

## THE FATE OF TUBERCLE BACILLI IN THE ORGANS OF REINFECTED RABBITS

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In a recent review of our present knowledge of immunity in tuberculosis Löwenstein (1) says, "What is the fate of the newly introduced tubercle bacilli in the tuberculous organism? Easy as it would seem to decide this question, yet how little do we now know about it with certainty." It is generally assumed that tubercle bacilli of reinfection are destroyed (2), although Krause and Willis (3) are of the opinion that immunity to reinfection is primarily due not to specific bacteriolysins, which destroy the bacilli, but to the interposition of an inflammatory barrier about them, which checks their spread in the body. There have been many experiments purporting to demonstrate a lysis of tubercle bacilli in the previously infected animal (4). These conclusions however have usually been based upon the reduction in numbers of stainable bacilli of reinfection and the tinctorial changes of those found. But when animal inoculation (5) has been used to determine the fate of tubercle bacilli of reinfection they have been found to persist in a virulent form.

In a previous study (6) cultural methods were used to determine the fate of both human and bovine tubercle bacilli in the organs of intravenously infected normal rabbits. It was shown that at first both the human and the bovine tubercle bacilli multiply in all the organs. The human tubercle bacillus grows faster at first than the bovine bacillus, but soon a change takes place, which causes its destruction, first in the liver, spleen, and bone marrow, and later in the lung and kidney. The bovine tubercle bacillus multiplies in these organs more slowly at first, but here too destruction takes place in the liver, spleen and bone marrow, though later than with the human bacillus, whereas in the lung and kidney multiplication continues without effective opposition until the death of the animal. It was also

shown that the time at which destruction of either type begins and the completeness of destruction depend upon the quantity of bacilli inoculated. The larger the number of bacilli inoculated the more rapid and the more complete will this destruction be, as could be seen in certain organs and with certain dosages.

Thus it would appear that as a result of the growth of both types of bacilli in the rabbit some change takes place in the body, which destroys the bacilli. One would expect that after such a change has once been brought about in the rabbit the introduction of fresh tubercle bacilli from without would be followed by immediate destruction without any preliminary growth. To test this hypothesis, and to ascertain the fate of tubercle bacilli in the organs of reinfected rabbits, the following experiments were performed.

#### *Method*

A series of rabbits was inoculated intravenously with 0.001 mg. of a human strain of tubercle bacilli, P-48A, per kilo of body weight. It was shown in the previous study that six months after such an infection the tubercle bacilli had practically disappeared from the liver, spleen, bone-marrow and kidney and had been greatly reduced in number in the lung. At about this time, therefore, these rabbits were given intravenously a reinfesting dose of 0.01 mg. of the same strain of human tubercle bacilli. Another series of rabbits was similarly infected with the human strain and reinfected with a bovine strain, Bovine C, in the same quantities and by the same route. At the same time two series of normal rabbits were injected intravenously with 0.01 mg. of the same strains used for reinfection, one with the human, and one with the bovine bacilli.

At intervals of 1 day, 1, 2, 4 and 6 weeks and 2 months, equal amounts by weight of suspensions of ground lung, liver, spleen, kidney and bone marrow in varying dilutions were seeded upon the surface of Dorset's and Petroff's media, both directly and after sodium hydroxide treatment. Care was taken to avoid tissue obviously affected by primary infection. At least 3 tubes of each medium were seeded with a given dilution of a given organ, both directly and after treatment. The results obtained were therefore based upon the readings of at least 12 separate tubes seeded with the same quantity of the same tissue or, when 2 dilutions were used, upon 24 tubes, except, of course, when contamination had occurred in some. The method has been described more fully in the previous publication; in the experiments described here larger tubes were used to afford a larger surface area and more accurate enumeration of colonies, and, for the same reason, instead of a strip of celluloid, a strip of spring metal, into which windows of accurately measured square areas had been cut, was used for estimating the number of colonies when these exceeded 200.

The number of colonies of tubercle bacilli appearing upon each tube was repeatedly determined, the final readings being made after 3 months' incubation.

It was shown in the previous study that there is no essential difference between the number of colonies derived from a given dilution of a given organ on either Dorset's or Petroff's media. Therefore for brevity's sake only the highest average figure obtained from the one or the other medium after direct seeding is tabulated. Where all the tubes of direct seeding were contaminated the number of colonies obtained after sodium-hydroxide treatment is given multiplied by 10, for it has been found that the number of colonies as a result of the manipulation in treatment is usually reduced ten times. However only occasionally have these adjusted figures been necessary.

