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Experimental Transmission of Human Leprosy to the Golden Hamster[1]

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Until recent years, attempts to inoculate animals with Hansen's bacillus never resulted in infection transmissible in series. Following Binford's hypothesis ([1]), according to which the pathogenic agent of leprosy finds ground suitable for its development in parts of the body with a relatively low temperature, several authors have carried out inoculation attempts on rodents in the foot pads, the ears, and the testes. Only inoculations in foot pads of mice regularly have shown bacillary multiplication localized at the injection point. No diffusion of Hansen's bacillus in the animal organism, however, is observed, even after several passages.

Since 1959 we have undertaken numerous human to hamster experiments, using principally intratesticular and intraperitoneal methods.

MATERIALS AND METHODS

Inoculation material was obtained always from cutaneous lesions of lepromatous patients (with the exception of one, all were patients from our department), either untreated or treated with DDS for one or two weeks (25 mgm. per day), with the exception of one under theoretic DDS treatment. These patients had contracted the infection in various parts of the world (the Antilles, Madagascar, Tahiti, Indochina). In some cases the disease had just appeared; in others there had been setbacks after cessation of treatment of about two to four years. The cutaneous lesions, which were relatively recent (several months to one year), were either papules or nodules, but in all cases the lesions contained numerous bacilli, mainly homogeneous.

In certain cases the material was stocked in the refrigerator at about 0°C and inoculated the same day; in others, it was stocked in the refrigerator for from two to seven days; finally, in one case, it was transported for 36 hours at normal temperature.

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The cutaneous fragments were cut into small pieces with scissors and then crushed in a porcelain mortar, and physiologic salt solution was added. The suspension thus obtained therefore contained, in addition to the bacilli (isolated bacilli, globi and clumps), tissue particles and often a large quantity of debris of porcelain or enamel from the mortar; this suspension was inoculated either as it was, or after decanting into a stem-glass, or after centrifuging for one or two minutes, or even after filtering through paper.

The quantity of bacilli in the suspension was highly variable, ranging from one bacillus to 100 bacilli per oil immersion objective field.

Experimental animals. Golden hamsters, weighing between 50 and 90 gm., whose diet was made up of wheat, maize, bread, cabbage and carrots, were used. During the first series of experiments, 20 male hamsters were inoculated, 16 intratesticularly and four intraperitoneally. During a second series of experiments, consisting of seven groups of animals inoculated with specimens from four different patients, five hamsters were inoculated subcutaneously in the testicular region and 27 males and six females intraperitoneally. Each experiment was made up of few animals, either because of the small quantity of material, or because the same material was used, in addition, for inoculation of 40 male and female hamsters by other routes.

Dose inoculated. 0.1 to 0.2 ml. of suspension was used for intratesticular inoculations, 0.2 to 0.25 ml. for subcutaneous inoculations in the testicular region, and 0.2 to 0.4 ml. for intraperitoneal inoculations.

Observation periods. These were from three and a half to 25 months.

Autopsy. A thorough search was carried out for bacilli in smear preparations, at the level of the primary focus, in the lymph nodes, and in the organs. Histopathologic examination of the testes was carried out in the majority of cases (staining by Fite, Cambre and Turner's methods). When there was sufficient material, we undertook passages, as well as cultures on Loewenstein-Jensen medium. In these respects it should be noted that it is impossible to carry out at one and the same time a close systematic examination, and passages and cultures as well, with slightly infected animals.

RESULTS

The first series of experiments had to be interrupted, as the inoculated hamsters were contaminated indirectly in the animal house by rats infected with murine leprosy. This contamination was shown by study of passage animals.

A verification, carried out by keeping uninoculated control hamsters in the animal house, showed that *M. lepraemurium* could not be found in all these animals after about 12 months in these premises, a fact demonstrating the hamster's extreme susceptibility to this bacillus. We recommenced our attempts, therefore, in premises where animals infected with *M. lepraemurium* had never been kept.

All of the male animals inoculated intraperitoneally or subcutaneously in the testicular region, and which survived for more than three and a half months, with the exception of two (that is, 29 out of 31), showed progressive infections, whose departure point was implantation of bacilli in the various tunics of the testis, and we have been able to specify the characteristic aspects of the infection.

On the other hand, except for very rare exceptions that will be dealt with later, animals in the second premises, inoculated by various routes, and females inoculated intraperitoneally, showed no infection; therefore they were used as control animals for the second series of experiments. we were thus able to verify the fact that this time there was no contamination by *M. lepraemurium* (in 25 hamsters that survived more than 12 months), and to observe that the inoculated bacilli, even if they could be destroyed after several months, could equally persist much longer, either at the inoculation site, or in the lymph nodes, without evolution.

Furthermore, animals that died soon enabled us to learn the quantity of germs

to be found after inoculation, together with their localization in the organism.

In all cases where we were able to carry them out, cultures on artificial media were negative, a fact excluding the possibility of superinfection by tubercle or paratubercle bacilli.

I. LOCAL INFECTION

A. Description of the Infection

1. Intraperitoneal inoculations. Our observations relate to 27 male hamsters of the second series of experiments (noncontaminated premises), 26 of which survived more than three and a half months, and to six females.

(a) *Fate of inoculated materials:*—In the first periods observed, in males and females that died early, the majority of the bacilli and the globi were first found dispersed at all levels of the abdomen; they were seen in smear preparations made by scraping the abdominal wall at various points, and in smears of the omentum and the mesentery (20 days); for the most part they were very rare and search for them was laborious. Histologic sections of a hamster that died after three and a half months enabled us to locate them as follows: in the subepithelial layer of the tunica vaginalis of the testis, and within the thickness of the latter, isolated histiocytes, or, more frequently, groups or strings of histiocytes, were found, frequently containing numerous highly granular bacilli, and mortar debris. These pictures were apparently of phagocytosis with destruction. A few epithelial cells of the serosa contained a very few bacilli.

