THE FATE OF BCG AND ASSOCIATED CHANGES IN THE ORGANS OF RABBITS

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PLATES 6 TO 8

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In view of the widespread use of BCG in the vaccination of infants against tuberculosis, it seemed desirable to determine to what extent this microorganism of low virulence multiplies in the various organs of the body and in the lymphatic tissue, for how long it remains viable within them, and what are the histological changes associated with the fate of the bacilli. Such a study would appear to be prerequisite to an understanding of the capacity of the BCG for protecting animals against a virulent infection. Furthermore, it was hoped that it might throw some light on the pathogenesis of the more complex disease incited by virulent tubercle bacilli, and upon the relation of the tuberculin reaction to the various phenomena observed.

Exhaustive reviews of the vast literature on the BCG have recently appeared (1). Schilling (2) noted clumps of acid-fast bacilli in the lungs of guinea pigs intravenously inoculated with BCG, which he interpreted as evidence of their multiplication. Ninni (3) recovered cultures of BCG from the cervical and bronchial lymph nodes of 3 out of 6 guinea pigs that had ingested the microorganism from 2 to 6 days previously. When emulsions of these nodes were injected into the cervical nodes of normal guinea pigs, acid-fast bacilli could be seen, and, in rare instances, macroscopic colonies were obtained from their lymph nodes from 4 to 8 days after inoculation but not on the 12th day. He concluded that the BCG may multiply for some time in the body.

As to their persistence in the body, Griffith (4) cultured the BCG from the lymph nodes of monkeys 7 months after inoculation, and Birkhaug (5) cultured them from the lymph nodes of guinea pigs after 577 days.

Material and Methods

The writer (6) has reported on the fate of a strain of BCG that was fatal to a certain percentage of rabbits. Since the overwhelming majority of studies have demonstrated that the BCG does not cause fatal tuberculosis, another strain, serial No. 376, was brought to this laboratory in 1928 by Dr. S. Mudd directly from the Pasteur Institute in Paris. This strain has been propagated according to Calmette's directions by monthly transplants on veal-glycerol-potato medium, with every ninth and tenth transplant cultured on bile-glycerol-potato medium. Up to this time this BCG has caused no progressive tuberculosis in any of the large numbers of guinea pigs and rabbits into which it has been inoculated.

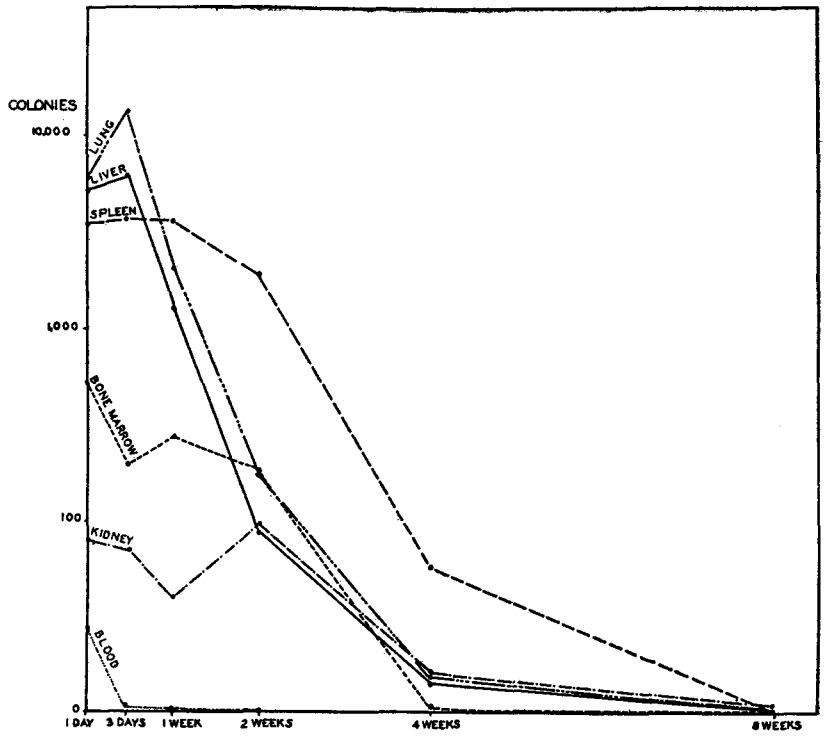
Three series, each consisting of 12 rabbits, were inoculated intravenously with 1.0 mg. of this strain of BCG. 1 and 3 days, and 1, 2, 4 and 8 weeks following inoculation, 2 rabbits of each series were killed; weighed amounts of ground lung, liver, spleen, kidney and bone marrow in various dilutions, and a measured quantity of blood from the heart, were seeded on egg media, directly and after treatment (7) with 6 per cent sulfuric acid. In Series II and III, weighed amounts of ground tracheobronchial and mesenteric lymph nodes, and in Series I, bile from the gall bladder, were also seeded in a similar manner.

Another group of rabbits were inoculated intravenously with 1.0 mg. and placed in a separate room apart from possible tuberculous contagion and kept there as long as 14 months. At the end of this time some of these were killed and cultures were made from the organs and lymph nodes.

In preliminary experiments difficulty was encountered in obtaining consistent colony counts of the BCG on the egg media hitherto used. Ninni (3) and others are of the opinion that there is no suitable medium for the cultivation of the BCG from the body. However it had been noted in previous studies with more virulent bacilli that the colonies that grew on egg media planted with infected bone marrow were more luxuriant than those planted with other infected tissue. Brown (8) also found that the addition of bone marrow infusion to Dorset's medium gave unusually good primary bovine type cultures. Accordingly the number of colonies obtained from seeding a given amount of infected tissue on Dorset's, Petroff's and Löwenstein's media were compared with that obtained on Löwenstein's medium in which bone marrow infusion replaced the distilled water required by the formula (9). It was found that from 100 to 200 per cent more colonies of BCG, and of virulent bovine and human type tubercle bacilli developed on the modified Löwenstein's medium. Therefore this medium, with malachite green substituted for Congo red in 0.034 per cent concentration to suppress contaminants, was used exclusively in this study.

