TUBERCULOSIS OF RABBITS INDUCED BY DROPLET NUCLEI INFECTION

I. Initial Response to Infection*

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Plates 9 to 14

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The significance of droplet nuclei infection in the spread of bacterial and virus diseases has been established by experimental and epidemiological studies (1–3). Experiments also have demonstrated that transport of droplet nuclei infection into the alveolar tissues of the lungs by the air currents of normal respiration depends upon the settling velocity of droplet nuclei (4). The experiments by which the relation of settling velocity of droplet nuclei to their transport by the air currents of normal respiration was determined used virulent tubercle bacilli as indicator organisms and rabbits as test animals. These animals inhaled the organisms as separated cells in droplet nuclei. Thus the tubercles which developed in their lungs were known to be induced by organisms derived from single cells, all of which were implanted upon alveolar tissue within relatively brief intervals.

During a period of about 6 weeks following infection these tubercles developed at a remarkably uniform rate which was not appreciably influenced either by the number of lesions or by their position in the lungs (4). These observations are consistent with the results of similar experiments which involved rabbits of families of high and low levels of resistance to tuberculosis (3). Thus it seems that the initial response of normal rabbits to virulent bovine tubercle bacilli, inhaled as separated cells in droplet nuclei, may be said to be homogeneous. The homogeneous phase of inhaled tuberculosis, as observed in these experiments, contrasts sharply with later stages of the infection which are well known to be strikingly heterogeneous, especially when rabbits of various strains are subjected to infection. This observation suggests that rabbits do not differ in their inherent resistance to bovine tubercle bacilli. Instead, they differ in their capacity to acquire resistance. Additional evidence which supports this hypothesis is provided by a study of the sequence of histological changes during the homogeneous phase of inhaled tuberculosis and of its transition into the

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heterogeneous phases of the reaction. These features of air-borne tuberculosis will be described in this paper.

Material and Methods

Animals were infected by organisms of the Ravenal strain of *Mycobacterium tuberculosis (bovis)* which had been grown in a liquid medium in rotating flasks containing glass beads. This method has been found to stimulate rapid growth of the organisms, to a large extent in the form of separated cells (5).

Infections were induced by newly developed methods and apparatus. This apparatus consisted of an aerosol flask, an inhalation chamber, and an incinerating chimney connected in series. Animals exposed in this apparatus inhaled virulent organisms, air-borne as separated cells in fine droplet nuclei, under standardized conditions (6). The procedure for a typical experiment was as follows:—

Aqueous suspensions of separated organisms were prepared by passing a liquid culture through No. 4 Whatman filter paper. Density of organisms in this filtrate was determined by the Breed method, which had been found to give results consistent with colony counts of suspensions planted on the Crumb medium (7). Then the filtrate was diluted to a level which would provide the desired concentration of bacilli in the aerosol, and the dilute suspension introduced into the aerosol flask.

The aerosol flask was made from a Florence flask by blowing an auxiliary neck tangential to its equator. This flask was attached in the horizontal position by its main neck to the intake portal of the system, with the auxiliary neck rotated above the level of the bacterial suspension. A Venturi nozzle and throat, carefully designed for efficient atomization, was fitted into the auxiliary neck of the flask. The base of this nozzle extended to the exterior of the flask through a rubber stopper to connect with a tank of compressed air. Adjustments being completed, the flask was rotated on its horozintal axis to bring the fluid into the auxiliary neck about the base of the Venturi throat to allow the bacterial suspension to enter. Opening the valve to the air line allowed a high velocity jet of air to draw fluid from the Venturi throat and whirl it into a cloud of droplets about the equator of the flask. Larger droplets impinged upon the wall of the flask and returned to the pool of liquid. Only the smallest ones evaporated rapidly enough to produce droplet nuclei which were carried by the air stream from the core of the cloud into the system through the horizontal neck of the flask. Occasional droplet nuclei contained tubercle bacilli.

The aerosol passed from the flask into the inhalation chamber through a large duct which entered the bottom of this chamber beneath a false floor. This floor was perforated at its periphery. The inhalation chamber, a hexagonal metal box, accommodated a modified Murphy respirator on each of its sides. Rabbits were placed in the respirators with the rubber collars about their necks. When the respirators were bolted in place, these collars served as gaskets to seal the apertures. This arrangement admitted only the heads and necks of the animals into the inhalation chamber in positions above the perforations in the false floor, while the air flowed from beneath upward and, after passing the noses of the rabbits, was drawn downward through a central outlet to the exhaust duct. The exhaust duct connected with the incinerating chimney, which served to sterilize the aerosol and to maintain negative pressure within the system.

The concentration of organisms in the aerosol and the length of exposure determined the intensity of infection. When an exposure had been completed and atomization was stopped, the system was cleared by ventilation and ultraviolet irradiation, after which the animals were removed and returned to their cages.

Fifty-six albino rabbits, all purchased from one source, were used in these experiments. These animals weighed about 2 kilos each when they were exposed to infection. They were

kept in individual cages and fed a commercial mixture supplemented by carrots. All of them harbored species of *Eimeria*, but accidental bacterial disease was not found.

Animals which were killed for study were bled from the carotid artery. When respiration had stopped, the trachea, lungs, and heart were removed with intercommunicating channels intact and the lungs were partially deflated by immersion in formalin diluted to 6 per cent in 0.85 per cent aqueous sodium chloride solution. Following partial deflation the lungs were moderately distended by introducing fixative into the trachea through a pipette and bulb. Repeated distention and immersion into the fixative removed the greater part of the alveolar air, after which the trachea was clamped and the organs fixed for several days. Lungs of rabbits that died of tuberculosis were also fixed in this way, but rarely could they be so completely filled by the fixative. As judged by histological appearances, however, fixation was equally satisfactory.

Sections were cut at 5 microns and stained by carbol fuchsin and Mayer's hemalum. The number of blocks of tissue taken from any set of lungs for section varied with the character of the disease. In the study of the early stages of the infection, for example, blocks of tissue were taken from each lobe of the lungs and 10 to 20 serial sections cut from each block. Each of these series was estimated to contain 5 to 10 c.mm. of tissue.

EXPERIMENTS

1. The Initial Reaction of the Lung to Air-Borne Tubercle Bacilli

This phase of the study utilized 16 rabbits, each of which was estimated to have inhaled upwards of 20,000 organisms. Six of the animals were infected as one exposure group and killed in pairs, 1, 2, and 3 days after infection. Other animals were infected in pairs and killed in pairs 2, 5, 6, 9, and 12 days after infection.

