INTRATESTICULAR MULTIPLICATION OF *MYCOBACTERIUM LEPRAE MURIUM* IN NORMAL AND SURAMIN-TREATED ANIMALS

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In a previous paper (2) were presented the results of a histological study of the early evolution of lepromata in the rat. The main object of that study was to obtain information likely to be useful in experiments on the *in vitro* cultivation of *M. leprae murium* by tissue-culture or other means. In particular we wished to know whether that organism, on entering a new host, passed through a lag period before beginning to multiply or whether division at the maximum rate began at once. The answer to this question has obvious implications as to the length of time tissue cultures might have to be maintained before multiplication became detectable. Our conclusion was that *in vivo* during the first six weeks or so after intraperitoneal inoculation in the rat, bacilli divided little if at all, but they increased in length; multiplication then became evident, and the average length of the bacilli diminished.

The work reported here is an attempt to confirm these findings more precisely by a quantitative technique. The same technique has also been used to determine the effect of suramin[1] on murine leprosy in mice and rats. This drug has been found by Rees and D'Arcy Hart (7) to enhance experimental tuberculous infection in mice, although it had previously been thought to have a beneficial effect in human tuberculosis (8). The method used is based on the description by Hobby *et al.* (5) of a technique for counting rat leprosy bacilli in mouse spleens. Hanks and Backerman (3) had previously shown that the testis of the rat or mouse is the most susceptible organ, and we therefore chose the intratesticular route of inoculation. Furthermore, since we intended to excise and homogenize the same organ as that into which we had introduced the bacilli, it seemed likely that very small inocula could be used and also that errors of sampling would be minimal. Intratesticular inoculation is simple to carry out with precision; and testicular tissue, being normally semi-fluid in consistency, is easily homogenized.

[1] Suranim is the British Pharmacopoeia name for the substance originally introduced as Bayer 205. It is the symmetrical urea of the sodium salt of *m*-benzoyl-*m*-amino - *p* - methylbenzoyl - 1 - aminonaphthalene - 4, 6, 8 - trisulfonic acid. It is a trypanocide, used for many years for the prophylaxis and treatment of trypanosomiasis in man.

MATERIALS AND METHODS

Suspensions of the rat leprosy bacillus.—The "Wells" strain described in our previous study was used throughout. Approximately 1 gm. of lesion tissue was removed from an infected rat or mouse with sterile precautions, cut into small pieces with sterile scissors, and ground to a smooth suspension in a hand-operated glass tissue-grinder, using 8-10 cc. of 5 per cent bovine albumin (Armour bovine plasma fraction V) in 0.9 per cent saline. The suspension was centrifuged at about 500 G for 5 minutes, to remove tissue fragments and clumps of bacilli. The milky supernatant, containing mainly single bacilli or aggregates of two to four, was used for inoculation, after a count of its bacillus content had been made as described below.

Mice and rats.—White Swiss mice and Wistar albino rats were used. The ages and weights varied in different experiments, and will be mentioned in the account of each.

Intratesticular inoculation.—Animals were anesthetised with a 1:2 mixture of ether and chloroform, except in Experiment 3 in which very young rats were used, which would only tolerate ether. The testes were brought into the scrotum by pressure on the abdomen and inoculated with an "Agla" micrometer syringe fitted with the fine-gauge "214 R" intradermal needle.[2] It was easy to feel first the resistance, then the "give," as the point passed through the tunica of the testis; the needle was then passed about 5 mm. further into the substance of the organ. Both testes were injected, that of a mouse receiving 0.01 cc. of the bacillus suspension, and that of a rat 0.02 cc.

Bacillus counts.—A batch of animals, usually 6-8 in number, was killed immediately after inoculation to provide the initial count, and other similar batches were killed at intervals up to 4 months. Both testes were removed and freed of epididymis, moesotestis and vessels by dissection with fine-pointed scissors. Usually the handling and homogenization were without sterile precautions because "stained counts" were to be made, but when testes were needed for further passage they were removed aseptically from the abdomen.

