STUDIES ON THE MECHANISM OF IMMUNITY IN TUBERCULOSIS

The Fate of Tubercle Bacilli Ingested by Mononuclear Phagocytes Derived from Normal and Immunized Animals

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Plates 5 and 6

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The mechanism of the increased resistance to reinfection in tuberculosis is still uncertain. It has been demonstrated that bacilli of reinfection are either destroyed at once (1), if small numbers penetrate the tissues, or fail to multiply in the immune animal if large numbers invade the body (2), whereas in the normal individual the bacilli, whether many or few, at first grow unhindered. As to the mechanism of this growth inhibition in the immune animal, it may be said that, while antibodies in low titer develop during the the course of tuberculosis, there is no evidence of specific bacteriolysins acting *in vitro* (3). However, *in vivo*, studies have shown that in the body fluids of the immune animal the growth of tubercle bacilli is much less than that taking place in the body fluids of normal animals (2, 4). Since the bacillus multiplies not only in the tissue fluids but especially within the cytoplasm of the cells of the normal animal (5), the apparent failure of the microorganism to multiply within the phagocytes of the immune animal (6) must be explained.

The increased rapidity of the mobilization of mononuclear phagocytes at the site of invasion of the parasite, which characterizes the response of the immune as contrasted with that of the normal animal, has been recently shown to be a function of a heightened physiological activity conferred upon these cells by the tuberculous process (7). This results in an increased rate of cell division on the part of these phagocytes in response to the microorganism in the immune animal. Furthermore, this heightened physiological activity of the mononuclear phagocytes of the infected animal expresses itself by an increased phagocytic capacity for a variety of particulate substances such as carbon particles, staphylococci, and collodion particles. Tubercle bacilli are also more readily phagocyted *in vitro* by mononuclears derived from actively tuberculous animals than by those obtained from normal animals. This is independent of the menstruum of normal or immune serum in which they are suspended and is a property of the cells themselves. It is noteworthy that

this property is not significantly increased in animals vaccinated with an avirulent microorganism.

Whether the observed failure of bacilli of reinfection to grow within the mononuclears of the tissues of the immune animal is also an expression of the altered physiological activity of these cells is the burden of the present study. It is obvious that mere increased phagocytosis of tubercle bacilli by "immune" phagocytes is no explanation for the failure of bacilli to grow within them. There is abundant phagocytosis of tubercle bacilli by the mononuclears of the normal animal (8) and it is precisely within the cytoplasm of normal phagocytes that the bacilli multiply.

Hanks and Brockenbrough (9) have recently reported that serum leukocyte mixtures from normal, infected, and immunized rabbits fail to show any bacteriocidal action on human tubercle bacilli *in vitro*. Rich (10) states that cells derived from immunized animals that ingest tubercle bacilli while growing in tissue culture fail to show a greater inhibitory effect on the multiplication of tubercle bacilli within them than cells derived from normal animals similarly cultivated.

Thus *in vitro* methods have failed to duplicate the inhibition of growth of bacilli that can be readily observed to occur in the living tissues of the reinfected animal. Since the bacteriostatic action of the body fluids of the immune animal cannot be demonstrated *in vitro*, but can be shown to occur by *in vivo* methods, it appeared possible that by *in vivo* methods one might demonstrate what appears to be the bacteriostatic effect of the cells on the growth of tubercle bacilli in the tissues of the reinfected animal. Greene (11), Lucké (12), and others have recently used the anterior chamber of the eye of the living animal as a culture medium for the growth of living tumor cells. It appeared possible that this method might also be applied to the problem on hand.

All previous studies have indicated that, even with the highest degree of immunity attainable, the bacilli of reinfection are rarely, if ever, completely annihilated; at least a few bacilli always remain. It is obvious therefore, that quantitative methods must be used in order to answer the question whether within the cytoplasm of cells derived from immune animals tubercle bacilli are inhibited in their growth as compared to that taking place within the cytoplasm of phagocytes derived from normal animals.

It appeared desirable at first to test the efficacy of the *in vivo* method for the solution of this question under the most advantageous, rather than the most crucial conditions.

Methods and Materials

Normal and immunized rabbits were given a subcutaneous injection of human or bovine type tubercle bacilli suspended in salt solution containing India ink. The lymph nodes draining the site of injection, both in the normal and in the immunized

animal, were removed 2 days following the inoculation. Each lymph node was then divided into three portions; one was weighed, ground, and cultured, to determine the number of living bacilli it contained; an adjacent portion of each node was prepared for microscopic study; the third weighed portion of the lymph nodes, derived from the normal and the immunized animal, respectively, was cut up into fine particles with sharp scissors, washed several times in Tyrode solution, and injected into each anterior chamber of the eyes of a normal, non-tuberculous, albino rabbit previously treated with novocaine. The cells derived from the lymph node of the normal animal, containing the bacilli and the carbon particles that had been carried to them by the lymph flow from the site of infection, were placed in the anterior chamber of one eye. The cells derived from the lymph node of the immunized animal, with their burden of tubercle bacilli and carbon particles originating at the site of reinfection, were placed, in the same amount, in the anterior chamber of the opposite eye of the same albino host. In each instance the chamber fluid was first withdrawn by means of a 26 gauge needle, attached to a tuberculin syringe, and inserted tangentially in the anterior chamber through the limbus of the cornea. Leaving the needle in place within the chamber, the syringe was removed and another tuberculin syringe, containing the desired cell suspension, was attached and the suspension injected into the chamber in 0.1 to 0.2 cc. volumes.

The growth of the implanted cells was observed for a period of 10 to 14 days. At the end of this time the rabbit host was killed. Both eyeballs were removed and fastened with sterile push pins on sterile wooden squares. The fluid from each chamber was withdrawn into a syringe containing a suitable amount of 3 per cent sodium citrate or a heparin anticoagulating solution. This fluid, after microscopic examination, was appropriately diluted and cultured to determine the number of living tubercle bacilli it contained. The cornea was then removed by a circular incision. The entire iris and the ciliary body was then ablated from the underlying lens and vitreous humor, together with any growth which was attached to these structures. This tissue was now divided into three portions: one was examined microscopically by direct smear; the other was fixed for histological study; and the third, constituting the major portion of these structures, was weighed, ground, and suspended in suitable amounts of fifteenth molar Na_2HPO_4, and cultured to determine the number of tubercle bacilli which it contained.

Identical experiments were done with bone marrow obtained from normal and immunized animals, respectively, that had received intravenously, 2 days previously, 0.1 to 10 mg. of human type tubercle bacilli mixed with India ink.

Fate of Tubercle Bacilli Ingested in Vivo within Cells of Normal and Immunized Rabbits

In this test the tubercle bacilli, both human and bovine, had been phagocyted *in vivo* by cells of normal and immunized rabbits in the menstruum of their own body fluids. These normal and "immune" cells with their ingested tubercle bacilli and carbon particles were washed to remove adhering body fluids and transferred into the environment of the anterior chambers of a normal rabbit. Since the rabbit hosts were albinos, no pigment was present in

their irides or ciliary bodies and the cells implanted in the chambers could be identified by the carbon particles which they contained. A correlation was made of the growth of these implanted cells on or in the iris and ciliary body of the host rabbit, the bacilli which they contained, as seen in their microscopic sections, and the number of tubercle bacilli cultured from an adjacent portion of these structures. A comparison of the number of bacilli contained in the lymph nodes or in the bone marrow originally implanted in each anterior chamber with the number of bacilli cultured from the corresponding chamber fluid, the iris and ciliary body after 10 to 14 days incubation, gave an estimate of the relative inhibition or multiplication of the bacilli that had taken place in each chamber.

In Table I*a*, are detailed the exact conditions under which each of 7 such experiments was done. The results obtained from these experiments are recorded in Table I*b*.

