# THE RELATIONSHIP OF HUMAN LEPROSY AND RAT LEPROSY

## A STUDY OF WILD RATS CAPTURED IN THE CULION LEPER COLONY [1,2]

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### INTRODUCTION

Rat leprosy exhibits certain pathological, bacteriological and clinical features in common with leprosy of man. The delicate rod-like morphology of the bacilli of Stefansky (26) and of Hansen (9), their strongly acid-fast and alcohol-fast staining reactions, and their resistance to cultivation on artificial mediums indicate a close relationship of these two generally accepted etiological agents. In consequence, a firm belief in their identity has been expressed frequently in the literature.

From the viewpoint of epidemiological considerations the unity of the two germs is rendered improbable, since a comparison of the known regional distribution of rat leprosy does not necessarily coincide with that of human leprosy. The original observations of Dean (6) indicated this fact. He diagnosed rat leprosy in a district outside of London which had been free from the human malady for centuries. The same situation exists in California today, where rats infected with Stefansky's bacillus are repeatedly encountered in the systematic campaign which is relentlessly waged against bubonic plague. This area would hardly be considered a focus of the human disease, although transients with the disease contracted elsewhere are not rare. Perhaps the most definite example of a lack of coexistence is to be found in the reports of studies from the Hawaiian Islands (4, 5, 17, 18) which is an endemic center of the human infection. In a careful examination of over 16,000 wild rats, the majority of which were trapped in Honolulu—where possibly due to congestion the in-

[1] This study is one of a series carried out under the auspices of the Leonard Wood Memorial in cooperation with the Philippine Bureau of Health.

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cidence of human leprosy is the greatest—no case of the rat disease was detected.

Efforts to transmit human leprosy artificially to various species of rats—the crucial test—have been legion, and with the exception of one isolated instance (3) have uniformly failed, whereas rat leprosy develops regularly in rats injected with the homologous virulent material. Attempts in the reverse direction, namely, the purposeful injection of man with infected rat tissue, have not been recorded.[3]

Patients with leprosy in its most advanced stages have been isolated at Culion continuously during the past twenty-nine years, and at present they number about 6,700. In a tropical community like this colony, in which there are numerous hospital buildings, and many dormitories for both sexes and for various conditions of people from active children to crippled "invalids," together with hundreds of individual private residences of inmates—the whole run on a budget that does not permit of very elaborate sanitary arrangements—it is inevitable that there are many rats. It would be futile to attempt to estimate the current rodent population, but as might be expected from the fact that the rat originated in the Far East it was sizeable and well established when the village originally there was taken over and the colony built,[4] and it certainly is large today. This is a matter to which the colony authorities have never been callous, but though they frequently make use of poisons and traps in an effort to keep down the rat population, its extermination is impossible and such relief as is obtained is temporary.

It is also inevitable under the circumstances in the colony that much material contaminated with the germ of human leprosy is accessible to rats. Waste-receptacles, mostly improvised boxes often in poor condition, are emptied but once or at most twice a day, and there is abundant opportunity, abundantly taken advantage of especially at night, for rats to scavenge among materials contaminated with

[3] Dr. Hans Zinsser (personal communication) states that he has knowledge of the accidental injection of a definite quantity of a rich suspension of infected rat tissue into and under the skin of a laboratory worker; over a period of twenty years no suggestion of a local or general reaction has been noted as the result of this inadvertent inoculation.

[4] Personal communication from Sister Calixte, one of the French nuns assigned to the colony at its inauguration. It may be added that rats are sometimes encountered wild in the forests at Culion, and that rat depredations seriously affect some of the cultivated crops, especially sugar cane.

the germ of human leprosy, such as discarded bandages, pus-soaked cotton dressings, blood-soiled pledgets, and even tissue fragments from the surgical dressing clinic, the operating room and elsewhere. In spite of sanitary regulations patients all over the colony are careless about discarding old materials used in dressing the ulcers so common in this disease. All in all there is thus a constant source of infected materials available to and utilized by the rats of the colony.

A further, occasional, source of contact lies in depredations of rats on dead bodies and even on the persons of patients. Several times a year inmates report that while they were asleep rats had actually gnawed tissue from extremeties so anesthetic that the molestation did not awaken them; and once or twice a year wounds so produced cause a patient to be hospitalized for treatment. Even if it be the fact that tissues so completely anesthetic are usually not rich in bacilli, this is not the case with tissues mutilated by rats in the morgue. Since the inauguration of the colony bodies awaiting burial (which occurs within twenty-four hours, by regulation), have usually been laid out on a slab in the morgue room. Though they are wrapped in blankets dampened with a malodorous disinfectant, rats sometimes get to them and remove pieces of tissue. These occurences may not be frequent, but they constitute another source of infectious materials for the rats.