Under the conditions of the experiment bovine tubercle bacilli, deposited in the lungs of previously uninfected rabbits, as individual cells, did not excite inflammatory response for about 12 days. During this interval the organisms became increasingly abundant within the alveolar macrophages, as seen in sections, without inducing visible changes in these cells or in the surrounding tissues.

Tubercle bacilli were found in each of the series of sections cut from the lungs of these rabbits. But, in the sections from animals killed within 7 days after infection, prolonged search was required. The lungs of these animals, when filled but not forcibly distended by fluid, displaced about 50,000 c. mm. Since about one-half of this volume was occupied by bronchi, bronchioles, and blood vessels upon which the tubercle bacilli apparently did not gain a foothold, the inhaled organisms were deposited in about 20,000 c. mm. of susceptible lung tissue.

1st Week.—During the 1st week after infection the tubercle bacilli invariably were found in isolated alveolar macrophages which were more or less loosely attached to the walls of otherwise normal alveoli, and recognizable by carbon particles in their cytoplasm. As a rule that part of the cytoplasm of the macrophage included in a section contained a single, solidly stained bacillus. Oc-

casionally, however, 2 or 3 organisms were found in a section of a cell. The average frequency of infected macrophages was upwards of one in 200 sq. mm. of tissue section. Since sections approximated 5 microns in thickness, this number corresponded to the number of bacilli estimated to have been inhaled; *i.e.*, one organism per cubic millimeter. The frequency of infected macrophages did not increase appreciably during this 1st week of infection.

2nd Week.—Between 6 and 9 days after infection, however, the numbers of infected macrophages seemed to have increased by about tenfold. In the lungs of animals killed 9 days after infection a majority of the infected cells still were isolated macrophages (Fig. 1). Less frequently the infected cells were found in small groups (Fig. 2). Alveoli containing the infected cells were unchanged otherwise.

Between 9 and 12 days after infection the collections of parasitized macrophages, as seen in serial sections of the lungs of 4 rabbits killed at these intervals, seemed to have increased rapidly in size. As judged by this material, the rate of increase in the size of these foci of infection was more pronounced during this interval than from 6 to 9 days, and the numbers of bacilli within these foci were increased correspondingly (Figs. 3 and 4). This apparently accelerated development of the infection was in keeping with progressive growth of the organisms, if this followed approximately the expected normal rate.

After 12 days of development a majority of infected foci occupied only one or two alveoli, but occasional ones had spread into several adjoining air spaces. Usually the alveoli were compactly filled by alveolar macrophages, which fused into dense masses and sometimes formed giant cells which resembled cross-sections of poorly defined tubules (Figs. 5 and 6).

At this time leukocytes and small monocytes had begun to accumulate about the developing tubercles. In some lesions numerous leukocytes clustered about the infected macrophages and penetrated into the masses of cells. The small monocytes were most conspicuous in the alveolar walls about the infected cells where they had become sufficiently numerous to thicken the tissue.

In addition to the reaction which has been described, scattered acute focal inflammatory lesions developed in the lungs of more than half of the animals in this series. These foci were well developed in 24 hours in one rabbit and seemed to be disappearing after 3 to 6 days, as judged by appearances in sections of lungs examined at these intervals (Figs. 7 and 8). In some animals they were found in all parts of the lungs; in others only one or two series of sections contained them. Their centers were composed of alveolar macrophages, with scattered leukocytes among these cells and about their outer borders. More abundant than the leukocytes were small, dark staining cells which resembled lymphocytes. These cells were most numerous about the periphery of the lesions, apparently having moved into the foci from blood vessels and capillaries.

An explanation for these lesions is not apparent. The rabbits inhaled from 10 to 100 uninfected droplet nuclei for every one which contained a bacillus.

If these lesions were reactions to the uninfected droplet nuclei they should have been much more abundant and more uniformly distributed. But they were sparsely developed and irregularly distributed. Hence they are considered to be non-specific reactions. They are described because it was inevitable that occasional ones would be infected, in view of the numbers of organisms inhaled by these animals. Apparently, even when infection occurred, these non-specific foci underwent some degree of regression, but the alveolar macrophages and the small, dark cells persisted in small numbers as the bacilli increased. The progress of these lesions seemed to correspond to that of ones which apparently developed from simple infection of isolated alveolar macrophages. This opinion is based upon study of infected non-specific foci found in the lungs of one rabbit killed 72 hours after infection, and of another killed 9 days after infection (Fig. 9).

2. Development and Progress of Initial Tubercles

Beyond the 2nd week of its development the response of the lungs to initial infection by separated bovine tubercle bacilli has been studied in 40 rabbits. Fourteen of these died or were killed between 16 and 28 days, 14 between 32 and 43 days, 6 between 56 and 72 days, and 6 after 100 or more days of infection. The number of organisms inhaled by these animals during the single exposure to which they were subjected varied, by the plan of the experiment, from less than 10 to more than 20,000.

3rd Week.—The tubercles became visible by the end of the 3rd week as translucent, pale foci, 1 to 2 mm. in diameter. The histological preparations suggested the following pattern of development during this period.

Growth of the bacilli in the alveolar macrophages continued unchecked and these masses of infected cells spread to adjacent alveoli through the alveolar ducts. With this growth an inflammatory reaction developed rapidly about the foci of bacterial growth. Within 18 to 20 days alveolar macrophages had disappeared as intact cells to leave the bacilli concentrated among their fragments while leukocytes and monocytes or histiocytes continued to move into the mass and distintegrated (Fig. 10).

The spaces which contained these centers of bacterial growth often were lined by flattened basophilic cells, apparently monocytes. Alveolar walls immediately about the centers became increasingly thickened and alveolar spaces filled by monocytes among which were smaller number of leukocytes and lymphocytes. Occasional monocytes near the inner border of the mass of inflammatory cells contained bacilli.

By the end of the 3rd week of growth the tubercles consisted of relatively wide inflammatory zones centering about the spaces in which the bacilli were concentrated. Toward the periphery the alveoli tended to be free of cells although alveolar walls were infiltrated well beyond this zone.

4th Week.—In the lungs of rabbits killed 28 days after infection tubercles

appeared as moist, translucent nodules, 4 or 5 mm. in diameter, outlined by narrow borders of injected capillaries. In sections the greater part of the nodules was composed of the zone of inflammatory cells. The necrotic centers seemed to have expanded less rapidly and, in expanding, seemed to follow alveolar ducts, so that their outlines became irregular. This expansion of the necrotic centers into alveolar ducts seemed to account for the contact between them and the lumina of the terminal bronchioles (Fig. 11). However, evidence of bronchial spread of the infection was not found until much later (Fig. 12).

