

A COMPARATIVE STUDY OF THE REACTION *IN VIVO*
AND *IN VITRO* OF RABBIT TISSUES TO INFECTION
WITH BOVINE TUBERCLE BACILLI.

PART II. OBSERVATIONS ON CULTURES OF SPLEEN AND
LYMPH GLANDS FROM INFECTED RABBITS

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(With Plates 9 and 10)

INTRODUCTION

In the preceding paper (Fell & Brieger, 1951), it was shown that when a droplet containing virulent bovine tubercle bacilli was placed on the surface of a fragment of rabbit spleen explanted *in vitro*, the macrophages ingested many of the organisms, distributed them throughout the explant and then broke down, liberating the bacilli which continued to multiply. In about 10 days the tissue was often completely infiltrated; no evidence of a toxic action was seen and there was no sign of the characteristic tuberculous tissue response.

These results suggested that there was an essential difference between the action of the bacilli in tissue culture and in the body. As already stated, we hesitated to draw a final conclusion from these findings, because the mode of infection was so different from that which occurs in the body. In the present investigation, however, this doubtful factor was eliminated, and the living animals were infected by intrapulmonary, intrasplenic or intravenous injection. They were then killed at intervals of 1–17 days, cultures of the spleen were made and the changes which appeared *in vitro* were studied; at the same time, the changes which took place during similar periods in the intact spleen left in the animal were investigated. In this way a critical comparison was made between the behaviour of the cells and bacilli in tissue culture and in the body.

MATERIAL AND METHODS

Young rabbits weighing about 2000 g. were used. They were killed at intervals of 1–32 days after infection, and the spleen or, in the case of intrapulmonary infection, (see below), some of the draining glands were removed aseptically; part of each organ was fixed in Susa or Zenker's fluids for histological examination, and usually explants for cultivation *in vitro* were prepared from the rest. The explants were grown by the hanging-drop technique described in our previous study (Fell & Brieger, 1951), and the same virulent bovine strains were used.

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Some of the cultures were stained and mounted whole as described in our previous study. Others were serially sectioned; the slides were either stained with carbol-fuchsin on a hot-plate or incubated in the solution for 2–3 hr. at 37–38° C. and counterstained with Delafield's haematoxylin. Some were incubated at 37° C. in Giemsa overnight and differentiated in 5% acetic alcohol.

The rabbits were infected in three different ways:

(1) *Intravenous injection.* Most of the animals were infected intravenously. A dose of 0.5–1.0 ml. of a suspension of tubercle bacilli was injected into the ear vein. In one experiment (Exp. 21, Tables 1 and 2), the effects of a dilute and of a more concentrated suspension were compared; the moist weight of the inoculum was determined, the more concentrated suspension contained roughly 0.01 mg. and the more dilute suspension 0.002 mg. of bacillary material. In the other experiments a rather denser suspension was used.

(2) *Intrasplenal injection.* In two experiments (Exps. 9 and 10B, Table 1), the animals were anaesthetized with 0.75% nembutal injected into an ear vein, and a small vertical incision was made in the abdominal wall on the left side; the spleen was pulled forward and the suspension injected into the tissue with a very fine needle. The wound was sutured in the usual way, and the rabbits made an excellent recovery.

(3) *Intrapulmonary injection.* One experiment (Exp. 3, Table 1) was made by injecting the suspension directly into the lung through the chest wall.

RESULTS

Most of the results described below refer to the spleens of rabbits infected intravenously or by direct intrasplenal injection, but one experiment (Exp. 10B) was made with the lymph glands of the animal which had received an intrapulmonary inoculation.

The histological changes in the spleen which follow infection by virulent mammalian tubercle bacilli have often been described, and have recently been discussed by Rich (1946). In general, the results of our experiments agreed with those of previous observers.

Up to the 12th day after injection the animals showed no clinical sign of infection, but at post-mortem examination the spleen was seen to be greatly enlarged. In sections of the infected organs, especially after intravenous inoculation, very few acid-fast bacilli were found even 17 days after injection, although the tissue showed advanced tuberculous changes; prolonged search often revealed only a few isolated organisms. This paucity of acid-fast rods in tissue with severe tuberculous lesions is a familiar phenomenon to pathologists and has been considered at some length by Rich (1946).

