ON THE MECHANISM OF IMMUNITY IN TUBERCULOSIS

THE HOST-PARASITE RELATIONSHIP UNDER THE CONDITIONS OF A LOCALIZED AGAR FOCUS OF INFECTION AND THE GENERALIZATION OF THE DISEASE IN NORMAL AND IMMUNIZED RABBITS*

BY MAX B. LURIE, M.D.

(From The Henry Phipps Institute, University of Pennsylvania, Philadelphia)

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There is still wide divergence of opinion as to the mechanism of immunity in tuberculosis. The extensive studies of Rich and his associates (1) on the rôle of inflammation in immunity to reinfection have cast much doubt upon the hitherto widely accepted view that allergy, which accompanies immunity in tuberculosis, is the essential mechanism thereof. On the other hand, the fundamental observations of Opie (2) on the fixation of foreign protein in the Arthus phenomenon and the illuminating experiments of Menkin (3) on the rôle of fibrin and thrombosed lymphatics in the fixation of a variety of substances, including bacteria, at the site of inflammation, would indicate that the increased inflammation incident upon reinfection may play a rôle in immunity to tuberculosis. It is almost universally accepted that the rôle of humoral substances in immunity to tuberculosis has not been demonstrated; hence immunity in tuberculosis is generally considered as entirely cellular in nature. The present investigation represents an endeavor to throw more light upon these questions.

In previous studies (4) it was shown that immunity to intravenous reinfection is a function of the increased capacity of the mononuclear phagocytes to digest tubercle bacilli. With the persistence of an

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extensive primary lesion, the bacilli of reinfection are completely destroyed and only inconspicuous nodules of mononuclear phagocytes develop wherever the bacilli had lodged, which soon disappear. If the primary lesion is largely healed, the immediate inflammatory reaction to reinfection is much more intense; accelerated formation of epithelioid and giant cell tubercles occurs associated with a less rapid destruction of the bacilli and a less rapid resolution of the lesion. This would indicate that the greatest degree of immunity is associated not with an increased but a decreased intensity of the initial inflammatory reaction. However intravenous inoculation does not correspond with natural infection. It was decided therefore to study the host-parasite relationships when the bacilli were introduced into the body in a localized area. After many trials, tubercle bacilli were finally incorporated in melted agar (5) and injected subcutaneously. The host-parasite relationships were studied as heretofore by correlating the fate of the bacilli as indicated by culture with the histological changes in adjacent tissue. This procedure affords certain advantages offered by the tissue culture method of approach and yet all the events occur within the body (6).

Methods and Materials

Sterile 6 per cent agar in saline at pH 7.4 was melted and when cooled to approximately 50°C. was intimately mixed with virulent bovine tubercle bacilli of the Ravenel strain suspended in a 1:100 dilution of Higgins' waterproof India ink in saline, or in a 1 per cent saline solution of trypan blue. The ink and the trypan blue served not only as a guide to the distribution of the bacilli within the highly viscous melted agar but also for the purpose of indicating the dissemination of these materials to the tributary lymph nodes.

The inoculation was executed as follows: To accurately measured amounts of melted agar was added a constant amount of the suspension of the bacilli. 5 cc. of the mixture was injected subcutaneously into each of the normal and immunized rabbits of a given series. One tube containing the identical mixture was reserved for culture in order to determine the number of living bacilli present in a given weight of the inoculum. Simultaneously, these rabbits received subcutaneously on the opposite side a mixture of melted agar and dye without bacilli. At varying intervals of time following inoculation a normal and a treated rabbit were killed. The number of living tubercle bacilli present in a given weight of the agar coagulum and its surrounding capsule, in the lymph nodes draining this focus, and in the internal organs, was determined by planting weighed amounts upon Löwenstein's egg medium supplemented with bone marrow infusion as previously described (7).

The number of colonies cultured from known amounts of a given tissue was correlated with the histological changes in the immediately adjacent tissue. The sections were stained with hematoxylin and eosin and by the Ziehl-Neelsen method for tubercle bacilli. The Gram and fibrin stains were used in some instances.

Two types of rabbits were used in these experiments. Some had received repeated intravenous inoculations of B C G. Others had been previously inoculated with virulent bovine tubercle bacilli of the Ravenel strain. The host-parasite relationships in these two groups were compared to those in a group of normal rabbits simultaneously inoculated with the same agar-tubercle bacillus suspension.

TABLE I

The Fate of Virulent Bovine Tubercle Bacilli in a Localized Agar Focus and Their Dissemination in the Body of Normal and BCG Vaccinated Rabbits, as Indicated by the Number Found (Series 1)

Agar suspension	Interval after inoculation	Rabbit No.		Agar focus		Draining axillary nodes		Spleen		Lung	
		Normal	Vaccinated	Normal	Vaccinated	Normal	Vaccinated	Normal	Vaccinated	Normal	Vaccinated
4,450	1 day	26-2	12-0	—	3,700	0	0*	0	0	0	0
	3 days	26-5	12-5	18,060	8,900	18	1	0	0	0	0
	1 wk.	26-8	12-3	27,800	2,650	176	19	1	3	1	0
	2 wks.	26-7	12-1	40,000	5,300	10,400	73	100	1	10	0
	4 wks.	26-4	12-7	12,360	1,960	9,360	164	30	1	60	0
	80 days	26-9	12-4	—	6,880	7,200	52	13	—	11,500	14†

* Control axillary node 1.

† Adjusted from colonies obtained after treatment.

The Host-Parasite Relationships in Normal and BCG Vaccinated Rabbits

Fate of the Bacilli.—A series of rabbits received two intravenous injections of 1.0 mg. living B C G at an interval of 132 days. 20 days after the last injection the rabbits showed a marked degree of hypersensitivity to the intracutaneous administration of tuberculin. 43 days later these vaccinated rabbits, together with a group of normal animals, received a trypan blue suspension of virulent tubercle bacilli in agar subcutaneously over the right chest wall. The number of colonies isolated from 10 mg. of the agar inoculum, the subcutaneous agar focus, the draining lymph nodes, the spleen and lung of the normal and vaccinated rabbits is recorded in Table I.

It will be noted that the bacilli multiplied unhindered in the agar focus of the normal animal to the end of the 2nd week. In the vaccinated animals on the other hand there was scarcely any multiplication in this location. With this

quantity of inoculum, containing 4,450 bacilli, no microorganisms could be cultured from the lymph nodes draining the agar focus of either the normal or the vaccinated animals 1 day after inoculation. Thereafter bacilli accumulated in increasingly larger numbers in the axillary lymph nodes of the normal animal, and a very much more retarded though continuous increase of the microorganism took place in the nodes of the vaccinated animal to the end of the 4th week. 80 days after inoculation the bacilli persisted in practically undiminished quantity in the draining lymph nodes and accumulated in large numbers in the lung of the normal animal. In the vaccinated animals on the other hand they persisted in small numbers in the draining lymph nodes and accumulated very slowly in the lung.

TABLE II

The Fate of Virulent Bovine Tubercle Bacilli in a Localized Agar Focus and Their Dissemination in the Body of Normal and BCG Vaccinated Rabbits (Series 2)

Agar suspension	Interval after inoculation	Rabbit No.		Agar focus		Superficial axillary node		Deep axillary node		Spleen		Lung	
		Normal	Vaccinated	Normal	Vaccinated	Normal	Vaccinated	Normal	Vaccinated	Normal	Vaccinated	Normal	Vaccinated
8,400	1 days	3	13-9	9,000	11,880	0	30*	—	—	0	0	0	0
	3 days	4	13-7	17,200	9,860	5	37	—	4	1	0	0	0
	1 wk.	2	13-0	131,000	29,640	1,850	3	190	0	19	1	21	1
	2 wks.	5	12-2	492,000	3,000	32,500	150	100	20	164	2	22	3
	5 wks.	1	13-1	1,044,000	5,700	31,900	3,430	35,700	0	840	1	6,000	96

* Control axillary node 0.

