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CONTENTS

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EDITORIAL

Year of Expectation

There are several things which make this year one of expectation of significant advances in knowledge in the leprosy field. The Seventh International Congress of Leprology is to be held in New Delhi from 8th to 16th November, 1958. Such international leprosy congresses are held only at intervals of five years; each previous one, seen retrospectively, was undoubtedly a giant's stride forward, and it is reasonable to expect the Delhi one will be no exception.

One of the most fascinating subjects will certainly be the reports on the wide scale campaigns to control leprosy in countries of high endemic prevalence. Since DDS was brought into wide use about 1949 or 1950 there has been a strong tendency to use this therapy as a means of control, perhaps even the main means of control. Does it work? It is hoped that many reports will come in, so this matter can be assessed. Already there is evidence that it does work, for the Nigerian campaign has shown that one can imbed a large component of mass treatment in the leprosy control scheme, and get remarkable results in the reduction of leprosy prevalence which have every appearance of being permanent.[1]

Another question about which expectation will gather is the use of BCG as a prophylactic against leprosy. Time is needed to prove that it does protect, and the five years since the last congress should have produced more evidence. Some countries have already used BCG on a large scale, and reports from them will be of the greatest interest. Controlled experiments have also been in progress.

There is also great interest and expectation in the possibility of new drugs becoming available for the treatment of leprosy. Since Davey [2] and others made their report on a three year trial of a diphenylthiourea compound called DPT, and indicated its freedom from toxicity allied to a certain calm efficiency, the results of trials in many parts of the world will be awaited eagerly, and some of them may mature in time for the Delhi Congress. Fuller information on the pharmacology and biochemistry of DPT may also then be available. Other drugs which may be said to loom over the horizon are the surface-active agents and the ethyl thiolesters. The surface-active agents (polyoxyethylene ethers) are represented by Triton WR 1339 and Macrocyclon, and Rees[3] has reported favourable results against murine leprosy with twice-weekly subcutaneous injections of them, and a trial elsewhere in human leprosy is in progress. The ethyl thiolesters, in particular diethyl dithiol*iso*phthalate, have been studied by Davies and Driver,[4] and have a marked action on experimental tuberculosis by injection and even by inunction; they have a marked effect in weekly doses and combined treatment with INH proved much better than either the

ethyl thiolester or INH alone, and there is no cross-resistance. A trial in human leprosy in due course seems indicated.

There is also reasonable expectation of developments in culture *in vitro* of *M. leprae murium*, which eventually may make possible the culture of the human leprosy bacillus. Rees and Wong[5] in February 1958 reported limited multiplication of the murine leprosy bacillus in tissue culture. Rees had found that the generation time of *M. leprae murium, in vivo*, was 13 days. [6, 7] Based on this, the authors devised a method for counting the total number of bacilli per culture before and after cultivation. In their earlier attempts to culture the bacillus monocytes were used, but even after 80 days no multiplication could be shown, though Wong[8] showed that bacilli recovered from some of these cultures were still infective. When they used tissues obtained from previously infected animals multiplication did occur. They used explanted spleen cells from infected mice and in five experiments showed there was a limited multiplication which occurred at the 15th day and which corresponds closely to that observed *in vivo*. They think that continued multiplication requires a regular supply of healthy cells, preferably in a medium containing a low concentration of serum, and are investigating methods to provide these culture conditions. Hawking, in this issue of *Leprosy Review* reports on an attempt to grow *M. leprae murium* in macrophages, following a suggestion of Rees. The macrophages were obtained from the rat peritoneum and the fluid medium contained 40% serum, and fresh supplies of uninfected macrophages were added every 3 to 4 weeks as required. The bacilli have been maintained in cultures of macrophages for over 6 weeks, and in the most favourable culture the number of bacilli doubled during a period of 52 days. Cultures were still infective to rats after incubation for 42 days.

A method of animal transmission has been tried by Bergel of Argentina[9] who fed rats on a pro-oxidant diet (low in Vitamin E and containing linseed oil) and inoculated them with human leprosy bacilli. There was a control series of inoculated rats kept on a normal diet, wherein there was no evidence of multiplication of the bacilli. The rats on the special diet, however, as well as testicular atrophy and disappearance of body fat, showed at first a phase of granulation and decrease in number of the inoculated bacilli, followed by an increase in number of the bacilli, which became again homogenous, and globi were seen. Material from the first series of rats was used to inoculate a second series, and again a third series from the second series, and multiplication seemed to occur throughout. This work awaits development and confirmation. Similarly K. R. Chatterjee of Calcutta, in a personal communication of 10th March, 1958, gives advance information of

his success in transmitting human leprosy to a species of laboratory bred hybrid black mice, and in establishing the infection in them by successive passages. The transmitted infection seems very heavy in the mice involving almost all tissues, including skin and peripheral nerves.

