INTRAPERITONEAL LYSIS OF TUBERCLE BACILLI.*

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Koch[1] observed, in 1891, that a tuberculous guinea pig is more resistant than a normal guinea pig toward inoculation with tubercle bacilli. This observation was not confirmed by Koch's contemporary workers.[2] It was not until seventeen years later, after investigators had learned to avoid conflicting results from anaphylactic phenomena, and mainly as a result of the careful quantitative work of Römer[3] and Hamburger[4] that the observation was finally established. A similar heightened resistance toward reinoculation was demonstrated by Arloing,[5] von Behring,[6] Calmette and Guerin,[7] and others for tuberculous cattle; by Kraus, Gross, and Volk[8] for tuberculous monkeys; and by Römer and Joseph[9] for tuberculous sheep.

Numerous attempts have been made to determine the mechanism of this heightened tubercular resistance. Thus, Römer and Joseph[10] studied the fate of tubercle bacilli injected subcutaneously in tuberculous guinea pigs and found that the bacilli were not destroyed at the site of the reinoculation, but were held in the tissues in a living

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1 Koch, R., *Deutsch. med. Wchnschr.*, 1891, xvii, 101.

2 For a summary of the negative work, see Hamburger, F., *Beitr. z. Klin. d. Tuberk.*, 1909, xii, 261.

3 Römer, P. H., *Ztschr. f. Infektionskrankh. d. Haustiere,* 1909, vi, 393.

4 Hamburger, F., *loc. cit.*

5 Arloing, S., *Compt. rend. Acad. d. sc.,* 1909, cxlix, 962.

6 von Behring, E., *Therap. d. Gegenw.*, 1907, xlviii, 145.

7 Calmette, A., and Guerin, C., *Compt. rend. Acad. d. sc.,* 1910, cli, 32.

8 Kraus, R., and Gross, S., *Centralbl. f. Bakteriol., Orig.,* 1908, xlvii, 298. Kraus, R., and Volk, R., *Wien. klin. Wchnschr.,* 1910, xxiii, 699.

9 Römer, P. H., and Joseph, K., *Beitr. z. Klin. d. Tuberk.,* 1910, xvii, 287.

10 Römer, P. H., and Joseph, K., *ibid.,* 365.

and virulent state, without the production of a tuberculous lesion. They even thought that an actual multiplication of the bacilli took place. The heightened resistance apparently depended upon a power of the tissues to inhibit the pathogenic properties of the bacilli rather than upon a power of destroying them. A rapid lytic process, such as is observed in the immunity of certain other infectious processes, was not demonstrated.

Contrary to this observation Deycke and Much,[11] Much and Leschke,[12] and Kraus and Hofer[13] have recently obtained evidence of lysis by injecting tubercle bacilli into the peritoneal cavities of tuberculous and artificially immunized guinea pigs. They noted a rapid disappearance of the bacilli from the peritoneal fluids, and the appearance in the fluids of atypical and non-staining forms and of granules resembling bacterial fragments.

A rapid decrease in the number of tubercle bacilli in the peritoneal fluids, however, is not necessarily evidence of a destruction of the bacilli, since the decrease might conceivably be brought about by such factors as absorption by the peritoneal lymphatics, or adhesion to the peritoneal surfaces. Non-staining bacilli and granules resembling bacterial fragments are also not necessarily evidence of lysis, since Markl[14] has shown that non-staining forms are often produced in the peritoneal cavities of normal guinea pigs, and Van Giesen[15] has pointed out that granules resembling bacterial fragments can result from tissue disintegration. The evidence cited in support of the belief that lysis takes place is, therefore, not adequate to establish the conclusion beyond doubt. Hence we have been led to review somewhat carefully the experimental evidence in support of this view.

INTRODUCTORY EXPERIMENTS.

In the preliminary experiments eight strains of tubercle bacilli were tested on normal and tuberculous animals. Most of the

[11] Deycke, G., and Much, H., *Beitr. z. Klin. d. Tuberk.*, 1910, xv, 277.

[12] Much, H., and Leschke, E., *Beitr. z. Klin. d. Tuberk.*, 1911, xx, 405.

[13] Kraus, R., and Hofer, G., *Deutsch. med. Wchnschr.*, 1912, xxxviii, 1227; *Wien. klin. Wchnschr.*, 1912, xxv, 1112.