It will be noted in Table I*b*, in Experiments 4 to 7 inclusive, that the number of bacilli recovered from a unit weight of bone marrow obtained from immunized rabbits that had received an intravenous injection of 0.1 to 10 mg. of human type tubercle bacilli 2 days previously, was invariably greater than that cultured from the marrow of the normal animal similarly inoculated with the same amounts of the same suspension of tubercle bacilli (columns 2 and 6). Since the large numbers of bacilli injected have not been destroyed in the marrow of either animal, the greater numbers cultured from the immunized animal may be ascribed to the possibility that more bacilli had been removed by the bone marrow of the immunized animal from its circulation than had occurred in the normal animal. This would be in accord with the previously demonstrated fact that the phagocytic capacity of mononuclears of tuberculous animals for tubercle bacilli is greater than that of normal animals. The phagocytosis in the tuberculous animal is here further enhanced over that in the normal animal by the immune body fluids of the former.

Ten to 14 days after incubation of these marrows in the anterior chambers of a normal host, the iris and chamber fluid implanted with cells derived from the normal animal contained many more living bacilli than the same structures implanted with "immune" cells, despite the greater original number of bacilli contained in the "immune" implants. In the three remaining experiments, in which lymph node tissue of normal and immunized rabbits, containing either human or highly virulent bovine bacilli drained from subcutaneous foci of primary infection and of reinfection, respectively, was incubated in the anterior chambers of the same normal host, there were again much greater numbers of bacilli in the chamber implanted with cells of normal origin than in that implanted with cells of immune origin. In all of these 7 experiments the multiplication of the bacilli implanted together with normal cells was from 2 to 10 times as much as that of bacilli implanted in association with cells derived

TABLE I

Fate of Tubercle Bacilli Ingested in Vivo within Cells of Normal and Immunized Rabbits, Implanted in the Anterior Chamber of Normal Animals

(*a*) *Conditions under Which Experiments Were Performed*

Experiment No.	Tissue tested	Dose, type, and route of inoculation of bacilli	Mode of immunization of donor of "immune" cells and interval, in days, between beginning of treatment and time of test	Days of incubation of implanted tissue in anterior chamber
1	Axillary nodes	1.0 mg., human, subcutaneously	0.1 mg. human bacilli intravenously followed 1 mo. later by 0.2 mg. and 1.0 mg. of the same strain intracutaneously and subcutaneously, respectively; 109	11
2	Popliteal nodes	4.0 mg., bovine, subcutaneously	0.0001 mg. virulent bovine bacilli intravenously; 84	14
3	Popliteal nodes	4.0 mg., bovine, subcutaneously	0.0001 mg. virulent bovine bacilli intravenously; 95	14
4	Bone marrow	2.0 mg., human, intravenously	Same mode of immunization as in Experiment 1;* 162	14
5	Bone marrow	0.1 mg., human, intravenously	4.0 mg., human, subcutaneously; 38	10
6	Bone marrow	0.4 mg., human, intravenously	1.0 mg., human, intravenously; 38	14
7	Bone marrow	10.0 mg., human, intravenously	1.0 mg., human, bacilli intravenously followed 4 mos. later by 0.2 and 1.0 mg. of the same strain intracutaneously and subcutaneously, respectively; 134	14

* An additional 1.0 mg. human bacilli given intravenously 15 days before test.

(*b*) *Results Obtained*

Experiment No.	Original No. of colonies cultured from 100 mg. of cells derived from normal animal	No. of colonies cultured from 100 mg. of iris implanted with normal cells	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with normal cells	Ratio between No. of colonies cultured from iris and from cells originally implanted	Original No. of colonies cultured from 100 mg. cells derived from immunized animal	No. of colonies cultured from 100 mg. of iris implanted with "immune" cells	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with "immune" cells	Ratio between No. of colonies cultured from iris and from cells originally implanted
1	5	202	1	40.40	6	28	0	4.66
2	5,360*	3,200	—	0.60	5,160*	1,700	55	0.33
3	39,000*	78,700	67,400	2.02	15,000*	4,300	7,100	0.28
4	872	41,800	2,730	47.93	1,118	14,900	64	13.33
5	960	20	0	0.02	1,790	20	0	0.01
6	9,470	8,300	672	0.88	25,740	1,900	408	0.07
7	59,800	66,000	2,950	1.10	91,800	16,100	1,450	0.17

* Ravenel (bovine).

from an immune animal, both implants growing in identical environment in the two chambers of the same normal rabbit host. (Compare column 3 with 7 and 5 with 9.)

Microscopic examination of the iris implanted with cells of normal animals showed variable accumulations of intact, large mononuclears growing diffusely or in nodular formation on the surface or in the stroma of the iris. In the cytoplasm of these cells, which was often greatly developed and composed of large vacuoles, there were found large numbers of long, deeply and uniformly stained tubercle bacilli (Fig. 1). These were frequently arranged in the form of skeins or packets of parallel rods. In the cytoplasm of the same cells were seen variable amounts of carbon particles in varying degrees of dispersion. These particles as well as the bacilli were at times found in the form of a circle about the centrosphere of the cells. The mononuclears were intermixed with variable numbers of polymorphonuclear leukocytes in varying degree of preservation. At the periphery of these mononuclear growths mitotic figures were frequently encountered. There was a variable degree of accumulation of mononuclears without carbon particles in the stroma of the iris, apparently of host origin, which seemed to multiply in proportion to the multiplication of the bacilli in the implanted cells.

By contrast, in the opposite iris of the same host, implanted with cells of the immunized animal, the multiplication of the implanted cells was much less pronounced. The carbon particles within the cells were often more numerous and less dispersed. In these cells the bacilli were usually much fewer than in the cytoplasm of the implanted cells of normal origin. They were usually very short, faintly stained, and often beaded (Fig. 2). The cytoplasm of the implanted cells was characteristically finely vacuolated and, occasionally, typical mature epithelioid cells were seen. There was usually little polymorphonuclear infiltration of the explanted cells. The multiplication of the host cells was usually slight corresponding with the slight multiplication of the bacilli in the implanted "immune" cells.

On the iris implanted with bone marrow the growths frequently retained their original, natural structure with preservation of the fat spaces. However, in these explants the hemopoietic cells and most of the polymorphonuclears degenerated. Only the reticular cells with their burden of carbon particles and bacilli remained (Fig. 2). In the iris implanted with lymph node tissue, plasma cells were encountered, presumably derived from the implanted lymphocytes.

By far the greatest number of bacilli seen in the chambers were within carbon-bearing, intact, living cells both in the iris and in the chamber fluid. Occasionally free, extracellular bacilli were encountered and some bacilli were seen in mononuclears not containing carbon. Most of the implanted cells whether derived from normal or immunized animals appeared well preserved and alive. Occasionally, however, necrobiotic cell collections were seen on the surface of the iris implanted with cells of both normal and immunized animals.

It is clear therefore that, while the bacilli cultured from the chamber fluid and the iris did not represent solely those contained within the implanted cells, there is no question but that most of the bacilli cultured were contained within the implanted cells. Therefore, it can be said with a reasonable degree of certainty that cells of immunized animals, that had ingested tubercle bacilli *in vivo* and had grown in the environment of the chamber fluid of a normal rabbit, greatly inhibited the multiplication of the microorganisms contained within them, as shown by comparison with the growth of similar bacilli ingested *in vivo* within cells of normal animals and incubated in the chamber fluid of the opposite eye of the same rabbit host. Whether the inhibitory effect of the "immune" cells on the growth of bacilli present in them is due to the properties of these cells themselves cannot be decided from this experiment. For it is conceivable that the greater inhibition of bacillary growth in these cells as compared with that in normal cells may be due to the film of immune body fluids that had coated the bacilli before their ingestion within the "immune" cells, for the bacilli that were contained within the normal cells were coated with normal body fluids before their phagocytosis. However it is plain from this experiment that no renewal of immune body fluids is necessary for the cells of immunized animals to continue to inhibit the growth of tubercle bacilli within them for 2 weeks after their transfer into the environment of a normal rabbit host.

