THE CORRELATION BETWEEN THE HISTOLOGICAL CHANGES AND THE FATE OF LIVING TUBERCLE BACILLI IN THE ORGANS OF TUBERCULOUS RABBITS

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PLATES 1 TO 4

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By means of cultural methods it was found in a previous study (1) that multiplication of tubercle bacilli follows intravenous inoculation of rabbits with either the human or the bovine type. At first the human bacillus grows faster than the bovine but soon a change takes place causing its destruction, first in the liver, spleen and bone marrow and later in the lung and kidney. The bovine bacillus multiplies in these organs more slowly; it is destroyed more slowly than the human type in the liver, spleen and bone marrow and it continues to multiply in the lung and kidney without effective opposition until the death of the animal.

Lubarski and Korshinskaja (2) using both similar and other methods have recently confirmed these results as far as they have studied them. Moreover an examination of the protocols of Lewis and Sanderson (3), who came to the conclusion from histological observations that the human tubercle bacillus fails to multiply appreciably in the lungs of rabbits, shows a definite though slight primary increase followed by a decrease in the number of stainable bacilli observed. As will be shown, there is a general parallel between the number of colonies isolated and the number of stainable bacilli, though the culture method, which indicates the viability of the bacilli, is essential to determine quantitative relations.

The study reported here is an attempt to correlate the histopathological changes in the various organs of rabbits with the number of viable bacilli contained in them as indicated by the number of col-

onies isolated from each organ at various intervals following infection. The end in view is to determine if possible what reactions of the host are associated with the multiplication of the parasite and what with its destruction. The following brief discussion will indicate how terms have been used and what significance has been attached to acid-fast particles.

Cells and Acid-Fast Material Observed

The most significant cell was designated the large mononuclear (Fig. 4). It is twice the size of a red blood cell, with a vesicular, rounded, oval or kidney-shaped nucleus containing sometimes one or several nucleoli, and pale transparent vacuolated cytoplasm with numerous processes.

Sabin and her coworkers (4) have separated this mononuclear phagocyte of the connective tissue into two types, the now well known monocyte and clasmatocyte, which, they have maintained, behave differently toward neutral red with the supravital stain and have different origins and different physiological functions. However numerous investigators, among these Lewis, Willis and Lewis, Carrel and Ebeling, Gardner and Smith, Clark and Clark (5), by the same and by different methods have come to the conclusion that they represent merely physiological states of the same cell. Recently Forkner (6), associated with Sabin, has stated that under certain conditions monocytes may be transformed into clasmatocytes. Injecting neutral red intravenously into rabbits according to the method of Cash and Gardner (7), we have found that at the periphery of advancing tubercles most of the large mononuclears showed the stain, like the monocyte, as fine, even sized granules, arranged spherically, of a uniform salmon pink, but that others showed granules characteristic of the clasmatocyte, being uneven in size, arranged in no definite pattern, and varying in color from red to yellow. In this paper the term mononuclear has been used without the subdivision into monocyte and clasmatocyte.

By gradual transitions the large mononuclear is transformed into what is designated here as the young epithelioid cell (Fig. 4). This is a much larger cell with abundant foamy or reticulated cytoplasm. The nucleus is usually round and very poor in chromatin. These cells are connected with one another by large processes. They are seen in the early tubercles. Later they become rounded and, as first described by Castrén (8), the cytoplasm becomes differentiated into an inner more eosinophilic, more compact, spherical area and a peripheral zone of more basophilic, often vacuolated cytoplasm. It is in the central area that the large accumulation of very finely divided neutral red granules is seen when the cells are stained supravitally, as shown by Lewis, Willis and Lewis (5) and by Sabin and her coworkers (4). This is the mature epithelioid cell (Fig. 5).

One other cell plays a part in the tuberculous process in the lung. This is the alveolar phagocyte, a large spherical cell with foamy, abundant, pale staining

cytoplasm, and an eccentric oval or kidney-shaped vesicular nucleus. Carbon particles and other ingested material are often found in the cytoplasm. Believing with Lang (9) and with Gardner and Smith (5) that this phagocyte is derived from the so called septal cell belonging to the general group of connective tissue phagocytes or clasmatocytes, we have designated it simply as a macrophage.

In these studies various forms of acid-fast material in addition to typical acid-fast rods were noted within the cells. These ranged from ovoid or globular, deeply or lightly staining forms of the size of large cocci, or larger, to irregular granular debris with all the outward characteristics of fragments of acid-fast bacilli, and to fine dust-like, scarcely visible, acid-fast particles. They showed a tendency to change in the order given.

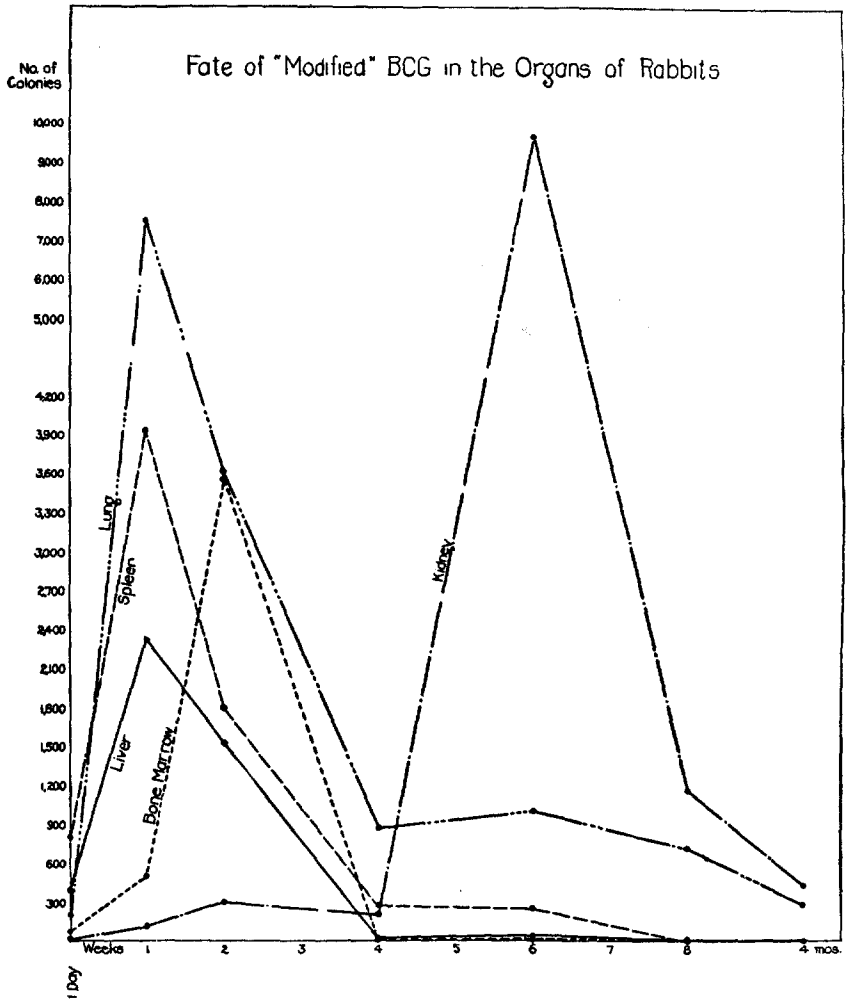
As to the significance of this material, little can be said with certainty at the present time. There are numerous observations in the literature suggesting that such particles are degeneration products of the tubercle bacillus. Amongst these are the experiments on "lysis" (10), showing that when tubercle bacilli are introduced into the tissues or cavities of tuberculous animals there is a rapid accumulation of acid-fast material and a marked reduction in the numbers of stainable bacilli associated with the heightened resistance of the infected animal to reinfection. In normal animals, similarly treated, intact bacilli persist and granular debris is much less in evidence but acid-fast material interpreted as degeneration products of the tubercle bacillus has been observed in the cells by numerous investigators (11, 12). An objection that has been urged to this interpretation even with the reinfected resistant animal, is that when inoculated into guinea pigs this material gives rise to progressive tuberculosis. It has been shown by the writer (13) however, that the tubercle bacilli of reinfection are in great part destroyed immediately, and that some few bacilli linger in the tissues. These would account for the virulence of such particles. On the other hand Spengler's "splitters" (14), the work of Sweany (15) and especially the recent single cell studies of Kahn (16) would suggest that they may be stages in the development cycle of the microorganism.

In the present studies the occurrence of acid-fast material is correlated with the number of living bacilli cultured. In general it has been found that the bacillus grows more slowly and is destroyed more rapidly in that organ in which more acid-fast material accumulates within a given time.

