EXPERIMENTAL IMMUNITY WITH REFERENCE TO
THE BACILLUS OF LEPROSY.

PART I.

A STUDY OF THE FACTORS DETERMINING INFECTION IN
ANIMALS.*

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Plates 20 and 21.

INTRODUCTION.

The discovery of microorganisms and their identification as caustive agents in the production of specific diseases has led in recent years to the newer science of immunology. It may be stated that our present conception of the defensive and offensive mechanism of the host toward parasitic invasion is the direct outcome of studies upon the biochemical properties of bacteria and protozoa. Furthermore, the cultivation and study of the biological characteristics of microorganisms are of the greatest value in rendering possible the study of immune processes. Immunology has presented in particular three problems for elucidation: (1) the factors determining the infection of man and animals, and the development of changes in the tissues—the result of bacterial and protozoal action; (2) the method through which parasites exert their detrital influence, whether by toxin or simply by mechanical injury to the tissues; and (3) the factors determining the manner of production of protective and curative substances on the part of the infected animal. Naturally, in such a study we must take into consideration the possibility of protective properties on the part of the invading parasites; that is, their immunity against injurious substances already present or subsequently produced in the body of the animal.

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host. This applies especially to a disease like leprosy in which the specific excitor lives and multiplies in the animal body over an indefinite period, and which, in the light of our present knowledge of parasitism, can be explained only upon the ground that a mutual relation exists between host and invader. To determine in what way this reciprocal relation of parasite and host is established may ultimately lead to a means by which such a mechanism can be disarranged and, in consequence, the disease controlled.

In the extensive experiments and observations during the past twenty years, the greater amount of effort has been expended in the consideration of the manner of the production of immune bodies upon the part of the host, together with quantitative and qualitative methods for the determination of such bodies. Thus specific substances have been recognized, to which the names antitoxin, agglutinin, precipitin, amboceptor, opsonin, etc., have been given. The presence and importance of complement or alexin is also being appreciated; and a more adequate knowledge of the part played in the defensive and offensive powers of the host by the cellular elements has been acquired.

In the present communication, the results of animal experiments which throw light upon the type of the immune bodies induced on the part of the host by the inoculation of leprosy bacilli will not be considered extensively, as the purpose of this paper is to draw attention more especially to certain experiments which seem to indicate the factors determining leprosy infection. The experimental development of different types of host immune bodies and their significance will be discussed in a future publication.

Until quite recently, difficulty has been experienced by investigators in producing experimentally leprous lesions in animals. With the exception of the Japanese dancing mouse, most animals were assumed to possess a natural immunity. One of us (Duval) has been successful in producing, with comparative regularity, lesions and even generalized leprosy in the monkey (Macacus rhesus). Couret has likewise been able to produce in many cold-blooded animals multiplication of the leprosy bacillus with reactive lesions

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on the part of the host. The guinea pig, goat, and horse, as well as ordinary white mice, have been found by us to be subject to infection. Clegg also reports the production of lesions in the guinea pig and in the rabbit.

The number of animals, then, regarded as naturally immune is diminishing. It may, therefore, be asked with reason, why up to the present time it has been found impossible to infect animals which more recently have shown themselves susceptible to inoculation. An analysis of the following experiments offers, we think, an explanation, and at the same time helps to explain the phenomena noted in the development of the disease in human subjects; namely, the apparent immunity of most individuals and the fact that, as a rule, only those living in long and intimate contact with leprous patients have become infected.

Before proceeding to a detailed description of the experiments, it is, perhaps, proper to state the impression which has been gained by us as a result of the study of these experiments. Briefly, it appears that two factors are of great importance in determining the successful inoculation of animals, if we leave out of consideration, for the present, the variation in virulence of bacilli from different sources. The cause of the unsuccessful attempts to infect animals seems to be the result of insufficient dosage and the fact that a second injection is necessary to establish the infection. We have found that constant takes occur in animals only when sufficiently large doses of the organism are used. Furthermore, second or third injections are much more potent to induce disseminated lesions and the multiplication of the bacilli in the tissues, than is the first, although occasionally with certain species of animals success follows the initial injection provided the dose is very large. Following second and third inoculations, the lesions appear more rapidly, and there is a greater tendency for the organisms to multiply and metastasize than with primary injections. Again, the lesions resulting from subsequent inoculations grow more readily and persist longer than those following the first injection and may

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be produced by smaller doses of bacilli. The primary injection, which, we assume, sensitizes the animal, may consist of dead or living bacilli; either one serves equally as a sensitizing agent.