It was evident that very rapidly the bacilli were captured by the lymphatic system and carried some distance away, to be found in various abdominal and thoracic lymph nodes (20 to 36 days). In the tissues, as well as in the nodes, the bacilli could be found for about four to six months. After about eight months they had almost disappeared.

(b) *Implantation of the bacilli in the tunica of the testis:*—This was observed fairly early, particularly at the level of the caudal end of the epididymis. One particularly clear example was noted on examination of sections of the hamster that died after three and a half months. In addition to the phagocytosis pictures, perfectly homogeneous and well-stained bacilli were found at the level of the epididymis, in the tunica vaginalis, in the free state, and dispersed here and there in the fibers or between the fibers of the stroma, usually isolated, but sometimes lined up end-to-end or grouped in small heaps, as well as in the fixed connective cells (approximately one to three bacilli per cell). They were rare, on the whole, but more numerous in places, particularly at the level of the scrotal ligament. Rare bacilli were visible, here and there, in the intermuscular connective tissue of the walls of the organ, and in the pellucid septa as far as the subcutaneous level. Apart from these localizations, the presence of a few disseminated histiocytes containing one to three homogeneous, well-stained bacilli could be noted.

(c) *Evolution of lesions:*—At six and a half months, infection was still developing in certain places at the level of the epididymis. In the connective tissue of the tunica vaginalis and the tunica albuginea, the appearance was similar to that just described, but the bacilli were more numerous and often localized in small groups. They were also somewhat more numerous in the intermuscular stroma, disseminated or in very small groups, as far as the subcutaneous level.

However, involvement of the various cells was also observed. The bacilli were frequent here in the endothelial cells of the capillary vessels and the venules. Preparations made from animals that died at this stage also revealed their presence in endothelium of the lymphatic capillaries. At this point a few isolated bacilli were observed in the striated muscular fibers of the organ walls.

The intrahistiocyte multiplication of the bacilli was now well established, although it was relatively scanty; it was noted particularly at the level of the muscle, and also at the subcutaneous level almost up to the upper part of the testis.

At eight and a half months, the picture

was comparable, but richer and more widespread. The bacilli free in the stroma were numerous and dispersed almost everywhere; at the level of the epididymis, dense groups and exceptional globi were found in the fixed connective cells of the tunica vaginalis and the tunica albuginea. A few, still homogeneous, were found rarely in some striated muscle fibers and in some nerve filaments. Histiocyte infiltration was greater and more widespread, and, in this case, rose along the tunics as far as the upper pole of the testis. The histiocytes contained numerous homogeneous or granular bacilli and several globi.

Among the animals as a whole the lesions were not always situated at the same place. Sometimes, instead of spreading toward the exterior, they predominated in depth immediately around the tubules of the epididymis. The bacilli were then situated in the stroma and the nonstriated muscular fibers of the walls of the tubules; they were chiefly within histiocytes in the interstitial tissue.

The more the infection was prolonged, the more the bacillary multiplication was intense and widespread locally. The nerve filaments might be widely invaded by free or intrahistiocyte bacilli. From 15 months onward, actual cavities made by the bacillary clumps could be observed in the striated muscular fibers. In two hamsters that died at 18 and 22 months, respectively, numerous bacilli were seen, forming compact clumps and even globi in the nonstriated fibers of the dartos muscle.

The bacilli situated in the stroma, the muscular cells, the lymphatic and blood endothelium, and free in the nerve filaments, were always perfectly homogeneous. The bacilli in the histiocytes might be homogeneous, particularly in recent infections, but were often granular, and at times highly so.

We have chosen to describe the most typical examples, which were also the most numerous. In several cases, although the cellular localizations were typical, infection remained minimum even after a long time. In other cases, special cellular localizations were very rare and local infection was chiefly within histiocytes.

Smear preparations made from subcutaneous and dermal tissues, the muscle of the organ walls, and the ligaments, also enabled early observation of bacilli at the level of the peritesticular focus. The quantity and aspect of the bacilli could also be appreciated. They were at times highly polymorphous, with a certain proportion of fragmentary or granular bacilli. The most typical aspect, however, and one always noted, was the presence of homogeneous, rectilinear bacilli, often thick, of varying sizes, but often very short and at times highly so. They might also take the form of coccobacteria or diplococci. They were often grouped in parallel formation, in small palisades, or in globi. These highly characteristic aspects distinguished them clearly from *M. lepraemurium* in particular.

2. Subcutaneous inoculations at the level of the testis. Only five hamsters were inoculated in this manner. The results were entirely similar to those already described. In the sections, juxtaposition of two phenomena was observed: pictures of phagocytosis formed by histiocytic areas or nodules, sometimes extensive, containing mortar debris, in which the bacilli were in a state of degeneration or destroyed, and also pictures corresponding to bacillary implantation and multiplication, with their special cellular localizations. Involvement of the nonstriated fibers of the dartos muscle was particularly frequent in this case because of the location of injection.

3. Intratesticular inoculations. These were made in hamsters of the first series of experiments, i.e., those that were later superinfected with murine leprosy. Observations made during the first stages (animals dying before 12 months), however, appeared noteworthy, as did the characteristic aspects of local lesions, observed again in more prolonged infections and noted to be quite different from those presented by infections with *M. lepraemurium*. We supposed, therefore, that *M. leprae* had become implanted in the organisms of these animals and there was, thus, without doubt, coexistence of the two infections.