Method

Three strains of tubercle bacilli were used, a modified strain of the bacillus of Calmette and Guérin (BCG), one of human type, P-48A, and one of bovine type, Bovine C. The last two have been described in a previous paper (13). The BCG was obtained from Dr. W. H. Park in March, 1926. At that time it caused no progressive tuberculosis in guinea pigs, but instead of its being propagated under the conditions prescribed by Calmette, it was transplanted monthly on glycerol agar, and when the animals were infected in the summer of 1927 it had attained

considerable virulence for rabbits. This result is in agreement with the observations of Petroff, Branch and Steenken, Sasano and Medlar, Dreyer and Vollum (17) and others. However, although some rabbits died between the 4th and 6th weeks



TEXT-FIG. 1

following the injection, the majority survived with almost complete resolution of their lesions by the 2nd to the 4th month.

The BCG was injected intravenously in doses of 1 mg. per kilo, the human and

bovine strains in doses of 0.01 mg. For the microscopic examination sections of all the organs were taken from the immediate neighborhood of those parts that were cultured. They were stained with hematoxylin and eosin, and by the Ziehl-Neelsen method for tubercle bacilli with hematoxylin for a counterstain.

At intervals of 1 day, 1, 2, 4 and 6 weeks and 2 months equal amounts by weight of suspensions of ground lung, liver, spleen, kidney and bone marrow in varying dilutions were seeded upon the surface of Dorset's and Petroff's egg media, both directly and after sodium hydroxide treatment. At least three tubes of each medium were seeded with a given dilution of a given organ, both directly and after treatment. The number of colonies of tubercle bacilli appearing upon the surface of each tube was repeatedly determined, the final reading being made after 3 months incubation. The method of culture has been more fully described in previous publications (1, 13).

EXPERIMENTAL OBSERVATIONS

The Lung after Infection with a Modified BCG

The accompanying graph (Text-fig. 1) pictures the fate of the modified BCG in the organs of rabbits. Each point plotted represents the average of two or three rabbits except in the 6th week and 4th month intervals, when only one was available. It is seen that the bacillus is at first distributed in the organs quantitatively in the order in which particulate matter accumulates after intravenous injection (18), namely, spleen, liver, lung, bone marrow and kidney. There is a brisk multiplication of the BCG in all the organs, the most rapid in the lungs. At the end of the 1st week the bacilli have attained their greatest numbers in the lung, liver and spleen. This phase is followed by a rapid destruction, seen in the 2nd and 4th weeks. From the 4th week on destruction of the few microorganisms left in the liver, spleen and bone marrow proceeds more slowly. After the 2nd month they are very slowly destroyed and persist in the kidney and also in the lung in considerable numbers even 4 months after infection.

The complex relationship between the host and the invading parasite as studied with the three strains, is shown in the simplest form by the least virulent strain with its rapid initial multiplication and subsequent destruction. The histological observations in the lung may be summarized as follows.

24 hours after infection there is a well defined inflammation with an accumulation of polymorphonuclear leucocytes in the lung. Multiplication of mononuclears in alveolar walls is indicated by mitosis. That some tubercle bacilli are being destroyed by the mononuclears is suggested by the acid-fast particles found within them.

In one rabbit 1 week after infection the lesion (Fig. 1) is observed from which the greatest numbers of viable bacilli are recovered (11,000 colonies). There is a general infiltration of mononuclears in the septa, especially about blood vessels, the lumen of which is often narrowed by the multiplication of mononuclears under the endothelium. The infiltrating cells are often gathered into nodules. Some of

the nodules are composed of a thick, closely packed ring of mononuclears, many in mitosis, surrounding young epithelioid cells; these are the early tubercles (Fig. 4). Tubercle bacilli are found within the young epithelioid cells in large numbers, and in smaller numbers within the mononuclears. The simultaneous occurrence of acid-fast rods and acid-fast granular debris in the same cell suggests that some bacilli are being destroyed.

The microorganisms have been greatly reduced in number (2,930 colonies) by the end of the 2nd week (Fig. 2). The epithelioid cells have assumed a more mature character, and the ring of mononuclears is very thin or has disappeared entirely. The first signs of caseation appear (Fig. 6): there is an accumulation of polymorphonuclears in some tubercles. Isolated tubercle bacilli may be seen in some of the mature epithelioid cells, especially in those undergoing pyknosis or necrosis, or they may be absent altogether. Exudation into the alveoli has become conspicuous.

The most extensive tuberculous changes are found in the lung 4 and 6 weeks after infection, when most of the bacilli have been destroyed (Fig. 3). At 4 weeks (655 colonies) the parenchyma is largely replaced by tubercles with or without central foci of caseation. Langhans' giant cells appear at the periphery. The tubercles are invested by lymphocytes, often permeating the epithelioid cells, and sometimes ingrowing capillaries, plasma cells and fibroblasts are seen. Mitosis is rare. The exudate into the alveoli is more widespread. The alveolar lining in contact with tubercles may show metaplasia with cuboidal and columnar cells. Tubercle bacilli are very difficult to find in the epithelioid cells.

At the 6th week (1,000 colonies) many tubercles are composed of widely spaced, disintegrating epithelioid cells, and they are often permeated by lymphocytes and polymorphonuclears, and canalized by capillaries in all directions. Giant cells are more numerous at the periphery and sometimes occur in the centre about the remains of caseous material (*cf.* Medlar (26)). The exudation at times assumes pneumonic proportions, and may undergo caseation. Tubercle bacilli are found in small numbers in epithelioid cells in contact with caseous foci of interstitial tubercles and at the edge of pneumonic areas undergoing caseation.

In one rabbit 2 months after infection only fourteen colonies were recovered after sodium hydroxide treatment of the tissue. The lung field has cleared considerably. The disintegrating epithelioid cells sometimes contain minute, faintly acid-fast globules staining dark brown with hematoxylin-eosin, often clumped in hollow spheres and having no resemblance to derivatives of tubercle bacilli. Often the entire tubercle is composed of such cells surrounded by a thick ring of lymphocytes. Tubercle bacilli in small numbers are seen in immediate contact with caseous areas. Caseous foci may disintegrate into microscopic cavities (Fig. 7).

The data show that although tubercle bacilli are being destroyed from the very beginning, multiplication continues to outstrip destruction until after 2 weeks, when mature epithelioid cells are formed.

In any two rabbits, even at the same interval, the more effective destruction of bacilli has occurred in that animal in which epithelioid cell and tubercle formation are further advanced. The most rapid growth of the microorganism was found in those tissues in which the most extensive formation of new mononuclears was observed. When the parasite stops multiplying, mitosis is seen less often and now giant cells appear at the periphery of the tubercle. Caseation and exudation into the alveoli are coincident, not with the greatest increase, but with the most rapid destruction of bacilli. Caseation is therefore not due to the mere accumulation of tubercle bacilli, though some are usually present in or about a caseous focus. Epithelioid cells persist for a considerable time after most of the bacilli within them have died, although their disintegration products may still be present. Lymphocytic infiltration and granulation tissue are seen after the greater part of the tubercle bacilli have been destroyed.

The Lung after Infection with Tubercle Bacilli of Human Type

With a dose of 0.01 mg. of human tubercle bacilli there is little multiplication of the microorganism during the 1st week. The associated slight histological changes are of the same character as those described for the BCG.

No gross tuberculosis is seen at the end of the 2nd week. The bacilli have increased ten and thirty times. In Rabbit 25–76, from which the larger number of colonies (1,580) were isolated, the histological changes are essentially those seen in the 1st week with the BCG except that cells lying free in the alveoli contribute considerably toward the tubercle formation. Tubercle bacilli are again found in large numbers in the young epithelioid cells, three or four in a cell, together with acid-fast particles. Macrophages in the alveolar space occasionally contain a bacillus. The lymph follicles are much enlarged. In them large mononuclear cells show frequent mitosis, and there are islands of young epithelioid cells, some containing numerous tubercle bacilli as well as carbon particles. There is no caseation.

In Rabbit 23–01, with 560 colonies at this interval, the infiltration into the alveolar septa is less, mitosis is rarer, the tubercles are fewer and composed more largely of epithelioid cells, in which tubercle bacilli are seen in smaller numbers than in the first rabbit.

In one rabbit 4 weeks after infection the bacilli had either remained stationary in number or had decreased considerably; in the other, they had increased to 7 and 22 times the numbers found in the 2nd week.

In the second rabbit (No. 25–79, 12,500 colonies) the tuberculous process is of two types: interstitial or productive, and intraalveolar or pneumonic. The for-

mer is seen in perivascular, peribronchial, intraseptal tubercles, and in tubercles within the lymph follicles. All of these are composed wholly or in part of mature epithelioid cells (Fig. 8). Some, showing no caseation, have no mononuclears and no mitosis at their periphery, but instead masses of lymphocytes and plasma cells; capillaries may begin to grow into them. Such tubercles do not contain intact tubercle bacilli although there may be acid-fast particles within the epithelioid cells. Other interstitial tubercles contain small foci of caseation in which moderate numbers of tubercle bacilli are found. They may perforate a bronchus. They are surrounded by mononuclears, some in mitosis, and show considerable exudation into adjacent alveolar spaces.