It is evident that vaccination with living B C G affords a pronounced protection against a virulent infection. While the bacilli are not destroyed at the portal of entry, they are markedly inhibited in their multiplication in this location. The bacilli that reach the draining nodes and internal organs are similarly affected.

In Table II is recorded a similar experiment with the exception that the vaccinated rabbits received three intravenous injections of 1.0 mg. of B C G, the intervals being 132 and 80 days. The virulent reinfection followed 81 days after the last B C G inoculation. 1 cc. of India ink in a 1:100 dilution in saline containing the virulent bovine bacilli was mixed with 9 cc. of agar; 5 cc. of this mixture was injected subcutaneously in the same location as in the previous experiment, into the vaccinated and a normal series of rabbits.

It will be noted that approximately twice as many tubercle bacilli were present in the original agar inoculum used for this series as in that of the previous experiment. There was no significant difference in the number of bacilli cultured 1 day after inoculation from the agar focus of the normal and vaccinated rabbits. The subsequent findings as concerns the microorganism in the agar focus of the two groups of this series were essentially the same as in the previous experiment, namely, a marked if not complete inhibition to the multiplication of the bacilli within the agar focus was apparent in the vaccinated animal, as compared with unhindered and tremendous accumulation of the bacilli in the normal animal. However, contrary to all expectations, while no tubercle bacilli had reached the draining lymph nodes of the normal animal 1 day after inoculation, 30 colonies were cultured from the superficial axillary node draining the agar focus of the vaccinated rabbit. The superficial axillary nodes on the opposite side examined at the same time contained no tubercle bacilli. It follows that instead of a retardation to the dissemination of the bacilli from the portal of entry to the draining lymph nodes there was an actual acceleration of the spread. This accelerated spread to the immediately draining lymph nodes, however, did not prevent the marked inhibition to their multiplication in the vaccinated animals, as was noted in the previous experiment. It is plain that the increased resistance to reinfection, as noted in this series, did not depend upon the fixation of the bacilli at the portal of entry but upon forces inhibiting their multiplication after their enhanced dissemination to the draining nodes.

As in the previous experiment BCG vaccination had retarded the multiplication of the bacilli at the portal of entry and in the immediately draining lymph nodes. It is noteworthy that while the deeper draining axillary lymph nodes remained practically sterile, some bacilli either passed this barrier or entered the circulation more directly, and the few that reached the lung slowly increased in number.

What now are the host-parasite relationships associated with these observations?

The Response of the Host

The Agar Focus in Normal Rabbits.—

1 day after inoculation of agar and tubercle bacilli into a normal rabbit, there was an extensive and widespread edema and polymorphonuclear infiltration of the

tissues surrounding the agar deposit. The blood vessels in its proximity were dilated and severely congested with a slight infiltration of mononuclears in their immediate vicinity. The agar itself was broken up into fine shreds with fluid and coagulated fibrillar strands between them. The fluid of the exudate penetrated the agar islands, which was indicated by a fine uniformly distributed eosinophilic precipitate. Well preserved polymorphonuclear and red blood cells infiltrated the spaces between the islands (Fig. 1). The carbon particles were uniformly dispersed in the agar islands as well as aggregated in loose clumps. The fragments of agar were smaller and the cellular infiltration more intense at the periphery than in the deeper regions of the agar focus. In the center no cells were present in the spaces between the large agar islands. Short granular tubercle bacilli were occasionally found throughout the agar mass. At times aggregations of polymorphonuclears with ingested bacilli were found.

On the *3rd day*, the accumulation of mononuclears about the blood vessels in proximity to the agar focus was pronounced, with an occasional mitotic figure among them. Sprouting capillaries began to extend into the agar mass from these vessels. The periphery of the focus was a dense fibrin network in the meshes of which the agar was contained. The contraction of the fibrin had apparently caused the fusion of many agar particles, as indicated by the enmeshed leukocytes in the center of some of these. Polymorphonuclears and mononuclears infiltrated the fibrin zone and penetrated along the fibrin threads for a considerable distance into the agar mass. Some of the mononuclears had phagocyted agar, carbon particles, tubercle bacilli or injured polymorphonuclears. Beyond this zone the cellular infiltration and the fibrin deposits were progressively diminished. Here necrotic polymorphonuclears predominated with occasional bacilli within them. The bacilli were also seen singly or in small groups lying free in the agar or adherent to fibrin threads. In general they were definitely rod-shaped with bulbous ends; granular, coccoid and transition forms were also seen. This appearance was associated with a multiplication of the bacilli, as disclosed by culture.

On the *7th day* the capsule surrounding the agar focus had greatly thickened by a massive accumulation of mononuclears intermixed with large numbers of granulocytes. Here and there were zones of macrophages, foreign body giant cells and syncytia engulfing and surrounding agar particles. Fibroblasts and granulation tissue were prominent in some sections of the wall. Tubercle bacilli in large numbers were often present along with agar globules in the macrophages and giant cells. Abutting against the main agar mass the dense fibrin was infiltrated by a vast accumulation of polymorphonuclears, many of which were necrotic. The penetration of the agar by the fibrin and various exudate cells was more widespread and pervasive than in the previous interval. The bacilli were present in particularly large numbers in the polymorphonuclear zone, lying free in the bits of agar between them. They were found, lying free in the agar at a considerable distance from any of the cells, as prominent colonies with rods radiating in all directions from the central mass of microorganisms. Bacilli arranged in packets

of distinct parallel rods, like bundles of cigars, were found both extra- and intracellularly. These, as well as dispersed individual rods, were also found lying free in the agar at a distance from the cells in the fibrin threads and exudate cells between the agar islands. They were deeply acid-fast, long rods with bulbous ends, and, as noted by culture, were actively growing.

2 weeks after inoculation nodules of young epithelioid cells infiltrated with polymorphonuclears and swarming with individual bacilli were found throughout the capsule and down to the very margin of the wall (Fig. 6). About these and about the blood vessels dense accumulation of mononuclears with hyperchromatic nuclei and scanty basophilic cytoplasm with numerous mitotic figures were seen. As noted above, together with these specific changes in response to the tubercle bacilli, there were foci of foreign body giant cells, granulation and fibrous tissue due to the agar in the focus. The zone of polymorphonuclears noted above had undergone complete necrosis. Here the bacilli were present in tremendous numbers forming large skeins and loose actively growing colonies; these were much larger and more numerous, and extended far more deeply in the acellular agar islands than in the previous interval. Vast numbers of discrete individual rods swarmed in the necrotic exudate separating the agar islands (Fig. 2). The colonies and the dispersed bacilli were much less prominent in the central regions of the agar mass, although small colonies and some dispersed bacilli were found throughout the agar focus in the acellular agar islands and especially in the fibrin shreds separating these.

In this series as well as in those to be reported later, the bacilli grew in the agar adjacent to the muscle layer to a much greater extent than in that portion of the agar which lay nearest the skin. This distinctive distribution of the bacilli was invariably associated with a much greater vascularity of the cellular wall in the former region. Therefore, the nonspecific foreign body reaction to the agar was always more pronounced in the wall surrounding the agar beneath the skin, whereas the proliferation of tuberculous tissue was more pronounced in that portion of the capsule which was adjacent to the muscle layer.

5 weeks after inoculation the capsule was a vast mass of epithelioid cells with central foci of caseation. Discrete tubercle bacilli were present in considerable numbers in these tubercles, especially in those undergoing caseation. Nests of new mononuclear cell formation with mitotic figures were still present. These specific changes were interrupted here and there by foreign body giant cells, granulation tissue and more mature connective tissue. Numerous macrophages, some aggregated into nodules and containing vast numbers of individual bacilli, apparently in active growth, as judged by their morphology, occurred at the very periphery of the capsule. The center of the lesion was undergoing softening, and individual well stained microorganisms were present in great numbers. The pleomorphism of the microorganism accorded with the continued multiplication of the bacilli as observed culturally.