Fate of Tubercle Bacilli Ingested in Vitro within Cells of Normal and Immunized Rabbits

In order to ascertain the rôle of the cells themselves and that of the immune body fluids of the immunized animal in the inhibition of growth of tubercle bacilli within them the ingestion of the microorganism must take place *in vitro* in the presence of both normal and immune serum, respectively. It must be first determined whether cells of normal origin will also inhibit the growth of tubercle bacilli within them if the phagocytosis of the bacteria takes place in a menstruum of immune serum. It must next be disclosed whether cells of immune origin will continue to inhibit the growth of the bacilli when the latter are ingested in the presence of normal serum. Finally, the relative inhibitory effect on the growth of tubercle bacilli must be estimated when cells of normal and immune origin both phagocyte the bacilli in a medium of immune serum. In all these three experiments it would be desirable to grow the cells with their ingested bacilli in the same type of body fluid in which the phagocytosis had taken place.

The following procedure was carried out. Normal or immunized rabbits received an intrapleural injection of about 15 cc. of a sterile 30 per cent acacia solution (13) containing 4.25 per cent sodium chloride. Two such preparations were employed, Lilly's and Endo's. Both are used clinically for intravenous injection after fivefold

dilution with distilled water. The rabbits received this material undiluted. Four to 5 days later the sterile exudate was withdrawn from each rabbit in a syringe containing a suitable amount of 0.4 per cent sodium citrate in 0.85 per cent sodium chloride (8). Both exudates were now centrifuged simultaneously at low speed for 4 to 6 minutes. The supernatant fluid was pipetted off and the cells were gently washed in 30 cc. of the citrated salt solution just mentioned, and again centrifuged. This washing was repeated three times in order to remove the body fluids in which the exudate cells were contained. The final cell sediment was then suspended in about 2 cc. of the citrated salt solution. Total and differential cell counts were made on each cell suspension. Both cell suspensions were then adjusted by proper dilution so as to contain the same number of mononuclears per cubic millimeter. The concentration of mononuclears in the various exudates varied to some extent. However, in the majority of instances, it constituted well over 75 per cent of the cells. The polymorphonuclears and the lymphocytes could not be equalized.

To 0.5 cc. of each cell suspension was added the same volume of fresh undiluted serum derived from the heart's blood of either the normal or the immunized donor of the exudate cells. The amount of serum added to the cells varied from 0.3 to 1.0 cc. in the different experiments. To each of these serum leukocyte mixtures was added the same amount of the same suspension of human type tubercle bacilli (P 15 B) containing India ink. The tubes were stoppered with sterilized corks coated with melted paraffine and rotated in the Robertson machine (8) in an incubator for 30 minutes at 37°C. to permit the phagocytosis of the tubercle bacilli and the carbon particles. At the end of this time the tubes were plunged into ice water to arrest phagocytosis and lightly centrifuged for 2 to 4 minutes. The supernatant fluid was removed as completely as possible in order to free the cell mixtures from unphagocyted bacilli present therein. The number of living unphagocyted tubercle bacilli present in the supernatant fluid was determined by culture.

To each cell sediment was now added 0.5 cc. of the same fresh normal or immune serum originally present in each phagocytic mixture. The chamber fluid from one eye was then withdrawn and one portion of the gently but thoroughly stirred cells with their proper serum fluid was injected into the emptied chamber, as previously described. The other cell suspension, containing the cell and serum combination to which the first was to be compared, was similarly injected into the opposite empty anterior chamber of the same albino rabbit host. From the remaining portions of each cell mixture smears were made on specially cleaned slides to determine the degree of phagocytosis of tubercle bacilli and carbon particles that had taken place *in vitro*. From the same portions of each cell mixture proper dilutions in fifteenth molar Na_2HPO_4 were prepared and cultured on modified Löwenstein medium (14) to determine the number of living tubercle bacilli present in each cell mixture at the time of implantation into each anterior chamber.

The cells were thus incubated in the anterior chambers for 14 to 20 days. At the end of this time the number of bacilli present in each chamber fluid and in each iris was again determined by culture as outlined above. This was correlated with microscopic studies of the same tissues. As in the first experiment, the implanted cells could be identified by their contained carbon particles. A comparison of the number of bacilli originally present in the cells implanted in each chamber with numbers

cultured from the corresponding chamber fluid and iris with its growing implanted tubercle bacilli-bearing cells after incubation, indicated the fate of the bacilli in the cytoplasm of each cell type. Since the chamber fluid was removed before the implantation of the cells and since each cell mixture thus introduced into each chamber was suspended in the same fresh serum in which the *in vitro* phagocytosis of the bacilli had taken place, it is clear that the implanted cells were exposed, for some time at least, to the same fluid medium in which the *in vitro* phagocytosis of the bacilli had occurred.

Fate of Tubercle Bacilli Ingested in Vitro by Cells Derived from Normal Animals in the Presence of Normal and Immune Serum.—As stated above, in evaluating the rôle of the cells themselves and that of the immune body fluids in the inhibition of growth of tubercle bacilli in the cells of the reinfected animal, it must first be determined whether cells of normal origin will also inhibit the growth of the microorganism within them if their phagocytosis and growth takes place in a medium of immune serum. In Table II*b* are presented the results of 6 experiments in which the growth of tubercle bacilli ingested by normal cells in the presence of normal serum is compared to that of bacilli ingested by the same cells but in a medium of immune serum. The experiments were executed as just outlined and the detailed conditions under which these experiments were performed are recorded in Table II*a*.

Column 4 gives the mode of immunization of the donors of immune serum. It will be noted in columns 5 and 6 that the number of mononuclears per cubic millimeter in each of the phagocytic mixtures greatly exceeded the number of individual tubercle bacilli estimated to be present in the same volume. This estimate was based on the weight of bacilli suspended in a given volume of the phagocytic mixture in question. It was assumed that there were approximately 100,000,000 individual bacteria in 1 mg. of pure culture.

In the majority of instances the cell suspensions contained more than 85 per cent mononuclears. There was no constant increment in the phagocytosis of tubercle bacilli or carbon particles *in vitro* by the normal cells when their ingestion occurred in the presence of immune serum as compared with that taking place in normal serum. (These data are omitted from the table to save space.) In the majority of instances after phagocytosis, fewer tubercle bacilli remained in the supernatant fluid of the mixture containing immune serum than in that containing normal serum. The difference however was slight and, in all but one instance, within the range of error of the experiment.

In Table II*b*, columns 2 and 6, it can be seen that there was no considerable, constant difference in the number of living bacilli present in the cells after ingestion of the microorganism *in vitro* in either serum at the time of their respective implantation in each chamber. After 14 to 15 days incubation of these normal cells in the chamber of each eye of the same rabbit there was no constant difference in the relative multiplication of the bacilli within the implanted cells in or on the iris, whether the original ingestion of the micro-

TABLE II

Fate of Tubercle Bacilli Ingested in Vitro within Cells Derived from Normal Rabbits in the Presence of Normal and Immune Serum, Planted in the Anterior Chamber of Normal Animals

(*a*) *Conditions under Which Experiments Were Performed*

Experiment No.	Inflammatory irritant	Interval between introduction of irritant and withdrawal of exudate	Mode of immunization of donor of immune serum and interval, in days, between beginning of treatment and withdrawal of serum	No. of mononuclears per c. mm. in phagocytic mixture	Estimated No. of tubercle bacilli per c. mm. in phagocytic mixture	No. of colonies in 100 c. mm. of supernatant fluid of phagocytic mixture containing:		Incubation in anterior chamber
						Normal serum	Immune serum	
		days						*days*
1	Lilly's acacia and glycerol broth	4	0.0001 mg. Ravenel intravenously; 116	41,000	3,600	1,040	700	14
2	Endo's acacia	4	Progressive bovine tuberculosis acquired by contact; 120*	29,000	1,500	3,520	4,630	14
3	Endo's acacia	4	0.1 mg. human bacilli intravenously followed 1 mo. later by 0.2 and 1.0 mg. of the same strain intracutaneously and subcutaneously respectively; 47	—	1,500	3,580	2,100	14
4	Endo's acacia	4	Same mode of immunization as in Experiment 3; 53	24,000	1,400	26	13	14
5	Endo's acacia	4	Same mode of immunization as in Experiment 3; 59	21,000	1,500	53	53	15
6	Endo's acacia	5	Same mode of immunization as in Experiment 3; 108‡	11,000	2,000	14,300	2,600	14

* Animal received 0.01 mg. bovine type bacilli 6 days before withdrawal of serum.