Out of 16 hamsters that died between five and 19½ months, three of which were

examined by smear preparations and 13 by histologic sections, only one, which died after 17½ months, showed bacillary multiplication inside the testis in smear preparations.

On the other hand, as early as five months after inoculation, we observed, in the sections, bacilli in the different tunics of the testis, from the albuginea as far as the tegument of the skin, and in the surrounding cellulo-fatty tissues; the lesions were situated most frequently between the testis and the epididymis or around the epididymis. The number of bacilli found, and the local extension of the infection, which was slight at the beginning, increased with time. Above all, we noted special cellular localizations of the bacilli, already described in the section on intraperitoneal inoculations; these we observed clearly in eight histologic examinations out of 13.

Locally, intratesticular inoculation therefore appeared to us to be the equivalent of subcutaneous, intramuscular and intraperitoneal inoculation at one and the same time, probably because the bacilli were deposited at the level of these tissues by the passage of the needle during the injection. This fact led us to use these different routes later.

4. Intradermic inoculation in the ear. Although it does not come within the framework of this report, but in order to make certain comparisons, we wish to call attention to local aspects observed after intradermic inoculation in the ears of a hamster that died after 18½ months. Free bacilli were numerous, isolated, and in dense clumps in the stroma. Some were found in a few striated muscle fibers, isolated or in clumps. Bacilli were numerous in the endothelium and also in the lumina of capillary blood vessels. We even found some isolated examples in the cutaneous epithelium as far as the stratum corneum, and also, although rarely, in the cartilaginous cells. All these bacilli were well stained and homogeneous. Granular or homogeneous bacilli were found also in histiocytes, but in smaller number. These characteristics were observed in foci between which there were fairly large areas free from infection; local dissemination, therefore, seemed less diffuse here than in the testicular tunics.

B. Overall Interpretation

1. Early fate of inoculated materials. Inoculation was followed by passive transfer of the bacilli over greater or lesser distances. Regardless of the place where they were injected, the inoculated products (isolated bacilli, clumps, globi, foreign bodies, such as tissues, mortar debris and, in certain cases, India ink) led to significant macrophage phagocytosis, unquestionably intensified by the presence of foreign bodies, on the one hand, locally in isolated histiocytes or grouped in areas, and, on the other hand, at a distance in lymph nodes, sometimes remote, to which they had been transported. It should be noted that after intraperitoneal inoculation, the inoculated products were first partially captured by the peritoneal epithelium and then transported into the subjacent stroma.

A small number of free bacilli were dispersed locally within the stroma, an invariable phenomenon due essentially to the movement of interstitial liquids in circulation. These isolated bacilli avoided phagocytosis, and therefore were enabled to survive.

2. Destruction and absence of evolution at the animal's normal temperature. At the point of inoculation, e.g., subcutaneously in the flank, the bacilli discovered, which were numerous at first, diminished with time and disappeared altogether after a variable but relatively long period. In fact, it was noted that they might persist up to 20 or 22 months.

In the lymph nodes to which they were transported at the outset, the bacilli were sometimes destroyed fairly quickly, i.e., within a few months, but in some cases they persisted, although few in number, up to 17 or 20 months.

Usually, most of these nonevolutive bacilli assumed a residual aspect, becoming granular or swollen, although some remained homogeneous for a long time, in apparently good condition. Inoculated globi of human origin were also noted,

which degenerated gradually until they were scarcely discernible.

3. Progressive evolution in regions of low temperature. In the region of the testicular tunics, the coexistence of two processes was observed, viz.:

(a) *Bacillary destruction in phagocytes:*—Destruction could be observed clearly after intratesticular and subcutaneous inoculation in the testicular region. In zones where there was mortar debris, the bacilli, which were numerous at first, degenerated gradually. After seven to eight months we found none in the interstitial tissue of the testes, and after 14 months none in the subcutaneous tissue. After intraperitoneal inoculation the same phenomenon was evident, but more difficult to detect because it was highly dispersed.

(b) *Adaptation to the animal; later local extension by contiguity and special cellular involvement in the primary focus.*—Free bacilli in the stroma, sheltered from phagocytosis, were able not only to survive but also to adapt themselves to their new host, and, afterward to multiply and spread locally. It is probable that this adaptation could take place only after sufficient time for the bacilli to (1) multiply in the stroma itself, a process revealed as early as at three months and a half, and (2) adjust themselves to new histiocytes. The latter process was perhaps longer; after three and a half months, whereas there were already several small clumps in the connective tissue fibers, only one to three bacilli per histiocyte were found. This was not a question of phagocytosis, since toward five to six months the bacilli were more numerous, and these histiocytes were situated at a distance from the regions of destruction.

It would be tempting to think that this adaptation to the histiocyte can occur only after this period of latency (three to four months) in the stroma, but we are not in a position to state this positively.

In time the bacilli became more numerous and spread along the connective tissue fibers following the direction of the tissues in all the tunics from the albuginea to the tegument. A special type of cellular affinity appeared. The bacilli began to penetrate the nonstriated or striated muscular fibers (after about five to six months) in the majority of cases observed; involvement of the endothelial cells of the lymphatic capillaries and blood capillaries (after about six to seven months) and the nerves (about eight months), occurred less frequently, perhaps partly because of the fact that they were seen less often in the sections. The tendency of the bacilli to infiltrate individually in the stroma was doubtless responsible for this invasion. In these various tissues the bacilli were homogeneous, and well stained, in contrast with the aspect of the intrahistiocyte bacilli, which were almost invariably granular. They multiplied, forming small clumps and sometimes even globi rather similar to globi in human tissue.