In the pneumonic areas the alveolar septa are very much thickened by an infiltration of mononuclears with numerous mitotic figures, and in places by polymorphonuclears as well. Within the alveoli there are numerous macrophages, epithelioid and giant cells, usually containing acid-fast particles and occasionally also tubercle bacilli. Frequently the collections of epithelioid cells and sometimes the adjacent septa undergo extensive caseation (Fig. 9). Tremendous numbers of tubercle bacilli are found within these caseous plugs in alveoli and distinctly fewer within the caseated septa (Fig. 10). This caseous pneumonia constitutes the most extensive phase of the lesion in the lungs. Metaplasia of the alveolar lining and mononuclear mitosis within alveoli are seen.

In Rabbit 23–06 (only 600 colonies) the lesion differs from the above only in that the interstitial process is far more widespread than the pneumonic. The lymphocytic infiltration is more extensive; giant cells appear at the periphery of the tubercle; caseation is less. Tubercle bacilli are fewer in all sections of the lung, and even in the infrequent areas of caseous pneumonia, where they are numerous, they are never found in such tremendous numbers as in the previous rabbit. On the periphery of the pneumonic areas the mononuclear infiltrations of the alveolar septa are transformed into epithelioid cells, which in turn become surrounded by lymphocytes.

Retrogressive changes seen in the 6th week (1,820 colonies) are of the same nature as with the BCG. 2 months after infection there is a considerable, consistent secondary increase of bacilli cultured, 2,970 colonies in one rabbit, 5,260 in the other. In the latter only a moderate number of tuberculous foci are seen. They contain extensive areas of caseation, which in places show excavation and numerous pyknotic lymphocytes and polymorphonuclears. There are hollow clumps of faintly acid-fast globules in epithelioid cells at the periphery of some tubercles. Tubercle bacilli are found in epithelioid cells not only about the caseous focus but also at the periphery of the tubercle, and within giant cells. These tubercles may be extensively permeated by lymphocytes and granulation tissue. Numerous bacilli may also occur in caseous material plugging the alveoli in pneumonic areas undergoing organization at the periphery.

Thus essentially the same processes observed in the lung with the less virulent BCG are seen again with the human type, but from the

beginning there is a more conspicuous accumulation of cells within the alveoli, and intact tubercle bacilli are more often found within these cells. Tubercle bacilli are effectively destroyed in the epithelioid cells of the interstitial tubercles. Within the alveoli the restraining influence of the epithelioid cells may be overcome and living bacilli may accumulate within these in tremendous numbers. They may then undergo extensive caseation. This caseous pneumonia is usually localized and does not become generalized. It is noteworthy that in the rabbit with the most extensive areas of caseous pneumonia tubercle bacilli are found in larger numbers, and caseation is more extensive even in the interstitial tubercles.

The microorganism persists in greater numbers 6 weeks after infection than with the larger dose of BCG at the same time. At 2 months there is a secondary increase in the numbers of both stainable and viable bacilli despite the permeation of the tubercle by lymphocytes and granulation tissue.

The Lung after Infection with Tubercle Bacilli of Bovine Type

The bovine bacillus like the human type proliferated very little in the 1st week and the accompanying histological changes were of the same nature except that the initial outpouring of polymorphonuclears was more intense and the subsequent infiltration of mononuclears more extensive.

At the end of the 2nd week the bacilli had increased a hundredfold and more (1,200 colonies). Even in the gross there was a moderate number of pin-point tubercles, some with scarcely visible caseous foci. Microscopically the interstitial circumscribed early tubercles, occurring often about blood vessels, are as described for the human infection. The more frequent lesion is a diffuse process consisting of alveolar septa thickened by an extensive infiltration of mononuclears and fused together by a conspicuous accumulation of macrophages in the alveoli. Mitosis is very common in both types of lesion and islands of young epithelioid cells containing tubercle bacilli are seen in the mononuclear infiltrations. The larger tubercles are in the first stages of caseation and accumulations of macrophages and polymorphonuclears are prominent in the alveoli about them. Tubercle bacilli are found in large numbers in epithelioid cells within such tubercles, and the macrophages in the alveoli, which at times undergo mitosis, may contain intact bacilli. Islands of epithelioid cells are again found in the lymph follicles with frequent mitosis of surrounding cells.

A marked retardation, or cessation, in growth had occurred at the end of the 4th week. From Rabbit 22–76 only 1,080 colonies were isolated; yet in the gross the lungs were almost entirely consolidated by discrete miliary tuberculosis. Mi-

croscopically the productive and pneumonic processes are more extensive and further advanced. Tubercle bacilli are difficult to find in the mature epithelioid cells of the interstitial tubercles; caseation when present is usually slight; the tubercle is surrounded by mononuclears with occasional masses of lymphocytes within vessels and free in the tissues. The greater part of the lesion involves both interstitial tissue and alveolar lumina. In the alveoli, macrophages contain a great deal of acid-fast globules, but the collections of epithelioid and giant cells show little if any. Tubercle bacilli are not found within these epithelioid cells, except occasionally, in moderate numbers, in tubercles undergoing caseation.

Thus with a far more extensive epithelioid cell and tubercle formation than in the 2nd week, viable bacilli have not increased and persist appreciably only in the alveolar pneumonic areas.

In one rabbit 6 weeks after infection the bacilli had increased to twelve times, in the other to only twice the number found after 4 weeks. In the first (No. 22–50, 12,000 colonies) the process is essentially as in the previous interval, but with caseous pneumonia predominating. Tubercle bacilli are found even in the interstitial tubercles, and not only in the epithelioid cells in the zone between the caseated and the intact cells, but also in the unaffected epithelioid cells in an area of slight extent, most of the tubercle having caseated. These tubercles are invested by a growing zone of mononuclears infiltrated with polymorphonuclears; giant cells are not found at the periphery; lymphocytic investment is only partial. Tubercle bacilli are numerous in the pneumonic areas, especially those undergoing caseation. There may be metaplasia of the alveolar lining in areas of tuberculous pneumonia and the bronchi may contain cells similar to those in the alveoli carrying tubercle bacilli.

In general there was extensive caseation, few intact epithelioid cells and a massive growth of mononuclears. The epithelioid cells have apparently failed to check effectively the growth of the microorganism, especially in the alveoli, where tubercle bacilli are numerous within epithelioid cells and mononuclears. The progress of the lesion has not been stopped.

In the second rabbit of this interval (No. 22–85) only 2,100 colonies were isolated. The process is largely interstitial with the pneumonic process and caseation much less extensive than in No. 22–50. Tubercle bacilli are found much less frequently, even in the pneumonic areas.

Essentially the same difference is found between the lungs of rabbits killed 2 months after infection, from one of which 200,000 colonies were isolated and from the other 3,100. The first (Fig. 11) showed a massive caseous pneumonia with much less extensive interstitial change. The productive process consists of multiple foci of caseation separated from one another by tuberculous granulation tissue, the advancing edge of this tissue being a mass of mononuclears with frequent mitosis and many polymorphonuclears. Tubercle bacilli are found in great numbers in the intact epithelioid and giant cells that have collected in the alveoli in the areas of caseous pneumonia. In the pneumonic areas the exudate is sometimes largely serum and polymorphonuclears intermixed with macrophages.

In the second rabbit, in which the growth of bacilli was held in check, the lung, though as massively consolidated as in the first rabbit, was the seat of an interstitial process (Fig. 12) with the tuberculous pneumonia usually limited to areas immediately about the tubercles, and even these few foci of caseous pneumonia had become the centers of a peripheral productive change.

As here shown the same defensive processes that have been observed in the lung with the BCG and human strains are overcome characteristically with the bovine. The initial reaction of the organ to the most virulent strain is more intense as expressed by a greater rush of polymorphonuclears and a more extensive multiplication of mononuclears. But also from the very beginning cellular accumulation within the alveoli, in which, as was seen before, tubercle bacilli are not readily destroyed, is more important in the bovine-type lesion, which is more diffuse and less nodular than with the human strain. These changes were accompanied by a sharp increase of viable tubercle bacilli in the 2nd week following a lag in the 1st week. They stopped multiplying between the 2nd and 4th week, again at a time of extensive formation of epithelioid cells and of tubercles, but they persisted in the epithelioid cells collected in the alveoli. From now on the lesion is increasingly pneumonic and bacilli are found in increasingly large numbers. Again they are fewer in the interstitial tubercles but even in these more numerous when the pneumonic areas are more extensive. The greater part of the parenchyma may become consolidated by caseous pneumonia, throughout which tubercle bacilli are found in tremendous numbers. On the other hand, they have been held in check in those rabbits in which the interstitial lesion predominates, even though the parenchyma may be as extensively involved.

