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

SECOND PAPER.*

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In a previous paper1 we presented experiments showing that splenectomized mice are definitely more resistant than intact mice to experimental infection with the tubercle bacillus of bovine type. We have continued our work in the hope of arriving eventually at some adequate explanation of this increase in the resistance of the animal. Our more recent results seem to have some measure of general interest, although they have by no means served to solve the problem under consideration.

Before proceeding to the immediate subject of this paper, some comment on the matter contained in our first paper is necessary. The increase in resistance shown by the splenectomized mice, while unmistakable, has proved to be of a relative character rather than an absolute immunity. In table III of that paper four mice are marked as still living in October, 1913. As regards animals 6 and 13 the table is erroneous. These mice died 120 and 127 days, respectively, after inoculation. No. 7 died in 306 days, and No. 8 in 314 days. In table IV of our previous paper animals 19 and 20 are marked "still living." These animals died 158 and 164 days after inoculation. All these mice had large numbers of tubercle bacilli in their organs, and there is no reason to doubt that they died as the result of this infection. Moreover, experiments which it is hardly necessary to present in detail have shown that in mice inoculated more than six months after splenectomy the resistance has diminished again to normal or nearly so.

In these animals, when infected for some time with the tubercle bacillus, we have usually found an accessory or perhaps, more properly, an hyperplastic accessory spleen, 1 or 2 mm. in diameter. We have considered it possible that this splenic tissue might be responsible for the loss of resistance occurring thus

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spontaneously after some months. With this in mind we have reopened a number of uninoculated mice several months after splenectomy. In these animals we have been unable to find gross evidence of splenic tissue, and it seems probable that these small organs only become evident when rendered hyperplastic by the infection. What influence such subvisible splenic tissue may have on the physiological condition of the animal is problematical.

In a recent paper Murphy and Ellis report experiments in confirmation of our observation of an increase in resistance after splenectomy. Studying mainly the changes in the lymphocytes of the blood in splenectomized and intact animals, with and without exposure to the X-ray, Murphy and Ellis are led to the belief that splenectomy increases resistance by causing an increase in lymphocytes. We have no data bearing on this interesting conclusion.

For the purpose of the present paper, therefore, we use as a point of departure the fact that mice after the removal of the spleen develop in the course of several weeks a well marked although transient increase in resistance to infection with Bacillus tuberculosis.

The present series of experiments were made to find out whether the function of the spleen could be wholly or partly replaced by feeding fresh sheep and mouse spleen to splenectomized mice infected with the tubercle bacillus.

In the present experiments the culture used, Bovine C, is one of those previously employed. The methods of splenectomy and infection are those outlined in our previous paper. It should be stated that the usual food of our mice is a mixture of stale bread and oats soaked in water. The animals have been kept in museum jars allowing six by eight inches of floor space. No more than six mice are kept in one jar. The jars are plentifully supplied with fresh wood shavings. The animals are changed at least three times a week to freshly scalded jars. In hot weather or when fresh


The tables of Murphy and Ellis show only the average duration of life, and are not particularly convincing. Dr. Murphy has informed us that some of the splenectomized animals lived much longer than any of the controls. It will be noted that in all our tables certain animals are recorded which lived but a few days. These seem to occur without relation to the nature of the experiment. If they are included they greatly disturb the averages except when the groups are very large. In the absence of any definite basis for excluding such animals from consideration, we feel that it is best for the present to discard the average and present the length of life of each individual.
meat has been fed in the jars this change has been made daily. The following tables (experiments I and II) present our more recent results.

**EXPERIMENT I.**

*Intraperitoneal Infection.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Mice</th>
<th>Amount of culture</th>
<th>Subsequent treatment</th>
<th>Days lived.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Intact</td>
<td>1 mg.</td>
<td>None</td>
<td>18, 20, 24, 20.</td>
</tr>
<tr>
<td>III</td>
<td>Splenectomized</td>
<td>1 mg.</td>
<td>None</td>
<td>3. 10, 50, 50.</td>
</tr>
<tr>
<td>IV</td>
<td>Splenectomized</td>
<td>1 mg.</td>
<td>Fed</td>
<td>10, 20, 30, 34.</td>
</tr>
<tr>
<td>V</td>
<td>Intact</td>
<td>5 mg.</td>
<td>None</td>
<td>17, 20, 20, 20.</td>
</tr>
<tr>
<td>VII</td>
<td>Splenectomized</td>
<td>5 mg.</td>
<td>None</td>
<td>40, 40, 40, 40.</td>
</tr>
<tr>
<td>VIII</td>
<td>Splenectomized</td>
<td>5 mg.</td>
<td>Fed</td>
<td>2, 2, 28, 28.</td>
</tr>
</tbody>
</table>

