THE BEHAVIOR OF BACILLUS LEPRÆ IN COLD-BLOODED ANIMALS.*

By MAURICE COURET, M.D.

Instructor in Pathology at Tulane University.

(*From the Laboratories of Pathology, Tulane University, New Orleans, La.*)

PLATE LXXVIII.

At the time the study of experimental leprosy in various species of mammals was begun by Dr. Duval, many species of cold-blooded animals were inoculated with the same strain of *Bacillus lepræ* that was used for the mammals. The culture was obtained in pure growth twelve months previously by Dr. Duval, who has described its characters in detail.[1] In order to afford a basis of comparison, a drawing of the normal organism as it appears in culture is appended (figure 1).

In the experiments presented here, seven tadpoles, nine frogs, four turtles, four snakes, twenty-six gold fish, and thirty-two salt water fish of various kinds were employed, either for inoculation or for feeding with cultures of the bacillus.

All the fish used were very active and apparently healthy when inoculated. With the exception of those employed in the feeding experiments, all were inoculated subcutaneously on the right side of the back, midway between the tail and the head. The culture emulsion was injected immediately below the skin, penetration into the muscle being avoided as much as possible. The amounts of the bacterial suspension used varied according to the size of the animal. In many instances, a slight swelling along the point of inoculation followed the injection, otherwise the animals showed nothing of note. From four to six days following the inoculation, there was sometimes seen an ecchymotic area at the site of puncture, which remained for a variable length of time, but had usually disappeared

*Read before the American Association of Pathologists and Bacteriologists, Chicago, April 14, 1911. Received for publication March 17, 1911.

[1] *Jour. Exper. Med.*, 1910, xii, 649.

by the tenth day. In no instance were there further macroscopic changes, either in the form of tumefaction, discoloration, etc., or in a lessened activity of the animals.

The tadpoles, frogs, and snakes were inoculated into the peritoneal cavity or subcutaneously. In these animals no external evidences of the inoculation could be seen at any time.

Two turtles were inoculated below the skin of the neck, and two intraperitoneally; one turtle injected intraperitoneally died two days later.

In the feeding experiments, the same strain of *Bacillus lepræ* was employed.

EXPERIMENTAL OBSERVATIONS.

PROPAGATION BY DIRECT INOCULATION FROM CULTURES.

Experiment I.—Six gold fish (*Carassius auratus*) 10 cm. long were inoculated subcutaneously with three minims each of a suspension of *B. lepræ* in normal saline solution. There was no perceptible effect from the inoculation. Fish A was killed seventeen days after inoculation; B, twenty days; C, twenty-three days; D, thirty days; E, thirty-nine days; and F, fifty days after inoculation.

There was nothing upon the skin or about the scales of the animals, when killed, to denote the existence of underlying lesions. The skin was found adherent along the track of inoculation and on each side extending to the spinous processes of the vertebræ above and to the abdomen below. Upon breaking the adhesions, bits of tissue were carried with it. The epidermis on the left side was not adherent. The ragged surface left after removing the skin was swollen and slighty darker in color than the corresponding area on the other side, and was covered with a gelatinous substance, which was greater in fish killed after twenty days than in those killed later. The appearance suggested a fibrinous exudate; but on microscopic examination no fibrin or other evidence of acute inflammation existed. The films showed, however, masses of bacilli and many large and smaller cells with a single reticulated nucleus, within the cytoplasm of which were large numbers of acid-fast organisms. The smaller cells, measuring about twenty microns contained few or no bacilli, while those measuring from 100 to 130 microns contained as many as one to two hundred. Films from fish killed early showed fewer cells containing bacilli than those killed after longer periods. In the muscle tissue on the injected side were several minute grayish irregular areas. These areas were round or oval, or they appeared as streaks radiating from the exudate below the skin. They were more distinct in the fish killed on the seventeenth, twentieth, and twenty-third days, and could hardly be seen in those killed thirty-nine and fifty days after inoculation. On the side opposite the inoculation, no lesions could be seen, although films showed cells similar to those found on the inoculated side. These cells, suggesting lepra cells, were smaller and less numerous, and contained fewer bacilli than those about the site of inoculation. The numbers of acid-fast bacilli were taken to indicate

multiplication. Multiplication was much more striking in the fish killed after the longer periods.