When fragments of the tuberculous spleens were cultivated *in vitro*, they usually grew well for some days. At first the outgrowth consisted largely of macrophages and lymphocytes but later fibroblastic cells predominated, although the other cell types were present until the end of the culture period or until growth ceased.

As in cultures of normal spleen, the interior of the explants usually became necrotic during the first 3 days, though much of this dead material was often

resorbed later. It was noteworthy that the bacilli were found almost exclusively inside the living cells enclosing the degenerate core and only a few sheaves of organisms appeared in the necrotic tissue. The number of infected cells increased during the first 7 days. Up to a point, their rate of increase was correlated with the duration of the preceding infection *in vivo*, and was much more rapid in spleen cultures from rabbits infected for 6–16 days than in those from rabbits infected for 1–3 days (see Table 2). On the other hand, the rate of increase was relatively slow in two of four rabbits killed 17 days after infection (Exp. 19 and 21B); this may have been due to regressive changes in the spleens possibly associated with a decline in the viability of the strain BY; the cultures of the BY strain used for these experiments were the last to show normal growth on Dorset medium.

Table 1. *Infection in vivo*

No. of exp.	Strain	Inoculation	Duration of infection	No. of cultures examined
			<i>in vivo</i> (days)	
1		Intrasplenal	Immediate fixation	5
3	BH	Intrapulmonary	33	50
9	BH	Intrasplenal	9	46
10A	BH	Intravenous	16	22
10B	BH	Intrasplenal	16	24
16A	BY	Intravenous	1	42
16B	BY	Intravenous	3	39
16C	BY	Intravenous	6	38
16D	BY	Intravenous	12	30
17	BY	Intravenous	17	24
19	BY	Intravenous	17	43
21A	BY, dilute suspension	Intravenous	1	17
—	BY, concentrated suspension	Intravenous	1	21
21B	BY, dilute suspension	Intravenous	17	13
—	BY, concentrated suspension	Intravenous	17	16

How the infection spread from cell to cell was not clear. As described in our previous study (Fell & Brieger, 1951), in cultures of spleen infected *in vitro*, the steady increase in the number of infected cells was readily understandable; there were many free bacilli to be ingested and the heavily laden macrophages soon broke down, liberating their bacilli into the surrounding tissue where they were soon phagocyted by other cells. In spleen cultures from newly infected animals, on the other hand, there were very few acid-fast rods in the original explants, and those encountered were in apparently healthy cells. During the first few days after explantation, extracellular organisms were usually rare, so that it was difficult to see how phagocytosis of free acid-fast rods could account for the increasing number of infected cells.

As in cultures infected *in vitro* (Fell & Brieger, 1951), the infected cells eventually broke down, liberating the parasites into the surrounding tissue where they continued to multiply. In this way the whole explant became densely infiltrated and finally replaced by tubercle bacilli.

The details of individual experiments are given below.

I. *Intravenous injection (Table 2)**One day's infection in vivo*

Experiment 16A. In the original spleen there was slight hyperplasia of the reticulum cells in the centre of the Malpighian bodies, but no major pathological change and few acid-fast rods were seen.

The explants grew vigorously. Tubercle bacilli were first observed in sections of 6-day cultures which contained scattered, infected epithelioid cells and a few sheaves of free bacilli. After 10 days, although cell outgrowth remained active, many explants had become permeated with ropes of bacilli which had a purplish colour when stained by the Ziehl-Neelsen method, owing to the presence of feebly stained rods and filaments mixed with strongly acid-fast forms. Some cultures were still growing well by the 13th day, in spite of a dense infection of the explant, but outgrowth had almost ceased after 17 days and the tissue was largely replaced by a solid mass of bacilli.

Experiment 21A. (a) Dilute infection (see p. 190). In the spleen there was some hyperplasia of the reticulum cells and a few giant cells, not necessarily pathological, were present in the red pulp. Bacilli were rare.

