

HISTIOCYTIC GRANULOMATOUS MYCOBACTERIAL LESIONS  
PRODUCED IN THE GOLDEN HAMSTER (*CRICETUS AURATUS*)  
INOCULATED WITH HUMAN LEPROSY

NEGATIVE RESULTS IN TEN EXPERIMENTS USING OTHER ANIMALS<sup>1</sup>

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In January 1956 a cooperative project was undertaken between the Armed Forces Institute of Pathology, Washington, D.C., and the U.S.P.H.S. Communicable Disease Center, Laboratory Branch, Chamblee, Georgia, for the purpose of attempting to infect laboratory animals with *Mycobacterium leprae*.

To date more than 35 inoculation experiments with *M. leprae* have been undertaken in approximately 1,500 small animals—guinea-pigs, hamsters, white rats, white mice and hairless mice—and in 31 monkeys. The only unusual animal was the hairless mouse, used in the hope that the absence of hair would make it suitable for the purpose, but the results have been negative.

All the small animals used in these experiments have been kept in a building at Chamblee used exclusively for the purpose. No work with the rat leprosy bacillus or any other etiologic agent has been carried on in this building.

The first aim was to produce progressive local lesions at the inoculation sites. Except for the testis the inoculations were intracutaneous or cutaneous, the latter signifying inoculation by scarification and/or multiple punctures.

The experiments were designed to employ certain methods of reducing host resistance, principally total body irradiation and/or cortisone administration. The animals for the typical full experiment have been divided into five groups: (1) irradiation, (2) irradiation plus cortisone, (3) cortisone, (4) inoculation only, and (5) control, injected with heat-killed inoculum. The cortisone dose for hamsters was 5 mgm. the first week, and 2 mgm. in succeeding weeks. Irradiation was applied once, usually one day before inoculation but sometimes longer, up to a week.

<sup>1</sup>Presented at the VIIth International Congress of Leprology in Tokyo, November 12-19, 1958. The manuscript made available, drastically slashed to keep within a time limit, had originally been prepared in large part as a commentary of slides (40 in total) to be shown on the screen, the audience being also provided with three mimeographed sheets of data. In the version here published an attempt has been made to assemble the essential data and to reduce the comments on slides to more orthodox text. The product is published with the approval of the author.—EDITOR.

Lepromatous leprosy selectively affects the prominences of the skin, the superficially-placed peripheral nerves, the anterior segment of the eye, the upper respiratory tract, and the testes. Thus, a feature common to all anatomic sites preferred by *M. leprae* is a temperature that is relatively lower than that of the internal organs. While undoubtedly there must be many factors required for the successful multiplications of *M. leprae* in the human being, it seemed obvious that in planning animal experiments the temperature factor should be given serious consideration. Consequently, the cooler parts of the body were chosen for inoculation.

The predilection of *M. leprae* for peripheral nerves has also been taken into consideration, by inoculating the ulnar and femoral nerves of monkeys and by inoculations through scarification of the skin in various animals.

(A slide was exhibited which showed the recorded average temperatures for small groups of golden hamsters kept under varying conditions. This showed the testis to be approximately 10°F cooler than the temperature of the body, and the ear approximately 26°F cooler.)

The sources of the material inoculated have been the Philippines and the United States. Drs. Rodriguez and Guinto of the Philippines assisted in obtaining skin specimens from untreated lepromatous cases, which specimens were solidified in CO<sub>2</sub> and preserved at very low temperatures until used. The material from the United States was kept in thermos bottles packed with wet ice until used.

The skin specimens were prepared by homogenizing with a Ten Broeck all-glass hand tissue grinder, the grinding facilitated by adapting the instrument to a cone-drive motor stirrer. The concentration of the bacilli in the inocula varied from 10 to approximately 100 per oil immersion field. A small amount of india ink was added to the suspension for purposes of identification of the injection sites later on.

In all of the experiments control animals were inoculated with material that had been boiled for at least 20 minutes.

## RESULTS

### EXPERIMENTS A TO J

Regarding the first ten experiments, A through J, which had been started in January 1956 and completed by August 1958, none of the 571 animals used—albino hamsters, albino rats, white mice, and white guinea-pigs—showed any suggestion of a progressive disease. The inocula used were prepared from lepromas from untreated cases in the Philippines, the specimens stored at -50° to -60°C until used. Other details are given in Table 1.<sup>2</sup>

Strikingly different results were obtained in two experiments designated by the letters N and Q: In each of these the male golden hamster were used, the inoculations being made into the ears and testes.