Other subjects about which it is fair to be expectant in 1958 are the morphological studies of the human leprosy bacillus by electronmicroscopy, closer histological study of the skin in leprosy and the fine nerves of the skin and the light this may throw on the path of invasion of the leprosy bacillus and the tissue reaction to it, studies of the lepromin test and of immunology, the increasing and beneficial interest of orthopaedic surgeons in the prevention and cure of leprosy deformities, and the growing plans for rehabilitation of leprosy patients when their disease is arrested. There are many others; it should be a good year.

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GROWTH OF RAT LEPROSY BACILLUS IN TISSUE CULTURE

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The purpose of this paper is to describe investigations on the cultivation of the organism of rat leprosy, *Mycobacterium leprae murium*, in tissue culture. The first attempt thus to cultivate this organism was made by Zinsser & Carey in 1912. Since then there have been many other attempts (see Gavrilov, 1939; Kudicke & Vollmar, 1937; Dharmendra & Lowe, 1937; and the review by Lowe, 1937), but it is usually considered that successful cultivation was not achieved.

The present paper deals with the attempt to grow the organism in macrophages, and the experiments were undertaken as the result of the suggestion of Dr. R. J. W. Rees of this Institute. Dr. Rees has in the meantime published a short description of successful experiments by another method (Rees & Wong, 1958).* Grateful acknowledgments are due to him for the stimulus of the initial proposal, for supplying the strain or organisms, and for advice about obtaining macrophages from the peritoneum.

Methods

Use was made of the strain of *M. leprae murium* which has been maintained at this Institute for many years. The method of tissue culture employed was similar to that used for cultivating various kinds of intracellular protozoa such as the exoerythrocytic forms of *Plasmodium gallinaceum* (Hawking, 1945, 1951). Briefly, small Carrel flasks (diameter 3.5 cm.) were used. A thin layer of fowl plasma was spread over the floor and 1-4 pieces of cover slip, 0.5 cm. square, were embedded upon it. Small pieces of tissue were mounted on top of the glass slips, being held in place with a drop of plasma. The fluid medium is described in the text. To examine the growth of the tissue the slips were removed from the flask, fixed, stained with Ziehl-Nielsen haematoxylin and mounted tissue downwards on a slide. In the later experiments the flasks were prepared with one or two pieces of cover slip which, together with the floor, were coated with a thin layer of plasma and embryo extract. Macrophages from the rat's peritoneum were suspended in fluid medium, and 3 ml. of the suspension were added to each flask.

Most of the cultures were incubated at 35°C, but some flasks were kept at 30°C. The cells grew well at this lower temperature

* Wallace, Elek, and Hanks (1958) have reported similar experiments in which limited multiplication of the organism was obtained. This information became available since the present paper was submitted for publication: see references.

and probably survived better than at 35°C, but presumably the growth of the bacilli would also be slower. In the later work the total number of bacilli in a flask was estimated by a method using citric acid as described by Sandford (1954). The Carrel flask was almost filled with about 12 ml. of 1.8 per cent citric acid. The floor was scraped with a spatula to loosen tissue and plasma clots and the flask was then shaken at 35°C. for several hours. In some cases, if shreds of tissue were still visible, the fluid was further ground in a tissue grinder until a homogeneous suspension was obtained. The fluid was then made slightly alkaline by adding concentrated caustic soda; unless this is done the bacilli do not take Ziehl-Nielsen stain. Horse serum (1 ml.) was added to improve adhesion of bacilli to the slides. Samples of 20 cmm. were withdrawn and spread on glass slides inside a square measuring 2.0 cm. The numbers of bacilli lying within three strips 0.1 mm. wide were counted with an oil immersion objective; and appropriate calculations were made to obtain the number in the original suspension.

In some experiments small quantities of mycobactin or of extract of rat leproma were added to certain flasks as 1-3 drops of fluid every time the medium was changed. The mycobactin was a partially purified preparation kindly provided by Dr. G. A. Snow. For use, 100 mg. was dissolved in 1 ml. of ethanol with heat and then 60 ml. Ringer solution was quickly added. The resultant opalescent fluid was sterilised by autoclaving. Concentrations of 0.3 mg. per 100 ml. tended to be toxic to the cells, but concentrations of 0.02 mg. per 100 ml. were well tolerated. The leproma extract was prepared by extracting about 100 g. of rat lepromatous tissue with methanol at 35°C. for 10 days. The fluid was then decanted and concentrated by evaporation until a yellow waxy mass remained. A small quantity of this (300 mg.) was redissolved in 0.5 ml. alcohol, which was then diluted with 40 ml. Ringer and sterilized by autoclaving. Concentrations of 10 mg. in 100 ml. were well tolerated by the cells. To test whether the bacilli in a culture were alive or dead, inoculations were made subcutaneously into rats. In the case of fluid, the inoculation was made directly, or after concentration by centrifuging. In the case of tissue, the whole piece of cover slip was inserted. If the material contained viable bacilli, lepromatous nodules usually became palpable at the site of inoculation after about 6 months, but all animals were observed for 12 months before being discharged as uninfected.

