TUBERCULOSIS OF RABBITS INDUCED BY DROPLET NUCLEI INFECTION

II. Response to Reinfection*

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Plates 15 to 17

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When normal rabbits were caused to inhale an aerosol suspension of virulent bovine tubercle bacilli, the rate and pattern of tubercle formation were highly uniform for about 5 weeks. Thereafter, the progress of the disease was not uniform; it varied with the animal.

Transition of this relationship from the homogeneous phase to one in which the progress of the disease varied with the animal has been attributed to the development of resistance (1, 2). As judged by differences in the rate of development of initial tubercles after the 5th week of growth, it appeared as if resistance developed slowly and the rate of its development varied widely. After the 5th week of infection the rate of growth of the initial tubercles and extension of the infection to other foci was proportional to the number of bacilli contained in the lesions. Thus the only demonstrable effect of developing resistance seemed to be upon the apparent growth rate of the bacilli (2).

Experiments on inhaled reinfection have provided another method by which the effects of developing resistance upon the rate of growth of the bacilli may be observed. These experiments have shown that demonstrable levels of resistance develop more rapidly and increase at a more uniform rate than the study of initial infection had indicated.

Material and Methods

The 36 albino rabbits which were used in these experiments were purchased from the dealer who had supplied animals for other studies of this series. These animals weighed about 2 kilos each when they were first subjected to infection. All of them harbored species of *Eimeria* but accidental bacterial disease was not encountered. Details of feeding and care of these animals, as well as the methods by which infections were induced and tissues prepared for study, have been described fully in earlier publications (1–5).

EXPERIMENTS

Preliminary experiments demonstrated that rabbits exposed to massive reinfection 6 or more weeks after initial infection did not respond as do normal animals. Accordingly the response to reinfection was further studied by rein-

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fecting rabbits at intervals from 2 to 7 weeks after initial infection. To this end, 18 normal rabbits, in 3 exposure groups of 6 animals each, were allowed to inhale about 30 bacilli per animal and divided into 6 groups for reinfection. Each reinfection group contained one rabbit from each of the 3 initial infection groups and one normal control.

The first of these groups was reinfected 2 weeks after initial infection. Each week thereafter another group was reinfected until all had had a second exposure, during which every animal inhaled upwards of 20,000 bacilli. This large dose invariably killed normal animals within 4 weeks. Following the second exposure to infection each group of rabbits was observed until the control animal had died. If, by this time, no other member of the group had died of tuberculosis, one of the reinfected rabbits was killed for examination. With 2 exceptions (see Table I), other reinfected rabbits were held until dead.

1. Time Response to Reinfection

The results of this experiment have been summarized in Table I in which the animals are arranged by reinfection groups, whereas they had been numbered in regular sequence for the initial infection. This table shows the intervals in days between infection and reinfection, and reinfection and death. Under the heading "Character of the disease" a brief description is given of the macroscopic features of the disease in each animal.

All control rabbits of this series died of tuberculosis within 4 weeks after infection. At death their lungs were fully expanded, deeply congested, and friable. Closely placed tubercles, many of which had fused, were distributed throughout these organs, and usually occupied more than half of the tissue (Fig. 1). With reference to size, cellular components, and numbers of bacilli, these lesions corresponded in all essential features to other initial tubercles of the same ages (Figs. 3 and 5). Their development apparently had not been influenced by the number of organisms inhaled.

The appearance of the lungs of rabbits of the first, second, and third reinfection groups which died or were killed within 32 days after reinfection, was much the same as that of the lungs of the control rabbits. The closely placed reinfection tubercles seemed to have developed at about the same rate as did initial tubercles. The larger initial tubercles, distributed at random through the lungs, also seemed to have progressed at about the rate that would have been expected, if these rabbits had not been reinfected (Fig. 2). However, histological changes in the lungs of these animals suggested that initial tubercles were affected by reinfection, and that reinfection tubercles were influenced by initial infection.

Initial tubercles of these animals were outlined by poorly defined, narrow borders of monocytes and leukocytes which surrounded relatively large irregular caseous centers. In contrast, initial tubercles, of the ages at which these lesions of reinfected animals were examined, ordinarily would have had much wider bor-

ders of inflammatory cells and relatively small compact caseous centers (2). Reinfection of the intensity used in this experiment, apparently had partially inhibited the inflammatory response of the initial tubercles and increased the extent of caseation without appreciably changing the rate at which these lesions had expanded.