Multiplication in the histiocytes intensified likewise. It was distinct, although relatively scanty, from five to five and a half months, a little more abundant at six and a half months, and toward eight to 10 months, it became more important than multiplication in the tissues.

Bacillary dissemination was insidious and diffuse, whatever its cellular localization. The histiocytes, particularly, were scattered, usually isolated or in small areas. In prolonged infections, the characteristic aspects increased in intensity; toward 15 months the bacilli were numerous, isolated and in small clumps, in the nonstriated muscles; the bacillary clumps could lead to formation of true lacunae in the striated fibers. Histiocytes also became more numerous and more loaded with bacilli; toward 15 months they might invade the nerve filaments in notable fashion. When infiltration was important, they were grouped in fairly large areas in places.

The local primary lesion could be considered as clearly established approximately between seven and ten months. It rarely went beyond the testes and might even remain belatedly confined at the level of the epididymis; it might, however, reach the tunics of the second testis when inoculation was carried out on one side only.

Macroscopic lesions were never observed, even in the most significant infections; exceptionally, minute noduli of encystation of the inoculated products could be found.

II. EXTENSION OF THE INFECTION AT A DISTANCE

Extension from the primary peritesticular focus occurred at a distance, and apparently in three ways: lymphatic system, blood stream, and step by step by contiguity.

1. Extension by lymphatic system. (a) *Lapse of time and intensity:*—Extension by the lymphatic route was constant when the lapse of time was sufficient, and was the first to appear. However, intensity and lapse of time were highly variable among animals. Satellite lymph nodes were obviously the first affected. The inguinal nodes were usually the first, becoming positive toward six or seven months; a little later, toward nine or ten months, the iliac and aortal nodes were affected. In the most unfavorable cases, however, none might be affected before 20 months. Between six and ten months there was a critical period when the presence of very small numbers of bacilli in the abdominal nodes had no significance, because of the possible persistence of inoculated bacilli.

Later, the first nodes were enriched in bacilli and the farthest nodes affected in turn. This distant extension usually occurred at the deepest level, around the thoracic nodes, which were affected in rare cases after about 15 months; it occurred less frequently by a superficial route toward the axillary nodes, which were rarely affected and then always slightly, at the earliest between 14 and 15 months. Later extension occurred toward the submaxillary nodes, which were more slightly and even more rarely affected from 22 months onward.

(b) *Appearance of the bacilli:*—In smear preparations the bacilli, which were in fact intrahistiocytic but seen rather in dispersed form as a result of crushing, had an appearance almost always polymorphous, but which recalled the appearance observed in the peritesticular focus, viz., homogeneous bacilli, rectilinear, well stained, of various sizes, but often short, and frequently grouped in small palisades or even globi. On the other hand, irregular or fragmented bacilli were often seen, or swollen or pale examples, apparently in degenerated state, which could be included in fuchsinophil cells or cytoplasmic fragments, or in refringent cytoplasmic inclusions. These appearances might correspond to a certain resistance on the part of the animal or rather to difficulty of adaptation on the part of the bacillus, which did not in any way prevent the infection from spreading to a distance.

2. Extension by contiguity. This was not always apparent and was late, since it occurred step by step along the ligaments and intermediate tissues, the cellulo-fatty tissues, the vasculo-nerve bundles, and perhaps by way of the peritoneum. In fact, bacilli could be found in smears prepared from the omentum, as well as smears of the paravertebral tissues at the level of the abdomen and as far as the thorax. These smears were at times positive from 14 to 15 months onward, but large numbers of bacilli were observed only later (from 22 months onward) and then rarely.

3. Involvement of organs. This occurred perhaps by continuity, and also without question by the blood stream. Whatever the route of inoculation, we have observed it very rarely, i.e., in six cases out of 23 hamsters in the series of experiments in which the animals were not contaminated and in which they survived more than 14 months, i.e., animals that died at 14½, 15½, 17, 18½, 22 and 25 months. The liver was the principal organ affected, but we have found bacilli several times in the lungs and spleen, and twice in the bone marrow (18 and 25 months). The bacilli, always very rare, had to be searched for minutely in the smears. Their appearance was variable, sometimes recalling that observed in the lymph nodes. The bacilli, homogeneous or in poor condition, were often short, and usually grouped in small clumps.

4. Arguments in favor of extension. Mitsuda tests carried out on six hamsters inoculated 21 to 22 months previously and later found to be positive for bacilli, were negative.

Tuberculoid histology was never observed, at least in the primary focus (nor in the lymph nodes, but there our histologic examinations were few). At the level of the primary focus, bacilli were found frequently and often precociously in the endo-

thelium and even in the lumina of the lymphatic and blood capillaries. Intrahistiocytic multiplication of the bacilli could lead to their disisemination by migration of the histiocytes.

The high temperature factor might prevent development of bacilli secondarily dispersed in the organism (in the same way as during inoculation). Since extension of the infection is a fact, it bears witness to a new possibility of adaptation of the bacillus, that of multiplying in warm areas of the organism, at least secondarily.

This extension should also take place in the hamster after inoculation in the foot pads and ears, but probably much later. We have observed, 18½ months after inoculation in the ears, that the retroauricular lymph nodes were affected, and in three hamsters and two mice, inoculated in the foot pads, slight involvement of the popliteal nodes at 10 and 11 months.[3]

The results of our experiments are summarized in Tables 1 and 2.