The number of colonies on the surface of each tube from the various dilutions of the different organs was repeatedly determined; the final reading was made after 3 months' incubation. The tissue immediately adjacent to that cultured was studied microscopically after staining with hematoxylin and eosin, and with carbolfuchsin to show tubercle bacilli.

To determine the relationship between the fate of BCG, the histological changes that they produce in the organs, and tuberculin sensitivity, a separate group of 13 rabbits were inoculated intravenously with 1.0 mg. of BCG and at various intervals after infection were tested by the intracutaneous injection of 0.1 cc. of a 1 in 5 dilution of tuberculin derived from the human type. This concentration of O. T. regularly gives rise to well marked inflammatory reactions 48 hours after injection in tuberculous rabbits, and causes none in animals free of tuberculosis. An inflammation extending over an area of 10 mm. in diameter with an edema of



TEXT-FIG. 1. Fate of BCG in the organs of rabbits. Series I.

1.0 to 2.0 mm. in thickness was designated a + reaction. An area of redness 20 mm. in diameter with an edema of 2 to 3 mm. in thickness was designated a ++ reaction. If the inflammation extended over an area of 30 mm. in diameter and the edema reached 4 mm. in thickness it was designated a +++ reaction.

The Fate of the Bacilli

Multiplication and Destruction.—In Text-figs. 1 to 3 is depicted the fate of the BCG in each of the three series of animals. Each point

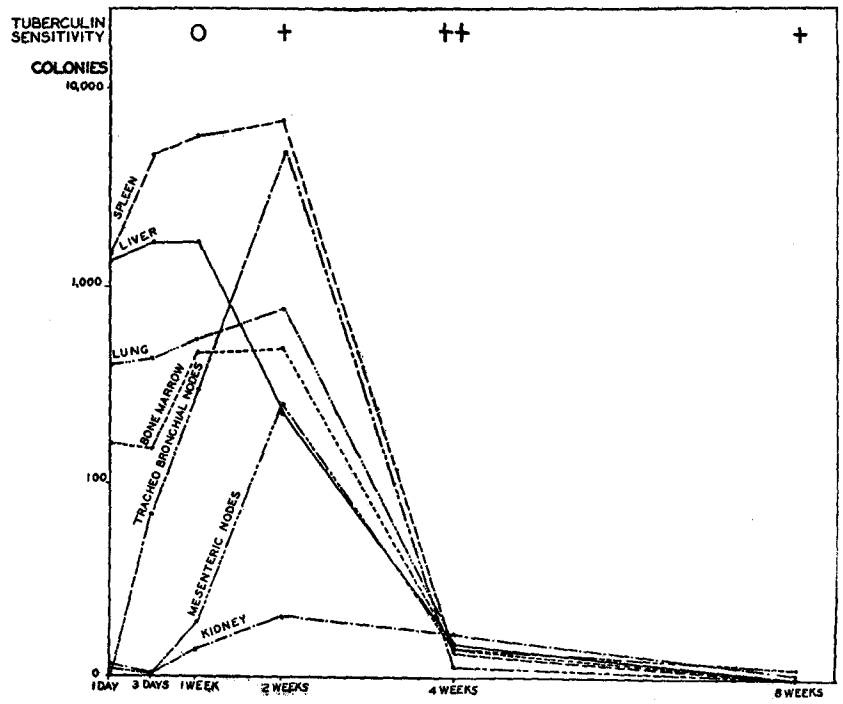
charted represents the average number of colonies obtained from 10 mg. of a given organ or tissue of 2 rabbits inoculated at the same time with the same suspension of BCG. It will be noted that widely differing numbers of colonies were cultured 24 hours after inoculation from the same organs in the three series. Thus in Series I, the primary deposition in the liver and spleen ranged between 3,000 and 5,000, in Series II, between 1,400 and 1,500, and in Series III between 700 and 900. Similar relations obtained in the blood and other organs. Thus 27 colonies were isolated from the blood in Series I, 5 in Series II and 2 in Series III. This difference may be accounted for in part by differences in the amount of moisture contained in the bacillary masses weighed out for each series.

The number of bacilli inoculated as indicated by the number of colonies cultured from the various organs after 24 hours influenced the subsequent fate of the bacilli and the course of the associated changes in the body. With the largest primary deposition, in Series I (Text-fig. 1), multiplication was apparent on the 3rd day in the lung and liver and to a very slight extent in the spleen. By the end of the 1st week there was a marked reduction in the number of bacilli in the lung and liver, while they remained in about the same numbers in the spleen. By the 2nd week the destruction of the bacilli was far advanced in the former organs and had definitely started in the latter. By the 4th week only small numbers of colonies remained in the lung and liver, and the destruction in the spleen was conspicuous, although still lagging. At the end of 2 months the bacilli had disappeared completely from all the organs, with the exception of a rare colony isolated from the kidney.

In Series II (Text-fig. 2), where there was a smaller primary deposition of the bacilli than in Series I, growth continued 11 days longer in the lung and spleen and destruction in the liver began a week later. However, again by the 4th week destruction of the bacilli was largely complete in all the organs.