One testis from each batch was set aside for histological study, and the other was cut in half with scissors and dropped into a small measuring cylinder; any testicular material adherent to the scissors was washed off into the cylinder with a few drops of distilled water. The volume of the material in the cylinder was then made up with distilled water to a convenient amount (between 3 and 6 cc.). The contents of the measuring cylinder were swiftly poured into the pyrex vessel of a Mickle tissue disintegrator (6), and the cylinder was washed if necessary with a small portion of distilled water, the washing being added to the rest of the testis suspension. A tally was kept of the total volume of the testes and their diluent. Approximately 2 cc. of glass beads (3 mm. diameter) were added to the testis suspension, which was then homogenized in the Mickle shaker for five minutes. Rat testes, being larger, were handled in pairs and were cut into several fragments. The total volume was made up to between 7 and 10 cc., and homogenization was carried out for one minute in a nylon tissue grinder (4). A homogenate was preserved for later counting, if necessary, by adding one drop of formalin per 5 cc. and storing at 4°C.

Homogenization by either method produced satisfactory rupture of tissue cells, with bacilli lying for the most part singly, or in twos and threes. If necessary, the homogenate was diluted suitably with formol-milk (see below). After thorough mixing, an aliquot was taken up in an Agla syringe fitted with a needle of rather

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[2] The Agla micrometer syringe, sold throughout the world by Burroughs Wellcome but not as widely known as it should be, is a precision-bore syringe whose plunger is actuated by a micrometer handle. Capacity, about 1 cc.; accuracy of delivery, ±0.00005 cc.

larger bore than is used for injections, the pointed tip of which had been ground off. Several drops were then expelled, the instrument set at zero, and any remaining fluid at the tip of the needle removed. The handle was then turned to expel a drop of one microliter which was touched, as a preliminary, on to the corner of a clean, grease-free micro slide, and eight further drops of one microliter volume were touched separately, but in a close group, in the center of the slide. Later in the investigation five drops were found to be sufficient. The close central grouping was in order to avoid variation in depth of staining of individual drops, which occurred if they were laid out along the length of the slide.

Without delay the slide was placed on a piece of white paper carrying an inked outline of a 0.5 cm.—side square. Each of the central drops in turn was brought over the square and spread to the same area with the end of a straight platinum wire. These operations were carried out as quickly as possible to prevent irregular distribution of bacilli due to drying. The slide was then dried quickly by placing it on the levelled lid of a boiling water-bath for 2 minutes. The slide was then cooled and flooded with a solution containing 0.5 per cent gelatine and 0.5 per cent phenol in distilled water; this was then poured off, the slide was allowed to drain for a few seconds, and it was again placed on the water-bath lid for 2 minutes. After cooling, the slide was held in formaldehyde vapor for 3 minutes, heated again to drive off formaldehyde, and stained by the Ziehl-Neelsen method with a very light methylene blue counterstain.

The bacilli in ten arbitrary microscope fields (2 mm. oil immersion objective with 5X binocular eyepieces) were counted across the equator of each drop in the following way: A low-power (2 cm.) objective, parfocal with the high power lens, was used to confirm that the homogenate had spread smoothly with a thicker central disc, which was then brought to a position concentric with the field. The oil-immersion lens was brought into use and the microscope stage moved laterally until the right- or left-hand edge of the drop was found. The bacilli in a field just within the edge were counted, and then those in nine other fields found with the stage micrometer at 0.5 mm. intervals across the drop. The last field was very close to the opposite edge. Counts were greater in the fields near the center of the drop, because of its greater thickness. When ten fields in every drop had been counted in this way, the average number of bacilli per field was determined, and the number of bacilli per cc. of dilution or per testis was calculated by the use of the calibration factors set out below.

Calibration and calculation.—A volume (v) of 10^{-3} cc. of material is spread over an area (A) of 25 sq. mm. in each square film. The average volume per sq. mm. is therefore 1/25,000 cc. The area (a) of the arbitrarily chosen microscope field was next determined, since the average number (n) of bacilli per field was counted. This was done by examining the smallest squares of an improved Neubauer hemacytometer using the high-power dry objective, when the ruled lines remain visible. In this work the field was 1/100 sq. mm. in area, and therefore the average volume per field was $\frac{1}{2.5 \times 10^6 \text{ c.c}}$

To determine the average number of bacilli per testis (N), the number of testes (t) in a homogenate of final volume (V) must be known, and also the extent of any further dilution (d) of the latter. Then,

$$N = \frac{nA}{av} \times \frac{Vd}{t} \frac{A}{av} \text{ is in fact } \frac{1}{\text{av. vol./field}}$$

and is a constant factor.