III. PASSAGES

1. Results during passages. Valid passages are still too few, because of lack of adequate material, and too recent. For this reason we cannot draw clear conclusions. The following are noted briefly:

(a) *Passages effected by the same favorable routes as inoculations from man:*—In our opinion they must, of necessity, succeed; if they are very early, they correspond to the transfer of the bacilli to another animal and therefore act as a primary inoculation.

We had three hamsters that died after five, seven and 13 months. The evolution of the infection and the time lapses were comparable to those observed in the primary inoculation.

(b) *Passages effected by various routes:*—These were negative in five cases and positive in two hamsters (one male, one female) inoculated subcutaneously in the groin. During these first few passages, the appearance of the bacilli was comparable to that observed in hamsters inoculated from man. Histopathologic examination also showed, in the tunics of the testis, all the characteristics already described.

2. Transformation of the bacillus by adaptation. The fact that the bacilli can, in the long run, multiply secondarily in the warm areas of the hamster's organism, as shown by bacillary dissemination, might lead to the belief that the bacillus is transformed. We can settle this question only later, especially if the passages by various routes are shown to result positively, either after sufficient duration of the infection in the hamster on primary inoculation, or after several passages by favorable routes.

IV. CASES EXCEPTIONAL TO THE RULE

These include several progressive infections in hamsters inoculated from man in any warm area of the organism. They were exceptional, trifling and late (21 to 26 months). We observed them only in three male hamsters inoculated subcutaneously, one in the flank and the other two in the groin.

It may be supposed that following inoculation, several bacilli were transported to the testes and became the departure point for infection. In fact, in the three cases, we found a few bacilli in the subcutaneous tissue in this area. It is possible too that, in the bacillary population inoculated, several "mutants" existed that were capable of adapting themselves directly to the warm area. This possibility would depend principally on the strain inoculated.

DISCUSSION

I. Value of Facts Acquired

1. Validity of experiments. The results of our experiments appear valid to us because of their constancy. In the second series of experiments, exempt from superinfection, out of the 31 hamsters inoculated in favorable ways, 29 were positive (one of the two negatives died after only four and a half months). The pictures observed were the same whatever the route of inoculation (intratesticular, sub-

[3] In mice inoculated in foot pads and examined at intervals between seven and 24 months, Rees also observed involvement of the inguinal nodes in 5 per cent of cases.

cutaneous in the testicular region, and intraperitoneal, in males). Out of 26 histologic examinations carried out on the positive hamsters, 24 had a characteristic appearance.

2. Factors likely to exercise influence on the progress of infection. Individual variations of susceptibility in hamsters appeared to us to be the most important factor concerned in differences noted up until now

TABLE 1. *Results in male hamsters after intraperitoneal inoculation.*

Experiment No.	Duration observation (mos.)	Multi-tissue involvement	Degree of bacillary infection			
			Primary focus	Satellite lymph nodes	Other lymph nodes	Liver
281	14½		++	+++	+	−
	22	yes	++	+	+	−
317-I	18		++	+	±	−
	24	yes	++	++	+	−
	24	0	−	−	−	−
317-II	14½	yes	++	++	±	+
	22	yes	+	+	±	−
	23	yes	++	++	+	−
	25	yes	++	+	−	−
318	3½	yes	+	−	−	
	6½	yes	+	+	−	
	12½	yes	++	++	−	−
	21½	yes	+	++	±	−
319-A	8½	yes	++	+	−	
	11	yes	++	++	−	−
	25	yes	+++	+++	+	+
319-B	17		+++	++	±	±
	23	yes	++	++	±	−
	24	histiocyte	++	++	+	−
319-C	4½	0	−	−		
	18	yes	++	+	−	−
	22	yes	+++	+++	+	−
321	15½	yes	+++	++	+	±
	19	yes	++	++	−	−
	22	yes	+++	+++	+	+
	25	yes	+	++	±	−

TABLE 2. *Results in hamsters after subcutaneous inoculation in the testes.*

Experiment No.	Duration observation (mos.)	Multi-tissue involvement	Degree of bacillary infection			
			Primary focus	Satellite lymph nodes	Other lymph nodes	Liver
	7	yes	++	+	−	
293	20	histiocyte	+	±	−	−
306	22½	yes	++	++	+	−
309	13½	yes	++	++	−	−
	18½	yes	++	+++	±	+

in our results. There are unquestionably individual animals that are only slightly receptive (five cases of slight infection out of 29 cases, even after lapse of from 18 to 25 months).

On the other hand, we observed no differences whether the patient providing the leproma had been treated for several weeks with DDS or not, whether the material was inoculated at once or after several days, whether the suspension was more or less rich in bacilli, or whether it contained foreign bodies or not.

However, in addition to the necessity for a good suspension containing a majority of homogeneous and viable bacilli, theoretically it should above all contain isolated bacilli, i.e., the only ones likely to spread in the stroma, and the least possible amount of tissue and foreign bodies in order to reduce histiocyte intake. Furthermore, with intraperitoneal inoculation, although our experiments were successful with fairly small quantities (0.2 to 0.4 ml.) it appears preferable to us to inoculate a larger quantity, at least 0.5 ml., in order to obtain satisfactory dispersion of the bacilli.

3. Low temperature factor. This factor, which is of first importance for implantation of the germ, seems to have been determinant in our experiences. In the case of animals inoculated in warm regions of the body infection evolved only exceptionally (three times in 20 cases of hamsters surviving more than 12 months), very late, and probably by initial passive transfer of the bacilli into a favorable region.

