RESULTS OF INOCULATION OF WHITE RATS WITH HUMAN LEPROSY BACILLI BY THE INTRANEURAL ROUTE PRELIMINARY REPORT

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INTRODUCTION

Since the discovery of the bacillus of leprosy by Armauer Hansen in 1873, experimental transmission of human leprosy to a suitable animal has remained an unsolved problem. Neither cultivation on artificial media nor transmission to living animals of this acid-fast organism has as yet been consistently successful. Innumerable attempts have been made to transmit it to each and all known experimental animals, employing almost all routes of inoculation. Successful transmission has been reported from time to time by various enthusiastic workers, but these reports have not been confirmed generally.

Dermatropism and neurotropism are two characteristic properties of *Mycobacterium leprae*; and, after its entry into the skin, neurotropism plays a dominant role over dermatropism. An attempt to transmit human leprosy to an experimental animal utilizing the latter property would therefore be worth undertaking. In the experiment here reported we chose a new method of inoculation, viz., the intraneural route by open dissection, using the white rat as the experimental animal. The principal inoculum was an albuminized suspension of leprous tissue rich in bacilli, because it has been reported by Hanks (1) that sterile bovine albumen enhances the infectiousness of *M. leprae murium*.

MATERIAL AND METHOD

Fifteen white rats were selected for the purpose. They were divided into three groups, each consisting of 5 animals. The first group was inoculated with normal saline (control), the second group with a saline suspension of the bacillus-rich leprous tissue, and the third group with the same suspension mixed with an equal amount of sterile egg albumen. The route of inoculation was intraneural, into the sciatic nerve by open dissection, ether being used as the anesthetic, and the dose of inoculum was 0.1 cc. in each animal. The dose of bacilli in the third group was one-half of that in the second group because of the dilution of the original suspension by the egg albumen. Each group of animals was kept in a separate cage, and the cages were housed in the same place.

OBSERVATIONS

Of the control group, two rats died 3 months after inoculation, and the remaining three died after another 2 months. The postmortem examinations did not reveal any significant changes, and smears from the lymph nodes, viscera and injected nerve were negative for acid-fast bacilli.

Of the second group, inoculated with the saline suspension of leprous tissue, two died after 2 months, and one was sacrificed 7-1/2 months after inoculation. The remaining two died 1 month later. Although a few bacilli were found in smears from the inoculation site in two animals—the one that was sacrificed, and one of the last to die—no evidence of multiplication of the bacilli inoculated was found, and no significant histologic change was observed in any organ.

Of the third group, inoculated with the albuminized suspension, one died 3 months after inoculation; smears and the histologic examination did not reveal any changes. A second one was sacrificed 3-1/2 months after inoculation. Enormous numbers of acid-fast bacilli were found in this animal, as described below. In two others (one sacrificed after 7-1/2 months, and one which died after 10 months) a few acid-fast bacilli were found only at the sites of inoculation. One rat of this group is still living.

The animal of this third group which was sacrificed after 3-1/2 months had developed on the right side of the chest a small, fluctuating, subcutaneous mass measuring about 4 by 2 cm., with depilation of the overlying skin. On opening the abdomen of this animal the internal viscera were found to be congested, the spleen enlarged and hemorrhagic. The axillary glands of both sides were also enlarged. Smears from the chest abscess, the lymph nodes, and the viscera showed enormous numbers of acid-fast bacilli. Histologic examination of sections of the different viscera and the chest abscess showed cellular infiltration of focal distribution and a tendency to form localized granulomas. The infiltrating cells were chiefly round and epithelioid ones. There was no evidence of giant-cell formation, and no vacuolated histiocytes were encountered.

Inoculation of material from the chest abscess onto Loewenstein-Jensen's, Petroff's, and glycerine agar media, yielded negative results.

To test the transmissibility of this infection, three white rats and two guinea-pigs were inoculated, on the day the cultures were made, intraperitoneally with a saline suspension (1.0 cc.) made from the caseating mass. This suspension was treated with acid and alkali before inoculation.

Of the three white rats inoculated in this first passage, two died 2 days after inoculation. The third rat was sacrificed after 6 months. One of the guinea-pigs died after 6 months, the findings in smears from the different organs negative; the other guinea-pig is still living.

Examination of the rat sacrificed after 6 months revealed, macroscopically, moderate-degree enlargement of the liver and spleen. Bilaterally in the axillae there were two small subcutaneous masses, each measuring about 2.5 x 1.25 cm. and there was enlargement of the regional lymph nodes. Smear examination from the different viscera, lymph nodes, and the subcutaneous masses showed a fair number of acid-fast bacilli indicating a slight degree of multiplication. Histologic studies of the viscera revealed cellular infiltration of focal nature.

A saline suspension was prepared from the axillary masses and the spleen of this animal. Three white rats and three white mice were inoculated with this suspension (0.5 cc.) intraperitoneally, and they were kept in separate cages. They have been under observation for only 1-1/2 months at the time of writing. Media commonly used for pathogenic acid-fast bacilli were also inoculated with the saline suspension, but yielded negative results.

The whole of the procedure described is summarized in Table 1.