In contrast to the initial tubercles in the lungs of these animals the reinfection tubercles usually were more sharply defined. The size and the extent of caseation within these lesions usually corresponded closely to initial tubercles of the same ages, but their cellular components sometimes did not. In about one-half of the animals reinfection tubercles contained appreciable numbers of epithelioid cells, whereas in the lungs of other rabbits these lesions, like initial tubercles, were composed chiefly of monocytes and leukocytes. This difference was not related to time of reinfection.

The one feature which distinguished reinfection tubercles from initial tubercles of the same age was the relatively small number of bacilli which could be demonstrated within the caseous centers. Differences between the numbers of bacilli in initial and reinfection tubercles were estimated to be of the order of one hundred to one (Figs. 4, 6, and 7). This difference did not change appreciably with the interval between exposure to infection.

One of the 3 rabbits which were exposed to reinfection 4 weeks after initial infection apparently did not develop reinfection tubercles. At autopsy of this animal, 62 days after the second exposure, the lesions found in its lungs could be attributed solely to progression of the initial tubercles. Likewise it seemed that death of all the rabbits exposed to reinfection at intervals greater than 4 weeks was the result of progressive growth of initial tubercles. Yet it was not so certain that all of them resisted completely the bacilli of reinfection. For example, one of the rabbits exposed to reinfection after 6 weeks died 21 days later. This animal, and an exposure mate which was killed 26 days after reinfection, had in their lungs numbers of small tubercles of sizes corresponding to reinfection tubercles of equivalent ages. But the kidneys of both animals also contained equally numerous small tubercles. Thus it seemed reasonable to suppose that the small tubercles in the lungs also developed from organisms which had spread by way of the blood stream from the initial tubercles.

2. Effects of Reinfection upon Progress of Initial Tubercles

In spite of the lack of convincing evidence of growth of the bacilli of reinfection or of a response to their growth when rabbits were reexposed after more than 4 weeks, the progress of the disease, in some animals of this series had been surprisingly rapid. Other instances of equally rapid progression, without evidence of developing reinfection tubercles, had occurred among rabbits which had been used in earlier experiments on reinfection. These animals had been exposed to reinfection 46 and 82 days after initial infection.

In order to allow ready comparison of the characteristics of the disease in

TABLE I

The Response of Rabbits to Massive Inhaled Reinfection at Increasing Intervals from 2 to 7 Weeks after Small Initial Inhaled Infection

Rabbit No.	Interval between infections	Time survived second infection	Character of the disease
	days	*days*	
12-0	14	20	Initial tubercles poorly outlined, largely necrotic, contain numerous bacilli; reinfection tubercles vary with the animal from well defined lesions containing epithelioid cells to poorly outlined accumulations of monocytes and leukocytes which surround necrotic foci. Bacilli scanty in all of these
12-6	"	22	
13-2	"	19	
13-8	Control	22	Tubercles poorly defined, composed of monocytes and leukocytes, and contain large necrotic foci in which bacilli are numerous
12-1	21	27 k.	Initial tubercles outlined by wide zone of monocytes, large center of necrosis; reinfection tubercles often fused but all largely cellular. Epithelioid cells, monocytes, and fibroblasts outline small necrotic centers in which there are few bacilli
12-7	"	32	All lesions largely necrotic, poorly outlined by monocytes; few bacilli in foci of reinfection
13-3	"	24	Reinfection tubercles similar to No. 12-7 but show less necrosis
13-9	Control	27	Tubercles poorly defined, composed chiefly of monocytes which outline relatively large necrotic foci in which bacilli are numerous
12-2	28	25	Initial tubercles poorly defined, largely necrotic; reinfection tubercles composed mainly of monocytes and leukocytes which form poorly outlined borders about necrotic centers. Necrosis more advanced in No. 12-2, corresponding to age of infection, few bacilli in reinfection tubercles
13-4	"	20	
12-8	"	62	No evidence of second infection. Disease limited to lungs and mucosa of the intestine
14-2	Control	24	Lesions similar to those of No. 13-9
12-3	35	86	No evidence of second infection. Subacute disease destroys much of lungs. Few small tubercles in kidneys. Mucosa of gut ulcerated