The bacilli recovered from the fish agreed in morphology with the organism inoculated. They remained short, unbeaded, and coccoid, even in animals killed fifty days after the inoculation (figure 2).

Experiment II.—Five spots (*Leiostomus xanthurus*), two spotted sea-trout (*Cynoscion nebulosus*), twenty croakers (*Micropogon undulatus*), and five striped mullets (*Mugil cephalus*), were inoculated below the skin of the side of the back with three minims of the same suspension of *B. leprœ*. There were no immediate effects. One spot, one trout, and three croakers died four days later. In all five, there was a marked diminution in the number of bacilli injected. In two animals, the spot and one croaker, the organisms were found with difficulty, but they retained their original morphology. Some were elongated with pointed ends but unbeaded, while a few showed polar stained granules or massed chromatin. Lepra cells were absent. The twenty-seven surviving fish were killed on the seventh, fourteenth, and eighteenth days after inoculation.

One trout, one spot, and four croakers killed on the seventh day showed no external evidence of infection until examined by transmitted light, when distinct opaque areas could be seen below the skin extending radially from the site of inoculation, which were more marked in the croakers than in the trout, and barely visible in the spot. Upon opening the fish, a thick gelatinous exudate was found below the skin extending for some distance along the sheath of the muscles. Some of the opaque areas seen by transmitted light were quite large (3 mm.), and some (about 1 mm.) appeared as small as pin-head sized areas in the outer border of the muscle of the bull croaker. The trout lesions were fairly well marked and appeared as opaque bands following the muscle sheaths and radiating from the gelatinous material below the skin.

While lepra cells were absent from fish dying four days after inoculation, they were numerous in the animals killed after longer periods.

The smears made from the jelly-like material showed innumerable smaller and many larger cells. The nuclei were reticulated, oblong, and pushed to the edge of the cell by the bacilli. They retained normal staining properties and showed no evidences of degeneration. The cells were similar to those found in the gold fish; the larger ones contained masses of acid-fast bacilli, while the smaller ones contained only a few or none.

While a few single bacilli were present in the stained preparations, the greatest numbers occurred as colonies within the lepra cells. It was not uncommon to find two or more large and smaller masses of leprosy bacilli in a single microscopic field. Extracellular bacterial masses, evidently mechanically dislodged from the cells, also occurred. A few of the bacilli were short and unbeaded, but the great majority were long, thick, and beaded. Some showed two, and others as many as five, distinct chromatin beads. Some of the clear spaces between the red staining granules were distinctly "bellied," suggesting spores (figure 8). A noteworthy change in the organisms relates to their thickening, some bacilli being fully one third thicker than usual. Acid-fast bacilli were found scattered throughout the whole body, but less in the spots than in the other fish. In the fish killed fourteen days after injection, the lesions, though

plainly to be seen by transmitted light, were more diffuse, and hence less opaque. Still they were similar in every way to those present in the fish killed a week earlier. The number of larger lepra cells had now greatly increased, although many smaller ones still occurred. That multiplication had gone on is shown by the larger size and greater number of the globi. The bacilli appeared the same as in the fish killed a week earlier, with the possible exception that fewer retained the coccoid form.

The remaining fish (five mullets, one spot, and eight croakers) were killed eighteen days after inoculation. At that time all were active and showed no external indication of infection.

The five mullets showed no acid-fast bacilli. Earlier examinations of this species of fish were not made, and it is therefore difficult to state definitely the time at which the organisms disappeared.

The spot and croakers were without external lesions. On section, the usual tissue changes were present, though apparently less developed than in the fish previously described. The spot showed very small areas quite deep in the muscle. But the inoculated side in the spot and in the ordinary croaker was darker and slightly larger than the opposite one.

Lepra cells and bacterial masses and single bacilli were numerous, and the former were large. The scattered bacilli were more numerous than in the seven and fourteen day fish, and the tissues of the opposite side of the spine of these croakers, contained scattered acid-fast organisms. In the bull croaker, infection seemed to be limited to the inoculated area. The morphology of the organisms remained unchanged (figure 8).

