ON THE MECHANISM OF OPSONIN AND BACTERIOTROPIN ACTION.

III. The Development and Effect of the Antibodies Found in Experimental Tuberculosis of Rabbits.

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This study is concerned with three questions: first, the development of circulating antibodies in primary tuberculosis in rabbits; second, the development of antibodies in rabbits reinfected with tubercle bacillus while still incompletely recovered from a primary tuberculosis; and third, as in the two foregoing studies, with the correlation between the effects on bacterial surface properties and on phagocytosis produced by the sera of the animals studied.

The animals followed were taken from among those used by M. B. Lurie in continuation of his study of the fate of tubercle bacilli in the organs of the rabbit (1). Bacteriological data on these rabbits will be published later by Lurie. The titers of the sera of three groups of rabbits have been followed, each containing primarily infected as well as reinfected individuals. The sera were tested in all cases with suspensions in 0.85 per cent sodium chloride solution of a living bovine tubercle bacillus, Bovine III. This is an avirulent and now slightly atypical strain[1] (2). The test suspensions were adjusted to match a turbidity standard. The methods used are those described in a foregoing paper (3), except that the interface reaction was omitted. The sera were heated for 30 minutes at 56°C. in all cases.

[1] It was not practicable to use the infecting strain (Bovine C) as routine antigen in these tests because of the danger of infecting the workers. In a special experiment, however, living Bovine C bacilli were shown to exhibit typical surface changes and increase in phagocytosis as a result of serum sensitization. For all practical purposes Bovine III was an homologous antigen.

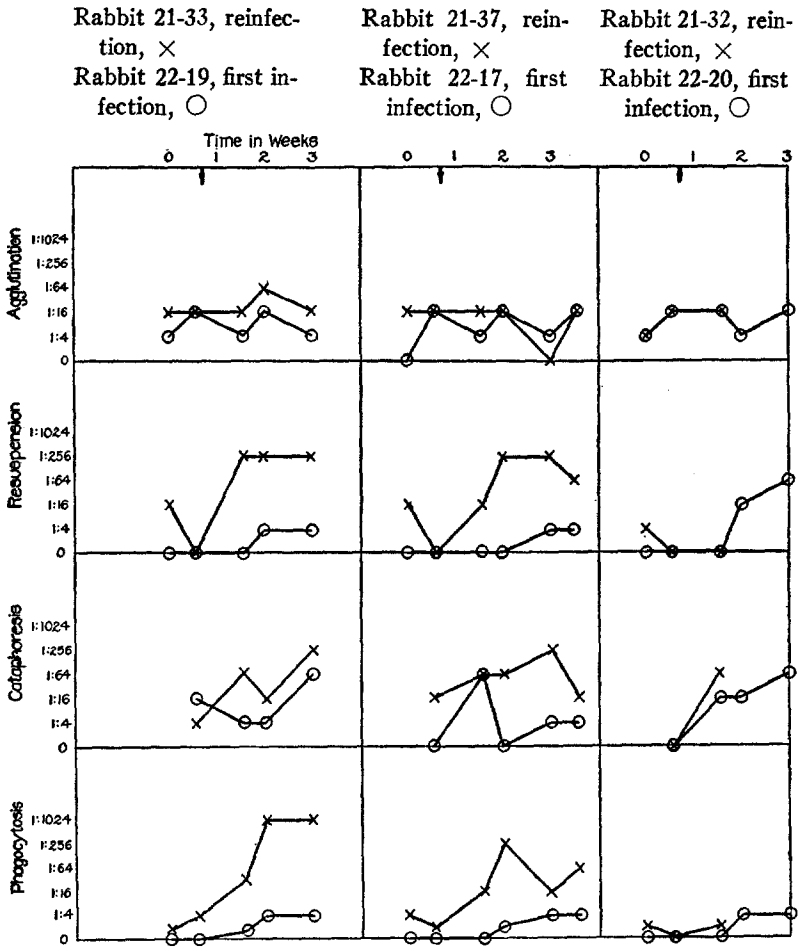

FIG. 1. Response in antibody production following a single injection (indicated by arrow at top of chart) of living, virulent tubercle bacilli. Three rabbits with residual foci of tuberculosis (crosses), and 3 previously healthy rabbits (circles). Ordinates represent the greatest dilutions of serum giving clearly positive reactions. It is shown that the reinfected animals promptly developed circulating antibodies strikingly in excess of those found in the course of primary infection. The changes in titer during tuberculous infection as indicated by the bacterial surface reactions (resuspension and cataphoresis) and by phagocytosis were in approximate correspondence.