*The Fate of Human Tubercle Bacilli of Reinfection in the Organs of Rabbits*

In Table I the fate of human tubercle bacilli in the organs of normal and of reinfected rabbits with the resulting pathological changes are compared. The number of colonies derived from the same quantity of a given organ of an infected and of a reinfected rabbit are placed in two columns in juxtaposition. As a rule 3 reinfected rabbits are compared with 2 infected animals for each interval.

In the *lungs* of the infected rabbits the human tubercle bacilli multiply at once, increasing in number continuously to about the 4th week, when the largest number, namely 12,000, was derived from 10 milligrams of tissue; thereafter there is a tendency for a diminution in numbers, though very considerable quantities of tubercle bacilli persist even after 2 months. In the lungs of the reinfected rabbits, on the other hand, neither growth nor destruction is discernible as a definite, continuous tendency, but tubercle bacilli are isolated in extremely variable numbers. These are ascribable to the residual lesions of the primary infection and not to the reinfection, for the normal rabbits primarily infected with 0.01 mg. of tubercle bacilli showed no macroscopic changes in any organ until the fourth week, whereas 7 out of the 8 reinfected rabbits killed within the first 2 weeks of reinfection showed considerable, and at times extensive, tuberculous lesions even 24 hours after reinfection. These lesions are obviously due to the primary infection and not to the reinfection, and the tubercle bacilli isolated in very variable numbers from the lungs of reinfected rabbits are therefore probably residual organisms from the primary infection. Moreover at each interval after reinfection there is at least one rabbit in which the number of tubercle bacilli in the lungs is extremely few. Thus rabbit 15, 24 hours after reinfection, showed 56 colonies; rabbit 17, after a week, 6 colonies; rabbit 20, after 2 weeks, showed 6; rabbit 22, after 4 weeks, showed 10; rabbit 27 showed 75 colonies 6 weeks after reinfection, and none could be isolated from rabbit 28 even 2 months after reinfection. On

TABLE I  
*Number of Colonies and the Pathological Changes Found in Rabbits Infected, and in Rabbits Reinfected† with 0.01 Mg. B. tuberculosis, Human Type*

Interval after infection or reinfection	Rabbit No.		Lung		Liver		Spleen		Kidney		Bone marrow		Pathological findings	
	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Infected	Reinfected
1 day	114	720	14	3*	18	6	0	2	2.5	0.3	Neg.			Num. discrete pulm. tubercles; 1 cortical lesion in left kidney
	215 16	56* 1,030	18 3*	12 0*	13 0	42 0	0 0	15 0	1.4	2	Neg.			2 isolated pulm. tubercles Small no. of discrete pulm. tuberc.; fibrocaseous tuberc. in cortex and medulla of left kidney
1 week	317 418	6 61	11 38	0* 0.3	40 76	2 142	2 0	0 1	17 21	1 1.5	Neg. Neg.			Single pulm. tubercle Localized caseous cavities in bases of both lungs; few tubercles at margins; no. of tuberc. in spleen
	19	40	8	8	11	11	3	4						Neg.; enlarged spleen
2 weeks	520	6* 123	110 83	1 8	243 1,380	1 10	1 0*	176 0	99 176	0 1	Neg. Enlarged spleen			Isolated marginal pulm. tuberc.; isolated cortical tuberc. in left kidney Several large localized lesions in both lungs, one of these enclosing a cavity
	621	1,580	83	8	1,380	10	0*	0	176	1				
4 weeks	722	10	60	0.3	596	0	10*	0*	72	0	Num. miliary tubercles with punctate caseation of lung; few to mod. no. of tuberc. in liver, spleen, kidney and bone marrow			2 or 3 isolated tuberc. at anterior margins of both lungs

	823	12,500*	3,220	7	4	416	40	2,200	4	0	0*	Num. miliary conglom. tuberc. in both lungs with caseous centers; occasional tuberc. in spleen; mod. no. of tuberc. in kidney	Numerous discrete pulm. tubercles; few cortical tuberc. in kidneys
	24		20	1.5			0		0	0	0	2 or 3 isolated tubercles in lungs; large cortical tuberc. and caseous pus in pelvis of right kidney	
6 weeks	925	1,820*	4,530*	4	0	105	0	76	22	10	0	Extensive miliary, caseous pulm. tuberc.; slight to mod. miliary tbc. of liver, spleen, kidney and bone marrow	Moderate no. of discrete nodular tubercles and pus containing cavities in both lungs
	1026	1,370	22,070	1	2	0	0.3	10	0	0	0.6	Moderate no. of tubercles in both lungs; 2 or 3 tubercles in cortex of kidneys	Localized ulcerative pulm. tbc.; 2 cortical tubercles in left kidney
	27**		75	0	0		0		3		0	Isolated fibrous pulm. tuberc.; isolated cortical tbc. of both kidneys	
2 mos.	1128	2,970	0*	1.5	0.3	9	0.3	170	120	0	0	Ext. conglom. miliary tbc. of lungs with multiple foci of caseation; mod. no. of cort. tuberc. in kidneys	2 isolated pulm. lesions, one of these an encapsulated cavity; sev. cort. fibr. lesions in kidney
	12	3,800*		0		0		1,170		0	0	Ext. conglom. discrete miliary tbc. of lungs with caseous foci. Few cortical tuberc. in kidneys	
	13	5,260		0		0.3		0		0	0	Numerous discrete tuberc. 2 to 4 mm. with caseation; single cortical tubercle in each kidney	