The difference between the human and bovine types is essentially one of degree. Usually with the former the process is chiefly interstitial; it is circumscribed and never becomes generalized. The pneumonic areas when present are localized and tend to become surrounded by a productive process. Caseation is limited; the regeneration of mononuclears soon ceases; giant cells appear at the periphery; lymphocytes and granulation tissue permeate the tubercle; in some instances after the lesion has persisted 2 months, softening and cavity formation overtake the isolated residual tuberculous foci. With the bovine infection, the lesion is chiefly of a mixed pneumonic and productive

character; it spreads over almost the entire lung; caseation appears early and becomes massive. The zone of intact epithelioid cells is slight and regeneration of mononuclears goes on unabated. Giant cells do not form at the periphery of the interstitial tubercles, though they have been seen in the pneumonic lesion. Lymphocytes do not permeate the tubercle to any considerable extent and the granulation tissue appears as islands between the multiple foci of caseation. The caseous pneumonia is widespread throughout the lung. Softening and excavation were not noted within the course of the experiment. Associated with these differences is the effective destruction of the human bacillus within the interstitial lesions. With the bovine on the other hand the bacilli are held in check but not destroyed in these lesions, whereas in the pneumonic areas they accumulate in far greater numbers than the bacilli of human type.

The Host-Parasite Relationship in Other Organs after Infection with the Three Strains

Essentially the same correlations between histological changes and the fate of tubercle bacilli as found in the lungs were seen in the other organs involved, and they are summarized here. When not otherwise specified the processes are like those in the lung and the reaction of each organ to the three strains differs only in degree. Significant differences are briefly described.

Liver.—The initial inflammation is very much less than in the lung despite the localization of larger numbers of bacilli in the liver. From the beginning bacilli are destroyed more effectively as shown by the much larger accumulation of acid-fast material in the Kupffer cells than in the pulmonary mononuclears. Associated with the slower multiplication of bacilli is the formation of fewer mononuclears and of smaller though more numerous tubercles, and the early appearance of giant cells (in the 1st weeks with the BCG and from the 2nd to 4th week with the human and bovine). The whole tubercle is often composed of giant cells; isolated giant cells are also seen. Caseation is not found with the BCG, presumably because little remains of the bacillus after the onset of extensive destruction. With the human infection, associated with the somewhat larger numbers of viable bacilli recovered, caseation and central softening with the formation of tissue defects are seen after 4 weeks, simultaneously with the greatest destruction of bacilli. The bovine infection differs from the human in the greater accumulation of acid-fast particles in the Kupffer and epithelioid cells and in the larger number and size of tubercles and their longer persistence, in association with the larger numbers and

longer persistence of viable bovine bacilli. With all three strains the bacilli are almost completely destroyed, and the liver tubercle, unlike the pulmonary, completely disappears between the 6th and 8th week.

Spleen.—In the spleen the initial growth of tubercle bacilli is more rapid than in the liver but slower than in the lung, and bacilli persist longer than in the liver but are more quickly destroyed than in the lung. This is explained, in part at least, by the different reactions of the splenic pulp and corpuscle. The macrophages of the pulp at first very conspicuously contain round acid-fast globules as big as large cocci, which later break up into irregular particles and finally appear in the epithelioid and giant cells as fine dust-like, scarcely visible debris. These particles are frequently found together with intact tubercle bacilli in the same cell. With a presumably greater localization of bacilli in the pulp corresponding with that of particulate matter injected into the venous system (19), the tubercles are at first more numerous than in the corpuscle. But the pulp tubercles mature earlier, show less active formation of mononuclears, are soon transformed into giant cells (Fig. 17) and tubercle bacilli are more rapidly destroyed within them. The tubercles remain small and stain darker than those in the corpuscle, the cells often containing acid-fast particles, red blood cells, hemoglobin pigment and other ingested material. Living bacilli persist longer and there is less acid-fast material in the tubercles of the corpuscle, which become massive, involving the whole structure.

With the BCG, slight caseation appears in the corpuscle tubercles 4 to 6 weeks after infection. With the less complete destruction of bacilli of human type, caseation is more extensive in the corpuscle and central softening appears 4 weeks after infection. With the bovine infection also tubercle bacilli disappear from the epithelioid cells in the corpuscle in some rabbits. In rabbits even of the same interval the destruction of bacilli is more complete in that animal in which, as indicated by the abundance of epithelioid cells, tubercle formation is further advanced (Figs. 15 and 16). In other rabbits the corpuscle tubercles, as well as tubercles in other organs, failed to destroy the microorganism and isolated tuberculous foci containing large numbers of living bacilli persisted even 2 months after infection. Caseation was not necessarily found with large numbers of bovine bacilli but often in association with a more mature tuberculous process and fewer bacilli. Central softening with the formation of tissue defects was noted in the splenic corpuscle 4 weeks after infection in tubercles permeated by lymphocytes, polymorphonuclears and capillaries, where numerous living tubercle bacilli persisted within the epithelioid cells.

Bone-Marrow.—With the BCG and human strains there is a considerable accumulation of acid-fast material in the reticular cells 1 week after infection. Growth and destruction of bacilli were seen with the same histological changes as in the other organs. No caseation was noted, again apparently because too few bacilli remained after the onset of extensive destruction.

With the bovine infection acid-fast granules are at first very prominent in the

reticular cells. From the 4th week on the bacilli are effectively destroyed in the epithelioid cells in some rabbits. In these caseation is seen synchronously with the greatest destruction of bacilli. Later these tubercles completely disappear together with the bacilli. In other rabbits the bovine bacilli are not destroyed, mitosis continues and, as in the lung with human infection, large numbers of living bacilli persist even 2 months after infection within the epithelioid cells of tubercles extensively permeated by lymphocytes and granulation tissue. There is little caseation in these tubercles and it is found not where intact bacilli are most numerous but where there are most acid-fast particles with all the characteristics of disintegrating bacilli.

Kidney.—The host-parasite relationship in the kidney is modified by two factors: the initial minimal localization of bacilli, and the anatomy and physiology of the organ. The first determines a long lag period in the progress of the disease. With maturation of the tubercle the bacilli are held in check for 4 weeks. However, due to the intertubular origin and extension of the tubercle, many tubules and glomeruli become involved. With the disintegration of foci of caseation in the cortex, tuberculous material may break into these channels and may be carried by the flow of urine to the medulla, where it sets up a new process (Fig. 19). Furthermore casts form within the involved tubules, in which tubercle bacilli may multiply as in a culture medium. Thus bacilli may persist in this organ for a long time, even when the cortical tubercles are healed. Central softening develops in the kidney except with the bovine infection.

With the human type the first stages of caseation are synchronous with the greatest accumulation of bacilli. However since this was not found until the 4th week, when caseation had occurred throughout the body, this observation is not evidence for an initial toxicity of the bacillus to the tissues. There was no constant relationship between the numbers of bacilli in the lung and in the kidney. (In 2 months, 5,260 colonies were cultured from the lung and none from the kidney, which contained only one minute cortical tubercle.) There was no evidence that these lesions in the kidney are caused by bacilli brought from the lungs or other organs to the kidney.

The bovine bacilli tend to increase from the 4th week, and they regularly persist within the epithelioid cells. Yet in some rabbits they may be largely destroyed by the epithelioid cells. Caseation occurred in the kidney 4 weeks after inoculation in the presence of relatively few bacilli (297 colonies).

SUMMARY AND DISCUSSION

It has been found that although there is some parallelism between the quantity of tubercle bacilli demonstrable histologically and the number of colonies that can be isolated from a given tissue, the culture method is far the more efficient in indicating quantitative relations. Tubercle bacilli were not perceived in the organs of rabbits

1 day after infection with the modified BCG although as many as 1,500 colonies were isolated from one of them. This may be solely because it is difficult to see widely dispersed single minute acid-fast rods in the diffuse infiltrations of mononuclears with their hyperchromatic nuclei and sparse cytoplasm. Later, with the formation of tubercle, the parallelism is much closer. The culture method gives evidence concerning the number of living tubercle bacilli in the tissue.

The significance of the accumulation of acid-fast particles in the tissues has been discussed. It has been seen that from the beginning this accumulation is greater in the Kupffer cells of the liver, in the macrophages of the spleen and in the reticular cells of the bone marrow than within the mononuclears of the lung, the organ where the bacilli grow with the greatest rapidity and are destroyed with the greatest difficulty. Acid-fast particles are more prominent with the bovine than with the human bacillus or the BCG, the microorganism that is destroyed with the greatest difficulty thus leaving more incompletely digested bacillary debris at a given time within the cells. Thus it seems permissible to conclude from the presence of acid-fast material that some tubercle bacilli are undergoing destruction even 24 hours after infection. The initial accumulation of polynuclear leucocytes corresponds with the subsequent severity of the infection. Despite the greater primary localization of bacilli in the liver, this initial inflammatory reaction with all three infections is much greater in the lung than in the liver. In each organ it is more intense with the bovine than with the less virulent strains.

The multiplication of the bacillus and its accumulation within large mononuclear and young epithelioid cells is accompanied by an intense formation of new mononuclears by mitosis. The more rapid the growth of the bacillus, the more conspicuous the regeneration of these cells. Thus with all strains mitosis is more intense in the more susceptible organ, as in the lung compared with the liver; with the most virulent strain the most extensive and diffuse accumulation of these new cells corresponds with the greater rise in the numbers of bovine bacilli after the lag of the 1st week.