Group 1.—Rabbits 21-32, 21-33 and 21-37 injected intravenously on May 17, 1928, with 0.001 mg. per kilo body weight of a living virulent human tubercle bacillus, Strain P-48A. X-ray of rabbits' lungs, Oct. 24, 1928, showed for Rabbit 21-32 slight, and for Rabbit 21-37 moderate disseminated pulmonary tuberculosis; lesions in Rabbit 21-33 were not certainly detectable by x-ray. These previously infected rabbits and normal Rabbits 22-17, 22-19, and 22-20 were injected intravenously on Nov. 6 with 0.01 mg. of a living virulent bovine tubercle bacillus, Bovine C. The titers of the sera of the rabbits of this group are given in Text-fig. 1; the injections on Nov. 6 are indicated by arrows at the top of the chart. The titers represent the greatest dilutions of serum giving clearly positive reactions; doubtful reactions and traces are omitted as in the first paper (3). Cataphoresis after sensitization with these relatively concentrated sera does not give a sharp end point; therefore in each experiment any value representing a change (decrease) of 20 per cent or more from the average cataphoretic velocity of the unsensitized bacteria of that experiment has arbitrarily been taken as positive.

The most striking feature of Text-fig. 1, and also of the charts following, is the difference in response to injection of the reinfection and first infection animals. The titers of the two groups do not differ greatly before the injections of Nov. 6, although those of the infected animals tend to be a little higher. But a marked difference appears at the first bleeding 6 days after injection; the titers of reinfection Rabbits 21-33 and 21-37 have clearly increased, whereas those of the first infection rabbits are essentially unchanged. Reinfection Rabbit 21-32, whose titers showed little change at the first bleeding on Nov. 12, was in bad condition at the time and died Nov. 13.

An increase in titers appeared in each of the first infection animals at the second (Nov. 15) or third (Nov. 22) bleeding, respectively 9 and 16 days after the injections. Although slight, this increase was so uniform in the 3 animals that we believe it to have been significant.

The potency of the sera in changing the properties of the bacterial surfaces (resuspension and cataphoresis reactions) and in promoting phagocytosis are parallel, within the experimental error, as was found to be the case with sera of animals under active immunization (3). The "agglutination" reaction, shown in the uppermost lines, does not render a satisfactory account of the changes in the sera shown by the other reactions. It has already been pointed out that the ordinary agglutination technique is not satisfactory with mammalian tubercle bacilli as antigen (4, 5), but that the resuspension reaction is a satisfactory detector of the binding of agglutinins by these bacilli. Our

experiments thus afford strong corroborative evidence of the parallelism of agglutinin and bacteriotropin content in sera (6, 7), not evidence contradicting such parallelism as might appear on first inspection of the charts.

The sera of Rabbits 21-33 and 21-37, 16 days after reinfection, *i.e.*, when active against Bovine III, were tested for specificity against *M. chelonei* (turtle bacillus). They did not react to a significant degree with *M. chelonei.*

The autopsy protocols of the rabbits of this group are given below.

Autopsies by M. B. Lurie.

Rabbit 21-32, died Nov. 13, 1928, *i.e.*, 7 days after *reinfection.* Lungs: moderate number of discrete, rather firm subpleural tubercles. Liver, spleen and bone-marrow: no tubercles. Kidney: discrete tubercles in cortico-medullary region.

Rabbit 21-37, killed Dec. 18, 1928, *i.e.*, 42 days after *reinfection.* Lungs: posterior portions have large caseous pus-containing cavities; anterior portions show discrete subpleural tubercles. Liver and bone-marrow: no tubercles. Spleen: enlarged, surface irregular, no clear-cut tubercles. Kidney: moderate number of cortical tubercles.

Rabbit 21-33, killed Jan. 8, 1929, *i.e.*, 63 days after *reinfection.* Lungs: a few scattered fibrocaseous tubercles, 2 to 3 mm. in diameter. Spleen: enlarged and mottled, no tubercles. Kidney, liver and bone-marrow: no tubercles.

Rabbit 22-20, killed Dec. 3, 1928, *i.e.*, 27 days after *first infection.* Lungs: numerous miliary tubercles about 2 mm. in diameter with punctate caseous foci. Liver: minute punctate tuberculous foci. Spleen: numerous subcapsular tubercles about 1 mm. in diameter. Kidney: moderate number of cortical tubercles about 1 mm. in diameter. Bone marrow: a few discrete tubercles.

Rabbit 22-17, killed Jan. 10, 1929, *i.e.*, 65 days after *first infection.* Lungs: anterior portions almost entirely consolidated (diffuse caseous pneumonia); posterior portions show massive conglomerate miliary tuberculosis; extensive communicating caseous foci. Liver: appears normal. Spleen: several tubercles 3 to 4 mm. in diameter. Kidney: moderate number of discrete cortical tubercles. Bone-marrow: no tuberculosis.