† The reinfected rabbits had a primary inoculation of 0.001 mg. B. tuberculosis, human type, about six months before reinfection.

\* Adjusted from figures obtained after treatment.

\*\* No direct cultures made; figures for this rabbit adjusted from those obtained after treatment.

the other hand the lowest numbers of colonies found in the primarily infected rabbits were 9, 48, 560, 600, 1370 and 2970 for the corresponding intervals. There is thus no clear direct evidence for the multiplication of human tubercle bacilli in the lungs of reinfected rabbits and similarly no evidence of any tuberculous changes in the lungs due to the reinfection.

In the *liver* of normal rabbits the human tubercle bacilli multiply somewhat more slowly than in the lung and reach their height by the second week. Their destruction is rapid from the 4th week, so that 6 weeks after infection they have practically disappeared. In the liver of reinfected rabbits fewer tubercle bacilli are recovered even 24 hours after reinfection than after primary infection. From then on only a few isolated colonies are found. It is noteworthy that although the bacilli of reinfection are destroyed immediately upon introduction without any preliminary growth this destruction is not complete; even 2 months after reinfection some few isolated organisms persist.

In the *spleen* of normal animals multiplication is more rapid than in the liver; this also reaches its height by the second week. Destruction begins about the fourth week; by the sixth week it is well under way and at the end of the second month the bacilli have practically disappeared. Again, as in the liver, the human tubercle bacilli of reinfection are destroyed at once without any preliminary multiplication. The 142 colonies recovered from the spleen of rabbit 18 are obviously due to tubercles found in the spleen and therefore, obviously, are derived from the primary infection, as no macroscopic tubercles are formed in the spleen within a week after infection, when this rabbit was killed.

In the *kidneys* of normal rabbits the bacilli accumulate very slowly; the highest number was obtained in the fourth week; there is a tendency to a slow decline thereafter, but numerous tubercle bacilli persist, in the kidney as in the lung, even 2 months after infection. In the kidney of reinfected rabbits the same result was obtained as in the lung. In the great majority of cases no evidence of growth of the bacilli of reinfection occurred in this organ, although occasionally considerable numbers of tubercle bacilli were found, probably due to the residual lesions of the primary infection.

In the *bone marrow* of normal rabbits multiplication is rapid at first, reaches its height by the second week and then rapidly declines, so that 6 weeks after infection the bacilli have practically disappeared. In the bone marrow of reinfected rabbits there is no preliminary multiplication. Destruction takes place from the beginning, and again as in the other organs, is not complete; a few lingering bacilli persist even 6 weeks after reinfection.

Associated with the immediate destruction in the organs of the bacilli of reinfection there is no evidence of any tuberculous lesions due to the reinfection. This is brought out clearly in the intervals of the fourth and sixth weeks after infection and after reinfection. Although there is a generalized miliary tuberculosis of variable degree

in all the organs of primarily infected rabbits, in the reinfected rabbits no tuberculosis developed in the liver, spleen and bone marrow and the variable lesions in the lung and kidney were undoubtedly residual lesions from the primary infection. Further evidence for the source of the bacilli and the tuberculous lesions in the lung and kidney of reinfected rabbits will be presented in connection with the fate of bovine tubercle bacilli of reinfection.

*The Fate of Bovine Tubercle Bacilli of Reinfection in the Organs of Rabbits*

In Table II the fate of bovine tubercle bacilli in the organs of infected and of reinfected rabbits as well as the tuberculous changes that they have induced are compared.

In the *lung* of normal rabbits the bovine tubercle bacilli multiply uninterruptedly to the end of the second month, so that by this time the lungs of some rabbits become virtually a pure culture of tubercle bacilli, and as many as 200,000 colonies have been isolated from 10 milligrams of tissue. In the lungs of reinfected rabbits however there is the same lack of a regular tendency to either growth or destruction as was noted above in rabbits reinfected with human tubercle bacilli. Here again extremely variable numbers were isolated. Here, too, the lungs of at least one reinfected rabbit of each interval can be seen to contain such numbers of organisms as can be ascribed to residual bacilli from the primary infection, as in rabbits 43, 45, 46, 48, 51 and 54. Again in the first 2 weeks after reinfection the lesions found in the lungs are unquestionably due to the primary and not to the secondary infection.