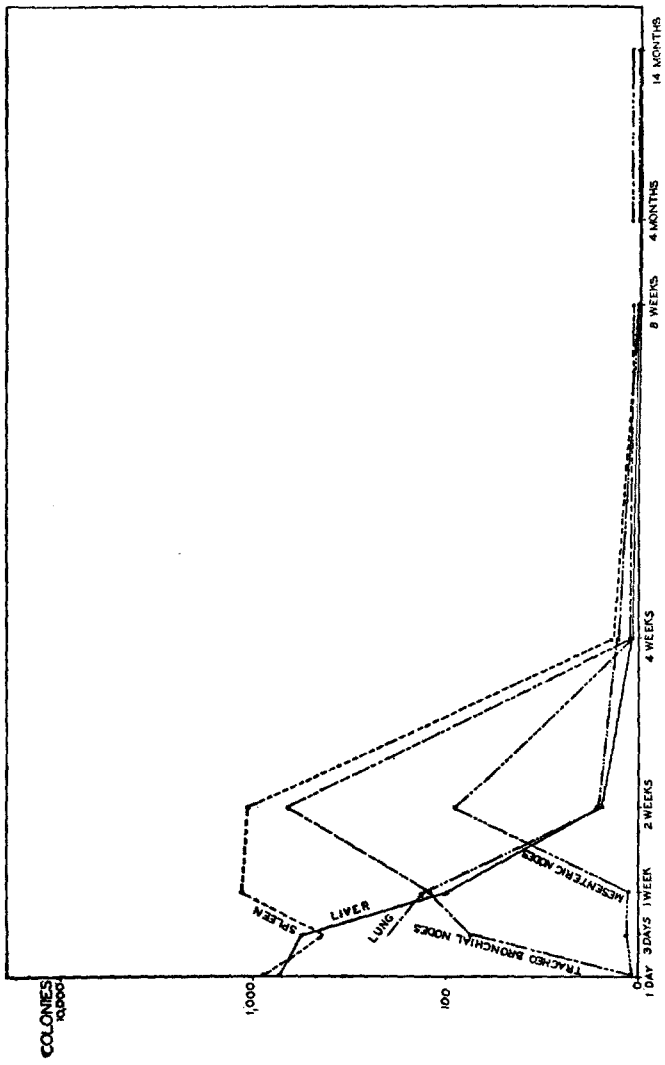
In Series III (Text-fig. 3), where the original deposition of bacilli was smallest, no preliminary growth at all took place in the lung and liver, but destruction began at once, although even in this series conspicuous multiplication occurred in the tracheobronchial and mesenteric lymph nodes, and the bacilli persisted in large numbers in the spleen. They were largely destroyed by the 4th week.

Thus with a large "dose" multiplication occurs in the first few days and is rapidly followed by destruction. With a smaller dose multiplication continues for a longer time and is followed by a later destruction. With the smallest primary deposition destruction begins at once without preliminary multiplication in the lung and liver. This is in accord with previous observations with virulent human and bovine type bacilli (10).



TEXT-FIG. 2. Fate of BCG in the organs of rabbits. Series II.

Correlation with Tuberculin Sensitivity.—In Text-fig. 2, depicting the fate of BCG in Series II, is indicated the average degree of sensitivity to tuberculin developed by 13 rabbits inoculated with the same strain of bacilli in the same quantity. It will be noted that simultaneously with the appearance of definite skin hypersensitiveness to tuberculin, destruction of the bacilli has started in the liver. By the 4th week, when tuberculin hypersensitiveness has reached its height, the destruction of the bacilli is practically complete in all the



TEXT-FIG. 3. Fate of BCG in the organs of rabbits. Series III.

organs in all the series. Unfortunately the skin sensitivity of the animals whose organs were cultured could not be determined at the time of killing the animal, for 2 days had to elapse between the testing and the reading of the reaction. However it is likely that in Series I, where the primary deposition was much greater, tuberculin sensitivity developed earlier. This follows from the work of Römer and of Lewis and Aronson (11), who found that the tuberculin reaction can be brought about more quickly in animals infected with larger doses of tubercle bacilli than in those infected with smaller doses. The development of skin hypersensitivity to tuberculin is thus synchronous with onset of the destruction of the bacilli.

It is noteworthy, moreover, that even in the relatively susceptible lymph nodes, the bacilli are destroyed at a time when the maximum sensitivity to tuberculin has developed. If tuberculin sensitivity be considered, in part at least, an indication of antigen-antibody reaction, as is widely held, there is thus a consistent correlation between the development of immunity to tuberculosis, as shown by the destruction of bacilli, and the production and accumulation of antibodies. However in the light of these data it can be observed only that the phenomena are parallel, and the one cannot be said to be the cause of the other.

Relative Immunity in Lymph Nodes and Other Organs.—The behavior of the BCG in the tracheobronchial and mesenteric lymph nodes and in the spleen is deserving of some discussion. It will be noted (Text-fig. 2) that in Series II an almost unhindered multiplication occurred in these lymph nodes at a time when the bacilli were being effectively destroyed in the liver and when the rate of multiplication in the lung was much slowed. In Series III destruction began without any multiplication in the lung and liver, but in the tracheobronchial and mesenteric lymph nodes extensive multiplication continued to the end of the 2nd week, and in the spleen the bacilli persisted without reduction throughout this period. These observations indicate that lymphatic tissue is a peculiarly favorable site for the growth of the BCG, confirming the opinion of Calmette (12).

It might be said that the accumulation of bacilli in the tracheobronchial nodes is ascribable to drainage from the lung rather than to any particular immunity or lack of immunity. But this interpreta-

tion cannot be applied to the mesenteric lymph nodes, for the intestinal tract was spared entirely by the infection studied. Moreover the growth and persistence of the bacilli in the spleen cannot be attributed to their segregation and accumulation in this organ from other sources, for the blood stream was always sterile a few days after inoculation. Further evidence was obtained in a study of another series of rabbits also inoculated intravenously with the same quantity of the same strain of BCG. In these animals the same observations were made with the axillary lymph nodes, in which there is practically no possibility of draining bacilli from other organs.

