EXPERIMENTS ON THE RÔLE OF LYMPHOID TISSUE IN THE RESISTANCE TO EXPERIMENTAL TUBERCULOSIS IN MICE.*

By JAMES B. MURPHY, M.D., and ARTHUR W. M. ELLIS, M.B.

(From the Laboratories and Hospital of The Rockefeller Institute for Medical Research.)

In the reaction to tuberculous infection the part played by the endothelioid cell or endothelial leucocyte (Mallory) has received much attention in recent years. This is probably due to the early appearance of these cells in the process of formation of the tubercle and because of their well known phagocytic activities.[1] The other conspicuous element in tubercle formation, the lymphoid cell, probably because of its later appearance and its lack of phagocytic power, has been almost entirely neglected. The small round cell infiltration about the tubercle has indeed been regarded by most observers as of very secondary importance and is often referred to as resulting probably from tissue destruction.

In spite of the lack of interest among pathologists concerning the rôle of the lymphocyte in the tuberculous process, attention has been called by a number of clinicians to the prognostic importance of the lymphocytes in the circulating blood.[2] These observers have noted that in rapidly fatal miliary tuberculosis the lymphocytes fall, often to below 10 per cent. of the circulating white cells, whereas in patients with early, healed, or healing tuberculous lesions these cells are increased, sometimes forming more than 50 per cent. of the total leucocytes.[3] Moreover, it is well known that in acute rapidly fatal

* Received for publication, August 18, 1914.

[1] For the literature see Goldmann, E. E., Neue Untersuchungen über die äussere und innere Sekretion des gesunden und kranken Organismus im Lichte der "vitalen Färbung," Tübingen, 1912, 52; and Evans, H. M., Bowman, F. B., and Winternitz, M. C., *Jour. Exper. Med.*, 1914, xix, 283.

[2] For the literature, see Brecke, A., in Brauer, L., Schröder, G., and Blumenfeld, F., Handbuch für Tuberkulose, Leipzig, 1914, i, 581.

[3] Wack, P., *Deutsch. Arch. f. klin. Med.*, 1914, cxv, 596.

miliary tuberculosis relatively few lymphocytes occur in the individual tubercles, while in the subacute form where a higher resistance of the individual may be assumed, the tubercles contain large numbers of lymphoid cells. These facts have a close analogy in the reaction of the polynuclear leucocytes in certain infections against which they are presumed to form the chief resisting factor.

LYMPHOCYTES AS FACTORS OF RESISTANCE.

Until recently, the round cell infiltration occurring about slowly growing or healing cancer, about cancer grafts in immunized animals, about failing tissue grafts in unsuitable or resistant animals, has been assigned, as in tuberculosis, a secondary rôle. The lymphoid elements here, too, are supposed to be present as a result of tissue disintegration. However, it has been shown that in the case of tissue grafts, the lymphocytes in all probability are the chief agents in causing the destruction of the introduced tissue. The chick embryo normally has no resistance against the growth of implanted tissues from a foreign species, and likewise shows a total absence of the round cell infiltration about the graft.[4] When, however, the chick embryo is provided with a graft of adult chicken lymphoid tissue, it becomes as resistant as the adult to the growth of implanted tissue from a foreign species, and, like the adult, shows an intense infiltration of small round cells about the foreign graft.[5] Furthermore, if the lymphoid system of an adult animal is depleted by means of X-ray, the animal loses its power of resistance to heterologous tissue and an implanted tissue from a foreign species will then grow readily. It is significant that in such animals there is a total absence of the round cell infiltration about the edges of the graft always present in resistant untreated animals.[6]

In the light of these results it seemed probable that the lymphoid cell might play a more important rôle in the resistance to certain infections than had previously been supposed. The fact of its presence in the reaction to tuberculous infections, its variation in the blood with the condition of the tuberculous individuals, and the recent ex-

[4] Murphy, Jas. B., *Jour. Exper. Med.*, 1913, xvii, 482.
[5] Murphy, Jas. B., *idem*, 1914, xix, 513.
[6] Murphy, Jas. B., *Jour. Am. Med. Assn.*, 1914, lxii, 1459.

periments of Lewis and Margot[7] suggested the advisability of investigating the part played by the lymphocyte in the resistance to tuberculosis. Lewis and Margot made the observation that rats and mice experimentally infected with tuberculosis developed large spleens. Splenectomized animals, however, lived longer after inoculation than normal animals. This anomalous result seemed difficult to explain. It is interesting to note in this connection that the inoculations were never made in less than two weeks after the splenectomy and were usually done in the third week. At this period the lymph glands and the lymphoid tissue of the body have shown considerable hypertrophy and many animals show a great increase over normal in the circulating lymphocytes.[8]

THE EFFECT OF X-RAY ON LYMPHOID TISSUE.