The Agar Focus in Vaccinated Rabbits.—

In contrast with the changes noted in normal animals, rabbits vaccinated with

BCG reacted to the inoculation of virulent tubercle bacilli in agar by a greatly increased intensity of the vascular reaction. A more widespread edema resulted, and a more intense cellular infiltration of the tissues about the agar focus, in which mononuclears were prominently present on the *1st day* after inoculation. The agar was broken up into larger islands, the fibrin threads between them were coarser and more pronounced, and the cellular infiltration, including the mononuclears, have penetrated more deeply into the agar mass. Many polymorphonuclears already showed loss of granules and pyknosis of the nuclei. The carbon particles were aggregated in denser masses and the remaining agar coagulum was as a rule free from dispersed particles. The bacilli were often obscured by the carbon masses; otherwise, they were found in the same condition as in the normal animal.

On the *3rd day* the differences between the normal and vaccinated animals were of the same character. The endothelial cells of the blood vessels were swollen and the mononuclear cells had accumulated in the areolar tissue, as well as about the blood vessels, their cytoplasm was more developed, their nuclei were more vesicular and mitotic figures were more frequently found than in the normal animal. Active migration of the mononuclears toward the center of the agar mass was apparent at the periphery of the lesion. Tubercle bacilli were found, but with difficulty, throughout the lesion.

1 week after inoculation in the vaccinated animal the vascular congestion had subsided. Granulation tissue, agar-containing macrophages, and fibroblasts were more prominent than in the normal animal. Nodular collections of epithelioid cells had already appeared in the capsule, whereas the active multiplication of mononuclears was less intense than in the normal animal. Polymorphonuclears were much less prominent in the entire lesion. There was no definite zone of polymorphonuclear infiltration at the point of junction between the main agar mass and the capsule. The destruction of the polymorphonuclears had advanced much further than in the normal animal. Deep within the agar mass the fibrin bands between the agar islands were more prominent than the agar itself, so extensive was the deposition of fibrin between them. This was the height of the multiplication of the bacilli, such as it was, in the vaccinated animal. Yet the bacilli were difficult to find. They were rarely seen as isolated microorganisms in the granulation tissue, extremely rarely in the dense network of fibrin abutting against the main agar mass or free in the agar (Fig. 4). Here they could be found as isolated microorganisms or as occasional minute dense clumps without any radiations, in which the individual bacilli could not be distinguished. The bacilli were short, often coccoid, and at times had the appearance of spores, as suggested by their elliptical shape with a centrally located unstained portion in a thin shell of acid-fast material. Dark staining polar bodies were seen in some of the rods (Fig. 4). It was evident both from the cytological appearance and the more definite cultural results that while the bacilli multiplied to some extent in the vaccinated animal they were definitely inhibited in their growth, both when situated in the cells and when free in the agar, as compared with the normal animal.

2 weeks after inoculation the difference between the normal and vaccinated animals was most pronounced. Whereas in the normal rabbit the proliferation of tuberculous tissue and the massive multiplication of the bacilli both intracellularly and free in the agar were conspicuous, in the vaccinated animal the foreign body reaction to the agar with the formation of foreign body giant cells and fibrous tissue was most prominent (Fig. 7). Associated with this there was not only no further multiplication of the bacilli in the vaccinated animal, but a marked reduction in their numbers had taken place. Whatever bacilli had lodged in the capsule had been destroyed by the epithelioid cells. In the latter they were found very rarely and only in immediate proximity to the main agar mass. They were not found in the peripheral regions of the capsule. Faintly acid-fast, beaded forms were occasionally seen free in the agar and at times, together with acid-fast globules, in the epithelioid and Langhans giant cells.

The same differences persisted *5 weeks* after inoculation between these two groups of animals. Caseation was very extensive in the normal animal. It was but slight or absent in the vaccinated. The bacilli were still multiplying within macrophages at the periphery of the capsule and in the central softened agar mass in the normal animal, whereas only rare isolated beaded forms persisted within the cells and in the thoroughly fibrin-permeated necrotic agar mass of the vaccinated rabbit.

The Draining Lymph Nodes.—

Except for some congestion of the capillaries in the superficial axillary nodes draining the agar focus in the normal animal the glands were entirely normal 1 day after inoculation. Both the marginal and intermediate sinuses were free of extraneous cells (Fig. 8). On the 3rd day large numbers of polymorphonuclears undergoing pyknosis and karyolysis were found in these sinuses and small numbers of tubercle bacilli were isolated from the node. 4 days later it yielded on culture 1,850 colonies, and large mononuclears with highly vesicular nuclei and reticulated cytoplasm, both isolated and in groups, some containing as many as 5 separate rod-shaped or coccoid forms of tubercle bacilli, were present in the cortex of the node. Agar globules were found in these cells, as well as in macrophages lying free in the peripheral sinuses. On the 2nd week after inoculation the multiplication of the bacilli had reached its height and young epithelioid cell tubercles, many containing long bacilli with bulbous ends, were found dispersed throughout the node, with frequent mitotic figures. There had been no significant change in the bacillary numbers in this node, 3 weeks later, when 31,900 colonies were cultured from it, and widespread epithelioid tubercles with rare giant cells and occasional caseous foci were found. Sparsely scattered bacilli were seen throughout.

In the B C G vaccinated rabbit in contrast, 1 day after inoculation the marginal sinus of the superficial axillary node was distended with fluid and contained large numbers of polymorphonuclears. These cells extended into the intermediate sinuses along the septa in the direction of the lymph flow and the medullary sinuses were filled with them. Macrophages with phagocytosed red blood cells and agar globules were also seen here (Fig. 9). In association with this evidence of the overflow of material from the agar focus into the lymph nodes, 30 colonies were

cultured. On the 3rd day the marginal sinus was practically free of polymorphonuclears, but numerous degenerating polymorphonuclears were found lying free or within swollen reticular cells in the cortex of the node. Small syncytia about agar globules were found in the medullary sinuses. Again 36 colonies were isolated. The bacilli, instead of multiplying unhindered as in the normal animal, had practically disappeared for the time, and 1 week after infection nodular collections of hypertrophied macrophages, some of which had assumed epithelioid forms, and some of which contained large agar globules, were found in the cortex of the node. However, this almost complete suppression of the invading bacilli in the lymph node of the vaccinated animal was not constant, and on the 14th day they were slowly increasing, associated with mature epithelioid cell formation and minute foci of caseation. In the 5th week this retarded growth of the bacilli continued associated with a liquefaction of the tuberculous foci.

The Lung.—

Bacilli reached the lung of the normal rabbit in the 1st week, while they were first cultured with regularity from the lung of the vaccinated rabbit in the 2nd week after inoculation, and then in significantly smaller numbers. In both instances the response in the lung was, from the very beginning, a combined interstitial and pneumonic reaction, as distinguished from the interstitial lesion that characterizes the initial response to a first infection by intravenous inoculation. In this connection the fact should be recalled that exudation into the alveoli of a normal animal is synchronous with the development of sensitivity to tuberculin and caseation as previously pointed out (7). In the present study the bacilli first reached the lung in significant numbers at a time when caseation had already started in the agar focus even in the normal animal. From then on, numerous conglomerate, extensively caseous, predominantly pneumonic lesions developed throughout the lung of the normal rabbit, with large numbers of microorganisms within the caseous foci and the epithelioid and giant cells within the alveoli. In the vaccinated animal, on the other hand, there developed rare, isolated lesions of the same nature, but with the distinction that the bacilli were present in very small numbers even in the intraalveolar epithelioid cells and the minute caseous foci. The latter observation was corroborated by the tremendous difference in the number of bacilli cultured from these lungs.

It was stated above that tubercle bacilli multiply in the acellular agar islands of the normal animal and that the fluid of the exudate penetrates these islands. To make certain that the growth in these foci was due to the fluid contained in the agar, experiments were performed of which the following will serve as examples.