EXPERIMENTAL PART.

In the experiments, eight monkeys (*Macacus rhesus*), four goats, two kids, eighteen guinea pigs, four white mice, and one horse were employed. For convenience of description, the monkeys, goats, and guinea pigs will be designated alphabetically. In the inoculations, both dead and living bacilli were used, the devitalizing of the organisms being accomplished by means of heat and by treatment with tricresol. It has been determined that heating of the leprosy culture, washed down in saline solution from the surface of agar slants, at 62° C. for one hour kills the organisms. One hour exposure to five per cent. tricresol has the same effect.

Experiment I.—Two monkeys (A and B) were inoculated subcutaneously with living lepra bacilli in doses, approximately, of 400 million organisms. No multiplication or lesions developed until the third injection, when lesions resulted not only at the site of inoculation but also at distant parts, and the animals presented many of the clinical manifestations of human leprosy.

The lesions which developed, however, disappeared after several months. Two months after the disappearance of all signs of the disease both animals were again inoculated upon the inner surface of the thigh with approximately ten billion dead bacilli which had been killed by heating. Twenty-four hours later both monkeys showed small firm nodules, 1 cm. by 5 cm. in size, about the point of inoculation. The following day, i. e. forty-eight hours after injection, the nodule in one monkey was retrogressing, while in the other it had increased in size and was red and tender. This nodule when excised was found to consist of a central area containing a purulent yellowish material which, upon microscopic examination, was found to be composed almost entirely of polymorphonuclear leucocytes and numerous acid-fast bacilli. Many of the bacilli were apparently phagocytosed by the pus cells. Three days after the inoculation the nodule in the monkey not operated upon had disappeared; while the only remains of the nodule in the other animal was a small granulating ulcer.

Experiment II.—On March 8, two monkeys (C and D) were inoculated with about ten billion dead bacilli into the subcutaneous tissue over the chest. No reaction was noted at any time following the inoculation. On March 22, both monkeys were again injected, one with dead, the other with living organisms, approximately ten billion bacilli being used in each case. Twenty-four hours after the second inoculation, both animals had small firm nodules upon the inner surface of the thigh at the site of inoculation. In the animal receiving the living organisms, the lesion was 1.8 cm. by 1.2 cm. in extent; while in the other, the
lesion was somewhat smaller. On the following day, the lesion in the monkey which had received the living bacilli had increased in size and was firmer; the nodule in the monkey which received the dead bacilli was smaller than at the previous observation.

*Experiment III.*—In this experiment, an effort was made to determine, if possible, the size of the dose necessary to infect monkeys by a single inoculation.

Two monkeys (E and F), were inoculated with approximately forty billion living bacilli into the subcutaneous tissue below the nipple. For the first three days, the animals showed neither local nor constitutional reaction, with the exception of a slight edema, which disappeared by the third day. On the fourth day, in both monkeys, small pulpy protuberant masses, which measured 1.8 by 2 cm. in diameter, could be palpated. These swellings were not red, and apparently not tender. The nodules increased slightly in size during the fifth and sixth days, but at no time were they more than barely visible to the naked eye; on palpation, however, they were readily demonstrable. From this time on, the masses decreased in size until the sixteenth day when they had entirely disappeared.

Three weeks after the original inoculation, these monkeys (E and F) were again inoculated, ten billion bacilli being used. Two days after injection, the nodules in the thigh were very prominent, firm, and apparently somewhat tender, measuring 1.5 cm. in diameter in both animals. The nodules continued to increase slowly for several days and remained firm and distinct and somewhat reddened for two weeks.

This experiment with others proves that, although it is possible to induce the production of lesions with the primary inoculation, a very large dose must be used. Such lesions are, moreover, transient and small, never reaching the size of those easily developed upon secondary inoculation by smaller doses.