For example: Number of testes, 2; volume of homogenate, 8 cc.; further dilution, 30x; av. count/field, 7.6:

$$N = 7.6 \times 2.5 \times 10^6 \times \frac{8 \times 30}{2}$$

= 2,280 million bacilli per testis.

This calculated derivation was checked by carrying out a routine red blood cell count with a known statistical error (1) on a sample of human blood and comparing it with the figure obtained by the method described. The sample was diluted 1/200 in formol-citrate and this dilution was used for both determinations. Two thousand cells were counted in the improved Neubauer hemacytometer, and the count gave a value of 4.93 ± 0.25 millions/cmm. The formol-citrate suspension was diluted with an equal volume of formol-milk and the "stained count" carried out in a way similar to the bacillus count, using dilute carbol-fuchsin for staining. By this method the value obtained was 5.15 millions/cmm.

Adjustment of bacillus concentration in a homogenate.—Bacillus counts, carried out in this way, are most accurate if the average field count is in the neighborhood of 10, and in this work homogenates were diluted, when necessary, to produce counts of between 5 and 15 per field. The diluent was formol-milk prepared by a simpler method than that described by Hobby *et al.* (5), but found on comparison to be equally effective. A 25-cc. screw-cap universal container was filled with milk and centrifuged at about 1,500 G for 15 minutes to separate the cream. With a Pasteur pipette the subnatant milk was drawn off, leaving the cream behind. Ten cc. of milk and 1.5 cc. formalin (40% HCHO in water) were brought to 100 cc. with distilled water and thoroughly mixed; the diluent was then ready for use.

Measurement of bacillus length.—The testis set aside from each batch was cut in half, and the cut surface was pressed lightly onto a slide to make an impression preparation—after which the tissue was fixed in 10 per cent formol-saline for histological study. The impression preparations were fixed by heat, and were used for measurements of bacillus length in preference to homogenates, as it was thought that homogenization might break up the longer forms into short segments and give a false impression of the state of the bacilli *in vivo.* (In fact, however, a number of parallel estimates did not demonstrate any significant difference between average bacillus lengths in a smear and in a homogenate made at the same time.) The smears were stained by the Ziehl-Neelsen method, and 200 consecutive bacilli in each were measured with an eye-piece micrometer.

Suramin solution for injection.—This solution, 1 per cent, was made up by dissolving the contents (1 gm.) of an ampule for human injections ["Antrypol," Imperial Chemical (Pharmaceuticals) Ltd.] in 100 cc. of sterile saline.

RESULTS

1. *Statistical assessment of the counting method.*—Twenty-four mice were each injected in both testes with 0.01 cc. of a bacillus suspension. They were killed the next day and the testes homogenized in 6 groups of 8. A slide for counting was prepared from each homogenate, using 8 drops per slide. Data on the replicate counts on the 6 sets, each of 8 testes, are recorded in Table 1.

The complete results were kindly analyzed by Drs. J. Knowelden and P. Armitage of the London School of Hygiene and Tropical Medicine. They concluded that the coefficient of variation among the 6 replicate homogenates is 14.3 per cent, giving a standard error (S.E.) of the difference between two estimates of 20.2 per cent of their mean. At the conventional 2 S.E. ($P. = 0.05$) level, the difference between two estimates would be significant if it exceeded 40.4 per cent of their mean. In practice, this means that if the number of bacilli per testis from one group of animals is 80 per cent higher than that in another group, the difference is significant. In the experiment whose description follows next, twice the number of testes per

homogenate were used, and it was calculated that an "increase" of 50 per cent is significant.

A comparison was also made between the number of bacilli injected and the number recovered 24 hours later. The original bacillus suspension was

TABLE 1.—*Data on replicate counts of bacilli in 6 homogenates, each of 8 mouse testes, made 24 hours after all 48 testes had been injected with the same inoculum.*