In previous studies (6, 10, 13), the special position of the lung and kidney in regard to immunity developing against a virulent infection has been emphasized. Nevertheless local immunity was not inferred, because it was felt that the multiplication or accumulation of bacilli in these organs when they were being effectively destroyed in the liver, spleen and bone marrow, might be ascribable to some other cause. In the lung and kidney spread through tubules was obviously one important factor. In the lymph nodes, however, this factor also is not operative.

Thus it appears that definite evidence in support of the conception of local immunity is available in the persistence of the BCG in the spleen and their growth in the lymph nodes when they are being effectively checked in other organs. In Series I and II it is apparent that this local immunity is acquired, for at first the bacilli multiply in these organs; later these organs acquire a resistance that destroys the bacilli. For a time the acquired immunity in the lymph nodes and spleen is distinctly less effective than in the other organs. By the 4th week, however, concurrently with the development of marked hypersensitiveness to tuberculin, the bacilli are destroyed almost completely even in the lymph nodes, although isolated living BCG persist in the lymph nodes as late as 14 months after inoculation.

The Histological Changes

With Multiplication and Destruction.—The tissue responses to the BCG differ in no qualitative way from those incited by bacilli of human or bovine type. The differences, largely of degree, are determined by the limited multiplication and subsequently rapid destruction of the less

virulent, and by the more prolonged multiplication and less efficient destruction of the more virulent microorganisms.

Calmette (12) originally held that the BCG is not tuberculogenic, although retaining the immunizing capacities of the more virulent tubercle bacilli. However Couland (14) showed that large doses intravenously inoculated into rabbits produced typical epithelioid and giant cell tubercles, which regressed without undergoing caseation. Schilling (2) found that guinea pigs inoculated intravenously with BCG developed macroscopic tubercles in the lungs. He observed the accumulation of polymorphonuclears about the clumps of bacilli; these cells died, but there was no caseation of tubercles. He noted the encapsulation of the tubercles by fibrous tissue, their gradual shrinkage and final disappearance.

Since the correlation between the fate of more virulent bacilli and the associated histological changes has been described in detail in previous studies (6), only representative animals of the present study will be discussed.

24 hours after inoculation, as in Rabbit 18-06 of Series I, there was a generalized infiltration of the alveolar septa of the lung by polymorphonuclears. Here and there, about clumps of bacilli surrounded by polymorphonuclears, many of which were necrotic, there were nodular accumulations of large mononuclears, some of which had phagocyted the nuclear debris of the polymorphonuclears and their contained bacilli. 7,230 colonies of tubercle bacilli were isolated from 10 mg. of lung of the rabbit described (Fig. 1).

3 days after inoculation, 13,180 colonies were isolated from Rabbit 13-09 of the same series. There was a moderate number of nodules in the septa and surrounding blood vessels, composed of closely crowded lymphoid mononuclears. In the center of some of these nodules there were large mononuclears with well developed foamy cytoplasms, in which very short acid-fast bacilli might be found. At times these mononuclears formed syncytia, in which the oval vesicular nuclei might be arranged at the periphery of the protoplasmic mass. Mitosis was sometimes seen in these nodules (Fig. 2).

1 week after inoculation, as in Rabbit 18-07 of this series, the nodules were larger; many of the mononuclears had assumed the typical structure of mature epithelioid cells; mitoses were less frequent; fewer colonies (1,900) were isolated. There was as yet no exudation into the alveoli (Fig. 3).

2 weeks after inoculation, the lungs of Rabbit 13-08 of the first series did not collapse, and were riddled by numerous macroscopic tubercles. Microscopically the tubercles were composed of typical mature epithelioid cells. There was a conspicuous exudation into the alveoli of fluid, epithelioid and Langhans giant cells, macrophages and polymorphonuclears. At times this exudation assumed pneumonic proportions. The larger tubercles were infiltrated with polymorphonu-

clears, many of which were necrotic. This acute inflammation was simultaneous with the development of hypersensitivity to tuberculin. Only 30 colonies were recovered from the lung of Rabbit 13-08 (Fig. 4). In the tracheobronchial lymph nodes the first stages of caseation were apparent.

In Series III, in which, as was noted above, destruction of the bacilli was far advanced in 2 weeks, the changes in the lung were essentially those seen in Series I at this interval, and caseous foci were found in the tracheobronchial lymph nodes, as in Rabbit 19-88 (Fig. 10).

In contrast to the changes in Series I at this time was the reaction in Series II, in which, as was noted above, multiplication continued to the 2nd week after inoculation. In the lungs, for example in Rabbit 20-95, there were numerous nodules composed of large mononuclear cells, many of which were in mitosis. Many of the nodules were situated about small blood vessels. On either side of the elastic membrane of some of these vessels there was a large accumulation of mononuclears. These pushed the endothelial lining ahead of them and encroached upon the lumen of the vessel. In some of the nodules were seen young epithelioid cells. Mature epithelioid cells were infrequent. There was no exudation into the alveoli (Figs. 5 and 6). No caseation was noted in the tracheobronchial lymph nodes (Fig. 9). This was the height of multiplication of the bacilli in the lungs of this series; 960 colonies were isolated from the lung described. However when the bacilli in Series II had been destroyed, 4 weeks after inoculation, there was a widespread tuberculosis with exudation into the alveoli.

Essentially the same correlation was found in the other organs. Thus in Rabbit 20-94 of Series II, 1 week after inoculation 11,360 colonies were isolated from the spleen, but except for frequent mitotic figures both in the hypertrophied corpuscles and in the pulp, and collections of large mononuclears in the latter, there was no indication of any tubercle formation (Fig. 7). However by the 4th week after inoculation, when only 1 colony was isolated from 10 mg. of the spleen of Rabbit 44 of the same series, the pulp was largely replaced by large epithelioid and giant cell tubercles (Fig. 8).