‡ Animal received 0.001 mg. bovine type bacilli 28 days before withdrawal of serum.

(*b*) *Results Obtained*

Experiment No.	Original No. of colonies cultured from 100 mg. of cells phagocyting tubercle bacilli in the presence of normal serum	No. of colonies cultured from 100 mg. of iris implanted with cells phagocyting tubercle bacilli in the presence of normal serum	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with cells phagocyting tubercle bacilli in the presence of normal serum	Ratio between the No. of colonies in implanted iris and in originally implanted cells	No. of colonies cultured from 100 mg. of cells phagocyting tubercle bacilli in the presence of immune serum	No. of colonies cultured from 100 mg. of iris implanted with normal cells phagocyting tubercle bacilli in the presence of immune serum	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with normal cells phagocyting tubercle bacilli in the presence of immune serum	Ratio between the No. of colonies cultured from implanted iris and from originally implanted cells
1	233,000	240,000	860	1.03	380,000	113,000	430	0.30
2	108,000	10,300	830	0.09	113,000	29,000	570	0.25
3	66,000	20,000	1,400	0.30	57,300	3,660	2,930	0.06
4	1,000	860	10	0.86	2,600	600	53	0.23
5	3,600	2,300	13	0.64	2,600	1,630	10	0.63
6	113,000	39,300	3,300	0.35	103,000	73,000	800	0.71

organism had taken place in a medium of normal or immune serum. Thus in Experiments 1, 3, and 4 the growth in the cells was 3 to 5 times less in the presence of immune than in the presence of normal serum (columns 5 and 9). In Experiment 5 the growth was the same in both and, in Experiments 2 and 6, the growth in immune serum was 2 to 3 times greater than that in normal serum (columns 5 and 9). Similar observations were made on the chamber fluid.

Microscopically there was usually little difference in the growth of the implanted cells and their contained bacilli in either iris. Their character was essentially the same as that described above for the growth of transplanted normal bone marrow or lymph node tissue that had ingested tubercle bacilli and carbon particles *in vivo*. In addition there was found in both chambers a fibrinous matrix attached to the iris in the meshes of which mononuclears bearing carbon and tubercle bacilli were present in diffuse or nodular growths intermixed with variable numbers of polymorphonuclears. Irrespective of whether the cells had originally ingested the bacilli in a menstruum of normal or immune serum, there was usually little difference in the number, size, shape, or staining characteristics of the bacilli within the implanted growing cells. In some instances, however, the number of bacilli visible within the cells that had ingested the microorganism in the presence of immune serum was less (Figs. 3 and 4).

It is clear that immune serum did not impart a constant inhibitory effect on the growth of tubercle bacilli within normal mononuclears when the ingestion of the microorganism took place in immune serum. The presence, for some indeterminate time, of immune serum in the medium of growth of these normal phagocytes also failed to endow them with constant bacteriostatic properties.

Fate of Tubercle Bacilli Ingested in Vitro by Cells Derived from Normal and Immunized Animals in the Presence of Normal Serum.—If the failure of the bacilli of reinfection to proliferate within the mononuclears of the tissues of the immunized animal is largely a property of the cells themselves, it would follow that cells of immune origin that had ingested the microorganism in normal serum should also inhibit the growth of the phagocyted bacteria when subsequently grown on normal body fluids.

Accordingly the growth of tubercle bacilli ingested by cells of immunized rabbits in the presence of normal serum was compared to that of cells from normal animals that had ingested the microorganism in the same medium. These normal phagocytes that had ingested the bacilli and the carbon particles *in vitro*, suspended in normal serum, were planted in the emptied anterior chamber of one eye. The "immune" phagocytes, with their similarly ingested carbon particles and bacilli, suspended in the same normal serum, were implanted in the emptied anterior chamber of the other eye of the same albino rabbit host. All the other measures involved were carried out as previously outlined.

In Table III*a* are given the conditions under which 5 such experiments were carried out. It may be mentioned that the cells and sera used in 5 of the preceding experiments were derived from the same animals as the cells and sera of the present 5 experiments. The original concentration of mononuclears in the pleural exudates of the normal animals varied between 30.1 to 89.5 per cent; that of the immune rabbits ranged between 63.5 and 95.0 per cent. Needless to say the percentage of mononuclears of both cell types was equalized before utilization for phagocytosis. The average phagocytosis of tubercle bacilli and carbon particles by cells of immune origin was greater than that of mononuclears of normal origin, as in previous experiments. However the number of living bacilli remaining in the supernatant fluid of the phagocytic mixture containing "immune" cells did not differ from that remaining in the supernatant fluid of the normal cell mixtures. Apparently the difference in the degree of phagocytosis between the normal and immune cells was insufficient to be detected by the number of bacilli remaining in their menstrua. It must be emphasized that these experiments were not set up for the purpose of determining the degree of phagocytosis. For this latter purpose the bacilli in the menstruum were too few; chance meeting of phagocyte and particle played too much of a rôle. The aim was to present so few bacilli for phagocytosis that even the normal cells would take up the bacilli in approximately the same numbers as the immune cells.

As seen in columns 2 and 6 of Table III*b* this goal was actually achieved in 4 out of the 5 experiments. For in these tests the number of viable bacilli in the normal phagocytic cells before implantation did not differ significantly from that contained in the "immune" cells. In these same experiments, the multiplication or rather the survival of the bacilli in the iris of the normal rabbit implanted with "immune" cells was from 5 to 12 times less than that of the iris implanted with normal cells, despite the fact that there was no immune serum in the medium in which either the ingestion of the bacilli or the growth of the phagocytic cells had taken place. Likewise in the majority of the chamber fluids implanted with "immune" cells there were much fewer bacilli after 14 to 15 days incubation than in the chamber fluid planted with normal cells. The single exception was Experiment 3 where the survival of the bacilli in the "immune" cells was greater than that in the normal phagocytes. It is interesting to note in this connection, that the normal and immunized donors of the cells of this experiment were subsequently infected with virulent tubercle bacilli. It was found that the normal rabbit showed greater resistance to tuberculosis than the immunized animal. The normal animal was a member of a naturally resistant family (15).

Microscopic examination of the growths of the implanted cells confirmed the observations previously detailed. The growth of the bacilli in the cells of

TABLE III

Fate of Tubercle Bacilli Ingested in Vitro within Cells Derived from Normal and Immunized Rabbits in the Presence of Normal Serum, Planted in the Anterior Chamber of Normal Animals

(*a*) *Conditions under Which Experiments Were Performed*

Experiment No.	Inflammatory irritant	Interval between introduction of irritant and withdrawal of exudate	Mode of immunization of donor of "immune" cells and serum and interval, in days, between beginning of treatment and withdrawal of serum and cells	No. of mononuclears per c. mm. in phagocytic mixture	Estimated number of tubercle bacilli per c. mm. in phagocytic mixture	Number of colonies in 100 c. mm. of supernatant fluid of phagocytic mixture containing: Normal cells	"Immune" cells	Incubation in anterior chamber
		days						*days*
1	Endo's acacia	4	Progressive bovine tuberculosis acquired by contact; 120*	29,000	1,500	1,310	1,880	14
2	Endo's acacia	4	0.1 mg. human bacilli intravenously followed 1 mo. later by 0.2 and 1.0 mg. of the same strain intracutaneously and subcutaneously, respectively; 47	—	1,500	4,540	3,010	14
3	Endo's acacia	5	Same mode of immunization as in Experiment 2; 108‡	11,000	2,100	7,000	8,600	14
4	Endo's acacia	4	Same mode of immunization as in Experiment 2; 53	24,000	1,400	90	90	14
5	Endo's acacia	4	Same mode of immunization as in Experiment 2; 59	21,000	1,500	50	30	15

* Animal received 0.01 mg. bovine type bacilli 6 days before withdrawal of serum and cells.

