A FREE GROWTH PERIOD OF TUBERCLE BACILLI IN THE GUINEA PIG OMENTUM AS RELATED TO THE HYPERSENSITIVE STATE *

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The study of immune processes in tuberculosis is complicated by the fact that true immunity, in the sense in which that term is used in diphtheria or smallpox, does not exist. The patient who, to all clinical appearances, has completely recovered from tuberculosis may suffer a relapse, just as the experimental animal which has been "immunized" may be fatally reinfected with a large enough dose of virulent tubercle bacilli. At the same time, as was first adequately demonstrated by Römer,[1] something happens, in the laboratory animal and, presumably, in man, after an initial tuberculous infection, which renders the individual or the animal somewhat more resistant to reinfection. Just what changes occur in the body cells and fluids following this initial sensitizing infection is not clear.

A useful approach to the problem of immunity in tuberculosis and one which has been employed by numerous investigators, is the attempt to discover how the tubercle bacillus itself, with its resistant, waxy structure, is affected by inoculation in the normal and the "immune" animal. Thus Markl,[2] Kraus and Hofer,[3] Manwaring and Bronfenbrenner,[4] Bergel,[5] Rist, Léon-Kindberg and Rolland [6] and Dworski, Smith and Gardner [7] all have studied peritoneal fluid withdrawn at intervals after intraperitoneal inoculation of the experimental animal, and Paterson [8] has made a similar study of pleural exudates. All of these studies, however, are subject to the limitations imposed by the use of inflammatory exudates, most of the investigators having noted the early appearance of so-called Much's granules and the complete disappearance of acid-fast bacilli from the fluid of the respective serous cavities within 4 or 5 days after inoculation. In every case the bacilli were found to have disappeared more quickly in the reinoculated than in the normal animal.

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In the study which follows use has been made of the well known fact that bacteria, when inoculated in the peritoneal cavity, accumulate in large part in the omentum. Spread preparations of the omentum have been employed, a simple technic having been developed for staining such preparations. This use of omental spreads has made it possible to study the inoculated bacilli in their normal relation to the fixed tissue cells and the developing tubercles, a study that cannot be made adequately either with histological sections or with smears of the body fluids.

Technic

Albino guinea pigs were employed in the major part of the experiments, since the reaction of this animal to tuberculous infection seems to approximate so closely that of man. In general, young animals from 200 to 300 gm. in weight were used, as the omentums of old guinea pigs contain too much perivascular fat to make good spread preparations.

In most of the experiments the well known H-37 human strain of *Mycobacterium tuberculosis* was used for the inoculum. A portion of the pellicle of a glycerine broth culture was weighed, after blotting off the excess fluid on sterile filter paper, and carefully ground with mortar and pestle in a few drops of sterile saline. Saline was then slowly added to give the dilution which was desired, usually 1 mg. of the culture per cubic centimeter of fluid.

Anyone who has attempted to prepare inoculums from cultures of the tubercle bacillus has experienced the difficulty of getting a uniform suspension of the organisms. Even after the most painstaking grinding relatively large clumps of bacilli remain in the triturated material. In the present experiments no attempt was made to remove these clumps by filtration since it was desired to maintain the known weight of inoculum. In most of the experiments the animals were given an amount of inoculum equivalent to 0.1 mg. of bacilli per 100 gm. of body weight, though in some series as much as five times this amount was employed. Whenever inoculums containing clumps of bacilli were employed, it was found important to take into the syringe sufficient inoculating fluid for only one animal at a time. If enough fluid for several animals is taken into the syringe the clumps of bacilli gravitate so rapidly that an uneven dose for the different animals results.

Another pitfall which must be guarded against in making intraperitoneal inoculations is that of losing all or a portion of the inoculum either by penetrating the bowel or by failing to enter the peritoneal cavity at all. In the present study the animals, with abdomen shaved, were lightly etherized and an incision 3 to 4 mm. in length made through the skin with scissors. By making use of this incision one could usually be certain of entering the peritoneal cavity with the inoculating needle. The best protection against penetration of the gut we have found to be an initial inoculation of 10 cc. of air with a separate syringe. If the air enters the peritoneal cavity the anterior abdominal wall will be bulged up uniformly, while if the air is forced into the appendix or some other portion of the gut a serpentine bulge is formed. Once the anterior abdominal wall has been separated from the underlying intestines by a layer of air the inoculating needle can be introduced with little fear of penetrating the bowel. If the inoculating dose should be lost in its entirety into the gut the omentum remains completely normal.

Omental Spreads

In order to follow the omental changes animals were killed at daily, or more frequent, intervals. During the first few days after inoculation omental spreads are easily made. After the first week, however, there is an increasing tendency for the free edges of the omentum to become matted together. Under these circumstances it is well to pull with forceps on the matted portion of omentum before it is removed from the animal, thus breaking most of the fibrinous adhesions. That part of the omentum which shows the maximum involvement is then freed with scissors and mounted on a clean glass slide, either directly or after first floating the tissue in a jar of normal saline. Using a pointed glass rod for manipulating the moist preparation, a single, thin layer of the omentum is isolated and stretched out from the major omental mass. By allowing this thin film of tissue to dry on the slide one obtains a point of attachment and can pull from this point on the main mass of omentum further to break apart its fibrinous adhesions. Finally one obtains a layer of omentum not more than one cell thick through most of its extent. The preparation is then allowed to dry in the air, after which it will be found firmly fixed to the slide. The steaming with carbol fuchsin completes the fixing process.

With minor exceptions the ordinary Ziehl-Neelsen technic for staining is employed. The preparation, flooded with carbol fuchsin, is heated to the steaming point on an aluminum plate, washed in tap water, flooded with acid alcohol, washed, and then immersed for 10 minutes in acid alcohol (3 per cent HCl in 95 per cent alcohol) in an attempt to decolorize the thicker portions of the preparation. After again washing in tap water the omentum is counterstained with methylene blue, washed and blotted. The preparation should then be allowed to dry for an hour at 37°C or for 12 hours at room temperature to allow the perivascular fat to "sweat out." Finally, any remaining portions which are too thick should be removed. The process is completed by merely immersing the dry preparation in absolute alcohol, then in xylol, and mounting in balsam. The result is a permanent preparation, easily and quickly available, which shows the entire developing tubercle with its content of bacilli and its surrounding cells. Such a preparation may be studied through most of its extent with the oil immersion lens.

Results

I. Inoculation of the Normal Guinea Pig

The results which we have obtained in the early hours after intraperitoneal inoculation corroborate those reported by other workers. There is an initial acute inflammatory reaction, the tubercle bacillus being taken up first by polymorphonuclear cells. One of the surprising findings was the rapid disappearance of the large clumps of bacilli (Fig. 1) which were included in the inoculum. A few hours after inoculation these clumps had been almost completely broken up by the polymorphonuclears, each cell taking up from one to a dozen bacilli (Figs. 2 and 3), and by the end of 24 hours the clumps had completely disappeared. At 2 hours there are relatively few large mononuclear leukocytes, but an occasional one will be found which has phagocytosed bacilli. In no case, however, have we found the mononuclear cells phagocytosing polymorphonuclears at this stage. Unfortunately, the Ziehl-Neelsen stain used for our preparations did not enable us to differentiate between polymorphonuclear neutrophiles and eosinophiles, the latter, according to Dworski, Smith and Gardner,[7] being the cell most actively phagocytic for the tubercle bacillus in the first few minutes after inoculation.

After 24 hours the peritoneal fluid and the omentum still show numerous polymorphonuclear leukocytes, but very few of these cells contain tubercle bacilli. Many of the polymorphonuclears which phagocytosed bacilli during the first few hours have now been taken up by large mononuclear cells (Figs. 4 and 5).* The bacilli, as seen after this double phagocytosis, exhibit marked irregularities in staining and in many cells non-acid-fast granules may be seen which probably represent the so-called Much's granules. Also, occasional mononuclear cells are seen which have evidently taken up bacilli directly (Fig. 6). It will be noted that these bacilli appear to lie within cytoplasmic vacuoles. Furthermore, they appear better preserved than the bacilli in Figures 4 and 5 which were first phagocytosed by polymorphonuclears.

While many damaged polymorphonuclear leukocytes are taken up by large mononuclear cells, as illustrated in Figures 4 and 5, a large portion of the polymorphonuclears bearing tubercle bacilli apparently gravitates to the milk spots (*tache laiteuse*) of the omentum or to the perivascular fat tissue. The dark areas shown about the blood vessel in Figure 7 are made up almost entirely of polymorphonuclears which have encroached upon the perivascular fat cells. Many of these polymorphonuclears still carry their burden of bacilli. Within 3 days after inoculation the perivascular areas have become a dense cellular mass composed largely of mononuclear cells (Fig. 8). It is the opinion of Gardner,[10] who has studied this phenomenon in regular histological sections, that the mononuclear cells proliferate *in situ* to form the tubercle-like nodules about the blood vessels. It is in these areas that caseation first appears.