Two females inoculated in the peritoneum and surviving for more than 12 months, showed no infection. The one male hamster inoculated intraperitoneally, completely negative after sufficient lapse of time (24 months) was from a batch of three hamsters inoculated at too early an age (weighing about 50 gm. at the time of inoculation).

Table 3 presents the details of three experiments.

The primary focus takes hold in the tunics of the lower pole of the testis and the caudal end of the epididymis. Extension by contiguity after inoculation at the level of one testis occurs toward the other rather than upward. The tunica vaginalis is known to be a favorable site in numerous experimental infections (e.g., mycosis, rickettsiosis); it is not, however, for this reason that the bacilli were able to develop in our animals, since they took hold similarly in the different tunics (muscle, subcutaneous tissue, dermis), and infection developed in comparable manner when inoculation was subcutaneous in this region.

4. Comparison of results in our experi-

TABLE 3. *Results showing that infection takes hold only in low temperature regions of body*

Experiment No.	Inoculation	Sex	Duration observation (mos.)	Result
281	Intraperitoneal 0.25 ml.	male	14½	+
	" "	"	22	+
	Subcutaneous, groin 0.20 ml.	male	19	−
	" " "	"	21	−
317-II	Intraperitoneal 0.3 ml.	female	17½	−
	" "	male	14½	+
	" "	"	22	+
	" "	"	23	+
	" "	"	25	+
319-A	Intraperitoneal 0.4 ml.	female	12	−
	" "	male	8½	+
	" "	"	11	+
	" "	"	25	+

ments with those with *M. lepraemurium* infection in the hamster and *M. leprae* infection in man. (a) *Essential characteristics observed in our hamsters:*—In addition to the necessity for implantation of the bacilli in a low temperature area, the most noteworthy fact in our observations is success in obtaining, in the primary peritesticular focus, bacilli having not only a characteristic morphologic appearance, but also the quite special cellular localizations already described. These localizations, furthermore, are not specific for the peritesticular region. We first found them scarce (at five months) and then in abundance (at 18½ months) in the ear sections of hamsters after intradermic inoculation in this region. Moreover, when the bacilli spread to the organs, the liver was the first and most frequently affected.

(b) *Comparison with* M. lepraemurium *of the hamster:*—In contrast, *M. lepraemurium*, being directly adapted to the animal, takes hold wherever it is inoculated. Thus, intraperitoneally infection takes hold immediately in various regions of the abdominal cavity in small foci, as may be seen in a preparation of the omentum, and in the abdominal lymph nodes, as well as precociously in the thoracic nodes. It rapidly reaches the abdominal organs, principally the spleen and the suprarenal glands, the spleen being affected before the liver and sometimes increasing to 30 or 40 times its normal weight.

Multiplication of the bacilli takes place immediately, very rapidly, especially in the histiocytes, and infiltration is usually massive. *M. lepraemurium*, especially in advanced infections, may certainly present many localizations in addition to that in histiocytes, and especially may be free in the stroma.[4] Nevertheless, in the tunics of the testis we have never seen bacilli in the nonstriated or striated muscular fibers, or in the nerves, and when they attack the muscle of the tunics it is through the medium of the histiocytes, which proliferate in the intermuscular stroma. When they are inoculated in the testes, they develop there, within histiocytes, with massive infiltration of the interstitial spaces.

Morphologically, *Mycobacterium lepraemurium*, in the hamster, is usually quite different from the organisms we observe after inoculation of *M. leprae*. The organisms are sinuous and often have the appearance of a string of grains. These differences are, of course, not absolute, and have only orientation value. Particularly *Mycobacterium lepraemurium* may assume the aspect of lacquered globi sometimes comparable to the globi of *M. leprae*.

(c) *Comparison with human leprosy:*—Without considering here all localizations of *M. leprae*, we would merely emphasize, with respect to human leprosy, those localizations that may be compared with what we have observed in our hamsters. In all forms of the disease, but especially in the lepromatous form, histologically the cutaneous lesions show free bacilli in the stroma, in the nonstriated fibers of the erector muscles of the hair, where they multiply, in the vascular endothelium, and in the nerves, where they settle in the Schwann cells, as well as in the histiocytes. Bacilli may be found also in the muscular fibers of the striated muscles themselves, where their homogeneous appearance contrasts with the granular aspect of the neighboring bacilli within histiocytes ([7]), and also in the tegumental malpighian epithelium.

Without wishing to give too nearly absolute a value to histologic peculiarities, we may note that experimental infection of our hamsters shows sufficient analogy with human leprosy to warrant the belief that it is a question of development of inoculated germs. The early stages could be compared with indeterminate leprosy in man; later stages resemble indeterminate, prelepromatous and, later, lepromatous leprosy, when intrahistiocyte multiplication clearly gains ground.

II. Comparison with Work of Other Investigators

1. Low temperature factor. In recent years, various investigators have obtained apparently valid results only after inocula-

[4] Recently Wiersema *et al.* ([36]) studied the manner in which *M. lepraemurium* can attack nerves after inoculation in the foot pads and the ears of mice.

tion of *M. leprae* in low-temperature regions of the animal (foot pads, ears, testis).

Inoculation in hamsters' ears was followed by local bacillary multiplication, as shown in experiments by Binford [2, 3], Convit *et al.* [8, 9], and Waters and Niven [25].