Regressive Changes.—All these changes, which at times are extensive, regress and by the end of the 2nd month have largely disappeared. This is brought about by the process of resolution (Fig. 11).

The epithelioid cells become highly vacuolated; they coalesce until only shreds of the cytoplasm remain, although the nucleus retains its viability. 2 months after injection there is almost complete *restitutio ad integrum*. Only about the blood vessels in the lung and in the portal canals are found collars of large mononuclears (Fig. 12), or there may be found rare collections of epithelioid cells undergoing regressive changes.

There is a complete correlation between the histological changes and the fate of the bacilli as indicated by the number of colonies

cultured from a given tissue. The growth of the bacilli is associated with the accumulation of mononuclears into nodules and their local multiplication by mitosis; the destruction of the bacilli with the formation of epithelioid and giant cell tubercles.

Caseation; Tuberculin Sensitivity.—It is noteworthy that the secondary invasion of the tubercles by polymorphonuclears with their subsequent necrosis, exudation of fluid and cells into the alveoli of the lung, and caseation of the tracheobronchial lymph nodes occur simultaneously, and are synchronous with development of hypersensitiveness to tuberculin. That the first three phenomena are closely bound together is clearly seen by a comparison of the fate of the bacilli and the associated changes in the lung and tracheobronchial lymph nodes 2 weeks after inoculation in Series II and III. In Series II, where multiplication continued for 2 weeks, there is no exudation into the alveoli, there is no invasion of the young tubercles of the lung by polymorphonuclears, and there is no caseation of the tracheobronchial nodes (Fig. 9), although the bacilli are at their height; 960 colonies were isolated from the lung and 8,360 colonies from the tracheobronchial nodes of one rabbit. In Series III, in which destruction of the bacilli began at once, only 16 colonies were isolated from the lung and 1,020 colonies from the tracheobronchial lymph nodes, but there is an infiltration of the pulmonary tubercles by polymorphonuclears, which are necrotic, exudation of serum and cells into the alveoli, and the tracheobronchial nodes show caseation (Fig. 10).

That the primary toxicity of tubercle bacilli is not responsible for these acute inflammatory changes is clearly indicated by the fact that in the spleen and in the lungs the bacilli may be present in great numbers 3 days and 1 week after inoculation without any of these changes; later, simultaneously with the development of hypersensitiveness to tuberculin, the presence of far fewer bacilli is associated with necrosis of invading polymorphonuclears, exudation into the alveoli and caseation in the lymph nodes.

It was noted above that the lymph nodes are a particularly favorable site for the growth of the BCG; that the immunity developed in them is at first less effective than that of other organs. Therefore it is understandable that caseation develops in them, whereas only necrosis of polymorphonuclears is observed in the lung. When hypersen-

sitiveness is sufficiently developed too few bacilli remain in the lung and liver to exercise in a sufficient degree their newly enhanced toxic effect on the tissues, but in the lymph nodes, where enough bacilli remain even after development of hypersensitiveness, there is not only death of the invading polymorphonuclears but also necrosis of the epithelioid cells and caseation.

Caseation and tuberculin sensitivity thus bear the same relation to the course of the disease. As the disease progresses tuberculin has a more toxic effect and at the same time the presence of relatively small quantities of bacilli in the tissues is associated with caseation. In an earlier stage of the disease much larger numbers of bacilli do not injure the tissues, and the same or a larger quantity of tuberculin does not cause inflammation.

It is evident, therefore, that the BCG produces the same histological changes in the tissues that more virulent tubercle bacilli do. The differences are due to the restricted multiplication and more rapid and complete destruction of the BCG, associated with the limited development of mononuclear infiltrations, their rapid transformation into epithelioid tubercles and their subsequent complete resolution. Only one phenomenon observed in virulent infection is lacking with the BCG; that is, caseation does not go on to softening.

Caseation, which, it has often been asserted, does not develop with the BCG, was regularly found in the lymph nodes, where the bacilli persist the longest. Softening, as is well known, develops only during a protracted course of a virulent infection. Since the BCG all but completely disappeared from the body in 2 months and the pathological changes, including the caseation that they had induced, completely resolved, it is obvious that softening could not take place.

The Persisting Bacilli; Disposal of the BCG

It has been seen that in the mesenteric and tracheobronchial lymph nodes and occasionally in the spleen and kidney isolated bacilli were cultured even 14 months after inoculation. At this time the organs were entirely normal in their histological structure. It was found, moreover, that after their long sojourn in the body, the BCG recovered from these organs had acquired no increase of virulence for rabbits.

Destruction of the BCG appears to occur *in situ*. Certain ob-

servers have concluded that bacilli of reinfection are eliminated from the body with the bile (15). However in the present study the BCG were never cultured from the bile except during the first days after inoculation. At that time they could be accounted for by contamination with blood, in which they were then present in considerable numbers.

SUMMARY

For some time after intravenous inoculation into rabbits the BCG multiplies in various organs of the body. As with more virulent tubercle bacilli, the greater the primary deposition of bacilli the more rapid is their initial growth and the earlier is the beginning of their destruction. Destruction sets in synchronously with the development of definite hypersensitivity to tuberculin. With the smallest deposition destruction begins at once in the lung and liver without preliminary multiplication. However multiplication always takes place in the lymph nodes. It continues here and in the spleen at a time when other organs have acquired sufficient immunity to reduce the number of bacilli considerably. It is seen that the relative susceptibility of the lymphatic tissue is not due to such factors as drainage. Thus the local immunity acquired by the lymph nodes against the BCG is for a time less effective than that developed in the other organs. However by the end of the 4th week, simultaneously with the development of marked hypersensitivity to tuberculin, even these structures acquire sufficient immunity to bring about the all but complete annihilation of the bacilli. Isolated microorganisms persist in the lymph nodes even 14 months after inoculation. These bacilli do not acquire any added virulence because of their long residence in the body.