In experiment IV, we sought to ascertain whether it might be possible to transfer from the serum of an inoculated animal the sensitizing substance which, from former experiments, seemed to be the determining factor in the production of lesions in animals upon second and subsequent injections.

*Experiment IV.*—Two monkeys (G and H) were inoculated in precisely the same manner. Ten billion living bacilli were injected into the subcutaneous tissue over the chest of both animals, and at the same time 12 c.c. of immune goat serum were given into the thigh. The serum was procured from a goat (Grey Billy) which had developed leprosy lesions after repeated inoculations, in spite of the fact that in vitro his blood had early showed fairly well marked bactericidal properties. At no time did monkey G present the slightest local or constitutional reaction to the bacilli. The other animal (H) showed only a small, firm, pea-sized nodule, which appeared upon the fifth and disappeared by the eighth day.
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This attempt to infect the animals with Bacillus leprae must, therefore, be considered unsuccessful, since practically no reaction developed in either monkey, although the dose of bacilli was sufficiently large to induce the production of definite nodules in the sensitized animals which were used as controls.

Experiment V.—Two goats (male (A) and female (B)) were inoculated subcutaneously on December 20 with approximately 500 million dead bacilli (killed with tricresol), and a week later a second injection of double the quantity of dead bacilli was given. Ten days after receiving the initial dose, the animals were injected at weekly intervals over a period of three months with doses commencing at 25 million and increasing to approximately 200 billion.

Each inoculation after the first three gave rise to small nodules 1.5 cm. in diameter, which were neither tender nor red. These nodules gradually increased in size for about two weeks, and then disappeared entirely in five or six weeks after their first appearance was noted. Unfortunately no note was made of the time of onset after inoculation, as our attention had not been attracted to the apparent significance and importance of this interval at that time.

After the inoculation on February 17, in which over 200 billion bacilli were injected, a hemispherical mass appeared upon the neck (the site of inoculation) of goat A, which gradually increased in size until March 18, on which date it had attained a diameter of 5.5 cm. On opening the nodule, it was found to contain friable but not purulent material, which was mottled greyish red in color. This material contained innumerable acid-fast bacilli. The organisms were for the most part clustered together in enormous masses (globi) within large mononuclear and multinuclear cells; and instead of being small coccoid bacilli, they now presented the long beaded appearance that is characteristic of the organism in the human tissues. A pure growth of B. leprae was obtained upon special media from the transplanted grumous material. It is noteworthy that the growth did not appear until six days after incubation at 32° C., and it required several subplantations before the bacilli regained their former rapidity of growth and unbeaded coccoid form. The nodule commenced to recede five weeks after its appearance, and by April 1 had practically subsided, leaving a small granulating ulcer at the site of incision. At no time did any material escape from the opening, so that the decrease in the size of the nodule cannot be considered as due to a discharge of its contents. Notwithstanding the progressive growth of the nodule, inoculations were continued, although in somewhat smaller doses, but no further lesions developed subsequently. Previous to the inoculation of February 17, and constantly since that time, the serum from this animal contained antibodies, but they have never increased in any considerable quantity. There was approximately as much immune body in the serum after the first two or three injections as at any subsequent time.

We wish here to draw attention to the point brought out by this experiment to which we shall have occasion to refer again. In the first place, we find that, in spite of the fact that the serum from the
male goat possessed immune bodies to a fairly high degree, the bacilli were able to grow and to develop characteristic lesions.

Experiment VI.—Two kids were born to goat B, which had received twelve doses of leprosy bacilli. The serum from both these young animals was found to fix complement in the presence of B. leprae as antigen, in dilutions and in quantities similar to those of the mother. Specific agglutinins, however, were not demonstrable in the serum, as no clumping of the bacilli occurred even in mixtures allowed to stand for twenty-four hours; whereas the mother’s serum tested for specific agglutinins gave a very positive reaction in the dilution of 1 to 50.

The lytic properties noted previously in the mother’s serum had disappeared, as was evidenced by the profuse growth of the bacilli, which was obtained from mixtures of the organisms and the undiluted serum kept for twenty-four hours at 37° C.