Inoculation in foot pads of mice of various races enabled Shepard [19, 20] to obtain results that have been confirmed, in mice and hamsters, by numerous authors [5, 10, 16] and considered valid by the Technical Committee of the VIIIth International Congress of Leprology [6]. Shepard's success with white mice, which we assume to be less susceptible to *M. leprae* than black mice or hamsters, may result from the fact that the temperature of the foot pads is lower than that of the testes. More recently [21] he demonstrated that the most favorable temperature of the foot pads of mice for bacillary multiplication, is about 30°C; it occurs with an environmental temperature of 20°C. At an environmental temperature of 30°C, multiplication is clearly weaker. These facts may explain why Mukherji and Sircar [12] did not obtain infection in mice inoculated in the foot pads, and maintained at a temperature of 30°C.

2. Interaction of various factors. In certain cases deficiency in the low-temperature factor seems partially compensated for by one or another of several possible intervening factors. Since Convit *et al.* [8, 9], who inoculated hamsters' ears (susceptible animal, favorable region), contrary to everyone else, never obtained infection with material taken from lepromatous patients, it may be presumed that his animals did not find a suitable temperature in the Venezuelan climate. However, since under the same conditions he succeeded with material taken from borderline patients, it seems reasonable to suppose that the bacilli from nonlepromatous patients adapted themselves more easily to the animal.

Inoculation in the dermis, a tissue probably favorable to bacillary implantation, if not to its dissemination, sometimes yields success despite normal temperature. For example, in mice inoculated by tattooing, Rees [15] observed a few bacilli in local lymph nodes, from which he was able to obtain, by subcutaneous and intraperitoneal passages, active infection. We have ourselves observed, in smear preparations, with one black mouse, inoculated 16 months previously by the subcutaneous route in the groin, a layer of globi localized solely in the dermis at the inoculation point, and nothing elsewhere.

3. Intratesticular inoculations. Here the results obtained by different authors appear less well defined. Most have been negative. However, in the light of our observations, it seems that certain criticisms can be made. For example, what appears to us an essential point is that the bacillus hardly ever multiplies in the testis itself; if the latter only is examined, without the tunics, a negative conclusion may be reached, particularly when this examination includes, as is often the case in published works, only a bacillus count without histologic control. The latter, in our opinion, is of first importance, revealing even a slight multiplication, e.g., in the albuginea or in the tunica vaginalis.

Then again, certain investigators have inoculated animals that were too young, under the age of puberty, in which, because of this fact, the low-temperature factor did not operate. Thus Kanetsuna *et al.* [11] carried out intratesticular and intraperitoneal inoculations in male hamsters, but they were either new-born or only one to two months old.

Another possible cause of failure is the individual resistance of certain hamsters.

Despite these reservations, we have noted certain observations of other authors that agree with our own. Rees [15], in three hamsters out of 84, found granulomata containing numerous bacilli in very good condition, noncultivable, inside the testes themselves. Binford [2, 4] observed in several hamsters bacilli in good condition in the tunica vaginalis. Shepard [20], using a testis of a first inoculation hamster, obtained positive infection in the second passage by the intratesticular method. Waters and Niven [25] found bacilli in good condition in the testes of one first

inoculation hamster and one hamster of the first passage.

4. Cellular localizations. Precise localization of bacilli in tissues affected by lesions has not been sought from the beginning by different authors. They first described particularly the histiocyte granulomata, and for the most part sought, and usually discovered, localization of the bacilli in the nerve filaments ([2, 5, 17, 20]).

Shepard, however, several times noted the presence of numerous free bacilli, without appreciable cellular infiltration. Recently, Palmer *et al.* ([13]) observed, also in the foot pads of mice, a high proportion of homogeneous bacilli having the appearance of "micro-colonies," in striated muscular fibers. For their part, Waters and Niven ([25]) have recently described, in the ears of hamsters, but in first inoculation animals and during first passages, all the cellular localizations that we have ourselves described.

5. Possible transformation of the germ. (a) *Long-term transformation of the bacillus apparently noted by some investigators:* —Convit *et al.* ([9]) observed, at the seventh passage, a highly hypertrophied spleen containing numerous bacilli, comparable, therefore, to spleens observed in murine leprosy. Lepromin prepared from these hamsters did not provoke reactions in man of the same character as those induced by human lepromin. Convit recorded his belief, moreover, that a new type of *M. leprae* developed in his hamsters by mutation.

(b) *Localized infection after inoculation:*—On the other hand, even after numerous passages, the infection developing in the foot pads of mice remains local, without generalization. Rees ([16]), however, observed involvement of the lymph nodes in 5 per cent of cases. Shepard and Rees demonstrated that the bacilli collected from the foot pads retained their biologic and antigenic characteristics; therapeutic attempts gave results comparable to those obtained in human leprosy ([14, 18, 22, 23]) and lepromins prepared from the foot pads reacted in man in the same way as human lepromin ([24]).

However, it should be noted that bacilli that have multiplied in the foot pads remain permanently, during successive passages, at a lower temperature than that of the rest of the body. It is, therefore, not impossible that the *M. leprae*, having become generalized in the animal after intratesticular or intraperitoneal inoculations, undergo from this and particularly after several passages a transformation that modifies their biologic behavior. We have noted this in a few cases only, but we cannot yet reach a definite conclusion. Only after several passages in series shall we be able to know if this transformation is real.

If the possibility of obtaining a transmissible and generalized infection adapted to the animal is confirmed, it would be of great interest. It is not, however, at all certain that this infection could then be used for the experimental study of *M. leprae*, as is the case with local infection developed in the foot pads.

SUMMARY

Binford's hypothesis, according to which growth of *M. leprae* will take place preferentially in the coolest parts of the body, has been confirmed by our experiments with the golden hamster. The animals were inoculated with suspensions of skin biopsy specimens from patients with lepromatous leprosy. Our results were tested histopathologically and in smear preparations.