Results

Various preliminary experiments were made to study the rate of multiplication of the bacilli *in vivo*, and also their survival outside the body under various conditions. In one experiment, a

standard dose of bacilli was injected intravenously into a large group of rats. After a few days 4 rats were killed and the number of bacilli in the spleen was estimated, and further groups of 4 rats were killed at fortnightly intervals. During the
of this experiment, the bacilli multiplied at the rate of one division every 9 days, but over a period of 7 weeks they multiplied at the rate of one division every 15 days. In a second experiment, bacilli were injected into the testes of rats and the rates of multiplication in the testis was estimated in the same way; over a period of 10 weeks, multiplication proceeded at the rate of one division every 13 days. Accordingly it could not be expected that multiplication *in vitro* would be quicker than this, and probably it would be much slower.

The number of bacilli required to produce an infection in rats was estimated by making up a suspension of bacilli which was counted in the usual way. Serial dilutions x 1/10 were made and standard quantities were inoculated subcutaneously into groups of 4 rats. The rats which received 10^8 bacilli showed slight lumps after 3 months, and marked nodules after 4 months; those with 10^4 bacilli showed nodules after 6 months; those with 10^3 or 10^2 bacilli showed nodules after 6 and 7 months, and those with nominally 10 bacilli all showed definite nodules after 8 months. Clearly a very small number of bacilli is sufficient to initiate an infection, and subcutaneous inoculation into rats is a sensitive test for the presence of viable bacilli.

The resistance of the bacilli to drying was tested by placing a drop of a suspension of bacilli (containing about 300 million bacilli per drop) on each of many small pieces of filter paper 0.5 cm. square and then storing them individually in open test tubes in a hot room at 35°C. After suitable intervals, the pieces of paper were embedded subcutaneously into rats. Rats which received paper immediately or after drying for 5 hours showed nodules after 4 months; those receiving papers after drying for 1 day showed nodules after 5-6 months; those receiving papers after drying for 3 or 8 days showed nodules after 7-11 months; and those receiving papers after drying 16 days showed no nodules even after 14 months. Apparently most of the bacilli are killed by drying for 1 day, a few resist drying for 8 days and none resist for 16 days.

The survival of bacilli in tissue culture media without living cells was investigated by making suspensions of peritoneal cells and bacilli as described below and incubating the cells in a dense suspension for 24 hours so that the cells would be killed. The mixture of dead cells and bacilli was then set up in Carrel flasks in the usual way and incubated at 35°C. At suitable intervals fluid from the flasks was injected subcutaneously into pairs of rats. In a few cases

fluid which had been incubated for 6 days produced nodules after 6 months, but in most of the experiments viable bacilli were not found after 6 or more days. The death of the bacilli might have been due to the absence of favourable conditions or to a direct toxic action of the serum in the medium. These results are in contrast to those obtained by proper tissue culture technique.

In other experiments investigations were made on the effect of cortisone (2 mg. per 100 g. s.c. on alternate days for 3 months) upon the development of nodules in rats. In one experiment the rats which received cortisone developed nodules earlier and larger than the controls without cortisone; in a second experiment, there was no significant difference between the two groups. Other experiments showed that *M. leprae murium* can develop in the cotton rat *(Sigmodon hispidus)* but the lesions are much smaller than in ordinary rats.

Early experiments with tissue culture

These were made with small pieces of tissue, approximately 1 cmm., cut from the edge of lepromata. The fluid medium consisted of 20 per cent rat serum, 3 per cent embryo extract, 67 per cent Tyrode and a small amount of phenol red. Fluid was changed every 4-5 days. The fibroblasts grew vigorously and many were still healthy after 7 weeks; they contained practically no bacilli and it was concluded that they were not desirable in the cultures. The macrophages were less vigorous, but in some cultures a few survived up to 7 weeks They usually contained great numbers of bacilli, but it was impossible to decide from the microscopic appearances whether these bacilli had multiplied in the cell, or whether they had merely accumulated by ingestion. In addition there were great numbers of bacilli lying extracellularly, presumably liberated by the breakdown of cells. In some experiments implants of clean tissue from the spleen or liver of baby rats were inserted into flasks containing implants from lepromata. When examined one or more weeks later the colonies growing around the clean implants included macrophages which contained bacilli; but it was impossible to tell from which colony these macrophages had originated. In some experiments baby rats were inoculated intraperitoneally with bacilli, and a week later tissue cultures were made from the surface of the spleen and liver. The cells grew well, and bacilli could be found in them after several weeks, but this type of culture seemed to offer no particular advantage. Inoculations into rats during these experiments showed that the bacilli survived and were still infective after the longest period studied (54 days), provided that living cells (macrophages) were also present. In flasks where the implant had failed to grow, bacilli were dead after 24 days, although they were still alive after 7 days.