An agar saline suspension containing 3,430 viable tubercle bacilli in 10 mg. of material was incubated at 37°C. simultaneously with a sample of agar mixed with normal rabbit plasma and containing 5,120 organisms. At the end of 11 days the

agar saline suspension was sterile, whereas the agar plasma suspension contained 60,000 colonies.

A suspension of tubercle bacilli in agar, 10 mg. of which yielded on culture 2,200 organisms, was placed within a Chamberland L3 filter. The open end was effectively sealed, and the filter was buried between the muscles. At the end of 14 days the contents of the filter were sterile.

It is plain that in the absence of plasma or body fluids tubercle bacilli incorporated in agar die in 11 to 14 days both *in vivo* and *in vitro*.

In summary, the mechanism of immunity as revealed by this series of observations was as follows:

The inflammatory reaction in the vaccinated animal is more intense, which leads to a greater outpouring of cells and fluid into and about the agar focus containing the bacilli of reinfection. This increased accumulation of cells and fluid operates to sweep the bacilli into the draining lymph node more rapidly than in the normal animal, if the dose on reinfection is large (Figs. 8 and 9). On the other hand there are factors which tend mechanically to fix the bacilli to a greater degree at the portal of entry in the vaccinated than in the normal animals. There is the larger and denser barrier of fibrin about the agar focus as a whole, and the individual agar islands of the vaccinated animals (Figs. 2 and 4). The greater agglutination of particulate matter, as shown by the carbon particles in the agar focus of the vaccinated, would also tend to immobilize the tubercle bacilli. However, it would seem that these factors are less important than those which tend to inhibit the multiplication and enhance the destruction of the bacilli in the vaccinated animal. For it has been clearly demonstrated that an extracellular factor is present which inhibits the multiplication of the bacilli in the agar islands away from the cells in the vaccinated animal, whereas in the normal creature the multiplication of the bacilli in these regions is unhindered (Figs. 2 and 4). Furthermore there is a greater and more rapid mobilization of the mononuclears which engulf and destroy the tubercle bacilli in the vaccinated animal. This destruction is indicated by the more rapid formation of epithelioid and giant cell tubercles containing little if any bacilli, and is associated with the suppression of their growth as determined by culture. Thus both the extra- and intracellular factors operate to inhibit the growth

and destroy the bacilli of reinfection. On the other hand in the normal animal the impotent polymorphonuclears persist and the mononuclears arrive later. Their capacity to destroy tubercle bacilli is markedly less than after vaccination, as indicated by the accumulation of bacilli within their cytoplasm, the more tardy formation of mature epithelioid tubercles and the vastly greater numbers of bacilli cultured. It is evident that in the normal animal both the extra- and intracellular factors operate to enhance the growth and disseminate the bacilli.

The proliferation of tuberculous tissue, its caseation and the massive multiplication of the bacilli, both intracellularly and free in the agar, are most prominent in the normal animal, whereas the reaction to the agar in the focus with the formation of foreign body giant cells and fibrous tissue is the most conspicuous features of the agar capsule in the vaccinated animal (Fig. 7).

It is the enhanced capacity of the mononuclear phagocytes of the B C G vaccinated rabbit to destroy tubercle bacilli that is the determining factor in the dissemination of the disease to the draining lymph nodes and internal organs. For when the bacilli reach these structures, even if they penetrate them more rapidly than in the normal animal, the phagocytes soon destroy them, as indicated by the more rapid epithelioid cell formation associated with suppression of their growth, as demonstrated by culture.

The Host-Parasite Relationships in Normal and Tuberculous Rabbits

It is generally held that a greater immunity to reinfection to tuberculosis is afforded by a virulent primary infection than by one of low virulence. With the hope of throwing further light on the mechanism of immunity the experiments described above were repeated on a series of animals primarily infected with virulent tubercle bacilli and reinfected as previously with melted agar containing virulent bacilli.

The Fate of the Bacilli.—

In order to produce a slowly progressive tuberculosis with minimal involvement of the lymphatic system a group of rabbits were vaccinated by an intravenous injection of 1.0 mg. of living B C G. 60 days later they received 0.0001 mg. of the Ravenel strain of bovine type tubercle bacilli by the same route. 97 days after the last injection these tuberculous rabbits, together with a group of normal animals, received subcutaneously in the right foreleg 5 cc. of an agar-bovine

tubercle bacilli suspension containing 0.1 per cent trypan blue. An identical mixture without the bacilli was injected in the opposite foreleg.

In Table III is recorded the fate of the bacilli and the trypan blue in these two groups of rabbits. The colonies cultured from the lungs and spleen are omitted, for bacilli from the primary infection remained in these organs. In the lymph nodes, however, no bacilli remained from the primary infection, as was evident from the regularity of the rise and fall of the number of bacilli cultured, from the microscopic studies of these nodes and finally from the sterility of the lymph nodes not draining the agar focus. There was no significant difference in the number of bacilli cultured from the agar focus of the normal and tuberculous rabbits 1 day after inoculation of an agar suspension containing 10,900 tubercle bacilli in 10 mg. of material. As with B C G vaccinated rabbits there was practically complete suppression of multiplication of the bacilli of reinfection in the agar focus of the tuberculous rabbits, as distinguished from the massive growth of the microorganism in the normal animals. Again, as was noted in that series, the axillary lymph nodes draining the agar focus of the reinfected animal were already invaded by the bacilli on the 1st day after reinfection, while the lymph nodes draining the agar focus of the normal animal were still sterile at this time. There was however one important difference. While in the B C G vaccinated rabbits the bacilli still slowly and continuously increased in numbers up to the 5th week, although definitely retarded in their multiplication in the superficial draining lymph nodes; in rabbits having a virulent primary infection, on the other hand, the bacilli that invaded these nodes experienced only an ephemeral increase, which was soon suppressed. An identical result was obtained in another series of rabbits treated intravenously with large amounts of a heated suspension of Ravenel bacilli containing but a small number of living organisms and similarly reinfected later with an amount of bacilli similar to that of this series. In all instances the microorganisms that invaded the deeper nodes were destroyed practically completely.

It will be noted that the amount of trypan blue retained both in the agar focus and the draining lymph nodes was uniformly greater in the tuberculous than in the normal rabbit. This greater retention of dye at the site of reinfection was noted for both trypan blue and India ink in the BCG series described above, and was also observed, and to a greater extent, in actively tuberculous rabbits reinfected with melted agar containing India ink and tubercle bacilli. Furthermore the presence of bacilli in the mixture was not essential for this phenomenon, for it occurred, though perhaps to a less marked degree, when bacilli were not present in the injected agar.

An indication as to the mechanism of the more rapid dissemination of bacilli to the draining lymph nodes in the sensitized animal was apparent from the condition of the efferent lymphatic of the deep

TABLE III

The Fate of Virulent Bovine Tubercle Bacilli and Trypan Blue in a Localized Agar Focus and Their Dissemination to the Draining Lymph Nodes in Normal and Tuberculous Rabbits (Series 3)

Agar suspension	Interval after inoculation	Rabbit No.		Agar focus				Superficial axillary node				Deep axillary node			
				Normal		Tuberculous		Normal		Tuberculous		Normal		Tuberculous	
		Normal	Tuber-culous	Colonies	Trypan blue	Colonies	Trypan blue	Colonies	Trypan blue	Colonies	Trypan blue	Colonies	Trypan blue	Colonies	Trypan blue
10,900	1 day	6	14-0	10,000	+	12,000	+	0	±	0	+	0	±	16	+±*
	3 days	7	14-1	76,400	+	1,800	++	39	±	164†	+	180	±	46	+
	1 wk.	8	14-2	69,600	+	23,900	+++	5,742	±	3,478	++	2,654	±	5	±
	2 wks.	9	14-3	260,000	±	14,200	+	9,300	+	242	+++	8,400	±	5	+++
	4 wks.	10	14-4	120,000	±	1,260	—	16,600	0	222	+	—	0	8	±

The intensity of coloration of the agar focus and draining lymph nodes is graded as follows: 0, no blue; ±, faintly blue; +, pale blue; ++, moderately blue; and +++, deep blue.