TABLE I—*Concluded*

Rabbit No.	Interval between infections	Time survived second infection	Character of the disease
	days	*days*	
13-5	35	105	Comparable to No. 12-3 but less rapidly destructive, and no evidence of spread by blood stream
12-9	35	23 k.	Initial tubercles well circumscribed, few ulcerated. Miliary tubercles scattered in lungs and kidneys. No evidence of second infection
14-6	Control	22	Lesions similar to those of No. 13-8
12-4	42	21	Initial tubercles circumscribed but excavated. Miliary tubercles numerous in lungs and kidneys. No evidence of second infection
13-6	"	26 k.	
13-0	"	50	Disease comparable to that in No. 13-6. Tubercles in kidney 3–4 mm. in diameter, gut ulcerated
14-3	Control	24	Lesions similar to those of No. 13-8
12-5	49	85	Moderate grade of blood stream spread. Disease in lungs chronic
13-1	"	121 k.	Infection limited to lungs except for few small lesions in kidneys
13-7	"	26 k.	Tubercles limited to lungs. No evidence of second infection
15-1	Control	23	Well defined small tubercles composed of monocytes and leukocytes contain small necrotic foci. Bacilli numerous

k., killed.

animals which were exposed to reinfection at intervals greater than 4 weeks, the records of 10 rabbits listed in Table I have been combined with those of 8 other animals of the 2 groups mentioned, to form Table II. In this table the animals have been arranged in the order of increasing intervals between exposures to infection. The table also shows the average number of organisms inhaled by each animal at initial infection, and the survival period following reexposure. A brief characterization of the disease in each animal is also given.

Progressive tuberculosis in the rabbits listed in Table II seemed to follow one general pattern. Certainly the rate of progress of the disease varied widely, but it involved the lungs chiefly and the length of life of the animals reflected the rapidity with which the lungs were destroyed. Hematogenous spread of

TABLE II

Survival Periods of Rabbits and the Character of Tuberculosis in Them Following Inhalation of about 20,000 Virulent Bacilli 4 or More Weeks after Initial Infection by Small Numbers of Air-Borne Tubercle Bacilli of Equivalent Virulence

Rabbit No.	Bacilli inhaled during initial exposure: approximate	Interval between exposures	Time survived second exposure	Characteristics of disease
		days	*days*	
12-8	30	28	60	Many large cavities, bronchial spread to dependent parts of lungs. No evidence of hematogenous spread
12-3	"	35	86	Large, thin walled cavities occupy greater part of lungs. Dorsal regions of lungs relatively free. Gut ulcerated and perforated
12-9	"	"	23 k.	Initial tubercles ulcerated, cavities small; many 1 mm., solid tubercles in lungs and kidneys. Extensive tuberculous pneumonia
13-5	"	"	105	Similar to No. 12-3, kidneys free
12-4	"	42	21	Similar to No. 12-9, but small tubercles in lung and kidneys 2–3 mm.
13-6	"	"	26 k.	Similar to No. 12-9
13-0	"	"	50	Initial tubercles, small, ulcerated. Many small solid, 2–3 mm. tubercles in lungs and kidneys. Tuberculous pneumonia in dependent parts of lungs; gut ulcerated
9-4	150	46	20	Similar to No. 12-4
9-2	"	"	52	Similar to No. 12-4, but less acute with few tubercles in kidneys
9-3	"	"	53	Similar to No. 12-4, without hematogenous spread
9-5	"	"	102 k.	About 75 initial tubercles 5–10 mm., about 25 ulcerated, others compact and caseous; gut widely ulcerated. Small tubercles in kidneys
13-7	30	49	26 k.	Many initial tubercles ulcerated, bronchial spread with solid tubercles clustered about bronchi, solidifying dependent parts. Kidneys free
12-5	"	"	85	Similar to No. 13-0, but less acute

TABLE II—*Concluded*

Rabbit No.	Bacilli inhaled during initial exposure: approximate	Interval between exposures	Time survived second exposure	Characteristics of disease
		days	*days*	
13-1	30	49	121k.	Few large cavities, many solid initial tubercles. Many small tubercles scattered in lungs and kidneys. Bronchial spread to dependent parts of lungs
8-5	100	82	6	Similar to No. 12-9, with more extensive tuberculous pneumonia
8-4	"	"	36	Similar to No. 12-9
8-6	"	"	40	Similar to No. 12-9
8-7	"	"	50 k.	Similar to No. 13-1

k., killed.

the infection was a frequent complication, especially of the more acute forms, but this could not be credited as a major factor in the death of any animal. Animals which exhibited chronic tuberculosis, usually had developed tuberculous enteritis also, but only one died of a perforated intestine.