As observed with more virulent strains in previous studies, the growth of the bacilli is associated with a local accumulation of mononuclears into nodules and their multiplication by mitosis, the destruction of the bacilli with the formation of epithelioid and giant cell tubercles. These changes may be extensive and at times involve a large part of the lung and lymph nodes. The secondary invasion of the tubercles by polymorphonuclears, their death, exudation of serum and cells into the alveoli of the lung, and caseation in

the lymph nodes occur simultaneously and are synchronous with the development of hypersensitiveness to tuberculin. At this time the presence in the tissues of numbers of bacilli that previously were innocuous to the cells is associated with caseation. It is noteworthy that both caseation and the tuberculin reaction are not elicited in an early stage of the disease, but in a later stage they are induced in response to smaller quantities of bacilli or tuberculin.

All the tuberculous changes, including the caseous foci in the lymph nodes, usually resolve completely by the end of the 2nd month after inoculation without leaving any fibrous scars, although perivascular accumulations of mononuclears may still remain. The few bacilli that persist in the lymph nodes after this period cause no tuberculous changes.

CONCLUSIONS

1. Under the conditions of experiments in which 1.0 mg. of BCG was introduced intravenously into rabbits, the BCG multiply in the body, but they are soon destroyed, completely in most organs, all but completely in the lymph nodes.

2. The remaining BCG persist in the lymph nodes for a long time, causing no tuberculous changes, and acquiring no added virulence for rabbits.

3. The BCG produce typically tuberculous changes, sometimes extensive, which resolve completely. They caused caseation, but no softening.

4. Acquired local immunity in tracheobronchial, mesenteric and axillary lymph nodes and in the spleen is shown to be less effective for a time than that in other organs.

5. The destruction of bacilli begins with the appearance of skin sensitivity to tuberculin, and is at its height with maximum sensitivity.

6. The secondary acute inflammatory reactions in and about tuberculous foci, caseation and hypersensitivity to tuberculin develop synchronously.

7. Caseation and tuberculin sensitivity do not occur early in the course of the disease in response to considerable amounts of bacilli and of tuberculin, but later they are incited by smaller amounts.

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EXPLANATION OF PLATES

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

PLATE 6

FIG. 1. Lung of Rabbit 18-06 of Series I, 24 hours after inoculation; 7,230 colonies were isolated from 10 mg. of tissue. The alveolar septa are infiltrated by polymorphonuclears. About a clump of bacilli not shown in figure are necrotic polymorphonuclears surrounded by a nodular accumulation of mononuclears. ×200.

FIG. 2. Lung of Rabbit 13-09 of Series I, 3 days after inoculation; 13,180 colonies were isolated. A nodule composed of closely crowded lymphoid mononuclears; in the center a syncytium with large oval vesicular nuclei. ×200.

FIG. 3. Lung of Rabbit 18-07 of Series I, 1 week after inoculation; 1,900 colonies were isolated. The nodule is larger than in Fig. 2. Many of the mononuclears have assumed the typical structure of mature epithelioid cells. ×200.

FIG. 4. Lung of Rabbit 13-08 of Series I, 2 weeks after inoculation; 30 colonies were isolated. Widespread tubercle formation with exudation of fluid and cells into the alveoli. ×100.

PLATE 7

FIG. 5. Lung of Rabbit 20-95 of Series II, 2 weeks after inoculation. The bacilli have attained their largest numbers; 960 colonies were isolated. Numerous mononuclear nodules are seen. There is no exudation into the alveoli. ×100.

FIG. 6. A higher magnification of another portion of the section shown in Fig. 5. A perivascular nodule with a large accumulation of mononuclears on either side of the elastic membrane. There are no mature epithelioid cells. To the right and above the elastic membrane is a mitotic figure. ×200.

FIG. 7. Spleen of Rabbit 20-94 of Series II, 1 week after inoculation; 11,360 colonies were isolated. There is no tubercle formation. A mitotic figure can be seen in the pulp in the center of the photograph. ×200.

FIG. 8. Spleen of Rabbit 44 of Series II, 4 weeks after inoculation; 1 colony was isolated. Extensive tubercle formation in the pulp. ×200.

