THE FUNCTION OF THE SPLEEN IN THE EXPERIMENTAL INFECTION OF ALBINO MICE WITH BACILLUS TUBERCULOSIS.

Third Paper.

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In our second paper under this title[1] we were able to state that the increased resistance which splenectomized mice show to infection with the tubercle bacillus is decreased again if the infected animals are fed continuously with fresh spleen. The natural conclusion would follow from this that the feeding of the spleen had served to restore some function of this organ which makes for the normal susceptibility to the infection. The situation was, however, extremely complicated by the fact that splenectomized, uninfected mice were found to suffer from a more or less severe intoxication when fed with fresh spleen. The administration of any poison to mice infected with the tubercle bacillus, even in doses which are considerably below those necessary to cause symptoms in the normal animal, is apt to shorten life. We were at that time of writing, therefore, unable to reach a satisfactory conclusion in regard to the issues involved.

Since then we have made a more extensive study of the intoxication to which splenectomized mice are subject when fed fresh spleen.[2] As a result of this study we attained command of a method which seemed to make it possible to determine whether or not feeding of fresh spleen would restore a function to the body which affects its susceptibility to infection in definite degree.

[1] Lewis, P. A., and Margot, A. G., *Jour. Exper. Med.*, 1915, xxi, 84.

[2] Lewis and Margot, *ibid.*, 1915, xxii, 347.

The facts on which we rely for the purpose of the present paper are the following. When recently splenectomized mice are fed with fresh spleen or with the fresh mucous membrane of the stomach and upper small intestine an acute intoxication is produced. In its immediate manifestations the intoxication is the same, whichever of these organs is administered. A number of weeks after splenectomy the mice are less susceptible to this intoxication and in time they can no longer be affected. This return to the normal condition of tolerance can be hastened by the continuous feeding of the fresh organ in sublethal doses, beginning a few days after splenectomy. In this way we have been able to produce groups of mice which between two and three weeks after splenectomy can be fed fresh spleen and fresh gastro-intestinal mucosa with entire impunity.

It is unimportant for the purposes of the present discussion to decide upon the nature of this tolerance: whether, for example, it is an acquired immunity or a selection of naturally resistant specimens. It is, on the other hand, absolutely essential to a proper interpretation of the results, that the tolerance exist at the time stated, two or three weeks after splenectomy, for the following reason.

We have previously stated that the resistance given against infection with the tubercle bacillus gradually disappears, being entirely gone in six months. We present figures in this paper (Experiment II, Group k) to show that in as short a time as ten weeks the specific effects of splenectomy have disappeared, or at least have been reduced to an insignificant residuum. It is obvious that experiments which depend for their interpretation on the presence or absence of this resistance must be carried out in such a way that the animals are infected from two to three weeks after splenectomy, at a time when the resistance is at a maximum and when, as we have repeatedly shown, it is almost uniformly present. On the basis of these considerations we have carried out the following experiment.

A number of mice were splenectomized and separated into three main groups. The first group (Table I, Experiment I, b) was retained on the regular diet. The second group was fed fresh spleen continuously in addition to the regular diet, beginning on the fourth

day after splenectomy and throughout the course of the experiment. A considerable number of this group died, following the first few feedings, of the acute intoxication we have considered. Those surviving in the third week appear in Table I (Experiment I) as Groups c, d, e, and f, according to the source of the spleen with which they were fed. The third group was fed in exactly the same way with stomach or small intestine, and the survivors appear in Table I (Experiment I) as Groups g and h. The animals of all the groups were now, in the third week, inoculated intraperitoneally with 1 mg. of a culture of the tubercle bacillus, Bovine C. A group of intact, normal mice was also inoculated in the same way, as controls. The feedings were continued during the course of the infection. The results of the experiment are shown in Table I (Experiment I).

TABLE I.

Experiment I. Intraperitoneal Infection with 1 Mg. of Culture Bovine C.

Group.	Mice.	Treatment.	Days lived.
a	Intact	None	19, 21, 29, 29, 32, 34, 39, 42.
b	Splenectomized	"	3, 9, 42, 44, 61, 87, 89.
c	"	Fed beef spleen	29, 38, 52.
d	"	" sheep "	26, 28, 30, 36, 37, 61.
e	"	" rabbit "	24, 28, 40, 41.
f	"	" human "	29, 30, 34, 37, 39.
g	"	" stomach (mouse)	9, 57, 71, 94.
h	"	" small intestine (mouse)	2, 78, 79, 90, 126, one still living.

The results of this experiment seem clear. The normal mice (Group a) were all dead by the forty-second day. Disregarding, as we are accustomed to do in this work, the animals dying in less than ten days, the splenectomized mice which received only the normal diet (Group b) lived much longer than this (forty-two to eighty-nine days). The results with these groups repeat our fundamental experiment showing the increased resistance following splenectomy, and furnish the standards for comparison with the following groups.

The mice fed with fresh spleen continuously, with two exceptions were dead in the same time as the normal controls. The resistance given by splenectomy is therefore abolished by feeding spleen. The two exceptions cannot be held of serious account. The experiment

contains so many possibilities for failure that it is rather surprising that a larger number of exceptional cases do not occur. The most obvious possibility in this connection is that these exceptional mice may not have eaten sufficiently or with sufficient regularity to produce the result attained with the larger number.

The animals fed with the gastro-intestinal mucosa lived as long as or longer than the unfed, splenectomized mice. This result seems to dispose entirely of the objection which might be offered on the basis of the spleen feeding alone that the shortening of life could be the result of an added low grade intoxication.

The experiment as a whole is convincing evidence that as a specific result of feeding fresh spleen the resistance to tuberculous infection is lowered to the normal level again.