The rate of progress of the disease did not appear to be related either to the length of the interval between infection and reinfection, or to the number of bacilli which were inhaled during initial infection. Instead it varied with the animal. For example the initial tubercles and the lesions associated with them were in much the same state of development in rabbits 8-4, 8-5, and 8-6, exposed to massive reinfection after 82 days, as they were in rabbits 12-4, 13-0, and 13-6 reexposed after 42 days.

In the more acute forms of the disease, as developed by rabbits 8-5, 12-4, 12-9, and 13-0, a large proportion, if not all, of the initial tubercles ulcerated. At autopsy these lesions varied from 1 to 1.5 cm. in diameter. They consisted of wide zones of inflammatory cells surrounding poorly defined cavities which were filled almost completely by soft necrotic material in which bacilli were exceedingly numerous. Masses of this material were found in adjacent bronchi. Apparently these lesions provided the intense infection of the dependent parts of the lungs which lead to the development of more or less extensive tuberculous pneumonia (Figs. 8 and 9).

The more chronic forms of tuberculosis, as illustrated by rabbits 12-8, 13-5, 8-7, and 9-5, were characterized by the development of relatively few large cavities from initial tubercles. Usually these cavities were 2 to 4 cm. in diameter, with their walls well defined by fibrous tissue. Within and about their

walls the inflammatory reaction was relatively inconspicuous and they usually contained only small amounts of exudate. Other initial tubercles in these animals apparently remained quiescent or regressed, although lesions secondary to the cavities usually were numerous in the dependent parts of the lungs (Figs. 10 and 11).

DISCUSSION

Under the conditions of these experiments the lungs of rabbits have exhibited a highly uniform response to reinfection with virulent bovine tubercle bacilli when reinfection occurred within 4 weeks after initial infection. With one exception rabbits reinfected within 4 weeks seemed to be just as susceptible to implantation of separated organisms upon alveolar walls as were normal animals. In its essential features the pattern of this response seemed to correspond closely to that of normal rabbits (1, 2). Reinfection tubercles developed at about the same rate as initial lesions, and, more often than not their histological pattern was identical with that of initial lesions. The occurrence of epithelioid cells in the reinfection tubercles of some animals was not related to time of reinfection. However, reinfection tubercles always contained far smaller numbers of bacilli than in initial tubercles of the same ages. This was the one constant difference between initial and reinfection tubercles and was no less pronounced in reinfection tubercles which developed in animals reexposed at 2 weeks than in animals reexposed at 4 weeks. Whether or not this difference in the number of bacilli resulted from a reduced rate of growth of the bacilli of reinfection, increased destruction, or from both factors has not been determined. Evidence presented by others also has been inconclusive, but has suggested, as does the present study, that the growth rate of the bacilli of reinfection was reduced (6–9).

While the apparent reduction in the growth rate of the bacilli of reinfection has not been explained, it must be taken as indicating a level of acquired resistance. It is evident that this level was developed rapidly. It was demonstrated within 2 weeks after initial infection with small numbers of virulent bacilli, inhaled as separated cells in droplet nuclei, but it did not increase further until about 4 weeks after initial infection. Then susceptibility to reinfection by methods used in this study seemed to end abruptly. Thus it may be suggested that acquired immunity of rabbits to reinfection with virulent bovine tubercle bacilli, inhaled as separated cells in droplet nuclei, corresponds in its essential features to that which develops in infections which terminate in "clinical crisis."

If, as indicated by these experiments, immunity to reinfection with virulent bovine tubercle bacilli, inhaled as separated cells in droplet nuclei, developed in slightly more than 4 weeks, then none of the bacilli of reinfection grew sufficiently to induce the development of tubercles in the lungs of animals which were exposed to reinfection at intervals of 5 weeks or more after initial infec-

tion. The present evidence is inadequate to establish this completely. In certain rabbits bacilli of reinfection may have induced the development of some of the small tubercles which were found in the lungs, although spread of organisms from initial tubercles could explain the development of these lesions. In other rabbits, however, it is evident that none of the bacilli of reinfection became established. Therefore, in spite of incomplete evidence, it is suggested that within 5 weeks after initial infection with virulent bovine tubercle bacilli, inhaled as separated cells in droplet nuclei, rabbits become immune to reinfection with these organisms.