Late in the 4th week of infection spread of the organisms from the initial lesions along perivascular and peribronchial lymph channels was found and, within this period, epitheloid tubercles developed in the tracheobronchial lymph nodes of some animals.

5th and 6th Weeks.—Within the 5th week after infection all tubercles underwent more or less extensive central caseation which separated the masses of bacilli and necrotic cells in the core of the lesions from the cells of the inflammatory zone. During this time the rate of peripheral expansion of the tubercles decreased; apparently with caseation fewer macrophages and leukocytes migrated into the mass, and the width of the caseous zone came to exceed that of the inflammatory zone. During the 5th week, also, differences in the rate of progress of the tubercles from animal to animal became evident, although in the lungs of any one animal all lesions were remarkably uniform in size.

On the whole, smaller tubercles seemed to be most completely caseous and, in these, the bacilli were more distinctly concentrated in the central zone. Often organisms could be found only in these central zones, enclosed in spaces which corresponded to alveolar ducts and were outlined by poorly stained reticulum fibers.

By the end of the 6th week of infection differences in the rate of progress of the disease from animal to animal were well developed. The reaction to infection had become heterogeneous. Thus in the lungs of some of the rabbits which were killed 43 days after infection, tubercles were scarcely larger than at 5 weeks. They were well circumscribed, with extensive central zones of caseation and relatively narrow borders of inflammation. Evidence of spread of the organisms by way of the bronchi was not found, nor had tubercles developed in other organs. Other animals at 6 weeks had well developed secondary tubercles both of the lungs and kidneys. In the lungs, caseous material containing large numbers of bacilli apparently had drained into the connecting bronchioles and had been deposited in adjacent parts of the lung. This resulted in the initial lesions being surrounded by clusters of satellite tubercles. Or the infection had spread by the blood stream and many small tubercles had formed where bacilli had chanced to lodge in the lungs and kidneys (Figs. 13 to 16). The size of the secondary tubercles which were found after 6 weeks

of infection suggested more than 2 weeks' growth but, without knowing the numbers of organisms by which such lesions were initiated, there was no basis for an estimate of age.

These differences in the rate of progress of the infection during the 5th and 6th weeks sometimes could be related to the intensity of initial infection. Three of 6 rabbits which had inhaled approximately 150 bacilli each, exhibited macroscopic secondary tubercles of the lungs and kidneys when they were killed 43 days after infection. In 6 other rabbits, each of which had inhaled about 10 bacilli, the infection was limited to the initial tubercles except for spread to the tracheobronchial lymph nodes.

Later Phases.—Progress of individual tubercles in the lungs of any rabbit varied after 6 or 7 weeks. Some tubercles in the 5 rabbits killed 7 to 10 weeks after infection had not expanded beyond diameters of 5 or 6 mm. Others had enlarged to about 10 mm. in 10 weeks (Figs. 17 and 18). The expanding ones, more often than not, were located beneath the pleura and tended to enlarge along this surface rather than in other directions. The pleura was considerably thickened by fibrosis but other surfaces of the lesions were bounded by dense zones of epitheloid cells among which were smaller numbers of lymphocytes, monocytes, and leukocytes.

The smaller tubercles were sharply circumscribed in the tissue while the larger ones were associated with numbers of satellite lesions. Unless there was obvious evidence of drainage of the caseous centers into bronchioles the numbers of demonstrable bacilli in the initial tubercles at 10 weeks were less than 10 per cent of the numbers present at 5 or 6 weeks. Likewise, the numbers of infected monocytes among the cells of the inflammatory zone seemed to have been reduced in these relatively inactive tubercles. These estimates were based upon study of serial sections taken through the centers of a number of representative lesions.

When, however, the tubercles were draining into bronchi, the numbers of bacilli within them were enormously increased. At the same time the zone of inflammation about the periphery of the initial lesions decreased and they became thin walled cavities into which leukocytes and monocytes migrated, to disintegrate among the masses of bacilli. Fibrosis about such cavities was very slight.

The 6 rabbits examined more than 100 days after infection all carried more than 30 initial tubercles, and all had developed a number of cavities (Figs. 19 and 20). Usually these were located in the dorsal parts of the lungs and the infection apparently spread, mainly by gravity, to the dependent parts of these organs. However, some of the cavities, also, were located on the ventral surfaces.

Among these 6 animals there were striking differences in the rate of spread

of the infection. However, the rate of progress of the infection did not seem to be related to the major pathway of spread, which, in these animals, was the bronchi rather than the blood stream.

DISCUSSION

Under the conditions of these experiments the reaction between the lungs of normal rabbits and virulent bovine tubercle bacilli, inhaled as separated cells in droplet nuclei, was completely homogeneous for about 4 weeks. The separated bacilli were ingested by alveolar macrophages and seemed to grow progressively in these cells without causing obvious damage to them for about 2 weeks. Apparently infected cells did not wander from alveolus to alveolus to an appreciable degree. Instead, other alveolar macrophages, recognizable by carbon particles in their cytoplasm, moved into the alveoli in which the bacilli had been deposited. How these cells came to contain organisms, unless the macrophages which were infected originally, disintegrated, cannot be imagined. Yet no cell which seemed to be degenerating was found in the infected alveoli before 12 days had passed.

Tubercle formation may be said to have started about the end of the 2nd week of the infection. At this time the alveolar macrophages had fused into imperfect giant cells or had become vacuolated. Progressive development of the inflammatory reaction and growth of the bacilli apparently continued unchecked until the tubercles underwent caseation during the 5th week of infection. Thereafter the reaction to the infection became heterogeneous. No evidence of delayed tubercle formation was encountered; this has been postulated to occur when air-borne infection was induced by another technique (8).

Differences in the rate of progress of the infection after 5 or 6 weeks did not seem to be related to differences in the character of the inflammatory response. Instead, heterogeneity of the pattern of disease could be related to the intensity of the inflammation which, in turn, seemed to correspond to the differences in the growth rate of the bacilli after the 4th week, these differences, of course, being estimated from the number of organisms found in the lesions. This, it must be admitted, was a crude measure at best. Yet careful study of serial sections of representative tubercles revealed differences in the numbers of demonstrable bacilli of such magnitude that more exact methods hardly seemed necessary. It appears worth considering that heterogeneity of progressive tuberculosis may be related to differences in the capacity of animals to change the composition of their tissues or blood in such a way that growth of the bacilli may be more or less inhibited.