During these experiments it became clear that by this technique it would be difficult to show whether successful culture was being achieved, since it was hard to estimate the number of bacilli inserted into a flask at the beginning of an experiment, in order to compare it with the number present at a later date. Accordingly later experiments were made with macrophages obtained from the rat's peritoneum.

Later experiments

Macrophages were obtained from the peritoneum of a rat by a method similar to that described by MacKaness (1952). Briefly, 10 ml. sterile liquid paraffin was injected intraperitoneally into a large rat. Two or three days later a suspension of bacilli was prepared by grinding down a suitable leproma, mixing with Ringer, centrifuging off the coarse deposit, and taking the supernatant fluid. About 15 ml. of this fluid (plain or diluted) was injected intraperitoneally into the rat which had received paraffin. Two to three days later still the rat was killed and the peritoneum was washed out with cold citrate saline. The washings were centrifuged and the deposit of cells was washed once in citrate saline and then suspended in nutrient medium. After counting the cells and adding more fluid to obtain a suitable density (8,000 cells per cmm.), 3 ml. of the fluid was placed in each Carrel flask. The flasks already contained a thin layer of fowl plasma (plus a little embryo extract to promote clotting) on the floor and one piece of cover slip for sampling. The flasks were then incubated at 35°C. as usual. The fluid medium used in these later experiments consisted of horse or rat serum 40 per cent, Tyrode's solution 60 per cent, 50-100 units of penicillin per ml., and enough phenol red to give a red colour. The fluid was changed three times a week. Enumeration of the bacilli was performed both by spreading 20 cmm. of the fluid inserted into the flasks on a slide as described above, and also by taking two of the flasks after one day and digesting the total contents with citric acid as described above.

The cells cultivated by this procedure were almost all macrophages. In some flasks fibroblasts did appear and occasionally they became numerous, but in most flasks they remained inconspicuous. The cells initially formed a close covering over the floor of the flask, but after three weeks they were less numerous, although still apparently healthy; and after 4-5 weeks they usually became degenerate and diminished considerably in number. In the later experiments of this series, when it was seen that the cells were diminishing in number and in health, a fresh supply of macrophages, *without bacilli,* was obtained by the method described above and added to the flask when the nutrient medium was being changed. The new cells seemed to settle down readily on top of

the old colony, ingesting any bacilli which might be available in distintegrating cells of the earlier generation. This addition of fresh macrophages can be repeated as often as is desired. One flask was maintained in this way for 4 months with 4 lots of new cells. Provided that there is no infection by bacteria resistant to permissible antibiotics, it seems that a substrate of vigorous macrophages can be maintained indefinitely by this technique. In order ot avoid undue loss of the bacilli during changing of the fluid medium, which takes place at an increased rate when the cells are degenerating and disintegrating, it is advisable to insert new cells as soon as the earlier cells show the least sign of diminution. During the maintenance of these cultures the fluid medium was changed three times a week (as said), and on each occasion there is the possibility of losing a smaller or larger number of bacilli. The importance of this factor was not appreciated until the latest period of this work. In one case, in a flask which had been maintained for over 2 months and which probably contained about 3 million bacilli at this time, the loss on each change (as estimated from examination of the fluid removed) was about 100,000 bacilli, i.e. about 3 per cent of the total number present. If the cells are degenerating, or if part of the plasma clot flakes off, the loss can be much greater. During most of this work no notice was taken of this possibility of loss, until one day fragments of fibrinous clot, which had been removed while changing the fluids from two flasks, were examined and were found to contain dense masses of bacilli.