It is noticeable at Culion that the inmates expectorate liberally and carelessly. The discharge impregnates the soil, as we have determined, with unlimited numbers of acid-fast rods. These are probably Hansen's bacillus, though it is also true that the tubercle bacillus has to be considered in this connection, since it is commonplace knowledge that the incidence of active pulmonary tuberculosis is quite high among the Filipinos in general and the colony inmates in particular. Therefore, in addition to the ingestion of the infected materials mentioned, the rats are in intimate contact with a heavily contaminated environment. Ideal conditions are thus furnished for the introduction *en mass* of the organisms of leprosy by at least two routes, the gastro-intestinal tract and the broken skin. One assumes the latter situation to be relatively common as a result of superficial injuries sustained by the animals in foraging for food and in fighting among themselves.

After more than a quarter of a century of such close association with human leprosy it would be only natural to expect that

its germ would have firmly entrenched itself in the rodent population if this were susceptible. This would surely be expected in the case of the introduction of rat leprosy into such a rodent community, for there is ample evidence from laboratory experience that that infection is readily transmitted from animal to animal by application of tissue suspensions to scarified areas of the skin as well as by their subcutaneous or intracutaneous injection.

## HISTORICAL

Stefansky (26), while associated with the investigation of plague in Odessa (1901), observed a naturally occurring disease of wild rats that closely resembled human leprosy. There were present in the lesions enormous numbers of acid-fast bacilli that were morphologically indistinguishable from the leprosy bacillus of man. Rabinowitsch (22), who had been associated with him at the time, on returning to Berlin in 1903 promptly confirmed the existence of this infection by finding rats in that locality which harbored the same organism. Across the Channel, working independently and without knowledge of these contemporary studies, Dean (6) in 1903 made similar discoveries in rodents captured in the environs of London. With the passage of time the disease now termed rat leprosy has been encountered in various parts of the world. It was found by Tidswell (27) in Australia in 1904, Wherry (29) in San Francisco in 1908, Kitasato (11) in Tokyo in 1909, Marchoux and Sorel (16) in Paris in 1912, Pas de Azevedo (1) in Brazil in 1913, and by many others working in these same areas and elsewhere.

The frequency of the naturally occurring infection, judged on the basis of macroscopic observations, has never been very great. In Stefansky's rats 5 per cent had definite lesions; those examined by Tidswell gave an incidence of 0.001 per cent; Wherry found 0.21 per cent infected and Marchoux and Sorel 5 per cent. These last two writers stated that the distribution of positive findings varied in different localities ("spotted" distribution) and that as many as 45 per cent of the rats in a certain district were infected. This is in keeping with the observations of McCoy (18) who examined about 200,000 rats in the San Francisco area and found 186 with leprosy-like conditions, an incidence of about once in 1,075 rats. The majority of the diseased rodents came from that section of the city in which the meat industry was segregated, and McCoy was inclined to account for this uneven distribution on the basis of a concentration

of rats in Butchertown with consequently a greater possibility of infection there.

Stefansky gave an excellent description of the disease as it occurred in wild rats. He pointed out that in the gross there were two chief types, the one affecting primarily the skin and the musculature; the other confined to the lymph glands. The line of demarcation between the two forms was somewhat indefinite.

A very extensive portrayal of the outstanding features of the malady was presented later by Dean (7). Two excellent photographs accompany his paper. His attention was first attracted to the diseased animals by the fact that they were abroad during the daytime in an obviously sick condition, hence easily caught. In his experience patches of alopecia were the most marked attribute of the disease.

> These may be extensive, affecting in some cases almost the whole thorax and abdomen. Above the surface of these bare patches of skin, elevated bosses, or even nodules, the size of a bean are frequently present. The surface of these elevations may be ulcerated.
>
> On incising an area affected in this manner the skin appears greatly thickened because of a pale yellow infiltration, which cuts with a clean, dry, cheese-like surface. The glandular enlargement is frequently considerable and widespread; the axillary, cervical, and inguinal glands are often involved, some even reaching the size of a small hazel-nut. Microscopic examination of the tissues in stained sections shows a close resemblance between this and the human disease. The chief characteristic common to both infections is the presence of large numbers of acid-fast bacilli within cells. When engorged with organisms these cells are referred to as lepra cells.

Dean in commenting on his own observations stated:

> The features of resemblance between these two diseases are so strong that they point to a close relation between the associated bacilli. The consideration that man and the rat are fellow victims of almost equal susceptibility to other disease germs such as the plague bacillus, might even suggest the possibility that in lepra and in this rat disease we are dealing with the same micro-organism affecting two species. There is no intention here, however, in the present state of our knowledge to uphold such a hypothesis, which is obviously rendered improbable from epidemiological considerations alone.

The rat disease was first transferred by Dean to the domesticated albino variety, and he found that it could be perpetuated in this species. By means of this transfer an ample quantity of experimental material was made available. In an attempt to determine the specificity of the organism Dean injected a number of labor-

atory animals such as rabbits, guinea-pigs, mice and monkeys with the comminuted infected tissue of wild rats, but he found all of them refractory to the disease. These findings were promptly confirmed by Wherry (30), and this led Brinkerhoff (4) to state:

> The leprosy-like disease of the rat is of great interest to leprologists because of its close similarity to the disease leprosy in man. Its practical importance... is increased by the fact that it can be artificially propagated under laboratory conditions from animal to animal. It is earnestly hoped that the investigation of this disease will be undertaken...as it is extremely probable that certain of the most difficult problems presented by leprosy in man can be studied in this disease of the rat, and if solved there, the information gained can be directly applied to the solution of the analogous problems in the human disease.