As we thought that perhaps the kids would have naturally a hypersensitivity to the bacilli, both were inoculated when three weeks old, one receiving about 15 million living, the other a similar quantity of dead bacilli into the under surface of the thigh. The day following the injection the animals were slightly lame, although no definite thickening could be made out. The lameness disappeared at the end of five days without any appreciable lesions having developed.

Apparently then the hypersensitivity which follows inoculation of the bacilli is not transmitted to the offspring, unless it be considered that in these animals the immunity developed by the mother was sufficiently great to protect the kids against infection.

Owing to the difficulty experienced in fixing foreign complement by means of amboceptor from the goat, after repeated experiments it has been found more satisfactory and trustworthy to utilize the normal or natural complement present in goat serum, and to use as hemolytic body the natural amboceptor against guinea pig corpuscles. The method used is similar to that described by one of us (Gurd4) in carrying out the Wassermann reaction. These two bodies (complement and amboceptor) have been found to be constant in their combined action upon guinea pig corpuscles, being active in quantities similar to those in human serum. By means of this method, it was found that the two immunized goats, as well as the kids, fixed large quantities of complement present in normal goat serum.

The following table gives the protocols of the reactions. It is

seen that 0.04 cubic centimeter of the immunized serum of all four goats possessed sufficient antibody to fix the complement present in 0.1 cubic centimeter of goat serum.

<table>
<thead>
<tr>
<th>Tested serum</th>
<th>Antigen+ 1.0 c.c. serum</th>
<th>Antigen+ 0.05 c.c. serum</th>
<th>0.05 c.c. serum</th>
<th>Antigen+ 0.05 c.c. immune serum + 0.05 c.c. normal serum</th>
<th>Antigen+ 0.025 c.c. immune serum + 0.075 c.c. normal serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal goat</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Normal goat</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td></td>
</tr>
<tr>
<td>Grey Billy (immune)</td>
<td>N</td>
<td>N</td>
<td>H</td>
<td>N</td>
<td>I</td>
</tr>
<tr>
<td>Nanny (immune)</td>
<td>N</td>
<td>N</td>
<td>H</td>
<td>N</td>
<td>I</td>
</tr>
<tr>
<td>Male kid</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Female kid</td>
<td>N</td>
<td>N</td>
<td>I</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

H = hemolysis; N = no hemolysis; I = incomplete hemolysis.

The leprosy bacilli used as antigen were killed by heating almost to dryness at 60° C. for forty-eight hours, after having been washed down from the surface of 1.5 per cent. alkaline fish agar in sterile distilled water. The bacilli were then suspended in salt solution so that each cubic centimeter contained about 800 million organisms. This material was standardized so that double the quantity necessary to fix complement with positive sera showed no hemolytic, and but slightly anticomplementary properties. Of the suspension used in the tests reported in this paper, six capillary drops were found to be satisfactory in every way.

Experiment VII.—On February 20, a horse was inoculated with approximately 100 billion living bacilli of B. lepra in the subcutaneous tissues of the neck. Six days after the injection, there developed a firm swelling, somewhat tender, about the site of inoculation, which increased in extent for three or four days until it attained a size of 12 by 10 cm. in diameter. The nodule then commenced to recede and had completely disappeared at the end of two weeks. A second injection was given on March 2, and in twenty-four hours a nodule developed which in size was equal to that resulting from the first injection. Within forty-eight hours the mass fluctuated, and on the third day it broke, discharging a purulent material containing necrotic tissue and innumerable acid-fast bacilli. The organisms were scattered and extracellular.

All attempts to cultivate Bacillus lepra from this material failed, which suggests that the bacilli, although maintaining their normal morphology, had been destroyed in the tissues.
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The third injection resulted in a lesion similar to that of the second. The dose given in all three injections consisted of approximately the same number of bacilli. The fourth and fifth injections gave rise, from the start, to proliferative lesions which increased steadily in size for several weeks. Microscopic examination of excised bits of the nodule showed the typical lesion of leprosy with large numbers of multinucleated cells containing enormous masses of viable acid-fast bacilli. This was proven by cultural tests.

The horse serum tested at this time showed no bacterioltyic substance; however, specific agglutinins were present in moderate amount, and there was clumping of the bacilli in the dilution of 1 to 200.

