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

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Experiments were begun in this laboratory three years ago to test the susceptibility of white rats and white mice to infection with *Bacillus tuberculosis.* It was expected that the animals would show a high degree of resistance to the infection, but it was hoped that they might react with such regularity as to make them available for testing various therapeutic measures. This expectation was in no way fulfilled. It developed that the animals were, on the whole, susceptible to infection, but that there was much irregularity in the reaction of individual animals.

The first experiments were made with rats. It was noted that a constant lesion in these animals was a great increase in the size of the spleen, which occurred whenever the animals survived two weeks or more. It was also noted that in several instances where the animals died in the second week after inoculation the spleen was small and extremely hemorrhagic. It seemed not impossible from a consideration of these facts that the spleen might be an important factor in the resistance of the animal to the infection, and that irregularity in the reaction of this organ might account in considerable degree for the irregularity of reaction of the animal as a whole. The same general facts were observed in a repetition of the experiments in mice.

A number of mice were splenectomized and later tested for their resistance, in comparison with intact controls. Contrary to expectation the splenectomized animals were found to have more than

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normal resistance. In fact a number of animals without spleen have survived for months after inoculation with amounts quickly fatal to normal animals. In this paper we shall present the data on which the foregoing statements are based, with such observations as we have so far made that bear upon the cause of the increased resistance.

We are aware of no literature bearing on the main points at issue although the virulence of the tubercle bacillus for rats and mice, and the pathological anatomy of experimental tuberculosis in these animals, has been the subject of considerable study.[1]

The removal of the spleen was carried out under ether anesthesia. After etherization the mice were tied out on a frog board. The abdominal region was shaved and the spleen delivered through a suitable incision. The main blood vessels were ligated in the omentum, and the organ was then cut off. In closing the laparotomy wound the muscles and skin were separately sutured with fine silk. The animals were kept for a number of hours after operation in a warm place, free from draught. The operation was well borne; a small proportion of the animals died, either under ether or, within a few days, from hemorrhage or peritonitis. Animals surviving beyond the first week seemed to be in good health. The inoculations have never been made in less than two weeks after operation, and have usually been done in the third week.

The cultures used for inoculation were of bovine type. Culture bovine C was isolated in 1912 from a case of spontaneous bovine tuberculosis. This culture is somewhat more virulent for mice than culture R_3, also of bovine type, isolated in 1911 from a human case of cervical adenitis.

The observations are presented in the following tables.

Table I illustrates the constancy and also the degree of enlargement of the spleen when rats are infected with fatal doses of tubercle bacilli.

Table II gives the results of all the inoculations made into normal mice with one, two, and five milligrams of culture bovine C. The culture has never failed to kill in these doses. The maxi-

[1] The literature is reviewed by Goldmann, E., Neue Untersuchungen über die äussere und innere Sekretion, Tübingen, 1912, 52.

TABLE I.

Relation of Spleen Weight to Body Weight in Rats.

Normal rats.		Tuberculous rats.[2]	
Body weight.	Spleen weight.	Body weight.	Spleen weight.
123 gm.	0.26 gm.	140 gm.	1.46 gm.
135 gm.	0.43 gm.	150 gm.	1.15 gm.
130 gm.	0.37 gm.	115 gm.	1.30 gm.
129 gm.	0.71 gm.	135 gm.	2.08 gm.
150 gm.	0.42 gm.	110 gm.	2.01 gm.
125 gm.	0.51 gm.	130 gm.	2.05 gm.
		115 gm.	1.60 gm.
		120 gm.	2.20 gm.
		125 gm.	1.01 gm.
		145 gm.	0.63 gm.
Average 132 gm.	0.45 gm.	128.5 gm.	1.57 gm.
Second Series. Average of Six Rats.			
70.6 gm.	0.20 gm.	Average weight lost.....	8.3 gm.

TABLE II.

Normal Mice. Culture Bovine C.

Dose.	Lived.	Exudate.	Spleen.	Liver.	Heart blood.	Lung.	Kidney.
1 mg.	14 dys.	+	+	−	−	−	−
1 mg.	5 dys.	+	+	−	−	−	−
1 mg.	13 dys.	+	+	+	o	+	+
1 mg.	13 dys.	+	+	+	+	+	+
1 mg.	13 dys.	+	+	+	o	+	+
1 mg.	14 dys.	++	+	+	+	+	+
1 mg.	14 dys.	++	+	+	+	+	+
1 mg.	19 dys.	++	+	+	o	+	+
1 mg.	19 dys.	++	+	+	o	+	+
1 mg.	19 dys.	++	++	+	+	+	+
2 mg.	10 dys.	+	+	+	o	o	o
2 mg.	13 dys.	+	+	+	o	+	o
2 mg.	14 dys.	+	+	+	o	+	+
2 mg.	20 dys.	+	++	++	+	+	+
2 mg.	20 dys.	++	++	+	+	++	+
2 mg.	33* dys.	++	+++	+	+	++	+
5 mg.	13 dys.	++	+	+	o	+	+
5 mg.	13 dys.	+	+	+	+	+	+
5 mg.	14 dys.	++	+	+	o	+	+
5 mg.	14 dys.	++	+	+	+	+	+
5 mg.	14 dys.	++	+	+	+	+	+