[14] Markl, G., *Centralbl. f. Bakteriol., Orig.*, 1905, xxxviii, 69.

[15] Van Giesen, I., *Med. Rec.*, 1910, lxxviii, 52.

strains were found to undergo marked degeneration when injected into the peritoneal cavities of normal guinea pigs. The bovine strains gave especially rapid involution forms, as early as the end of the first hour after the injection a large number of faintly staining and non-staining bacilli being seen, as well as club-shaped and spindle-shaped organisms and bacilli containing one or more blue staining granules. We finally selected two cultures that gave comparatively few degenerations in normal guinea pigs. These were:

H_{24}, a virulent strain of the human type, originally obtained from Dr. Theobald Smith, and

H_{39}, a slightly more virulent human strain, originally obtained from Dr. E. L. Trudeau.

They were used in the inoculations and tests reported in this paper.

Guinea Pigs.—Half grown guinea pigs were inoculated subcutaneously. By making the inoculations in the axilla, it was found possible to render the guinea pigs tuberculous, without producing tubercular changes in the peritoneal surfaces. A few metastatic nodules usually formed in the lungs and occasionally in the spleen. The liver was usually not involved and the omentum and other peritoneal surfaces usually remained normal. The immunity test was made five or six weeks later by intraperitoneal reinoculation.

In making the intraperitoneal test four cubic centimeters of a carefully filtered or sedimented suspension, containing approximately 80,000,000 tubercle bacilli per cubic centimeter, were injected and a sample of the peritoneal fluid was withdrawn at intervals by means of capillary pipettes, and stained by the Ziehl-Neelsen and Much methods.

The changes observed in the morphology and staining reaction of the withdrawn bacilli were less striking in our hands than those described by Kraus and Hofer. There was usually a more rapid appearance of beaded and club-shaped forms in the tuberculous animal than in the control, as well as bacilli nearly or completely devoid of acid-fast properties and bacilli containing one or more large, blue staining granules. Free granules ("Kügelchen") staining like the intrabacillary granules, on which Kraus and Hofer laid greatest stress, were, however, rarely seen.

The bacilli taken up by the leucocytes were less altered than the extracellular bacilli. Apparently the process of phagocytosis, instead of accelerating the degenerative changes, actually protected the bacilli. In this we incline to agree with Deycke and Much,[16] who look upon phagocytosis as shielding the tubercle bacilli from the curative processes of the body.

We observed with Much and Leschke the most striking difference between the tuberculous and normal guinea pig to be the rapid decrease in the number of bacilli in the peritoneal fluids of the tuberculous animal. In the normal guinea pig the number of bacilli remains large for several days. In the tuberculous guinea pig more than nine tenths of the bacilli disappear within an hour, and all but an occasional bacillus within three hours.

Differences were also noted between the resistance of the normal and tuberculous guinea pigs toward the intraperitoneal reinoculation. The normal guinea pigs all died in from three to four weeks from a fulminating type of visceral tuberculosis, while most of the tuberculous guinea pigs lived from six to twelve weeks. A few of the latter, however, showed immediate toxic symptoms, from which some died within from twenty-four to forty-eight hours in a manner suggestive of the anaphylactic condition described by Römer[17] and Hamburger.[18]

White Rats.—Subcutaneous inoculation of white rats with virulent tubercle bacilli usually produces a slight local induration lasting but a few days. There is generally no subsequent enlargement of the neighboring lymph glands and no formation of secondary lesions in the internal organs.

Tubercle bacilli injected into the peritoneal cavities of normal rats undergo rapid degeneration. Granular, faintly staining and non-staining forms soon appear, both in the extracellular fluids and in the phagocytes. The number of bacilli rapidly decreases, though numerous acid-fast organisms can be found several days after the injections.

In rats previously inoculated the same changes take place, only they are more rapid and more pronounced. Within three hours

[16] Deycke, G., and Much, H., *München. med. Wchnschr.*, 1909, lvi, 1985.
[17] Römer, P. H., *loc. cit.*
[18] Hamburger, F., *loc. cit.*

after the injection the number of bacilli is distinctly less than in the controls, and the bacilli usually completely disappear by the end of twenty-four hours. Inoculated rats, therefore, show the same general phenomenon as tuberculous guinea pigs, though the contrast between the normal and inoculated rats is less striking than that between normal and tuberculous guinea pigs.