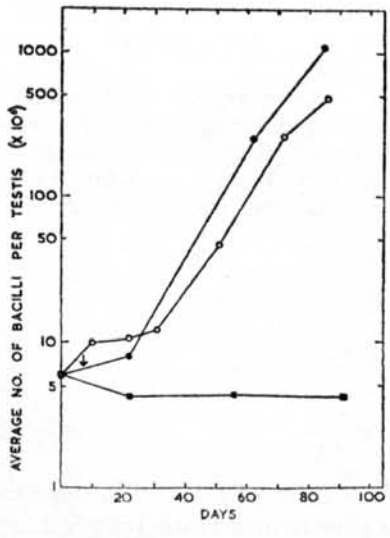
Homogenate No.	Volume (cc.)	Average field count	Bacilli x 10^6 per testis
1	2.30	8.00	5.75
2	2.85	5.82	5.17
3	2.95	5.39	4.98
4	2.70	7.91	6.67
5	2.60	7.20	5.85
6	2.60	5.48	4.46
Mean of all six homogenates			5.49

suitably diluted with formol-milk and a count was made, also based on 8 drops. This was compared with the average of the 6 estimates derived from the homogenates (Table 1), from which it was possible to calculate that the technique recovered 86-89 per cent of the bacilli injected 24 hours earlier.

2. *Multiplication of bacilli in the mouse testis.*—In this determination 148 mice each weighing 30-35 gm. were used. The bacillus suspension was obtained from an 18-month-old subcutaneous leproma in a Wistar rat. It was diluted with 5 per cent bovine albumin in saline to give each testis an inoculum of approximately 6 million bacilli, and was used within three hours. A heated control suspension was prepared in the following way: An aliquot of the diluted suspension was centrifuged at 1,000 r.p.m. for 30 minutes, the clear supernatant removed, and the deposit resuspended to the same volume with normal saline. The saline suspension was heated at 75°C for an hour in a water-bath to kill the bacilli. (The albuminous diluent was replaced by normal saline to avoid its coagulation by this heating process.)

One group of mice received the heated inoculum, and a batch of eight was killed for counting immediately. A large number of mice received the unheated living material, and a similar batch was killed at once. There was no significant difference between the counts on testes containing heated or unheated bacilli at this time. The larger group of mice was later divided into two subgroups, one of which was treated with suramin. The dosage was 0.1 cc. of solution (i.e., 1 mgm.), given subcutaneously to each mouse twice a week, starting on the seventh day after inoculation.

The results of counts on the testes of mice in the three groups are shown in Text-fig. 1. It can be seen that with the killed bacilli there was an early small reduction in numbers, but that later there was no change. In the other two groups, on the other hand, there was evidence of multiplication; the increase in number was rather slow over the first five weeks, and then entered a logarithmic phase in both cases. The mean generation time during this phase in untreated mice was 10 days. In the suramin-treated group the rate of multiplication was significantly higher, so that the two growth curves diverge with increase in time. After the 50th day the number of bacilli per testis in the treated group was twice as great as in the controls, and the difference had become even greater by the 90th day.

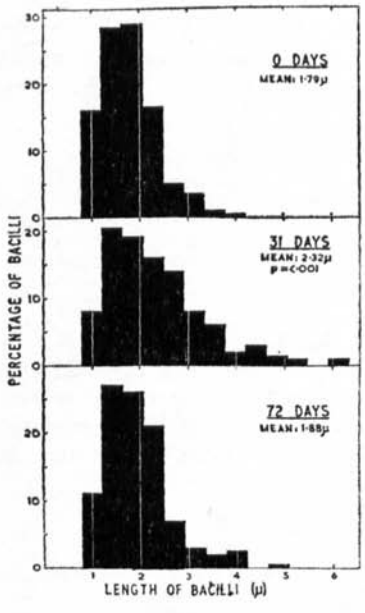


TEXT-FIG. 1. Numbers of bacilli in the testes of mice. Open circles: living suspension in untreated animals; solid circles: living suspension in suramin-treated animals; solid squares: heat-killed suspension. The start of the suramin treatment is indicated by an arrow.

Histologic sections showed that the bacilli were taken up by phagocytic mononuclear cells lying in the intertubular spaces. At the outset, most of these infected cells contained 1-3 bacilli. In the case of the heat-killed inoculum this appearance remained unchanged throughout. Sections of the testes which had received a living inoculum showed a progressive increase in the average number of bacilli per infected cell during the first two months, later accompanied by an increase in the number of infected cells. Their accumulation produced an increase in the total amount of intertubular tissue, with separation of the tubules and a moderate increase in the size of the testicle. These changes were greater in the testes of the suramin-treated animals than the others; furthermore, in this group at the end of the experiment the infected cells appeared swollen as compared with those of the untreated animals, and bacilli were both more numerous and more loosely packed within them. No inflammatory response could be seen in any preparation.