The Appearance of Freely Growing Bacilli: Gardner [11] has pointed out the remarkable clearing of the acute inflammatory reaction within 3 days after intraperitoneal inoculation of the normal guinea pig with tubercle bacilli. We have observed the same thing in spread preparations of the omentum. At 4 days the preparations show the dense perivascular infiltration, the enlarged milk spots and occasional clumps of mononuclear cells which apparently proliferate *in situ* on the surface of the omentum to form miliary tubercles (Fig. 9). At 4 days, also, a phenomenon which we have not hitherto

* A recent paper by Vorwald [9] indicates a similar transference of bacillus-containing polymorphonuclears to the large mononuclear cells in the lung of the rabbit after intravenous inoculation with H-37.

seen reported makes its appearance. In association with certain cells may be seen masses of bacilli growing in parallel strands just as they might grow on the surface of glycerine broth (Figs. 10 and 11). These masses may be immediately adjacent to, or some little distance from, the groups of cells that are forming tubercles (Figs. 10, 11, 12 and 13) and, surprisingly enough, show no sign whatever at this stage of attracting inflammatory cells of any type (Figs. 14 and 15). The bacilli commonly follow the cytoplasmic outline of the cell with which they are associated and at times are looped about the cell nucleus (Fig. 16). However, they appear to be growing on the surface of, rather than within, the cytoplasm. From all appearances the cells in which, or on which, the bacilli are growing are the ordinary ones which make up the major portion of the omental network (Figs. 17 and 18). They show no deleterious effect from the symbiotic growth. These organisms, because of the capacity which they exhibit for growth without the least interference from either the cells or body fluids of the animal in which they are proliferating, have been termed *freely growing bacilli*.

That the microörganisms which have just been described have actually proliferated and do not represent merely clumps of bacilli from the inoculum is indicated, first, by the fact that no such clumps are found in the omentum in the interim between inoculation and the 4th day and that all large clumps have disappeared from the peritoneal fluid by the end of 24 hours; secondly by the fact that the bacilli are in parallel strands just as they grow on culture media, while the inoculated bacilli, after phagocytosis by either polymorphonuclears or monocytes, are found helter-skelter in the cell without any regular arrangement. Thirdly, only a few small colonies of freely growing bacilli are found in an occasional omentum at the 4th day, while, on the 6th day the colonies are larger and more numerous and are found with uniformity in the omentums of most of the animals. Fourthly, the freely growing forms do not appear in the omentum of "immunized" or secondarily inoculated guinea pigs. Finally, various control inoculations (to be described in detail later) made with caseous material, heat-killed bacilli, and timothy grass bacilli prove that the tubercle bacillus does actually proliferate in the animal body at a certain period after inoculation without any inflammatory response on the part of the diseased animal. The great regularity with which the free proliferation of tubercle bacilli

occurs in the body of the normal guinea pig is indicated by the accompanying table.

TABLE I

Normal Guinea Pigs Inoculated with H-37 from Glycerine Broth Cultures *

Days after inoculation	1	2	3	4	5	6	7	8	9	10	11	12	13–78
Total No. pigs sacrificed	24	13	12	15	15	26	18	18	14	11	13	12	103
No. of pigs *positive* for free bacilli ..	0	0	0	6	11	21	16	6	2	1	1	0	0
No. of pigs *negative* for free bacilli ..	24	13	12	9	4	5	2	12	12	10	12	12	103

* Based upon 21 series of guinea pig inoculations.

As indicated, the freely growing forms were found first after an interval of 4 days. Four days is about the length of time required for the H-37 strain to establish itself on favorable culture mediums and this fact probably explains why freely growing bacilli were not found before the 4th day after inoculation. From the 4th day the number of animals showing the freely growing bacilli rises to 81 per cent at 6 days* and 89 per cent at 7 days, falling off abruptly after the 7th day.

The Use of Caseous Material as Inoculum: Five series of animals were inoculated with caseous material obtained from cold abscesses, the latter being the result of inoculating guinea pigs subcutaneously with H-37. This caseous material can be readily triturated and diluted with saline to give a uniform suspension. The amount of inoculum used varied from 10 mg. of caseous material per pig in 1 series to a maximum of 50 mg. per pig in another series. Smears of the inoculum showed only scattered acid-fast bacilli, while in 2 series the caseous material contained no demonstrable acid-fast organisms whatsoever. In every series, however, masses of freely growing tubercle bacilli made their appearance, Table II presenting the accumulated data from the 5 series.

* A careful reading of one of Krause's [12] early papers indicates that he must have observed freely growing bacilli in the iliac lymph nodes of guinea pigs after subcutaneous inoculation in the groin. In describing the masses of bacilli which have proliferated in these nodes at 6 days Krause says, "In none of these particular fields did the bacilli appear to lie within the cells. . . ."

It will be noted from Table II that the freely growing bacilli in these series were somewhat more tardy in making their appearance than when the inoculum was obtained from broth cultures. When they eventually appeared, however, the freely growing forms were perfectly typical in arrangement (Figs. 19 and 20).

TABLE II

Normal Guinea Pigs Inoculated with H-37 Caseous Material obtained from Cold Abscesses *

Days after inoculation	1	2	3	4	5	6	7	8	9	10	11	12	13–47
Total No. of pigs sacrificed	4	2	3	2	3	2	3	1	6	1	1	1	47
No. of pigs *positive* for free bacilli	0	0	0	0	0	2	3	1	2	0	0	1	0
No. of pigs *negative* for free bacilli	4	2	3	2	3	0	0	0	4	1	1	0	47

* Based upon 5 series of guinea pig inoculations.

A single series of animals was inoculated with the bovine strain, H-61, and another series with caseous material obtained at the autopsy table from a case of generalized tuberculosis in man. In both series beautiful examples of freely growing bacilli were found.

The Use of Dead Bacilli and of M. Phlei as Inoculum: Three series of pigs were inoculated intraperitoneally with H-37 killed by boiling and 1 series with the same organisms killed by exposure to direct sunlight. In the 4 series 26 guinea pigs were employed. Tubercles were formed in the omentums of these guinea pigs and, in the tubercles, faintly staining acid-fast bacilli were found for as long as 22 days after inoculation. Though the inoculums — in each case 1 mg. per pig — contained large clumps of bacilli, in no instance was anything found in the omentum suggestive of the freely growing form of the bacillus.

In 4 series, utilizing 33 pigs, the timothy grass bacillus, *M. phlei*, was employed as inoculum. The relatively small lipoid content of this bacillus, as compared with the pathogenic acid-fast strains, has been pointed out by Anderson,[13] a fact that may explain the early disappearance of the inoculated grass bacilli from the omental preparations. However, tubercles are formed sooner than after in-

oculation with H-37 (Figs. 21 and 22). Acid-fast bacilli can be found in these tubercles in large numbers during the first 24 hours, but by 3 days and thereafter bacilli can no longer be found. The tubercles do not progress to caseation.

II. The Inoculation of Hypersensitive Guinea Pigs and of Normal Rabbits

The results from the inoculation of hypersensitive pigs contrast strikingly with those obtained from the normal animals. In 3 series the pigs were given a subcutaneous sensitizing dose of H-37 from 3 to 5 weeks before the secondary intraperitoneal inoculation. As far as the exudative reaction in the hypersensitive animal is concerned our findings again corroborate those of Gardner.[11] The initial outpouring of polymorphonuclears is greater than in the normal animal. Tubercles form more rapidly, and the omentum, within 4 days after the second inoculation, is matted together in a dense sausage-shaped mass which cannot be separated without tearing the tissues. Within this matted omentum may be found accumulations of creamy caseo-pus, while a dense fibrinopurulent exudate is frequently found adhering to the surfaces or edges of the liver and spleen. If, at daily intervals, smears are made of either the pus or the fibrinopurulent exudate they will be seen to contain the same large clumps of acid-fast bacilli that were included in the original inoculum (Fig. 23). These clumps are closely surrounded by polymorphonuclear leukocytes, the cells being literally plastered against the edges of the bacillary mass. An occasional cell in the smear (either polymorphonuclear or mononuclear) may contain a few bacilli, but there is no sign of the breaking up of bacillary clumps through the phagocytosis which occurs in the normal pig.