Experiment III.—Nine leopard frogs (*Rana pipiens*) were inoculated as follows with three minims of the suspension: four below the skin of the back, three below the skin of the hind leg, and two into the peritoneal cavity. The two frogs injected intraperitoneally died in twenty-four and forty-eight hours respectively.

After five days, two frogs were killed. They had been injected into the back and the hind leg respectively. In both a few leprosy bacilli were found near the point of inoculation. All lesions were absent and the few bacilli present had, in this short time, undergone a complete metamorphosis; the short unbeaded forms had become long, broad, and distinctly beaded, many containing four chromatin masses each.

Two frogs inoculated in the back were killed fifteen days later. They gave no external evidences of infection; but a gelatinous exudate existed, which was, however, not abundant. Smears from the exudate showed many masses of intra- and extracellular acid-fast bacilli. The cells were small, and very few contained more than twenty to thirty, and many less than ten bacilli. Judging from the number of bacilli present it was evident that the organisms had multiplied. The organisms were still fairly long and thick and some showed polar stained granules. In a few bacilli there were three to four granules, although the greatest number had two.

One frog injected subcutaneously into the leg, and two injected into the back were killed thirty days later. No external lesions existed. Upon removing the skin, a thick, steel gray exudate was seen, and the underlying muscle tissue was moderately congested. Smears made from the exudate showed numerous very

large bacterial masses and many large cells containing masses of bacilli. Several thousand organisms were found in almost every field. The great number of organisms compared to that found in animals killed at shorter intervals indicates that they have multiplied profusely. The morphology of the bacilli had almost completely changed to the short unbeaded forms (figure 4). In both of these frogs, the bacilli and a few lepra cells were found in the omentum, and bacilli had invaded still other distant parts.

Experiment IV.—Seven tadpoles (*larvæ, Rana pipiens*) received 0.2 c.c. each of the same culture used in the previous experiments. Four were injected beneath the skin of the back, and three into the peritoneal cavity. All survived the inoculations. Three days later two were killed. The one injected in the back showed locally many acid-fast bacilli, but no exudate or other lesion. In the other tadpole injected intraperitoneally, there was a slight amount of gelatinous exudate covering the viscera which contained many acid-fast bacilli, but no lepra cells with bacillary inclusions. The bacilli in both animals were confined to the inoculated site, and appeared normal. The five remaining tadpoles were killed twenty-eight days later. Two had in this time developed into frogs. In the exudate were large and small masses of bacilli, the former predominating. The masses were contained chiefly inside of lepra cells; there were a few extracellular colonies. Some of the latter consisted of several thousand bacilli, nearly all of which were of the short unbeaded variety. The longest ones were about two thirds as long and slightly thicker than those found in human lesions. Some bacilli contained as many as three or four chromatin masses (figure 6). It was evident, on comparing these animals with those killed previously, that the bacilli had multiplied.

Experiment V.—Four turtles, one being a box-turtle (*Cistudo triunguis*) 10 cm. long, the others geographic terrapins (*Malacoclemmys geographica*) 15 cm. long, were inoculated. The box-turtle and two terrapins were injected intraperitoneally, and the other terrapin subcutaneously. One of the terrapins died two days later. The peritoneal cavity contained the injected organisms of normal appearance.

A terrapin inoculated beneath the skin of the neck was killed on the twentieth day. A viscid exudate was found extending along the entire length of the neck, but no other lesions. However, films made from the peritoneum and the subcutaneous tissues of the hind legs showed a few acid-fast bacilli. These were short, unbeaded, and coccoid. The viscid exudate found in the subcutaneous tissues of the neck consisted chiefly of mononucleated cells, many of which contained acid-fast bacilli, besides which many large bacterial masses occurred.

The box-turtle and the two remaining terrapins were killed thirty-five days after inoculation. The injection site showed a viscid exudate, and in it were found large cells with bacterial inclusions, besides enormous numbers of extracellular bacterial masses and scattered bacilli. The bacilli retained their ordinary appearance (figure 5).