Rabbits.—Subcutaneous inoculation of rabbits with virulent human strains produces lesions that are generally strictly local. With the bovine strains the neighboring lymph glands are usually involved and a few secondary tubercles usually form in the lungs.

The reaction of normal rabbits toward intraperitoneal inoculation resembles that of white rats. Phagocytosis is slow, and there is the rapid appearance of degeneration forms. Numerous acid-fast bacilli, however, can be demonstrated several days after the injection.

Tuberculous rabbits vary greatly in their reactions. Phagocytosis is usually slower than in normal rabbits, though in one series it was apparently more rapid. The bacilli slowly decrease in number, and usually disappear within twenty-four hours after the injection. In one of the animals the decrease was exceptionally rapid, a noticeable diminution being observed by the end of one hour. Tuberculous rabbits, therefore, exhibit the same general phenomenon as tuberculous guinea pigs, though here also the contrast between the normal and tuberculous animals is less striking.

Dogs.—Subcutaneous inoculation of dogs with human strains of tubercle bacilli usually produces a slight local induration, disappearing within a few days and giving rise to no glandular or visceral involvement. Bovine strains usually produce a permanent local induration occasionally leading to ulceration. The neighboring lymph glands are usually slightly enlarged, but no visceral lesions result within the first few weeks.

Normal dogs injected intraperitoneally with large doses of the bacilli show a rapid phagocytosis, but almost no immediate change in the morphology and staining reaction of the injected organisms. By the end of four hours phagocytosis is complete, the intracellular bacilli being acid-fast. This condition persists for three or four days, after which the number of leucocytes and bacteria gradually

decreases. Both leucocytes and bacteria usually disappear from the peritoneal fluid by the end of the seventh day.

In the peritoneal cavities of tuberculous dogs phagocytosis is less rapid. A rapid decrease in the number of extracellular bacilli, however, takes place. Within six hours a marked diminution in the number is noticed, and by the end of twenty-four hours the extracellular forms have completely disappeared. By the end of forty-eight hours, the intracellular forms also have disappeared, only an occasional leucocyte being seen containing blue staining granules.

The contrast between the normal and tuberculous dogs is, therefore, similar to that between normal and tuberculous rabbits, being less marked than in guinea pigs.

Monkeys.—In one tuberculous monkey tested by the intraperitoneal method a distinct decrease in the number of tubercle bacilli occurred in the sample withdrawn at the end of one hour and a complete disappearance of the bacilli in the sample withdrawn at the end of two hours. The control monkey showed numerous acid-fast bacilli for several days after the injection. If this experiment is typical, the contrast between normal and tuberculous monkeys is similar to that between normal and tuberculous guinea pigs.

PHENOMENON ANALYZED.

The above experiments show that the phenomenon hitherto interpreted as intraperitoneal lysis can be reproduced in all laboratory animals thus far tried. The phenomenon, however, is more sharply defined in guinea pigs than in other laboratory animals, with the possible exception of the monkey. Guinea pigs were therefore selected for the analysis of the reaction.

The number of bacteria present in an aspirated sample of peritoneal fluid depends upon several factors, among which are the total volume of the peritoneal fluid, the amount of bacterial absorption by the peritoneal lymphatics, and the amount of bacterial adhesion to the peritoneal surfaces.

Volume.—When suspensions of tubercle bacilli are injected into the peritoneal cavity of normal guinea pigs, a rapid absorption of the injected fluid takes place (AN, figure 1). One half of the fluid

disappears by the end of three hours, and the peritoneal cavity becomes practically dry by the end of ten hours.

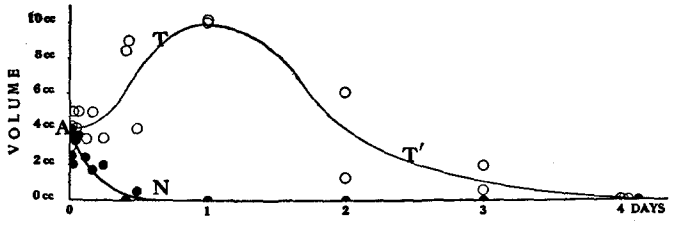

FIG. 1. VOLUME OF PERITONEAL FLUID. Uniform amounts (4 c.c.) of a suspension of tubercle bacilli were injected into the peritoneal cavities of a series of guinea pigs. The volume of the peritoneal fluid was afterwards determined at autopsy. AN = approximate changes in the volume of the peritoneal fluid in normal guinea pigs; ATT′ = approximate changes in tuberculous guinea pigs.