Experiment VIII.—On March 7, six guinea pigs (series A) of approximately the same weight (250 grams) were inoculated subcutaneously in the region of the groin with 800 million dead leprosy bacilli. Another lot of six guinea pigs (series B) were, on the same day, injected intraperitoneally with a similar amount of killed leprosy bacilli. Subsequently these twelve animals showed no evidence of either peritonitis or local reaction at the site of inoculation. Six of the guinea pigs, three of which had received the primary dose intraperitoneally and three subcutaneously, were given a second injection of eight million killed leprosy bacilli on April 7, one month after the first inoculation. Two of the animals developed a well marked inflammatory reaction at the site of the second injection. However, the swelling induced in the subcutis, the result of the second injection, had completely subsided in forty-eight hours after its first appearance. These six guinea pigs were inoculated for a third time on May 9, a suspension of approximately two million living leprosy organisms being used instead of killed bacilli. The viable bacilli were obtained by washing down in normal salt solution a sixty-four hour growth from an alkaline fish agar slant.

On this date, the six guinea pigs which had received but one injection of dead bacilli were inoculated with two million viable leprosy bacilli, the same route being used in each case as in the first injection.

All six guinea pigs injected intraperitoneally, half of which had received but one injection, and half two injections of dead organisms, showed signs of peritonitis within twenty-four hours after the inoculation. In two of the animals signs of acute peritonitis continued for three days. During the second day, the animals were so ill that it was thought they would not recover. However, both animals recovered, and in five days after the injection appeared to be in normal health.

Two other pigs of the series injected intraperitoneally gradually lost weight and died, one on May 14, the other on May 20, two and three weeks after the injection of the living bacilli.

Autopsies showed disseminated leprosy in practically all organs of the body (figure 3). In general, the gross appearance of the lesions simulated closely that in guinea pigs dying of tuberculosis. The most striking difference was the
color of the larger caseated areas, which were distinctly golden yellow. The
spleen and liver showed the most extensive involvement. Both organs were
greatly enlarged and studded with leprous nodules ranging from 1 mm. to 1 cm.
in diameter. The smaller areas were firm and greyish white in appearance,
while the larger lesions showed central caseation. The spleen was the seat of
large anemic infarcts in addition to multiple leprous tubercles of varying size,
which were readily distinguished microscopically as leprous lesions by the
characteristic appearance of the large cells containing globi and acid-fast bacilli.
Pure cultures of \textit{B. lepra} were recovered on special medium from the lesions
in both animals.

The four remaining guinea pigs that were injected intraperitoneally were
killed at intervals of two, four, ten, and fourteen days after the injection of liv-
ing bacilli. All of these animals showed more or less extensive leprosy lesions in
the abdominal viscera.

\textit{Experiment IX.}—Eight full-grown guinea pigs were inoculated on March 7
with living leprosy bacilli, four subcutaneously and four intraperitoneally. The
dose was approximately 400 million organisms suspended in 5 c.c. of normal
saline solution. Twenty-four hours after the injection, the animals appeared to
be in perfect health; even those which had received the dose subcutaneously
showed no evidence of swelling or tenderness at the site of inoculation. Two
weeks after the first injection, four of the animals (two of which had received
the bacilli intraperitoneally and two subcutaneously) were given a second injection of 400 billion viable leprosy bacilli. The other four animals of this series
were kept for controls.

The two guinea pigs which received the second injection of living bacilli into
the peritoneum showed early signs of peritonitis. Twenty-four hours after the
injection, the animals refused food and remained huddled in their cages, showing
signs of distress. Four days after the second injection the pigs had apparently
recovered from the acute symptoms.

The two guinea pigs which had received the second dose of living bacilli
subcutaneously developed a tumefaction at the site of inoculation. The nodule
in each instance appeared on the third or fourth day and from this time on
gradually increased in size. On the fifth day after the injection, the mass in the
subcutis of one guinea pig had attained the size of a hen’s egg (figure 2). The
overlying skin was purple and on palpation the swelling gave evidence of central
fluctuation. This animal was found dead in the cage seven days after the
injection.