3. *Morphology of bacilli in the mouse testis.*—There was little variation among the three groups, or at different time intervals, with regard

to depth of staining, granularity, or beading of bacilli. There was, however, considerable variation in average length of the bacilli in the untreated group during the earlier phase of multiplication. The changes taking place are illustrated in Text-fig. 2, in which each histogram shows the percentage distribution of bacillus lengths among groupings of arbitrary micrometer units; and the calibration of these in microns is also indicated.



TEXT-FIG. 2. Histogram showing percentage distribution of bacillus lengths in 200 consecutive bacilli measured in stained smears of mouse testes, immediately after inoculation of a living suspension and after one and two months.

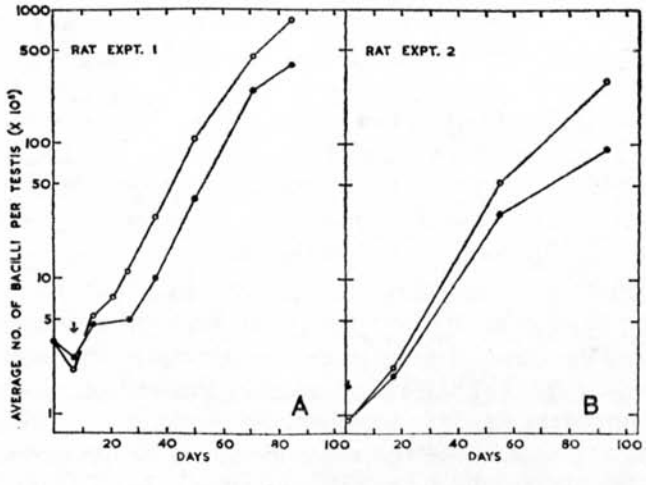
It can be seen from the upper histogram that most of the bacilli derived from the 18-month-old rat leproma (day 0 in the mouse testis) were very short, 93 per cent being between 1-3 μ in length. A month later, however, when the inoculum had nearly doubled, there was a "shift to the right" in distribution of length: 25 per cent were more than 3 μ long, and approximately 10 per cent were 4.5 μ or more in length. No bacilli of this size were detected in the original inoculum. The increase in mean length from 1.79 μ to 2.3 μ is highly significant. Just over a month later, when multiplication was well into the logarithmic phase, bacilli had, on the average, become shorter again. No significant change in the average length of killed bacilli at different time intervals was found.

4. *Multiplication of bacilli in the rat testis.*—Two experiments were designed to find out whether suramin produced an enhancement of the infection in rats similar to that seen in mice. In the first experiment 86 well-grown young rats ($\pm$200 gm.) were used. The inoculum was obtained by homogenizing the heavily-infected spleen of a mouse 18 months after inoculation; counted in the testes of rats killed immediately after inoculation, the inoculum was 345 x 10^6 bacilli per testis. Suramin treatment was started in one-half of the rats one week after the intratesticular inocula-

tion, 5 mgm. being given subcutaneously twice a week. Batches of 4 from each group were killed at approximately weekly intervals.

In the second experiment 50 young rats (±50 gm.) were used. The inoculum was prepared from a rat testis leproma 7 months after inoculation. The average number of bacilli injected into each testis, based on the findings in 5 rats killed 24 hours after inoculation, was 93 x 10^6. Suramin treatment of one-half of the animals was started on the same day as they received their intratesticular inoculation—unlike the previous experiments. Each treated rat was given in each week 5 mgm. subcutaneously followed 4 days later by 2.5 mgm. (7.5 mgm./week). Batches of 5 from each group were killed on the 17th, 55th and 93rd days.

The results of testis counts in these two experiments are shown in Text-fig. 3. The growth curves in both are essentially similar, with a few minor differences. It appears, for instance, that the initial drop in testis bacillus content seen in Text-figs. 1 and 3A did not appear in Text-fig. 3B, because most of this kind of loss occurs in the first 24 hours after injection, i.e., *before* the first point in this figure. In the first rat experiment the lag phase was shorter and less marked than in the mouse experiment, and in the second experiment no lag was detectable. In both cases, in contrast to what was found in mice, bacilli multiplied *less* readily in the suramin-treated groups, the most marked divergence being seen in the younger rats. The mean generation times during the logarithmic phase in untreated rats was 7-8 days.