At 10 days the clumps are still found, somewhat more rounded or oval in shape, with a ring of polymorphonuclears still pressing tightly against the bacilli (Figs. 24, 25 and 26). The latter appear to have undergone partial lysis, no longer staining as sharply as in the early days after inoculation. Thus, in the hypersensitive guinea pig the clumps of bacilli which may be included in the inoculum are not broken up by phagocytosis, as they are in the normal animal. Furthermore, at no time in the hypersensitive animals is the phenomenon of free proliferation of the inoculated tubercle bacilli ob-

served. In none of the 31 animals used in the 3 series was there any suggestion of that free growth of tubercle bacilli which occurs in the normal guinea pig.

Since the normal rabbit has a high degree of natural resistance to infection with the human type of tubercle bacillus, it was decided to inoculate some of these animals. Accordingly, a small group, 9 rabbits in all, was inoculated intraperitoneally with H-37 on the same basis as the guinea pigs, namely, 0.1 mg. of bacilli per 100 gm. of body weight. None of the inoculated rabbits showed at any time any free growth of tubercle bacilli.

III. The Fate of the Freely Growing Bacilli

Up to 6 days after inoculation the freely growing bacilli show no evidence of attracting phagocytic cells (Figs. 10 to 18), although by the 7th day they usually begin to exert a chemotactic influence upon the polymorphonuclear leukocytes. Frequently in a single omental preparation at this stage one finds a few phagocytic cells hovering about at a little distance from the bacilli (Figs. 27 and 28), while another colony of bacilli may have a polymorphonuclear directly applied to it (Fig. 29). Still another colony may be completely broken up by the invasion of polymorphonuclear leukocytes (Figs. 30 and 31). The bacilli at this period show a marked degree of beading (Fig. 32).

At 7 days, then, the omentum of a single animal may show considerable variation in the status of the masses of freely growing bacilli, though by the 8th day the freely growing forms have disappeared in the majority of the animals. In their place we find evidence of the formation of a secondary series of tubercles, and by 9 days this phenomenon is well established. At this time the omental preparations show two definite vintages, so to speak, of tubercles — large ones which have formed about the original bacilli of the inoculum, and minute ones which are in the process of forming about the once freely growing bacilli (Fig. 33). It is frequently impossible to make out the bacillary content of the large tubercles at this stage, even when they are teased apart, while in the minute tubercles bacilli are clearly visible (Fig. 34).

During the period from 10 to 14 days after inoculation one gains the impression that the number of acid-fast bacilli is definitely less,

occasional preparations showing beaded, non-acid-fast forms. Acid-fast bacilli, if found at this stage, are frequently bizarre in shape, as shown in Figure 35. At much later stages the omentum still shows acid-fast bacilli which are irregular in length and in staining reaction (Fig. 36). Some of these bacilli appear to lie free on the surface of cells, but there are no freely growing colonies such as characterize the 4 to 7 day interval. Any phagocytosis at this stage is apparently by cells of the mononuclear series (Fig. 36).

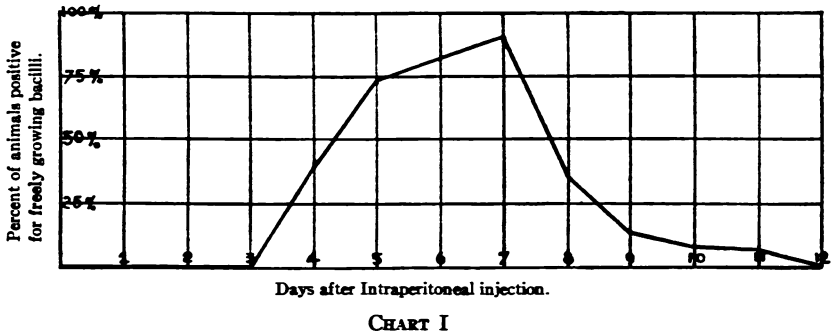
Discussion

From the data presented it is evident that tubercle bacilli of the H-37 strain, after intraperitoneal inoculation in the normal guinea pig, pass through certain definite changes, as far as their chemotactic relation to cells of the host animal is concerned. The inoculated bacilli attract and are taken up by polymorphonuclear leukocytes, which then either move to the perivascular tissues or milk spots, or are phagocytosed in turn by large mononuclear cells. At 4 to 6 days the freely growing forms appear and show no sign of chemotactic influence until the 7th day, when they again attract the polymorphonuclear cells. It seems probable that this variable picture, as regards chemotaxis, is due to one of two things — either to changes that occur in the cells and (or) fluids of the infected animal, or to changes that occur in the biochemistry of the inoculated microörganisms. In favor of the former hypothesis is the fact that hypersensitiveness, as indicated by a positive tuberculin reaction, appears within 6 to 12 days after experimental inoculation of the guinea pig with tubercle bacilli.[14] To determine whether there is any correlation between the appearance of a positive tuberculin reaction and the disappearance of freely growing bacilli in a given animal, a small series of pigs was tested by the Mantoux method. In this small series there was no absolute correlation observable, the freely growing bacilli having usually disappeared several days before the appearance of a positive tuberculin reaction. It was noted, however, that whenever the tuberculin test did become positive in an animal there was no longer any sign of freely growing bacilli in that animal's omentum.

In favor of the hypothesis that some change in the biochemistry of the bacillus is responsible for the chemotactic cycle is the abrupt

falling off in the percentage of animals positive for freely growing bacilli.

As indicated in the curve below, the proportion of animals showing the freely growing forms falls from 89 per cent at 7 days to 9 per cent at 10 days, a rather more abrupt drop than one would expect if the decrease were due merely to the gradual development of hypersensitiveness in the animals during the interval from 6 to 12 days. The abrupt appearance of freely growing bacilli at 4 days we have attributed to the latent period required for the H-37 strain to adjust



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itself to culture mediums of any type. Possibly the abrupt disappearance of these freely growing forms is again dependent upon some change in the characteristics of the bacilli themselves. Kahn [15] has demonstrated in beautiful manner that the H-37 strain of *M. tuberculosis* may pass through a complicated pleomorphic cycle when grown on certain culture mediums. The marked beading of the freely growing bacilli (Figs. 29, 30 and 32) is suggestive of the zoning within certain bacilli, which Kahn describes as occurring in his microdroplet cultures, or of the granules to which Gróh [16] attaches such significance.

It is impossible, at the present time, to decide definitely whether the abrupt reappearance of positive chemotaxis and the concomitant disappearance of the freely growing bacilli is due to the development of immune bodies in the infected animal or to changes in the bacillus itself, or to both. It seems certain, however, from our experience with the 31 secondarily inoculated animals, that the phase of free growth does not occur at all in an animal once it has been rendered hypersensitive. Thus, whatever else it may mean,

the positive tuberculin reaction in a guinea pig indicates that that animal is no longer susceptible to the type of free, unencumbered growth of tubercle bacilli, which takes place with uniformity in the normal animal.

Since we have noted that the freely growing bacilli disappear *before* the tuberculin test becomes positive, it seems possible, if not probable, that some reaction more delicate than the tuberculin test, which will indicate this changed condition of the animal, may be discovered. A further basis for this hypothesis is to be found in our results with the series of normal rabbits which failed to show free growth of the inoculated tubercle bacilli at any time. The normal rabbit does not, of course, exhibit a positive tuberculin reaction. It is interesting to speculate upon the possibility that the natural resistance of rabbits to infection with the human type of *M. tuberculosis* is related in some way to this failure of the free growth of the inoculated bacilli.

In the occurrence of the free growth of tubercle bacilli within the body of the normal guinea pig and the failure of that type of growth to occur in the hypersensitive animal we find the refutation of those workers who maintain that the difference between the reactions of the two types of animals is merely quantitative.[17] To be sure, the inflammatory reaction in the hypersensitive animal is quantitatively greater at the outset, but this reaction is maintained, instead of clearing up completely, as in the normal pig. This difference seems to us qualitative in nature, particularly when viewed in the light of the different effect upon the growth of the tubercle bacillus during the first week after inoculation. Rich,[18] in arguing against the acute inflammatory reaction of the allergic animal as a factor in "localization" of injected bacilli says: "Certainly, we ourselves have never been able to discover any difference between the number of stainable bacilli at the site of inoculation into the skins of normal and immune animals during the first few days after the inoculation; and after that, the number of bacilli in the area in the normal animals surpasses that in the immune animals — quite the reverse of what one would expect to find if the bacilli were quickly drained from the site in the normal animals, and actually held there bodily for days in the immune animals." Though the given data, particularly with regard to time intervals, are incomplete, it is our impression that Rich, in finding an increased number of organisms in his normal guinea pigs

after several days, was dealing with freely growing forms of the tubercle bacillus. In a series of normal animals which we inoculated subcutaneously, evidence was obtained that the free growth of tubercle bacilli occurs 6 or 7 days after subcutaneous inoculation, as well as after the intraperitoneal route of infection.*

An impression which inevitably follows working with omental preparations from several hundred infected guinea pigs is that one must have a very definite respect for the function of the polymorphonuclear leukocyte in the immune processes of tuberculosis. The polymorphonuclear has fallen into low esteem in this disease due, in part, to the well known fact that the opsonic index is lower in the animal rendered hypersensitive to tuberculosis than in the normal animal. To Zinsser [19] ". . . it is perfectly plain at the present time that polymorphonuclear phagocytosis has no protective value in tubercle bacillus infection. Indeed the tubercle bacilli are carried by the polynuclears throughout the body and any intra-cellular destruction that takes place is the function of clasmatocytes and giant cells."