‡ Animal received 0.001 mg. bovine type bacilli 28 days before withdrawal of serum and cells.

(*b*) *Results Obtained*

Experiment No.	Original No. of colonies cultured from 100 mg. of normal cells phagocyting tubercle bacilli	No. of colonies cultured from 100 mg. of iris implanted with normal cells	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with normal cells	Ratio between the No. of colonies in implanted iris and in originally implanted cells	Original No. of colonies cultured from 100 mg. of "immune" cells phagocyting tubercle bacilli	No. of colonies cultured from 100 mg. of iris implanted with "immune" cells	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with "immune" cells	Ratio between the No. of colonies in implanted iris and in originally implanted cells
1	122,000	26,000	1,530	0.21	111,000	5,000	360	0.04
2	56,000	7,300	4,100	0.13	65,000	860	130	0.013
3	153,000	2,000	0	0.013	80,000	5,300	130	0.066
4	2,300	2,300	26	1.00	1,600	33	0	0.02
5	3,000	166	0	0.05	2,600	10	16	0.004

normal origin was greater in 4 out of the 5 experiments than that in the implanted cells of immune origin. In some of these the difference was very great.

The normal cells actually contained skeins of tubercle bacilli, deeply stained and long (Fig. 5); while in the "immune" cells they were difficult to find and those seen were very short, poorly stained, and beaded (Fig. 6). Furthermore the cytoplasm of the normal cells consisted of large irregular vacuoles. That of the "immune" cells was much finer and more regular. Typical mature epithelioid cells with differentiation of the cytoplasm into an external dark periphery and an internal lighter zone were also seen in the "immune" cells growing in or on the iris of the normal host.

It is plain therefore that cells obtained from an immunized animal, washed free of their immune body fluids, which had ingested tubercle bacilli *in vitro* in the presence of normal serum and had been planted and grown *in vivo* in the environment of a normal animal bathed either in the normal serum introduced into the chamber or in the natural chamber fluid, inhibited the growth of tubercle bacilli within their cytoplasm, an average of 10 times more effectively than cells of normal animals that had ingested the microorganism and had grown under identical conditions. This clearly indicates that the inhibition of growth of tubercle bacilli within the cells of the reinfected animal is a property chiefly of the phagocytic cells themselves and that immune body fluids and the organ environment of the immune animal are not essential for this function.

Fate of Tubercle Bacilli Ingested in Vitro by Cells Derived from Normal and Immunized Animals in the Presence of Immune Serum.—It was found above that, while immune serum does not constantly impart a significant inhibitory effect on the growth of tubercle bacilli ingested by cells of normal origin, the immune sera of some inmmuized animals did apparently exercise such an effect, to some degree. It appeared desirable, therefore, to test further the rôle of the cells and sera by determining the relative inhibitory properties of both agents acting simultaneously. Accordingly the growth of tubercle bacilli ingested by cells of normal animals in the presence of immune serum was compared to that of bacilli ingested by cells of immune origin in the same medium.

It will be noted in column 4 of Table IV *a*, which details the conditions under which this series of experiments was performed, that all the donors of the "immune" cells and the immune sera used in this series had undergone progressive tuberculosis caused by the highly virulent bovine bacillus, Ravenel. At the time when these sera and cells were obtained, this tuberculosis had affected the donors for 44 days to one year. This was done in order to elicit a high immune state in these donors.

As in the previous series, the average phagocytosis of tubercle bacilli and carbon particles by the "immune" cells was greater than that by the normal cells. However, here too there was no constant difference in the number of

TABLE IV

Fate of Tubercle Bacilli Ingested in Vitro within Cells Derived from Normal and Immunized Rabbits in the Presence of Immune Serum, Planted in the Anterior Chamber of Normal Animals

(a) Conditions under Which Experiments Were Performed

Experiment No.	Inflammatory irritant	Interval between introduction of irritant and withdrawal of exudate	Mode of immunization of donor of "immune" cells and immune serum and interval, in days, between beginning of treatment and withdrawal of cells and serum	No. of mononuclears per c. mm. in phagocytic mixture	Estimated No. of tubercle bacilli per c. mm. in phagocytic mixture	No. of colonies cultured from 100 c. mm. of supernatant fluid of phagocytic mixture containing:		Incubation in anterior chamber
						Normal cells	"Immune" cells	
		days						*days*
1	Lilly's acacia and glycerine broth	4	0.001 mg. Ravenel intratracheally; 44	58,000	590	500	130	14
2	Same as in Experiment 1	4	Progressive bovine tuberculosis; 1 yr.	23,000	140	530	430	20
3	Lilly's acacia	2*	0.001 mg. Ravenel intratracheally; 71‡	—	140	250	706	14
4	Endo's acacia	4	0.05 mg. human type intravenously and 0.001 mg. Ravenel, by the same route, 59 days later; the serum and the exudate were withdrawn 58 days after the last injection; 117	52,000	1,000	170	940	14
5	Endo's acacia	5	Same procedure as in Experiment 4; the serum and the exudate were withdrawn 70 days after the last injection; 129	21,000	1,000	9,600	9,100	15
6	Endo's acacia	4	Same procedure as in Experiment 4; the serum and the exudate were withdrawn 78 days after the last injection; 137	100,000	1,000	360	120	14

* The exudate from the corresponding normal rabbit was withdrawn 4 days after injection of the irritant.

‡ This rabbit died with tuberculosis and snuffles pneumonia 2 days after the serum and exudate were obtained.

(b) Results Obtained

Experiment No.	Original No. of colonies cultured from 100 mg. of normal cells phagocyting tubercle bacilli in the presence of immune serum	No. of colonies cultured from 100 mg. of iris implanted with normal cells phagocyting tubercle bacilli in the presence of immune serum	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with normal cells phagocyting tubercle bacilli in the presence of immune serum	Ratio between the No. of colonies in implanted iris and in originally implanted cells	No. of colonies cultured from 100 mg. "immune" cells phagocyting tubercle bacilli in the presence of immune serum	No. of colonies cultured from 100 mg. of iris implanted with "immune" cells phagocyting tubercle bacilli in the presence of immune serum	No. of colonies cultured from 0.1 cc. of chamber fluid implanted with "immune" cells phagocyting tubercle bacilli in the presence of immune serum	Ratio between the No. of colonies cultured from implanted iris and from originally implanted cells
1	28,000	22,000	160	0.80	31,000	2,300	16	0.07
2	8,800	15,100	500	1.70	8,600	2,400	230	0.28
3	35,000	3,000	126	0.09	60,000	34,000	1,440	0.57*
4	19,000	63,000	440	3.31	29,000	4,700	100	0.16
5	137,000	209,000	1,800	1.52	120,000	19,000	240	0.16
6	93,000	810,000	1,370	8.71	93,000	5,300	140	0.057

* The donor of these cells had snuffles as well as tuberculosis and died 2 days after cells were tested.

viable bacilli remaining in the supernatant fluid of either cell mixture after phagocytosis. Nor was there any significant difference in the number of living bacilli present in the normal and "immune" cells after phagocytosis in the same immune serum at the time of implantation of these cells in each chamber. (Table IV *b*, columns 2 and 6.) These normal and immune phagocytes, each containing approximately the same number of living bacilli and suspended in the same immune serum, were introduced into each emptied anterior chamber respectively of the same normal albino rabbit host.