After various preliminary trials an experiment consisting of 29 flasks was set up, 19 with horse serum and 10 with rat serum. Two flasks were sacrificed after one day and showed that there were 270 million bacilli per flask present. Forty-two days later a small floating piece of fibrin, which had been removed from one of the flasks (166) during changing, was examined and it was found to contain enormous numbers of bacilli. This flask and two others were then terminated for examination by removal of the cover slip for microscopical inspection and by digesting the remaining contents with citric acid. One of these flasks (169, which had received leproma extract) was found to contain 354 million bacilli, plus a clot containing dense masses of bacilli; no new cells had been added to this flask. The second (116, which had received mycobactin) contained 210 million bacilli, plus a clot which contained enormous numbers of bacilli, mostly still inside cells. The third (161) contained 132 million bacilli, plus a big clot with large masses of bacilli. Since these flasks initially contained only 270 million bacilli and since a large but unmeasurable number of bacilli were known to have been lost during changing, it was considered that multiplication of the bacilli had occurred certainly in flask 169 and most probably

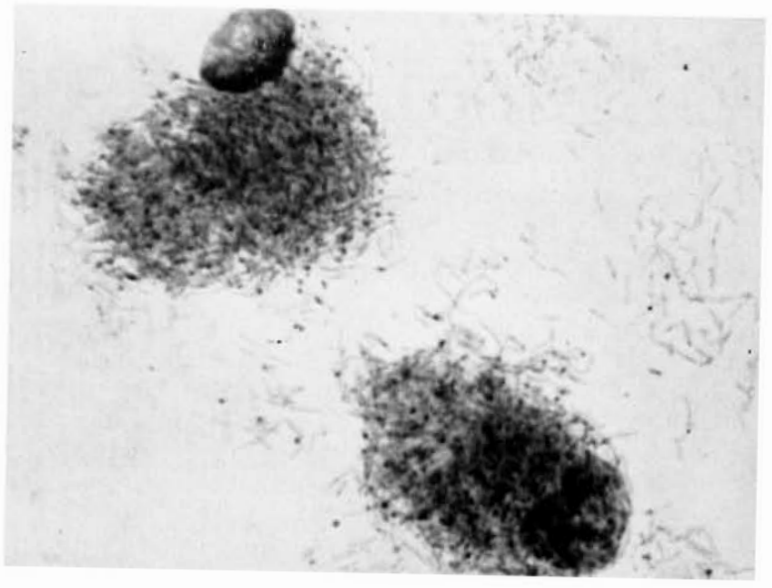

Fig. 1. Two large macrophages full of bacilli. Smear of clot from flask 166 after culture for 42 days.

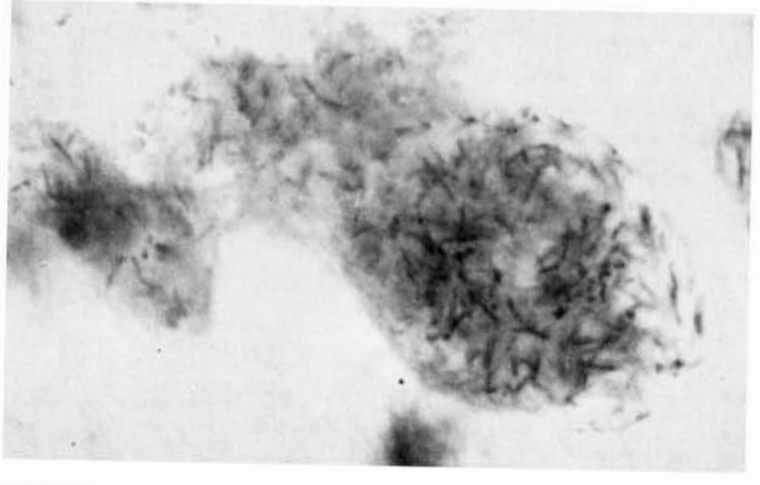

Fig. 2. Bacilli in large cell, after cultivation for 44 days (flask 161).

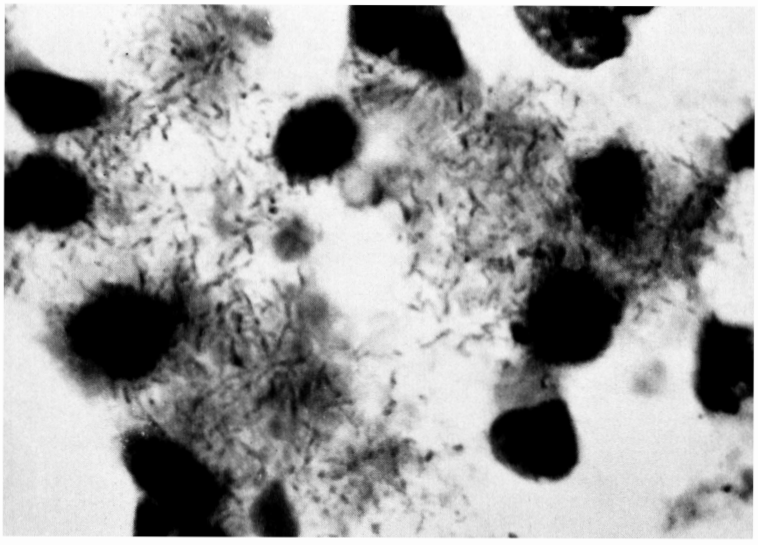

Fig. 3. Bacilli in cells after cultivation for 53 days (flask 171). Stained with Ziehl-Nielsen and haematoxylin. Magnification x 2,200.

in the others also. The microscopic appearances on the cover slip (described below) also supported this interpretation.