The cultures grew well. Necrotic matter which was seen in the interior of the 3-day cultures, was largely resorbed by the 6th day. Many epithelioid cells were present, some of which contained bacilli. In sections of 10-day cultures, the epithelioid cells were very abundant, and many multinucleate giant cells of both the Langhans and foreign body type were present (Pl. 9, fig. 1). After 13 days' cultivation many of the infected cells had broken down so that the tissue was diffusely infiltrated with few bacilli. Growth was still fairly profuse after 17 days, although the explant was largely replaced by bacilli.

(b) Concentrated infection. The histology of the spleen was similar to that in the previous experiment.

The cultures closely resembled those of the preceding series infected with the diluted suspension.

Three days' infection in vivo

Experiment 16B. The histological picture of the spleen was similar to that described above. Bacilli were rare but bundles of filamentous, acid-fast organisms were present in a few apparently healthy macrophages.

The cultures resembled those of the spleens infected for 1 day. Some were still growing actively by the 14th day, although the explants were densely infiltrated with purplish stained, convoluted ropes of bacilli, and many of the outwandering fibroblastic cells, though apparently healthy, were heavily infected.

Six days' infection in vivo

Experiment 16C. The spleen showed severe oedema and congestion. Tubercles had not yet appeared and there was no obvious necrosis. There were many large reticulum cells of the epithelioid type. Bacilli were rare in the sections examined.

Control explants fixed at the beginning of the experiment contained a very small number of infected epithelioid cells, but after 3 days' growth *in vitro* many infected

Table 2. The progress of infection in explants of spleens removed at various intervals after intravenous infection, and cultivated in vitro

Spleen histology	1 day <i>in vivo</i>			3 days <i>in vivo</i> Exp. 16 B	6 days <i>in vivo</i> Exp. 16 C	12 days <i>in vivo</i> Exp. 16 D
	Exp. 16 A	Exp. 21 A (dil. susp.)	Exp. 21 A (conc. susp.)			
Zero explants or 1 day <i>in vitro</i>	Nearly normal; hyperplastic reticulum cells; very few bacilli	Similar to 16 A	Similar to 16 A	Picture of severe, acute infection; no tubercles or necrosis; bacilli in very few cells	Largely replaced by epithelioid cells; many giant cells; very few bacilli	
3 days <i>in vitro</i>	Interior necrotic; no bacilli seen	Interior necrotic; bacilli rare	Bacilli rare (zero)	Very few infected cells (zero)	Very few infected cells (zero)	
6-7 days <i>in vitro</i>	Few bacilli in necrotic tissue; some infected macrophages	Necrotic tissue nearly resorbed; some infected cells	Similar to 21 A (dil.) but more infected cells	Few bacilli in necrotic interior; very many living cells infected	Slight internal necrosis; more infected cells than at zero	
10 days <i>in vitro</i>	Permeated by purplish ropes of bacilli	Many epithelioid cells, some infected; Langhans cells	Similar to 21 A (dil.) but more infected cells	Few bacilli in necrotic tissue; high proportion of living cells infected	Much necrosis; few bacilli in necrotic tissue; many in living cells	
13-14 days <i>in vitro</i>	—	Diffuse infiltration of free bacilli	Diffuse infiltration	Heavy infiltration	Heavy infiltration	
17 days <i>in vitro</i>	Nearly replaced by bacilli	Nearly replaced by bacilli	Nearly replaced by bacilli	Nearly replaced by bacilli	Nearly replaced by bacilli	
			17 days <i>in vivo</i>			
Spleen histology	Exp. 10 A	Exp. 17	Exp. 19	Exp. 21 B (dil. susp.)	Exp. 21 B (conc. susp.)	
Zero explants or 1 day <i>in vitro</i>	Almost replaced by epithelioid cells; few necrotic areas; bacilli in very few cells	Mainly epithelioid cells; necrotic areas; many giant cells; bacilli rare	Many tuberculous foci; some free bacilli	Some intact Malpighian bodies; tubercles; few necrotic areas; no bacilli seen	Dilatation of sinuses; areas of normal spleen tissue; tubercles; some necrosis; no bacilli seen	
3 days <i>in vitro</i>	Necrotic interior; variable number of infected cells	Some infected epithelioid cells (1 day)	Largely necrotic (1 day)	Many bacilli in necrotic tubercles and elsewhere	Crust of living epithelioid cells; interior necrotic; very few bacilli	
6-7 days <i>in vitro</i>	Infection widespread and sometimes dense	Living cells infected with bacilli	Little change	Many living cells infected	Little change; no bacilli seen	
10 days <i>in vitro</i>	—	Necrotic and nearly replaced by bacilli	Few round colonies in some cultures	Much necrosis; few bacilli in necrotic areas, but many living cells infected	Few infected cells	
13-14 days <i>in vitro</i>	—	—	—	Heavy infection	Variable number of infected cells	
17 days <i>in vitro</i>	—	—	Little change; no general infiltration	—	Some free colonies; variable number of infected cells	