Heineke has shown that X-ray has an almost specific and immediate destructive action on the lymphoid system, and in small doses seems to have little, if any, effect on other cells of the body.[9] These results have been adequately confirmed by other observers. We have found that by carefully regulated doses of X-ray, repeated at intervals, a gradual atrophy of the lymphoid tissue may be accomplished without any appreciable effect on the other tissues, or on the general health of the animal.

TUBERCULOUS INFECTIONS IN X-RAYED MICE.

This specific effect of X-ray on the lymphoid tissue offers an excellent experimental method for testing the value of the lymphocyte in various conditions. Animals whose lymphoid tissue has been destroyed should be highly susceptible to those infections against which the lymphocytes play a part in the defense, while their resistance to those infections met by the polymorphonuclear leucocytes should be unaffected. With this idea in view the following experiments were planned.

[7] Lewis, P. A., and Margot, A. G., *Jour. Exper. Med.*, 1914, xix, 187.

[8] Dr. Linda B. Lange has made a series of differential counts on mice before and at intervals after splenectomy. These will probably be reported later.

[9] Heineke, H., *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1905, xiv, 21.

Experiment 1.—Fifty mice of about the same age and size were selected. These were divided into lots of ten each and subjected to the following treatment.

A. Ten mice were splenectomized and then given a daily 5-minute exposure to X-ray for 2 weeks. Average dosage less than ¼ unit (Holzknecht scale), average penetration No. 6, milliamperes 3–4. Spark gap 1 to 1½ inches.

B. Ten normal mice were given daily exposures to X-ray for 2 weeks in the same dosage as group A.

C. Ten mice were splenectomized on the date of inoculation 4 to 9 hours before this procedure.

D. Ten mice were splenectomized 8 to 10 days before the date of inoculation.

E. Ten normal animals served as controls.

All the animals were in good condition at the time of inoculation.

They were divided into small groups in order to prevent the spread of epidemics if any should develop, and were then inoculated with an emulsion of a 6 weeks' glycerin veal bouillon culture of bovine tubercle bacilli, each animal receiving a dose of 1 mg. of dry tubercle bacilli in 0.8 c.c. normal salt solution.[10] As control to the X-ray effect numerous mice have been given the same or larger doses and have shown no bad effects while under observation for several weeks afterward.

TABLE I.

Animals.	Average time of survival.	Percentage of animals with tubercle bacilli in various organs.					
		Exudate.	Spleen.	Liver.	Heart's blood.	Lungs.	Kidney.
Group A.....	7.3 dys.	100		100	40	80	100
Group B.....	7.0 dys.	100	100	100	60	80	100
Group C.....	9.1 dys.	100		100	50	80	100
Group D.....	19.7 dys.	100		100	90	100	100
Group E.....	18.5 dys.	100	100	100	80	70	100

Group A = 10 splenectomized mice given 14 daily exposures to X-ray. Group B = 10 normal mice given 14 daily exposures to X-ray. Group C = 10 mice splenectomized a few hours before inoculation. Group D = 10 mice splenectomized 8 to 10 days before inoculation. Group E = 10 normal mice as controls.

Table I shows the average number of days the various groups of animals lived and the percentage of animals showing tubercle bacilli in the blood, peritoneal exudate, and the various organs. The two X-ray groups, A and B, lived on an average about seven days after the inoculation, and group C, splenectomized a short time before inoculation, averaged only about nine days. Group D, the ani-

[10]This culture was kindly provided by Dr. Paul A. Lewis of the Henry Phipps Institute. It is termed by him Bovine C. The organisms were dried by pressing between filter papers, weighed, and then made into an emulsion by long grinding in normal salt solution.

mals splenectomized about ten days previous to the inoculation, averaged 19.7 days which is a little more than a day longer than the normal group E, which survived 18.5 days as an average. The widespread distribution and great number of organisms leave little doubt that the tubercle bacilli were the cause of death. To rule out epidemics of mouse typhoid, cultures were always taken from the heart's blood and the character of any organisms obtained was studied.

Experiment 2.—This experiment confirms and adds a further control to experiment 1. The groups of mice used were as follows:

A. Eight mice were splenectomized and given 12 daily exposures to X-ray in the same dosage as in experiment 1.

B. Nine normal mice were given the same X-ray exposures as those in group A.

C. Sixteen small normal mice were given 17 daily exposures of X-ray of the same intensity as those in groups A and B. The exposures were discontinued 4 weeks before the inoculations were made.

D. Ten mice were splenectomized 3½ weeks before inoculation.

E. Ten normal mice.

All these animals except 8 of group C were given 2 mg. of a 9 weeks' old culture of bovine tubercle bacilli of the same strain as that used in the first experiment. These animals were isolated in individual glass jars so as to prevent the spread of epidemics, should any develop.