Rabbit 22-19, killed Jan. 11, 1929, *i.e.*, 66 days after *first infection.* Lungs: massive conglomerate miliary tubercles, 1 to 10 mm. in diameter. Spleen: about 4 times normal in size; numerous reddish subcapsular tubercles, 2 to 3 mm. in diameter. Liver: a few minute tubercles. Kidney: moderate number of cortical and corticomedullary tubercles. Bone-marrow: ill-defined tubercles.

Two of the reinfection rabbits, Nos. 21-32 and 21-37, showed a moderate number of discrete lesions in lungs and kidney, in all probability residual from the primary infection with tubercle bacilli of

human type. In the third reinfection rabbit, No. 21-33, the primary infection had been almost overcome, leaving only a few scattered fibro-caseous tubercles in the lung. All 3 animals were highly resistant

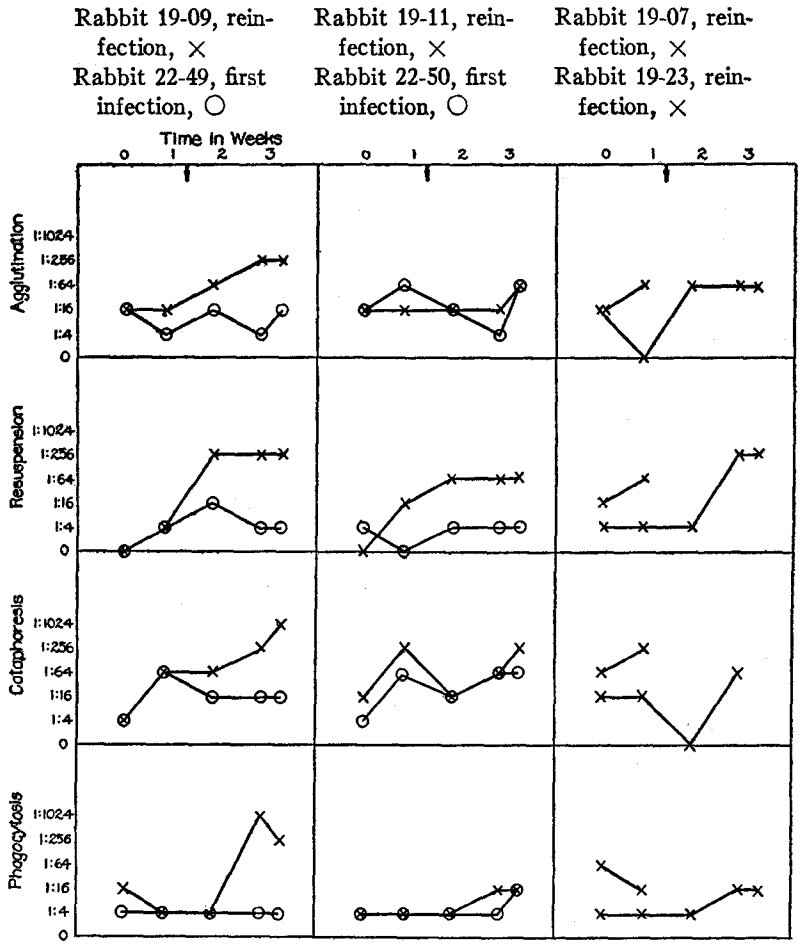

FIG. 2. Antibody production following single injection (indicated by arrow at top of chart) of living, virulent tubercle bacilli. Four rabbits with residual foci of tuberculosis (crosses), and 2 previously healthy rabbits (circles).

to the secondary infection with bovine tubercle bacilli; no lesions certainly referable to the second infection were found.

A marked contrast to the reinfection animals was afforded by the

rabbits infected with bovine tubercle bacilli without previous infection with bacilli of human type. In these the bovine infection ran its usual malignant course, causing after 2 months massive confluent tuberculosis of the lungs and involvement of the abdominal organs.

Group 2.—Rabbits 19-07, 19-09, 19-11 and 19-23 injected intravenously on Feb. 3, 1928, with 0.001 mg. per kilo body weight of a living, virulent, human tubercle bacillus, Strain P-48 A. These infected rabbits and normal Rabbits 22-49 and 22-50 injected intravenously on Oct. 11, 1928, with 0.01 mg. of living virulent bovine tubercle bacillus, Bovine C. Injections on Oct. 11 indicated in Text-fig. 2 by arrows at top of chart. In Groups 2 and 3 cataphoresis values are taken as positive if they represent a decrease of 10 per cent or more from the average cataphoretic velocity of the unsensitized bacteria of the experiment in question.

