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THE EXPERIMENTAL TRANSMISSION OF RAT LEPROSY TO THE GOLDEN HAMSTER (*CRICETUS AURATUS*)*

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These investigations were carried out with the object of ascertaining if the golden hamster was susceptible to the disease in rats caused by *Mycobacterium leprae murium* when inoculated experimentally with infected tissue.

Sugai (1908-09) records the transmission of human leprosy to Japanese dancing mice and Duval (1910) confirms this. Reference is made by Muir (1930) to the transmission of rat leprosy to this species of mice by both these workers. Marchoux (1922) describes the case of a patient suffering from a disease resembling although not identical with human leprosy, material from whom, when inoculated into white rats, caused a disease identical with rat leprosy. Muir and Henderson (1927-28) were unable to infect Japanese dancing mice and they record also the insusceptibility of the Chinese hamster to experimental infection with rat leprosy. Other workers for the most part report disappointing results.

Douglas (1935, personal communication) found the hamster to be susceptible to both human and bovine types of tubercle bacilli. The author was able to confirm these findings. A dose of 5 mg. of an active culture of either type, injected by the intraperitoneal route, killed two out of three hamsters in 5 weeks in each of two groups, the organism being recovered from the liver and spleen in each case. The third hamster in each group showed marked evidence of infection when killed 8 weeks after inoculation.

Four hamsters were inoculated with 50 mg. each of the avian tubercle bacillus and killed at intervals ranging from 2 to 6 months

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after inoculation: these showed no signs of infection, either macroscopically or in direct smears and sections of the liver, spleen and lung. All cultures proved sterile.

The hamsters used in all the experiments described in this paper were from the stock bred at this institute since May 1933. The strain of rat leprosy used was isolated by Douglas in 1922 from a natural case of the disease in a wild rat caught at Winchester and was kept alive by inoculation into albino rats.

EXPERIMENT 1

On 9th May 1935 two young hamsters were inoculated intraperitoneally with 0.25 cc. of a saline suspension of fresh material from an infected rat. The material injected consisted of omentum, liver and spleen, ground up in a sterile mortar without sand. Sufficient saline was added so that 1 cc. of the suspension contained approximately 0.4 g. of infected tissue. Two young albino rats were given the same dose of the same material to act as a control to the viability of the material used.

HAMSTER 1

Twenty-seven weeks after inoculation this hamster was found in a comatose condition and was killed with chloroform. The general condition was poor, skin dry and scaly, hair scanty. Post-mortem examination showed lesions very similar to those seen in advanced cases in the rat, except that the omentum was not so immensely thickened. The liver was enlarged, pale and firm, with a hard white line round the edges of the lobes, and the interlobular connective tissue was visible to the naked eye. The spleen, which was enlarged to 4 or 5 times its normal size, was paler in colour and firmer than normal. The omentum was thickened and contained numerous nodules composed of large groups of acid-fast organisms. In the thoracic cavity similar nodules were found on the surface of the diaphragm. The anterior lobes of both lungs were dark red in colour and showed a considerable degree of consolidation. Smears made from the cut surfaces of the liver and spleen showed very numerous acid-fast organisms.

Microscopical appearances. Sections were made from the liver, spleen, lung, kidney and precrural gland, and examined for acid-fast organisms. All sections in these experiments were stained for 15 minutes with warm carbol-fuchsin, decolourised with 25 per cent sulphuric acid and counterstained with Ehrlich's haematoxylin.

Liver: The liver cells had been pushed aside by large clumps of organ-

isms and in some instances had been destroyed by the pressure; where the organisms were not so numerous the cells appeared normal. Numerous so-called "lepra" cells were present, consisting of endothelial cells packed with leprosy bacilli.

Muir, Henderson and Landeman (1927-28) note the destruction of the liver cells by the pressure of organisms in leprosy in the rat.