With the maturation of the epithelioid cells and the formation of tubercles the bacilli have already been greatly reduced numerically

and the speed of this process diminishes with the virulence of the three strains used. The faster the development of tubercle the faster the destruction of the bacillus and the earlier the resorption of the tubercle.

Tubercle bacilli never accumulate in such large numbers in the mononuclears of the liver as they do in the lung. Though at first the tubercles in the liver may be more numerous than those in the lung they never attain the same size. The formation of new mononuclears by mitosis is restricted and Langhans' giant cells appear very early (1st and 2nd weeks). In the lung, giant cells are not found until much later with the BCG and the human bacillus (4th week); they were not noted in the interstitial tubercles with the bovine type, but the extension of these tubercles was accompanied by an unabated mitosis of mononuclears until the death of the animal. The liver tubercles are resorbed early even with the bovine infection. Associated with these histological differences are the slow initial growth and the early and complete destruction of the tubercle bacilli even of bovine type in the liver, and the more rapid initial growth in the lung, with the later destruction of the BCG and the human bacillus and the unabated growth of the bovine bacillus. Similar differences were observed between the splenic pulp and corpuscle. In the former the accumulation of acid-fast particles was much greater and the tubercles developed earlier. Mitosis of mononuclears was less frequent and giant cells appeared earlier. Tubercle bacilli, always intracellular, disappeared from the tubercles in the pulp sooner than from those in the corpuscle, and the tubercles themselves first disappeared from the pulp. Consequently with the persistence of bacilli mitosis continued in the tubercles of the corpuscle and these attained a much larger size.

Moreover individual resistance is linked with the ability to form mature tubercles early. In two animals simultaneously infected with the same strain and killed at the same time, the destruction or retardation of the bacillus is greater in that rabbit in which maturation of the tubercle and of epithelioid cells has proceeded further (Figs. 15 and 16).

These observations indicate that the mononuclears of different organs or even of the same organ, as in the different parts of the spleen, have a different capacity to destroy the tubercle bacillus, and that the

transformation of the mononuclear into the mature epithelioid cell follows its destruction of the tubercle bacilli.

In the lung the more virulent types of bacillus are destroyed within the epithelioid cells of interstitial tubercles but persist in foci of tuberculous pneumonia. In this organ in rabbits infected with the human strain and to a lesser degree in rabbits infected with the bovine strain, the parasite largely disappears from the epithelioid cells of interstitial tubercles. But with both strains tubercle bacilli in large numbers may accumulate within epithelioid cells lying free in the alveoli. With the human type they are numerous within the cells and free in caseous material in the localized foci of caseous pneumonia. With the bovine infection, this caseous pneumonia is more often widespread and in the areas of caseous pneumonia the greater part of the vast accumulation of bovine bacilli in the lungs is found; as many as 200,000 colonies have been isolated from 10 mg. of tissue (Fig. 11). Flooding of the respiratory passages by the caseation of tuberculous lesions into the bronchi plays an important rôle in dissemination of tubercle bacilli through the lung. The process on the contrary is predominantly interstitial when the bovine bacillus is held in check (Fig. 12).

Thus there is apparently some factor acting in the alveoli that favors the growth of the parasite. The accumulation of tubercle bacilli is seen especially in the peripheral epithelioid cells in immediate contact with the alveolar space. In the same lung the bacilli are much fewer in the interstitial tubercles.

The accumulation in human tuberculosis of large numbers of tubercle bacilli in the tissues lining cavities is well known. Novy and Soule (20) have shown that within certain limits the growth of the bacillus *in vitro* is proportional to the oxygen tension of its environment. Corper, Lurie and Uyei (21) have confirmed these observations and have noted further that a difference in the gaseous environment of the bacilli equal to the difference between the conditions existing in the alveolar air and the venous blood is sufficient to cause a considerable increase in the growth of the microorganism *in vitro*. Loebel, Shorr and Richardson (22) by the use of Warburg's manometer have found that the oxygen consumption of tuberculous tissue is such that a tubercle 0.5 mm. thick would completely exhaust the oxygen of the air before it reached the center.

These observations suggest that a factor responsible for the greater multiplication of the bacillus in the cells of the alveoli may be the greater oxygen tension of the alveolar air.

In the liver, spleen and bone marrow even with the bovine infection many instances were found of the effective destruction of the parasite synchronously with the maturation of epithelioid cells and the formation of tubercle. On the other hand, in the spleen and bone marrow of some rabbits, living bacilli persisted within the epithelioid cells of isolated tubercles even 2 months after infection, a condition never found with the human type or BCG infection. Thus the epithelioid cell is the means of defense for the rabbit against the bovine type bacillus, and as such it is usually adequate in the liver, spleen and bone marrow though ineffective in the lung and kidney. In the latter, descending infection, and the occasional colony-like multiplication of bacilli in unorganized material, tubular casts, determine the long persistence of large numbers of bacilli in this organ.

In differentiating the mononuclear phagocyte of the connective tissues into the monocyte and clasmatocyte Sabin and her coworkers (23) have maintained that the clasmatocyte can efficiently destroy the tubercle bacillus but that the monocyte and its derivatives, the epithelioid and Langhans' giant cells, cannot. With the progress of the disease they have noted that the monocytes accumulate in great numbers in the foci of infection and overflow into general circulation (4). White (24) and Sabin and her coworkers have concluded that tuberculosis is specifically a disease of the monocyte, and that this cell and its derivatives act as incubators for the tubercle bacillus. Doan and Sabin (25) have therefore sought, with indecisive results, to protect the body against tuberculosis by an antimonocytic serum. However it has been shown here that although an intense multiplication of mononuclears is associated with the growth of the tubercle bacillus, their transformation into mature epithelioid cells is constantly associated with its destruction, and the rapidity of the destruction varies with the rapidity of the maturation of tubercle. Even in the bovine infection the epithelioid cells destroy the bacilli in the liver, spleen and bone marrow as a rule, and even in the lung, keep them in check in the interstitial tubercles.

The appearance of giant cells is associated with cessation or diminu-

tion of mononuclear regeneration by mitosis, and is coincident with cessation of multiplication or marked reduction in the number of living bacilli. They therefore appear earlier and in larger numbers in these organs or parts of organs that first destroy the bacillus (Figs. 16 and 17). They were not observed even 2 months after the bovine infection in the interstitial tubercles in the lung. Their absence and the continued mitosis of mononuclears, which accounts for the massive pneumonic and interstitial consolidation of the lung with this infection, were associated with the failure of the lung to destroy effectively the bovine parasite. The formation of giant cells in the pneumonic foci in the bovine infection would seem to be an exception to this rule.

The Langhans giant cells have often been considered an indication of the chronicity of the pathological process. It would appear that they are formed from existing epithelioid cells when the multiplication of the bacillus has ceased and the stimulus for the formation of new cells has decreased or stopped. Giant cells were most conspicuous in the liver and splenic pulp where, with the BCG infection, no caseation ever developed, and in the liver before caseation was seen anywhere in the body. In the human and bovine infections, giant cells formed in the liver before caseation appeared. Hence caseation is not a necessary requirement for giant cell formation, as maintained by Medlar (26), though these cells frequently form about caseous material.

Lymphocytes and granulation tissue do not cause the destruction of tubercle bacilli, these being destroyed in their absence. They usually appear about tubercles due to all strains and in all organs, after the greater part of the microorganisms have been destroyed (Fig. 18). The bacilli are not destroyed in the lung with bovine infection where the tubercles are usually little permeated by lymphocytes and granulation tissue. There is however, no constant relation between granulation tissue and destruction of tubercle bacilli, for in the lung after the human infection and even in other organs after the bovine infection isolated tubercles may be surrounded and penetrated by lymphocytes and granulation tissue at a time when considerable numbers of living bacilli are still histologically demonstrable within the epithelioid cells.

Caseation is usually not caused by the local accumulation of tubercle bacilli. At first, when the BCG (after 1 week) and the human microorganism (after 2 weeks) are present in the cells in very large numbers as demonstrated both histologically and by culture (Figs. 4 and 13) there is no necrosis of these cells. An exception to this rule found in the lung with the bovine infection is considered below. Later, after the bacilli have been destroyed to a great extent and even though the number of bacilli is small, caseation appears (Fig. 14). After this preliminary destruction the extent of caseation apparently varies with the number of residual bacilli. With the least virulent microorganism, the BCG, few bacilli remained in the liver in the 4th week and no caseation was seen. In the tubercles of the splenic corpuscle at the same time bacilli were somewhat more numerous and there was scant caseation. On the other hand with the human bacillus after 4 weeks more bacilli survived and caseation was more extensive in both organs; with the bovine microorganism tubercle bacilli were much more numerous and caseation was far advanced.