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<sup>2</sup>This table contains the essential material embodied in one of the mimeographed sheets distributed by the author before the paper was read.—EDITOR.

TABLE 1.—Summary of inoculation experiments A to J, completed after two years with negative results.

Experiment; date begun	Animal (and sex)	Sites and methods of inoculation	Regimen groups						Radi- ation and date	Experi- ment com- pleted
			Radiation	Radiation/ cortisone	Cortisone	Inoculum only	Control (killed)	Total animals		
A 1/25/56	Hamster, albino (M&F)	Back, intra- cut. and cutan. <sup>a</sup>	—	—	—	33 <sup>b</sup>	33 <sup>b</sup>	33 <sup>b</sup>	—	
B 1/25/56	Mouse, white (F)	Back, intra- cut. and cutan.	—	—	—	54	26	80	—	6/24/58
C 2/16/56	Rat, albino (M&F)	Back & tail Intracut. & cutan.	—	—	—	30	10	40	—	3/21/58
D 3/7/56	Rat, white (M&F)	Back & tail Intracut. & cutan.	10	10	10	6	6	42	200r 3/5/56	3/21/58
E 4/11/56	Hamster, albino (M&F)	Back Intracut.	10	10	10	10	10	50	250r 4/10/56	9/24/58
F 5/10/56	Guinea- pigs (M&F)	Back & ears Intracut. & cutan. <sup>c</sup>	10	10	10	10	9	49	250r 5/9/56	7/14/58
G 3/23/56	Mouse, white (M)	Back & tail Intracut. & cutan.	15	15	15	15	30	90	200r 3/22/56	6/2/58
H 6/5/56	Mouse, white (M&F)	Back & tail Intracut. & cutan.	15	20	15	15	15	80	300r 6/4/56	7/14/58
I 5/26/56	Mouse, white (M)	Tail, cutan., Intrates- ticular	16	20	15	15	15	81	300r 5/21/56	7/14/58
J 7/25/56	Hamster, albino (M)	Testes only	—	5	—	15	6	26	300r 7/18/56	4/18/58

<sup>a</sup> Cutaneous = inoculation by scarification and/or multiple puncture.

<sup>b</sup> Left side of back inoculated with live inoculum, right side used for the control inoculation with heat-killed inoculum.

<sup>c</sup> On one side of the back the inoculation was made into an area of skin previously infiltrated with mineral oil, and the other side was inoculated in an area previously infiltrated with cottonseed oil.

#### THE N EXPERIMENT

There were 10 of the hamsters in each of the 5 groups of this experiment, their weights 100-165 gm. The inoculations, made on March 7, 1957, were of a suspension of three Carville specimens refrigerated with wet ice for 72 hours, and one Philippine specimen that had been preserved with solid carbon dioxide. A graph (slide) illustrating the survival rate of these animals showed that the animals receiving cortisone, Groups N2 and N3, died relatively quickly.

The first of these animals which exhibited granulomas on histopathologic examination was one in the N4 group which died 24 weeks after inoculation, the lesion in the testis. This hamster had received nothing except live inoculum.

The first of 3 slides of a section from the testis of this animal showed minute darker areas which, under higher magnification, were histiocytic granulomas consisting of a round mass of uniform cells surrounded by smaller and darker cells. These uniform cells are histiocytes (which, if preferred, might be called macrophages or reticuloendothelial cells).

Histologically this lesion was entirely different from anything that I had seen previously in any of the animals of other experiments. With the Fite-Faraco stain, many of these histiocytes contained from 1 to 5 short, thick, well-stained acid-fast bacilli.<sup>3</sup>

The next granuloma was seen in an animal of the N4 group which died 32 weeks after inoculation. This was a solitary lesion, much larger than that seen in the slides from the previous animal. Under higher power it was again composed of uniform large cells of the histiocyte type, a few lymphocytes to be seen in the periphery of the picture. On oil-immersion examination of a section stained for acid-fastness there were found, as in the previous granuloma, short, thick bacilli in small numbers.