Fourteen to 20 days later the growth of the bacilli in the normal cells, proliferating in a medium which, for a time at least, contained immune serum was, in 5 out of the 6 experiments, from 6 to 150 times as great as in cells derived from immume animals that had phagocyted the bacilli in the same immune serum and had grown in the same medium. Compare column 3 with 7 and 5 with 9. Likewise the chamber fluid implanted with cells of normal origin contained from 2 to 10 times as many living bacilli in these 5 experiments as the corresponding chamber fluid implanted with "immune" cells. In only one experiment, No. 3, were the results reversed. The donor of the "immune" cells and immune serum for this experiment had extensive snuffles pneumonia superadded upon his tuberculosis. This animal died 2 days after removal of its cells and serum for the test. It is possible that the intercurrent disease affected the immune state of this rabbit's cells and serum.

Microscopic examination of the irides with their implanted, carbon-bearing cells confirmed the cultural results and showed the same differences in the character of the growth of the two cell types and their contained bacilli as had previously been described in those experiments where cells of normal and immune origin were compared. These differences are illustrated in Figs. 7 and 8. The large numbers of the long tubercle bacilli in the cells of normal origin are contrasted with the single, very short bacillus seen in cells of immune origin. It is noteworthy that the carbon particles are in large masses in the cells of immune origin. They are much fewer and more dispersed in the cells of normal origin. Apparently the normal cells had greatly multiplied together with the multiplication of their contained bacilli and subdivided the burden of carbon, originally ingested *in vitro*, among the daughter cells. The "immune" cells, in which the bacilli not only failed to multiply but, as the cultures indicated, had been greatly reduced in number, proliferated but slightly and hence did not reduce the original carbon present in each cell at the time of implantation. Furthermore the normal mononuclears were intermixed with large numbers of polymorphonuclears. Many of the former had undergone injury from the large numbers of bacilli they contained and chemotactically attracted the granulocytes from the host. The "immune" cell growths on the other hand were not intermixed with polymorphonuclears. They were not injured,

as the bacilli had not grown in their cytoplasm, and hence exerted no chemotactic effect on the polymorphonuclears of the host's vessels.

It is thus again clearly shown that the inhibition of growth of tubercle bacilli by cells of the immune animal is a function of the cells themselves and that immune serum cannot impart to normal cells an equal inhibitory property for the multiplication of tubercle bacilli within their cytoplasm.

Essentially similar results were obtained in a few experiments with guinea pig cells and sera, using normal albino guinea pigs as hosts for the normal and immune cells that had phagocyted tubercle bacilli *in vitro* in normal or immune sera.

SUMMARY AND DISCUSSION

An endeavor was made to determine whether phagocytic mononuclears of immunized animals, removed from the influence of the organs in which they are naturally situated, will inhibit the growth of tubercle bacilli in their cytoplasm more effectively than similar cells obtained from normal animals. It was also undertaken to ascertain to what extent the inhibition of growth of tubercle bacilli in the immunized animal is due to the cells themselves and to what degree it is accounted for by the immune body fluids which bathe them. To answer these questions the anterior chambers of normal albino rabbits were used as *in vivo* incubators. One anterior chamber was host to one type of cell or cell-serum mixture, while the other anterior chamber of the same rabbit was host to a cell type or a cell-serum mixture to which the first was to be compared. The fate of the bacilli in each cell type and in each fluid menstruum was culturally determined by comparing the number of living bacilli present in the cells originally implanted with the number of bacilli that were contained in these cells after they had grown in the chambers. The implanted cells were identified by carbon particles that had been ingested by these cells at the same time that the bacilli were phagocyted.

It was found that cells of immunized animals, which had phagocyted tubercle bacilli *in vivo* in lymph nodes or bone marrow and had been transferred 2 days later into the environment of the chamber fluid of a normal rabbit, inhibited the growth of the microorganism within their living cytoplasm for 2 weeks in the absence of immune body fluids. Similarly transferred normal cells, under these conditions, permitted the bacilli to grow within them to a much greater extent. To what degree the inhibition of growth in these "immune" cells was due to the immune body fluids which had formed a film about the bacteria before their phagocytosis, or to the mere sojourn of the bacilli in these phagocytes in the body of the immune animal for 2 days before their transplantation to a normal environment, could not be determined by these experiments.

To answer the latter question exudative mononuclears derived from normal

and immunized animals, washed free of their body fluids, were permitted to ingest tubercle bacilli and carbon particles *in vitro* in the serum of the normal or of the immunized donor of cells. Before incubating these phagocytes in the anterior chambers, the supernatant fluid of each phagocytic mixture was replaced by the same fresh serum in which the phagocytosis had taken place.

The presence of antibodies against the tubercle bacillus in the immune sera was not determined. It is certain however that in the tissues of such donors of "immune" cells and sera bacilli of reinfection fail to multiply, as had been abundantly demonstrated. It is not known how long the immune or normal serum introduced into the chambers with the phagocytic cells persisted there. It cannot be said with certainty therefore that implanted cells grew for the entire period of their sojourn in the chamber in the serum introduced with the cells. However, there is presumptive evidence to indicate that such introduced fluids remained in the chamber for a considerable time. It has been demonstrated by Seegal and Seegal (16) that protein antigens introduced into one anterior chamber sensitize the corresponding eye but not the opposite eye of the same rabbit. If proteins introduced into one chamber readily leave it there is no explanation for this phenomenon. Under these conditions, it was found that "immune" cells that had ingested tubercle bacilli *in vitro* in the presence of immune serum inhibited the growth of tubercle bacilli in their cytoplasm to a much greater extent than normal cells that had phagocyted the bacteria in the same medium. Furthermore, the absence of immune serum in the medium of phagocytosis and growth of cells obtained from immunized animals did not rob them of their greater bacteriostatic properties on the growth of tubercle bacilli within them. Nor did the presence of immune serum during phagocytosis impart to cells of normal origin a significant and constant bacteriostatic effect.

It is clear therefore that "immune" phagocytic cells possess in themselves, apart from the immune body fluids, a greater bacteriostatic property on the growth of tubercle bacilli within them than do normal cells. This demonstration of the inherent bacteriostatic property of the "immune" cells is in harmony with the previously demonstrated increased capacity for phagocytosis of tubercle bacilli possessed by cells of actively tuberculous rabbits, which is also independent of the medium in which the phagocytosis takes place. It is significant that this enhancement of phagocytosis parallels the immunity of the possessor of such cells. Treatment of rabbits with tubercle bacilli of low virulence confers little immunity to the infection and little or no enhancement of the inherent phagocytic capacity of their cells for tubercle bacilli (7).

The demonstration by Moen and Swift (17) that mononuclears derived from animals sensitive to tuberculin retain that property even after several transplants in tissue culture, shows that allergy, the development of which is so

regularly associated with immunity, is also a property inherent in the cells themselves.

These results also support the conclusion drawn from many years' study on the fate of tubercle bacilli and the associated cellular responses in normal and immunized animals, namely, that the destruction or inhibition of multiplication of tubercle bacilli in the reinfected animals rests chiefly upon an increased capacity of the mononuclear phagocytes of the immunized animal to destroy or inhibit the growth of tubercle bacilli. The present study makes this conclusion more nearly certain. For, in the former studies it was found that the fate of the bacilli of primary infection or of reinfection is greatly dependent upon the organ in which they are focalized. Thus, when immunity develops in the course of a first infection, which had invaded all the organs simultaneously *via* the blood stream, the bacilli may be effectively destroyed in the liver, spleen, and bone marrow but not in the lung and kidney. It is conceivable that forces, apart from the inherent properties of the phagocytic cells that harbor the bacilli, resulting from the structures or functions of the organs and acting as environmental factors affect the behaviour of these cells and account for the observations. In the experiments here recorded it has been clearly demonstrated that immunity in tuberculosis is associated with an increased bacteriostatic property acquired by the mesenchyme cells in general that is independent of the organ environment in which the cells are situated, for it is also present outside of their influence.