The increase in rate and quantity of antibody production in the reinfection rabbits as compared with the first infection rabbits again appears in Text-fig. 2. This difference is marked between Rabbits 19-09 and 22-49, less apparent between Rabbits 19-11 and 22-50. The reinfection rabbits in each case showed increase in titer by 11 days after injection; this increase was not consistently apparent 4 days after injection. No unequivocal increase in the titers of the 2 first-infection rabbits was detected within the 2 weeks after injection during which the experiment was continued.

Rabbit 19-07 died 3 days after reinfection. The titers before reinfection were unusually high. Also the amount of pulmonary disease was unusually great for rabbits infected with so small a dose of human bacilli. This association may have been coincidental, however.

The serum of Rabbit 19-11, 18 days after reinfection was tested for specificity against *M. chelonei.* It did not react significantly with *M. chelonei.*

Autopsies by M. B. Lurie.

Rabbit 19-07, died Oct. 14, 1928, *i.e.*, 3 days after *reinfection.* Lungs: extensive tuberculosis with cavitation; confluent subpleural lesions toward anterior margin. Pneumonia superadded in left lung. Liver: no tubercles. Spleen: no tubercles. Kidneys: 2 isolated tubercles in each.

Rabbit 19-09, killed Oct. 26, 1928, *i.e.*, 15 days after *reinfection.* Lungs: fibrocaseous consolidation of anterior margins. Lung elsewhere has a few discrete lesions. Liver, no tubercles. Spleen: enlarged and studded with firm grayish nodules. Kidney, no tubercles. Bone marrow: epiphysis of right tibia, caseous focus.

Rabbit 19-11, killed Nov. 8, 1928, *i.e.*, 28 days after *reinfection*. Lungs: moderate number of small glossy tubercles, 1 mm. in diameter, in both lungs; no caseation. Liver: fibrosis, but no tubercles. Spleen: enlarged; no gross tubercles. Kidneys: small number of pin-point tubercles. Bone marrow: no tubercles.

Rabbit 19-23, killed Nov. 23, 1928, *i.e.*, 43 days after *reinfection*. Lungs: numerous discrete, subpleural tubercles, 3 to 4 mm. in diameter with ill defined caseous centers. Liver: no tubercles. Spleen: no tubercles. Kidneys: small number of ill-defined cortical tubercles. Bone marrow: no tubercles. Fat depots remarkably well filled.

Rabbit 22-50, killed Nov. 21, 1928, *i.e.*, 41 days after *first infection*. Lungs: massive conglomerate miliary tubercles, 3 to 4 mm. in diameter, with marked caseation in centers. Liver: punctate tubercles. Spleen: extensive miliary tuberculosis. Kidney: moderate number of cortical tubercles with central punctate caseation. Bone marrow: moderate number of tubercles.

Rabbit 22-49, killed Dec. 12, 1928, *i.e.*, 62 days after *first infection*. Lungs: massive, conglomerate, miliary tubercles with conspicuous caseous centers; almost complete solidification. Liver: 1 or 2 questionable pin-point foci. Spleen: riddled with tubercles about 2 mm. in diameter. Kidneys: extensive cortical tubercles, 3 to 4 mm. in diameter, raised as hemispheres above the surface; tubercles have conspicuous caseous centers. Bone marrow: several discrete tubercles about 3 mm. in diameter. Animal well preserved.

Group 3.—Rabbits 16-13, 16-14, 16-20 and 16-22 injected intravenously on Oct. 17, 1927, with 0.001 mg. per kilo body weight of a living virulent human tubercle bacillus, Strain P-48A. Rabbits 16-13 and 16-14 and normal Rabbits 20-93 and 20-94 were injected intravenously on Apr. 18, 1928, with 0.01 mg. of living P-48A. Rabbit 16-22 and normal Rabbits 20-95 and 20-97 were injected intravenously on Apr. 18, and Rabbit 16-20 on Apr. 20, 1928, with 0.01 mg. of a living virulent bovine tubercle bacillus, Bovine C. Injections on Apr. 18 and 20 indicated in Text-figs. 3 and 4 by arrows at top of chart.

The rabbits of Group 3, (Text-figs. 3 and 4) were followed for a longer time, 6 or 7 weeks, after injection. Unfortunately the titrations made before injections are not available for comparison and are not indicated in the figures; human tubercle bacilli (P-48A), heated to 56°C. for 75 minutes, were used as antigen in the first titrations, and proved not to be satisfactory detectors. In the titrations plotted living Bovine III was used as antigen as in the other groups.