* Lymph trunk between deep axillary node and axillary vein distended to 3 mm. in diameter and faintly blue.

† Inguinal nodes 0.

axillary node. This vessel was greatly distended and was colored with trypan blue in the tuberculous animal 1 day after reinfection. A similar observation is recorded below in another group of tuberculous animals. This evidence of increased lymph flow from the site of inoculation was not encountered in normal animals.

In Table IV are recorded the results of a similar experiment with the difference that the reinfecting dose was about ten times less than that in the preceding series.

All the tuberculous rabbits in this series except rabbit 9-6 received 1.0 mg. B C G intravenously, followed 99 days later by 0.001 mg. of the Ravenel strain by

TABLE IV

The Fate of Virulent Bovine Tubercle Bacilli in a Localized Agar Focus and Their Dissemination in the Body in Normal and Tuberculous Rabbits (Series 4)

Agar suspension	Interval after inoculation	Rabbit No.		Agar focus		Superficial axillary node		Deep axillary node		Popliteal node	
		Normal	Tuberculous	Normal	Tuberculous	Normal	Tuberculous	Normal	Tuberculous	Normal	Tuberculous
1,070	1 day	21-2	9-6	4,200	700	0	10,216*	0	0†	—	0
	1 wk.	21-1	F-1	3,000	100	47	0	80	‡	—	0
	2 wks.	21-5	F-2	265,000	2,400	1,520	0	760	1	—	0
	4 wks.	21-4	G-1	20,000	230	12,500	0	1,400	1	—	0

* Signs of acute inflammation; no tuberculous changes.

† The lymph trunk between the deep axillary node and the axillary vein is distended and faintly blue.

‡ 106 colonies were cultured from this node; there was a large regressive tubercle undergoing organization; obviously due to the primary infection.

the same route. 34 days after the last injection, these rabbits, together with a group of normal animals, received a mixture of agar, bovine tubercle bacilli and trypan blue subcutaneously over the right chest wall. Rabbit 9-6 received its subcutaneous reinfection 72 days after an intravenous inoculation of 0.001 mg. of the Ravenel bacilli.

It will be noted that the control popliteal lymph nodes were sterile in each of the reinfected tuberculous animals. Again the axillary nodes draining the agar focus of the normal animal were sterile 1 day after inoculation, but large numbers of tubercle bacilli were isolated from the superficial axillary nodes draining the agar focus of the

reinfected animal. These nodes were definitely stained with trypan blue and the efferent lymphatic from the deeper node was slightly distended and contained faintly bluish lymph. There were no microscopic tuberculous changes in this node but there was a great accumulation of polymorphonuclears and red cells in the sinuses. All this makes it certain that the bacilli were not residual bacilli from the primary infection. After this period the draining lymph nodes in the reinfected animals were practically sterile, while bacilli were present in increasingly large numbers in the normal animal. Identical observations were made in other tuberculous rabbits likewise reinfected with small doses. If the dose was small enough, so that the agar inoculum contained less than 500 bacilli per 10 mg. of substance, they never reached the draining lymph nodes of the reinfected rabbit. In the agar focus of these rabbits only small numbers of tubercle bacilli persisted after 4 weeks.

These results again emphasize the greater immunity afforded by a virulent primary infection as compared with that conferred by a primary infection of low virulence. For even with a small reinfecting dose the bacilli were still increasing slowly in the superficial axillary nodes of BCG vaccinated rabbits at the end of 4 weeks, whereas in tuberculous rabbits the bacilli of reinfection were completely destroyed in the draining lymph nodes at the end of the 1st week.

The Reactions of the Host.—

What is the host expression of this greater immunity of rabbits affected by a virulent tuberculosis as compared with that of animals vaccinated with BCG? The difference is quantitative rather than qualitative. There was no conspicuous difference in the intensity of the initial inflammation to reinfection in these two groups. In fact in one series of reinfected rabbits, treated intravenously with large amounts of heated bacilli containing a few living virulent microorganisms, the initial inflammation on reinfection was actually less than that of the normal animal. However in the other two series reported here in detail the intensity of inflammation was much greater in the tuberculous animals than in the normal controls. This was associated with evidence of an increased lymph flow from the infected agar focus, which swept the bacilli out more rapidly so that they

reached the draining lymph nodes before those did in the normal animals. It is noteworthy that in the series in which the inflammation of reinfection was not intensified the dissemination of bacilli to the draining lymph nodes was not accelerated, nor did these nodes show any evidence of overflow of leukocytes into the nodes from the agar focus, which was so conspicuous in the other series.

As against this the factors that act mechanically to fix bacilli *in situ* were more pronounced in the tuberculous than in the B C G vaccinated animals. The carbon was agglutinated in denser masses (Figs. 12 and 13). The phagocytosis of trypan blue by the macrophages that surrounded the agar focus was more intense, and the individual globules of dye were much coarser than those in the B C G vaccinated rabbits. Likewise the agglutination of tubercle bacilli was more pronounced in this group and was particularly evident in the series of rabbits treated with a mixture of killed and a few living virulent tubercle bacilli which, incidentally, showed specific agglutinins in a 1:64 dilution of their serum before reinfection. The fibrin barrier about the agar focus, while much more pronounced than in normal animals, was not consistently more marked in the tuberculous than in the B C G series.

The same humoral inhibitory influence was apparent on the growth of the bacilli in the acellular agar as had been noted in the B C G series (Figs. 3 and 5). The mobilization of mononuclears was more rapid and their physiological development was more accelerated than in the B C G series. Also the necrosis and rapid lessening in number of the polymorphonuclears was conspicuous in the reinfected rabbits. The increased capacity of the phagocytes to destroy tubercle bacilli was clearly more in evidence in the tuberculous animals, and epithelioid cell formation was much further advanced at the end of the 1st week. There resulted from all these circumstances a more pronounced foreign body and fibrous tissue reaction in the capsule surrounding the agar focus of the tuberculous rabbits. Similar observations were made in the lymph nodes. Large masses of agar enclosed within syncytia could be found in the practically sterile nodes of the reinfected tuberculous animals (Fig. 11), whereas in the normal animals these syncytia contained large numbers of tubercle bacilli both microscopically and on culture (Fig. 10).

DISCUSSION

By introducing agar impregnated with tubercle bacilli in the tissues of an animal conditions are produced which afford an excellent opportunity for studying extracellular effects on the growth of the parasite. For the body fluids penetrate the agar masses, whereas the cells invade the solid agar islands slowly. By this means it was shown that in the normal animal the bacilli grew unhindered in the acellular agar at a great distance from the cells (Figs. 2 and 3). In the vaccinated or tuberculous animal on the other hand a marked inhibition of their growth was evident (Figs. 4 and 5). If the body fluids are prevented from entering the agar mass, as was accomplished by burying Chamberland L3 filters filled with agar and tubercle bacilli between the muscles, the bacilli die completely in 14 days. Tubercle bacilli impregnated in saline agar mixtures such as were used for inoculation, and left at incubator temperature die in 11 days. Since even in the vaccinated animals tubercle bacilli were cultured from the agar it is evident that their persistence was due to the fluids that penetrated the agar. It would appear, therefore, that in a normal animal the fluids penetrating the agar focus support the growth of tubercle bacilli, whereas the fluid from a vaccinated or tuberculous animal is so altered that it permits slight or no multiplication of the bacilli.

