THE LEPROSY OF RATS *

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Stefansky, in 1903, while searching among rats for those carrying the *Bacillus pestis* of Yersin, discovered a certain number of them which were distinguished by enlarged lymph nodes, the hypertrophy of which was not due to that organism but to an acid-fast bacillus that was present in great quantities. In some of these animals the skin was also covered with nodules, with ulcers, with areas of alopecia; some of them could hardly move and they could be caught with the hands.

This disease, which resembles human leprosy very closely, was soon found again in Berlin by Lydia Rabinowitch; in England by George Dean; in Australia by Tidswell and Bull; in the United States by Wherry, McCoy, and Walker; in Rumania by Mezincescu and Alexandrescu; in Japan by Kitasato; in New Caledonia by Leboeuf; in Brazil by Meyer and de Azevedo; in India at first in the Punjab, later in Calcutta by Muir and Henderson. It is, in short, spread all over the world as is human leprosy.

It is found most commonly in the sewer rat, *Mus norvegicus,* more rarely in *Mus rattus* and *Mus alexandrinus.* Dujardin-Beaumetz in Paris and Borrel in Strasbourg have reported it in white rats, but the contamination of these laboratory animals is exceptional and is due to frequent and too easy intercourse with wild rats.

The disease manifests itself in two forms. One is limited to the superficial—inguinal or axillary—lymph nodes, which are large and hard and may attain a length of 3.0 cm. by 1.5 cm. in width. The other spreads over the skin, to the muscles, and invades the whole organism. The cutaneous covering is rough with tubercles appearing principally on the head and on the limbs, with areas of alopecia

* Professor Marchoux has kindly provided the Journal, in lieu of the anticipated "review" of the publications that have appeared on this subject, a valuable summary which appeared in the *Revue Française de Dermatologie et de Vénéréologie,* in June, 1933. A translation, by Mr. M. Zialcita, is here printed in full.

scattered preferably on the back and on the flanks, and with more or less extensive ulcers.

The cause of this disease is found in the multiplication in colossal numbers of an acid-fast bacillus. This is 3 to 5 microns in length and on the average 0.5 micron thick, but with both shorter and longer elements, typically curved, sometimes with a terminal swelling at one or at both extremities. It is clearly acid- and alcohol-fast, and is Gram positive. Like the Hansen bacillus— as, indeed, like all acid-fast bacilli—the Stefansky bacillus may break up into granules. All attempts to cultivate this organism have, as yet, been followed with no more success than have the attempts to cultivate that of Hansen.

The inoculation of this disease into rats is easy, and if Stefansky and Lydia Rabinowitch did not succeed in transmitting it that was because they did not wait long enough for it to develop. To cause an infection it is not necessary to make the injection into the peritoneal cavity, as George Dean did; subcutaneous injection succeeds just as well. More than that, it is enough to apply some of the virulent pulp to slight scarfications of the skin, or simply to a place which has just been depilated, in order to see at the end of a few months the development of an infection of the corresponding lymph nodes. Muir and Henderson have produced, as we did, infection by means of scarifications. On the other hand, penetration of the virus is prevented by healthy skin, even by the pink and smooth skin of young rats in the first days that follow their birth.

Outside of the mouse, to which we have been able to transmit it, the rat is the only laboratory animal susceptible to the infection. We have tried in vain to pass it to other animals, such as the monkey, the rabbit, and the guinea pig.

Markianos has demonstrated in my laboratory that, like the tubercle bacillus, that of rat leprosy possesses elements which pass through porous bougies, so that injection of the filtrate infects young rats.

The inoculation of the rats with any of the procedures cited succeeds in 100 per cent of cases, provided that the virus is used under conditions which do not alter its vitality. This germ is very delicate, and drying kills it. It does not resist a temperature of 60°C. for fifteen minutes. Kept moist on agar in an incubator at 37°C., it has lost its vitality at the end of 12 days. Planted on the medium of Shiga or of Wherry it ceases to be infectious at the end of the same period of time, though after one or two transplantings there

are found on the culture media a considerable number of bacilli that still seem to be good condition, as we have observed with Markianos.

On the other hand, if the pulp of organs infected with rat leprosy is preserved in a medium with 40 per cent glycerin, in refrigerator at between 0° and 6°C., the germs infect rats even after more than two years, as we have recently seen with Chorine.

Staining in the cold for an hour with Ziehl's carbol-fuchsin, or the action of 15 per cent sulphuric acid or antiformin, does not prevent the bacilli from developing in rats.

When the Stefansky bacillus is injected into the peritoneal cavity of a rat and peritoneal fluid is taken periodically in order to follow the progress of the infection, it is seen that the isolated bacilli are very promptly taken up by the polymorphonuclear leucocytes, which seem rapidly to bring about the destruction of the greater part of them. However, the cells themselves are also very much affected and the nuclei become pycnotic, after which they are engulfed by the large mononuclears, in which we can see both polynuclears and bacilli.