Although these granulomas occurring in testes of the two N4 hamsters which had died at 24 and at 32 weeks were of interest, no attempt was made to interpret their meaning at the time of the histopathologic examination. As time went on granulomas appeared in the testes of two other hamsters—one of which (of the N4 group) died at 37 weeks, the other (of the N1, irradiation group), at 52 weeks. They were of about the same size and composition as the previous ones. The testis of another N1 animal, which died 56 weeks after inoculation, showed three granulomas of fair size (slides). These appeared histologically like the others, and the number of bacilli was essentially the same.

Seventy-two weeks after inoculation another animal of the N1 series died. A section of the testis revealed a number of granulomas, some solitary and others coalescent. There is (in slides) the same monotonous histiocyte pattern seen in other lesions, but many more acid-fast bacilli in the histiocytes of this 72-weeks hamster than had been seen in the earlier granulomas. Nearly all of the histiocytes contained bacilli, and in some as many as a dozen were seen.

At this time there remained one animal in the N4 series, and this was killed at 76 weeks primarily for passage. One of the testes showed a lesion, and a block for histologic study was removed. A section (slide)

<sup>3</sup>The staining process referred to as "Fite-Faraco" is the one reported by Fite, Cambre and Turner in 1947 [*Arch. Path.* **43** (1947) 624-625], fundamentally different from the Faraco process and sometimes called the Fite II method [WADE, H. W. *Stain Technol.* **32** (1957) 287].—EDITOR.

taken through the midpart of the testis shows that the granulomatous process had replaced all of the testis with the exception of a thin fringe of persisting tubules. Histologically this granulomatous process was similar to the others in the N series. The Fite-Faraco stain revealed numerous bacilli.

One of the ears, also, was found to contain a sessile small nodule, a histiocytic granuloma, but (higher magnification) of a somewhat different cell pattern than before. The cells were somewhat more spindle shaped, probably due to the increased tissue pressure of the ear as compared to that of the testis. Oil-immersion examination revealed numerous intracellular acid-fast bacilli. In two small nerves on the opposite side of the ear cartilage from the granuloma, up to 40 intracellular bacilli were to be seen.

In a picture (slide) of a high power field of the inoculation site of the ear of an animal in which growth did not occur, made approximately one year after inoculation, no persisting bacilli are to be seen. There is only the carbon pigment used to mark the inoculation site. Another picture (slide) is from the testis of a control animal, also approximately one year after inoculation. That it represents the site of the inoculation is shown by the small foci of persisting india ink particles, but there is no evidence of an infection and no acid-fast bacilli were demonstrated.

In summary of Experiment N: In 2 of 10 animals of the irradiation-only group (N1), and in 6 of the 10 animals in the group that had received nothing other than the live inoculum (N4), granulomas were demonstrated. These granulomas had developed very slowly. The first one was observed 24 weeks after inoculation. There was no appreciable increase in the number of demonstrated intracellular bacilli until a year and one-half post-inoculation, at which time practically all of the histiocytes contained bacilli, and in some of them as many as 12 bacilli were observed. In the animal that had survived 76 weeks, one testis was completely replaced by a granulomatous process in which nearly all of the histiocytes contained numerous bacilli. This animal demonstrated in one ear a granulomatous nodule composed of histiocytes heavily populated with bacilli. Intraneural invasion was seen in two tiny nerves nearby, but not within the lesion. No granulomata were seen in the animals of the control group (N5), which had a survival rate approximately the same as the animals receiving only the live inoculum.

*Transfer inoculations.*—After a block for histologic study had been removed from the lesion-bearing testis of the 76-weeks N4 hamster, the rest of that organ was ground up in 10 cc. of saline. A smear of this suspension contained innumerable acid-fast bacilli. Passage was made to the testes of 20 hamsters, late in August 1958. I expect to wait four or more months before sampling these animals to learn the results of the transfer inoculation.



## THE Q EXPERIMENT

The Q experiment was patterned along the same lines as the N one. Again male golden hamsters were used, and the animals were divided into 5 groups of different regimens, although in somewhat different proportions. The inoculations were begun nearly three months after those of the N experiment.