TABLE 1.—*Summary of the observations reported.*

I.	Original experimental group inoculated with an albuminized leproma suspension; 5 white rats (intraneural).		
	Rat 1	Died after 3 months	Negative
	Rat 2	Sacrificed, 3½ mos.	Generalized infection with subcutaneous abscess on chest
	Rat 3	Sacrificed, 7½ mos.	A few acid-fasts at site of inoculation
	Rat 4	Died after 10 months	Negative
	Rat 5	Living	—
II.	First, passage, from subcutaneous mass of Rat I 2; 3 white rats and 2 guinea-pigs (intraperitoneal).		
	Rats 1 & 2	Died after 2 days	—
	Rat 3	Sacrificed, 6 mos.	Small subcutaneous axillary masses, bilateral.
	Guinea-pig 1	Died after 6 months	Negative
	Guinea-pig 2	Living	—
III.	Second passage, from axillary masses and spleen of Rat II 3; 3 white rats and 3 white mice (intraperitoneal).		
	Rats 1-3	Living	—
	Mice 1-3	Living	—

DISCUSSION

From the observations reported it is evident that an acid-fast bacillus multiplied in one of the white rats inoculated intraneurally with an albuminized suspension of leproma, and that this organism was

passaged to one of a second group of white rats inoculated by the intraperitoneal route, with the production of subcutaneous lesions in the axillae and with bacilli found in smears made from the viscera. There was no evidence of multiplication of bacilli in any of the other four original rats inoculated in the same manner with the plain saline suspension of the same leproma material.

Noteworthy is the fact that none of the culture media favorable for the acid-fast group of microorganisms that were inoculated showed any trace of growth of this organism, nor did it cause lesions in the two guinea-pigs included in the first-passage experiment. This excludes the possibility that the infection observed was due to *M. tuberculosis*.

The problem remaining to be solved is whether the organism encountered is *M. leprae* or some other acid-fast organism, especially the Stefansky bacillus. There is some indication that it is Stefansky's bacillus, as it caused an infection in the only one of the first-passage rats which survived for a sufficient length of time—although it did not cause lesions in the peritoneal cavity, the site of the inoculation. The result of the second passage into white rats and mice is awaited.

If the organism proves really to be the Stefansky bacillus, that will indicate that chance infection of experimental animals inoculated with human leprosy material is possible in laboratories where work with both the human and the Stefansky bacilli is being carried out; and that the greatest caution should be exercised in carrying out inoculation experiments in rodents with human leprosy bacilli and pronouncing any or every acid-fast bacillus encountered in such experiments to be the human bacillus. This also brings out the importance of verification of a supposed strain of human organisms in such experimental animals by attempting to pass it on to a second batch of white rats or mice. If the isolated organism is readily transmissible to these animals, it indicates the probability that the Stefansky bacillus is being dealt with. It is possible that other methods, for example electron microscopy of the bacilli, may also reveal their true nature.

SUMMARY

1. An attempt has been made to transmit the human leprosy bacillus to white rats by injecting the exposed sciatic nerve with a suspension of bacillus-rich leprous tissue mixed with an equal amount of sterile egg albumen.

2. One of the five rats so inoculated showed evidence of massive infection with an acid-fast organism when sacrificed three and one-half months after inoculation. In an attempt to ascertain the actual nature of this acid-fast organism, material from this rat was passed by intraperitoneal inoculation to other rats and to guinea-pigs; and a second similar passage has been made, too recently for the results to be known,

to rats and mice from lesions which developed in one of the first-passage rats.

3. Negative results of cultures and of the guinea-pig inoculations indicate that the acid-fast bacillus encountered is not the tubercle bacillus or any other readily-cultivated mycobacterium. The occurrence of an infection by acid-fast bacilli in the rat of the first passage group which survived sufficiently long suggests that the bacillus may be the Stefansky bacillus and not the human leprosy bacillus.

4. This observation brings out the great importance of excluding chance infection with Stefansky bacillus of rodents inoculated experimentally with human leprosy material, especially in laboratories where work with both human and rat leprosy is being carried out.

RESUMEN

1. Se trató de transmitir el bacilo de la lepra humana a las ratas blancas inyectándoles en el nervio ciático mayor exteriorizado una suspensión de tejido leproso rico en bacilos con una cantidad igual de ovialbúmina estéril.

2. Una de las cinco ratas inoculadas en esa formal, al ser sacrificada a los tres meses y medio de la inoculación, reveló signos de infección masiva por un microbio ácidorresistente. Tratando de averiguar la verdadera naturaleza de este microbio ácidorresistente, se pasó material de esta rata por inoculación intraperitoneal a otras ratas y a cobayos; y se ha hecho otro pase semejantes, aunque demasiado recientemente para conocer los resultados, a ratas y ratone usando material procedente de las lesiones que aparecieron en una de las ratas del primer pase.

3. Los resultados negativos de los cultivos y de las inoculaciones en el cobayo indican que el bacilo ácidorresistente encontrado no es el tuberculoso ni tampoco es ninguna otra micobacteria de fácil cultivo. La existencia de una infección producida por bacilos ácidorresistentes en la rata del grupo del primer pase que sobrevivió por tiempo suficiente sugiere que el germen puede ser el bacilo de Stefansky y no el bacilo de la lepra humana.

4. Esta observación pone de relieve la mucha importancia que reviste la exclusión de la infección fortuita por el bacilo de Stefansky en los roedores inoculados experimentalmente con material de lepra humana, sobre todo en laboratorios en que trabajan con lepra tanto humana como murina.

REFERENCE

1. HANKS, J. H. Relationship between the metabolic capacity and the infectiousness of *M. leprae murium;* refrigeration studies. Internat. J. Leprosy **22** (1954) 450-460.