The inoculation of human leprosy tissue into the various species of readily available animals has been repeated time and time again, with disappointing results. The inoculation of Hansen's bacillus from man to man, though purposefully attempted on a small scale as early as 1844 by Danielssen (who was struggling to prove the non-contagious nature of the disease), has likewise given negative results.[5] Obviously, the latter procedure is too replete with danger to permit of exhaustive trials.

It has been repeatedly said and generally accepted that rat leprosy is closely allied with human leprosy, but the concensus of opinion is that they are distinct. Nevertheless, a comparative study of the cultures from the rat, man and the soil convinced Walker and Sweeney (28) that the strains obtained from all three sources were identical. These authors hold that rat leprosy has the same etiology and endemiology as human leprosy; that it is an actinomyces infection from the soil. They conclude:

> These lights on some of the obscure phases of leprosy represent only the beginning of a new progress in leprosy research that should be initiated by the recognition of the identity of the disease in rat and man. Of especial importance for this advance is the fact that the discovery of this identity of the two leprosies provides an experimental animal for the study of the human disease, of which the lack has hitherto constituted an insurmountable barrier to experimental investigation.

Five years later Salle (23), reporting in 1934 the results of his cultivation studies in tissue cultures, in which he used materials from

[5] Professor Marchoux (15) has recently reported an accidental infection in man. The patient had pricked his skin with a needle during the closing of a wound following the removal of a leprotic nodule. The incubation period was between eight and ten years.

human and rat sources, reaffirmed the belief of Walker and Sweeney. "On the basis of these results, it must be concluded that human and rat leprosy are caused by one and the same organism." However, in the summary of his work Salle makes no mention of the actinomyces form.

Nearly three decades have elapsed since the statement of Brinckerhoff was made, and in the meantime a number of leprologists have accepted the obligation he imposed upon them and have undertaken exhaustive studies of artificially transmitted rat leprosy. A careful survey of the many publications which have appeared as a result of these investigations fails to reveal that the desired insight into the enigma of human leprosy has been attained.

## METHODS EMPLOYED

The rats studied in this investigation were caught in baited traps by inmates whose cooperation was enlisted. The traps were distributed to those places where the activities of the rodents were most annoying, such as the dormitories for men, the hospital wards, the morgue and some of the private cottages. A small fee was paid for each rat brought in, and the catchers were particularly urged to be on the alert for sick animals. This seemed of unusual importance in view of Dean's statement that the majority of the rats which on subsequent examination were found to be infected with Stefansky's bacillus were observed prowling around in the daytime in more or less of a stupor and were easily captured. A suitable premium for such rats was offered our catchers, but none was forthcoming. It was learned after a rather extensive inquiry that it is most unusual at Culion to encounter an apparently diseased rat aimlessly wandering about in the daytime. This is particularly true during the rainy season, the period we were in residence.

The rats were captured without injury in the ordinary commercial wire cage traps, or in crudely improvised instruments of the same general type. The baited traps were distributed late in the day, and on the following morning the catch was delivered to the laboratory animal house and transferred to empty stock cages, where they were temporarily housed. In the laboratory the species and sex of each rat were determined, and the age was estimated. A thorough inspection was made, noting particularly the condition of the hair (alopecia), and of the skin (roughness, ulcerations). Search was made for mutilations of the ears, tail or feet, and for the presence or absence of a secretion from the nose and affections of the eyes. The animal was then deeply anesthetized in a glass jar fitted with a tight cover and containing cotton saturated with ether, and after re-examination of the skin was stretched on a board, anesthesia being

maintained by a cone containing cotton saturated with ether. The hair over the abdomen and thorax was now thoroughly moistened by rubbing with cotton saturated with 1 : 1,000 mercuric bichloride. Since by this treatment the ecto-parasites were driven out, lice, fleas and scabies were regularly observed but no attempt was made to identify the varieties encountered.

With sterile instruments and strictly aseptic technique each animal was examined. The skin over the lower abdomen was raised with forceps and nicked with scissors, and the incision was extended along the median line to the lower edge of the mandible, care being taken to avoid cutting through the underlying structures. At each end of the incision lateral cuts were made well out to the paws, and the two flaps of the integument were reflected, thus exposing the entire abdominal and thoracic walls along with the axillary and inguinal glands. With another pair of forceps the lower abdominal wall was elevated and a small cut made in it with scissors. Keeping the wall taut, the lower blade of the instrument was introduced through this opening which was then continued to the diaphragm which was cut close to the ribs. The costal cartilages on both sides were severed with a pair of shears and the tissue so freed was pulled forward over the head and fastened out of the way. By this procedure the entire abdomen and thorax were exposed. The lower end of the abdominal incision was extended out toward the extremities and the walls reflected. Particular care was taken to avoid cutting into the intestines or other internal organs.