At this point circumstances necessitated the termination of the work, and the existing cultures were killed for examination. Among the flasks which were thus terminated 52 days after the start, one flask (168, which had received leproma extract) contained 550 million bacilli, which was twice the number initially present; also many bacilli had presumably been lost during the 23 changes of fluid medium. Other flasks contained 235, 212, 214 million and similar numbers. All these flasks had been set up with horse serum. One flask which had been set up with rat serum contained 280 million; apparently the use of rat serum (instead of horse serum) is not sufficiently advantageous to outweigh the greater trouble of collection. On the other hand, 3 of the 4 flasks which received leproma extract had higher counts than those which did not, and all of the 4 flasks which received mycobactin had high counts; therefore the presence of these two substances probably promoted the multiplication of the bacilli, but it was not essential since other flasks which did not receive them also yielded high counts. Similarly the addition of new cells, although highly advantageous in most cases, is not essential, since flask 169 (mentioned above) showed clear evidence of multiplication although no new cells had been added.

On microscopic examination of the slips removed from the flasks, most of the cells were found to be macrophages. Giant cells with several nuclei occurred fairly frequently; fibroblasts were sometimes present in small numbers. The macrophages and giant cells contained varying numbers of bacilli; in some cultures and some areas the cells were distended by great masses of them. The bacilli appeared healthy. Unless the cells were degenerating (or unless they had been broken by making a smear) all the bacilli were found inside the cells. In some places the plasma clot was much thicker than elsewhere, e.g. the original layer of plasma clot had become folded over; in other places the macrophages had invaded the plasma clot underneath the cover slip, between the slip and the floor of the flask. In both these places the cells contained especially large numbers of bacilli, and it seemed that they constituted particularly favourable sites for the development of the bacilli. Probably the cells were protected from movements of the fluid at these places, so that bacilli liberated by disintegration of a cell would have a good chance of being ingested by another cell before they were washed away; alternatively, bacilli might be favoured by the lower concentration of oxygen which might be expected in such sites. (A low concentration of oxygen is favourable to the survival of trypanosomes *in vitro* and the same may well

be the case in the cultivation of *M. leprae murium*). As regards the infectivity of the bacilli found in these flasks, fluid removed from flasks 158 and 163 after 40 days culture was injected into a rat and produced a leproma after an incubation period of 5 months; similarly fluid removed from flasks 152 and 153 after 42 days produced a leproma after a period of 6 months.

Discussion

In the experiments described above, the bacilli of rat leprosy have been maintained in cultures of macrophages for over six weeks. At the end of this time the bacilli appeared numerous and healthy, and they were infective to rats. Judging by the counts they had doubled their numbers in one flask, (even though considerable quantities had presumably been lost during the changes of nutrient fluid). The following suggestions may be derived from these experiments:—

(1) The addition of fresh macrophages at suitable intervals permits cultures of this type to be maintained practically indefinitely and the bacilli pass readily from one crop of cells to the next.

(2) A medium containing 40 per cent of serum is beneficial to the cells, and the bacilli can probably multiply in the presence of this amount of serum. It has been reported by Hanks (1952) and Hanks & Gray (1954) that when bacilli are suspended *in vitro*, their respiration is impaired by substances present in serum; and Hanks has recommended that the presence of any, or of much, serum should be avoided during attempts to grow the bacilli in tissue culture. The above experiments suggest that this view is not wholly correct, as multiplication was probably obtained in the presence of 40 per cent serum. Since the bacilli are contained inside the cells and can pass from one cell to another (during the phagacytosis of degenerate cells by healthy ones) without significant exposure to hypothetical antibacillary substances in the serum, it is probably more important to provide conditions which are favourable to the growth of the cells than to provide those favourable to extracellular survival of bacilli. As intracellular parasites, the leprosy bacilli presumably depend upon the metabolism of the host cell for many important products and processes; and probably the multiplication of the bacilli will not occur unless the metabolism of the host cell is healthy and vigorous.

(3) The growth of the bacilli appears to be favoured by the presence of a thick plasma clot around the cells (see above); it might be advantageous to add fresh layers of plasma at suitable intervals during the maintenance of these cultures.

(4) During experiments of this kind it is important to estimate the number of bacilli removed from the flasks by the frequent changing of fluid medium and especially to examine any small clots

which may escape during this procedure.