At autopsy, the subcutaneous nodule was found to contain a thick, yellowish
white, caseous material which, on microscopic examination, was found to consist
almost entirely of mononucleated cells, many of which were crowded with acid-
fast bacilli. A pure culture of \textit{B. lepra} was obtained from this material on special
medium. There was no mixed infection. Aside from a slight enlargement of the
inguinal glands on the same side as the nodule, no other lesions existed.

The subcutaneous mass at the site of inoculation in the second guinea pig
measured 6 by 2 by 4 cm. in diameter two weeks after the injection. The over-
lying skin was firmly adherent, of a purplish red color, and greatly thickened.
Seventeen days after the inoculation the mass ruptured, discharging a thick,
creamy yellow material. From this day, the animal lost weight progressively, and eventually died of the infection twenty-eight days after the second inoculation.

At autopsy the spleen, liver, and lungs were thickly studded with leprous nodules. The spleen was three times its normal size and showed extensive involvement. Large anemic infarcts together with small, yellowish white nodules practically covered the whole organ. Here, as in the other animals, the gross appearance of the lesions greatly resembled tuberculosis. It may be mentioned in this connection that this orange yellow color of the necrotic material in the experimental lesions is also characteristic of the pus in the subcutaneous leprous abscesses in human cases. The color is due in both instances almost entirely to the chromogenic property of the leprosy bacilli.

The four control guinea pigs were killed at varying intervals, and at autopsy were found to be negative with respect to leprous lesions.

These experiments with guinea pigs tend to show that animals which were previously thought to be unsusceptible to leprosy are readily infected provided the animal receives a sensitizing dose of either living or dead bacilli. They also prove that the first dose must be large and that an interval of two weeks at least should elapse before giving the second injection. The second or infecting dose may be relatively small as compared to the initial sensitizing dose. Furthermore, these experiments show that after sensitization the animals develop extensive disseminated leprous lesions throughout the body. The lesions have the same general gross appearance, except in color, as those of tuberculosis. Microscopically, however, the picture is distinctly different. Here the areas contain large numbers of mononucleated cells, in addition to the epithelioid type of cell, which are filled with viable leprosy bacilli.

Experiment X.—Four full grown white mice were inoculated on March 10, two intraperitoneally with approximately two million dead leprosy bacilli, and two subcutaneously with the same number of living organisms. The mice injected intraperitoneally with dead bacilli received, two weeks later, eight million viable lepra bacilli, one intraperitoneally and one subcutaneously. The animals were killed on April 12, and at autopsy they showed multiple lesions in the internal organs. The lesions were most extensive in the mouse which had received the intraperitoneal injections (figure 1). White mice appear to be more readily infected than any of the ordinary animals used in laboratory experiments, with the exception of the Japanese dancing mouse; in our experience, however, they, too, are more constantly infected by second inoculations.
SUMMARY AND DISCUSSION.

Repeated experiments have proven that few, if any, of the ordinary laboratory and domestic animals are immune against infection by *Bacillus leprae*. As previously reported, the goat, horse, guinea pig, and many cold-blooded animals (Couret⁵) have been found susceptible to invasion by this organism.

Two factors are of great importance in effecting infection. In the first place, a sufficiently large number of organisms must be employed, and, what is still more important, second and subsequent inoculations are more liable to produce leprous lesions than are primary injections. Moderate doses used in the first inoculation of animals are comparatively harmless as regards their ability to induce lesions. Such preliminary doses, whether they consist of living or dead organisms, produce a condition of hypersensitiveness or allergy, which renders it possible by a second injection of viable bacilli to induce the development of a reactionary lesion. Lesions arising as the result of a second inoculation develop more rapidly, increase in size more quickly, and persist for a longer period than those taking place as the result of a single inoculation, even though very large doses are used. Moreover, the bacilli in these lesions are more liable to lead to metastasis and to a generalized infection. We regard the results of these experiments as having considerable bearing upon the development of the disease in human cases, since we find that it is chiefly among those living in prolonged intimate contact with leprous patients that leprosy develops.