Nevertheless some animals which were exposed to reinfection at 5 weeks or more developed more rapidly fatal disease than would have been expected of rabbits inbred for low levels of resistance to tuberculosis. Others lived as long as highly resistant rabbits which carried equal numbers of initial tubercles (10). In the more quickly fatal infections exposure to massive inhaled reinfection seemed to augment the progress of the initial tubercles which ulcerated early in their development and caused death by rapid bronchogenic spread of the infection.

It is difficult to imagine how some 20,000 separated bacilli, inhaled into the lungs and deposited upon alveolar surfaces without apparent growth, could influence the spread of the infection from scattered initial foci. However, it is certain that the progress of the initial tubercles in the lungs of rabbits reinfected before the end of the 4th week was influenced by this experience. It is perhaps possible that massive inhaled reinfection after 4 weeks so saturated the defense mechanism of some animals that, although the bacilli of reinfection were destroyed, those in the initial foci increased more rapidly than otherwise would have occurred. It is also possible that rabbits, in which rapidly progressive disease followed exposure to reinfection, had become sensitized to the aerosol by the initial infection, and the course of the disease in them was influenced by sensitization.

This phase of reinfection tuberculosis, like the details of the reaction to inhaled reinfection, seems to demand further exploration, a task for which the apparatus and techniques employed in this and earlier studies of this series are adequate.

SUMMARY AND CONCLUSIONS

At intervals from 2 to 11 weeks after normal rabbits had inhaled small numbers of virulent bovine tubercle bacilli as separated cells in droplet nuclei, groups of these animals received a single exposure to reinfection during which each animal inhaled about 20,000 separated bacilli.

Normal control rabbits which inhaled this large number of bacilli died within 4 weeks thereafter. Their deaths were attributed to destruction of the lungs by developing initial tubercles.

Eleven of 12 rabbits which were reinfected within 4 weeks after initial infection seemed to respond as normal animals. Their lungs were largely replaced

by developing reinfection tubercles when they died or were killed within 32 days after reinfection.

The inflammatory response of the reinfection tubercles was not consistently different from that of initial tubercles, although reinfection tubercles contained fewer bacilli than initial lesions of the same age.

Within 5 weeks after initial infection rabbits apparently had developed immunity to reinfection with virulent bovine tubercle bacilli inhaled as separated cells in droplet nuclei. In some of them, however, exposure to massive inhaled reinfection seemed to stimulate the progress of initial infection.

It is suggested that in rabbits the development of resistance to tubercle bacilli does not bear a linear relationship to time, but progresses in steps and within 5 weeks after small initial infection by inhalation is adequate to prevent the growth of separated bacilli when these are deposited upon alveolar walls.

It is suggested also that the basic effect of acquired resistance of rabbits to tubercle bacilli is inhibition of multiplication of the bacilli.

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EXPLANATION OF PLATES

Photography by Mr. Basil Varian, Department of Anatomy, University of Pennsylvania. Photographs are natural size. Photomicrographs are × 620. All sections from which these photomicrographs were taken were cut at one time, and stained in one lot, by one technician.