++ = many tubercle bacilli; + = few tubercle bacilli; o = no tubercle bacilli; − = examination for tubercle bacilli not made; * = nodules in lungs and heart muscle.

[2] The tuberculous animals were killed twelve days after the intraperitoneal inoculation of 10 mg. of the culture.

mum length of life was thirty-three days. At autopsy the animals frequently showed a small amount of thick yellow exudate in the omentum. The spleen was greatly enlarged. The one mouse that lived over thirty days showed numerous miliary nodules in the lungs. The nodules were softer and showed less organization than tuberculous masses of the same relative size as they are found in other species of experimental animals. The organs and frequently even the heart blood contained enormous numbers of tubercle bacilli.

TABLE III.

Splenectomized Mice. Culture Bovine C.

Mouse No.	Dose.	Lived.	Peritoneal dry exudate.	Peritoneal fluid.	Liver.	Kidney.	Lung.	Heart blood.	Remarks.
1	1 mg.	5 dys.	o	+	o	o	o	o	
2	1 mg.	18 dys.	o	o	o	o	o	o	
3	1 mg.	32 dys.	o	o	o	o	o	o	
4	1 mg.	34 dys.	+	−	+	o	−		
5	1 mg.	37 dys.	o	o	o	o	o	o	
6	1 mg.								Still living.
7	1 mg.								Still living.
8	1 mg.								Still living.
9	5 mg.	2 dys.	o	+	o	o	o	o	
10	5 mg.	29 dys.	+	−	+	o	+	+	Nodules in lungs and heart.
11	5 mg.	32 dys.	+	−	+	o	+	o	Nodules in lungs.
12	5 mg.	32 dys.	+	−	+	+	+	o	Nodules in lungs and liver.
13	5 mg.								Still living.

+ = few tubercle bacilli; o = no tubercle bacilli.

Table III gives the results of the first series of inoculations of splenectomized mice. It will be noted that two of the mice died within the first few days after inoculation. It is unlikely that the tubercle bacilli inoculated were responsible for the deaths. However, even if we include these presumably accidental losses, it is seen that the length of life in the splenectomized mice is much longer on the average than in the case of the normal mice, as shown in table II. Moreover, at the present writing (October, 1913), more than six months after inoculation, four of the animals are still alive and seem to be in perfect health. The increase of resistance, as brought out in this series, has been repeatedly demon-

strated in similar experiments or in slight modifications of them. It is of interest that these splenectomized mice, dying after the thirtieth day, show the same formation of nodules in the lungs that is found in the occasional normal mouse, surviving for an equal time. The bearing of this fact will be considered later.

It will further be noted that, as shown in tables II and III, the presence and distribution of the tubercle bacillus in the splenectomized animals that died was much more limited than in the intact animals. This suggested the possibility that in the splenectomized animals there might be a rapid destruction of tubercle bacilli which did not prevail in the intact animal. With this idea in mind we have studied the distribution of the tubercle bacillus in the first hours after intraperitoneal inoculation in splenectomized as compared with normal animals. The result of this study has been to show that in the normal animal, tubercle bacilli appear in the substance of the spleen, in the portal vein, in the liver, in the bile, and in the lumen of the small intestine within four hours after inoculation in sufficient numbers to be found readily. In splenectomized mice, on the contrary, we have never observed them outside the peritoneal cavity in less than seven days.

Within the peritoneal cavity there is no evidence of rapid lysis or increased phagocytosis. The splenectomized mice show more fluid exudate than the controls, but the significance of this fact is by no means apparent. It would seem, therefore, that the removal of the spleen has interrupted a path by which the tubercle bacillus is excreted from the normal mouse with some rapidity. These observations tend to emphasize the increased resistance brought about by the splenectomy, but in no way explain the cause of it.

In searching for an explanation of the increase of resistance, several possibilities demand consideration. In the first place it might be considered that the spleen formed a most favorable focus of infection as compared to the other organs of the body, and that with the removal of the organ much of the available food stuff is removed. Direct evidence as to the value to be assigned to the organ on the basis of these considerations is difficult to obtain.