To what extent immune body fluids can impart to normal cells increased bacteriostatic effects is not entirely answered by these experiments for there was no continuous renewal of immune body fluids in the medium of growth of the normal cells that were originally introduced into the chambers with immune serum. One especially hesitates to accept the inconstant results of this experiment in view of the fact that it has been clearly demonstrated that when bacilli situated extracellularly are continuously exposed to the body fluids of immune animals *in vivo* (2, 4) they are definitely inhibited in their growth by comparison to bacilli similarly exposed to normal body fluids.

CONCLUSIONS

1. Mononuclear phagocytes of immunized animals that had ingested tubercle bacilli *in vivo* and had subsequently been transplanted and grown in the environment of a normal animal continue to inhibit the multiplication of the microorganism in their cytoplasm in the absence of immune body fluids.

2. Mononuclear phagocytes of immunized animals that had ingested tubercle bacilli *in vitro* in the presence of immune serum inhibit the multiplication of the microorganism in their cytoplasm to a much greater extent than cells of

normal animals that had ingested the bacteria in the same medium and had grown in a similar environment.

3. The presence of immune serum during the *in vitro* ingestion of tubercle bacilli by mononuclear phagocytes of normal animals does not regularly endow them with increased bacteriostatic properties for the microorganism. Whether or not continued sojourn of normal cells in immune body fluids will confer upon them such properties has not been determined.

4. Mononuclear phagocytes of immunized animals that had ingested tubercle bacilli *in vitro* in a medium of normal serum and had subsequently grown in an environment devoid of immune body fluids inhibit the multiplication of the microorganism in their cytoplasm to a much greater extent than do normal cells under the same conditions.

5. Active tuberculosis confers on the mononuclear phagocytes themselves increased bacteriostatic properties for the tubercle bacillus which are independent of the immune body fluids or of the organ environment in which they grow.[1]

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[1] Preliminary reports of the present work were presented before the Eastern Pennsylvania Chapter of the American Society of Bacteriologists on Nov. 28, 1939 (*J. Bact.*, 1940, **39**, 339) and before the American Association of Pathologists and Bacteriologists, April 10, 1941 (*Am. J. Path.*, 1941, **17**, 636). Since its completion Kallós (18) has published qualitative tissue culture studies confirming these quantitative results.

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EXPLANATION OF PLATES

All microphotographs were prepared from tissues stained by the Ziehl-Neelsen method and counterstained with hematoxylin. The magnifications are about × 1400.

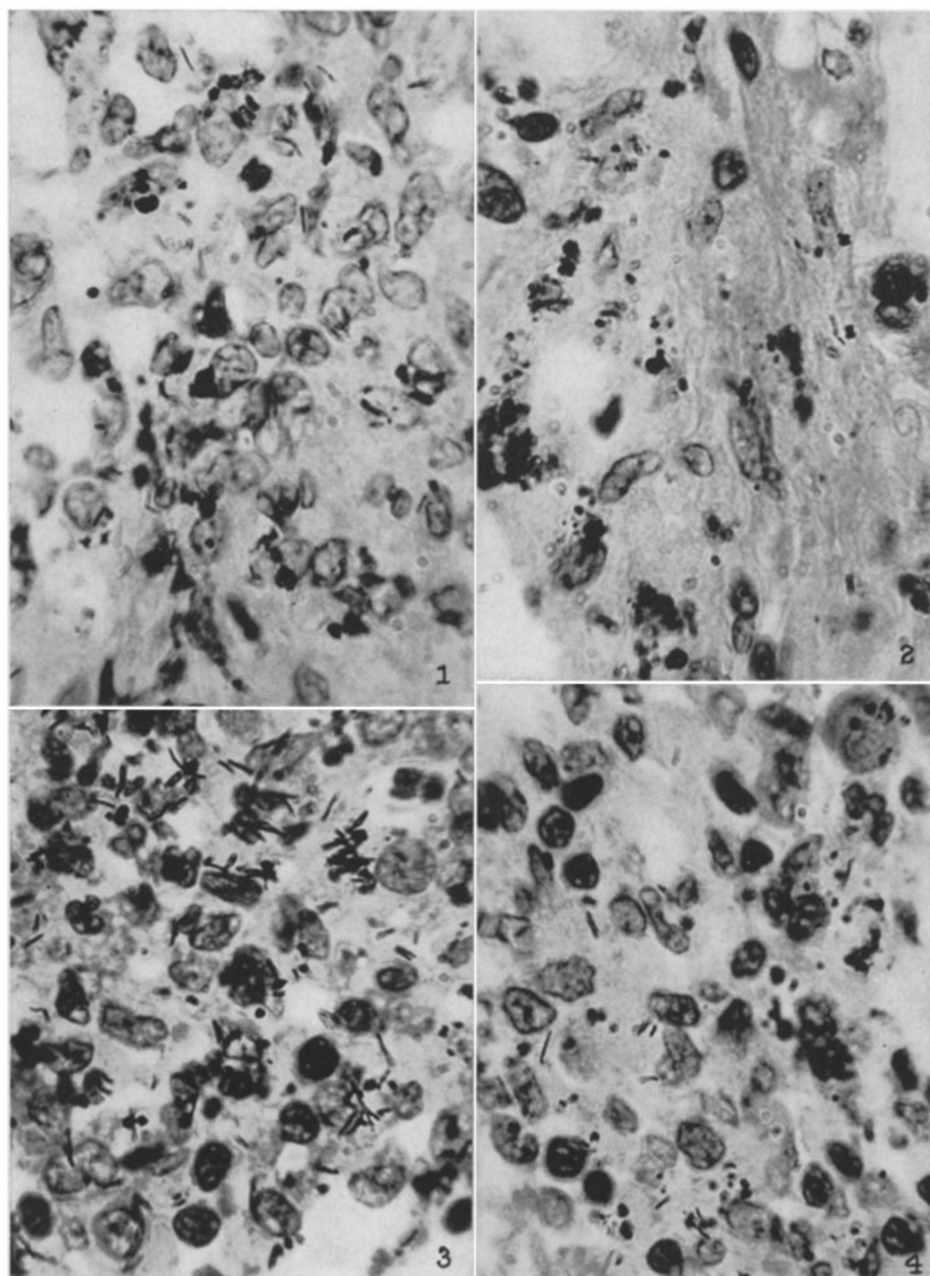
Plate 5

Fig. 1. Bone marrow of normal rabbit, the reticular cells of which had ingested tubercle bacilli and carbon particles *in vivo*, growing in the anterior chamber of the eye of a normal rabbit (Experiment 7, Table I*b*). 59,800 colonies were cultured from 100 mg. of this tissue at the time of implantation. After 14 days incubation, 66,000 colonies were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and that in the original inoculum, 1.1. Rapidly and diffusely growing mononuclears containing carbon particles and numerous, long, well stained tubercle bacilli in their cytoplasm.

Fig. 2. Bone marrow of immunized rabbit, the reticular cells of which had ingested tubercle bacilli and carbon particles *in vivo*, growing in the opposite anterior chamber of the same host that harbored the normal cells shown in Fig. 1. 91,800 colonies were cultured from 100 mg. of this tissue at the time of implantation. After 14 days incubation, 16,100 colonies were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and that in original inoculum, 0.17. The reticular cells of the surviving marrow contain carbon particles; one such cell, at the left margin of the photograph below its center, contains a mass of such particles. The original fat spaces of the marrow are retained and may be seen in the lower left and both upper corners of the microphotograph. Tubercle bacilli are very rare. Two microörganisms, poorly stained and short, are seen lying at an obtuse angle to each other near the right margin of the photograph, at about its center.