cells were seen. All the bacilli seemed to be intracellular, except a very few organisms in the small necrotic area in the centre of the explants. The outgrowth, at first profuse, had diminished by the 10th day in most cultures, and in section the explants were found to be permeated with bacillary ropes and to be largely necrotic. By the 14th day the tissue was completely degenerate and packed with bacilli.

Twelve days' infection in vivo

Experiment 16D. Tuberculous foci of various sizes and stages of development were present in the spleen, but few bacilli were seen. The normal spleen tissue was almost replaced by epithelioid cells, and Langhans cells, which seldom contained bacilli, were encountered.

In control explants, fixed at the beginning of the experiment, a few isolated bacilli were found after careful search. After 3 days the number of infected cells had increased, and by the 7th day the explants were usually extremely necrotic and fairly heavily infected; as usual most of the bacilli were in the living tissue and there were relatively few in the necrotic regions. After 10 days' cultivation growth had almost or completely ceased, and the degenerate tissue was heavily infiltrated with free bacilli.

Sixteen days' infection in vivo

Experiment 10A. The spleen was composed mainly of large epithelioid cells, and its normal texture was only recognizable in places; there were a few isolated areas of necrosis. Acid-fast bacilli were seen in a very small proportion of the cells.

The cultures grew actively. They were not maintained beyond the 7th day, when the outgrowth was usually still vigorous, although infection was widespread (Pl. 10, fig. 5). In some cultures, which contained tuberculous foci carried over from the parent spleen, the bacillary infiltration was remarkably dense (Pl. 10, figs. 6 and 7), and the explants thus presented a striking contrast to the original organ in which acid-fast bacilli, though present, were hard to find (Pl. 9, figs. 3 and 4).

Seventeen days' infection in vivo

Experiment 17. The spleen resembled that of Exp. 16, and acid-fast bacilli were rare.

Large macrophages began to wander out of the explants during the first 24 hr., but the outgrowth did not increase much and had nearly stopped by the 10th day. In sections of 6-day cultures most of the bacilli, as usual, were seen to be associated with the living cells near the surface of the explants and only a few small sheaves of organisms were scattered here and there in the necrotic material. By the 10th day the explants were almost completely necrotic and were occupied by a dense mass of organisms.

Experiment 19. The rabbit had been infected with a more dilute suspension than in most of the previous experiments. In the spleen, the Malpighian bodies were not completely obliterated and some were involved in the many tuberculous foci.

There were necrotic areas and many Langhans giant cells. Few acid-fast bacilli were present.

The cultures grew well for the first 3 days; outgrowth then declined and by the 7th day few cultures showed even slight growth. In section the explants were found to be largely necrotic. Multiplication of the bacilli was comparatively slow, and it was not until the 9th day that there was any heavy infection. Even then there was rarely a general infiltration of the explants, which contained a number of large round colonies.

Experiment 21B. (a) Dilute infection (see p. 190). Some of the Malpighian bodies in the spleen were still intact. Epithelioid and giant cells predominated and there were tubercles in various stages of development. Only a few small necrotic areas were seen. No acid-fast bacilli were found in the sections examined.

The explants grew profusely for the first 3 days, but by the 5th day growth had begun to decline in most of the cultures, though in some it remained fairly active until the 10th day. By the 5th day small sheaves of bacilli were scattered throughout the explants and the infection increased steadily, mainly in the living tissue, up to the 10th day when the remaining cultures were fixed.