Experiment VI.—Four king snakes (*Ophibolus getulus sayi*) 50 to 75 cm. long were inoculated, two into the peritoneal cavity and two into the subcutaneous tissue. Twelve days later two of these were examined. Neither showed external evidence of infection. The skin of the animal injected subcutaneously was removed, but no lesion could be found about the point of inoculation. Films

made from the subcutaneous tissue and muscle showed a large number of scattered acid-fast bacilli, and a moderate number of lepra cells containing leprosy bacilli. The cells were chiefly of the small type and many showed a few acid-fast bacilli. A few of the single bacilli, and most of the intracellular ones, were short and unbeaded; while most of the extracellular ones varied in size and morphology from the fairly long bipolar variety with pointed and rounded ends to the long and heavily beaded variety. Many of the latter showed four to five beads of chromatin (figure 7).

There was no question that multiplication had taken place, but what should especially be emphasized is that in this short sojourn in the body the morphology of the organism had so completely changed. The peritoneal cavity of the second snake contained no exudate; but films made from the omentum and gut showed large numbers of acid-fast bacilli. Very few short unbeaded bacilli were present; the greater number were long, thick, distinctly beaded, and pleomorphic. Many large globi and bacterial masses occurred. The most striking feature again was the morphological changes of the bacillus.

Experiment VII.—Two gold fish (*Carassius auratus*) received subcutaneous injections of the suspension of *B. leprœ*. The culture was one recently isolated from a case of human leprosy and still retained its long, slim, beaded appearance, and did not differ in morphology from the bacillus found in human leprous lesions. Its multiplication was very slow in the culture tube, in which it had been growing for two months when used in this experiment.

The first fish was examined at the end of twelve days, at which time it gave no evidence whatever of infection. Films from the point of inoculation, and at some distance within the muscle tissue, showed a few small masses of acid-fast granular detritus, and a few long and beaded bacilli similar to those injected, and other scattered ones in transition towards the small unbeaded and coccoid forms. The morphology, therefore, was changing from the type in human leprous lesions to the short unbeaded and coccoid forms of the leprosy bacillus grown *in vitro*. The second fish was killed thirty days after inoculation; it appeared normal. No lesions were found, but scattered and small clumps of bacilli were present. A few were contained inside of cells. Multiplication was evident, and the organisms were chiefly of the short, coccoid variety.

THE TRANSMISSION OF THE BACILLUS FROM FISH TO FISH.

Experiment VIII.—Three gold fish were inoculated below the skin of the back with 0.5 c.c. of an emulsion made from the tissues of a gold fish killed twenty-three days after primary inoculation. One died three days later, showing nothing of note.

One was killed thirty days after inoculation and several small opaque points, barely 0.5 mm. in diameter, appeared over the area covered by the injected fluid. The areas were superficial, and adhered to the skin. Films from the areas showed many cells resembling lepra cells containing organisms. The bacilli present were single and in small clumps of from six to ten. They all presented the characteristic short and unbeaded morphology.

The third fish was killed forty days after inoculation. External or internal evidence of infection was absent, the condition resembling the previous description.

THE TRANSMISSION OF BACILLUS LEPRÆ TO FISH BY WATER CONTAMINATED WITH THE ORGANISM.

Experiment IX.—Four day old slant cultures of the short unbeaded forms of *B. leprœ* were washed with sterile water and poured into an aquarium containing three gold fish, which were allowed to remain in the infected medium for seven days. They were then washed with several changes of water and transferred to a clean aquarium. The water was changed daily and the feces of the animals were examined every second or third day for acid-fast bacilli; thirteen days after the animals had been changed to uncontaminated water, the feces were found free from bacilli. Seventeen days after they had been removed from the source of infection, the first animal was killed. External evidences of infection were absent. The gills, as well as the skin and muscle tissue, were carefully examined for lesions or leprosy bacilli, but none were found. However, the omentum was covered with a gelatinous exudate, which contained large numbers of mononuclear cells and a few scattered acid-fast bacilli. The kidneys contained lepra bacilli, but none were found in the other organs. The organisms were short, unbeaded, and coccoid, and some showed deep staining bipolar granules, while a few were spindle-shaped.