The original purpose of this study was to evaluate tubercle bacilli as indicator organisms in investigations of the dynamics of droplet nuclei infection (4). When it became evident that, under the conditions of these experiments, the rate of development of initial tubercles did not vary with the animal until after 5 or 6 weeks of growth, a more complete study of the early phases of air-borne tuberculosis seemed advisable.

The homogeneous phase of tuberculosis, as disclosed by this study, seems to have been overlooked in most investigations of the immediate response to infection (9–12). Possible reasons for this are: (1) methods used to induce infections may have implanted more than single organisms in the average focus, (2) the rate of tubercle development is not the same in all tissues, and (3) the rate of tubercle development may differ with the species. Furthermore, animals may react to the medium in which the bacilli are suspended, or non-certain specific reactions may be confused with the response to the infection. Therefore it seems unwise to attempt to compare the results of different experiments, unless infection techniques be equally precise, and the response to infection studied in the same tissue. However, by the use of methods which approximated those of the present experiments, the early response of guinea pigs to inhaled tubercle bacilli has been found to correspond to that of rabbits as here reported (13).

SUMMARY AND CONCLUSIONS

Rabbits were caused to inhale known numbers of virulent bovine tubercle bacilli as separated cells in droplet nuclei.

For approximately 5 weeks after infection the growth of the bacilli and the response of rabbits to their growth was homogeneous; *i.e.*, all reacted in the same way and to the same degree.

After 6 weeks individual differences in the rate of progress of the initial tubercles and of the infection as a whole became evident. These variations in the response seemed to be influenced by the number of initial tubercles and by the number of bacilli found in the lesions.

It is concluded that, as evidenced by the homogeneous phase of infection, rabbits do not differ in their resistance to initial growth of bovine tubercle bacilli. However, the later, heterogeneous pattern of response suggests that these animals vary widely in their capacity to acquire resistance.

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EXPLANATION OF PLATES

Photography by Mr. Basil Varian, Department of Anatomy, University of Pennsylvania.

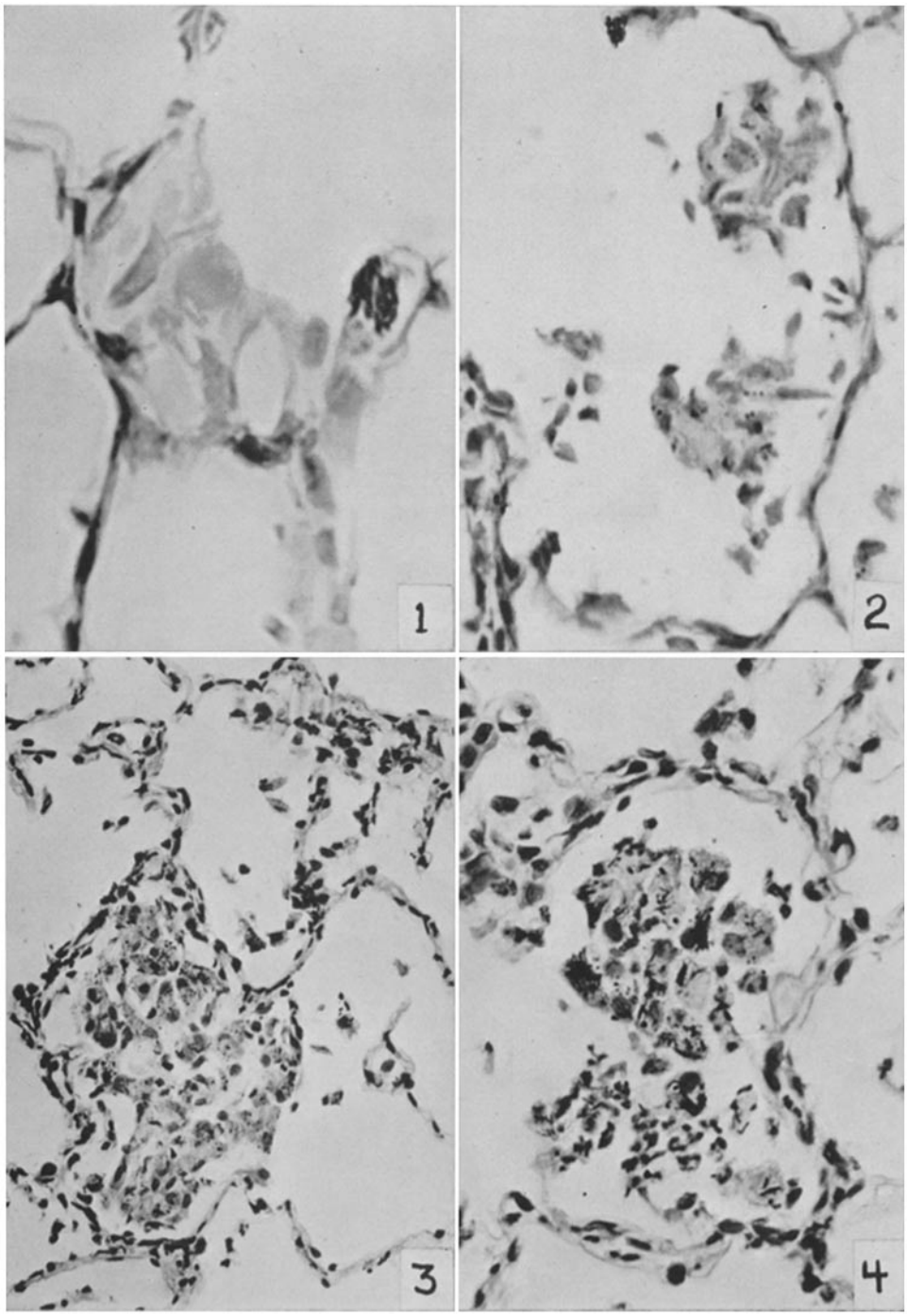
PLATE 9

FIG. 1. Rabbit 14-8. Tubercle bacilli in the cytoplasm of an alveolar macrophage 9 days after inhaled infection. × 1100.

FIG. 2. Rabbit 14-7. A group of infected alveolar macrophages 9 days after inhaled infection. The bacilli are distributed as solid black rods among these cells which also contain carbon particles. The small cells of this alveolus appear to be monocytes. Note that the alveolar walls about the infected cells in Figs. 1 and 2 are unchanged. × 700.