The pericardial sac was opened, the apex of the heart grasped in a pair of broad-pointed forceps, and the sterile, unpolished open end of a Pasteur bulb pipette was pushed with a twisting motion through the cardiac muscle into the right ventricle. Holding the heart lightly out-stretched, by the application of suction as much blood as possible was withdrawn, the usual yield being 8 to 12 cc. Without taking time either to defibrinate it or to add anti-coagulants the blood was distributed to tubes of sterile rabbit-blood agar and Petragnani's medium. These inoculated mediums were later placed in Novy jars, the contained atmospheres enriched with 10 per cent of $CO_2$, and the jars incubated at 37.5°C. Controls were kept at room temperature. Several slide smears of each blood sample were also made for purposes of direct examination and for permanent stains, the last drop from the pipette being mounted with a cover-glass and examined for the presence of trypanosomes.

Attention was then directed again to the skin and subcutaneous tissues. A careful search was made for evidences of nodular thickening of the skin, described as characteristic of the naturally occurring infection, and for a diffuse subcutaneous infiltration, often confused with fat, which is practically always associated with the disease as it occurs in nature. The peripheral lymph glands were thoroughly examined for the presence of fine white or slightly yellow granules.

The pleural cavity and its contents were next studied, particularly for evidence of broncho-pneumonia. Currie and Hollman (5) have reported that this involvement is the earliest and most constant lesion of rat leprosy, though their statement was based on observations in purposefully infected animals. The liver, spleen, and kidneys were examined for color, size and consistency,

and these organs were then carefully removed and placed in sterile dishes. McCoy (18) stated that nephritis was observed in 53.8 per cent of the leprous rats he had examined, the kidneys being large, yellowish-brown in color and friable. On several occasions slide preparations were made from the various lymph glands and these were later examined for the presence of acid-fast organisms.

In this manner each animal was thoroughly inspected for evidence of naturally occurring rat leprosy. However, it was felt that here was an excellent opportunity to detect the incidence, if present, of other diseases of rodents, so before discarding the carcasses a careful search was made for infections with *Bacillus pestis, B. whitmori, Spirochaeta icterohemorrhagiae, Sp. morsus muris, Trypanosoma lewisi* and for infestations with worms.

## OBSERVATIONS

A total of 212 rats was examined. They were fat, healthy, sleek-looking animals, in marked contrast to the mangy, half-starved appearance of the vermin-infested dogs and cats seen about the colony. They belonged to two species: *Mus norvegicus* (the barn, sewer, wharf, or gray rat) and *M. rattus*, the black rat. No members of the species *M. alexandrinus* were seen. *M. rattus* was easily distinguished from the other because of its smaller size, bluish-black color, small head, large rounded ears and long tail. It is rather common in the Far East to find two species of rats, not necessarily these two, living side by side in the same locality, the one burrowing in habit, the other arboreal. Owing to the antagonism of the larger and more ferocious barn rat these two types are rarely found together in the temperate zone.

The representatives of *M. rattus* are generally considered to be much more fastidious in their preference for food than the scavenging *M. norvegicus*. The latter undoubtedly feeds freely upon all infected materials that are accessible. One hesitates to believe that the black rat will prey upon man, but the inmates assured us that such is the case, and they had the feeling that it more prone to devour human tissue than the barn rat.

The data for the species and sex with percentages are recorded in Table 1. Of the 212 animals 83.5 per cent were barn rats, and 16.5 per cent black rats. There was a total of 92 males (43.4 per cent), and 120 (56.6 per cent) females. This slight excess of females is in accord with the proportion in rats caught in San Francisco (17), where out of 28,656 examined 64.7 per cent were of this sex.

Any attempt to estimate the age of rats leads into difficulties, but 46 of the barn rats were unquestionably old animals. Of the remainder, all except 12 were considered to be full grown, these latter being recorded as half grown. With the black rats the difficulty was even greater, but it was concluded that only 10 were half grown, the others fully mature.

No suggestion of rat leprosy, either incipient or advanced, was encountered either when making the gross inspection of the animals or in the subsequent examination of the tissues and organs. The hair was in excellent condition; there were no signs of alopecia or ulcerations. In every instance but one the tail, feet and ears were intact; the exception was the loss of a front paw of an old male, probably the result of an injury caused by a trap. This absence of deformities seemed rather remarkable, particularly in view of the many efforts made in the past to hold down the rat population, and

TABLE 1.—*Species and sex of the rats examined at Culion.*

| Species | Males | | Females | | Total | |
|---|---|---|---|---|---|---|
| | Number | Per cent | Number | Per cent | Number | Per cent |
| *Mus norvegicus* | 78[a] | 36.7 | 99[b] | 46.6 | 177 | 83.5 |
| *Mus rattus* | 14 | 6.6 | 21 | 9.9 | 35 | 16.5 |
| Total | 92 | 43.4 | 120 | 56.6 | 212 | 100.0 |

[a] Including twenty-four old males.
[b] Including twenty-two old females. Twelve of the total examined were gravid.

the fact that during these campaigns snap traps have played an important part.