(b) Concentrated infection. There was a very marked dilation of the sinuses in the spleen. The normal texture of the organ was preserved in places but the epithelioid cells had proliferated extensively to form tubercles. There was some focal necrosis but no acid-fast bacilli were found.

The cultures grew very well up to the 14th day, when they were fixed, and there were many epithelioid and giant cells in the outgrowth. After 1 day *in vitro*, a few bacilli were found in one culture after careful search; more infected cells were present by the 7th day, but there was no general infiltration of the tissue even after 10–14 days.

II. *Intrasplenic injection*

Immediately after infection

Experiment 1. The spleen was removed immediately after inoculation. Clumps of acid-fast bacilli were seen in the tissue along the path of the injection and there was some oedema.

The cultures grew well. Many infected macrophages were seen after 3 days' cultivation; by the 10th day the tissue was heavily infiltrated by ropes of bacilli, and by the 15th day was completely overgrown.

Nine days' infection in vivo

Experiment 9. The spleen contained necrotic areas consisting mainly of degenerating polymorph leucocytes surrounded by epithelioid cells. There were tubercles in various stages of development, which contained a good many bacilli. A few clusters of free organisms were seen in the tissues, a phenomenon which was not observed in any other spleen except in the necrotic areas.

Nearly all the cultures grew well, some until the 10th day, when they were fixed. In 3 days the epithelioid tubercles had become infested with bacilli; and infected cells were scattered throughout the tissue. By the 10th day all the cultures were heavily infected, and some were almost replaced by bacilli.

Sixteen days' infection in vivo

Experiment 10B. The spleen was much congested. Epithelioid cells predominated and there were tubercles and necrotic areas which contained a few acid-fast bacilli.

The cultures grew well for 7 days when they were fixed. All the explants became heavily infected. The tubercles were permeated with purplish stained ropes of bacilli and some explants were densely infiltrated.

III. *Intrapulmonary injection**Thirty-three days' infection in vivo*

Experiment 3. Histological examination of the lungs showed a large-scale consolidation with a few areas of necrosis. No acid-fast bacilli were found in spite of careful search.

In the axillary region there were several glandular tumours of about the size of a small pea. They were embedded in fibrous tissue and were fairly easily decapsulated. Sections showed necrotic areas and a few bacilli. Cultures were made from one of these glands.

The tracheal lymph glands looked almost normal. There were a few areas of epithelioid cells and no bacilli were found. Explants were taken from one gland.

The thoracic cavity presented a very characteristic feature of bovine infection. The mediastinal pleura was covered with 'pearls', i.e. very defined, almost colourless nodules, not more than 2 mm. in diameter and of a shiny, translucent appearance; explants from one of these 'pearls' were cultivated *in vitro*. In section the nodules were found to contain only a few areas of normal lymphatic tissue, and consisted mainly of large epithelioid cells and necrotic matter. At first no bacilli were seen, but after prolonged search through a number of sections, two acid-fast rods were found.

All the cultures were mounted whole in this experiment, which was made before the importance of sectioning the explants was fully appreciated.

Some of the explants of the axillary lymph gland grew well, but others feebly or not at all. Bacilli were seen in all the cultures fixed after 5 days, and by the 9th day the explants were heavily 'peppered' with rounded colonies and cell growth had almost or completely ceased.

The cultures of the tracheal lymph gland grew well, some until the 16th day when they were fixed. Bacillary colonies were found in the explants after 9 days, and were fairly large in some 12- and 16-day cultures; in others no infection was distinguishable.

Some of the explants of the mediastinal 'pearl' formed a large fibroblastic outgrowth which was maintained until fixation on the 16th day. Small bacillary colonies were noted in two 6-day cultures, and after 12-16 days' cultivation all the explants were densely infected (Pl. 10, fig. 8).