In the lung, however, caseation appeared with the first considerable accumulation of the bovine bacilli present 2 weeks after inoculation. That the bovine bacillus is primarily more injurious to the lung of rabbits than the BCG or the human bacillus is suggested by the greater intensity of the initial inflammation and by the more conspicuous accumulation of cells in the alveoli evident from the very beginning of infection. Maximow (27) showed that bovine bacilli even in small numbers cause the death of cells in tissue cultures of rabbit lymph nodes whereas the BCG or the human bacillus may accumulate within the cells in tremendous numbers without injuring them. Nevertheless in the liver, spleen and bone marrow of the living animal, caseation does not appear at the time when bovine bacilli are most abundant, but after they have been greatly reduced in numbers.

Large numbers of the less virulent types of tubercle bacilli accumulated in different organs a short time after infection do not cause caseation, and with the bovine infection caseation under the same conditions occurs only in the lung. Later when the animal is sensitized caseation occurs in various organs in the presence of the small numbers of tubercle bacilli that remain in the tissues after most of

them have been destroyed, and the extent of this caseation varies with the numbers of residual bacilli. These observations suggest that a large number of bacilli fail to cause necrosis soon after infection whereas a few bacilli produce caseation in the animal that is sensitized.

Many investigators have held that caseation is due to sensitization. Krause (28), Huebschman (29) and Pagel (30) think that caseation is caused by the action of tuberculin-like substances on the sensitized tissues of the allergic animal. Rich and McCordock (31) view the process in essentially the same light. Recently Schleussing (32) has suggested that caseation is a coagulation necrosis in Weigert's sense of an allergically inflamed tissue, and is similar to the necrosis of the Arthus phenomenon.

CONCLUSIONS

1. The mononuclears of the liver, splenic pulp and bone marrow destroy tubercle bacilli more readily than those of the lung, kidney or splenic corpuscle.

2. The multiplication of tubercle bacilli in an organ and their accumulation within mononuclears is accompanied by active new formation of these cells by mitosis.

3. When these mononuclears are transformed into mature epithelioid cells and tubercles have reached their maximum development the bacilli have already undergone extensive destruction and are disappearing.

4. Tubercle bacilli of moderate virulence (human type and BCG) are usually effectively destroyed within epithelioid cells of all organs. In the lung and kidney bovine bacilli persist within epithelioid cells but in other organs they are usually destroyed.

5. Tubercle bacilli are less effectively destroyed within epithelioid cells collected in the alveoli of the lung than in those forming tubercles in the interstitial tissues.

6. After multiplication of tubercle bacilli has ceased regeneration of mononuclears by mitosis becomes less active and now Langhans' giant cells may be formed from preexisting epithelioid cells.

7. Lymphocytes and encapsulation of tubercles by granulation tissue do not cause the destruction of tubercle bacilli.

8. Immediately after infection accumulation of the less virulent

types of tubercle bacilli in the tissues does not cause caseation, and the more virulent bovine bacillus produces this change only in the lung. Later caseation occurs in the presence of a small number of bacilli, and must be thought of as due, in part at least, to sensitization.

REFERENCES

1. Lurie, M. B., *J. Exp. Med.*, 1928, **48**, 155.
2. Lubarski, W. A., and Korshinskaja, A. F., *Z. Tuberk.*, 1931, **59**, 252.
3. Lewis, P. A., and Sanderson, E. S., *J. Exp. Med.*, 1927, **45**, 291.
4. Sabin, F. R., Doan, C. A., and Cunningham, R. S., *Carnegie Institution of Washington, Pub. No. 82, Contributions to Embryology*, 1925, **16**, 125. Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwal, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37**, 231.
5. Lewis, M. R., Willis, H. S., and Lewis, W. H., *Bull. Johns Hopkins Hosp.*, 1925, **36**, 175. Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1926, **44**, 285. Gardner, L. U., and Smith, D. T., *Am. J. Path.*, 1927, **3**, 445. Clark, E. R., and Clark, E. L., *Am. J. Anat.*, 1930, **46**, 149.
6. Forkner, E. F., *J. Exp. Med.*, 1930, **52**, 385.
7. Cash, J. R., *Proc. Soc. Exp. Biol. and Med.*, 1926, **24**, 193. Gardner, L. U., *Proc. Soc. Exp. Biol. and Med.*, 1927, **24**, 646.
8. Castrén, H., *Arb. path. Inst. Helsingsfors*, 1923, **3**, 191; as quoted by W. Pagel, Die Allgemeinen pathomorphologischen Grundlagen der Tuberkulose, Berlin, 1927.
9. Lang, F. J., *J. Infect. Dis.*, 1925, **37**, 430.
10. Kraus, R. and Hoffer, G., *Deutsch. med. Woch.*, 1912, **38**, 1227. Manwaring, W. H., and Bronfenbrenner, J., *J. Exp. Med.*, 1913, **18**, 601. Rist, E., Kindberg, L., and Rolland, J., *Ann. méd.*, 1914, **1**, 310, 375.
11. Evans, H. M., Bowman, F. B., and Winternitz, M. C., *J. Exp. Med.*, 1914. **19**, 283.
12. Rogers, J. C., *Am. Rev. Tuberc.*, 1928, **17**, 192.
13. Lurie, M. B., *J. Exp. Med.*, 1929, **50**, 747.
14. Spengler, C., *Z. Hyg. u. Infectionskrankh.*, 1905, **49**, 541.
15. Sweany, H. C., *J. Am. Med. Assn.*, 1926, **87**, 1206.
16. Kahn, M. C., *Am. Rev. Tuberc.*, 1929, **20**, 150.
17. Petroff, S. A., Branch, A., and Steenken, W., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1927, **25**, 14. Sasano, K. T., and Medlar, E. M., *Tubercle*, 1931, **12**, 214; *Am. Rev. Tuberc.*, 1931, **23**, 1215. Dreyer, G., and Vollum, R. L., *Lancet*, 1931, **1**, 9.
18. Drinker, C. K., and Shaw, L. A., *J. Exp. Med.*, 1921, **33**, 77. Lund, C. C., Shaw, L. A., and Drinker, C. K., *J. Exp. Med.*, 1921, **33**, 231.
19. Corper, H. J., and Lurie, M. B., *Am. Rev. Tuberc.*, 1926, **14**, 680.
20. Novy, F. G., and Soule, M. H., *J. Infect. Dis.*, 1925, **36**, 168.

21. Corper, H. J., Lurie, M. B., and Uyei, N., *Am. Rev. Tuberc.*, 1927, **15**, 65.
22. Loebel, R. O., Shorr, E., and Richardson, H. B., *Tr. Nat. Tuberc. Assn.*, 1930, 26th Annual Meeting, 196.
23. Sabin, F. R., and Doan, C. A., *J. Exp. Med.*, 1927, **46**, 627.
24. White, W. C., *Arch. Path. and Lab. Med.*, 1927, **3**, 84.
25. Doan, C., and Sabin, F. R., *J. Exp. Med.*, 1930, **52**, suppl. 3, 113.
26. Medlar, E. M., *Am. J. Path.*, 1926, **2**, 291.
27. Maximow, A., *Ann. Inst. Pasteur*, 1928, **42**, 225; *J. Infect. Dis.*, 1924, **34**, 549.
28. Krause, A. K., *Am. Rev. Tuberc.*, 1927, **15**, 137.
29. Huebschman, P., *Beitr. Klin. Tuberk.*, 1927, **67**, 186.
30. Pagel, W., *Jahreskurs ärztl. Fortbild.*, 1928, **19**, 9.
31. Rich, A. R., and McCordock, H. A., *Bull. Johns Hopkins Hosp.*, 1929, **44**, 273.
32. Schleussing, H., *Beitr. path. Anat. u. allg. Path.*, 1929, **81**, 473.

EXPLANATION OF PLATES

All microphotographs were made from sections stained by the Ziehl-Neelsen method and counterstained with hematoxylin.

PLATE 1

FIG. 1. Lung of Rabbit 15–53, 1 week after infection with BCG; 11,000 colonies were isolated. There is a thickening of the alveolar septa and an occasional early tubercle. The alveoli are free from exudation. Magnification about ×70.

FIG. 2. Lung of Rabbit 15–51, 2 weeks after infection with BCG; 2,930 colonies were isolated. There are numerous tubercles with exudation into the alveoli. Magnification about ×70.

FIG. 3. Lung of Rabbit 15–54, 4 weeks after infection with BCG; 655 colonies were isolated. There is extensive tuberculosis with caseation and a conspicuous exudation into the alveoli. Magnification about ×70.

FIG. 4. Higher magnification of an early tubercle in the lung shown in Fig. 1. The core of young epithelioid cells, one of which contains a number of tubercle bacilli in its cytoplasm, is surrounded by numerous mononuclears. There is no caseation. Magnification about ×900.

FIG. 5. Portion of a tubercle in the lung of Rabbit 15–83, 2 weeks after infection with BCG; 4,300 colonies were isolated. Note the well defined cell boundaries, the compact, almost homogeneous structure of the cytoplasm and, in one cell, the differentiation of the latter into a central light and a peripheral dark zone. No tubercle bacilli could be found in these mature epithelioid cells. Magnification about ×900.

FIG. 6. Portion of a tubercle in the lung of Rabbit 15–51, shown in Fig. 2, 2 weeks after infection, showing caseation with accumulation of polymorphonuclear cells.

