THE RÔLE OF THE RETICULO-ENDOTHELIAL SYSTEM IN IMMUNITY.

IV. The Action of Diphtheria Toxin in Splenectomized and Blocked Mice.

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While the relation of the reticulo-endothelial system to the production of an artificially acquired immunity has been studied in the past from various angles, it is only recently that attempts have been made to bring phenomena of natural immunity into connection with the same system of cells. Our knowledge of the mechanism of natural resistance in certain animals to certain infections and intoxications has so far been limited almost entirely to the observation that such insusceptibility, with a few exceptions, is not due to humoral immune bodies and hence is not transmissible in most cases. In some instances, well defined physiological properties, such as a higher or a lower body temperature, can be held responsible for this phenomenon. Ehrlich did not actually advance our knowledge on this subject by attempting to explain the phenomenon on the basis of lack of suitable receptors in the tissue of the respective animals. In 1914, Lewis and Margot (1) published experiments which seemed to show that removal of the spleen from albino mice greatly increased their resistance to infection with *B. tuberculosis*. Later, Murphy and Ellis (2), however, were able to explain the anomalous results of the above mentioned authors. They found that mice splenectomized a short time prior to inoculation with *B. tuberculosis* were distinctly more susceptible than normal animals, while, with the increase of the interval between splenectomy and infection, the mice gradually showed a greater resistance than the controls. This increased resistance they explained by a secondary

hypertrophy of the remaining lymphoid tissue. Kyes (3) first definitely connected natural resistance with the fixed phagocytic cells of the endothelial system by his observations on the rôle of the hemophages of the liver and spleen in the natural immunity of the pigeon to pneumococcus infection. In 1925 Singer (4) reported on a breach of natural immunity effected by blockade of the reticulo-endothelial system. He was able, after blocking the reticulo-endothelial system of hens by intravenous injections of India ink, successfully to infect the fowls with *B. anthracis*, while the non-blocked controls remained refractory. Jelin's (5) recent publications likewise emphasize the importance of the reticulo-endothelial system for the mechanism of natural immunity against saprophytic organisms and more particularly for the conditions which determine the course of typhoid fever infection and intoxication in rabbits.

The relative insusceptibility of rats and mice to diphtheria toxin is well known. In the case of mice, it was first observed by Loeffler (6) in 1884 and has since been confirmed by a great number of authors. Roux and Yersin (7) and Behring and Kitashima (8) have been able to kill mice only by employing extraordinarily large doses of diphtheria toxin, while Loeffler failed entirely to infect them. Glenny and Allen (9) found that, when administered intravenously, the M.F.D. of a particular diphtheria toxin for mice was 60 times the subcutaneous M.F.D. for the guinea pig, while 100 times this dose had to be given to kill mice by the intramuscular route. Kolle and Schlossberger (10) reinvestigated the question and concluded that mice were practically insusceptible to large doses of diphtheria toxin, but were generally susceptible to fairly small doses of living bacilli. These authors also found no antitoxin in the blood of mice as had previously been described for rats by a large number of authors (Aronson (11), Koudrevetzky (12), Kuprianow (13), Cobbett (14), Goodman (15), Petitt (16), Coca, Russel, and Baughman (17)). Hippke (18) has been able only partially to confirm the one contention of Kolle and Schlossberger since he found but six strains, out of fifteen, to be virulent for mice. Members of the British Medical Research Council (19) also repeated Kolle and Schlossberger's work, using the same and even larger doses of diphtheria bacilli without obtaining regular results. Wolff (20), on the other hand, corroborates Kolle and Schlossberger's statements regarding the susceptibility of mice to the infection with living bacilli, and furthermore reports that he has succeeded in killing mice by repeated intravenous or intramuscular injections of fairly large doses of diphtheria toxin.

From a review of literature it would appear that the mouse is, to a large extent, insusceptible to diphtheria infection and intoxication,

although this immunity is by no means complete. It has seemed of interest to investigate whether the natural immunity of the mouse to diphtheria toxin and diphtheria bacilli is dependent upon the presence of an intact reticulo-endothelial system, by comparing the action of a highly potent diphtheria toxin and of a highly virulent diphtheria strain in normal mice with their action in mice which have been (1) blocked by intravenous injections of India ink, (2) splenectomized,[1] (3) blocked and splenectomized.

EXPERIMENTAL WORK.

Diphtheria toxin with and without phenol was used as well as diphtheria toxin concentrated by ultrafiltration. For the preparation of the latter we are indebted to Mr. D. Roelkey of this laboratory. The toxin was given by the intraperitoneal and the intravenous route in different experiments. The mice ranged in weight between 24 and 30 gm. Inasmuch as the pathological lesions at autopsy were not as characteristic as seen in other species, cultures were made on a blood plate from the heart's blood and the peritoneal exudate of the dead animals in order to disclose any intercurrent infection.