(5) Extract of lepromatous tissue and mycobactin probably promote the multiplication of the bacilli, although their presence is not essential; they deserve further investigation.

(6) The optimal temperature, whether 37°, 35°, or 30° should also be investigated, since *in vivo* the bacilli multiply best in the superficial and, therefore, cooler parts of the animal.

(7) Penicillin, e.g. 100 units per ml., seems not to interfere with the growth of the bacilli and it is certainly valuable in preventing the growth of contaminants.

(8) In the above technique, liquid paraffin was used to facilitate the collection of macrophages from the rat's peritoneum; although such a foreign substance would appear to be disadvantageous to the cells, it is conceivable that globules of paraffin inside the macrophages might promote the multiplication of the bacilli therein.

The cultivation of the bacillus of rat leprosy is of importance in so far as it may help in the cultivation of the bacillus of human leprosy. Judging by the present experiments it is suggested that in attempts to grow *M. leprae* a substrate of macrophages should be used, presumably obtained from the blood, and that this should be renewed at suitable intervals by adding fresh cells to the containers. It would be essential to count the number of bacilli at the start of the experiment (either before or after addition to the cells) and again at its conclusion. The concentation of serum in the medium should be adjusted so as to favour the survival of the cells, i.e. it should probably be high. A thick plasma clot surrounding the cells would probably be advantageous; leproma extract or mycobactin might be advantageous. Even under favourable circumstances the growth of the bacilli seems to be slow, so it would be necessary to maintain the cultures for long periods. Since *M. leprae* grows as an intracellular parasite in macrophages, and since macrophages can be easily grown in tissue culture, experience with other intracellular parasites suggests that the cultivation of the human leprosy bacillus ought eventually to be possible by a suitable tissue culture technique.

Summary

Attempts were made to grow the bacillus of rat leprosy in tissue culture, using a Carrel flask technique with macrophages from the rat's peritoneum as a substrate. In the most favourable culture the number of bacilli present in a flask doubled during a period of 52 days; in addition an unknown (but probably large) number of bacilli had been lost during 23 changes of the fluid medium. The microscopic appearances also supported the belief that multiplication of the bacilli had occurred in these cultures. After incubation for 42 days cultures were still infective to rats. The growth

of the bacilli was favoured by the following factors in the technique: the fluid medium contain 40 per cent serum (horse or rat) to promote good growth of the cells; fresh supplies of uninfected macrophages were added as required (every 3-4 weeks), which would allow such cultures to be maintained indefinitely; a thick plasma clot around the cells; presence of mycobactin or of extract of leproma.

Other experiments showed that the bacilli doubled *in vivo* every 13-15 days; very small numbers (nominally 10 bacilli) were enough to infect a rat; most bacilli were killed by drying for 1 day, but some were not killed until after 8 days; they did not survive in tissue culture media at 35°C. for more than 6 days unless viable cells were present.

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*TUBERCULIN AND LEPROMIN SENSITIVITY IN E. NIGERIA

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Introduction

Outside the sphere of chemotherapy, the most important contribution to leprosy control in recent years has probably been the discovery of an immunological relationship between leprosy and tuberculosis. The existence of this relationship is not seriously in doubt. It has repeatedly been shown that in suitable circumstances BCG inoculation can induce sensitivity to lepromin as well as to tuberculin, and evidence is beginning to develop that children rendered lepromin sensitive by this method have in fact some protection against clinical leprosy. The nature of this relationship and the circumstances in which it is manifested are, however, not so clear. Increasing knowledge of the tuberculin and lepromin reactions has revealed new complexities not only in their relationship, but in the character of the reactions themselves. While it is generally agreed that high grade sensitivity to tuberculin is induced specifically by *M. tuberculosis*, the tuberculin reaction is subject to a variety of influences which have been discussed by Pepys (1954), and it is possible that low grade sensitivity may be induced by local and non-specific factors, of which one may indeed be *M. leprae* itself (Fernandez and Cabanillas 1955). Recent work on the lepromin reaction, notably that of Kooij and Gerritsen (1956) and Floch (1956) has drawn attention to non-specific elements in it. These matters would be mainly of academic interest were it not for the possibility that under field conditions the relationship between the two infections can be much more distant than it appears when stimulated by BCG. Thus Guinto, Doull and Mabalay (1956) in the Phillippines found a significant minority in whom no relationship between the two sensitivities could be detected. Kuper (1955) could detect no simple direct relationship between them in 110 South Africans. There is also the unresolved anomaly in the findings of Lowe and McNulty (1953a) that a relationship evident among healthy persons was not found among leprosy patients. It is clearly desirable to clarify the circumstances governing the operation of the relationship between the two infections and more extensive study of the subject is called for.