While it is undoubtedly true that tubercle bacilli are disseminated by the polymorphonuclear leukocytes following a primary infection, it is also evident that the bacilli grow at a certain period in perfect symbiosis with the omental cells — cells of the connective tissue series which are, presumably, closely related to the clasmatocytes. Furthermore, it is the polymorphonuclears that first attack and break up the freely growing bacilli and initiate the process of renewed tubercle formation. Also, it is the polymorphonuclears that we find, in the hypersensitive pigs, plastered about the clumps of inoculated bacilli — not phagocytosing them but preventing the clump from breaking up, helping to localize the bacilli, as Krause and Willis [20] have shown and, we surmise, helping to prevent the free growth which occurs in the normal animal. This reaction of the polymorphonuclear cells in the hypersensitive animal is perfectly compatible with the existence of a low opsonic index, the low index, in fact, being an indication of the new function of the polymorphonuclear leukocyte in these animals, in which as Krause [21] says, "An almost immediate inflammatory outpouring hems in the bacilli more

* The possibility that these freely growing bacilli may represent an R dissociant has been considered, though no actual experimental work bearing on this point has been performed.

or less effectively and thus delays or prevents their spread which is so facile and rapid in the non-tuberculous, non-allergic animal."

We can agree with Rich's [18] conception of the local fixation of bacilli as a phenomenon "separate and dissociable" from allergy. At the same time, however, we believe that the polymorphonuclear leukocyte plays an important rôle in determining whether or not inoculated tubercle bacilli may grow freely for a certain period in the body of the host, and that this cell, therefore, is a factor which must be given due consideration in any discussion of immunity in tuberculosis.

Summary

1. After intraperitoneal inoculation in the guinea pig the H-37 strain of tubercle bacillus is first subject to phagocytosis by polymorphonuclear leukocytes. Then certain of the bacilli grow freely for a period on, or in, the cells of the omentum without exhibiting any chemotactic influence. At the end of this period the bacilli again attract polymorphonuclear leukocytes.

2. In guinea pigs that have been rendered hypersensitive to tuberculosis, and in normal rabbits, free growth of inoculated tubercle bacilli does not occur.

3. The relation of free growth and of polymorphonuclear phagocytosis to resistance in tuberculosis is discussed.

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DESCRIPTION OF PLATES

PLATE 162

FIG. 1. One of the large clumps of tubercle bacilli which characterized inoculum employed in most of the experiments. Photomicrograph taken with blue light. × 1500.

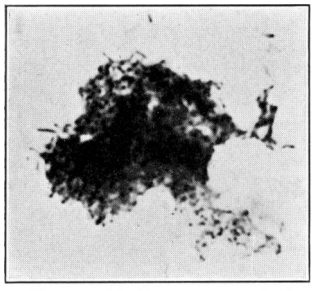
FIG. 2. Numerous polymorphonuclears in omental spread. Two hours after inoculation. Most of the cells have phagocytosed tubercle bacilli. Blue light. × 330.

FIG. 3. Polymorphonuclear cells with phagocytosed bacilli. From omental spread made 4 hours after inoculation. Blue light. × 1500.

FIG. 4. Large mononuclear cell showing phagocytosis of polymorphonuclears which had taken up bacilli. Found in smear of peritoneal fluid 24 hours after inoculation. Blue light. × 1500.

FIG. 5. Cell from same preparation as Fig. 4. Blue light. × 1500.

FIG. 6. Cell of mononuclear series which has phagocytosed bacilli directly. From omental spread made 48 hours after inoculation. Blue light. × 1800.



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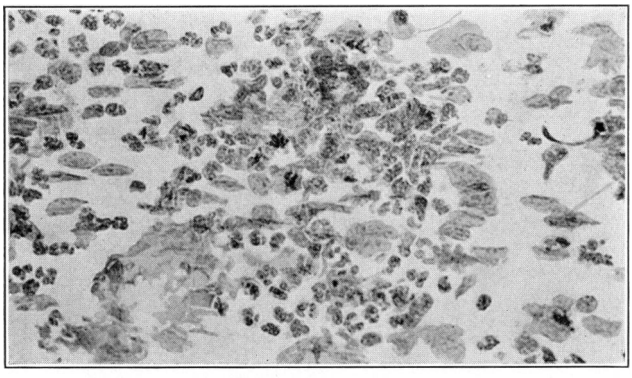

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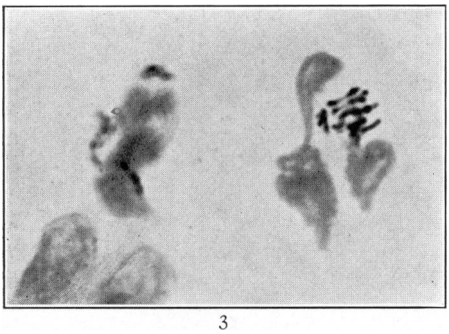

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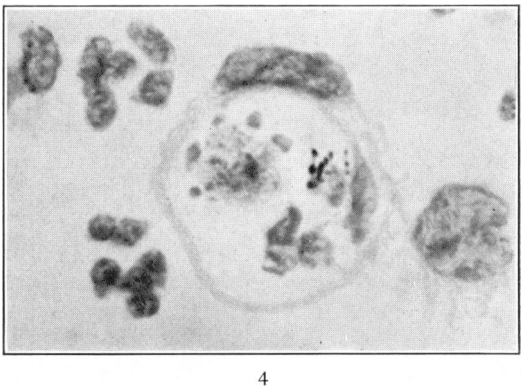

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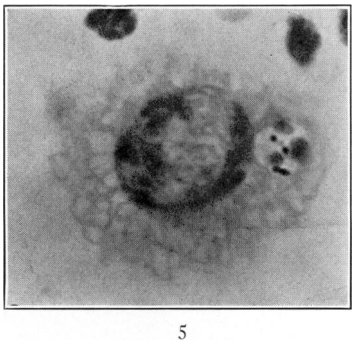

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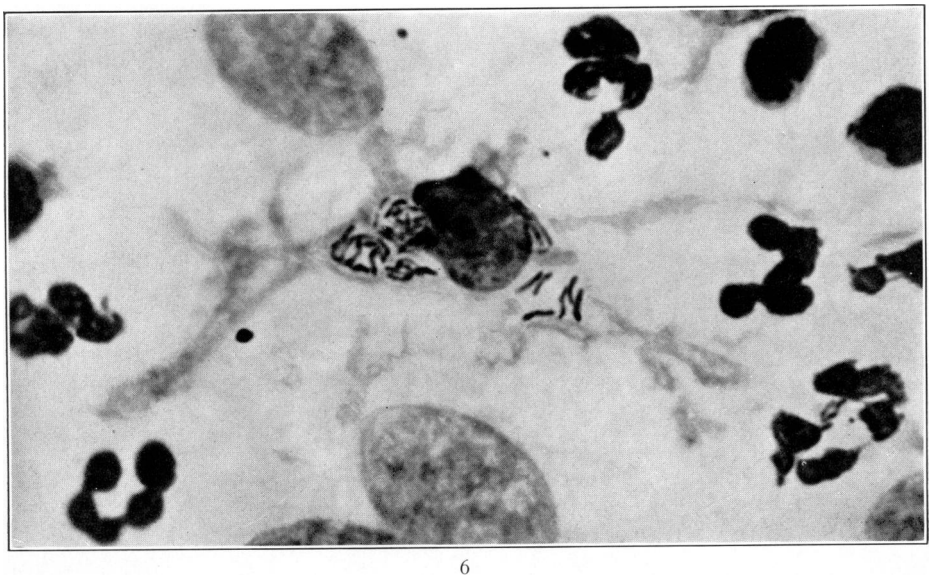