The fibrin barrier thrown about the agar focus in the immunized animal is more pronounced than that about the focus of the normal animal (Figs. 2 and 4). It is conceivable therefore that the differences just noted may be attributable in part to a greater hindrance to the penetration of nutritive substances into the agar islands of the immunized animal. However, in the normal animal at the height of the multiplication of the bacilli, there is a vast necrotic zone impregnated with fibrin separating the main agar mass from the capsule; yet the interposition of this barrier does not hinder the massive multiplication of the bacilli in the acellular agar, while in the immunized rabbit the bacilli fail to multiply in immediate juxtaposition to the living macrophages surrounding and infiltrating the main agar mass. It would seem therefore that the inhibitory influence of the fluid penetrating the agar of the vaccinated animal must be attributed to an inherent difference in its constitution. This demonstrates a rôle of humoral bacteriostatic substance in immunity to tuberculosis. That the observa-

tion has significance under other conditions than those afforded by the introduction of agar would seem indicated by the studies of Woodruff (8) on omental spreads of guinea pigs. He noted freely growing bacilli immediately adjacent to or some little distance from the cells of normal animals, a picture absent in reinfected animals.

The agar focus demonstrated in striking fashion extracellular factors which would tend to immobilize and limit the spread of the surviving bacilli in the immunized animal. Carbon particles and trypan blue were retained more effectively at the site of inoculation in the reinfected than in the normal animal. The retention of carbon at the site of introduction was obviously due to the clumping of these into dense large masses whose mobility in the tissues was obviously retarded (Fig. 13). In the normal animal this clumping was much less in evidence (Fig. 12).

Pagel (9) noted a greater retention of carbon at the site of reinfection than at the site of primary injection of mixtures of tubercle bacilli and carbon into guinea pigs. The phagocytosed trypan blue particles of our experiments were much coarser in the immunized than in the normal animal. Somewhat parallel observations were made with tubercle bacilli growing in the acellular agar. In the normal animal they accumulated as freely dispersed rods or as large loose colonies (Figs. 2 and 3). In the immunized animal they either persisted as isolated inactive individuals (Fig. 4) with structures often suggesting spore forms or, if colonies did form, they were minute and very dense so that the individual rods could not be distinguished (Fig. 5). This *in vivo* agglutination, as well as the denser and broader fibrin barrier thrown about the individual agar islands and about the agar mass as a whole, tended to immobilize the bacilli. *In vivo* agglutination of bacteria in the immunized animal has been noted by Rich with pneumococci (10) by Cannon and Pacheco with staphylococci (11) and by Woodruff with tubercle bacilli (8).

The various extracellular factors are effective in confining the bacilli at the site of reinfection when small doses are introduced. This was found to be true in tuberculous rabbits reinfected with small doses. However with larger doses and in association with a greatly intensified inflammation of reinfection all these factors tending to immobilize the bacilli are overcome by the greatly increased flow of lymph, which

sweeps the polymorphonuclear leukocytes and agar particles, some of which contain bacilli, more rapidly into the draining nodes than in a normal animal (Figs. 8 and 9).

Field, Drinker and White (12) found that a sterile inflammation produced by the application of heat caused a markedly increased flow of lymph. Hudack and McMaster (13) noted that in the human skin recently inflamed by heat, by ultraviolet light or by bacterial vaccine or toxin the lymphatic capillaries become far more permeable to vital dyes than in the normal skin. Menkin (14) found that the fixation of trypan blue at the site of inflammation depends upon the character of the irritant. The powerful necrotizing agent of *S. aureus* produces rapid fixation. Mild irritants on the other hand produce only delayed fixation. It is obvious that the inflammation caused by the tubercle bacillus of reinfection in the rabbit belongs in the latter category.

Despite the lack of immediate fixation in the vaccinated animal immunity is none the less effective. For the other and perhaps more important agent in immunity to tuberculosis, the increased capacity of the mononuclear phagocytes to destroy tubercle bacilli, asserts itself at the site of reinfection as well as in the draining lymph nodes. The polymorphonuclear leukocytes which are unable to destroy tubercle bacilli soon die and are replaced by the rapidly mobilized mononuclear phagocytes in the immune rabbit, in whose interior the bacilli are rapidly destroyed with accelerated formation of epithelioid tubercles (Fig. 7). In the normal animal the impotent polymorphonuclears persist and the mononuclears are tardy in their appearance. Moreover their capacity for destroying tubercle bacilli is slight, so that the microorganisms at first accumulate in large numbers within their cytoplasm (Figs. 6 and 10).

These observations permit a definite view on the question of the rôle of allergy in tuberculosis. With small doses on reinfection, such as those from exogenous sources in man under natural conditions, the immunity acquired from the primary lesion, which accompanies the allergy under these conditions, brings about the fixation of the bacilli at the portal of entry and the inhibition of their multiplication as described above. With larger doses however, such as can be derived only from endogenous spread, the heightened inflamma-

tion of reinfection will bring about a more rapid dissemination of the bacilli, if man reacts as a rabbit does; but even in such case as soon as the invading bacilli reach a new territory the forces of immunity, both humoral and cellular, tend to fix and destroy them.

It has been demonstrated in the course of the present work that vaccination of rabbits with BCG affords a pronounced protection against a virulent infection. This is not surprising in view of the fact that the BCG produces a disease in no way different fundamentally, both from the standpoint of the host and that of the parasite, from that of a virulent tuberculosis, with the all important exception that the lesions regress very rapidly and soon disappear completely, while the bacilli of the vaccination disappear with the exception of an occasional lingering microorganism in the lymph nodes (7). Many experimental and clinical studies point in the same direction.

In accord with our previous studies (4) the bacilli of reinfection are more effectively destroyed by an animal with residual lesions from a primary infection than by one without lesions. It is important to emphasize these observations, for recently Selter and his coworkers (15) in an attempt to repeat our experiments, came to the conclusion that a progressive primary tuberculosis exercises no greater inhibitory effect on the bacilli of reinfection, than a slight nonprogressive primary infection. An analysis of their experiments reveals that the guinea pigs which they used for the reinfection experiment had received 1 month previously a dose and strain of tubercle bacilli which killed them within 2 to 3 months with generalized tuberculosis. Only 2 to 5 days after reinfection these pigs showed macroscopic tubercles in the lung, liver and spleen. These lesions Selter himself attributed to the primary infection. Under these conditions they found no significant difference in the number of bacilli cultured from the reinfected and the control animals. It is obvious that some, at least, of the bacilli cultured from these reinfected animals were most likely from the primary infection and not the reinfection, and, while these authors state that it is desirable to determine the number of bacilli remaining in the organs from the primary infection, this consideration did not deter them from drawing a conclusion that seems questionable.

The greater immunity afforded by a virulent primary infection expressed itself as a quantitative increase of those factors which operate

in the mechanism of resistance of animals immunized with a less virulent infection.

CONCLUSIONS

1. There is an extracellular factor which inhibits the growth of tubercle bacilli in immunized rabbits.

2. Extracellular factors localize carbon particles, trypan blue and tubercle bacilli at the site of introduction to a greater extent in the immunized than in the normal animal.

3. This greater fixation is brought about by an increase in the density and extent of the fibrin barrier formed about the focus of the immunized animal. The more pronounced *in vivo* agglutination of tubercle bacilli and carbon particles in the vaccinated or tuberculous rabbit also tends to immobilize them in the tissues.

4. The growth inhibitory and localizing agents are effective in the fixation of small doses on reinfection at the portal of entry.

5. With large doses on reinfection, the increased lymph flow resulting from the intensified inflammation in the immunized animal brings about a more rapid dissemination of the bacilli to the draining lymph nodes than in the normal animal.

6. The most significant factor in immunity is the increased capacity of the rapidly mobilized mononuclear phagocytes to destroy tubercle bacilli. The impotent polymorphonuclear leukocytes quickly disappear from the site of reinfection.

7. The invading bacilli that reach the draining lymph nodes of the immunized animal are retarded in multiplication or destroyed by these phagocytes.

8. Vaccination of rabbits with BCG brings into play the factors tending to immobilize the bacilli of reinfection, inhibit their growth and destroy them with a resulting significant immunity.

9. A virulent primary infection affords a greater immunity than one of low virulence and the host reactions are expressed by a quantitative increase in those immunity factors which operate in a vaccinated animal.