No cutaneous or diffuse subcutaneous infiltration was observed, nor was there any involvement of the cervical, axillary or inguinal lymph glands. On several occasions these lymph nodes were removed and studied histologically,[6] though no evidence of the presence of acid-fast forms was found. All sections studied were normal. Lesions of the internal organs were detected in but one rat, in which the spleen was enlarged and hemorrhagic; the pathological report stated that this abnormality was the result of a hemangioma. McCoy (18) has called attention to the common occurrence of nephritis in animals infected with rat leprosy, but although particular attention

[6] These examinations were kindly made by Dr. J. O. Nolasco, senior pathologist of the colony staff.

was directed toward this involvement it was not observed. This absence of renal pathology was somewhat unexpected, as the writer has frequently seen enlarged kidneys, yellowish-brown in color and distinctly friable, when prosecting white rats in routine laboratory work.

### ATTEMPTS TO INFECT WILD RATS WITH HUMAN LEPROSY

The failure to detect the presence of a spontaneous infection with Stefansky's bacillus among the animals examined suggested an investigation of the possibility of the rats having acquired an immunity to Hansen's bacillus as a result of the protracted intimate association that has been discussed. This suggested possibility is of course based on the assumption that the causative agents of human and rat leprosy are identical. While no doubt existed regarding the frequent ingestion of infected materials by rats at Culion, there was no direct evidence of contact between infective human material and open skin lesions of the rats. If it is essential for the germ to enter the body by way of the broken skin in order to incite the disease, relevant data should be obtained to assure that such close contact had actually occurred. In an attempt to answer this question the following experiments were undertaken.

Thirty young rats, of both species—eight of them still nursing—were injected both intracutaneously and subcutaneously with saline emulsions rich in human leprosy bacilli. These were obtained from: (1) a three-months old leproma removed from the forearm of a young man; (2) material aspirated from a broken-down nodule on the back of the hand of an old man; (3) subepithelial pus that had appeared in large quantities in the integument over the extremities and face of a young girl as a result of lepra reaction approximately one week after a Mantoux text with tuberculin (O. T.). With a tuberculin syringe each animal was given a total of 1 cc. of the macerated tissue suspension, one-half of the contents being injected subcutaneously and the remainder intradermally. An area in the region of the inguinal lymph glands was selected as the site for administration in each instance.

In the first two series of experiments a total of 23 rats was involved (plus 11 controls) and the treated animals were carefully observed over a period of three months, after which they were sacrificed and examined. The third series, those receiving the reaction pus, were autopsied after an interval of six weeks. The species and sex of the rats employed are recorded in Table 2.

No changes suggestive of an adaptation of the parasite to the new host were noted either at the places of injection or in the regional lymph nodes. Stained smears of the latter frequently showed acid-

fast organisms, but such findings are without significance in the absence of suggestive histological changes. It is well known that when dead organisms are injected as our material was they are frequently encountered in smears of the glands made several months later.

These negative findings, which were anticipated because of previous experiences, left unanswered the question of an acquired immunity to the human leprosy bacillus on the part of the local animals. In an attempt to gain some information on this question 11 young rats had been imported from one of the neighboring islands

TABLE 2.—*Young rats injected intradermally and subcutaneously with human leprosy material.*

| Species | Comminuted nodule | | Broken down nodule | | Reaction pus | |
|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females |
| *M. norvegicus* | 4 | 5 | 4 | 2 | 2 | 2 |
| *M. rattus* | 1 | 2 | 2 | 3 | 2 | 1 |
| Controls [a] | 3 | 2 | 4 | 2 | — | — |
| Total | 8 | 9 | 10 | 7 | 4 | 3 |

[a] These animals came from a neighboring island and were used as controls to detect, if present, an acquired immunity on the part of the local rats.

(Cuyo), where the incidence of the human disease is relatively low, and they were included as controls in the foregoing experiments. Examination of their tissues showed that they remained normal in every respect.

These experiments confirm our earlier studies, and the experiences of a host of other investigators, that under the conditions encountered in nature human leprosy is not transmissible to rats. In this connection the possibility of an unknown intermediate host which might so modify the human parasite that it would later infect rats can not be excluded.

## OTHER OBSERVATIONS

The notations with reference to pathological conditions other than leprosy that were made during the autopsies of the wild rats examined will now be given.

*Bacillus whitmori.*—Melioidosis (25) is a disease of increasing interest and importance. The causative agent, *B. whitmori*, has been readily isolated from well over a hundred fatal human cases, thus affirming the diagnosis. At present the geographic distribution has been confined to Burma, Malaya, French Indo-China and Ceylon. The malady is primarily an affliction of rodents, and there is considerable evidence that the rat is the natural host. The source of the human ailment is still shrouded with mystery. It is believed with some basis that the organisms enter by way of the alimentary tract, possibly through the consumption of food which has been contaminated with animal excreta. Since the rat is a notorious traveler, it is the opinion of the workers in the so-called endemic area that a general search of these animals for the organism elsewhere will reveal a rather widespread distribution of the bacillus. If this prediction is ultimately established the disease will be of more than academic interest to the general clinician. In the present investigation *B. whitmori* was sought for both in the lungs and the heart's blood of the rats, but in every instance the cultures were negative.