FIG. 3. Rabbit 14-4. A focus of infection after 12 days' development. The alveolus is filled chiefly by alveolar macrophages, a majority of which contain bacilli. Monocytes are present in small numbers among and about these cells and have accumulated in the walls of the adjacent alveoli. × 400.

FIG. 4. Rabbit 14-4. An infected focus after 12 days' development. This illustrates the accumulation of leukocytes and monocytes about the cluster of infected macrophages. × 600.

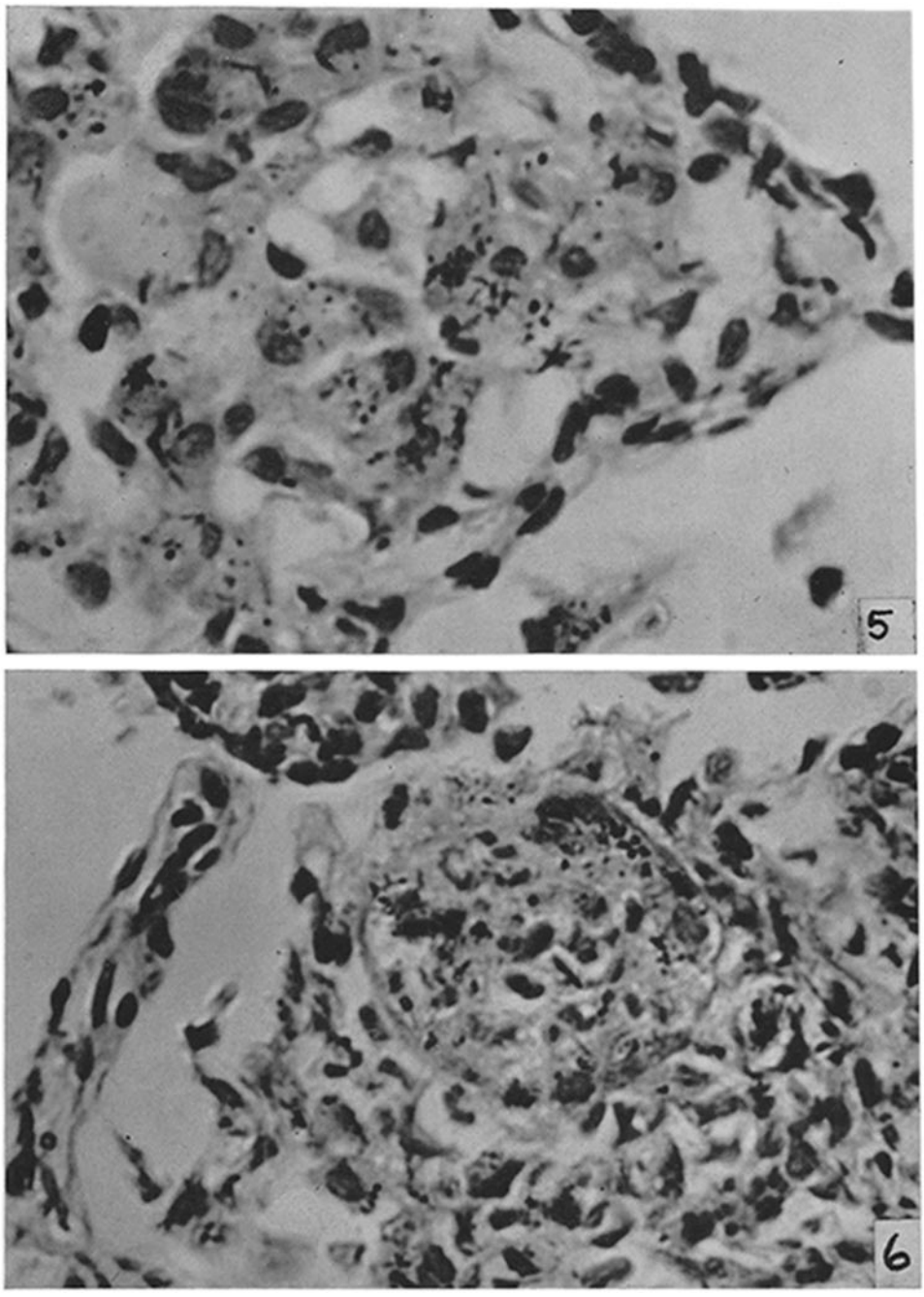


(Ratcliffe and Wells: Tuberculosis of rabbits. I)

PLATE 10

FIG. 5. A part of the focus of infection shown in Fig. 3, at a higher magnification. Bacilli and carbon granules are seen within the cytoplasm of the fused mass of alveolar macrophages. The upper and lower right edges of this mass of infected cells are bordered by the thickened wall of the alveolus. × 1100.

FIG. 6. Another infected focus from rabbit 14-4, 12 days after infection, illustrating the fusion of infected alveolar macrophages to form giant cells. Carbon granules and bacilli are scattered through this fused mass of cells. Monocytes and leukocytes surround the mass. × 1100.



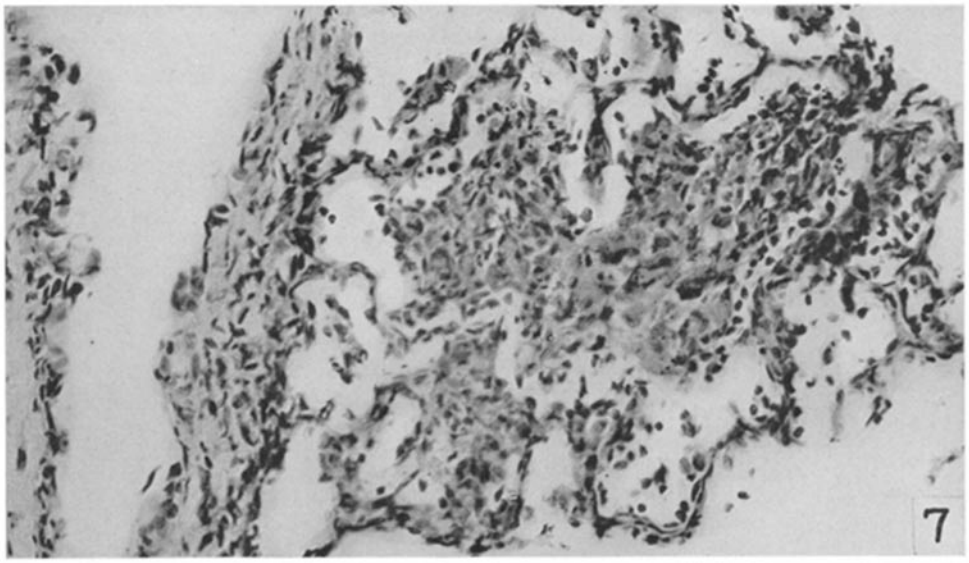
(Ratcliffe and Wells: Tuberculosis of rabbits. I)