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EXPLANATION OF PLATES

All sections were prepared from tissues stained either with hematoxylin-eosin or by the Ziehl-Neelsen method, and counterstained with hematoxylin. The magnifications given are approximate.

PLATE 52

FIG. 1. The agar focus of normal rabbit 3 of B C G series 2, 1 day after inoculation; 9,000 colonies were isolated. The agar is broken up into small particles with coagulated fibrillar strands between them. Polymorphonuclears infiltrate these spaces. × 100.

FIG. 2. The agar focus of normal rabbit 5 of B C G series 2, 2 weeks after inoculation; 492,000 colonies were isolated. Large, loose actively growing colonies are present in the acellular agar. Vast numbers of long discrete rods are swarming in the necrotic exudate separating the agar islands. × 200.

FIG. 3. The agar focus of normal rabbit 9 of tuberculous series 3, 2 weeks after inoculation; 260,000 colonies were isolated. Loose aggregates of individual tubercle bacilli are present in the agar at a great distance from cells of any kind. × 200.

FIG. 4. The agar focus of vaccinated rabbit 12-2 of B C G series 2, 2 weeks after inoculation of the same suspension of bacilli as in rabbit 5 of Fig. 2; 3,000 colonies were isolated. The agar islands are separated by a very prominent dense fibrinous exudate. No tubercle bacilli are found in the agar. In the center of the figure, as indicated by the arrow, a tubercle bacillus with a polar body can be seen in the fibrinous exudate. × 200.

FIG. 5. The agar focus of tuberculous rabbit 14-3 of tuberculous series 3, 2 weeks after reinfection with the same suspension of bacilli as in rabbit 9 of Fig. 3; 14,200 colonies were isolated. A minute dense clump of short bacilli in the acellular agar is indicated by the arrow. × 200.

PLATE 53

FIG. 6. The capsule surrounding the agar focus of normal rabbit 5 shown in Fig. 2; 492,000 colonies were isolated. A nodule of young epithelioid cells containing numerous tubercle bacilli surrounded by actively multiplying mononuclears. × 200.

FIG. 7. The capsule surrounding the agar focus shown in Fig. 4 of the BCG vaccinated rabbit 12-2; 3,000 colonies were isolated. Foreign body giant cells and mature epithelioid cells are prominent. × 200.

FIG. 8. The superficial axillary lymph node draining the agar focus of normal rabbit 3 of B C G series 2, 1 day after inoculation; no tubercle bacilli were obtained on culture. The lymph node is normal and in a resting state. The intermediary sinuses are free of infiltrating cells. × 200.

FIG. 9. The superficial axillary lymph node draining the agar focus of vaccinated rabbit 13-9 of B C G series 2, 1 day after inoculation; 30 colonies were isolated. The intermediary sinuses are engorged with fluid and polymorphonuclear leukocytes; macrophages and red blood cells are to be seen. × 200.

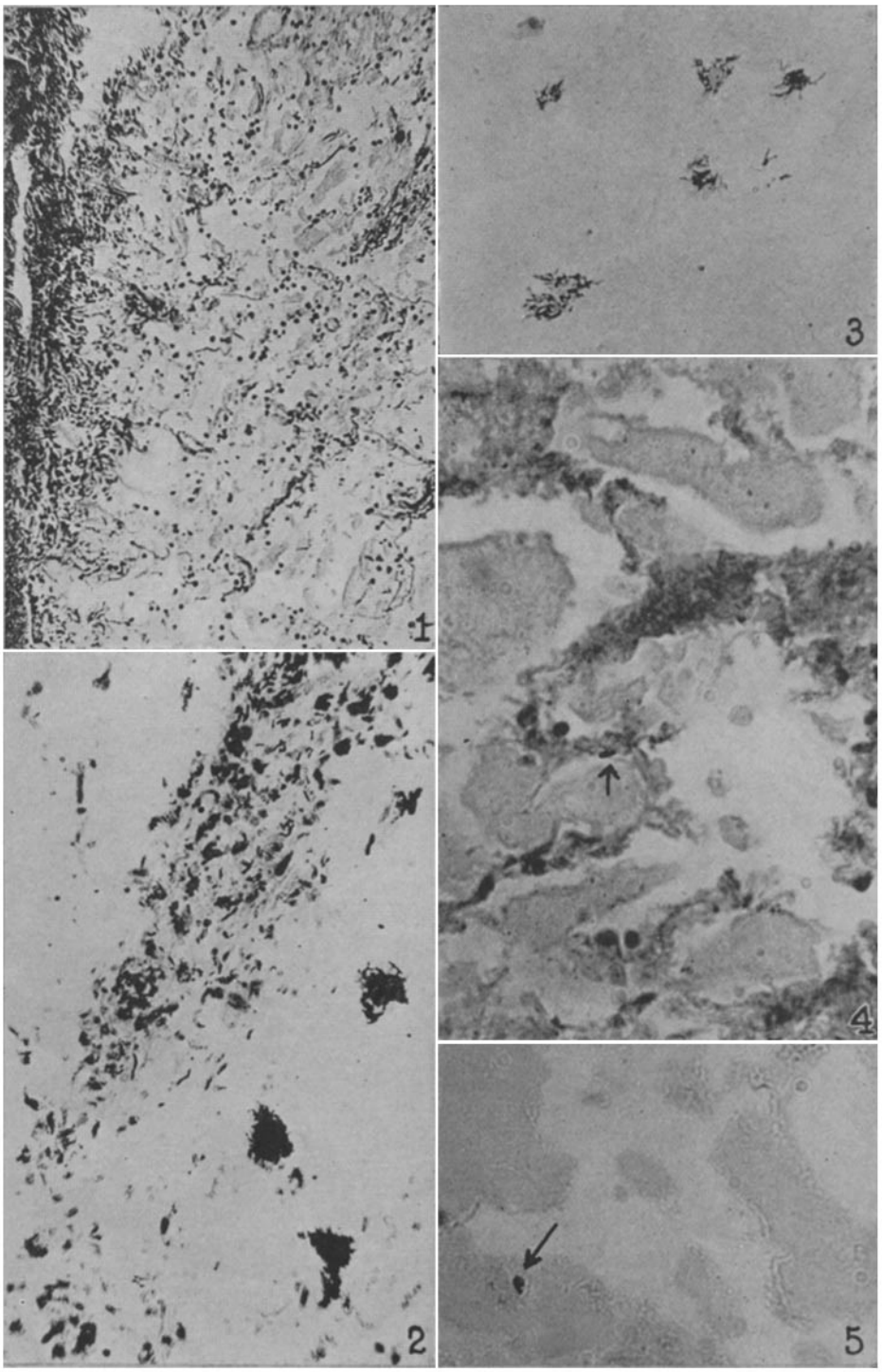
PLATE 54

FIG. 10. The deep axillary node draining the agar focus of normal rabbit 9 of tuberculous series 3, 2 weeks after inoculation; 8,400 colonies were isolated. Large giant cells enclosing agar globules, and young epithelioid cells undergoing the first stage of caseation with numerous tubercle bacilli. × 200.