In tuberculous guinea pigs, in contrast, there occurs at first an increase in the volume of the peritoneal fluid (AT). The volume may be doubled during the first ten hours, and the increase usually continues during the first twenty-four hours. Later the volume gradually decreases (TT′), and the peritoneum usually becomes dry by the end of the fourth day.

A rapid decrease in the number of tubercle bacilli would therefore be expected in samples of peritoneal fluid withdrawn from tuberculous guinea pigs, due solely to this initial increase in the volume of the peritoneal fluid, while an increase in the number of bacilli would be expected in normal guinea pigs due to the volumetric decrease.

Leucocytes.—If four cubic centimeters of Ringer solution are injected into the peritoneal cavity of a guinea pig and a sample of the peritoneal fluid is immediately withdrawn, this sample will usually be found to contain approximately 4,500 leucocytes per cubic millimeter. Or, calculating the total number of leucocytes free in the peritoneal cavity, allowing one cubic centimeter as the original volume of the peritoneal fluid before the introduction of the Ringer solution, we obtain about 22,000,000 as the total number of leucocytes present. Differential counts show approximately 10 per cent. polymorphonuclear leucocytes, 50 per cent. large mononuclear cells, and 40 per cent. lymphocytes.

Immediately after the introduction of the suspension of tubercle bacilli there usually takes place a rapid decrease in the number of

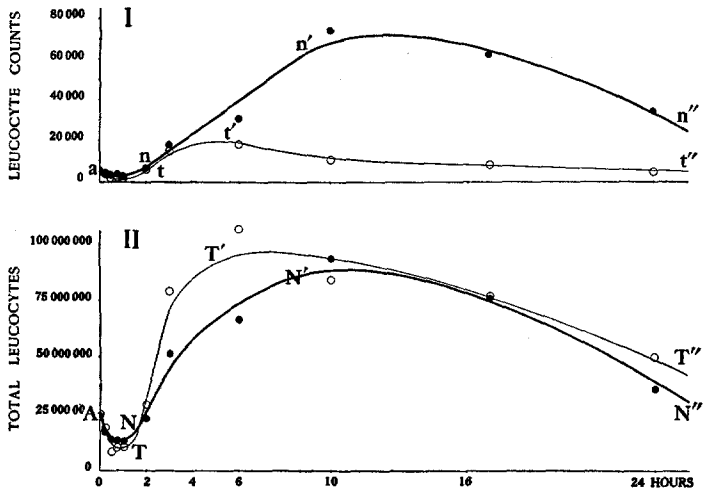

FIG. 2. PERITONEAL LEUCOCYTES. I = changes in the leucocytic count per cubic millimeter of peritoneal fluid, following the introduction of tubercle bacilli. ann' n'' = normal guinea pig; att' t'' = tuberculous guinea pig.

II = calculated changes in the total number of leucocytes free in the peritoneal fluid of the above animals. ANN' N'' = normal guinea pig; ATT' T'' = tuberculous guinea pig.

these leucocytes (I, figure 2). In normal guinea pigs (an, figure 2) the average leucocyte count falls within fifteen minutes to about 2,500 per cubic millimeter, and reaches 2,000 per cubic millimeter by the end of one hour. In tuberculous guinea pigs an even more rapid fall takes place (at, figure 2), the average count being reduced to 1,500 per cubic millimeter within thirty minutes. In particular instances counts as low as 150 per cubic millimeter have been observed.

This initial fall is due to an agglutination of the leucocytes and an adhesion of the resulting leucocytic masses to the omentum and adjacent gastrohepatic membranes. Agglutinated masses are occasionally seen in samples of the peritoneal fluid withdrawn at the end of fifteen minutes. At autopsy the omentum is found studded with these masses, many of which are sufficiently large to be visible

to the naked eye. The leucocytes thus mechanically removed from the peritoneal fluids are mainly the large mononuclear and polymorphonuclear cells (figure 3).