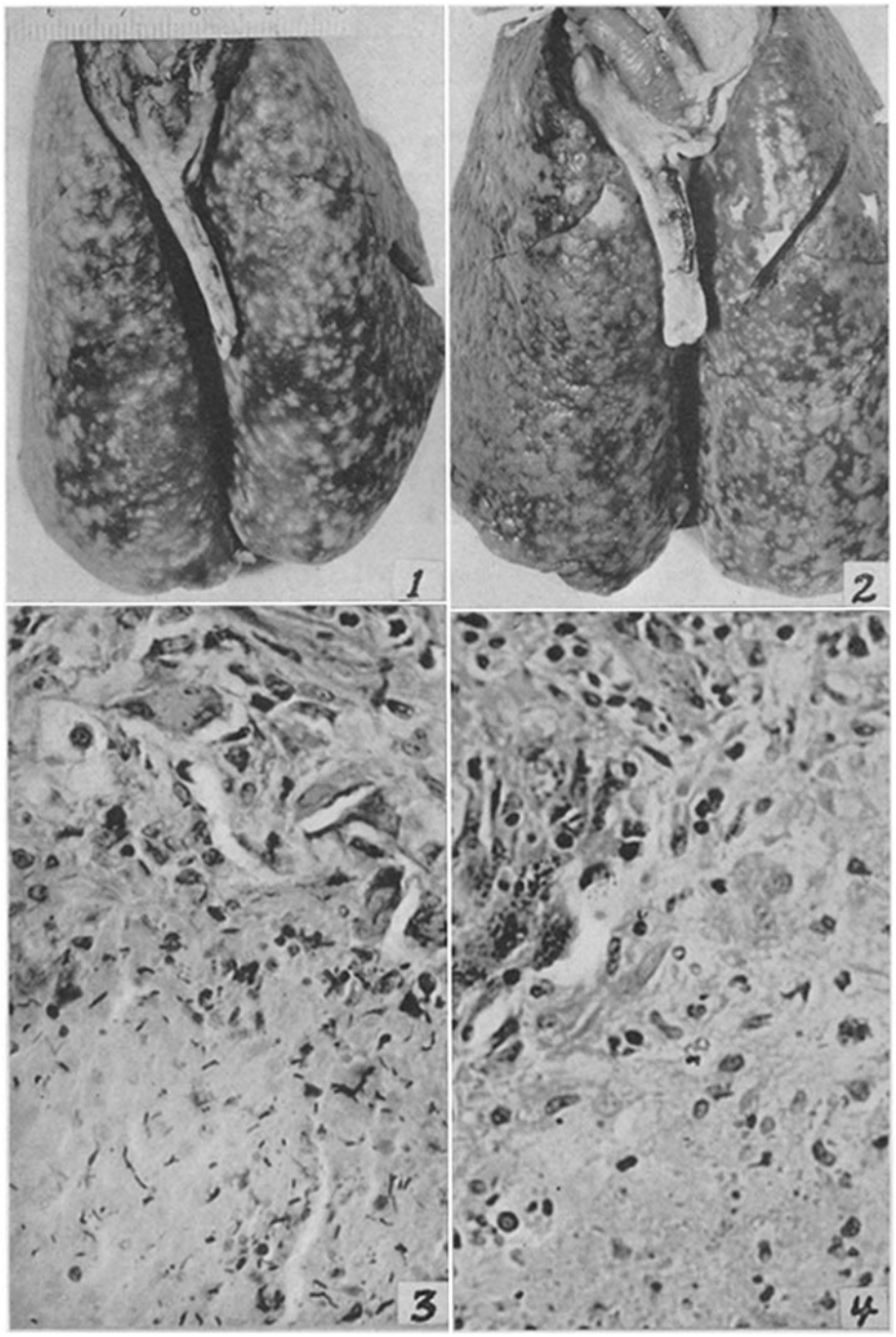
PLATE 15

FIG. 1. Dorsal view, lungs, rabbit 13-9, which died 27 days after initial infection by inhalation of some 20,000 separated bovine tubercle bacilli. These lungs are representative of the effects of massive inhaled infection. When deaths occurred earlier, tubercles were smaller and, of course, less often fused.

FIG. 2. Dorsal view, lungs, rabbit 12-2, which died 25 days after reinfection by inhalation of some 20,000 bacilli; initial infection with about 30 bacilli 21 days before reinfection. Initial tubercles seen as white spots, 5 visible. These lungs are representative of the effects of reinfection 2, 3, and 4 weeks after initial infection.

FIG. 3. A representative tubercle, rabbit 13-9. Note the numbers of bacilli in the necrotic center and the types of cells of the inflammatory zone.

FIG. 4. A representative reinfection tubercle, rabbit 12-2. This lesion seems to be identical with that shown in Fig. 3, except that bacilli are scanty in the necrotic center.