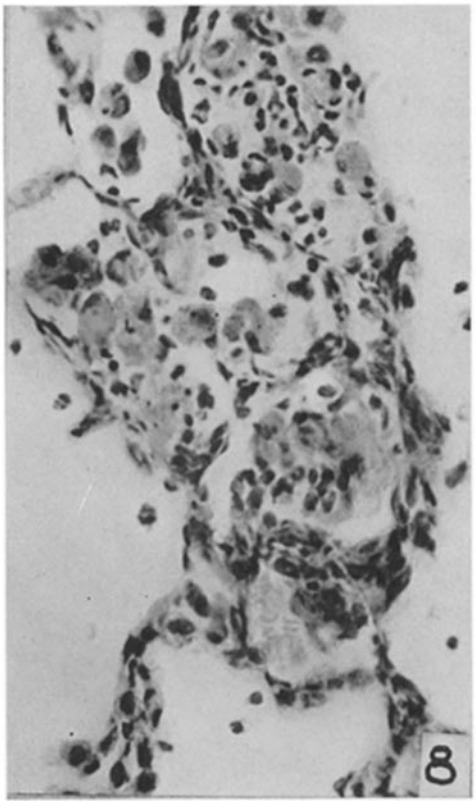
PLATE 11

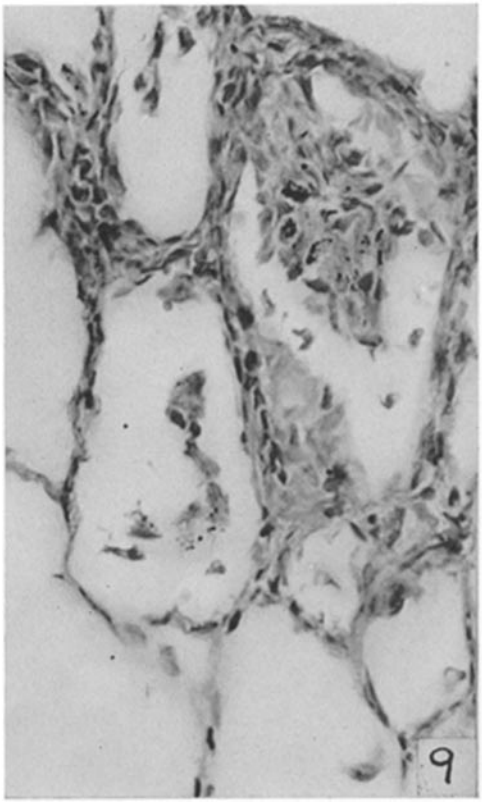
FIG. 7. Rabbit 10-1. A focus of non-specific reaction, 24 hours after the animal began inhaling the aerosol suspension of bacilli. Note that this focus is more extensive than a developing tubercle 11 days older. Serial sections of this focus did not reveal bacilli. × 400.

FIG. 8. Rabbit 10-4. A focus of non-specific reaction, 72 hours after the animal began inhaling the aerosol suspension of bacilli. At this time these foci were less compact and appeared to be breaking up. This focus did not contain bacilli. × 500.

FIG. 9. Rabbit 14-8. Tubercle bacilli in the cytoplasm of alveolar macrophages 9 days after infection. This lesion, and several others like it in the lungs of this animal, were attributed to chance infection of a focus of non-specific reaction. Compare the thickened alveolar walls about this group of cells with those shown about the average infected focus after 9 days' development (Figs. 1 and 2). × 500.







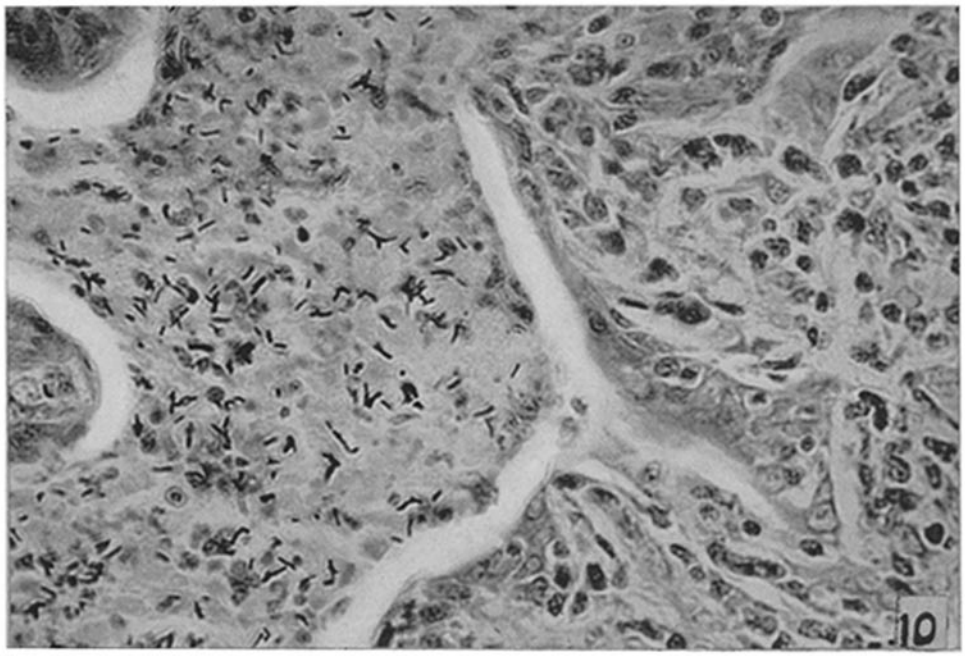
(Ratcliffe and Wells: Tuberculosis of rabbits. I)