Series I.—Phenolized diphtheria toxin, No. 411a, having a M.F.D. of 0.0025 cc. for the guinea pig, was employed in this series. It was given intraperitoneally in amounts of 400 M.F.D. (1 cc.), 300 M.F.D. (0.75 cc.), 200 M.F.D. (0.5 cc.), and 100 M.F.D. (0.25 cc.), the volume of the dose in each case being 1 cc. Four groups of mice received these doses of toxin, each group consisting of eight animals: two normal controls, two animals which had received an intravenous injection of India ink (1 cc. 1:15) 2 days previously, two animals which had been splenectomized the day before, and two animals in which the ink injection had been combined with splenectomy. The eight mice which were injected with the 400 M.F.D. died in from 25 to 51 hours, those injected with 300 M.F.D. were found dead after from 34 hours to about 10 days. It should be stated, however, that in the latter group culture from the animal dying after 34 hours revealed the presence of hemolytic streptococci in both the heart's blood and the peritoneal exudate. In the group which received 200 M.F.D. death occurred in from 8 to 17 days, while all the mice which had received 100 M.F.D. survived. There was no definite and uniform difference between the control and the experimental animals in the length of time they lived after injection of the various doses of toxin. Death occurred rather irregularly within each group, demonstrating the wide margin of individual varia-

[1] The operations were performed by using ether as an anesthetic.

tion in susceptibility of mice to diphtheria toxin as compared with the uniform reaction of guinea pigs to this poison.

Series II.—Diphtheria toxin without phenol was used in this series in which the effect of intravenous injections was studied. Toxin without preservative was selected, because it was found in preliminary experiments that phenolized toxin (0.5 per cent) killed mice immediately in intravenous doses of 0.75 cc., and even doses of 0.5 cc. provoked an extremely violent, immediate reaction. Broth, without phenol, injected in similar amounts intravenously, caused only a very slight transitory peptone shock. This toxin, which had a M.F.D. of 0.002 cc. for the guinea pig, was given in a uniform volume of 1 cc. in amounts of 200 M.F.D. (0.4 cc.), 150 M.F.D. (0.3 cc.), 100 M.F.D. (0.2 cc.), and 75 M.F.D. (0.15 cc.) intravenously to four similar groups of mice arranged as in the first series. The eight mice which had received the 200 M.F.D. died in from 4½ to 7½ days, while death occurred in the second group which had received 150 M.F.D., in from approximately 5 to 9 days. With the mice injected with 100 M.F.D. death was prolonged until about 11 days, while in the last group which had received 75 M.F.D., only one normal mouse died after 9½ days. Again, death occurred within each group rather irregularly among duplicate animals, and no significant difference between experimental and control animals became apparent in the length of time they lived.

Series III.—Finally, a last series of experiments was carried out with diphtheria toxin (411a) which had been concentrated by ultrafiltration from an original volume of from 60 cc. to 6 gm. The original M.F.D., for guinea pigs, of 0.0025 cc. was thus decreased, as determined by animal tests, to 0.00025 cc. By using this concentrated and refined product it was possible to inject larger amounts intravenously without fear of a reaction induced by the excess of protein and phenol. Doses of 600 M.F.D. (0.15 cc.), 400 M.F.D. (0.1 cc.), 200 M.F.D. (0.05 cc.), and 100 M.F.D. (0.025 cc.) were injected intravenously in a uniform volume of 0.5 cc. into thirty-two mice, arranged in four similar groups as described before. With the first group which had received 600 M.F.D., death occurred in from 20 to 88 hours, in the second group (400 M.F.D.), the animals lived from 29 hours to 6 days. 200 M.F.D. caused death in from 5½ to 8½ days, while in the last group (100 M.F.D.) one splenectomized and one normal mouse survived; the others died in from 8½ to 13½ days. This series was likewise marked by the absence of any consistent difference in the time of survival between controls and experimental animals.

A small series of experiments (six animals) in which the course of infection with a highly virulent diphtheria strain[2] was studied in normal mice and in mice which had been either injected with India ink or splenectomized, disclosed no difference of susceptibility, between the normal and experimental animals. It was found that

[2] This strain killed guinea pigs weighing from 230 to 280 gm. in a dose of 1/20 slant within from 2 to 3 days.

doses as large as a whole slant of a 24 hour culture on Loeffler's medium were ineffective in producing in either group of animals any symptoms more marked than a small nodule at the site of injection.

DISCUSSION.

The results of the experiments described indicate that such elimination of the reticulo-endothelial system in the mouse as is accomplished by means of one blocking injection of India ink, or splenectomy, or a combination of both operations, has no influence on the natural resistance which this species possesses against diphtheria intoxication and infection. The conclusion which suggests itself is that in this case the mechanism of natural immunity differs fundamentally from the processes of artificially acquired immunity.

SUMMARY AND CONCLUSIONS.

1. The minimum amount of diphtheria toxin which killed normal mice of from 24 to 30 gm. in weight upon intravenous injection, was found to be between 75 and 100 times the M.F.D. for the guinea pig. When given intraperitoneally, the fatal dose for mice was as high as 200 M.F.D.

2. There was no significant difference in the lethal action of diphtheria toxin for normal mice and mice in which an elimination of the reticulo-endothelial system had been attempted by means of blocking injections of India ink, or splenectomy, or a combination of both operations.

3. Attempts to infect normal mice and mice treated as described with large doses of a highly virulent diphtheria strain were unsuccessful with both groups of animals.

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