The increased antibody content of the sera of the reinfection rabbits as compared with the first infection rabbits is strikingly manifest in the majority of the titrations 7 days after reinfection and persists throughout the experiment. Although the titrations were quite irregular in this group, the difference between reinfection and first infection animals was brought out both by the bacterial surface re-

actions and by phagocytosis. Surface changes and phagocytosis again seem to be essentially concordant within the rather large experimental error.

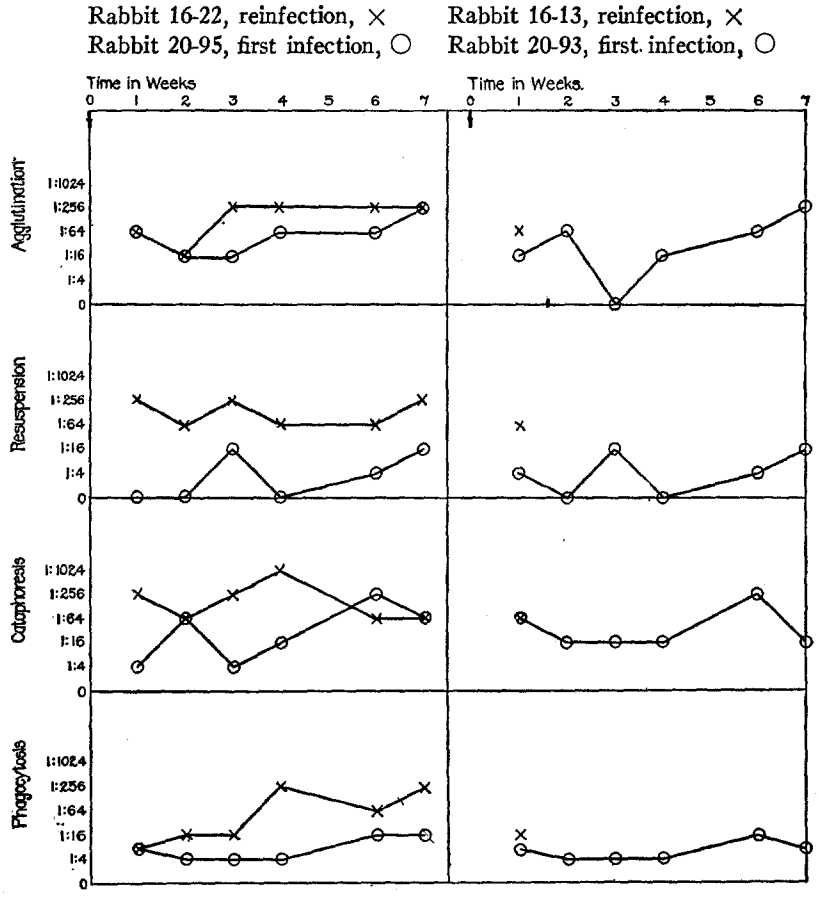

FIG. 3. Antibody production following a single injection (indicated by arrow at top of chart) of living, virulent tubercle bacilli. Two rabbits with residual foci of tuberculosis (crosses), and 2 previously healthy rabbits (circles).

The titers of the first infection animals seem to show a slight but nevertheless definite upward trend especially during the latter weeks of the experiment. Supporting data for this conclusion are afforded

by a comparison of the titrations of the sera of these first infection rabbits on the 6th and 7th week after infection with the titrations of 6 normal rabbits included in the same experiments. The results of