DISCUSSION

The results described above confirmed those of the previous investigation, and, in addition, showed that there were two important differences between infected tissue growing in the body and in tissue culture. First, true tubercles did not develop in

spleen explants cultivated *in vitro*, though they appeared in the spleen left *in vivo*. The only sign of a tubercle-like reaction in culture was encountered in explants of two spleens removed 1 day after intravenous infection, in which unusually many epithelioid cells and giant cells, some of the Langhans type, were present (Exp. 21A, Tables 1 and 2; Pl. 9, fig. 1). Secondly, whereas acid-fast bacilli were hard to find in spleens left in the body for as long as 3 weeks after inoculation, explants of spleens removed at various periods after intravenous infection, like those infected *in vitro* by a droplet of bacillary suspension, became densely infiltrated after 10 days in culture, although only a few infected macrophages, and no free bacilli were present in the spleens when first removed for explantation.

As already indicated in our previous communication (Fell & Brieger, 1951), there are certain factors in the intact animal which influence the course of the tuberculous reaction *in vivo*, and which are absent *in vitro*. Many workers consider allergy to be responsible for some of the characteristic features of tuberculosis *in vivo*. Allergy is thought to develop gradually in the infected animal, and according to the theory put forward by Lurie (1938), the tissue cells which at first live symbiotically with the ingested bacilli later become hypersensitive to the invaders and destroy them, thus liberating endotoxins which produce the tuberculous lesions. On this hypothesis Lurie explains the rarity of bacilli in tissues which are heavily damaged or completely replaced by epithelioid cells, tubercles and necrotic foci.

The view that special inhibitory forces are mobilized *in vivo* is largely based on the fact that as soon as infected tissues are removed from the body and incubated on artificial bacteriological media, bacterial colonies grow from the disintegrating material. Thus Woodruff & Kelly (1940) showed that free bacilli disappeared rapidly from the omentum of guinea-pigs after the intraperitoneal injection of human tubercle bacilli, but that when the omentum was removed and transferred to artificial medium masses of organisms appeared; on the other hand, there was no such multiplication of free bacilli when the omentum was left in the animal for the same length of time. Brieger (1944) obtained similar results with embryonic membranes of chick embryos infected with avian tubercle bacilli. There were few bacilli in the tissues, but when the membranes were removed after 3 days and incubated on a bacteriological medium, they reappeared in enormous numbers. It is difficult to see how allergy could account for this observation, since it is doubtful whether it develops in chick embryos, and in any case it is unlikely to do so in 3 days.

The results of the present study agreed with those quoted above. When rabbit spleens and lymph glands were removed from the body 6–17 days after infection and fragments were cultivated *in vitro*, they often became infiltrated with bacilli at an astonishing rate and much more rapidly than those taken only 24 hr. after infection *in vivo*. This indicated that the bacilli multiplied in the infected spleen (see Table 2) and agreed with recent observations by Woodruff, Kelly & Leaming (1945), who made bacteriological cultures of spleens removed at various intervals after infection, and found that the colony count increased rapidly with the duration of infection *in vivo*.

There was one important difference, however, between our experiments and those of other workers. In the latter the bacilli appeared in tissue which had been transplanted to a bacteriological medium where it disintegrated, whereas in our investigation the infected tissue was transplanted to a physiological medium in which the cells could live and grow. As described above, during the first few days of cultivation *in vitro*, the bacilli proliferated much faster in living cells than in necrotic areas. From this it appeared that *in vitro* the living cells promoted rather than inhibited the growth of the organisms (cf. Lurie, 1942), in much the same way as intracellular protozoan parasites in tissue cultures grow best when the host cells are alive and vigorous (Meyer, 1950). If the allergy rendered the cells hypersensitive to the bacilli in the body, it is surprising that this hypersensitivity should have been so quickly and completely lost when the cells were transplanted *in vitro*.

The explants of two spleens removed from rabbits killed 17 days after infection (Exp. 19 and 21 B (conc.), Tables 1 and 2) behaved rather differently from those discussed above. Bacilli appeared and multiplied in these explants, but their proliferation *in vitro* was far less than in the other spleen cultures. It is possible that in these organs regressive changes had taken place due to a reduced viability of the strain (see p. 191); according to Rich (1946), rabbit spleens are supposed to possess some degree of 'native resistance' to tuberculous infection.