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PLATE 163

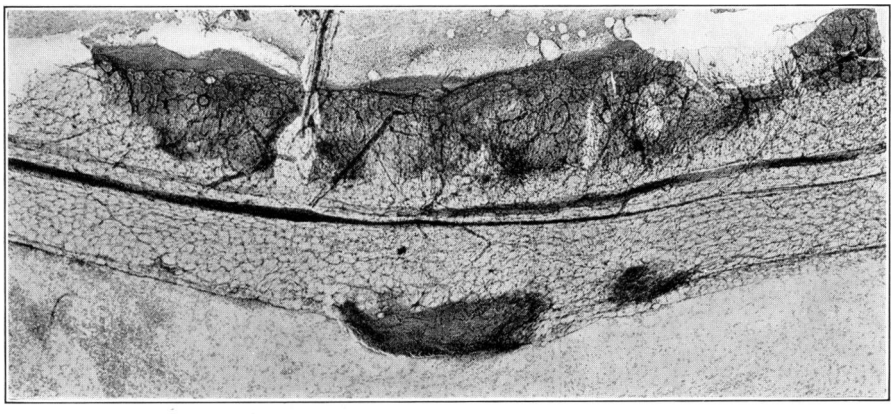
FIG. 7. The dark areas in the photograph are masses of polymorphonuclear leukocytes which have migrated to the perivascular fat tissue. From omental spread made 24 hours after inoculation. White light. × 25.

FIG. 8. Omental spread made 3 days after inoculation. Large mononuclear cells replace the polymorphonuclears and almost completely obliterate the perivascular fat cells. Arrow points to a circle of cells formed as the result of the localization of a bubble of air introduced at time of intraperitoneal inoculation. White light. × 25.

FIG. 9. Omental spread 4 days after inoculation showing clump of large mononuclear cells. These cells seem to proliferate locally, by amitotic division, to form miliary tubercles. Blue light. × 600.

FIG. 10. More advanced stage of miliary tubercle formation, with freely growing bacilli on cell outlined in rectangle. Omental spread made 4 days after inoculation. Blue light. × 165.

FIG. 11. Higher magnification of cell outlined in Fig. 10. Freely growing bacilli are clearly shown. Blue light. × 1500.



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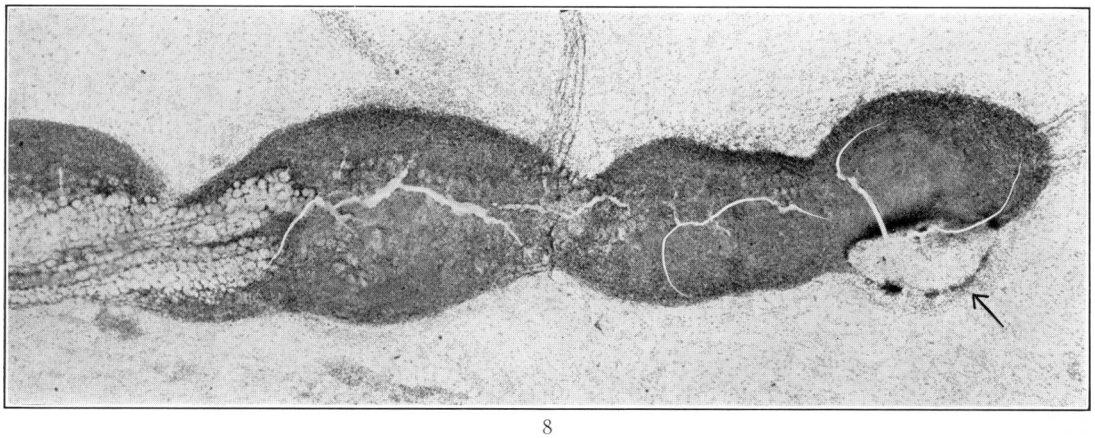

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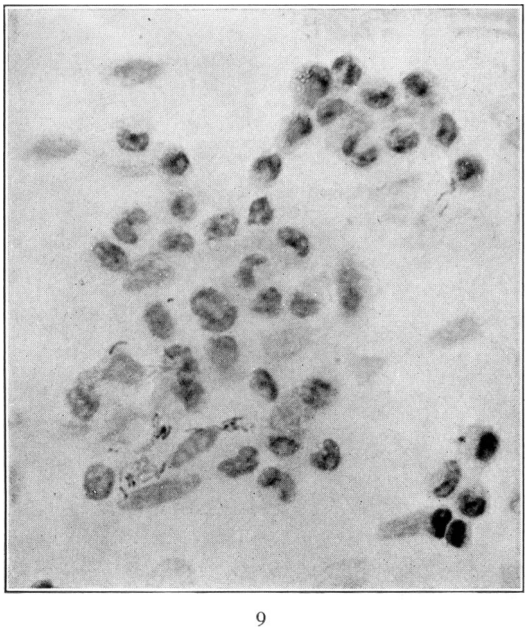

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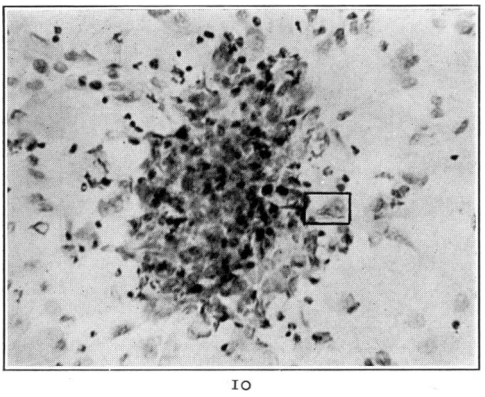

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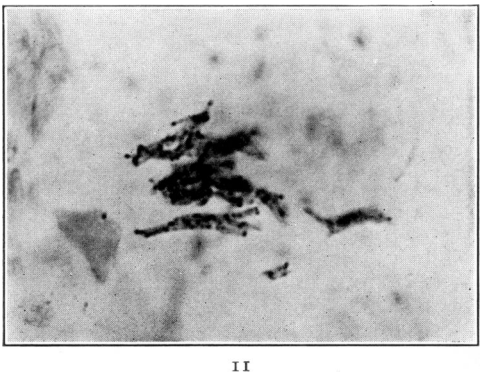

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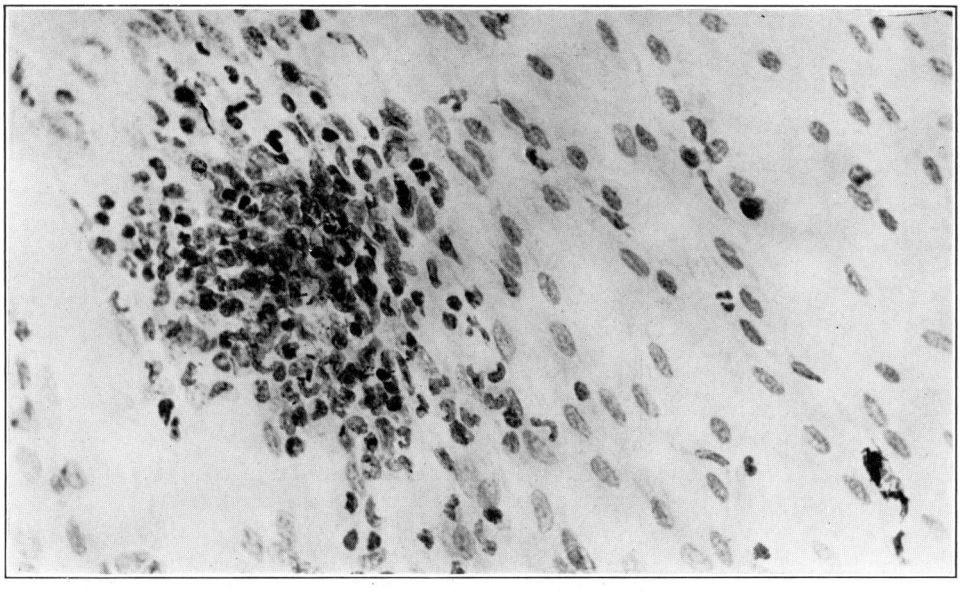
PLATE 164

FIG. 12. Freely growing bacilli, in lower right hand corner, at some distance from a developing tubercle. Omental spread made 6 days after inoculation. Blue light. × 330.

FIG. 13. Same tubercle and bacilli as shown in Fig. 12. Photomicrograph taken with green light to bring out the elastic network which characterizes the guinea pig's omentum. Green light. × 300.

FIG. 14. Numerous colonies of freely growing bacilli on omental cells 6 days after inoculation. Blue light. × 600.