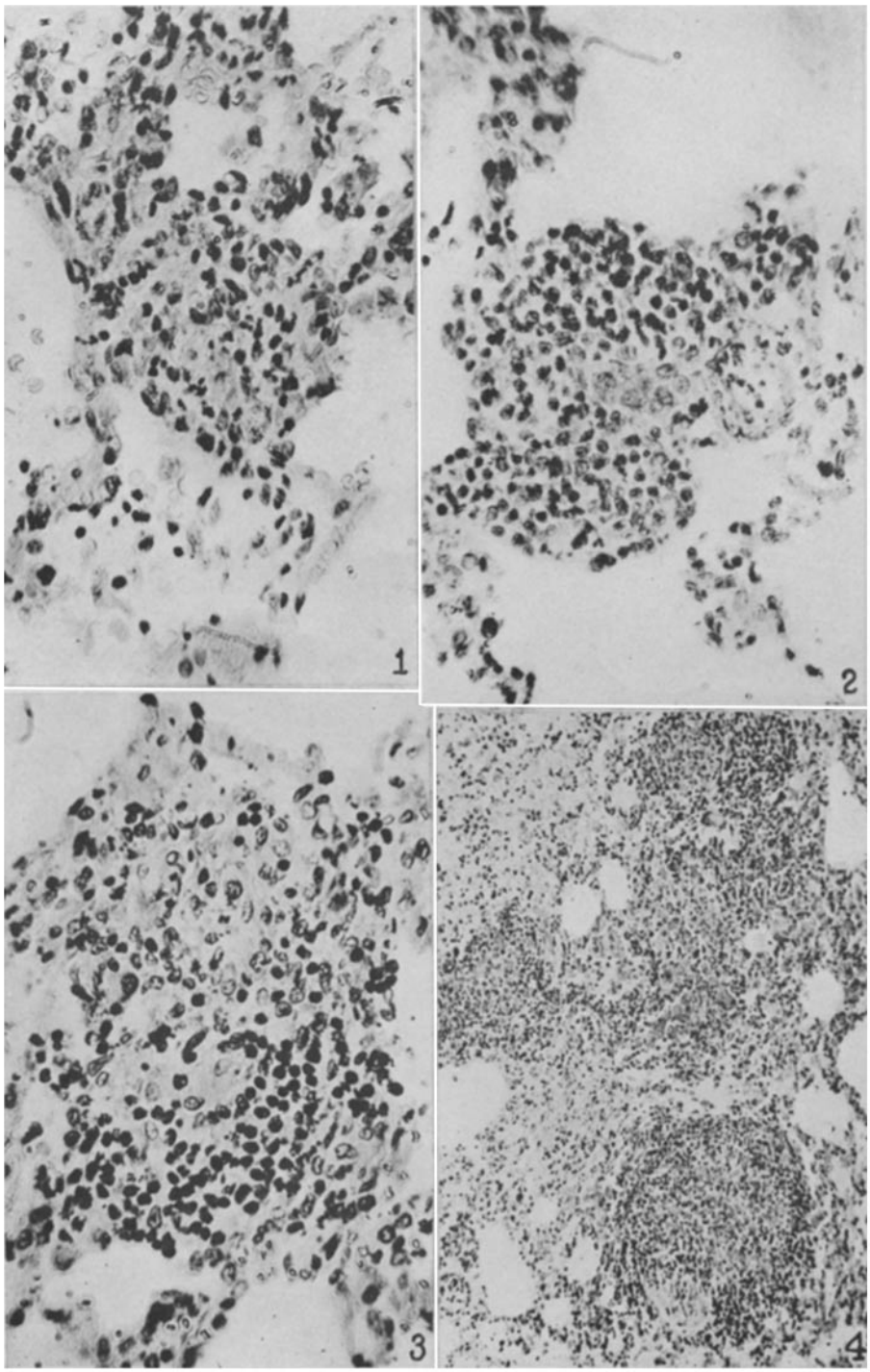
PLATE 8

FIG. 9. Tracheobronchial lymph node of Rabbit 20-95 of Series II, 2 weeks after inoculation; 8,360 colonies were isolated. There are islands of young epithelioid cells. A mitotic figure can be seen in the upper third of the photograph. There is no caseation. ×200.

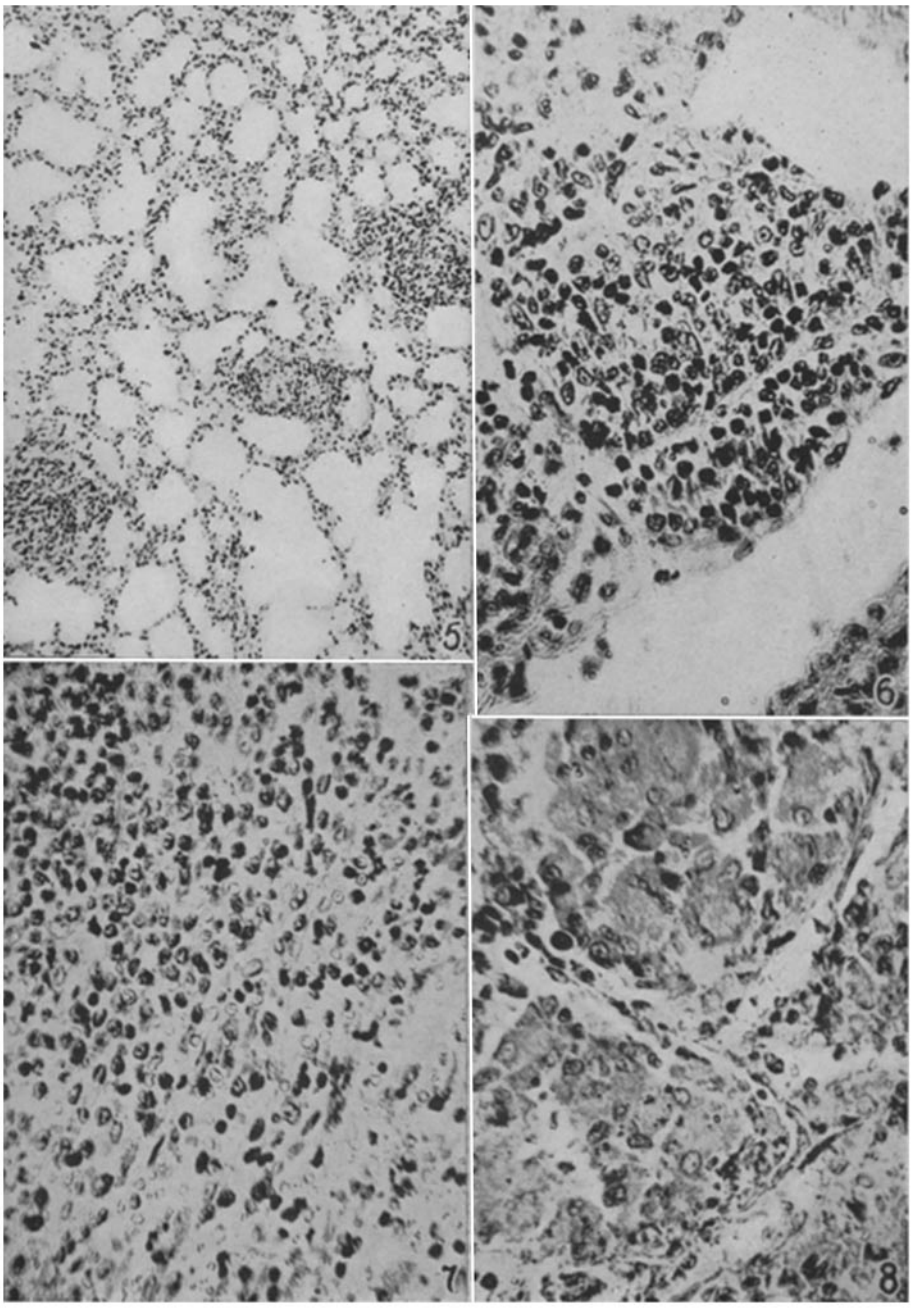
FIG. 10. Tracheobronchial lymph node of Rabbit 19-88 of Series III, 2 weeks after inoculation; 1,020 colonies were isolated. Caseation and edema of surrounding epithelioid cells. ×200.