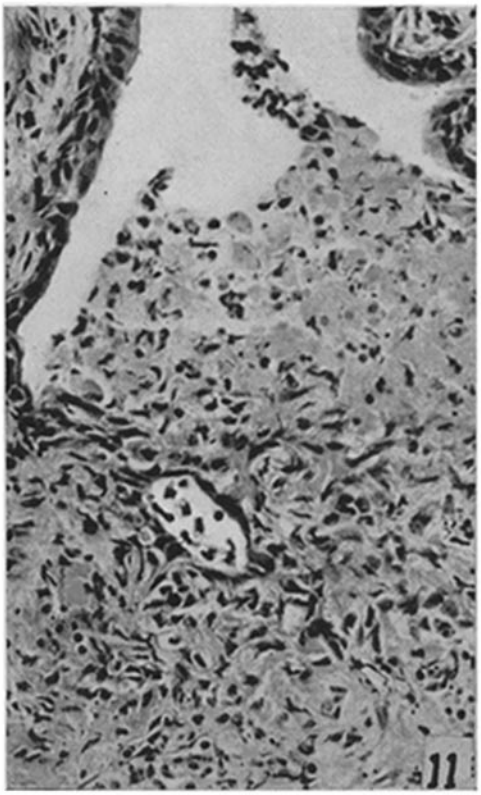
PLATE 12

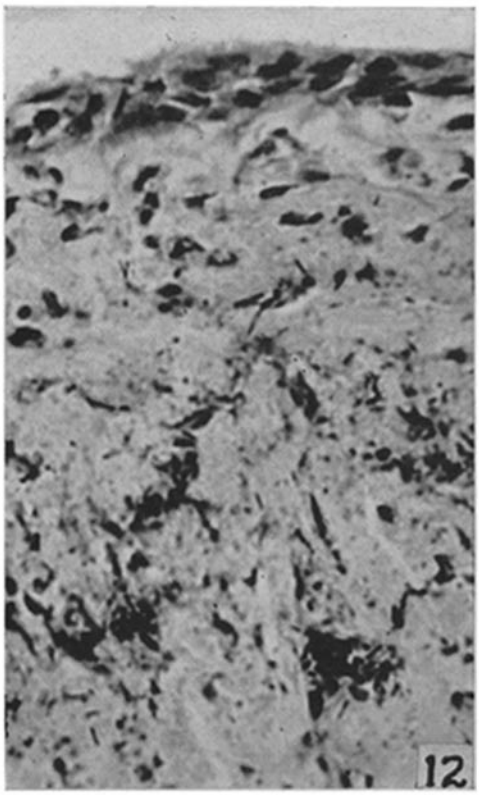
FIG. 10. Rabbit 5-4. Killed 20 days after inhaling 50 to 75 bacilli. This field is representative of the centers of initial tubercles during the latter part of the 3rd week and early part of the 4th week of development. Bacilli were found mainly among the cells which filled alveolar spaces and alveolar ducts. × 600.

FIG. 11. Rabbit 10-8. Killed 28 days after inhaling about 150 bacilli. This field illustrates the communication between the necrotic center of the tubercle, which contained abundant bacilli, and a patent bronchiole. × 350.

FIG. 12. Also from rabbit 10-8. This illustrates the lack of reaction in the wall of a large bronchiole as the expanding tubercle encroached upon it. Bacilli make up the black masses scattered through the lower two-thirds of the field. × 660.







(Ratcliffe and Wells: Tuberculosis of rabbits. I)

PLATE 13

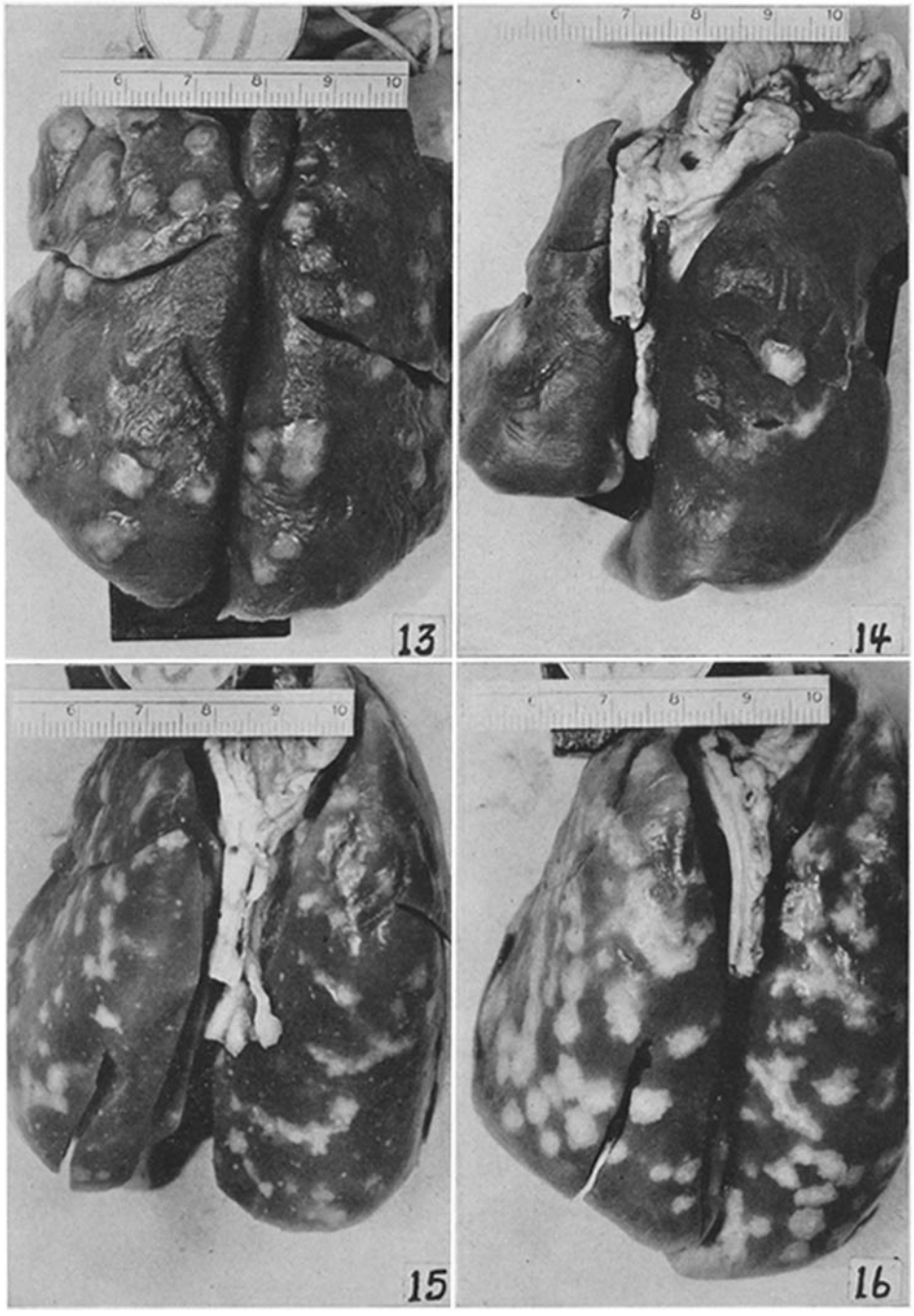
Photographs of lungs are natural size.