Spleen: This showed much the same picture as the liver; massive clumps of organisms were seen but there was a more general tendency for them to be fairly evenly distributed. There were large areas with a moderately thick but even distribution of bacilli, in which the cell structure had been almost destroyed, only the edges of the cells and the nuclei faintly retaining the stain. Small uninvaded areas showed little or no histological change.

Lung: While showing by no means the extreme invasion seen in the liver and spleen, the lung showed considerable thickening of the interalveolar connective tissue, which contained numerous small clumps of acid-fast organisms. In some places alveoli were occluded with exudate. The involvement of the lung as seen in this hamster was certainly more extensive than lesions seen by the writer in experimentally infected rats.

Lung lesions in the rat have not always been described. Dean (1905) mentions small lesions in the lungs of naturally infected animals. Currie and Hollmann (1911) found consolidated lungs in most natural cases of the disease but only in some did smears show a few acid-fast organisms, the lesions in many being small abscesses containing creamy pus. Other rats which were not affected with leprosy showed similar lesions but without the presence of acid-fast organisms. Marchoux (quoted by Muir, 1930) describes small lesions in the apices of the lungs in rats infected experimentally by the gastro-intestinal tract.

The author has observed lesions in the lungs of rats infected by the subcutaneous or intraperitoneal routes. The lesions observed consist of small dark red patches less than 2 mm. in diameter. Under high magnification these patches are seen to consist of one or more occluded alveoli. Clumps of acid-fast organisms are to be found in the surrounding connective tissue; these clumps may vary from two or three organisms to large masses completely surrounding the alveolus. Invasion of the epithelial lining of an otherwise normal alveolus has also been noted.

Kidney: This organ showed the most interesting differences from the lesions observed in the infected rat. In sections of the kidney from this hamster, stained by the usual method and examined under a high power, there were several areas of thickening of the interstitial tissue. These thickenings were chiefly due to clumps of organisms. Other small clumps were scattered through the interstitial tissue. These organisms were for the most part extracellular, but some endothelial cells of the glomeruli had been invaded.

McCoy (1908, 1913) mentions that nephritis is common in the rat in naturally occurring rat leprosy, the kidneys being enlarged, brown and friable, but no acid-fast organisms are present. Muir (1930) mentions that interstitial disease of the kidney is not seen in the rat infected with leprosy although nodular thickening of the serous covering is sometimes observed. Dean (1905) had previously noted this involvement of the serous covering only.

The author has noted that the kidney is usually normal in appearance and has never found acid-fast organisms in the substance of the kidney in a series of rats artificially infected and carefully examined over a period of three years.

With infected material from hamster 1 two further hamsters (nos. 8 and 9, *q.v.*) and two albino rats were inoculated.

HAMSTER 2

This hamster, inoculated subcutaneously at the same time and with the same material as hamster 1, was found dead 30 weeks after inoculation. The bodily condition was poor, the skin dry and the hair scanty. The lesions observed *post mortem* were well marked, although not so advanced as in the case of hamster 1. Sections were prepared from the liver, spleen, heart muscle, precrural gland, lung, kidney and testes.

The liver, spleen and gland all showed invasion by massive clumps of organisms. The lungs and kidneys also showed invasion to a lesser degree, lesions being similar to those seen in hamster 1.

Testis: While for the most part large clumps of organisms were situated just under the tunica, small clumps were also found distributed throughout the gland.

Heart: Sections of the myocardium showed small clumps of leprosy organisms situated between the muscle fibres. The fibres themselves were not involved.

Small nodules in the myocardium were observed by Dean in naturally infected rats.

HAMSTER 8

This hamster was one of the two inoculated (intraperitoneally) with material from hamster 1. Thirty weeks after inoculation it was killed in an emaciated condition. The skin was dry and scaly, hair scanty and the skin of the elbows and scrotum very much thickened, dry, wrinkled and grey in colour. Sections were examined of the liver, spleen, kidney, testis and skin of the elbow and scrotum.