Here, however, the bacilli find themselves in a suitable place, where they maintain themselves and multiply. The bacillus of rat leprosy, like that of human leprosy, is a parasite of the macrophages. It lives in harmony with these cellular elements, which even become hypertrophied in order to contain a greater number of them. The rat bacillus differs from that of man in respect to its distribution in the cellular protoplasm. Instead of being arranged in bundles, like packages of cigars, as are the bacilli in human leprosy, they are scattered irregularly, like a box of pins; and instead of repulsing the nucleus they distribute themselves without order all around it. However, that is a condition that depends more on the cellular host than on the nature of the bacillus, for when well-separated bacilli of human leprosy are injected into rats we find them also scattered loosely in the macrophages.

From the moment when a germ has infected a cell it develops little by little a small focus. The number of parasitic elements increases until they fill the cell, forcing it to increase in volume or, sometimes, to join with neighboring cells to produce a giant cell. The cells thus filled are distended until finally they rupture. Immediately the neighboring cells take up the liberated organisms, and thus produce the nucleus of the leprous tubercle.

This lesion, whatever its dimensions, is constituted like that of human leprosy; it consists of infected cells in juxtaposition without

any dividing line from the healthy cells, a characteristic which distinguishes it clearly from the tubercle caused by the bacillus of Koch.

Beyond this stage diffusion of the infection is effected in an insidious way. From the tubercle a slightly infected cell is detached and moved away to establish a new focus where it is stopped. From migration to migration, from focus to focus, the infection travels; it leaves the ganglion, spreads in the dermis or the subcutaneous tissue, surrounds the hair bulbs, provokes the formation of nodules, invades the intermuscular connective tissue, and forces aside and replaces the parenchymal cells in the organs to which it spreads.

In some rats which are very much affected we have seen the eye macroscopically diseased and the animal become blind. Muraro Uchida has found bacilli in the tissues of the eyeball in an earlier stage. In our laboratory Guilliny and Montastruc found some bacilli in this organ in two out of seven rats which were not severely affected.

Muir and Henderson believed that after subcutaneous injection, the cellular infection could be perceived in 8 days, but it seems to us very hard to decide in such case whether the findings are due to multiplication or to an accumulation of the injected material.

Lately we have directed analogous researches by Messrs. Guilliny and Montastruc, who have inoculated a little leprous material into the anterior chamber of the eye of rats. The quantity of fluid contained in this part of the eye is extremely small, hardly sufficing to fill the barrel of a very fine and short needle. In order to introduce bacilli we had to withdraw and inject several times in order to mix the aqueous humor with a little of the septic material, so that the quantity of bacilli inoculated was very small. An injected eye was removed every five days and examined in sections. In the first three eyes we could find only very small but increasing numbers of germs in the cells of the ciliary processes and of the iris, but after twenty days we found many large cells filled with organisms at the base of the ciliary processes. This experiment, difficult to perform elsewhere with the same surety, shows that the bacillus of leprosy multiplies with the same rapidity as the tubercle bacillus as soon as it has become established in the organism.

In nature, and in particular among the rat population of the sewers of Paris, we find on the average that 5 per cent of the captured rats carry the leprosy bacilli in one or several lymph nodes, but the number of those in which the infection has become generalized

is infinitely smaller. Hardly 0.6 per cent have been found in a very great number of rats examined.

Two problems which are of as much interest in human leprosy as in that of rats present themselves, and are susceptible of a solution. How is the infection contracted? What are the conditions that favor its generalization?

Rats are savage animals that devour each other; the bacillus may therefore penetrate through the digestive organs. We have caused a series of these animals to eat pulp full of bacilli. At the end of a few months we found in every one of them an infection limited to the thoracic lymph nodes. We could also find some infected cells, but without any definite focus, in the apices of the lungs. Lépine and Markianos have obtained the same infection in young rats to which they fed the virus. However, under these conditions the infection did not have the characteristics which it has under natural conditions, when it first affects the superficial nodes almost exclusively.

Are not the parasites with which these animals are covered—fleas, lice, acariens (*Lelaps echidninus*), sarcoptes—capable of transporting these germs in passing from a diseased to a healthy one? If these insects are collected by combing and then are ground up and injected, leprosy is surely produced. But in this case one inoculates hair bulbs and scales which carry an enormous number of bacilli. Things happen quite differently in experiments in which, at the death of a highly infected rat, the parasites transfer spontaneously to healthy rats; these have never become leprous. We must therefore discard the idea of transmission by means of the action of biting insects.

The sewer rat, as we have said, is ferocious and fond of fighting. Traces of bites are common, so much so that it is impossible to capture one absolutely unscathed. On the other hand the slightest fear causes these animals to huddle in a corner, where they pile up on top of each other. It is in this same fashion that they rest in burrows where they nest when they are not out looking for food. Under these conditions an ulcer easily comes into contact with a fresh wound, and the virus thus quite frequently finds a very favorable way for transfer.