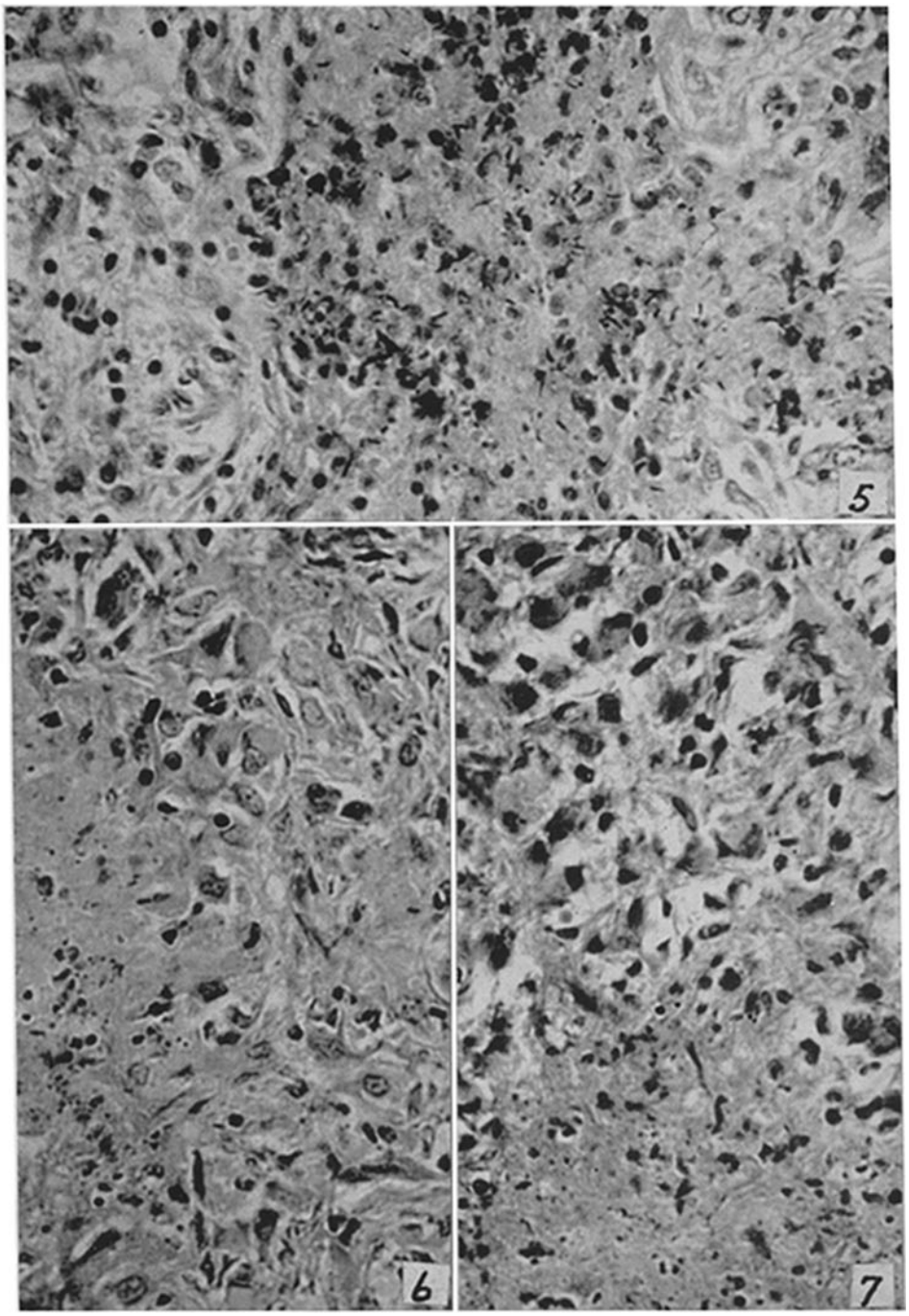
(Ratcliffe and Wells: Tuberculosis of rabbits. II)

PLATE 16

FIG. 5. A representative tubercle from the lungs of rabbit 13-8, dying 22 days after initial infection. The necrotic part of this lesion, about which the photograph centers, contains considerable numbers of bacilli either singly or in masses.

FIG. 6. A representative tubercle from the lungs of rabbit 12-6, reinfected after 14 days, in the exposure group with rabbit 13-8, and also dying 22 days after this experience.

FIG. 7. A representative tubercle from the lungs of rabbit 13-2, reinfected with rabbit 12-6. Figs. 6 and 7 illustrate variations in the cellular response found in reinfection tubercles. The cells which make up tubercles such as are illustrated by Fig. 7 correspond to initial tubercles. Lesions such as are shown in Fig. 6 resemble secondary tubercles as seen in this series of animals. Bacilli are equally scanty in the necrotic centers of these lesions.



(Ratcliffe and Wells: Tuberculosis of rabbits. II)