Because of its ability to produce in splenectomized mice an intoxication with the same general character and intensity as that produced by the administration of spleen, the feeding of the gastro-intestinal mucosa forms the most convincing evidence that the effects produced are in fact the restoration of a true splenic function as related to the specific infection. This being clear, another experiment which we had carried out at an earlier date may be reported, as it is now susceptible of interpretation. In this case, reported here as Experiment II (Table II), other important organs were fed, in comparison with the spleen and certain spleen products.

TABLE II.

Experiment II. Intraperitoneal Infection with 1 Mg. of Culture Bovine C.

Group.	Mice.	Treatment.	Days lived.
a	Intact	None	10, 15, 17, 20, 21, 22, 22, 24.
b	Splenectomized	"	40, 48, 48, 50, 58, 89.
c	"	Fed sheep spleen	14, 19, 22, 26, 26, 29.
d	"	" watery extract of sheep spleen	13, 29, 32, 36, 36.
e	"	" residue after water extraction	28, 30, 32, 34, 36, 87.
f	"	" sheep liver	1, 1, 58, 58, 68, 80.
g	"	" " thymus gland	1, 3, 48, 58, 73, 84.
h	"	" " thyroid "	5, 13,* 17,* 29,* 47, 50.
i	"	" " lymph nodes	1, 3, 51, 54, 58, 59.
j	"	" " pancreas	5, 40, 48, 64, 82, 90.
k	"	None	10, 19, 21, 24, 24, 25, 28, 30
	10 weeks before		

* Died immediately after feeding.

This experiment differs from the first one in that no effort was made to create a tolerance to spleen before the infection was established. The result shows that while the tolerance is a necessary feature in eliminating an important objection to our interpretation of this sort of experiment, it is not at all essential to the success of the experiment itself.

Table II shows that the loss of resistance as a consequence of spleen feeding is due to the restoration of a function probably peculiar to that organ. The resistance is not affected by feeding liver, thymus gland, lymph nodes, pancreas, or thyroid gland. On the same point our first experiment (Table I) shows that it is not affected by feeding gastro-intestinal mucosa. In our earlier papers it was shown that when fresh muscle was fed the resistance was likewise unaffected. It would be desirable from this point of view to feed certain other organs or tissues, notably the bone marrow.

The results of Experiment I and those reported in our second paper[3] show that the source of the spleen is a matter of indifference; whether it is derived from mouse, sheep, rabbit, beef, or human being, the result is essentially the same.

DISCUSSION.

We have reached a point in our consideration of the relation of the spleen to the tuberculous infection in the mouse where it seems possible and advantageous to discuss the subject in more abstract terms. Until it is shown by experiment to be otherwise, we shall in the future attribute the specific properties of the spleen in its relation to tuberculosis to the activity of a single substance. For convenience we may call this substance tuberculosplenatin, a name suggesting merely its origin and its apparent relationship to tuberculosis.

Tuberculosplenatin we consider to be a substance peculiar to the spleen in the same way that adrenalin is peculiar to the suprarenal gland. It is found in the spleen of several different mammals. Its action can be demonstrated by the use of mice as we have described, either by removing the organ and following the course of the infec-

[3] Lewis and Margot, *ibid.*, 1915, xxi, 84.

tion, or by following the course of the infection in splenectomized mice to which the substance is restored by feeding. While tuberculosplenatin exists in other mammals than the mouse we have not so far been able to demonstrate its activity when these other mammals are infected, presumably because of the presence of other factors which obscure its action.

Since the feeding experiments bring results, we must conclude that tuberculosplenatin is able to resist the activity of the gastric juices to a certain extent at least. We have made a beginning in the study of its physical and chemical properties. The results reported in Experiment II above with Groups d and e indicate that the substance is soluble in cold water but that it is difficult to extract completely in this way.

Tuberculosplenatin acts by producing an increased grade of susceptibility or by diminishing the resistance of the animal to infection. There is very little if any evidence as to the mechanism of the action. Murphy and Ellis[4] believe that the increase in resistance following splenectomy is a consequence of an increase in the circulating lymphocytes that follows the operation. If this is true, the substance we are considering might possibly act in restraining the freedom of action of these cells. We have made some blood counts to control this point in connection with Experiment I. We find, as did Murphy and Ellis, that there is a definite lymphocytosis following splenectomy. This has persisted, however, up to the time of infection in spite of the feeding of spleen, and while our observations are not extensive enough to be final we cannot at present attribute the loss of resistance in our experiments to a depression of the number of circulating lymphocytes. The enlargement of the lymph nodes as a result of splenectomy has not been sufficiently marked in our experience to be significant. It has certainly been no less marked in those animals receiving spleen in the food than in the others. For the present, in view of the almost unlimited possibilities for experimental observation in connection with this line of work, we are disposed to regard attempts to explain the reactions we have encountered as of secondary interest.

[4] Murphy, Jas. B., and Ellis, A. W. M., *Jour. Exper. Med.*, 1914, xx, 397.

SUMMARY.

Experiments are reported which show that in all probability the increased resistance to tuberculous infection which is imparted to mice by the removal of the spleen is a consequence of the loss of a function of the organ. This function can be restored by the feeding of fresh spleen. For the present we attribute these changes to the removal and restoration, as the case may be, of a particular substance for which the designation tuberculosplenatin is suggested. This substance is assumed to be related to the spleen as adrenalin is related to the adrenal gland. It is peculiar to the organ but not to the species. It is not found in other organs of the body so far as our observations have extended. The absence of the substance from the lymphatic glands seems of especial importance in this connection.