The second fish was killed twenty-nine days, and the third fish thirty-eight days after inoculation. The appearances and the findings in these were similar to the previous one, except that there were probably a few more bacilli present in the latter.

THE TRANSMISSION OF BACILLUS LEPRÆ TO FISH BY FEEDING HUMAN LEPROSY NODULES AND THE FLESH OF INFECTED FISH.

Experiment X.—Two gold fish were fed teased nodules removed from a leper. No other food was allowed the animals until they had disposed of the leprosy tissue, after which they were cleansed and transferred to a clean aquarium.

The first was examined twenty-four days later; acid-fast organisms had not been found in the feces for three days before the animal was killed. No evidence of infection was apparent externally or internally. Films made from the omentum and other organs showed a few scattered acid-fast bacilli. The average number of bacilli to the slide was about four, and no change in morphology was seen. The second fish was examined after thirty-seven days. The findings were similar to those in the first one.

Experiment XI.—Bits of flesh from salt water fish infected with *B. leprœ* were fed to two gold fish. Three days after they had eaten the food, they were washed and transferred to a clean aquarium.

The first fish was killed twenty days later, and two days after the feces had been found free from acid-fast organisms. There was no microscopic evidence of lesions. A few scattered acid-fast bacilli were found in the omentum and other viscera. They were chiefly short and coccoid in form, although a few were slightly longer and showed distinct polar staining chromatin masses.

CONTROL EXPERIMENTS.

The possibility suggested itself that cold-blooded animals might harbor an acid-fast organism similar in appearance to the leprosy

bacillus. One or more specimens of each species was killed and examined in sections, and films were made from all the tissues, in order to find such an organism, if present. Moreover, the tap water and the food used for the fish were carefully controlled and often examined. At no time was it possible to demonstrate an organism which could have been confused with the leprosy bacillus used in this study.

SUMMARY AND DISCUSSION.

Before proceeding to a discussion of the experiments upon cold-blooded animals, it is necessary to review briefly some of the work recently done with the bacillus of leprosy. The appearance of the bacillus in man and its behavior under artificial cultivation, and in the tissues of lower animals, should be considered in order that comparisons may be drawn.

In their studies with the organism under cultivation, Duval and Gurd[2] pointed out that the long, slender, and beaded appearance of the leprosy bacillus described by Hansen,[3] in 1872, is lost when removed for several generations from the parent stem, and under artificial cultivation the organism becomes unbeaded, short, and coccoid. Duval also noted that these changes in morphology were always followed by rapid multiplication of the organism. Duval argues, *a priori,* that the bacillus is not in a favorable environment in the human tissues. If these deductions are correct, the morphology of the leprosy bacillus should vary according to the resistance offered by the tissues of different animals.

The resistance of the human host to the leprosy bacillus becomes more evident in the light of the clinical aspect of the disease. The long period of incubation, the duration of the disease, and the disappearance of the bacilli preceding the healing of the infected foci show that the resistance offered to the bacillus by the human tissues is not to be overestimated. This opinion is confirmed when the behavior of the leprosy bacillus under cultivation and in the tissues of various mammals is compared.

When cats, rabbits, bats, guinea pigs, and rats are inoculated either below the skin or into the peritoneal cavity with large quanti-

[2] *Arch. Int. Med.,* 1911, vii, 230.
[3] *Norsk. Mag. f. Laegevidensk.,* 1872, ii, 1.

ties of *Bacillus leprœ,* a slight local reaction follows within twenty-four to forty-eight hours, but no definite lesions are produced and the bacilli soon disappear. The resistance of some animals to *Bacillus leprœ* is well illustrated by two cats which were inoculated subcutaneously and intraperitoneally with a heavy suspension of *Bacillus leprœ.* These animals were killed and examined three days later, but the bacilli were not demonstrable from the regions about the sites of inoculation.

Pigeons are likewise refractory. It is impossible to cause a local reaction in these birds, and the injected bacilli disappear rapidly. Hence, probably no multiplication takes place in them.

Goats, young pigs, and white and dancing mice are in a degree susceptible to injections, and though undoubted lesions are produced, and multiplication of the bacilli occurs, the lesions and bacilli disappear after a limited time. Acid-fast bacilli which are recovered from the lesions are long, slim, and beaded, though the organisms used in the inoculations were short, unbeaded, and coccoid.