More direct evidence was obtained for the origin of these lesions and bacilli, first in the x-ray pictures of the lungs of the rabbits taken just before reinfection, and secondly in the cultural behaviour of these bacilli.

It was found that if a rabbit showed slight or no lesions by x-ray previous to reinfection, slight or no lesions were found in the lungs at autopsy whether the animal was killed 24 hours or 2 months after reinfection. Rabbit 45 showed no lesions detectable by x-ray before reinfection and showed no lesions at autopsy 1 week after reinfection. Rabbit 54 showed no distinct lesions by x-ray previous to the second inoculation and only a few discrete tubercles in the lungs when he was killed 2 months after reinfection.

On the other hand, if the rabbit showed extensive lesions detectable by x-ray before reinfection these were found at autopsy. Rabbit 44 showed extensive pulmonary tuberculosis before reinfection and showed discrete pus-containing cavities when killed 1 week after reinfection. Similarly rabbits 47, 49 and 52, which were killed 2, 4 and 6 weeks after reinfection respectively. Moreover the

TABLE II  
*Number of Colonies and the Pathological Changes Found in Rabbits Infected, and in Rabbits Reinfected† with 0.01 Mg. B. tuberculosis, Bovine Type*

Interval after infection or reinfection	Rabbit No.		Lung		Liver		Spleen		Kidney		Bone marrow		Pathological findings	
	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Infected	Reinfected
1 day	29	42	3*	8,300	13	0*	7.3	0	0.3	0	2.3	0.3	Neg.	Tuberculous bronchopneumonia with cavitation; caseous pus in pelvis of one kidney
	30	43	?	20	3*	11	16*	12	0	405	2.5	11	Neg.	Few residual pulm. tuberc.; caseous tbc. of medulla of kidneys; tbc. of joints; x-ray free of tbc. before re-infection
1 week	31	44	6*	30,000	3	28	200	69	0.2	1,630	18	16	Neg.	Discrete pus containing pulm. cavities. Tbc. of joints; bacteremia? T.B. of human type. Ext. pulm. tbc. before re-infection
	32	45	6*	70	?	31	537	8	6	0.3	0	3	Neg.	2 or 3 residual tuberc. in both lungs; practically free of tbc. before re-infection by x-ray. Tbc. of one joint



2 weeks	33	46	1,010	300	150	1	2,410	0.3	0	4	64	0	Minute pulmonary miliary tubercles	Fibrocaceous consolidation of anterior pulm. margins with few discrete pulm. tuberc. over remainder of lung; tbc. of tibia
	34	47	1,200	1,270	380	0.3	1,425	0	27	300	480	0.6	Punctate miliary tubercles of lung, liver, spleen and kidney	Extensive ulcerative pulm. tbc.; miliary tubercles in cortex of kidneys; tbc. of one joint; ext. tbc. before reinfection
4 weeks	35	48	1,080	10*	?	0	466	0	297	10	170	0	Ext. discrete miliary tbc. of lungs; miliary tuberc. in liver, spleen, kidney and bone marrow	Moderate no. of miliary tubercles in lungs; few pinpoint tubercles in kidneys
	36	49	1,000*	6,000	305*	18	1,650	54	12	517	420	3	Numerous pulm. miliary tuberc. with caseation; slight to moderate miliary tbc. of liver, spleen, kidney and bone marrow	Ulcerative caseous pulm. tbc. Cortical and cortico-med. tbc. of kidneys; tbc. of joints; tbc. of both lungs before reinf.; t.b. of human type. Bac-teremia?
	50			60*		0.2		0		20		7	Discrete, sparse tubercles in both lungs. With localized lesions in anterior margin and apex of rt. lung. Few cortical tuberc. in kidney; localized lesions in lung before reinfection	