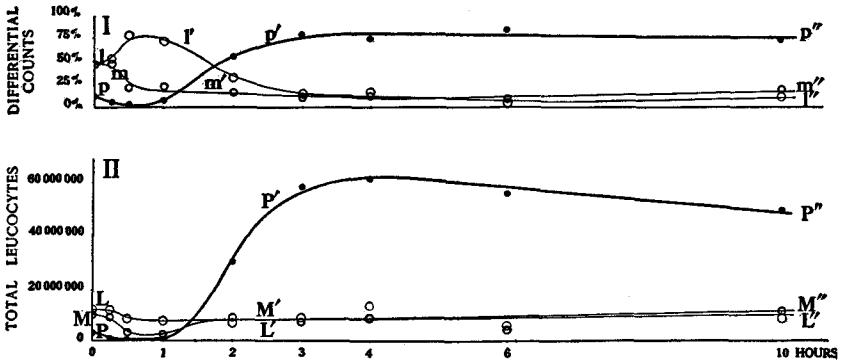

FIG. 3. TYPE OF LEUCOCYTES. I = changes in the differential leucocytic counts; composite picture obtained by taking the average count from four normal and four tuberculous guinea pigs. pp' p'' = polymorphonuclear leucocytes; mm' m'' = large mononuclear cells; ll' l'' = lymphocytes.

II = calculated changes in the total number of free peritoneal leucocytes of each type. PP' P'' = polymorphonuclear leucocytes; MM' M'' = large mononuclear cells; LL' L'' = lymphocytes.

Following this initial drop, there is a secondary rise in the leucocytic count (I, figure 2), the original number as a rule being restored within two hours, and the number increasing to three or four times the original number by the end of the third hour. In tuberculous guinea pigs this increase tends to reach its maximum by the end of the sixth hour (tt', figure 2). In normal guinea pigs the increase continues for at least ten hours (nn', figure 2), when the count may be from ten to fifteen times the original number.

This secondary increase is not due to a breaking up of the leucocytic masses adherent to the omentum. In normal guinea pigs a part of the increase is due to a diminution in the volume of the peritoneal fluid; but that an actual increase in the leucocytes occurs in both normal and tuberculous animals is brought out by calculating the changes in the total number of leucocytes free in the peritoneal fluids (II, figure 2). The leucocytes thus added to the perito-

neal fluid are for the most part polymorphonuclear cells (figure 3).

Tubercle Bacilli.—To determine the changes in the number of tubercle bacilli, smears were stained by the Ziehl-Neelsen and Much methods, and the number of bacteria per hundred leucocytes were counted. From this number the number of bacteria per cubic millimeter, and the total number free in the peritoneal cavity, were calculated. The changes in the total bacterial content, in a typical case, are shown in figure 4.

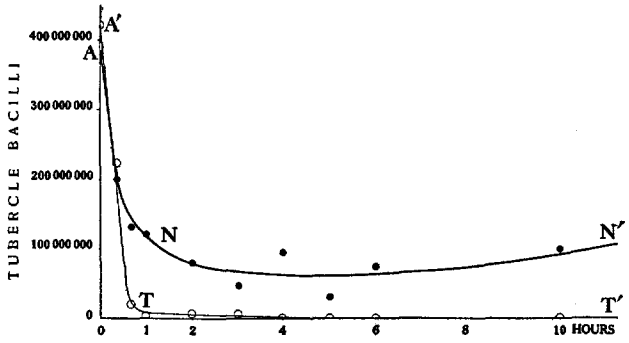

FIG. 4. CHANGES IN THE TUBERCLE BACILLI. Changes in the total number of tubercle bacilli free in the peritoneal fluid. ANN′ = normal guinea pig; A′ TT′ = tuberculous guinea pig.

In this experiment approximately 400,000,000 tubercle bacilli were injected into the peritoneal cavity of each animal. In the normal guinea pig over 50 per cent. of the bacteria disappeared from the peritoneal fluid within twenty minutes, and 75 per cent. within the first hour. The number remained approximately 100,000,000 in this animal from that time till the end of the experiment.

In the tuberculous guinea pig the same initial decrease took place, the number falling 50 per cent. during the first twenty minutes. Here, however, the rapid decrease continued, the number being reduced to about 1 per cent. by the end of the first hour, and bacilli completely disappearing by the end of the fourth hour. In most of the experiments, however, the contrast between the normal and tuberculous guinea pigs was not as great as this. Numerous bacilli were occasionally demonstrable in the peritoneal fluids of the tuber-

culous animals, five or six hours after the injection, particularly in cases in which the abdomen had been thoroughly massaged immediately before withdrawing the peritoneal sample.