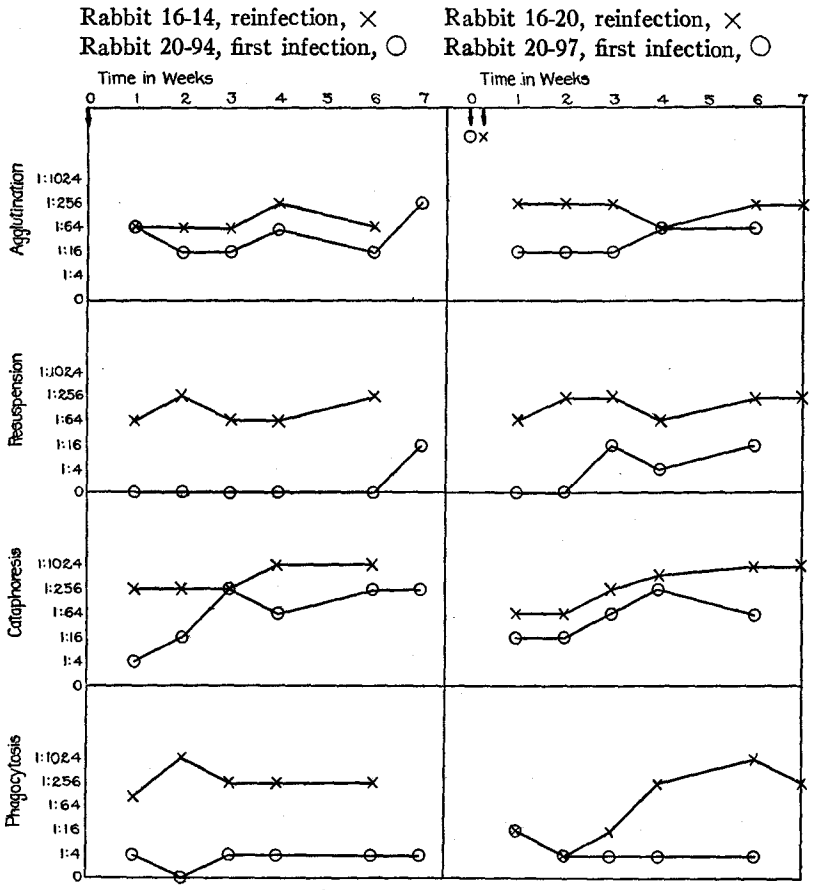

FIG. 4. Antibody production following a single injection of living, virulent tubercle bacilli. Two rabbits with residual foci of tuberculosis (crosses), and 2 previously healthy rabbits. Rabbit 16-20 injected 2 days later than the others (as indicated by arrow at top of chart).

these experiments are given in detail in Tables I and II. A definite phagocytosis-promoting power is present in the highest concentrations of serum from the first infection animals which is absent in the nor-

TABLE I.

Course of Tuberculous Infection (6th Week).

Details of experiment showing difference of response to virulent tubercle bacilli with respect to antibody formation. Three groups of rabbits are used: with residual foci of tuberculosis (reinfection), with primary infection (first infection), and normal animals, serving as controls.

	Serum dilutions					Sodium chloride control
	1:4	1:16	1:64	1:256	1:1024	
Reinfection Rabbit 16-14.						
Agglutination	++ to +	++ to +	++ to +	tr.	0	0
Resuspension	+++	++	++ to +	+	0	0
Cataphoresis	0.56	0.95	1.18	1.37	1.47	1.69
Phagocytosis	39	18	16	10	7	4
Reinfection Rabbit 16-20.						
Agglutination	++ to +	++ to +	++ to +	+	0	0
Resuspension	+++	++	+	+	0	−
Cataphoresis	0.82	0.97	1.22	1.08	1.46	1.65
Phagocytosis	27	16	19	13	10	4
Reinfection Rabbit 16-22.						
Agglutination	++ to +	++ to +	++ to +	+	sl. tr.	
Resuspension	++	++ to +	+	0	0	
Cataphoresis	0.78	0.93	1.23	1.68	1.67	1.54
Phagocytosis	26	18	14	7	7	
First Infection Rabbit 20-93.						
Agglutination	++ to +	+	+	sl. tr.		
Resuspension	+	sl. tr.	0	0		
Cataphoresis	1.19	1.21	1.33	1.46		1.74
Phagocytosis	14	10	4	3		
First Infection Rabbit 20-94.						
Agglutination	++ to +	+	tr.	sl. tr.		
Resuspension	tr.	0	0	0		
Cataphoresis	1.20	1.33	1.32	1.43		1.55
Phagocytosis	12	5	3	2		

TABLE I—*Continued.*

	Serum dilutions				Sodium chloride control
	1:4	1:16	1:64	1:256	
First Infection Rabbit 20-95.					
Agglutination	++ to +	++ to +	+	sl. tr.	
Resuspension	+	0	0	0	
Cataphoresis	1.20	1.38	1.36	1.47	1.71
Phagocytosis	12	10	3	1	
First Infection Rabbit 20-97.					
Agglutination	++ to +	++ to +	+ to tr.	sl. tr.	
Resuspension	++ to +	+ to tr.	0	0	
Cataphoresis	1.31	1.34	1.40	1.63	
Phagocytosis	10	5	1	3	
Normal Rabbit 21-28.					
Agglutination	+	+	+	sl. tr.	
Resuspension	0	0	0	0	
Cataphoresis	1.11	1.42	1.42	1.32	
Phagocytosis	2	4	1	3	
Normal Rabbit 21-56.					
Agglutination	++ to +	++ to +	tr.	0	
Resuspension	+	tr.	0	0	
Cataphoresis	1.15	1.35	1.43	1.54	
Phagocytosis	4	3	3	1	

mal sera. A very slight and indefinite difference in the same direction is suggested in the titers of the other tests. The loss of phagocytosis-promoting power of heated normal rabbit serum, which, however, is still able to alter the bacterial surfaces, has been discussed in the second paper of this series (8).