FIG. 13. Rabbit 9-1. Killed 39 days after inhaling 175 to 200 bacilli.

FIG. 14. Rabbit 11-6, killed 43 days after inhaling 10 to 20 bacilli.

FIGS. 15 and 16. Rabbits 10-9 and 11-2, killed 43 days after inhaling 150 to 175 bacilli. Fig. 14 illustrates the average character of initial tubercles at 6 weeks, when small numbers of bacilli were inhaled.

Figs. 13 and 16 illustrate progression of the infection by the air passages, and Fig. 15, progression by both air passages and blood stream at 6 weeks when initial infection was relatively intense. The irregular borders of the initial tubercles of Figs. 13, 15, and 16 could be traced to local spread of the infection through alveolar ducts and bronchioles.



(Ratcliffe and Wells: Tuberculosis of rabbits. I)

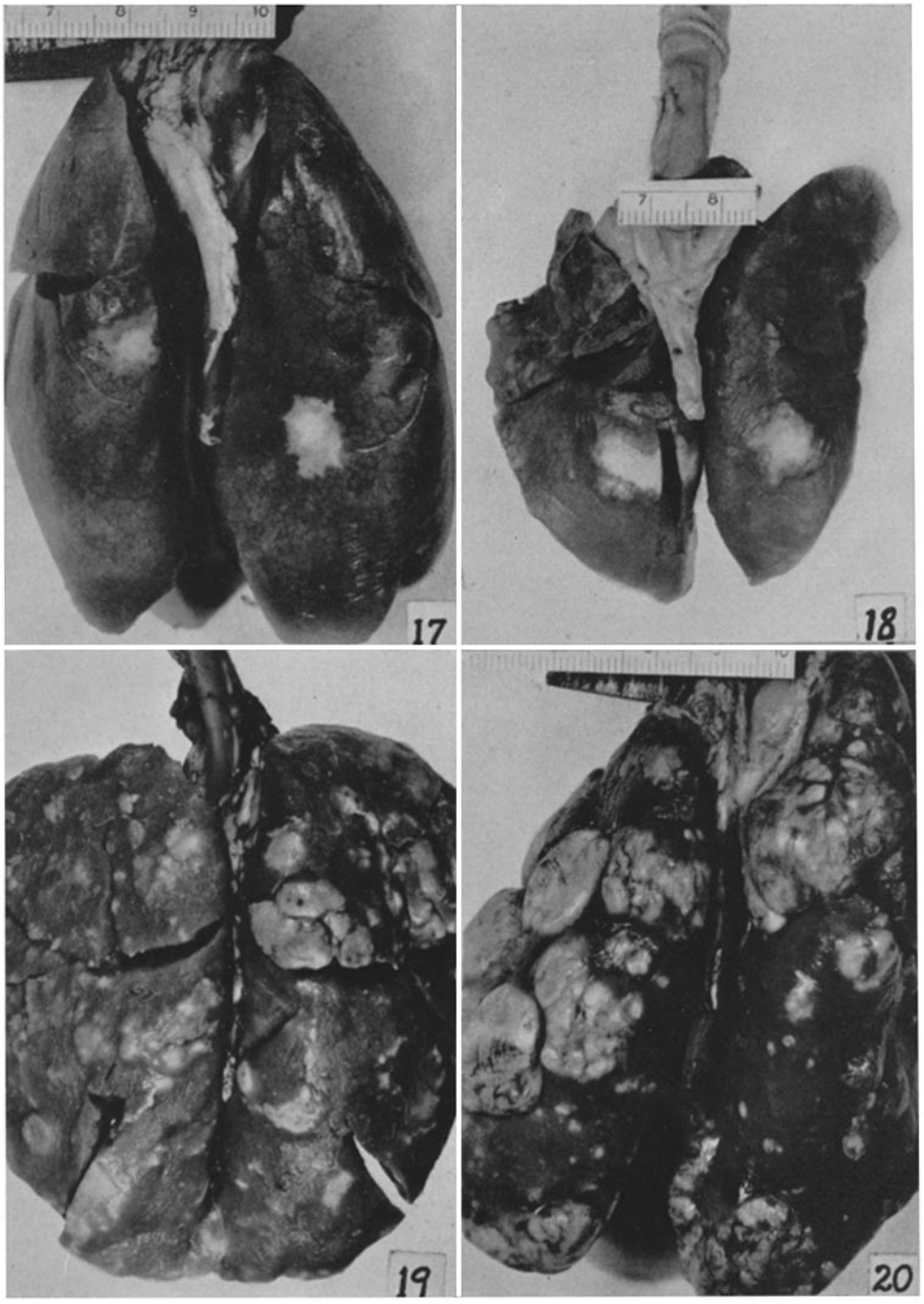
PLATE 14

FIG. 17. Rabbit 15-8, killed 56 days after infection by 5 air-borne bacilli. The tubercles in this animal are representative of the relatively slow progress of the infection at this time interval, when small numbers of bacilli were inhaled. Spread of the infection along the pleural lymphatics from the initial tubercles is clearly illustrated.

FIG. 18. Rabbit 7-4, killed 71 days after infection by 5 air-borne bacilli. The initial tubercles are correspondingly larger than those of Fig. 17, and bronchial spread of the infection is shown by satellite tubercles near the initial lesions and by small tubercles scattered through the lungs. Other organs were not involved.

FIG. 19. Rabbit 9-5, killed 132 days after infection by 175 to 200 air-borne bacilli. This animal was an exposure-mate of rabbit 9-1, Fig. 13. Some initial tubercles had advanced to form cavities; others were no larger than at 6 weeks; and still other initial tubercles seemed to have regressed. In spite of relatively intense initial infection this animal apparently developed a high grade of resistance to progression of the infection.

FIG. 20. Rabbit 12-3, died of perforated tuberculous ulcer of the gut 121 days after inhaling about 30 bacilli. Cavities account for about one-half of the initial tubercles. The remainder apparently regressed.



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