In this experiment the groups marked under “subsequent treatment” as “fed” received sheep spleen daily. The fresh spleen cut in small pieces was given to them early in the day before they had had other food and was left for several hours so that the animals ate their fill. The reaction of the animals to this method of feeding will be commented on at length in later paragraphs.

**EXPERIMENT II.**

*Intraperitoneal Infection.*

<table>
<thead>
<tr>
<th>Mice</th>
<th>Amount of culture</th>
<th>Subsequent treatment</th>
<th>Days lived.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>1 mg.</td>
<td>None</td>
<td>28, 29, 30, 31, 32, 33, 37, 38.</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>1 mg.</td>
<td>Fed fresh sheep muscle</td>
<td>37, 38, 43, 47, 50, 54, 70, 87.</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>1 mg.</td>
<td>Fed sheep spleen</td>
<td>4. 30, 33, 37, 37, 37, 36, 41.</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>1 mg.</td>
<td>Fed mouse spleen</td>
<td>4. 30, 33, 37, 34, 34, 33, 37.</td>
</tr>
</tbody>
</table>

In this experiment the group which was fed with muscle received all that they would eat each day before being offered other food. Those fed with sheep and mouse spleen received an unmeasured amount, but a much smaller one than that available in experiment I. Before receiving other food each mouse in these groups was placed in a separate jar and given a bit of spleen roughly equivalent to one fourth of the usual mouse spleen. The pieces did not exceed the bulk of a small pea.

These experiments have produced additional evidence that splenectomy increases the resistance of mice to infection with the tubercle bacillus. The extreme prolongation of life noted in the experiments reported in the previous paper has not occurred in the present series, but the differences developed in experiment I between the time of survival of groups III and VII and their respective control groups I and V are none the less striking.
It is evident also from the results of experiment I, on comparing group I with II and group V with VI, that the feeding of fresh sheep spleen to intact mice has no influence on the course of their infection. Groups IV and VIII, on the other hand, show that feeding fresh sheep spleen to splenectomized mice leads to the loss of the resistance afforded by the removal of the spleen. Experiment II done at another time with another lot of animals confirms this result and shows further that the feeding of fresh muscle does not affect the consequence of splenectomy.

The feeding experiments were undertaken with the idea that if the resistance could thus be lowered it would be convincing evidence that the spleen was playing a functional part in relation to the infection, and it might be concluded from these experiments that some function affecting the progress of the disease was removed with the spleen and restored with the feeding. There are, however, certain facts in regard to the feeding experiments which lead us to refrain, for the present at least, from regarding the results as decisive. These factors may be discussed briefly under two heads.

1. The gross anatomical features of the disease in the splenectomized mouse fed with spleen differ from those found in the intact mouse. In the intact mice which die in less than thirty days one seldom encounters striking gross lesions in the lungs. Occasionally there are definite gray nodules from one to three millimeters in diameter. Early there may be no visible changes or there may be minute scattered gray points. In the mice which live longer than thirty days, the large nodules are found more frequently, although irregularly. There has seemed to be somewhat more exudation of this type in the splenectomized mice than in the intact mice which have lived longest, but we have been inclined to attribute this to the greater length of life, and to assume that the factors influencing exudation were not grossly changed by splenectomy. The most regular occurrence of large nodules in the lung has, however, been in those splenectomized animals which have been fed with spleen. The significance of this is not clear, and the observation is recorded merely as suggesting that feeding with spleen, while it lowers resistance, does not restore an entirely normal reaction.

2. When normal intact mice are fed daily with fresh spleen, they
eat with apparent relish and maintain their condition perfectly. The intact mice which were fed with spleen after inoculation with tubercle bacilli also ate with relish as long as they could be expected to retain desire for food.