The facts presented in regard to the limitation of the distribution of the bacilli in the splenectomized mice suggest that perhaps the

chief effect of the operation is to convert the general infection into a local one by interrupting mechanically a path of transmission. In order to test this supposition we have infected comparative series of animals in the pleural cavities. The results of this experiment as presented in table IV show that the increased resistance also prevails under these conditions. The infection tends to remain localized in the pleural cavities in this case.

TABLE IV.

Controls. Culture R_3. Intrapleural Inoculation.

Mouse No.	Dose.	Lived.	Pleural fluid.	Peritoneal exudate.	Spleen.	Liver.	Kidney.	Heart.	Lung.	Remarks.
1	1 mg.	24 dys.	+	+	+	+	+	o	++	Killed. Nodules in lungs.
2	1 mg.	27 dys.	++	+	+	+	+	o	++	Killed.
3	1 mg.	36 dys.	++	++	+	+	+	+	+	Pleural adhesions. Nodules in lungs.
4	1 mg.	37 dys.	++	++	+	+	+	+	+	Nodules in diaphragm. Adhesions of pleura.
5	1 mg.	39 dys.	+++	+	++	++	++	+	++	Nodules in mediastinum.
6	1 mg.	49 dys.	++	+	++	++	++	o	++	
7	1 mg.	49 dys.	+	+	++	++	++	o	++	Nodules in pleura.
8	1 mg.	49 dys.	++	+	++	+	++	+	+	Nodules in diaphragm.
9	1 mg.	61 dys.	++	+	++	+	+	+	++	Nodules in lung and heart.
10	1 mg.	61 dys.	++	+	++	+	+	o	++	Nodules in right axillary region surrounding a blood vessel.

Splenectomized Mice. Culture R_3. Intrapleural Inoculation.

11	1 mg.	25 dys.	++	o		o	o	o	+	Killed.
12	1 mg.	27 dys.	+	+		o	o	o	+	
13	1 mg.	48 dys.	++	o		o	o	o	+	Killed. Few nodules in lung.
14	1 mg.	70 dys.	+	o		o	o	o	++	
15	1 mg.	70 dys.	++	o		o	o	o	++	Few nodules in lung.
16	1 mg.	125 dys.	++	o		o	+	+	++	Many nodules in lung.
17	1 mg.	125 dys.	+	o		o	o	o	++	
18	1 mg.	133 dys.	++	+		o	o	o	+	Nodules in lung.
19	1 mg.	Living								
20	1 mg.	Living								

We are, therefore, forced to the supposition that following the removal of the spleen there is a general physiological effect which

increases resistance. It has been mentioned in commenting on the results presented in table III that the mice that die after several weeks in the splenectomized series show the formation of gross nodular lesions comparable to those seen in the occasional normal mouse which survives for an unusual period. It is also notable that in the peritoneal and pleural cavities there is a plastic exudate similar to that found in the intact animals. This plastic exudate is accompanied by more fluid in the case of the splenectomized animal, whether the infection is intrapleural or intraperitoneal, but this does not furnish an obvious clue to the cause of the changed resistance.

The character of the various exudates found in the splenectomized series of animals seems to indicate that the removal of the organ does not radically change what we may call the capacity for exudation possessed by the body. The infection in the animals without spleen tends to remain localized. Experiments in progress may, we hope, show on what this limitation of distribution depends.

One control experiment should be mentioned. It is a well known fact in clinical medicine that tuberculous peritonitis is often greatly benefited and sometimes apparently cured by laparotomy without continuous drainage. As a comparable operation we have therefore removed one kidney from a number of mice by transperitoneal operation. When subsequently infected with tubercle bacilli the nephrectomized mice have reacted as do normal animals.

The change of reaction that is produced in mice by the removal of the spleen is one of the few striking instances of an increased resistance to an experimental infection with *Bacillus tuberculosis*. It would be of great importance if it should be proven that the resistance of other species of animals could be similarly altered. Experiments on guinea pigs and dogs to test this point have so far failed to bring out any change in resistance.

SUMMARY.

Infection of rats and mice with *Bacillus tuberculosis* (bovine type) develops a splenic tumor as a typical lesion.

Removal of the spleen from mice (albino) greatly increases their resistance to the infection. This increased resistance cannot

be explained at present. The infection in the splenectomized mice tends to remain localized as contrasted with an almost septicemic type of disease which is usual in the normal animal. The animals of each group that live more than thirty days are apt to present typical exudative lesions. The removal of the spleen does not therefore grossly change what may be called the capacity of the body for exudation.