*Bacillus pestis.*—The presence of plague in rats is always of vital importance to workers in the Far East. It has been repeatedly established that these animals are directly responsible for the cases of the human infection in a community and, in addition, that they are the medium through which the malady is carried from one locality to another. The gross lesions of this disease are characteristic and, if present, diagnostic. The lack of these cardinal manifestations in the rats examined is conclusive evidence of the absence of the disease. Plague has not been epidemic anywhere in the Philippines for over twenty years, and has never occurred at Culion.

*Spirochaeta icterohemorrhagiae.*—This organism, the causative agent of Weil's disease, has been found in wild rats in various parts of the world. *M. norvegicus* and *M. rattus*, as well as other species, are known to serve as reservoirs for it. The involvement usually persists for long periods of time, and during this interval the urine of the animal is highly infectious. In an attempt to detect the spirochaete during the autopsies the blood was examined; furthermore, composite samples of the urine of several animals were made by withdrawing each specimen with a syringe directly from the bladder, and these were injected into the peritoneal cavity of guinea-pigs. Sp. *icterohemorrhagiae*, if present, incites a characteristic disease. No organisms were encountered in the blood specimens and the injected guinea-pigs remained normal.

*Spirochaeta morsus muris.*—Rat bite fever is widely distributed, and though it is often considered endemic in the tropics the frequency of its occurrence does not seem to be affected appreciably by climate. The rat is commonly considered to be the vector of *Sp. morsus muris*, the germ associated with the disease. In this animal the infection simulates syphilis in man, and its course may be divided into well differentiated stages. During the secondary stage, which may persist for weeks and months, there is a synchronous bacteriemia. In the absence of lesions in the rats examined it was felt that a direct examination of the blood might reveal the presence of this germ, but no spirochaetes were observed in any of the blood preparations.

*Trypanosoma lewisi.*—This trypanosome is a common inhabitant of the blood stream of rats in all parts of the world. Its wide distribution is ample evidence of the propensities of the rat for traveling. The most satisfactory method for the diagnosis of trypanosomiasis in these animals is that which was employed, namely to examine a droplet of fresh blood and observe the violent agitation of the red blood cells resulting from the lashing of the body and flagellum of the organism. Dark field illumination is a valuable aid but not absolutely essential for the rapid examination of such preparations; with some experience a worker can readily detect the presence of spirochaetes and protozoa by using only a regulation Abbé condenser and a 1/12 oil immersion objective.

The specimens from the first 73 animals were monotonously free from organisms, but that from rat No. 74, a half-grown female *M. rattus,* showed the presence of myriads of trypanosomes morphologically similar to *Tr. lewisi.* Blood films treated with Wright's stain clearly revealed the structure typical of this species. The tubes of NNN medium inoculated with the blood of this rat, after incubation in air enriched with 10 per cent $Co_2$ (21) gave luxuriant growths of the cultural forms (Herpetomonas) of *Tr. lewisi.* With this stimulus the blood examinations and cultures were continued and in total fourteen infected rats were detected. They were distributed as follows: *M. norvegicus,* 1 male and 3 females; *M. rattus,* 3 males and 7 females. The parasitized animals were practically all half-grown, and the records indicated that they came from only five areas. This spotted distribution is in keeping with our experience in the findings of *Tr. lewisi* in wild rats in Ann Arbor and in Puerto Rico. A number of the old rats were injected with blood containing the organisms but they proved to be immune, perhaps as the result of an early infection. Several young rats born in captivity were found susceptible.

*Sarcocystis muris.*—Considerable time was devoted to a search of the skeletal muscles for the presence of this parasite, but it was not detected.

*Helminths.*—It is to be expected from the habits of rats that they will harbor many worm infestations. The relation of these internal parasites to the diseases of humans has been unequivocally established in perhaps only one instance, the trichina worm. The rat is the permanent reservoir for this form, and were it completely eradicated a substantial contribution would be made to the control of this scourge. In the present survey no nematodes or round worms were found, though it should be said that *Trichina spiralis* was the only representative diligently searched for. As an interesting corollary the local patients are quite generally infested with hookworms and representatives of ascarids. The cysticercus of *Taenia crassicollis* (*Cysticercus fasciolaris*) was observed encysted in the livers of 28 of the rats (13.2 per cent). The actual numbers of parasites varied; one rat harbored 7, four contained 3 each, eight had 2, and the remaining fifteen 1 each. When ingested by cats this cysticercus develops into an adult tapeworm, and the devouring of infested rats may account for the deplorable appearance of the cats at Culion. There is no evidence that man has been parasitized by the form in question. The larval cestode *Cysticercus cellulosae* when introduced into the alimentary tract of man develops into the tapeworm *Taenia solium.* This parasite has been reported as encysted in the peritoneum of wild rats, but it was not encountered in this study.