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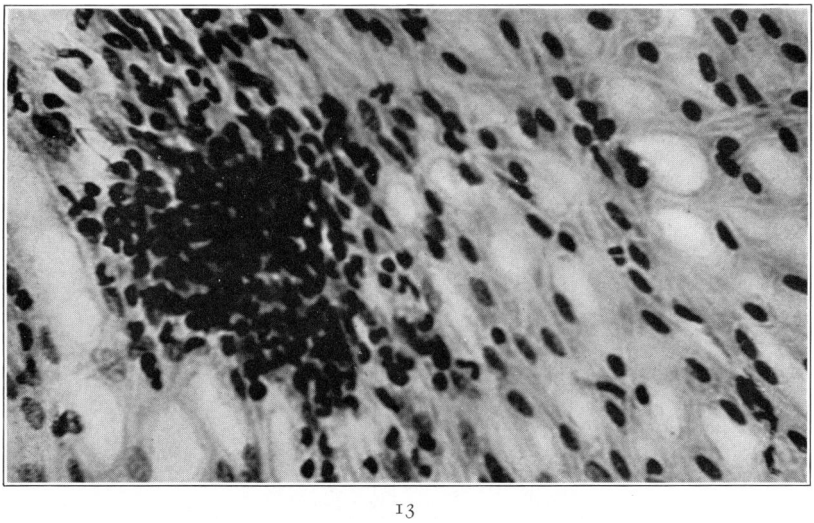

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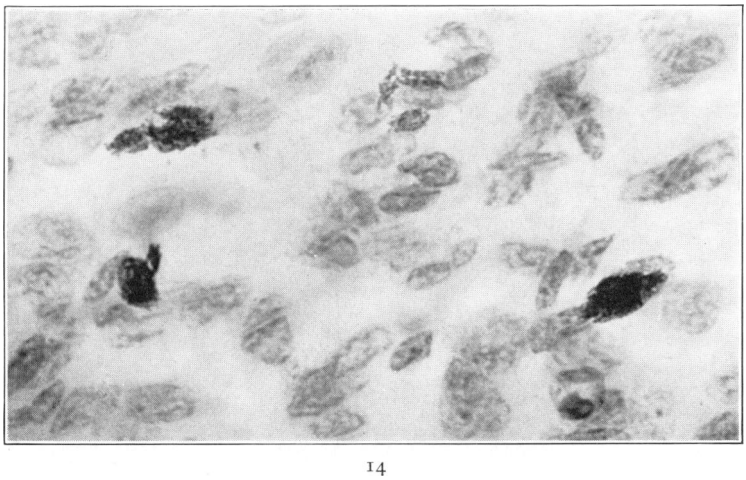

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PLATE 165

FIG. 15. Freely growing bacilli on omental cells 6 days after inoculation. Blue light. × 1500.

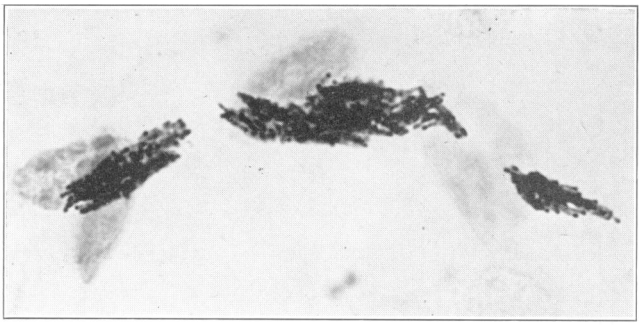
FIG. 16. Freely growing bacilli on omental cell 6 days after inoculation. Frequently the bacilli curve about the cell nucleus as shown. Blue light. × 1800.

FIG. 17. Freely growing bacilli on omental cells 6 days after inoculation. To all appearances the affected cells are no different from the surrounding cells. Blue light. × 350.

FIG. 18. Higher magnification of cells outlined in Fig. 17. Blue light. × 1800.

FIG. 19. Freely growing bacilli at some distance from a large tubercle formed 7 days after inoculation with caseous material. Tubercle formation was more rapid in this series than after inoculation of H-37 from culture. Blue light. × 165.

FIG. 20. Higher magnification of bacilli outlined in Fig. 19. Blue light. × 1500.



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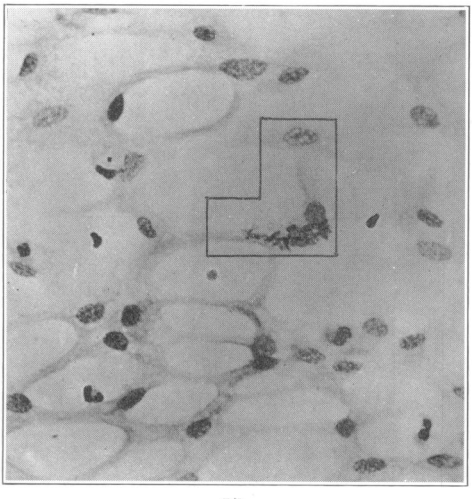

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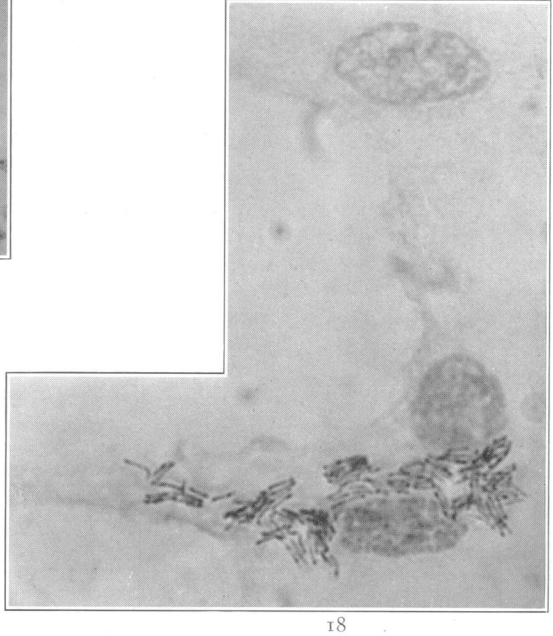

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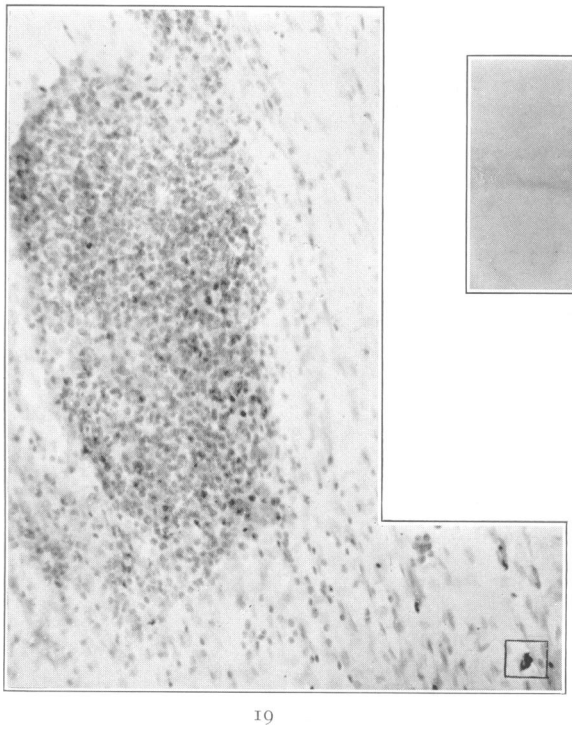

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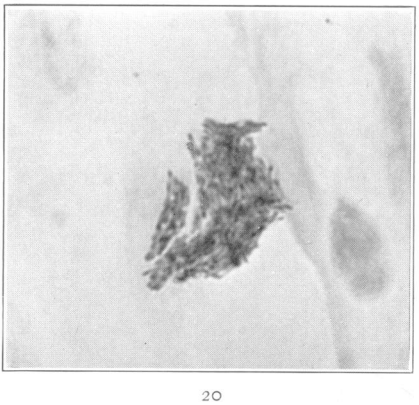

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PLATE 166

FIG. 21. Tubercles in omental spread made 48 hours after inoculation with timothy grass bacilli. Tubercle formation in this series, too, proceeded more rapidly than after H-37 inoculation. White light. × 165.