Distribution.—An examination of the abdominal cavity of a normal guinea pig, made two hours after the injection, shows numerous tubercle bacilli in the scrapings from all the peritoneal surfaces. The peritoneal surfaces of a tuberculous guinea pig, in contrast, at this time usually show few tubercle bacilli except upon the omentum. On the omentum numerous bacilli are found, adherent for the most part to the leucocytic masses previously described.

Later examinations, as, for example, at the end of twenty-four hours (table I), give even more marked contrasts. In the normal guinea pig numerous bacilli are demonstrable on all the peritoneal surfaces, both by the Ziehl-Neelsen and the Much methods. In the tuberculous guinea pig the peritoneal surfaces, with the exception of the diaphragm, the omentum, and the gastrohepatic membranes, are commonly free from tubercle bacilli, as determined by the Ziehl-Neelsen method. Numerous granules, however, can be demonstrated on the various surfaces by the Much method. After forty-eight hours, the contrast is still more striking, since by this time most of the Much granules have disappeared.

TABLE I.

Peritoneal Distribution.

Smears made from the peritoneal surfaces of normal and tuberculous guinea pigs, twenty-four and forty-eight hours after the injection of tubercle bacilli. + = bacilli, . = granules, — = no examination.

Surface.	24 hours.				48 hours.			
	Tuberculous.		Normal.		Tuberculous.		Normal.	
	Ziehl.	Much.	Ziehl.	Much.	Ziehl.	Much.	Ziehl.	Much.
Peritoneal fluid.....	o	.	+	+.	o	o	++	+++
Parietal peritoneum	o	....	+++	++..	o	o	++++	++++
Intestine...	o	o	+++	++..	o	o	++	+++
Mesentery..	o	—	++	++..	o	o	++	+++
Stomach ...	o	—	+	−	o	o	++	+++
Liver.....	o	—	++++	+++...	o	o	++	+++
Diaphragm.	++	++..	+++	++..	+	+.	++	+++
Omentum ..	++	+++..	+++	+++..	++	++	++++	++++

Autopsies made at the end of four or five weeks usually confirm these findings. While all peritoneal surfaces of the normal guinea pig are usually closely studded with miliary tubercles, most of the surfaces of the tuberculous animal are usually free from macroscopic lesions, at most half a dozen isolated tubercles being visible. The omentum, however, is always severely affected.

Agglutination.—One of the factors that assists in confining the tuberculous process to the omentum is an adhesion of the injected bacilli to the leucocytes. The agglutinated leucocytes seen in the early samples of peritoneal fluid usually carry with them from ten to a hundred tubercle bacilli adherent to each leucocyte.

Absorption.—A second mechanical factor that conceivably assists in freeing the peritoneal cavity of tubercle bacilli is absorption by the peritoneal lymphatics. An attempt was made to estimate the importance of this factor.

Through the kindness of Dr. Dochez a strain of pneumococcus was secured, which, injected into the peritoneal cavity of a normal guinea pig, undergoes comparatively little change during the first three hours. When a mixture of tubercle bacilli and this pneumococcus was injected into the peritoneal cavity of a tuberculous guinea pig, the tubercle bacilli usually disappeared from the fluid within thirty minutes, while the number of pneumococci was not appreciably reduced. This effect cannot be due to a mechanical absorption of the peritoneal fluid, since, if due to such a cause, the reduction in the number of tubercle bacilli and pneumococci would be proportional. Peritoneal absorption, therefore, plays a small part in the immediate decrease in the number of tubercle bacilli.

Granules.—The existence of mechanical factors tending to remove the bacilli from the peritoneal fluid and to cause their accumulation on the omentum, renders the proof or disproof of lysis difficult. The only direct evidence of lysis we have thus far obtained was from smears made from tuberculous guinea pigs, about thirty minutes after the introduction of the bacilli. In one case numerous acid-fast granules were seen, unmistakable fragments of tubercle bacilli. Non-acid-fast granules (Much granules) are usually seen in both normal and tuberculous animals, and cannot be taken as evidence of specific lysis.