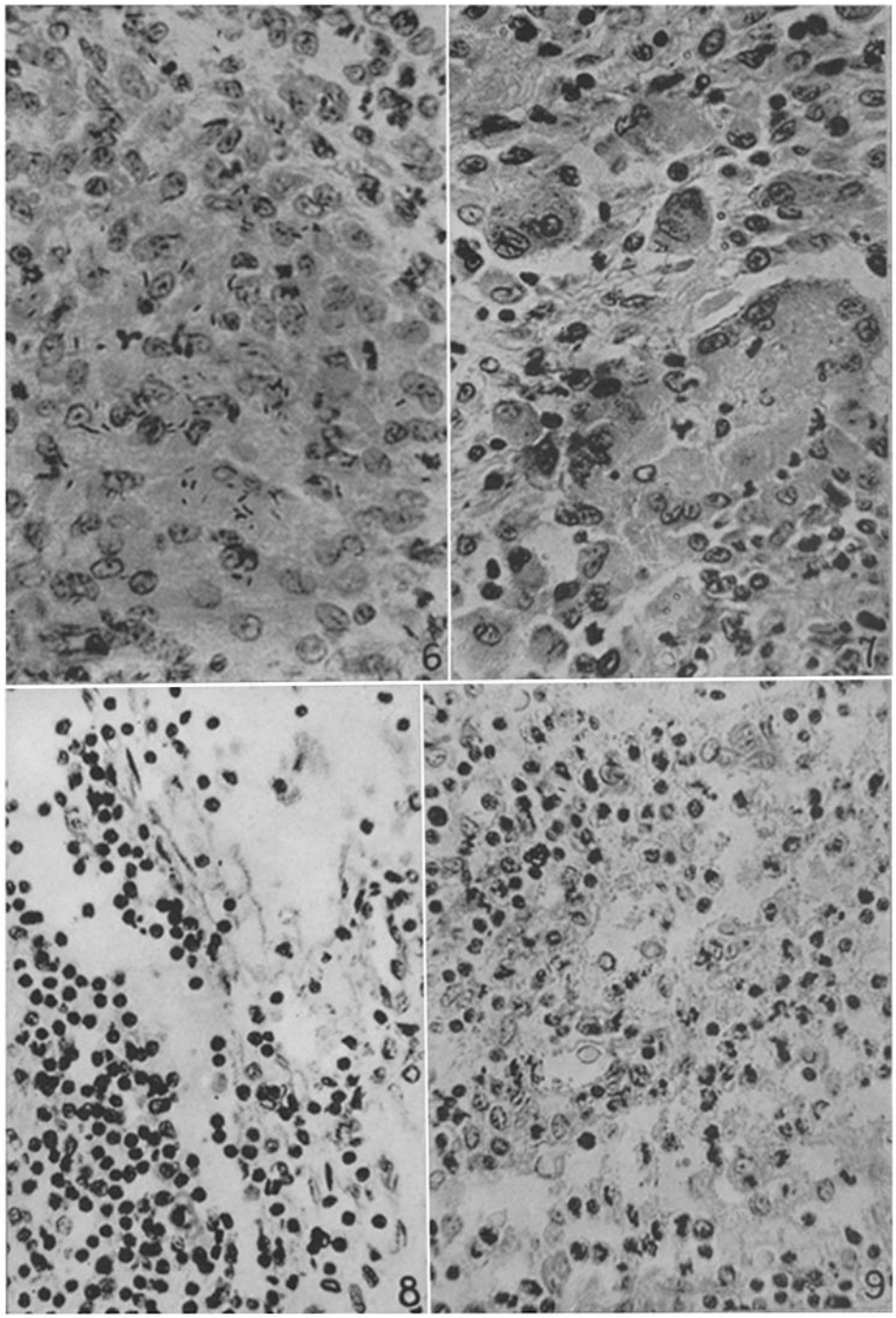
FIG. 11. The deep axillary node draining the agar focus of tuberculous rabbit 14-2 of tuberculous series 3, 1 week after reinfection with the same suspension of bacilli as in rabbit 9 of Fig. 10; 5 colonies were isolated. A syncytium surrounding a large agar mass. No tubercle bacilli could be found. × 200.

FIG. 12. The agar focus of normal rabbit Ar, 13 days after inoculation of an agar, India ink and tubercle bacilli suspension; 155,400 colonies were isolated. The bacilli and carbon particles are scattered in the acellular agar. The agglutinated masses of carbon are slight. × 200.

FIG. 13. The agar focus of tuberculous rabbit 14-4, 13 days after reinfection with the same suspension of agar, tubercle bacilli and India ink as in rabbit Ar of Fig. 12; 2,700 colonies were isolated. The carbon is clumped in large dense masses. In the center of the field as indicated by the arrow are 2 dense clumps of tubercle bacilli surrounded by necrotic cells. One of the latter has the silhouette of a poodle dog. × 200.



(Lurie: Mechanism of immunity in tuberculosis)



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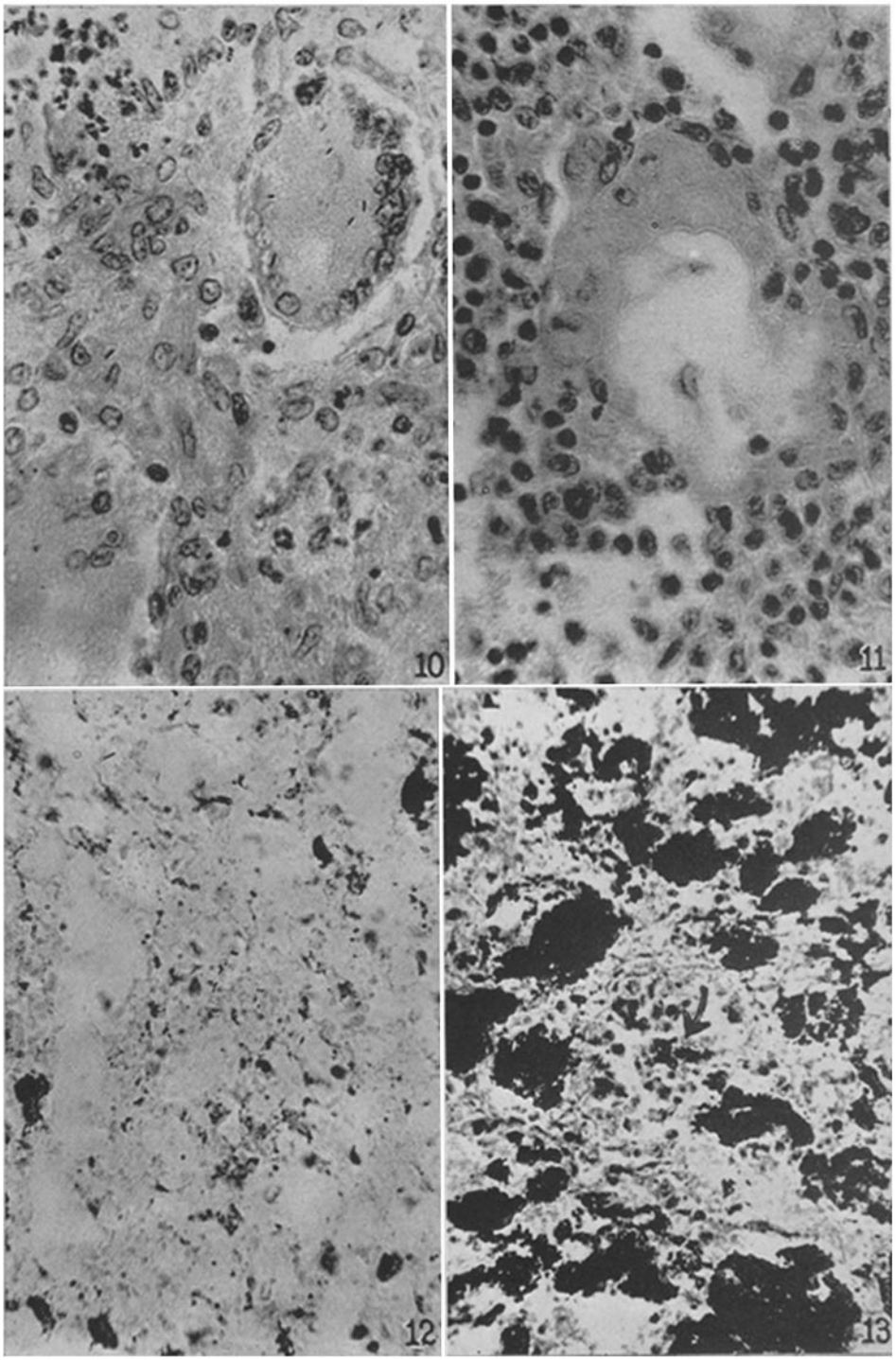

(Lurie: Mechanism of immunity in tuberculosis)