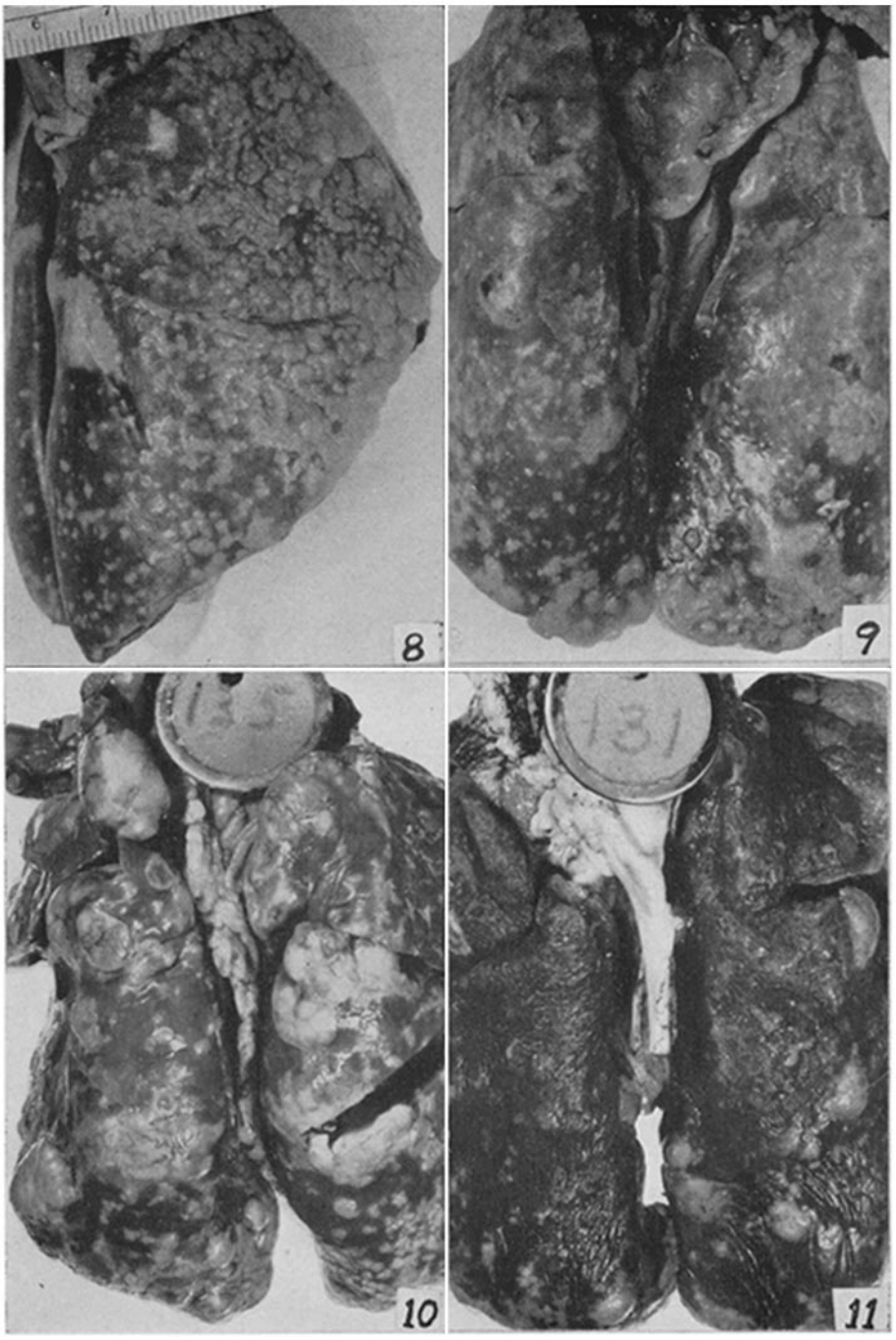
PLATE 17

FIG. 8. Lateral view, lungs, rabbit 13-7, reinfected after 49 days, killed 26 days later. Two initial tubercles are visible in dorsal parts of lungs. Dependent parts are largely occupied by secondary tubercles. Ulceration of initial tubercles into bronchi is believed to account for all small lesions. Other organs were not involved.

FIG. 9. Dorsal view, lungs, rabbit 13-6, reinfected after 42 days, killed 26 days later. Initial tubercles ulcerated, small tubercles equivalent in size to those in lungs were equally numerous in kidneys. Hence all small tubercles in lungs are believed to have developed from organisms spreading by bronchi and blood vessels.

FIG. 10. Dorsal view, lungs, rabbit 13-5, reinfected after 35 days, and dying 105 days later. Disease confined to lungs and mucosa of the intestine.

FIG. 11. Dorsal view, lungs, rabbit 13-1, reinfected after 49 days, killed 121 days later. Disease confined to lungs and mucosa of the intestine except for occasional small tubercles in kidneys. These 4 animals are believed to represent extremes of rapid and slow progression of the infection.



(Ratcliffe and Wells: Tuberculosis of rabbits. II)