Quantitative Analyses.—A quantitative determination of the residual bacteria in the peritoneal cavity was attempted. To make this determination, the peritoneal surfaces were repeatedly scrubbed with citrate solution and afterwards with 5 per cent. antiformin. The washings were united and allowed to stand over night in 10 per cent. antiformin. In the morning the undissolved tubercle bacilli were thrown down by centrifugation, an equal volume of alcohol being added to favor sedimentation. The alcohol forms a fine albuminous precipitate, which tends to hold the sedimented bacteria together. The sediment was washed with 0.2 per cent. hydrochloric acid and thoroughly emulsified in a known volume of serum, the serum being added to cause an adhesion of the bacteria to the microscopic slide. In estimating the number of the bacteria, homologous leucocytes were added to the suspension and the number of these leucocytes per cubic millimeter was determined, together with the average number of tubercle bacilli per 100 leucocytes.

By this method it was found that the bacteria could be recovered practically quantitatively from the peritoneal cavities of normal guinea pigs from one and one half to two hours after the injection. In a few determinations an apparent increase in the number of bacilli was noted, the average recovery being 106 per cent. of the original number injected. In tuberculous guinea pigs, in contrast, the analysis uniformly showed a considerable decrease in the number of the bacilli, the average recovery being but 65 per cent. of the original number injected. A definite decrease in the number of tubercle bacilli, therefore, does take place in the peritoneal cavity of tuberculous guinea pigs, independent of the apparent decrease due to changes in peritoneal distribution.

Lysis in Vitro.—That this decrease is due to an actual lysis was shown by experiments with isolated peritoneal tissues. If the small intestine of a fasting normal guinea pig is tied off, transferred to a warmed test-tube containing a known number of tubercle bacilli, and the preparation incubated from three to ten hours, no diminution in the number of tubercle bacilli can be demonstrated by the antiformin method (table II). There is often even a slight apparent increase in the number of bacilli thus recovered. With the intestine of a tuberculous guinea pig, however, there is uniformly

obtained a considerable decrease in the number of the bacilli, the average recovery being only 55 per cent. of the original number placed in the test-tube. In one experiment only 14 per cent. of the original number was thus recovered.

TABLE II.

Lysis of Tubercle Bacilli in Vitro.

A uniform number of tubercle bacilli were exposed at 37° C. to the action of peritoneal tissues *in vitro* for the lengths of time specified. The residual bacteria were determined by the antiformin method (Ziehl-Neelsen stain). The culture used in the tests was a six weeks' glycerin agar growth of H_{24}.

Time.	Bacilli recovered.			
	Normal.		Tuberculous.	
3½ hrs.	122%		62%	
4 hrs.	97%	Average	98%	Average
4½ hrs.	91%	109%	30%	73%
5 hrs.	127%		93%	
9 hrs.	100%		44%	
10 hrs.	107%		60%	
10 hrs.	97%	100%	33%	38%
11 hrs.	97%		14%	
Average		105%		55%

The number of tubercle bacilli destroyed by these tissues *in vitro* apparently depends upon the age and virulence of the culture tested. With a fresh culture of virulent organisms fewer bacilli are destroyed than with an older culture of a less virulent strain.

MECHANISM OF THE LYSIS.

The above studies clearly indicate that an actual lysis of tubercle bacilli is possible in tuberculous animals. The question now arises as to the mechanism of this tuberculolysis.

Circulating Antibodies.—The first factor examined was the possibility that lysis was due to substances present in the circulating blood. Tests were therefore made with sera, peritoneal fluids, and leucocytes of tuberculous and normal guinea pigs. No distinct lytic effects could be produced *in vitro* with any of these substances, either when tested alone, or in combination. Involution forms of the tubercle bacillus were often seen after exposure to the fluids

and cells of tuberculous animals; but similar involutions were produced by the products of normal guinea pigs, and often appeared in control suspensions in Ringer solution.

Aside from a slight agglutinating action occasionally seen with the sera of tuberculous guinea pigs, the only evidence of circulating antibodies thus far obtained was in certain quantitative determinations with large amounts of freshly drawn defibrinated blood. If a large amount of normal blood is mixed with a small number of tubercle bacilli, and the mixture incubated for twelve hours, a slight apparent increase (10 to 20 per cent.) in the number of tubercle bacilli is observed by the antiformin method. When the blood of tuberculous guinea pigs is used, this increase is not noted, suggesting a possible inhibition of multiplication by the tuberculous blood.