This mode of contagion can be reproduced artificially by putting scarified and tied rats into the same cage with infectious material

and flies. The insects flying alternately from the pulp of infected organs to the fresh wounds of the rat contaminate them every time. On the other hand, if these insects are separated for twenty-four hours from any infectious material they cannot infect wounded rats in that manner even though their intestines had been filled with the virus. Therefore, the fly only transmits the virus attached to its feet or to its proboscis, and can do that only on condition that the time elapsing between the moment it is contaminated and the time it contacts the wound is not too long (24 hours).

We have placed diseased males without ulcers in contact with healthy females, and healthy males with diseased females, without perceiving any contamination through sexual intercourse. However, by placing some fresh bacilli on the prepuce of healthy males, without causing any erosion, we found that the virus penetrated and affected the corresponding lymph nodes.

Both females and males injected intraperitoneally become sterile very rapidly. After subcutaneous injection cohabitation gives rise to some pregnancies. These, if the infection is recent, succeed perfectly and the young do not become leprous. If the subcutaneous infection is a bit old at the time of delivery the young die at the end of a relatively short time, because the infected mammary glands do not furnish milk to nourish them. If, as Borrel and Larousse have shown, the young of a leprous mother are fed by a healthy female they grow perfectly and have never been found to be carriers of the Stefansky bacillus. Therefore, congenital transmission of the infection, if it exists, is extremely rare.

Once, while injecting germs taken from rats with ulcers, we happened to inject at the same time a staphylococcus which is abundantly found in these septic erosions. We observed that under these conditions the infection frequently became generalized. In the same way, if infected rats are injected with this staphylococcus the leprosy departs from its narrow limits and spreads throughout the organism. Therefore, secondary infections, and also diseases of nutrition, have a big part in the development of the disease after infection.

The following experiment serves to strengthen this opinion. A wild rat, in the inguinal nodes of which we had found leprous bacilli by puncture, was kept in a cage in the laboratory and fed well and cleaned as it should be. At the end of a few months this rat, which had become very fat, was sacrified. There was no trace of infection

in any of its lymph nodes. Good nourishment and good hygiene, therefore, are capable of stopping a mild infection and of eradicating it.

Without wishing to make at this time a comparison between human and rat leprosy, which anyone can do from the descriptions just given, we do wish to recall that we have published an observation which raises the question of the possibility of transmission of the disease of the rat to man. A young Haitian, affected with leprosy having certain special characteristics, died in the Hôpital Pasteur with purulent streptococcic pleurisy. Some of the pulp of the spleen of this man, taken soon after his death, was injected into six rats. Five of them became infected. The bacilli found in them were somewhat special, rigid, shaped like pins with a head and a point, often swollen with a mass that stained more intensely than the rest. From the second passage in the rat this organism became indistinguishable from the bacillus of Stefansky, and since then it has behaved like this latter through the successive transfers that we have made in the laboratory, where we continue to maintain it under the name of *Mycobacterium pulviforme*. Since this time no attempt to transmit the Hansen bacillus to the rat has succeeded.

There would, therefore, seem to be several kinds of leprosy bacilli, as there are several kinds of tubercle bacilli, the bacillus of rat leprosy being under certain conditions transmissible to men.

CONCLUSIONS

Rat leprosy exists throughout the whole world.

It is limited to the superficial ganglia in 5 per cent of the sewer rats of Paris, and generalized in the proportion of 0.6 per cent.

It is due to an acid- and alcohol-fast bacillus, which it has not yet been possible to cultivate artificially but which easily infects laboratory rats.

This organism is very delicate; it dies on drying, or on heating for fifteen minutes at 60°C. It has been kept alive for 51 months under refrigeration in a strongly glycerinated medium. Zieh's carbol fuchsin, or 15 per cent sulphuric acid or antiformin, do not kill it.

It is a parasite of the macrophages which spreads from place to place through the rupture of the cells that contain them. It multiplies in the tissues as rapidly as does the bacillus of Koch.

Infection through the digestive tract is limited to the deep lymph nodes.

Parasites of the rat, in passing from a diseased animal to a healthy one, do not carry with them any of the germs transmissible by biting.

Transmission is effected by juxtaposition of an infected ulcer and a recent wound. Flies also effect this contagion indirectly. Genital contagion seems possible.

The young of a leprous rat die of inanition because invasion of the mammary glands supresses lactation, but they survive and remain healthy if they are nourished by a normal female.

Secondary infections favor the generalization of the bacilli of rat leprosy. Good nutrition and good hygiene eliminate a mild infection.

In one instance a leprosy bacillus taken from a man has been transmitted to the rat. There would therefore seem to be several leprosy bacilli, as there are several tubercle bacilli.