PLATE 2

FIG. 7. A tubercle in the lung of Rabbit 15–06, 2 months after infection with BCG; 40 colonies were isolated. There is a thick wall of lymphocytes investing

the tubercle. The epithelioid cells are disintegrating and some contain faintly acid-fast globules clumped in the form of hollow spheres. Magnification about ×200.

FIG. 8. Two interstitial epithelioid cell tubercles in the lung of Rabbit 25–79, 4 weeks after infection with tubercle bacilli of the human type; 12,500 colonies were isolated from this lung. No bacilli could be found in these tubercles. Magnification about ×200.

FIG. 9. An area of caseous pneumonia in another part of the lung shown in Fig. 8. Magnification about ×200.

FIG. 10. Part of Fig. 9 under higher magnification, showing numerous tubercle bacilli in a plug of caseous material within an alveolus. Magnification about ×900.

PLATE 3

FIG. 11. The lung of Rabbit 22–17, 2 months after infection with tubercle bacilli of the bovine type; 200,000 colonies were isolated. Massive tuberculous pneumonia with very extensive caseation of interstitial tubercles. Magnification about ×200.

FIG. 12. The lung of Rabbit 20–95, 2 months after infection with tubercle bacilli of bovine type; 3,100 colonies were isolated. The interstitial process predominates. Caseation is less extensive, fibroblasts and lymphocytes surround the tubercle. Magnification about ×200.

FIG. 13. Malpighian corpuscle of the spleen of Rabbit 25–76, 2 weeks after infection with tubercle bacilli of human type; 1,380 colonies were isolated. Mitosis is frequent; there are no epithelioid cells. Magnification about ×900.

FIG. 14. Spleen of Rabbit 25–79, 4 weeks after infection with tubercle bacilli of the human type; 416 colonies were isolated. Extensive tuberculosis of Malpighian corpuscle with caseation. Magnification about ×140.

PLATE 4

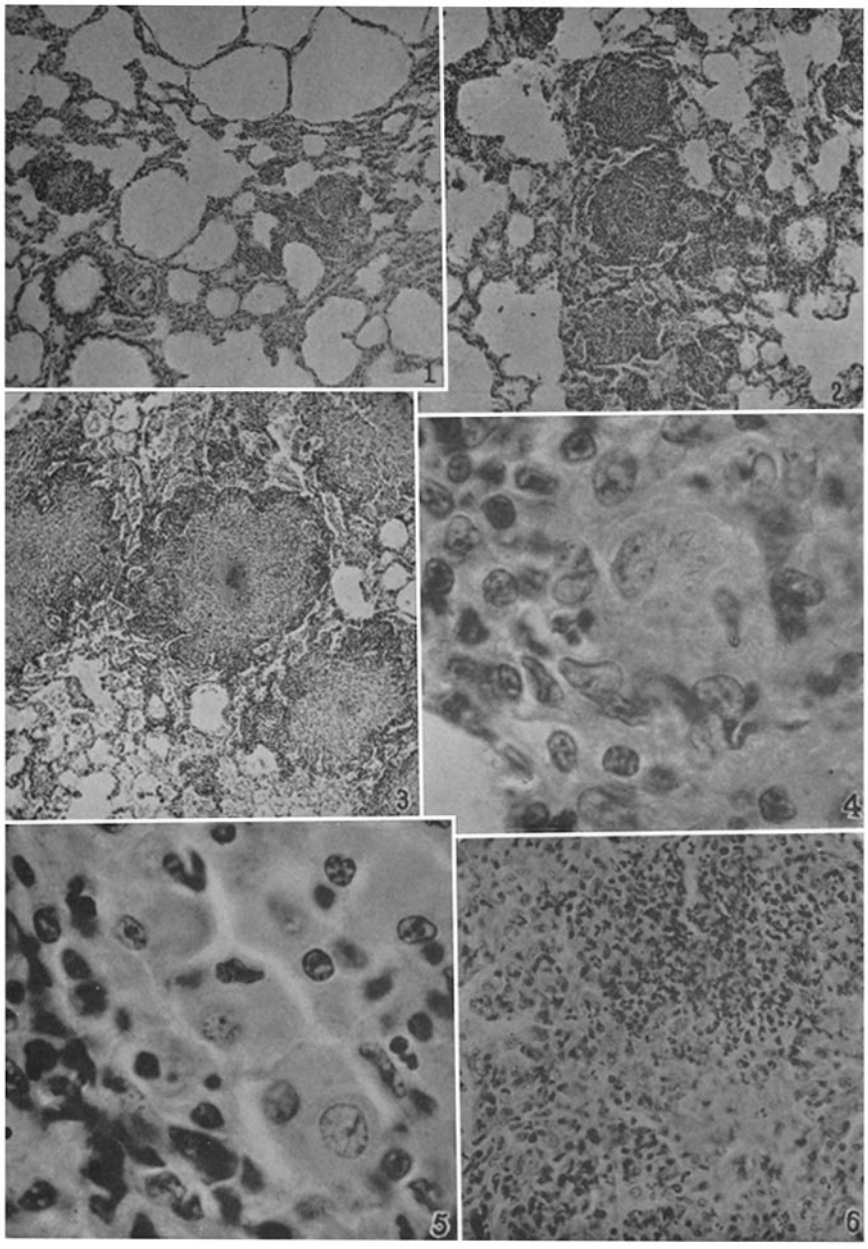
FIG. 15. Malpighian corpuscle of speen of Rabbit 22–75, 2 weeks after infection with tubercle bacilli of the bovine type; 2,410 colonies were isolated. There are no epithelioid cells. Magnification about ×200.

FIG. 16. Malpighian corpuscle of spleen of Rabbit 25–86, 2 weeks after infection with tubercle bacilli of bovine type; 1,425 colonies were isolated. Note extensive formation of epithelioid cells. Magnification about ×200.

FIG. 17. Mature epithelioid and giant cell tubercle in the pulp of the spleen adjacent to the Malpighian corpuscle shown in Fig. 16. Acid-fast particles were present within the cells. Magnification about ×200.

FIG. 18. Liver of Rabbit 22–50, 6 weeks after infection with tubercle bacilli of the bovine type; 2 colonies were isolated. Lymphocytes and giant cells are conspicuous. Magnification about ×200.

FIG. 19. The cortex of the kidney of Rabbit 22–76, 4 weeks after infection with tubercle bacilli of the bovine type; 297 colonies were isolated. The process of descending infection by way of the tubules is shown. Magnification about ×200.



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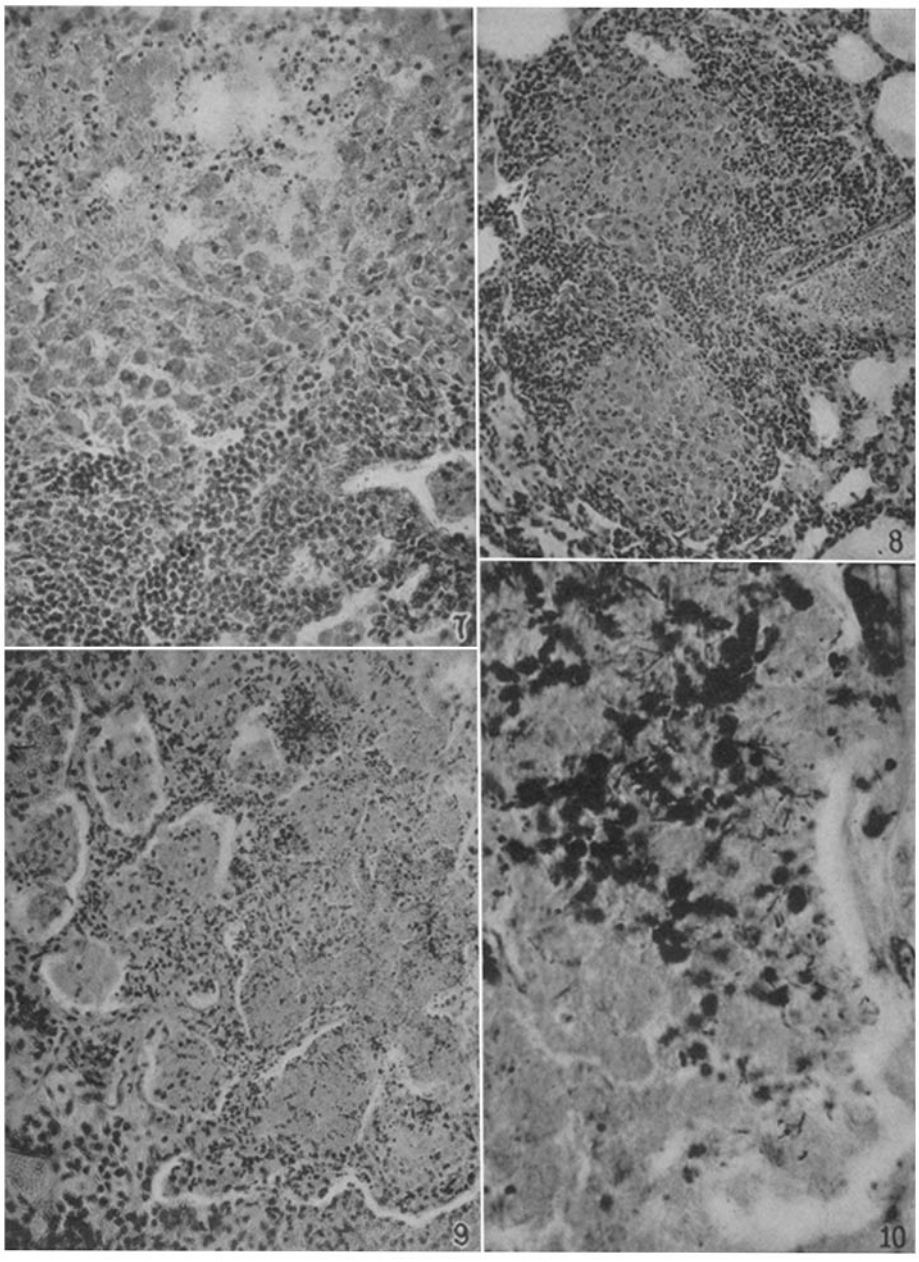