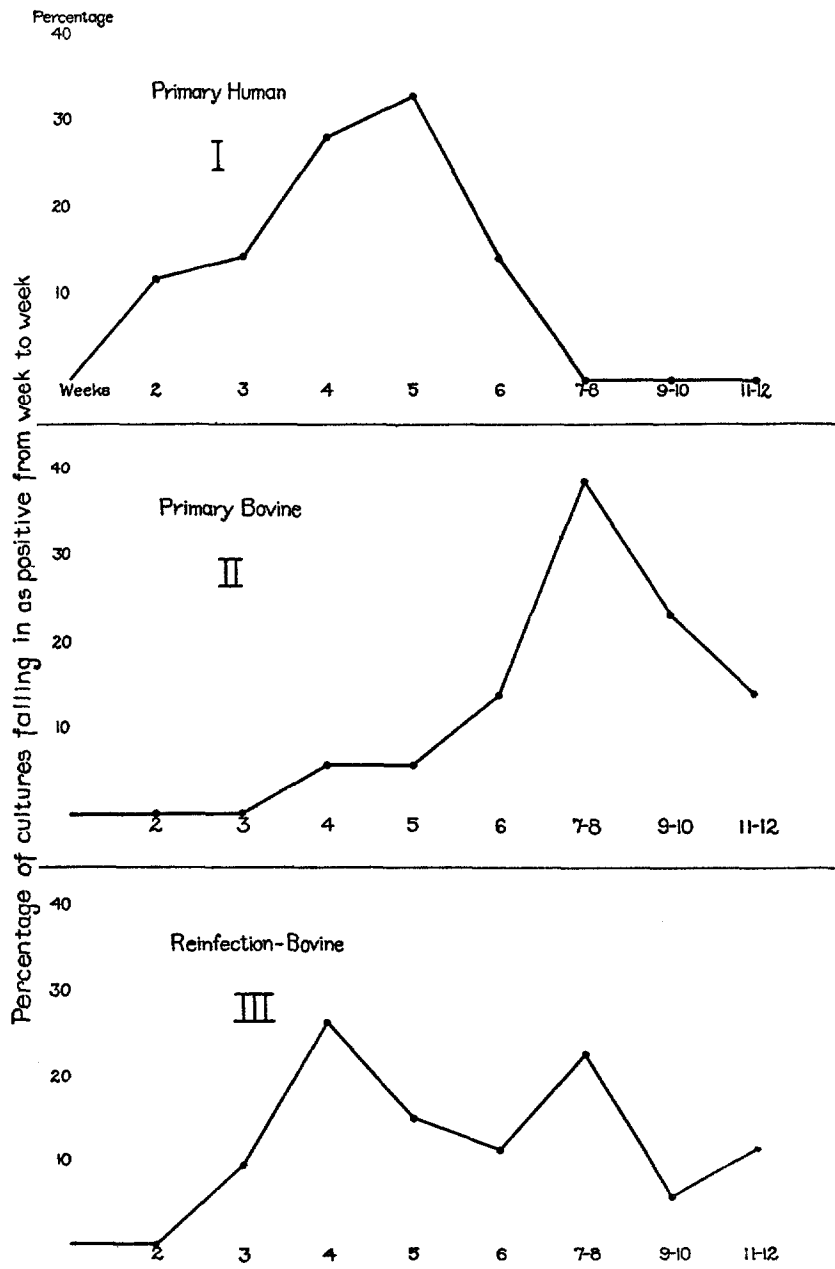
† The reinfected rabbits had a primary inoculation of 0.001 mg. B. tuberculosis, human type, about six months before reinfection.

\* Adjusted from figures obtained after treatment.

TABLE II—*Continued*

Interval after infection or reinfection	Rabbit No.		Lung		Liver		Spleen		Kidney		Bone marrow		Pathological findings	
	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Inf.	Reinf.	Infected	Reinfected
6 weeks	37	51	12,000	320*	1.3	0	4,000	0	1,530	2	1,575	0	Massive miliary conglom. pulm. tbc. with caseation; slight ext. miliary tbc. in liver, spleen, kidney and bone marrow	Numerous discrete subpleural tuberc. 3-4 mm. in diameter
	38	52	2,100*	2,560	38	0.3	82	0.3	111	130	23	1	Ext. miliary conglom. pulm. tbc. with caseation, with miliary tuberc. in spleen, liver and kidney	Ulcerative pulm. tbc. with discrete tuberc.; few cortical tubercles in kidneys. Disseminated pulm. tbc. before reinfection
				540*	0			0		3.5		180		Discrete tuberc. uniformly distributed over both lungs; few tuberc. in kidneys and bone marrow; free of tbc. before reinfection
2 mos.	39	54	3,100	260*	2	0	130	0	620	0	1,300	0	Massive consolidation with conglomerate tuberc. with multiple foci of caseation in both lungs; slight to ext. miliary tbc. of liver, spleen, kidney and bone marrow	Few discrete tubercles in lungs which were practically free of tbc. before reinfection

40	55	6,300*	11,000*	0	0	1,530	1	505	366	690	6*	Massive conglom. miliary tbc. with caseation of both lungs; miliary tbc. of spleen, kidney and bone marrow	Ulcerative pulm. tbc.; tbc. of cortex of left and caseous pus in right kidney
41	56	200,000	1,100	0.7	0.5	2,900	1	1,168	70	3	0.5	Massive caseous pneumonia and conglom. miliary tbc. of both lungs; several tubercles in kidneys and spleen	Numerous discrete subpleural tubercles; few cortical tubercles in kidneys; tbc. of joints



FIGURES I TO III

more extensive the lesions shown by x-ray before reinfection, the more tubercle bacilli were isolated.