The splenectomized mice when fed with spleen after inoculation took the first one or two feedings freely. After this time it was noted that they seemed not to care for it, and when they did eat of it they were apt to be sick. The illness took no very distinctive character. The animal would go to the corner of the jar and sit huddled up, with rough fur. This usually passed off in a few hours. On the following day, sometimes on the following two days, they would refuse to eat the spleen, although hungry for other food. After two or three days they would again eat spleen, show evidence of illness, and refuse it again on succeeding days. Several mice died in the course of a few hours after eating spleen. Some small hemorrhages in the serous membranes were noted in these animals, but as they were advanced with their infection the cause of death was not clear.

We have fed spleen to uninoculated, splenectomized mice. Of six mice so fed, two died within a few hours after eating spleen. These also showed a hemorrhagic condition of the serous membranes of the peritoneal cavity. The remaining mice showed the signs of illness with a distaste for the food which has been described, but lived and remained in good condition for upwards of two months, when the feeding was stopped. This indicates that there is an irregular, but more or less severe reaction to the feeding of fresh spleen in the case of splenectomized mice.

In view of the fact that uninfected splenectomized mice show some evidence of poisoning when fed with fresh spleen, a final opinion as to the reason for the loss of resistance as developed in the experiments reported in the above tables can hardly be drawn at present. It may be that a true function of the spleen controlling the reaction to the tubercle bacillus is removed by splenectomy and restored by feeding spleen. But it is not impossible that the shortening of life as manifested in the case of the splenectomized, infected, spleen-fed animals is due to some additional poison not directly related to the factors influencing the increased resistance after sple-
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ectomy. It is apparently the rule that the resistance of normal animals to infection with the tubercle bacillus is lowered by the continued administration of any poison that we are so far familiar with.

In the hope of getting further light on the reaction of the splenectomized animal to ingested spleen, we performed an experiment with dogs.

Two dogs were splenectomized and allowed to recover from the operation. They were kept for about two weeks on a mixed diet. Then with a normal dog for comparison the feeding was begun. The normal dog was fed entirely for over one month with fresh sheep spleen. He maintained his appetite and condition perfectly. One of the splenectomized dogs gave no reaction to spleen at any time. He was fed in alternate periods of several days with fresh chopped beef and with fresh ground sheep spleen.

The second splenectomized dog was fed from May 27 to June 4 with sheep spleen. On the 3d, 4th, 5th, and 6th days of this period, about twenty minutes after feeding, the animal had what appeared to be a slight chill. He lay down, the hair was roughened, and he had a general shivering tremor. The temperature remained normal. The tremor passed off in a few minutes but the animal remained quiet and indifferent to call for an hour or two. On the seventh day the animal became quiet but did not have the tremor. On the eighth day he had a slight tremor.

From June 5 to June 12 the dog was fed on fresh ground beef. He ate well and remained in good condition.

From June 13 to June 23 he was again fed sheep spleen. The first two days of the period he ate with apparent relish and showed no reaction. On the third day of the period he showed the same symptoms as those described during the first period. On the fourth day he ate, but showed no symptoms. On the fifth day he refused the food at first, but finally ate it and reacted as previously. On the sixth day he again ate with apparent distaste, but completed the meal very soon. He became very quiet and much depressed. For a time he seemed likely to vomit. On the following days of the period he refused to eat at first, but during the twenty-four hours consumed about half the quantity given. At the end of the period he had grown thin and was definitely out of condition.

From June 24 to 29 he was again fed fresh beef with the addition of boiled spleen, ate freely, and condition was rapidly regained. From June 27 to July 9 he was again fed sheep spleen alone, but showed no reaction at any time. He ate freely and maintained good condition.

While the result of this experiment is not entirely convincing, it increases the evidence drawn from the experiments with mice to the effect that fresh spleen is somewhat toxic for splenectomized animals.
SUMMARY.

The resistance to an infection with the tubercle bacillus which can be given to mice by the removal of the spleen is lost when fresh spleen, either of mouse or sheep, is added to the diet. The logical conclusion that splenic function in its relation to the specific infection is restored by feeding spleen cannot, however, be drawn; because, in the first place, the character of the disease in the splenectomized spleen-fed animals differs somewhat from that in intact animals; and, secondly, because fresh spleen when fed to splenectomized animals apparently gives rise to an acute intoxication.

This intoxication occurs quite regularly in mice. It is manifested in the infected animals and in those not inoculated. It has been seen in one of two splenectomized dogs experimented upon. Up to the present it can hardly be definitely characterized, but it seems to be best marked out by the specific anorexia associated with it.