Passive Immunization.—The second factor examined was the possibility that the body fluids contain tuberculolytic substances which require for their action the coöperation of the fixed cells of the body. To test this possibility sera and peritoneal fluids of tuberculous guinea pigs were introduced into the peritoneal cavities and circulatory system of normal guinea pigs, and the guinea pigs subsequently tested with intraperitoneal injections of tubercle bacilli. No lytic phenomena, however, were detected, though in one experiment a slight intraperitoneal agglutination was observed.

Transfusions.—On account of this negative result, the effect of transfusing large quantities of unaltered blood was tested. The initial transfusions were made with dogs and rabbits. In these animals it is easy to pass large quantities of blood from one animal to another, by using ordinary paraffined cannulæ and rubber tubing. By selecting animals of different sizes, or by using two or more tuberculous animals as the source of the blood, one can readily replace practically the entire volume of blood in the normal animal with that of tuberculous animals. The transfused animals were subsequently tested by intraperitoneal injections of tubercle bacilli, but in no case was lysis detected.

Transfusions were then made with guinea pigs, the guinea pigs being tested from twenty hours to three days after the operation. Here also in no instance was lysis observed. The subsequent histories of the transfused guinea pigs confirm this finding, the trans-

fused animals and the normal controls all dying, about four weeks later, from a fulminating type of visceral tuberculosis. The autopsies showed identical lesions in the two groups.

Fixed Cells.—It would appear from these experiments that the power of intraperitoneal lysis of tubercle bacilli does not depend primarily upon substances present in the circulating blood. Hence it would seem to follow that it depends largely or wholly upon certain properties of the local fixed peritoneal cells.

This conclusion agrees with the elaborate theory of partial antibodies recently put forth by Deycke and Much,[19] who state that they have obtained evidence, from complement deviation and anaphylactic studies, that tuberculous animals possess four distinct groups of partial antibodies, directed respectively against the neutral fats, the lipoids, the proteids, and the specific toxins of the tubercle bacillus. They also believe that some of these antibodies exist as circulating antibodies, while others are held in the fixed cells. Our experiments do not exclude the possibility that the fundamental lytic power of the fixed peritoneal cells is assisted or supplemented by circulating antibodies.

TUBERCULAR IMMUNITY.

The phenomenon of intraperitoneal lysis of tubercle bacilli is of importance because of the light it may eventually throw upon the nature of tubercular immunity. It would seem probable that the lytic property is not confined to the peritoneal cells alone and that it may also be acquired by other fixed cells.

Just how widely distributed throughout the body the cells are that acquire this power is a problem on which we are at present engaged. If the cells are found to be widely distributed it would suggest that tubercular immunity is essentially a specific adaptation of fixed local cells to destroy tubercle bacilli brought into immediate contact with them.

SUMMARY.

1. Tubercle bacilli injected into the peritoneal cavities of tuberculous guinea pigs, rats, rabbits, dogs, and monkeys, rapidly disappear from the peritoneal fluids, while persisting in the peritoneal fluids of normal control animals.

[19] Deycke, G., and Much, H., *München. med. Wchnschr.*, 1913, lx, 119, 190.

2. This disappearance is in part due to an adhesion of the injected bacilli to the peritoneal leucocytes and a fixation of the leucocytes on the omentum.

3. The injected tubercle bacilli can be recovered quantitatively from the peritoneal cavities of normal guinea pigs from one and one half to two hours after the injection, while from tuberculous guinea pigs only 65 per cent. of the bacilli can be recovered at this time.

4. Isolated peritoneal tissues from tuberculous guinea pigs have the power of destroying tubercle bacilli *in vitro.*

5. A second factor reducing the number of tubercle bacilli free in the peritoneal fluid is therefore an actual lysis of the bacilli.

6. The intraperitoneal lysis is not due solely to substances present in the circulating fluids, since the phenomenon cannot be produced by these fluids *in vitro,* and since a lytic power cannot be passively conferred even by a direct transfusion of blood from tuberculous to normal animals.

7. The intraperitoneal lysis is apparently due to specific changes in the fixed peritoneal cells of the tuberculous animals.