Further evidence pointing to the primary infection as the source of the bacilli cultured from the lungs of reinfected rabbits is as follows. It is well known that bovine tubercle bacilli on isolation from the body are dysgonic, that is, that they grow more slowly in the test tubes than human bacilli. Now although both the human and bovine strains of tubercle bacilli used in these experiments grew equally fast when transplanted on glycerol agar in the usual way, the dysgonicity of the bovine strain appears when the bacilli are reisolated from the organs of the rabbit. From the organs of numerous rabbits given a primary inoculation of human bacilli, 86 per cent of the colonies that were finally present after 3 months' incubation were already grown by the fifth week of incubation and all had appeared by the sixth week. On the other hand, from the organs of rabbits given a primary bovine infection, only 25 per cent grew in the first 6 weeks, whereas the great majority appeared between the 8th and 10th week, and a considerable number had not yet appeared before the twelfth week. See figures I and II.

Now from the organs of the rabbits that had been given a primary inoculation of human tubercle bacilli and had been reinfected with bovine tubercle bacilli two distinct groups of organisms were isolated. Of these, 61 per cent appeared within the first 6 weeks after incubation and 39 per cent appeared between the sixth and twelfth week after incubation. Thus according to their rapidity of growth on culture they are seen to be of two types, some behaving as human bacilli, others as bovine bacilli. See figure III.

It appears that the tubercle bacilli isolated from the lungs of all the reinfected rabbits except two, namely rabbits 51 and 53 of the 6-weeks interval, had grown in the first 6 weeks. Thus by far the largest number of tubercle bacilli isolated from the lungs of the rabbits reinfected with bovine bacilli behaved not like the bovine organism but like the human tubercle bacillus and therefore probably have been derived from the primary infection.

Thus the x-ray evidence before reinfection and the cultural behavior of the bacilli recovered, as well as the very low numbers of the organisms found in at least one rabbit at each interval after reinfection, as contrasted with their continuously increasing and finally tremendous numbers after primary infection, all argue strongly for the conclusion that the bovine bacillus does not grow as a rule in the lungs of reinfected rabbits.

That some growth of bovine bacilli of reinfection can take place in the lungs, though unquestionably a restricted one, is probably shown in rabbits 51 and 53. Rabbit 51 had about 320 colonies in 10 mg. of lung, which appeared in culture on the 12th week of incubation. Rabbit 53 showed no detectable lesions in the lung by x-ray before reinfection, yet when this rabbit was killed 6 weeks after reinfection there were discrete uniformly distributed tubercles in both lungs and the organism cultured from this rabbit appeared on the 8th week of incubation.

That the growth of bovine bacilli of reinfection in the lungs of even these rabbits

is greatly suppressed as compared with bacilli from a primary infection is seen from the fact that the average number of colonies recovered from 2 normal rabbits killed 6 weeks after infection was 7050, in contrast to the average of only 430 colonies derived from these two rabbits.

It is noteworthy that this, the only evidence obtained of multiplication of tubercle bacilli in the lungs of reinfected rabbits, was found in rabbits having practically no residual lesions from the primary infection.

In the *liver* of normal rabbits multiplication of the bovine bacilli is slow at first, reaches its height in the fourth week and thereafter declines rapidly. In the liver of reinfected rabbits fewer bacilli are isolated even 24 hours after the second infection than are found in primarily infected rabbits, showing a tendency to immediate destruction. Thereafter, as a rule, only a very few organisms are found, showing again as after reinfection with human bacilli, that destruction is never quite complete.

The presence of from 18 to 31 colonies in the liver in 3 rabbits of this group requires some explanation. Of these, rabbit 44 of the first-week interval, and rabbit 49 of the fourth-week interval, had extensive pulmonary tuberculosis before reinfection. At autopsy both showed an extensive ulcerative tuberculosis of both lungs with very large numbers of tubercle bacilli. Both were apparently suffering from a bacteremia derived from the extensive pulmonary foci, as evidenced by the tuberculosis of the joints, the distribution of bacilli in the liver and spleen, which was like the distribution following immediately upon a primary inoculation, and the cultural characteristics of these organisms, which were of the human type; unfortunately no blood cultures were made. These colonies therefore are due not to a growth of the bovine tubercle bacilli of reinfection but to a flooding of the circulation with human tubercle bacilli from the old foci in the lungs and their filtration in the various organs. In rabbit 45, however, the 31 colonies isolated are possibly due to an actual multiplication of the bovine bacilli of reinfection, for they were culturally of the bovine type. It is noteworthy that this rabbit was free of tuberculosis before reinfection and showed only 2 small residual tubercles in the lung when it was killed one week after reinfection.