TEXT-FIG. 3. Multiplication of bacilli in testes of rats. Open circles: control rats; solid circles: suramin-treated rats. Start of suramin treatment indicated by arrow. A: Bacilli from an 18-month-old mouse spleen leproma. B: Bacilli from a 7-month-old rat testis leproma.

DISCUSSION

In quantitative assessments of this kind, in which the growth of a bacterial inoculum within an organ is estimated, it is a frequent practice to relate the bacterial number to the weight or volume of tissue from the organ. This was, in fact, the approach of Hobby *et al.* (5) in their study, in which mouse spleens were removed at intervals after intraperitoneal

inoculation of the bacilli. This appears reasonable as a basis for estimation of initial bacillus numbers, since a larger spleen is likely to filter out larger numbers of circulating bacilli. However, if later in the experiment the average spleen size were to increase as a result of tissue reaction to infection, an artefactual appearance of slower bacillus growth would be produced. For this reason, and also because uniform inocula were injected into the testes in our experiments, we thought it correct to estimate the numbers of bacilli per testis rather than per unit volume or weight of tissue.

The further question arises as to whether the growth curves recorded here represent the actual state of affairs, or whether they are the resultant of two opposing conditions, namely, multiplication on the one hand, and on the other hand decrease by physical removal and intracellular digestion of a proportion of the bacilli. In the latter case the true rate of increase would be greater than is illustrated. We believe, however, that the two suggested loss factors are relatively unimportant.

There can be no doubt that some proportion of the bacilli injected are lost at the outset of an experiment, probably by drifting out of the testis in the lymphatic fluid before they can be taken up by phagocytic cells. This physical loss is illustrated in the initial drop in numbers seen in the first two experiments; in the third, no drop is seen, probably because the loss was largely complete before the first estimate was made one day after inoculation. This point was not investigated in these series, but some studies with staphylococci and tubercle bacilli showed a similar initial decrease limited to the first two days after inoculation. The steadiness in the numbers of killed bacilli at later periods shows that infected cells do not leave the testis to an appreciable extent. Physical loss might also result from the bursting of cells stuffed to the point of endurance by intracellular bacilli; the histological preparations show that this factor is hardly likely to play a significant role before about the third month. At that time, in fact, our curves and those of Hanks and Backerman (3) and of Hobby *et al.* show a flattening which may be accounted for in this way.

The factor of loss by intracellular digestion appears to be no more important than that of physical loss. In the first place, such digestion is usually partial, leading to the accumulation of cells containing amorphous acid-fast material. Scanty cells of this kind were seen in the sections, but they did not increase in numbers as time went on. Secondly, the heated bacilli, which were also intracellular, showed no evidence of destruction. Hobby *et al.* found that in the mouse spleen the rate of destruction of heated *M. leprae murium* was proportional to the amount of heat treatment applied.

To sum up, then, it seems likely that the data referring to a period between the end of the first two days after inoculation and about the twelfth week are a fairly true representation of the intracellular growth of rat leprosy bacilli in the testis.

The first question which we set out to answer in this investigation was whether a lag phase after inoculation of bacilli, observed by histological means in the peritoneum of the rat, could be confirmed by this more precise method. The results indicate that in the first two experiments such a lag phase did occur. In the mouse the numbers of bacilli had only doubled at the end of five weeks, although after this time doubling occurred at about 10-day intervals. In the first rat experiment the lag was shorter and less definite; in the second it could not be detected. A possible explanation for this difference may lie in the fact that a very much younger leproma, and, moreover, one from a rat testis, was used to provide the inoculum in this experiment. The bacilli, therefore, were still hardly out of their "tissue logarithmic phase" and were being transferred to an identical medium—circumstances which, *in vitro,* lead to the elimination of a lag phase. This is a more hopeful finding for attempts at tissue culture, as it suggests that, if bacilli in the right physiological state are used, it may be possible for them to continue multiplying at the maximum rate and therefore to show a detectable increase in numbers within the short period of observation available.