TABLE II.

Animals.	Average time of survival.	Percentage of animals with tubercle bacilli in various organs.					
		Exudate.	Spleen.	Liver.	Heart's blood.	Lungs.	Kidney.
Group A.....	8.4 dys.	100		80	20	40	40
Group B.....	9.7 dys.	100	100	66	11	44	55
Group C.....	7.1 dys.	100	88	71	42	57	100
Group D.....	26.0 dys.	75		87	25	37	62
Group E.....	23.3 dys.	100	100	80	10	90	70

Group A = 8 mice splenectomized and given 12 daily exposures to X-ray. Group B = 9 normal mice given 12 daily exposures to X-ray. Group C = 16 mice given 17 daily exposures to X-ray; 8 of them were inoculated 4 weeks after X-ray was discontinued. Group D = 10 mice splenectomized 3½ weeks before inoculation. Group E = 10 normal mice.

Table II shows the death rate and distribution of the organisms. The age of the culture explains the longer survival of the animals even with twice the dose used in the first experiment. Groups A and

B, the splenectomized X-rayed and the normal X-rayed animals, lived an average of 8.4 and 9.7 days, respectively, after inoculation. Of the sixteen mice in Group C, the life of the eight which were inoculated averaged only 7.1 days after inoculation. Although these animals had had a month to regenerate their lymphoid tissue, they still showed a completely depressed resistance. As a matter of fact, the autopsies showed the spleens and lymph glands to be atrophic, with little sign of regeneration. The early death in these animals compared with the other X-rayed animals may perhaps be due to the fact that they were much smaller mice and had been given more frequent exposures to X-ray. Eight mice of this lot which were not inoculated but kept as a control to X-ray effect are still living and in perfect condition almost three months after X-ray treatment. The mice splenectomized three and a half weeks before inoculation, in agreement with the results of Lewis and Margot, lived longer than the normal animals.

The experiments were planned with the idea of testing the resistance of the animals to tuberculosis when the amount of the lymphoid tissue was varied. The X-rayed animals are at the bottom of the scale, having a system greatly depleted, and they were the first to die of the disease. Next come those splenectomized shortly before the inoculation; these may be considered as having a reduced amount of lymphoid tissue. The animals inoculated eight to ten days after splenectomy, having an active proliferation of the lymphoid cells in the glands and elsewhere, at this stage apparently have about the same resistance as the normal animals. The animals, however, splenectomized three or more weeks before inoculation outlive the normal animals by a number of days and probably represent an increased activity of the defensive agents.

DISCUSSION.

The question naturally arises: Do these treatments cause variations in factors other than the lymphocyte, which might play a part in the resistance to tuberculosis? X-ray in the amount used in these experiments does not affect the general health of the animal. The polymorphonuclear leucocytes are not decreased in number and may be increased. The X-rayed animals have a normal resistance to

certain infecting agents against which these cells form the defense and in some cases they may even have an increased resistance. The circulating large mononuclear cells are not appreciably affected. As evidence that the endothelioid cells are not destroyed, great numbers of these are found in the spleen and lymph glands after X-ray treatment, actively phagocyting the remains of the lymphocytes.[11]

The well known association of the lymphocytes with tuberculous lesions tends to support the conclusions indicated by these experiments. In the acute miliary type of the disease where it may be supposed that little resistance is being offered, the lymphocytes are relatively few in the tubercle, while in the subacute miliary tuberculosis these cells occur in masses about the lesion. As has already been mentioned, the lymphocytes in the blood fall in the rapidly advancing cases, while individuals with localized and well controlled lesions will show a marked increase in the circulating lymphocytes. It would seem, therefore, that these facts taken in conjunction with our experiments strongly suggest that the lymphocyte plays an important rôle in the animal's resistance to tuberculosis.

SUMMARY.

Mice either normal or splenectomized after exposure to X-ray are markedly more susceptible to bovine tuberculosis than are normal animals. Animals splenectomized a short time prior to inoculation are also more susceptible than normal, while those splenectomized eight to ten days before inoculation have about the same resistance as normal. The mice splenectomized three to four weeks before inoculation have a resistance increased over the normal, as has already been shown by Lewis and Margot. As X-ray in the doses used apparently affects only the lymphoid tissue and as the hypertrophy of the remaining lymphoid tissue after splenectomy is so rapid that the circulating lymphocytes may be much above the normal by the third week, it is concluded that this evidence, taken with the well known association of the lymphocytes with tuberculous lesions, points strongly to the lymphocyte as an important agent in the defensive mechanism against tuberculosis.

[11] Heineke, H., *loc. cit.* See illustrations.