FIG. 11. Liver of Rabbit 30 of Series III, 4 weeks after inoculation. 3 colonies were isolated. A resolving tubercle. ×200.

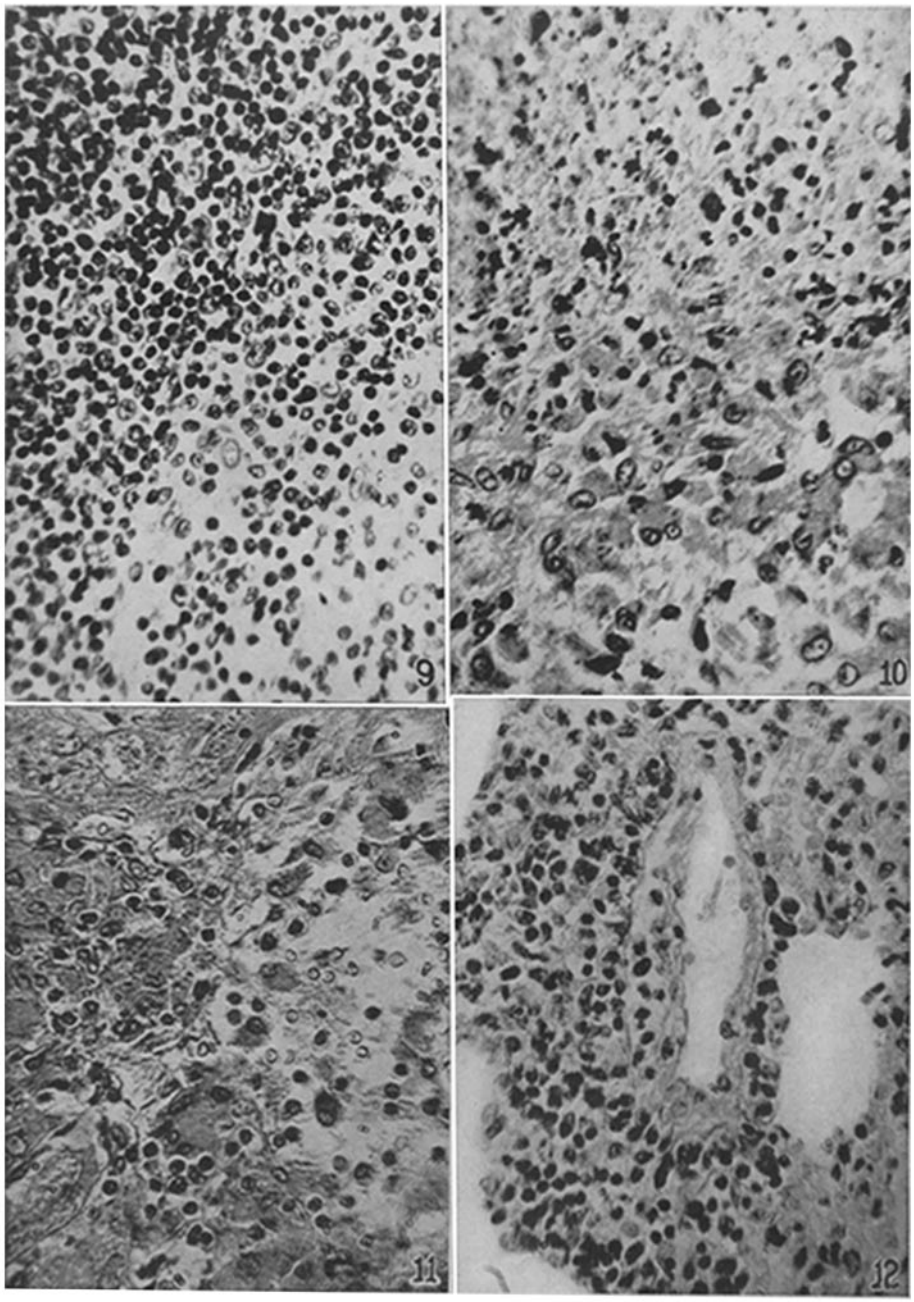
FIG. 12. Lung of Rabbit 34 of Series III, 2 months after inoculation. No tubercle bacilli were cultured. A perivascular collar of mononuclears. × 200.



(Lurie: Fate of BCG and changes in rabbit organs)



(Lurie: Fate of BCG and changes in rabbit organs)



(Lurie: Fate of BCG and changes in rabbit organs)