The quantitative determinations of bacillus length at intervals during intratesticular growth in the mouse confirmed our previous visual impression of a transient increase of length corresponding in time with the lag phase, followed by shortening when active multiplication was established. This is a phenomenon familiar in the growth of other species of bacilli *in vitro,* and provides confirmatory evidence that the observation of the lag phase is a genuine one and not just an artefact produced by counting a small proportion of rapidly multiplying bacilli among a mainly nonviable population. An alternative explanation for the change in percentage distribution of bacillus lengths might be a selective removal of shorter forms. This explanation seems put out of court, however, by the finding that the killed inoculum did not vary significantly with time and that, among the living bacilli, it was not difficult after a month to find rods which were longer than any seen in the inoculum.

It was a matter of some surprise that the mean generation time of our strain of *M. leprae murium* was rather less in the rat (7-8 days) than it was in the mouse (10 days), since it was expected from the work of Hanks and Backerman that the bacilli would multiply more rapidly in the latter species than in the former. These workers found a slightly longer mean generation time with their strain (Hawaii). However, we have found that the Wells strain used in this work produces lepromata very much more readily in our white Wistar rats than does the Hawaii strain. It seems likely that this discrepancy can be attributed simply to strain variation in virulence for different host species and strains.

The action of suramin on tuberculosis in mice was investigated by Rees and D'Arcy Hart (7) by means of counts of viable bacilli in spleen and liver homogenates after intravenous inoculation, and they found it to be the

most active of a number of substances, including cortisone, in enhancing the growth of tubercle bacilli in this species. The findings reported here show that this drug has a similar action on a testicular infection of *M. leprae murium* in the mouse. Rees and D'Arcy Hart have also found a similar effect in mice after intravenous infection with this mycobacterium (personal communication). However, suramin has an opposite effect on testicular leprosy in the white Wistar rat, since we have found that there is a significant reduction in the rate of increase of bacilli in treated animals in comparison with untreated controls. In this respect the influence of this drug on rat leprosy appears to resemble its action in human tuberculosis. In the absence of any fundamental knowledge of the basis of the action of suramin in mycobacterial disease, it is difficult to speculate on how close an analogy there may be in this resemblance. We may conclude that the influence of suramin on mycobacterial infection appears to be governed mainly by the reaction of the host, varying from one species to another.

SUMMARY

A technique is described for the intratesticular inoculation in mice and rats of suspensions of *M. leprae murium*, and for subsequent quantitative assessment of bacterial multiplication by counting the numbers of stained bacilli in homogenates of testes from animals killed at intervals. The method is convenient to carry out, and a statistical assessment shows that considerable accuracy is attainable.

Bacilli from an 18-month-old rat leproma, transferred to a different host species, the mouse, showed a "lag" phase of 2-5 weeks in its multiplication, succeeded by a logarithmic phase which continued for a further 2-3 months. On the other hand, when rat testes were inoculated with bacilli from a 7-month-old rat-testis leproma, the logarithmic phase was entered at once. The mean generation time in this phase was 7-8 days in rats, and 10 days in mice.

During the "lag" phase a transient but significant increase in average bacillus length occurred.

Suramin treatment of mice enhanced the growth of the bacilli in them. In rats, however, its effect was to decrease the rate of multiplication.

RESUMEN

Se describe aquí una técnica para la inoculación intratesticular en ratones y ratas de suspensiones de *M. leprae murium* y para la subsiguintee valuación cuantitativa de la proliferación bacteriana contado los números de bacilos teñdos en los homogeneatos de testículos procedentes de los animales sacrificados a ciertos plazos. El método resulta conveniente en su ejecución y una valoración estadística demuestra que puede alcanzar considerable exactitud.

Los bacilos procedentes de un leproma de rata de 18 meses, trasladados a un huésped de especie distinta, el ratón, revelaron en su multiplicación una fase de "retardo" de 2-5 semanas, seguida de una fase logarítimica que continuó por otros 2-3

meses. En cambio, al inocularse testículos de rata con bacilos de un leproma de testículo de rata de 7 meses, se entraba en el acto en la fase logarítmica. El tiempo medio de generación en esta fase fué de 7-8 días en las ratas y de 10 días en los ratones.

Durante la fase de "retardo" hubo un aumento pasajero, pero importante, en el largo medio de los bacilos.

El tratamiento con suramina de los ratones acentuó la proliferación de los bacilos en ellos. Sin embargo, en las ratas el efecto consistió en decrecer la velocidad de la multiplicación.

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