Monkeys inoculated with cultures of the short unbeaded forms react promptly. The lesions resulting, though confined in most instances to the site of inoculation, occasionally appear at distant points. The number of bacilli present in the nodules and their arrangement within typical lepra cells show that multiplication has taken place. The organism has, however, changed from the short coccoid form to the long, slender, beaded form. Though the lesions induced and the bacilli present are in every way similar to those found in man, their tendency to disappear gradually after a quiescent stage clearly denotes that the tissues of the monkey, although less refractory than the tissues of the animals previously mentioned, still offer resistance to invasion.

While mammals react but poorly to inoculations of the leprosy bacillus, this reaction manifests itself in various ways in different species. For example, while multiplication of the organism with the production of lesions occurs in some species, in others that are more refractory, the injected bacilli assume the involuted or beaded forms and do not multiply or produce lesions; in others, still more resistant to the action of the leprosy bacillus, the organisms quickly undergo granular metamorphosis and disappear. Furthermore, in

some species the lesions are, in most instances, limited to the site of inoculation, and though presenting all the characteristics of the lesion in man, the nodules and the bacilli disappear after a variable time. This behavior of the leprosy bacillus can be accounted for only by the degree of resistance offered by the tissues of the individual host.

Since the morphology of the organism invariably changes from the short coccoid to the large beaded form when placed in insusceptible animals, and convèrsely, from the long beaded forms to the short coccoid forms when placed in susceptible animals, the deduction can be drawn that the organism varies in morphology and rapidity of growth according to the susceptibility of the host.

Examples of similar behavior of *Bacillus leprœ* in the human subject are known to all investigators of leprosy. Ulcers and nodular areas often heal, and the bacilli disappear with little or no treatment. It is true that while older lesions are healing, new ones are constantly appearing, yet the duration of the disease and its undoubted tendency towards healing shows that conditions in the human subject are variable, and suggests that the organism has its natural habitat in some other host.

The experiments presented here serve to show that the bacillus of leprosy meets but little or no resistance in the tissues of cold-blooded animals, multiplies in their tissues, and may be harbored by them without apparent discomfort or external evidence of the disease.

That no appreciable resistance is offered to the multiplication of the leprosy bacillus by many species of cold-blooded animals is shown by the fact that aside from the trauma produced by the inoculation and the slight initial reaction of the tissues, the organism continues to grow profusely, and to invade the tissues without further reaction. Quite the opposite condition occurs in mammals: in some of these the leprosy bacillus degenerates into a granular mass shortly after inoculation; in others that are less refractory, typical lesions appear, but they seldom extend from the point of inoculation; and while the bacilli multiply slowly, they do not infiltrate the tissues, but disappear after a short time, the lesions healing.

That multiplication of *Bacillus leprœ* occurs in the tissues of cold-

blooded animals is shown by the fact that while animals examined a few days after inoculation show but a few scattered organisms, those killed at longer intervals show a proportional increase in the number of bacilli. Furthermore, the few bacilli found at the early period are extracellular and scattered, while after longer periods they tend to be massed and enclosed in large lepra cells.

The supposition that these lepra cells are phagocytes has naturally arisen. Duval[4] holds that they are not phagocytes in the true sense of the term, that the bacilli penetrate the cells rather than that the cells engulf them, after which, finding conditions for growth favorable, they multiply without causing serious injury to the cell. The size of the cell depends upon the size of the colony within. The experimental work bears out this view since the decrease in number of the organisms observed in animals killed shortly after inoculation depends not upon phagocytic action nor upon cells which appear later when active lesions are established. In early lesions, the lepra cells are smaller, barely measuring twenty to thirty microns in diameter, and contain but few bacilli; whereas in older ones, they attain a diameter of 100 microns or even more, and contain enormous numbers of bacilli. Were this increase in size due to phagocytic action, some cells would be found in which the limit of their capacity had been reached; and they would either contain a mass of dead and disintegrated bacteria or would themselves show evidence of disintegration. On the contrary, the bacilli, though they occupy most of the cell, show no signs of disintegration, and the nucleus and the cytoplasm of the cell retain normal staining properties. That the invasion and multiplication of the bacilli cause an irritation is evident by the amitotic divisions of the nucleus which occur in the larger cells.