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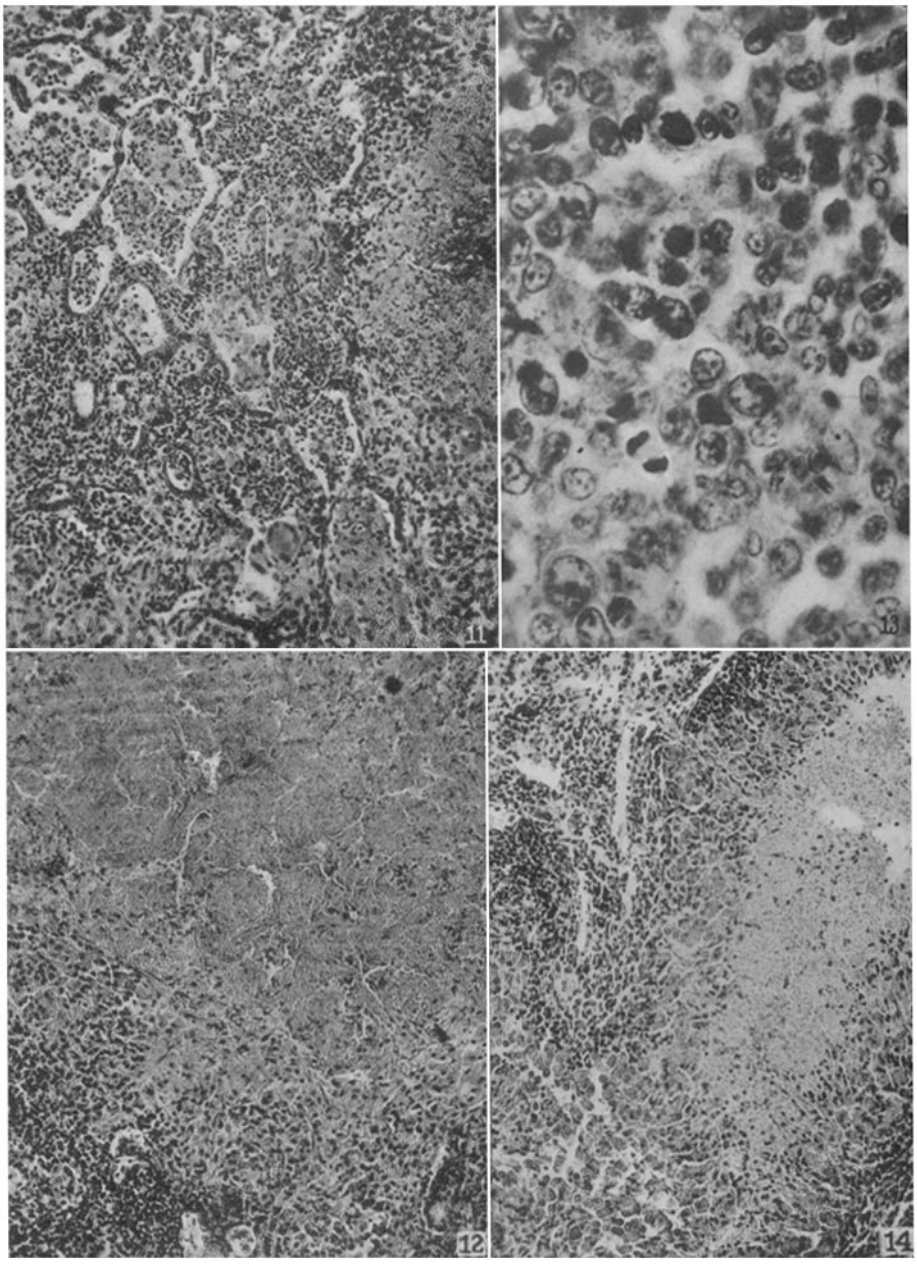

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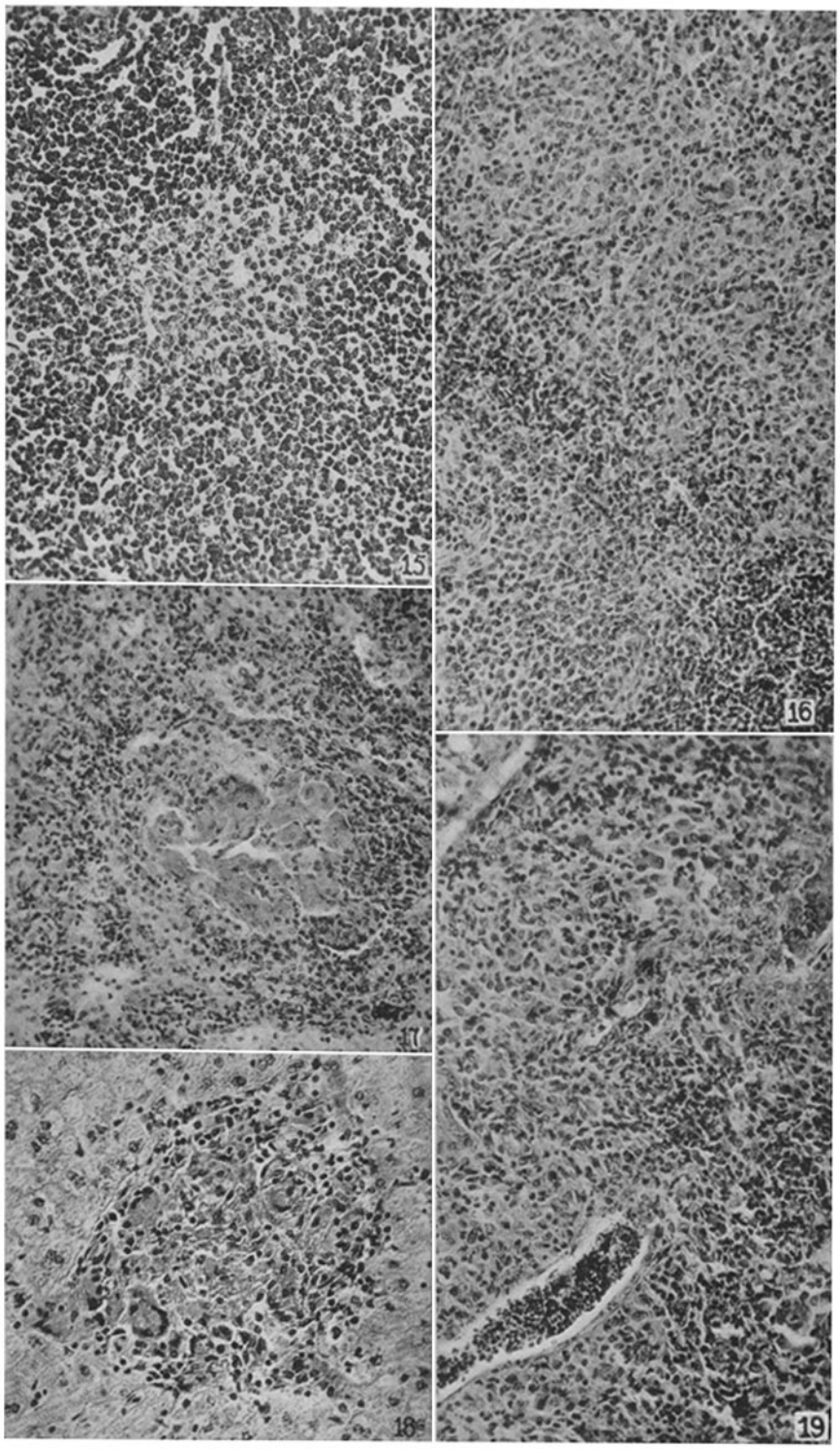

(Lurie: Tubercle bacilli)