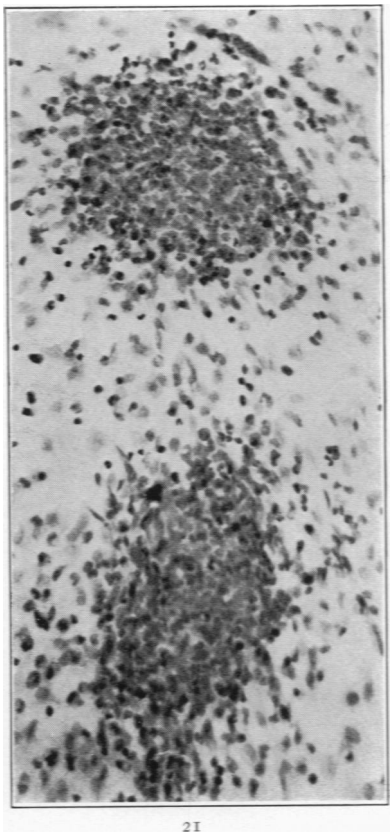
FIG. 22. Tubercle 6 days after inoculation with timothy grass bacilli. White light. × 165.

FIG. 23. Omentum of hypersensitive guinea pig 48 hours after inoculation. A clump of bacilli from the original inoculum still exists. Blue light. × 330.

FIG. 24. Smear of caseo-pus found encapsulated in omentum of hypersensitive guinea pig 10 days after inoculation. The smear showed numerous clumps of bacilli closely surrounded by polymorphonuclear cells. Blue light. × 330.

FIG. 25. Same clump of bacilli as shown in Fig. 24. Photomicrograph taken with white light to bring out the polymorphonuclears. White light. × 1500.

FIG. 26. Same bacilli as in Fig. 25. Photomicrograph taken with blue light. × 1500.



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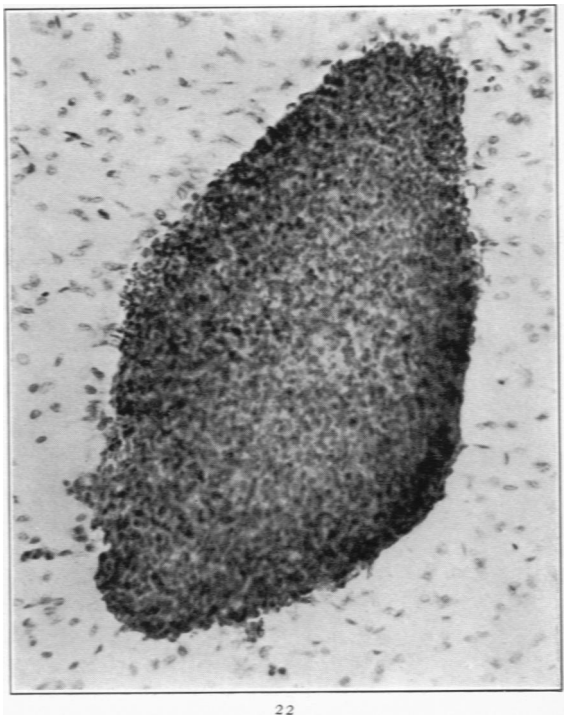

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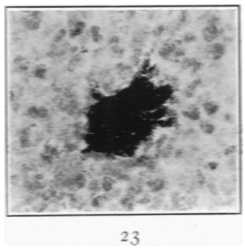

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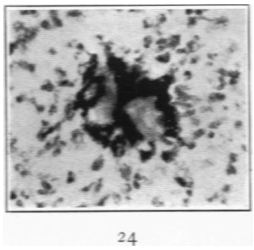

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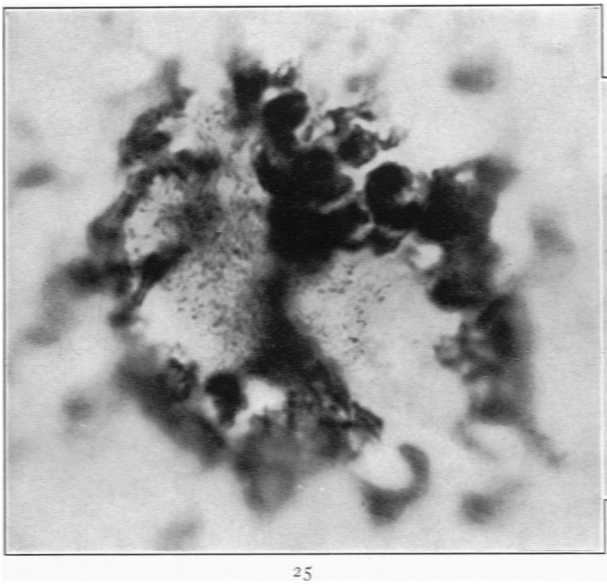

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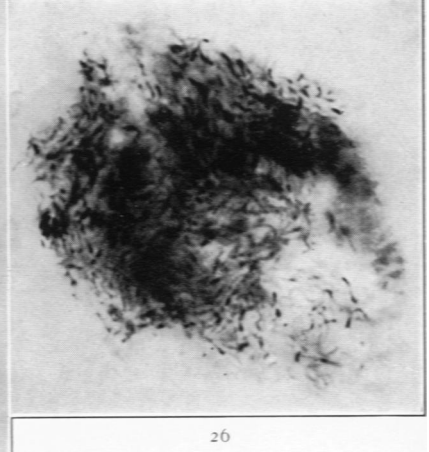

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PLATE 167

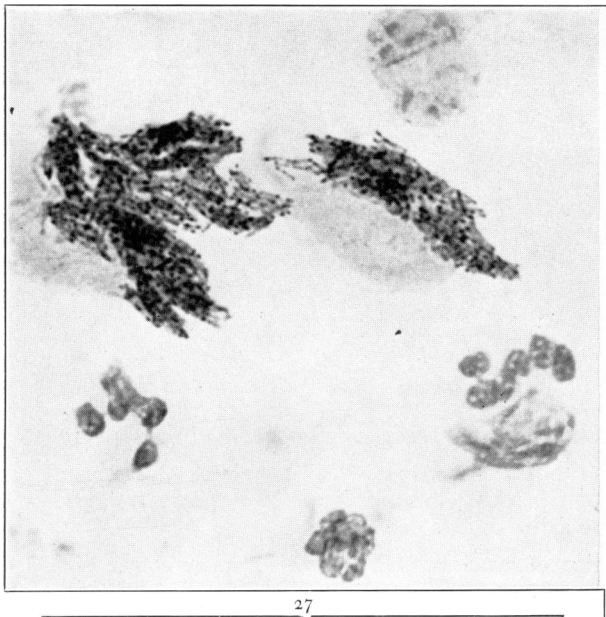
FIG. 27. Freely growing bacilli with adjacent polymorphonuclear cells. Omental spread made 7 days after inoculation. Blue light. × 1500.

FIG. 28. Freely growing bacilli with adjacent polymorphonuclear cells. Seven days after inoculation. Blue light. × 1500.

FIG. 29. Freely growing bacilli 7 days after inoculation. A polymorphonuclear leukocyte has applied itself to right hand edge of the colony. Blue light. × 1500.

FIG. 30. Colony of freely growing bacilli being invaded and broken up by polymorphonuclear leukocytes. Omental spread made 7 days after inoculation. Blue light. × 1500.

FIG. 31. Same group of bacilli as shown in Fig. 30. Photomicrograph taken with white light to bring out polymorphonuclears. White light. × 1500.



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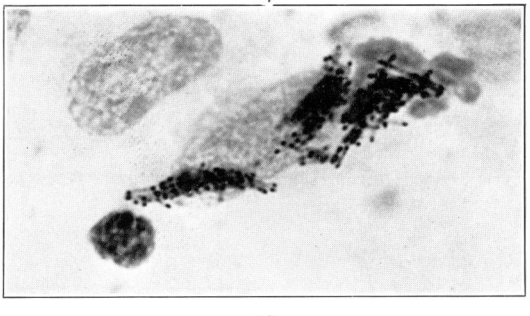

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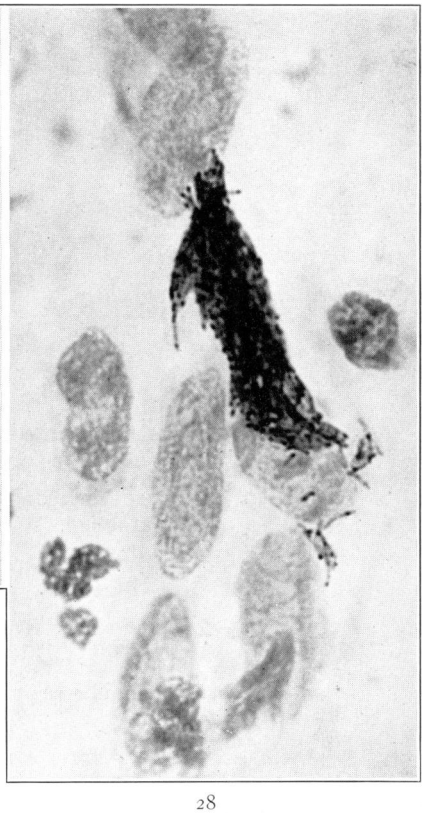