The absence of external evidence of invasion by *Bacillus leprœ* in cold-blooded animals, and the apparent lack of discomfort caused by the presence of the organism within their tissues, are points which should be remembered in considering the sources from which leprosy may be transmitted. In not a single instance in the numerous experiments presented here would it have been possible, from

[4] *Jour. Exper. Med.*, 1910, xii, 649.

any external sign, to suspect that the animals were harboring multitudes of leprosy bacilli.

While the evidence in support of the opinion that leprosy may be transmitted from man to man appears sufficiently strong to warrant this belief, the number of cases in which infection can be actually traced to this source is small. Since leprosy is known to be prevalent where fish and sea-food are plentiful, and since the experiments here recorded prove that fish can be infected by being fed cultures of *Bacillus lepræ,* or nodules from human lepers, or bits of fish previously infected with the leprosy organism, account should be taken of the possibility that leprosy, in certain localities, may arise from this source of infection.

The question as to how and from what source leprosy bacilli enter the human body may be still regarded as an open one. Isolated examples of direct infection of healthy human beings from lepers have been reported by Arning and Nonne,[5] by Manson,[6] and others. The notion that the agency of infection is already infected human beings, that is lepers, is at the foundation of the modern practice of the isolation and segregation of lepers, which would seem to have brought about a definite decrease in the prevalence of the disease. It is an acknowledged fact, however, that the lepers confined in institutions practically never cause infection of nurses, etc. Some other factor than the human agency may therefore be considered as affecting this issue.

It is well known that Jonathan Hutchinson[7] has brought forward the idea that fish are the source of the infection, basing the view on the high prevalence of the disease along the coast countries of Norway and Sweden, and in the Pacific Islands, and in the countries bordering the Mediterranean and Black Seas, in all of which fish furnish the chief food material. No convincing proof was ever adduced in support of this contention. But now that it has been shown that the leprosy bacillus survives and multiplies in cold-blooded animals, at least at room temperature in a warm climate,

[5] *Arch. f. path. Anat.,* 1893, cxxxiv, 319.

[6] Tropical Diseases, 1900, 448.

[7] *Verhandl. d. X. internat. med. Congr.,* Berlin, 1890, iv, Abt. 13, p. 42.

and since methods have been devised for cultivating and identifying the leprosy bacillus, the question has been opened up to accurate investigation.

Duval has shown that the leprosy bacillus in cultures grows better at room temperature than at 37° C., so that growth in cold-blooded animals kept at room temperature is perhaps in some way connected with this phenomenon. What must now be ascertained, in order to test the Hutchinsonian theory more accurately is whether such growth takes place at a temperature corresponding with the average mean temperature of such a body of water as the North Sea and that of the fiords of Norway.[8] Since cold-blooded animals possess the same temperature as their surroundings, they would be suitable media for the cultivation of leprosy bacilli at those temperatures. For the waters of the Mediterranean Sea and the tropical Pacific Ocean, this consideration would count less. But the theory will stand or fall according as it can account for the whole, and not only for a part, of the phenomena to be explained.

As the length and shape of the bacilli and the number of chromatin masses are constant for a given species of cold- or warm-blooded animals, which features are governed by the resistance of the individual species, the following conclusions seem justified: that the morphology and rapid multiplication of the leprosy bacillus in cultures and in some species of cold-blooded animals indicate that *Bacillus leprœ* under natural conditions is short, coccoid, and unbeaded, and that the long, slender, beaded variety which occurs in the mammalian species is atypical and the product of an unfavorable environment.

In conclusion, I wish to thank Dr. C. W. Duval for valuable suggestions during the course of this work.

EXPLANATION OF PLATE LXXVIII.