The sera of Rabbits 16-20, 16-22, 20-93, 20-94 and 20-95 were tested on June 6, in serial dilution for specificity against typhoid bacilli. They did not agglutinate the typhoid bacilli, which were shown to be agglutinated by anti-typhoid serum.

The autopsies in this group are in essential accord with those in Groups 1 and 2 and need not be given in detail. The reinfection

TABLE II.

Course of Tuberculous Infection (7th Week). See Legend of Table I.

The experiment here shown gives details of results obtained 1 week after the experiment presented in Table I.

	Serum dilutions						Sodium chloride control
	1:4	1:16	1:64	1:256	1:1024	1:4096	
Reinfection Rabbit 16-20.							
Agglutination	++ to +	++ to +	++ to +	+	0	0	0
Resuspension	+++	+++	++	++ to +	0	0	0
Cataphoresis	0.88	1.07	1.38	1.28	1.34	1.61	1.62
Phagocytosis	37	17	14	13	6	2	1
Reinfection Rabbit 16-22.							
Agglutination	++ to +	++ to +	++ to +	+	0	0	0
Resuspension	+++	+++	++	++ to +	sl. tr.	sl. tr.	0
Cataphoresis	0.67	0.90	1.28	1.50	1.52	1.59	1.35
Phagocytosis	37	21	19	10	5	1	2
First Infection Rabbit 20-93.							
Agglutination	+	++ to +	++ to +	+ to tr.	0		
Resuspension	+++	++ to +	sl. tr.	sl. tr.	sl. tr.		
Cataphoresis	1.32	1.27	1.58	1.51	1.42		1.57
Phagocytosis	15	7	4	6	3		
First Infection Rabbit 20-94.							
Agglutination	++ to +	++ to +	++ to +	+ to tr.	tr.		
Resuspension	++	+	sl. tr.	tr.			
Cataphoresis	1.26	1.28	1.36	1.36	1.41		1.34
Phagocytosis	10	4	2	1	3		
First Infection Rabbit 20-95.							
Agglutination	++	++ to +	+	+ to tr.	tr.		
Resuspension	++ to +	+	sl. tr.	sl. tr.	sl. tr.		
Cataphoresis	1.09	1.44	1.28	1.53	1.31		1.80
Phagocytosis	13	10	5	2	1		

TABLE II—*Continued.*

	Serum dilutions			
	1:4	1:16	1:64	1:256
Normal Rabbit 21-69.				
Agglutination	+	+	+	0
Resuspension	++ to +	+	sl. tr.	sl. tr.
Cataphoresis	0.91	1.27	1.35	1.39
Phagocytosis	6	2	5	
Normal Rabbit 21-73.				
Agglutination	+	++ to +	+	+ to tr.
Resuspension	+ to tr.	+ to tr.	sl. tr.	sl. tr.
Cataphoresis	1.32	1.30	1.36	1.42
Phagocytosis	4	2	4	
Normal Rabbit 21-75.				
Agglutination	++	++ to +	tr.	sl. tr.
Resuspension	++ to +	+	tr.	sl. tr.
Cataphoresis	1.09	1.23	1.39	1.38
Phagocytosis	2	3	0	
Normal Rabbit 21-77.				
Agglutination	+	+	+	+ to tr.
Resuspension	+	+ to tr.	sl. tr.	sl. tr.
Cataphoresis	1.05	1.28	1.36	1.53
Phagocytosis	5	3		

animals showed some residual tuberculosis in the lungs and kidneys, but no evidence of lesions due to the second infection. In the primarily infected rabbits, the infection ran its typical course.

DISCUSSION.

An augmented capacity of animals to produce antibodies against antigens to which they had previously been immunized was demonstrated by Cole in 1904.

Cole (9) immunized rabbits by intravenous injection of living typhoid bacilli and kept them until the resulting serum agglutinin titers had fallen to low values. Such rabbits on reinoculation with typhoid bacilli promptly produced antibodies in

considerable quantity in response to dosages which produced only negligible effects in normal rabbits. V. Liebermann and Acél (10) later demonstrated an augmented response in agglutinins and bactericidal antibodies in guinea pigs reinjected with typhoid bacilli. Dean and Webb (11) have reported the production of high titer antihorse sera in rabbits by two or more courses of injections at 2 or 3 months intervals. A single course of 8 or 10 or more injections gave sera of inferior titers. Schneider (12) revaccinated (with vaccinia virus) rabbits whose antivirus titers from the first vaccination had fallen to low values. Although revaccination led only to negligible visible symptoms, prompt and considerable increase in antivirus titers was demonstrated. Opie and Freund (13) found that the production of precipitin against crystalline egg albumin was accelerated and augmented in rabbits which had previously been immunized with crystalline egg albumin and had no precipitins in their blood at the time of reimmunization. It has also been found that antibody titers in previously vaccinated or infected men or animals may rise in response to heterologous antigens (14, 15, 16, 17), a phenomenon termed by Conradi and Bieling the "anamnestische Reaktion."