In the *spleen* of normal rabbits the bovine bacilli multiply at once, and continue to multiply to the sixth week; they are then slowly reduced in number, although many are still present at two months. In the spleen of the reinfected rabbits the tubercle bacilli are destroyed from the very first, so that even 24 hours after reinfection they are already less in number than after a primary infection with bovine bacilli. Thereafter only an occasional organism is isolated, except in rabbits 44 and 49, where there are some tubercle bacilli due to the bacteremia from the lungs in this organ as in the liver.

In the *kidneys* of normal rabbits the bovine bacillus accumulates very slowly at first and continues persistently with scarcely any abatement to the end of the experiment. In the kidneys of reinfected rabbits on the other hand there is the same irregularity as was noted in the lungs. Here again there is at least one

rabbit at each interval with only few residual tubercle bacilli and with no evidence of any multiplication even 2 months after reinfection. There is however another group of rabbits in which variable and at times considerable numbers of tubercle bacilli have been isolated from the kidneys. These all behaved in culture like the human type, and like those in the lungs of the same rabbits are most probably due to bacilli from the primary infection. It is noteworthy that the kidneys of rabbit 44, from which the largest number, 1630 colonies, of tubercle bacilli were isolated, showed no tuberculosis at all. Are these bacilli explained by bacteremia in this rabbit and are they being excreted by the kidney?

In the *bone marrow* of normal rabbits the bovine tubercle bacillus multiplies slowly at first. This continues to the sixth week when their numbers slowly decline. At two months considerable numbers of tubercle bacilli are still present. In the bone marrow of reinfected rabbits only occasional tubercle bacilli can be isolated; they are destroyed without any preliminary growth.

There was only one exception to this rule, rabbit 53, in which unquestioned though restricted growth took place, for 180 colonies were isolated from the bone marrow of this rabbit 6 weeks after reinfection. These were of the bovine type and could scarcely be residual bacilli from the primary infection. It is noteworthy that this rabbit also showed restricted multiplication of the bacilli of reinfection in the lungs, and, as was stated above, was free of tuberculosis before reinfection.

Associated with the immediate destruction of the bovine tubercle bacilli of reinfection in the organs of most of the rabbits, there was no evidence of any lesions due to the bacilli of reinfection; whereas the normal animals similarly infected showed a massive pulmonary miliary tuberculosis with extensive to moderate tuberculosis in the liver, spleen, kidney and bone marrow. In no reinfected rabbit was there any tuberculosis of the liver and spleen and in only one were there a few tubercles in the bone marrow. In the lungs of reinfected rabbits the lesions were different in character from those found in infected rabbits. The latter showed consolidation of the entire lung by a uniformly distributed massive miliary tuberculosis; in the former, the pulmonary lesions were often very unevenly distributed, irregular in size, extremely few in number or they consisted of more or less extensive pus cavities, such as are found in rabbits that are permitted to live a long time after a single injection of human tubercle bacilli. These lesions were present in the rabbits before reinfection, as the x-ray photographs showed. There were only 2 reinfected rabbits in which some pulmonary tuberculosis developed later, which however was very slight in comparison with that in the infected rabbits.

## SUMMARY AND DISCUSSION

In agreement with previous work (6) it was found that in normal rabbits after a primary inoculation of 0.01 mg. of human tubercle bacilli the organism shows a preliminary growth, which commences at once in all the organs, and reaches its height in the second week in the liver, spleen, and bone marrow; thereafter destruction begins, and is practically complete by the sixth week in the liver and bone marrow and less complete in the spleen. In the lung and kidney the largest number are isolated in the fourth week and thereafter there is a tendency to destruction, but to destruction far less complete than in the other organs, for even two months after infection large numbers of tubercle bacilli persist. Associated with this multiplication of the human tubercle bacilli were found extensive or moderate tuberculous lesions in the lung and kidney with moderate or slight changes in the liver, spleen and bone marrow.