The question arises as to the origin of the enormous numbers of bacilli that appeared so quickly in the cultures of most spleens removed 6–17 days after infection (Table 2). It is possible that tissue culture conditions so greatly favoured the growth of the very few acid-fast bacilli present in the original explants that they were able not only to overcome whatever inhibitory effect the cells had exercised *in vivo*, but also to multiply *in vitro* at enormous speed.

Alternatively we could adopt the view, expressed by many previous workers, that in the animal the tuberculous tissue contains many organisms which are not demonstrable by conventional staining methods; if this is so, we must assume that in our experiments such organisms acquired the usual bacillary form when transplanted *in vitro*. We ourselves, however, were unable to find any method that would demonstrate pre-bacillary forms in the infected rabbit spleens, but the cells contained so many granular inclusions of various kinds, that it might have been impossible to distinguish pre-bacillary bodies if any were there.

At present, therefore, we have no conclusive evidence as to the origin of the massive bacillary infiltration which appeared in the explants; the problem is being attacked by other methods (Brieger, Miles, Cosslett & Horne, 1951).

SUMMARY

1. Rabbits were infected with virulent bovine tubercle bacilli in three ways: by (1) intravenous, (2) intrasplenic and (3) intrapulmonary injection. In series (1) and (2) part of the spleen, and in series (3) part of the draining lymph glands were sectioned for histological examination, and usually explants for tissue culture were made from the rest of the organ.

2. A histological study of the infected spleens and lymph glands fixed after

different periods of infection showed the usual picture of progressive tuberculous infection; acid-fast bacilli were rare and often difficult to find.

3. After a few days' cultivation *in vitro*, explants of the infected spleens often became densely infiltrated with bacilli.

4. Previous investigators have expressed the view that acid-fast bacilli are so few in tuberculous lesions *in vivo*, because large numbers become unstainable. If this is true, and if the unstained bacilli remain viable, it would readily account for the extraordinarily rapid bacillary infiltration of tissue cultures of infected organs. Alternatively, it must be assumed that the few scattered acid-fast rods in the original tissue grow at a prodigious rate under the conditions of life *in vitro*.

5. These alternatives and other theoretical implications of the results are discussed.

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EXPLANATION OF PLATES 9 AND 10

Photographs by Mr V. C. Norfield

PLATE 9

Fig. 1. Ten-day spleen culture from a rabbit killed 1 day after intravenous infection (Exp. 21A; dilute). Note multinucleate giant cells of the Langhans type. (Section, $\times 300$.)

Fig. 2. Cell containing tubercle bacilli, in normal anaphase. From a 17-day spleen culture made from a rabbit killed 1 day after intravenous infection (Exp. 21A; concentrated). The interior of the explant was heavily infiltrated with bacilli. (Section, $\times 1330$.)

Fig. 3. Spleen from a rabbit killed 16 days after intravenous infection (Exp. 10A), showing a tuberculous focus. (Section, $\times 95$.)

Fig. 4. Same spleen, showing the necrotic interior of a tuberculous focus. Only one bacillus (b) is present in the field. (Section, $\times 2000$.)

PLATE 10

Fig. 5. Seven-day culture from the same spleen as that shown in Figs. 3 and 4 (Exp. 10A). Note the heavy infection of all the cells in the field; their nuclei are pycnotic. (Section, $\times 1200$.)

Fig. 6. Seven-day culture from the same spleen as that shown in Figs. 3 and 4 (Exp. 10A). Part of a tuberculous focus is seen, densely infiltrated with ropes of tubercle bacilli (cf. Fig. 3), which were stained a reddish purple by Ziehl-Neelsen's carbol-fuchsin and haematoxylin. (Section, $\times 200$.)

Fig. 7. Field marked with rectangle in Fig. 6, showing ropes of bacilli under high magnification. (Section, $\times 800$.)

Fig. 8. Twelve-day culture from a mediastinal 'pearl' of a rabbit killed 33 days after intrapulmonary infection (Exp. 3). Note many round colonies of tubercle bacilli; there is still some outgrowth on one side. (Whole mount, $\times 25$.)

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