While up to the present time investigators have obtained for the most part localized lesions by inoculation into the foot pads and the ears, we have induced progressive lesions after intratesticular inoculation, and particularly after intraperitoneal inoculation, in male hamsters.

It is exceptional for *M. leprae* to develop within the testis itself, but lesions becoming more and more important with the passage of time (observation periods from three to 25 months) are seen in the different tunics of the organ. Between seven and ten months after inoculation these lesions are clearly established.

Although infection reaches a maximum in the tunics, it does not remain there, but extends to the satellite lymph nodes (in-

guinal and lumboaortic) and, less often, involves the mediastinal, axillary and even submaxillary nodes. In some cases the infection may reach the liver.

While certain mycobacteria, and especially *M. lepraemurium*, are generally found particularly in the histiocytes, bacilli from leprosy patients are also observed, at the level of the peritesticular focus of hamsters, and in a wide variety of tissues, including connective, muscular, nerve and vascular (not only intracellular but also extracellular between the connective fibers), as in the case of human leprosy.

Some first hamster-to-hamster transfers turned out very well when routes of inoculation that had been successful with human material were used. First passages by subcutaneous inoculation into the groin were positive in only two out of seven cases.

It is necessary for several passages in series to be accomplished in order to ascertain whether the germ retains its initial characteristics or not.

RESUMEN

La hipótesis de Binford, según la cual el *M. leprae* crece de preferencia en las partes mas frías del cuerpo, ha sido confirmado en nuestros experimentos hechos en criceto dorado (hamster dorado). Los animales fueron inoculados con suspensiones de muestras de biopsias de la piel de enfermos con lepra lepromatosa. Los resultados de nuestra experiencia fueron comprobados histopathologicamente y en frotis.

Hasta el presente los investigadores han obtenido lesiones localizadas, mediante la inoculación en los colchones plantares y en las orejas, nosotros hemos producido lesiones progresivas después de la inoculación intratesticular y especialmente después de la inoculación intraperitoneal en los cricetos (hamster) machos.

Es excepional que el *M. leprae* se desarrolle dentro del testículo mismo; pero las lesiones se hacen mas y mas importantes con el tiempo (período de observación de tres a 25 meses) y son vistas en las diferentes túnicas del órgano. Entre siete y diez meses después de la inoculación, estas lesiones están definitivamente establecidas.

Si bien la infección alcanza su maximum en las túnicas, no permanece ahí, sin embargo, sino que se extiende a los nódulos linfáticos satélites (inguinales y lumboaorticos) y con menos frecuencia, compromete los nódulos del mediastino, axilares y aún los submaxilares. En algunos casos la infección puede alcanzar el hígado.

Mientras ciertos mycobacterias, especialmente el *M. lepraemurium*, son generalmente encontrados especialmente en los histiocitos, bacilos de los enfermos de lepra se observan también en focos peritesticulares en los cricetos (hamsters) y en una variedad amplia de tejidos, incluyendo el connectivo, muscular, nervioso y vascular (no solo intracelular sino, tambien, extracelular, entre las fibras del tejido connectivo), como ocurre en la lepra humana.

Algunos primeros pasajes de criceto a criceto (hamster-to-hamster), se hicieron muy bien cuando fueron usadas vias de inoculación que habían sido exitosas con material humano. Los primeros pasajes por medio de inoculación subcutánea en la ingle fueron positivos solamente en dos de siete casos.

Es necesario realizar varios pasajes en serie para determinar si el germen conserva o no sus características originales.

RÉSUMÉ

L'hypothèse de Binford, selon laquelle le bacille de Hansen se développerait de préférence dans les régions les moins chaudes de l'organisme, a été confirmée par nos expériences sur hamsters, inoculés avec du matériel provenant de biopsies cutanées prélevées sur des malades lépromateux. Les résultats ont été appréciés sur frottis et par examens histopathologiques.

Alors que, jusqu'à présent, par inoculation à la plante des pattes et aux oreilles, les expérimentateurs ont obtenu surtout des infections localisées, nous avons réalisé des infections progressives par inoculation intra-testiculaire, et surtout, intra-péritonéale au hamster mâle.

Dans le testicule même, le germe ne se développe qu'exceptionnellement. En revanche, les différentes enveloppes sont le siège d'infections devenant de plus en plus importantes avec le temps (les durées d'observation allant de 3 à 25 mois), et que l'on peut considérer comme nettement installées entre 7 et 10 mois.

Si l'infection est maximale dans les enveloppes, elle ne s'y cantonne cependant pas: elle s'étend aux ganglions satellites (inguinaux et lombo-aortiques) et, plus rarement, elle touche les ganglions médiastinaux, axillaires et même sous-maxillaires. Dans quelques cas, l'infection a pu atteindre la foie.

Alors que d'autres mycobactéries, et tout spécialement le bacille de Stefansky, se retrou-

vent en général surtout dans les histiocytes, les bacilles provenant de hanséniens se rencontrent aussi, au niveau du foyer péri-testiculaire des hamsters, dans les tissus les plus variés: conjonctifs, musculaires, nerveux, vasculaires (en position non seulement intra-cellulaire mais aussi extra-cellulaire entre les fibres conjonctives), comme cela s'observe en pathologie humaine.

Quelques premiers passages effectués par les mêmes voies qui ont permis, à partir de l'homme, l'implantation des bacilles dans les tuniques du testicule, ont réussi de la même façon. Les premiers passages effectués par voie sous-cutanée à l'aîne n'ont été positifs que dans 2 cas sur 7.

Il faudra attendre plusieurs passages en série pour savoir si les germes conservent ou non leurs caractères initiaux.

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