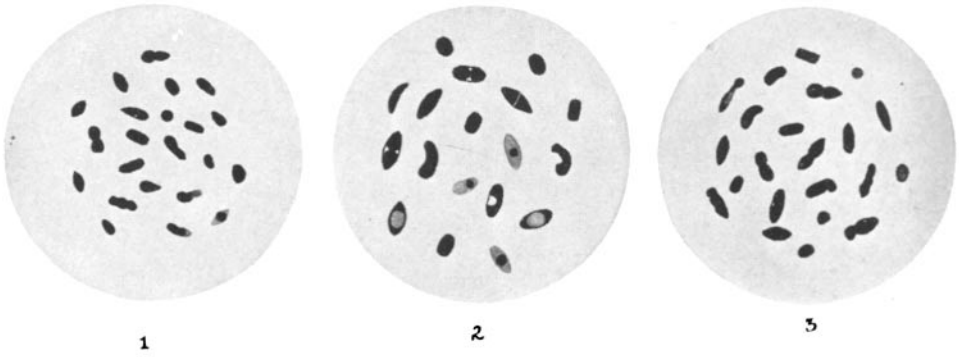
FIG. 1. Leprosy bacilli from the culture used in these experiments, growing upon fish agar. Note the short, unbeaded, coccoid forms.

FIG. 2. Bacilli from the same stem as figure 1, cultivated on alkaline glycerine agar.

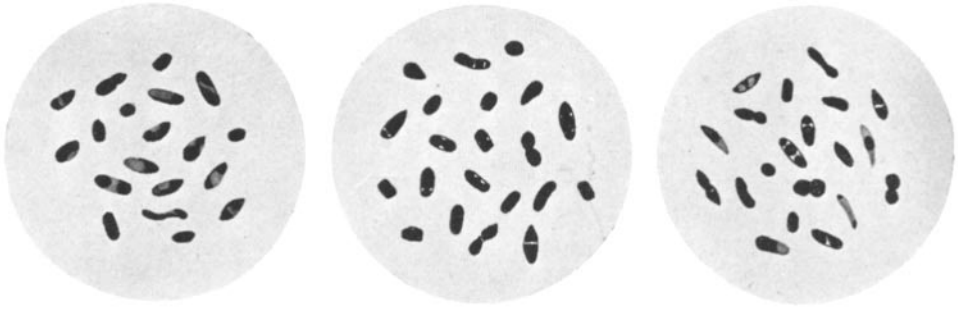
FIG. 3. Leprosy bacilli from a gold fish killed thirty-nine days after inoculation.

FIG. 4. The bacillus in the frog thirty days after inoculation.

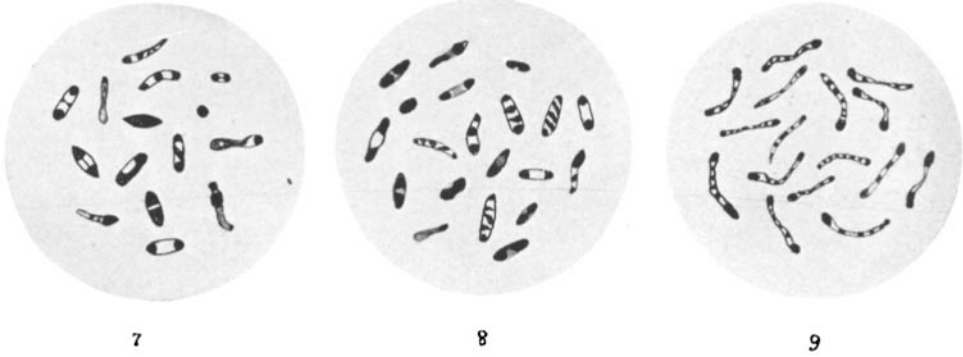
[8] Duval has recently found that *Bacillus leprœ* multiplies readily at 10° C.



1 2 3



4 5 6



7 8 9

FIG. 5. *Bacillus lepræ* in turtles thirty-five days after inoculation.

FIG. 6. Leprosy bacillus in tadpoles killed twenty-eight days after inoculation.

FIG. 7. The organisms in the king snake twelve days after inoculation.

FIG. 8. *Bacillus lepræ* in salt water fish, showing the changes in morphology fourteen days after inoculation. Note the pleomorphic appearance of the organisms.

FIG. 9. The appearance of the bacillus in human tissues and in cultures recently isolated from leprous lesions of man and other mammalian species.