In contrast to these observations, it was found that in rabbits reinfected with the same quantity of human tubercle bacilli, the organism was destroyed immediately without any preliminary multiplication in the liver, spleen and bone marrow, though a few bacilli persisted in these organs even two months after reinfection. Neither could definite evidence of multiplication of the tubercle bacilli of reinfection in the lung and kidney of these rabbits be obtained; although variable and at times very large numbers of tubercle bacilli persisted in these organs, probably due to the primary infection. Nor were tuberculous changes found in any of the organs due to reinfection.

Again in rabbits that received a primary inoculation of 0.01 mg. of bovine tubercle bacilli the organism multiplied in all the organs. This multiplication reached its height in the liver, spleen and bone marrow between the fourth and sixth week, instead of in the second week as with human bacilli. Thereafter destruction was practically complete in the liver and much less complete in the spleen and bone marrow; in the lung and kidney multiplication continued unabated to the second month. Associated with these bacteriological data, there developed a massive pulmonary tuberculosis with extensive or moderate disease in the kidney, spleen, bone marrow and liver.

In rabbits reinfected with the same quantity of bovine tubercle bacilli after a primary infection with human tubercle bacilli, the



organism, in all but a few instances, was destroyed immediately in the liver, spleen and bone marrow. Here again, with the bovine type as with the human type, after reinfection as after primary infection, the destruction was not quite complete and a few isolated bacilli persisted even two months after reinfection. As to the lung and kidney, evidence was obtained that the variable and at times very large numbers of tubercle bacilli that were found in these organs were of human type in their cultural characteristics, and that in these organs also, the bovine tubercle bacilli of reinfection failed to grow. There was also as a rule complete absence of any tuberculous lesions in the liver, spleen and bone marrow. Nor were there any tuberculous changes found in the lung and kidney due to the reinfection, but those variable residual lesions that were found were present before reinfection, as shown in the lung by x-ray photographs. There were a few instances in which restricted multiplication of the virus took place and slight tuberculous lesions developed after reinfection in rabbits in which the primary lesions had all but disappeared.

Thus in rabbits having considerable residual lesions from a primary human infection the tubercle bacilli of reinfection, whether human or bovine, are destroyed immediately though incompletely without any preliminary multiplication. Yet these rabbits, which so efficiently destroy the more virulent bovine bacilli of reinfection introduced from without, at times harbor tremendous numbers of human tubercle bacilli in the old lesions of the lung and kidney.

Parallel with the bacteriological and pathological studies of these rabbits, the serum of some of them was studied for the presence of circulating antibodies by McCutcheon, Strumia, Mudd (S), Mudd (E. B. H.) and Lucké (7). These investigators found that on primary infection there was only a slight and slowly developing production of agglutinating and phagocytosis-promoting antibodies whereas, in the reinfected rabbits, which showed an immediate destruction of the bacilli, these antibodies rose promptly and in relatively large amount. They suggest that the prompt rise of antibodies in reinfected animals may play a rôle in the immunity to tuberculosis.

In the previous study, which was performed in 1927, emphasis was placed upon the comparatively slower original rate of growth of the bovine bacillus in the rabbit as compared with that of the human type,

and in this delay was seen a partial explanation of the greater virulence of the bovine organism for this species. The more rapid the original growth the more rapid was the following destruction. In this series of experiments, which were performed early in 1929, although the delayed destruction of the bovine type in the rabbit was amply confirmed, the original rate of growth is not any slower than the human type. Coincident with this increasingly more rapid rate of growth of the bovine bacillus in the body is a gradual decrease in the virulence of the organism. In 1925 and 1926 0.01 mg. of this bovine strain regularly killed rabbits in about 30 days after intravenous inoculation (8), but in this experiment none of the 3 rabbits died within 65 days after a similar infection and 2 rabbits were still living 110 days after injection.

#### CONCLUSIONS

1. In the presence of a certain amount of residual primary lesions, human or bovine tubercle bacilli of reinfection are destroyed in all the organs of rabbits without any preliminary multiplication. This destruction is not quite complete; a few organisms persist even two months after reinfection.
2. No macroscopic tuberculous lesions due to the reinfection develop in these rabbits.
3. With the practical disappearance of the primary lesions and their enclosed organisms, restricted multiplication of the bovine bacillus of reinfection takes place but not the extensive growth of the virus observed in rabbits after a primary infection.
4. In these rabbits slight tuberculous lesions develop as compared with the massive tuberculosis of primarily infected rabbits.
5. The human tubercle bacillus of reinfection does not multiply at all, nor does it cause any lesions, even when the lesions of the primary infection have almost disappeared.
6. Although the rabbit destroys efficiently considerable quantities of bovine tubercle bacilli of reinfection introduced from without it may harbor innumerable human bacilli in the residual primary lesions of the lung and kidney.

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