This experiment differed from the previous one in that most of the animals were given 3 inoculations, approximately one month apart, late in May, June and July. The first inoculation, made into the ears and testes, was of a suspension of 3 frozen skin specimens from the Philippines preserved in CO<sub>2</sub> ice and a low-temperature electric refrigerator for approximately 10 months before use. One month later the testes were inoculated with a preparation of a dimorphous lesion from Washington preserved approximately three days in a thermos bottle with wet ice. One month following this, the testes of the Q1-2-3 animals (only) were inoculated with a preparation composed of three other frozen specimens from the Philippines.

All of the animals receiving cortisone (Q2 and Q3) died relatively rapidly, but granulomas of the testis similar to those seen in the N experiment were found in two of the Q2 (cortisone-irradiation) animals which died at 20 and 21 weeks. After 32 weeks the death rate in this experiment remained quite stationary until, for some reason not evident, the Q1 (irradiation only) animals began dying after 65 weeks. On routine histologic examination granulomatous lesions were observed in the majority of the animals dying after that time. There are several animals of this Q experiment still living. At this time 6 of the 10 Q1 animals have demonstrated granulomas.

The two earlier granulomas of the 20- and 21-weeks Q2 animals were histologically very similar to those that appeared in the N series, and contained relatively few short intracellular bacilli.

A section of the testis of a Q1 animal that died after 68 weeks (slides) shows coalescing granulomas composed as usual of histiocytes. These lesions were practically identical with those appearing in the N series, and in the number of bacilli and general appearance they were very similar to that one in the N series found in an animal dying at 72 weeks. There were, however, probably fewer bacilli in the testis of this animal.

Of special interest was one ear of this animal, in which there was a barely perceptible sessile nodule, composed of histiocytes containing numerous acid-fast bacilli (slides). In many cells the cytoplasm was almost completely replaced by globular masses or packs of these organisms. Bacillus-containing histiocytes extended between the muscle and cartilage of this ear for a distance approximately 1 cm. from the principal nodule. The sections (slides) show in this area several tangential sections of a nerve, in which intracellular bacilli are demonstrated. One field (slide) shows these bacilli to be numerous, some appearing as globular masses.

Also of interest is a section taken through the granuloma of a testis of a Q4 animal that died at 70 weeks, the lesion cells with numerous intracellular bacilli. The ear of this animal contained two small (3 mm.) nodules, they again being of granulomas rich in bacilli. Another Q4 animal that died at about the same time had a testicular lesion only.

In summary of Experiment Q: To date, 6 of the 20 Q1 (irradiation) animals have shown granulomas. In 4 of them there have been granulomas in the testis, and all 6 have shown granulomas of the ear. Two of the 20 Q2 animals had microscopic granulomas in the testis. Two of the 5 Q4 animals showed granulomas of the testis, and in one of them there was also a granuloma of the ear.

#### COMMENT

The monotonously uniform histiocytic composition of the granulomas in these animals, and the paucity of other inflammatory cells, affords a close parallel to developing lesions of human lepromatous leprosy. The extremely slow progression of the lesions was another feature resembling human leprosy. Intraneural invasion by bacteria was noted in a few instances.

There was no appreciable vacuolization of the histiocytes, nor were there any giant globi. However, if *M. leprae* grows in the histiocytes of an animal as slowly as it does in man, one would not expect to find foam cells or giant globi in a lesion only 18 months after infection.

#### SUMMARY

In two experiments reported in which golden hamsters were used, histiocytic mycobacterial lesions have been demonstrated at the inoculation sites, testes and ears. No such lesions were observed in ten other experiments, in which other animals (including albino hamsters) were used.

There was no evidence that irradiation as used enhanced the growth of granulomas. The cortisone animals died too quickly to permit weighing the results.

While the lesions demonstrate much similarity to those of human lepromatous leprosy, the ultimate evaluation must await the critical study of the mycobacteria of the lesions.

#### RESUMEN

En los dos experimentos descritos en que se emplearon roedores del género *Cricetus auratus*, se descubrieron lesiones micobacterianas histiocíticas en los sitios de inoculación, los testículos y las orejas. No se observaron tales lesiones en otros diez experimentos en que se usaron otros animales (incluso *Criceti* de la especie albina).

No hubo signos de que la irradiación acrecentara el crecimiento de los granulomas. Los animales tratados con cortisona murieron demasiado pronto para poder avalorar los resultados.

Aunque las lesiones muestran mucha semejanza a las de la lepra lepromatosa humana, la justipreciación definitiva tiene que aguardar el estudio analítico de las micobacterias de las lesiones.