The most detailed account of these phenomena now available seems to be that of Bieling (18), who studied the production of antibodies in rabbits in response to injections of typhoid and of dysentery bacilli. Antibodies corresponding to the antigen first injected reappeared on later injection of either homologous or heterologous antigen; heterologous antibodies reappeared at once, homologous antibodies after a latent period.

One feature of the reactions described by Bieling suggests a relationship of these phenomena to that described by Lewis and Loomis (see below). According to Bieling (18) rabbits previously immunized with dysentery bacilli formed typhoid agglutinins in response to small injections of typhoid bacilli which were without effect in normal animals. At the same time the dysentery agglutinins again increase. The first specific immunization leaves behind therefore an increased capacity to respond to later injections which is non-specific.

It has been established, then, that an animal which has once produced antibodies in response to a specific antigen may reproduce these antibodies more quickly or in greater amount or both in response to a later injection of the homologous antigen, and may even produce such antibodies in response to injection of a heterologous antigen.[2] It seems clear that this phenomenon is at least a factor in the augmented

[2] In the present study the preliminary and reinfecting antigens used were essentially homologous. For although tubercle bacilli of human type were necessarily used for the preliminary infection, whereas reinfection was in most instances with bovine bacilli, human and bovine tubercle bacilli have repeatedly been found to be serologically indistinguishable. See (2) in which earlier references are cited.

production of antibodies against the tubercle bacillus which we have demonstrated in animals with residual tuberculous foci.

A phenomenon which may in part rest upon a common basis with the "anamnestic reaction" and which doubtless contributed to the augmented antibody production in our reinfection rabbits has been described by Lewis and Loomis (19). These authors found in tuberculous animals a greatly increased capacity for antibody production in response to a cellular and also a bacillary antigen. Other conditions than tuberculosis also provoked to some degree this heightened "allergic irritability."

In the present experiments there has been found a slight and slowly developing production of antibodies in first infection rabbits which showed (20) a moderate and slowly developing resistance to tubercle bacilli, and a prompt production of antibodies in quantity in reinfection animals found (20) to be highly resistant to the reinfecting tubercle bacilli.

The relation of antibodies to resistance in tuberculosis has recently been discussed by Opie (21). The present experiments suggest that an increased capacity to produce antibodies may be a factor in the heightened resistance of previously infected tuberculous animals. However, definite conclusions cannot be drawn at the present time.

The chief purpose for which these experiments were conducted was the testing under still another set of conditions of the relation between the changes brought about by sera in the surface properties and the phagocytosis of bacteria. The experimental error in these titrations was somewhat larger in proportion to the titers themselves than in the foregoing papers, but was not so large as to invalidate the interpretation of the results, even in the case of first infection animals. Within this error, complete correlation was again found between the effects of the sera in modifying the bacterial surface properties and in promoting phagocytosis, except in the case of heated normal sera; this exception had already been discussed. In experimental tuberculosis, as in the other conditions studied, whenever a serum has promoted phagocytosis it has produced in roughly corresponding degree the characteristic alterations in the bacterial surfaces,—and, with the exceptions noted,—whenever the surface changes have been produced phagocytosis has been promoted.

We believe that we have shown, then, that serum sensitization of acid-fast bacteria consists in or involves the combination with and deposition on the bacterial surface of a component or components which increase the cohesion, reduce the surface charge, alter the wetting properties and promote the phagocytosis of the bacteria. Further analysis of the process is in progress.

Throughout this work we have had able technical assistance from Mr. H. J. Henderson.

SUMMARY.

Rabbits infected intravenously with virulent mammalian tubercle bacilli have in a majority of cases developed circulating antibodies to a slight but appreciable degree. The increase in titer was detected in one group of rabbits within the 2nd or 3rd week, in others during the 2nd month of infection.

Rabbits with residual pulmonary foci, resulting from infection 6 months or more previously with human tubercle bacilli, on reinfection with bovine tubercle bacilli promptly developed circulating antibodies strikingly in excess of those found during the course of primary infection. Such antibodies were present 6 or 7 days after reinfection.

The changes in titer during tuberculous infection as detected by the bacterial surface reactions and by phagocytosis were again, within the experimental error, in quantitative correspondence. The loss of phagocytosis-promoting power in heated normal serum involves an exception to this correspondence between surface and phagocytosis effects. This exception has already been discussed in an earlier paper (8).

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