The proper interpretation of these findings is difficult and becomes, apparently, more complex the longer they are studied. We are accustomed to similar phenomena of anaphylaxis or allergy with protein materials and with certain bacteria, especially the tubercle bacillus. It is not surprising that a specific allergy or altered reaction should take place in animals previously injected with leprosy bacilli, either alive or dead; why, however, lesions should develop in which the bacilli continue to grow in animals which had recovered from previous injections, or the serum of which showed bactericidal properties and contained other specific antibodies, is not so easily understood.

⁵ Couret, loc. cit.
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It is unnecessary in this paper to discuss the relative value of the different theories brought forward to explain the phenomenon of anaphylaxis. It must, nevertheless, be assumed that either as the result of the splitting of certain essentially non-toxic substances in the bacillus by specific ferment-like substances in the blood, which increase after a sensitizing dose, or by the joining of some body in the serum with certain substances in the bacilli, a toxic body is produced. Following the setting free or development of these injurious bodies, there results a cellular reaction, an area of local necrosis followed usually by the appearance of cells of the lymphoid and epithelioid type, and, especially if dead bacilli are used, by polymorphonuclear leucocytes. Such a reaction is usual in all allergic or anaphylactic conditions; but why the reaction should predispose to the more or less permanent development of parasitic powers on the part of the bacilli is not so plain. The idea has been expressed by us, as the result of observations based chiefly upon the study of the changes in the tissues of human leprosy, that the presence of bacilli within the large multinucleated cells that are characteristic of the lesion is the result of active multiplication of the bacilli in these cells rather than of phagocytosis by the cells, although it is possible that their original entrance may be the result of phagocytic action. If this be the case, it is possible that, through the cellular reaction plus the necrosis of certain of the fixed tissue cells, a pabulum of split protein products results upon which the bacilli feed, and in which they find protection from the antibodies present in the blood serum.

In our attempts to induce infection in various mammalian species with cultures of human leprosy, positive results have been obtained in almost every instance after the second injection of large numbers of the organism. In this manner, we have been successful in the production of lesions in the monkey, goat, horse, guinea pig, and mouse. The results of these experiments lead us to believe that the mechanism through which invasion and multiplication follows in these lower animals is similar to if not identical with that in man.

A careful study of the progress of the disease in man together with the behavior of the organism in the monkey and the goat
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would suggest, at least, that the function of toxin production is, after all, of little use to the leprosy bacillus because it has seemingly acquired a highly parasitic existence; instead of which it is not only possible, but highly probable, that its defensive protection, whatever it may be, is enormously developed.

After the inoculation of animals with either dead or living bacilli, the production of specific antibodies is induced; these do not develop in any considerable amount and apparently show no marked tendency to increase upon repeated injections. The tests of the sera in vitro indicate that the antisubstances are produced chiefly by the first few injections. Either the existence of some specific bacterial protective body, or what seems more probable, the protection afforded by the host cells in which the bacilli become ensconced accounts, we believe, for the difficulty of producing in animals an antiserum of high potency. The idea, too, that the bacilli do not multiply to any great extent until they have entered certain cells of the host has, in a large measure, been verified by the artificial cultivation experiments.

We know from our studies upon the biology of the organism that it will live for years in the most unfavorable conditions. This would suggest that during their sojourn in the tissue cells death as the result of autolysis rarely occurs, and as long as the cells can withstand the multiplication of the bacilli within and remain alive, so long are the bacilli protected from outside injurious influences, in consequence of which no disintegration of bacilli occurs to induce the production of immune bodies. On the other hand, death of the cells carrying the bacilli must occur from time to time, and this exposes large numbers of the organisms to the action of the body fluids, and, in consequence, to the condition of formation of specific antibodies.

EXPLANATION OF PLATES.

PLATE 20.

Fig. 1. The gross appearance of leprous lesions in the peritoneal visera of the white mouse. Note the distribution of nodules along the course of the mesentery vessels.
Fig. 1.
PLATE 21.

Fig. 2. A subcutaneous leprous nodule in a guinea pig, at the site of inoculation. The mass had attained a size of 2 cm. in diameter five days after the second injection of a pure culture of *B. lepra*.

Fig. 3. A fatal infection with *B. lepra* sixteen days after the second intraperitoneal injection. Note the extensive involvement of the liver, omentum, and under surface of the diaphragm. Note also the nodules in the pericardium and old fibrous adhesions attaching the organ to the sternum.