DISCUSSION

The discovery in 1903 of a condition in wild rats resembling leprosy was immediately envisaged as the prelude to the unravelling of the many mysteries shrouding this age-old affliction of man. The likeness of rat leprosy to the human malady is striking in its bacteriological, pathological, clinical and, to some extent, its endemiological characters. Until this condition was observed no disease had been recognized or artificially incited among the lower animals which had any similarity to leprosy, and exhaustive search during the past 32 years has failed to displace this infection from the unique position it occupies.[7]

Stefansky, in the title to his original paper, refers to this condition as a leprosy-like disease, and Dean's early interest in it resulted from the seeming counterpart it bore to the disease of man. Both of these authors were quite guarded in suggesting that the two entities might be identical. Nevertheless, such an interpretation was somewhat strengthened by their inability to cultivate artificially from the rat disease a germ which retained the morphological and tinctorial attributes possessed by the tissue-invading parasite.

Dean did succeed, however, in isolating from two of the affected rats growths of a diphtheroid bacillus. This observation interested him because the bacilli had a marked morphological likeness to cultures isolated from leprosy by Babes (3) and a number of other investigators, but he did not believe the two diseases to be caused by the same agent. This attitude was rather firmly intrenched in the minds of the students of that period and was aptly expressed by Wherry in 1908 (30): "There is no evidence that human leprosy and rat leprosy are identical or that human beings need fear infection from leper rats." Kitasato (11) in the following year reiterated the same opinion: "From our present knowledge we cannot say whether human and rat leprosy are identical."

The discovery of a lower animal susceptible to the organism of human leprosy would be of great significance. The lack of a susceptible laboratory animal has been, and still is, one of the chief obstacles to an understanding of this infection. In the absence of such an animal leprologists naturally welcomed the findings of a readily transmissible disease in rodents that simulated leprosy, and considerable optimism was attached to investigations of this naturally-occur-

[7] Wade, in a personal communication, has called my attention to a recent monograph by L. W. M. Lobel, of Buitenzorg, Java, describing "Lepra bubalorum," a leprosy-like disease of water buffalo in that region (Lepra bubalorum, Veeartsenijkundige Mededeelingen No. 81, Department van Economische Zaken Nederlandische-Indië. Utrecht, 1934), and says that the condition must be very limited in distribution because he has seen no previous report of anything like it, and that veterinary authorities in Manila who are familiar with the buffalo in the Philippines and Indo-China state that they have never encountered any such malady.

ring infection of rats in the belief that information might be gained which would explain the many enigmas associated with the disease in man.

Dean demonstrated the ease with which the animal strain could be perpetuated in rats, which is in distinct contrast to the refractoriness of all animals, including man, to artificial infection with the human disease. He also found the rodent strain to be species-specific; tissue emulsions regularly virulent for rats failed to infect guinea-pigs, rabbits, mice and monkeys. Several experiments, not prejudiced by opinions, attempted to show the unity of the two diseases by serological tests. Mezincescu (19) and later Schmitt (24) obtained positive complement-fixation reactions with human leprosy serum and antigens prepared from tissue that contained Stefansky's bacillus. It is only fair to state that their conclusions were never generally accepted because of the lack of consistent results in the fixation tests with pure cultures of acid-fast organisms.

In a recent publication Asami (2) reported the finding of 17 leprous rats among a total of 2,157 collected in Japan. He cultured specimens of tissue from all 17 and obtained 12 strains of acid-fast bacilli from 11 of them. These cultures were virulent for wild rats as well as for white rats, white mice, guinea-pigs and rabbits. Inasmuch as these data are not in accord with the usual observations in this disease it will be best to appraise them conservatively until they have been corroborated.

Walker, from epidemiological and bacteriological studies, construed human leprosy and rat leprosy to be the result of invasion by a common organism—an actinomyces—which is widely distributed, the soil being its natural habitat. Strains of this microorganism isolated from patients with leprosy, from infected rats, and from the normal soil were identical. He found support for this theory in the well established fact that in leprosy the patients often designate the feet or legs as the location of the initial lesions. According to this view man is infected by contaminated soil through breaks in the skin of the lower extremities, and the causal agent finds entrance into the tissues of the rat in a similar manner.

It was to obtain more definite information on the relation of human and rat leprosy that the experiments herein reported were undertaken. There was ample evidence of intimate exposure of the rats to infected material, both by ingestion and by contact with soil, sampling of which demonstrated a heavily infected environment. On purely theoretical grounds, therefore, it was believed that the local rats, after many generations in these fertile surroundings where they have been constantly exposed to the agent of the human disease, should be heavily infected if it were infectious to them. But our examination failed to detect a single case.[8] If, as Professor Marchoux (14) states, rat leprosy is world-wide in its distribution, one would expect ultimately to find the usual incidence of rodents infected with

[8] Denney (8) says: "Ten gray rats, caught at this hospital, were autopsied. They showed no sign of leprosy."