Fig. 3. Phagocytic mononuclears of a normal animal, that had ingested tubercle bacilli and carbon particles *in vitro* in the presence of normal serum, growing in the anterior chamber of a normal host (Experiment 1, Table II*b*). 233,000 colonies were cultured from 100 mg. of cells at the time of implantation. After 14 days incubation, 240,000 were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and in the original inoculum, 1.03. Diffusely growing, carbon-containing, mononuclears swarming with long, deeply stained, tubercle bacilli.

Fig. 4. Phagocytic mononuclears from the same normal animal, as those shown in Fig. 3 that had ingested tubercle bacilli and carbon particles *in vitro* in the presence of immune serum growing in the opposite anterior chamber of the same normal host that harbored the cells shown in Fig. 3. 380,000 colonies were cultured from 100 mg. of cells at the time of implantation. After 14 days incubation, 113,000 colonies were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and in the original inoculum, 0.30. Diffusely growing, carbon-containing mononuclears with fewer and less well stained tubercle bacilli in their cytoplasm than those shown in Fig. 3.



(Lurie: Mechanism of immunity in tuberculosis)

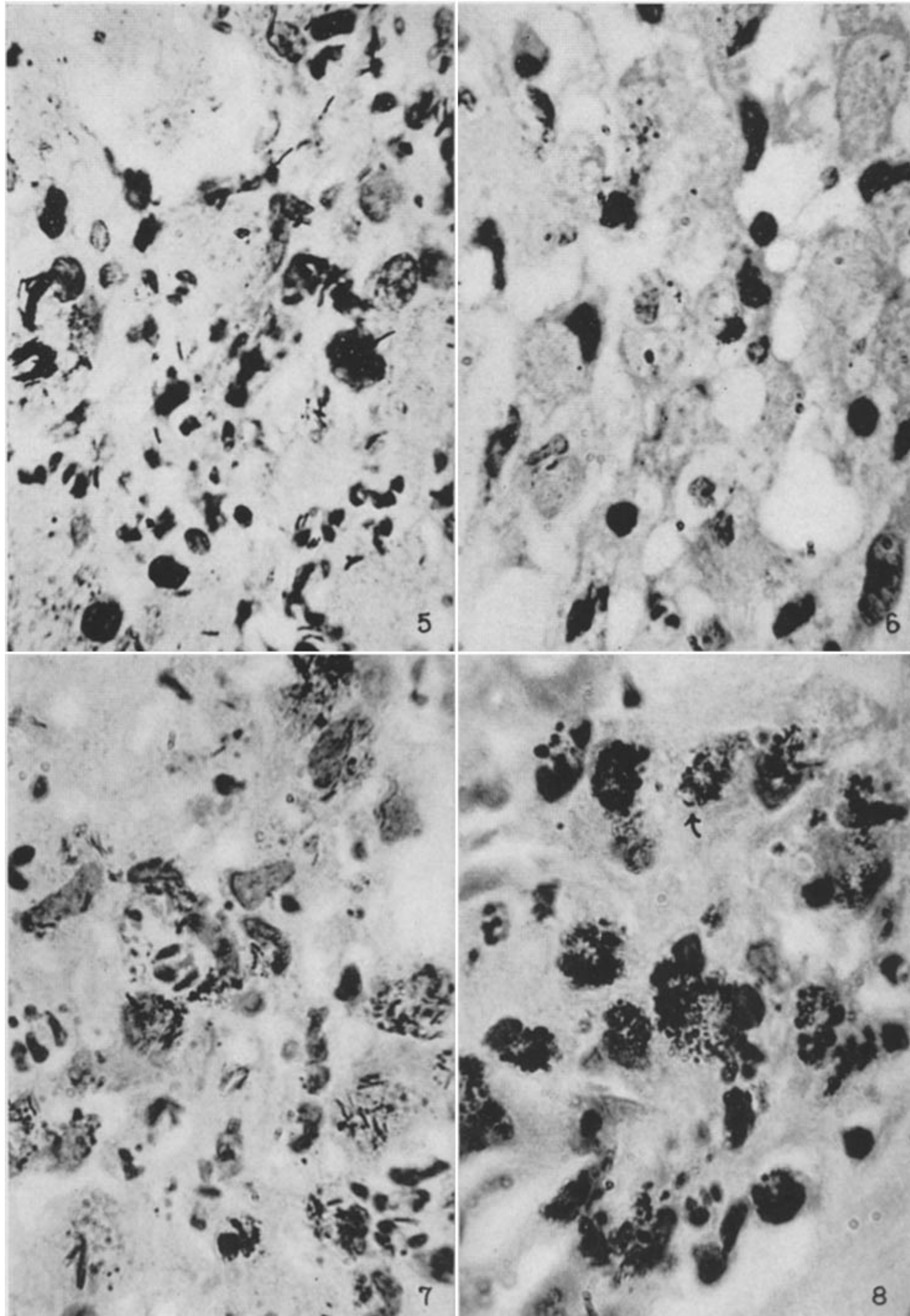
Plate 6

Fig. 5. Phagocytic mononuclears from a normal animal that had ingested tubercle bacilli and carbon particles *in vitro* in the presence of normal serum, growing in the anterior chamber of a normal host (Experiment 2, Table III *b*). 56,000 colonies were cultured from 100 mg. of cells at the time of implantation. After 14 days incubation, 7,300 colonies were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and in original inoculum, 0.13. Large mononuclears with large vacuoles in their carbon-containing cytoplasm, infiltrated with necrotic polymorphonuclear leukocytes. The bacilli are numerous. Two large skeins of bacilli, one of which is U shaped, can be seen in the mononuclears near the left margin of the microphotograph at about its center.

Fig. 6. Phagocytic mononuclears from an immunized animal, that had ingested tubercle bacilli and carbon particles in the presence of normal serum, growing in the opposite anterior chamber of the same normal host that harbored the cells shown in Fig. 5. 65,000 colonies were cultured from 100 mg. of cells at the time of implantation. After 14 days incubation, 860 colonies were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and in original inoculum, 0.013. There are large mononuclears with very small round vacuoles, some containing carbon particles with a structure characteristic of epithelioid cells. Such a cell, with a peripheral dark staining and a central light staining cytoplasmic zone, is seen near the left of the photograph at about its center. In the cytoplasm of a cell below the former one can see a very short, diploid tubercle bacillus, the only one that could be found in this field. There are no infiltrating polymorphonuclears.

Fig. 7. Phagocytic mononuclears from a normal animal, that had phagocyted tubercle bacilli and carbon particles *in vitro* in the presence of immune serum, growing in the anterior chamber of a normal host (Experiment 6, Table IV *b*). 93,000 colonies were cultured from 100 mg. of cells at the time of implantation. After 14 days incubation, 810,000 colonies were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and in original inoculum, 8.71. Mononuclears, infiltrated with and phagocyting polymorphonuclears containing numerous tubercle bacilli and dispersed carbon particles in their cytoplasm are seen.

Fig. 8. Phagocytic mononuclears from an immunized animal, that had ingested tubercle bacilli and carbon particles *in vitro* in the presence of the same immune serum in which were suspended the normal cells shown in Fig. 7, growing in the opposite anterior chamber of the same normal host that harbored the normal cells also shown in Fig. 7. 93,000 colonies were cultured from 100 mg. of cells at the time of implantation. After 14 days incubation, 5,300 colonies were cultured from 100 mg. of implanted iris. Ratio between the number of colonies in iris and in original inoculum, 0.057. Large mononuclears, heavily laden with masses of agglutinated carbon particles in their cytoplasm may be seen. Tubercle bacilli are very difficult to find. One very short, comma shaped bacillus, indicated by the arrow, can be seen in the cytoplasm of the cell near the upper right corner of the photograph just below the oval mass of carbon particles.



(Lurie: Mechanism of immunity in tuberculosis)