Spleen: This was enlarged and showed very extensive invasion by large clumps of organisms.

Liver: This was normal in appearance macroscopically but when examined for the presence of leprosy organisms showed very numerous small clumps of acid-fast bacilli scattered through the tissue.

Kidney and testis: Both appeared normal and sections failed to reveal the presence of any acid-fast organisms.

Skin: This showed very considerable thickening and acid-fast organisms in large clumps were found in the subcutis. In this hamster these lesions are interesting as they were not adjacent to the site of inoculation nor was there any lesion at that site.

Dean (1905) mentions similar findings in the rat. Lampe, De Moor and Van Veen (1936) were able to infect rats by rubbing soil which had become contaminated from naturally infected rats into the freshly shaved abdomen.

HAMSTER 9

The second hamster, inoculated intraperitoneally with material from hamster 1, died thirty-two weeks after inoculation. Post-mortem examination showed involvement of both liver and spleen.

RATS 1 AND 2

The first of the two rats inoculated with material from hamster 1 was killed thirty-seven weeks after inoculation. A large swelling was found at the site of inoculation, with enlargement of the axillary lymphatics, gross thickening of the omentum and enlargement of the liver and spleen. Smears and sections all showed acid-fast organisms in very large numbers. The second rat, killed a fortnight later, showed similar lesions.

EXPERIMENT 2

Two hamsters were inoculated, one intraperitoneally, the other subcutaneously, with 0.25 cc. of a suspension of spleen from an artificially infected rat. Both were killed 20 weeks later and showed enlarged spleens and pale firm livers. Smears and sections from these organs showed numerous small clumps of acid-fast organisms scattered throughout the tissue. The kidneys were not infected.

EXPERIMENT 3

In February 1936 three hamsters were inoculated with 0.03 g. of material from an artificially infected rat. Two died within a month from injuries received while fighting. The survivor was found comatose sixteen weeks after intraperitoneal inoculation and was killed. Post-mortem examination revealed the presence of

about 15 cc. of clear watery fluid in the peritoneal cavity. There was also a small quantity in the thoracic cavity. Numerous acid-fast organisms were found in the centrifuged deposit of the fluid.

Sections of the liver and spleen showed extensive invasion by acid-fast bacilli. Sections of the kidney showed nodules on the serous covering containing acid-fast organisms, but the kidney itself was not apparently involved.

ADDITIONAL EXPERIMENTS

Four other small groups of hamsters were inoculated and most proved susceptible to infection with rat leprosy except when death intervened within two months of inoculation, in which case no visible lesions were seen nor were any acid-fast organisms seen in smears or sections of skin or organs. The following are the details of these small groups.

Group 6.—Three hamsters were inoculated with material from an artificially infected rat. Two died within 6 weeks from injuries: they were apparently uninfected. The third died 4 months after subcutaneous inoculation and smears and sections from its liver and spleen showed evidence of leprosy.

Group 7.—Two hamsters were inoculated, one intraperitoneally, the other subcutaneously, as second "passage" from hamster 2. One died 17 weeks and one 19 weeks after inoculation. Both showed leprosy lesions in the spleen and liver.

Group 8.—Three hamsters were inoculated, two intraperitoneally, one subcutaneously, with material from the hamster described in detail in experiment 3. All were killed 20 weeks after inoculation and all showed leprosy lesions in the liver and spleen.

Group 9.—Three hamsters were inoculated with material from one of the hamsters in group 7. One died from injuries, the other two, which had been inoculated intraperitoneally, showed leprosy lesions in the liver and spleen.

In none of the hamsters in these four groups were kidney lesions noted.

SUMMARY

1. The golden hamster has been found to be susceptible to experimental inoculation with rat leprosy by either the subcutaneous or intraperitoneal route.

2. The most constant lesions were in the liver and spleen but

in some instances the kidneys and lungs were also involved and interstitial nephritis has been noted.

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