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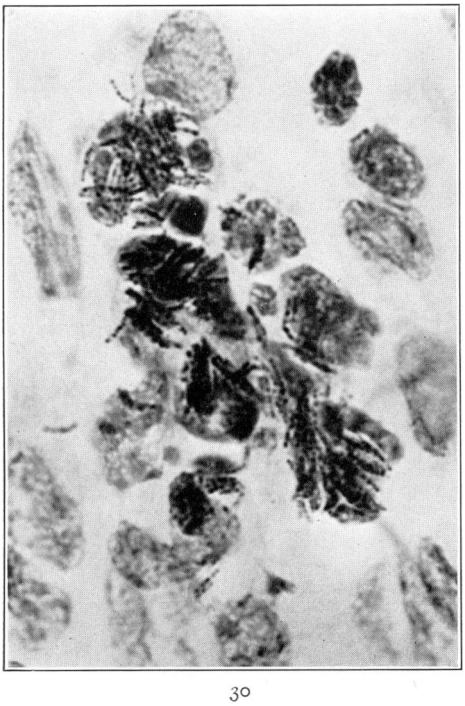

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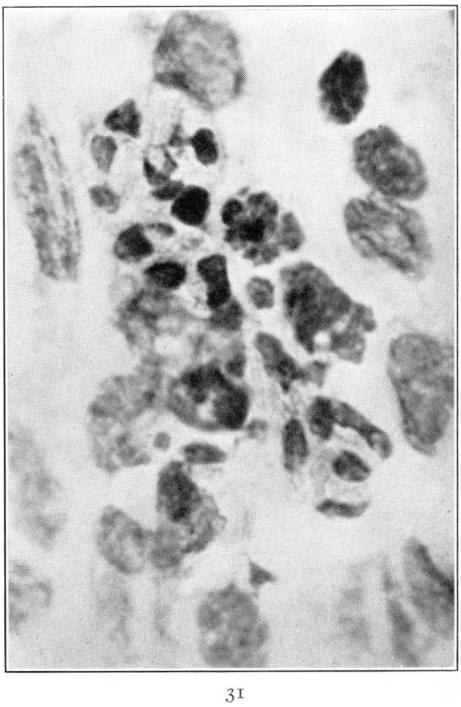


31

Woodruff

Free Growth Period of Tubercle Bacilli

PLATE 168

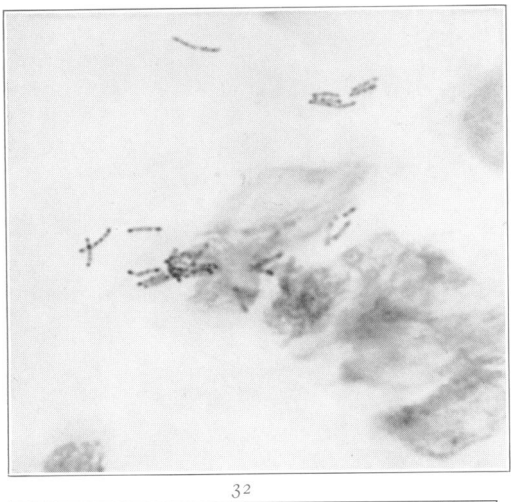
FIG. 32. Showing marked beading of the freely growing bacilli, 7 days after inoculation. Blue light. × 1500.

FIG. 33. Two "vintages" of tubercles commonly found in omentum 9 days after inoculation. White light. × 165.

FIG. 34. Higher magnification of small tubercle shown in upper corner of Fig. 33. Blue light. × 1500.

FIG. 35. Tubercle bacilli of bizarre shape. From omental spread made 11 days after inoculation. Blue light. × 1500.

FIG. 36. Bacilli in omental spread made 37 days after inoculation. Blue light. × 1800.



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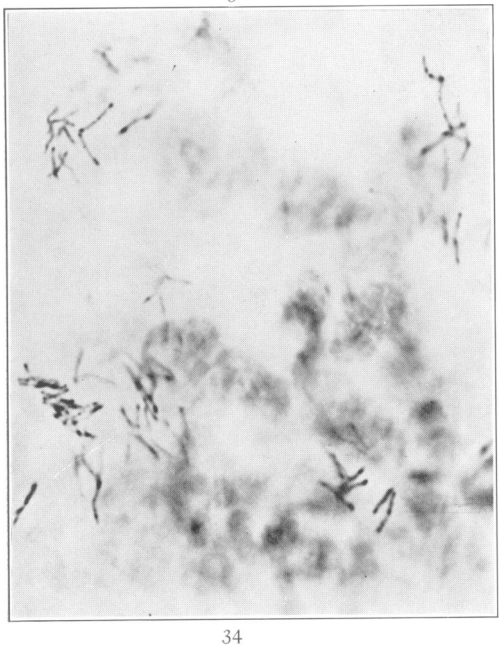

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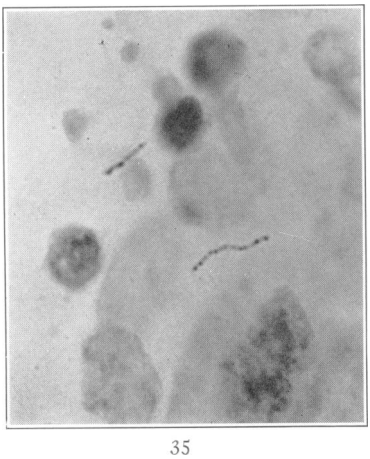

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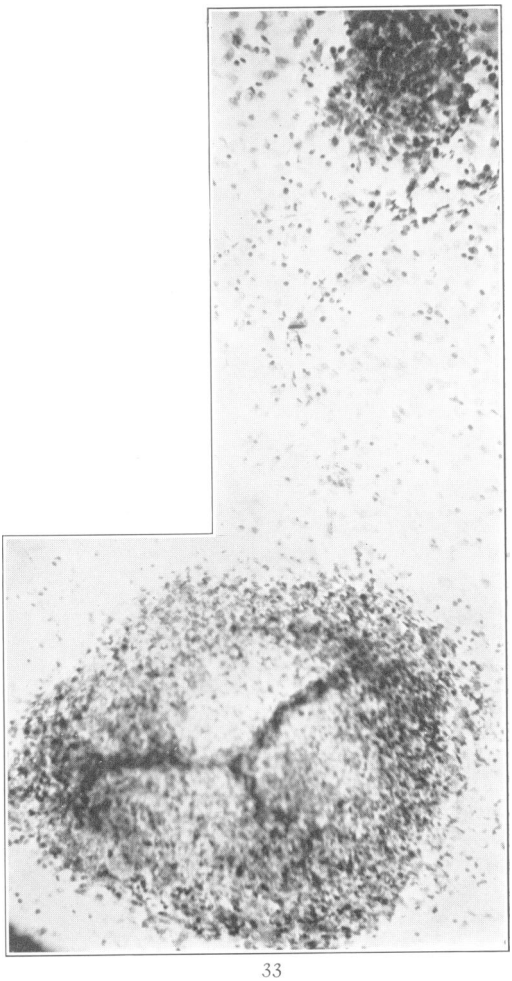

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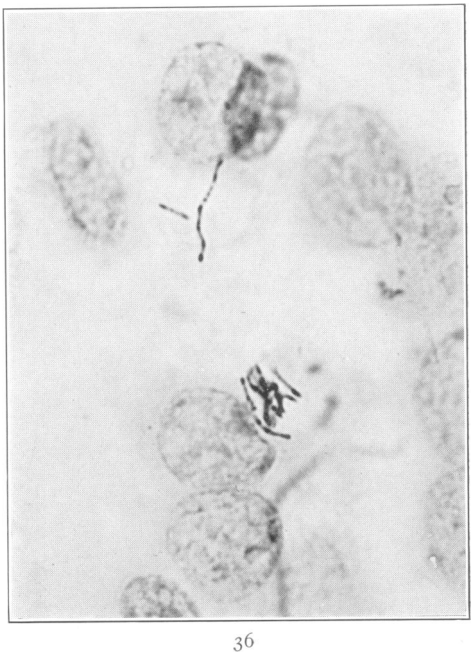

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Woodruff

Free Growth Period of Tubercle Bacilli