanation of certain facts, does not seem to have a universal application.

Organ cells of a mouse do not possess, nor do they require any specific food of the rat, which is the reason why a rat tumor fails to grow indefinitely in a mouse. When a rat is immunized against growth of a rat tumor by previous treatment with mouse tissue, such a failure to grow cannot be ascribed to the lack of proper nourishment within the host, since the previously inoculated mouse cells could not have absorbed such food.

Another important conclusion which may be reached from this series of experiments consists in the fact that this immunization cannot be due to life activities of the cells, since alien tissue could not remain alive for thirteen days after it was introduced under the skin. Furthermore, this phenomenon is of practical value in the further pursuance of this investigation. It is extremely difficult, as was stated above, to obtain the necessary amount of tissue...
from these small animals for autolysis. Since it was found that alien tissue may be used for immunization, organs of large animals (dogs) are being utilized for autolysis, and on addition of tumor tissue of a rat, heterolytic action is obtained. This is of great importance in a number of pathological processes and also in cancer, as is shown by the investigations of C. Neuberg and others. But these experiments are not yet sufficiently far advanced for conclusions to be drawn. The same is true for the experiments undertaken with the aim in view of obtaining in large animals autolytic antiferments and possibly, therefore, of inducing a passive immunity to the growth of the transplantable tumors.

The results obtained in this investigation are not final; a great deal more work must be done on different tumors. The difficulties encountered in such an investigation is well illustrated by a very recent statement of Abderhalden and Pringsheim (33), that though using the best methods for liberating endocellular enzymes, they occasionally do not find any enzymes in the solution, but the latter apparently become adherent to the filtrate and sand. Consequently, while positive results in our research are convincing, negative results may always be due to insufficient technique. Different means then must be employed to liberate the greatest amount of ferments and entirely new methods will probably be created before conclusive results will be reached, but the viewpoint appears to be correct and the work seems to be pursued in the right direction. Expressions like "life activity of the cell" or "cellular activity" hardly have any significance in biology; the active constituent part of the cell must be found, and autolysis may be the road to it. The idea, at least, is capable of stimulating further research.

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