Stefansky's bacillus at Culion, irrespective of the presence of the patients. Since leprous rats are regularly encountered in Japan, it will be merely a matter of time before the infection appears among the rodents throughout the Philippines, if it is not already present.

The etiologic relation of Hansen's bacillus to leprosy rests on circumstantial evidence only, since all attempts to fulfill Koch's postulates have failed. However, endeavors to meet the requirements of these rules have not been destitute of reward, as the germ is quite regularly associated with the disease and lacking in its absence. Proof regarding the significance of Stefansky's bacillus in rat leprosy has been more readily established, notwithstanding the failure of artificial cultivation, because of the ease with which it can be transmitted serially in its host.

Many fruitless attempts have been made to transfer the human disease to the rat by the injection of large quantities of comminuted tissue containing countless numbers of organisms. Because of this fact the majority of workers have rejected the theories of Walker (Muir, 1930, [20]) as well as those subsequently advanced by Salle. These negative results have again been confirmed in the present study. The administration of similar tissue emulsions obtained from three sources failed to incite an infection, though the presence of a postulated acquired immunity was eliminated by proper controls. Admitting the plausibility of the statement that most of the acid-fast forms one finds in the tissues in human leprosy are dead, the viability of the organisms found in rat leprosy is equally problematical, yet it is recognized that the amount of tissue suspension required to induce the infection in rats is rather small.

Walker and Sweeney obtained cultures of their organism from 24 out of 37 animals (65 per cent) without gross lesions of disease. Our own cultures proved sterile as regards bacterial growth in every instance.

The absence of the spontaneous condition at Culion would seem to answer the question: Should rats fear infection from man? This is the corollary of the early query relative to the danger of rat leprosy to man. My observations apply to healthy, evidently well-fed wild rats. Rats on deficiency diets, as Kingsbury ([10]) has recently shown, exhibit the same resistance to the human disease as do ordinary animals.

When leprosy is compared with tuberculosis, a concept which has gained favor as a logical analogy, it is very probable that human

and rat leprosy had a common etiology in the distant past, as no doubt did human and bovine tuberculosis. But with the passage of time this affliction of man and rodent was differentiated; thus each has become a specific disease entity.

## SUMMARY AND CONCLUSIONS

Patients with leprosy have been segregated continuously for the past twenty-nine years at the Culion Leper Colony, on one of the small isolated islands of the Philippine archipelago. The inmate population at the present time numbers about 6,700. Throughout this period the local rats have been intimately exposed to the human infection through scavenging in infected waste material from wards, clinics, dormitories and private residences, occasionally even by actual ingestion of pathological tissue from dead bodies or even sleeping patients, and by contact with the heavily infected soil.

It has been frequently alleged that the causative agents of human leprosy and of rat leprosy are identical. An examination of rats captured at Culion should reveal unequivocal proof of the validity of this contention as regards the existence of the disease in nature. Accordingly, 212 rats were trapped alive; 83.3 per cent were identified as *Mus norvegicus,* the remainder *M. rattus.* Of the former group 46 were old animals, 12 were half-grown, the others full-grown; all but 10 of the black rats were recorded as fully mature. No external gross pathological lesions were found, though each animal was scrutinized for alopecia and ulcerations, also for mutilations and for evidence of affections of the nose and eyes. Under deep anesthesia the abdominal and thoracic walls, including the axillary and inguinal glands were examined, and the cavities were opened and their contents removed. No suggestion of broncho-penumonia or nephritis (both cardinal involvements in naturally occurring rat leprosy) or other abnormalities were present in the gross or in sections examined histologically. Blood was withdrawn from the heart, direct smears and stained preparations were made, and the blood was cultivated on Petragnani's medium and rabbit blood agar.

There were no macroscopic or microscopic findings suggestive of the presence of an infection with Stefansky's bacillus. This study would therefore indicate that, under what might be considered ideal conditions in nature, rats are not subject to infection with the causative agent of human leprosy.

Comminuted human leprous tissue from a leproma and a broken-down nodule, and also acute reaction-pus, were injected into the skin and subcutaneous tissues of 23 half-grown rats (15 *M. norvegicus* and 8 *M. rattus*) in an attempt to transfer the human disease to the rodents. The possibility of immunity on the part of the local animals was controlled by including in the series injected 11 young rats caught on a neighboring island; these controls suffered no untoward effect.

A careful search was made for evidence of infection with *Bacillus pestis, B. whitmori, Spirochaeta icterohemorrhagiae and Sp. morsus muris,* direct microscopic examination and cultures of the blood being made; all were negative. *Trypanosoma lewisi* was detected in the direct blood smears of 14 of the animals (4 *M. norvegicus,* 10 *M. rattus*). This protozoön was cultured from all of the parasitized animals on NNN medium in an atmosphere enriched with 10 per cent of carbon dioxide. *Cysticercus fasciolaris* was dissected out of the livers of 28 of the rats (13.2 per cent) in which it was found encysted. *Cysticercus cellulosa* was not found. Neither *Trichina spiralis* nor *Sarcocystis muris* was observed.

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