E. Nigeria is a very interesting field for this study at the present time. In many localities the incidence of leprosy is declining. Along trade routes tuberculosis is becoming a major problem. If the two diseases are antagonistic to one another by virtue of an immunological relationship, a demonstration of the form of that relationship

may be taking place before our eyes. Lowe and McNulty (1953a, b,) working in this area found a significant correlation between tuberculin and lepromin sensitivity in 357 healthy persons, mostly secondary school boys and the staff of the Uzuakoli leprosarium. In this work tuberculin was used in both high and low dosage. Further analysis by Lowe and Davey (1956) indicated that there was still significant agreement if the results from a low dosage of tuberculin (10 TU) were alone considered. In a further group of 621 schoolchildren Lowe and McFadzean (1956) again found significant correlation between the two reactions, but it was less marked, and could have been produced by the simultaneous exposure of those concerned to tuberculosis and leprosy. As these children came from a country town on the railway in close proximity to a large leprosarium, this is at least possible.

The extension of this study to larger groups and a wider range of age and locality is here described. It was hoped thereby not only to broaden the basis on which conclusions were reached, but also to explore the very important dynamic aspects of any relationship found between the two sensitivities. In a country where tuberculosis is spreading, tuberculin sensitivity should show considerable variation between one locality and another, high in townships and along main routes of communication, low in localities distant from them. If tuberculin sensitivity induces lepromin sensitivity, this too should vary in harmony with the tuberculin findings regardless of its basic level in the communities concerned. Part I of this paper reports the results of simultaneous tuberculin and lepromin tests undertaken in schoolchildren in six different localities. There is also much to be gained by extending such a study to complete communities in the form of a tuberculin and lepromin survey. Thereby not only could the progress of each sensitivity be observed, but the relations between the two could be followed through succeeding age groups over the whole span of life.

Facilities for mass radiography are not yet available, but a survey would also make some estimate of the amount of clinical tuberculosis in the community. Part II of this paper gives the results of such a survey. Each part covers approximately 2,500 individuals.

Material and Technique

TUBERCULIN. The tuberculin used in this work was standard PPD manufactured at Weybridge, England. Undiluted, it keeps well in tropical conditions, but once diluted was used immediately, Mantoux tests being undertaken throughout in a strength of 10 TU per 0.1 cc. dose. Reactions were measured after 48 hours, and those 6 mm. in diameter or over regarded as positive, with 12 mm. and

18 mm. indicating high grades of sensitivity.

LEPROMIN. Lepromin prepared by the modified Dharmendra technique used by Lowe, and identical with that employed in previous studies in Nigeria, was used in this work. The technique of its preparation and reading has been described by Lowe and McNulty (1953b). The early (Fernandez) reaction was recorded after 48 hours, the late (Mitsuda) reaction after 21 days.

The three attendances necessary for obtaining the basic information sought were all that could be expected in the circumstances. It would have been valuable if tuberculin could have been used in both low and high dosage, but this would have involved another attendance on the part of those involved, and wastage would have been considerable. We were concerned primarily with sensitivity induced specifically by *M. tuberculosis*, and selected a 10 TU dose as a generally accepted standard for this, with little likelihood of non-specific reactions being obtained.

While referring where necessary to the findings in the early lepromin reactions, we have followed other workers in Nigeria in laying greater emphasis on the Mitsuda reaction, as more easily read and of greater practical significance. As Lowe pointed out (1955) the Mitsuda reaction is a reaction to the whole bacillus, and indicates resistance to it, and it is the development of this resistance that we are seeking to examine.

The team engaged in this work was kept as small as possible Lepromin reactions were read by two of us (T.F.D. or S.E.D.) together with Mr. G. O. Okezie, Senior Technician at the Uzuakoli Research Unit. Tuberculin reactions were measured by two of us (S.E.D. or C.S.). Assistance in the inoculations was also given by Mr. A. Eden and Dr. G. Moneta. Estimations of age were frequently necessary, and were the responsibility of one person (T.F.D.).

PART I.
TUBERCULIN AND LEPROMIN REACTIONS IN SCHOOLCHILDREN

In order to investigate the variations in tuberculin sensitivity between one locality and another, and its relation to variations in lepromin sensitivity, six schools were chosen, the first in a rapidly developing township, the others in rural areas of varying distance from main lines of communication. Tuberculin and lepromin tests were carried out on all the children attending these schools, a total of 3,052. Absentees on one or other of the days when tests were read accounted for a wastage of approximately 16%. Schools concerned, with numbers completing the tests are as follows, the school in an urban area heading the list, with rural schools listed in order of increasing remoteness from main roads, rail and river communications.

School	Status	Numbers completing test
C.M.S. School, Umuahia	Urban	455
C.M.S. School, Akwete	Rural 1	341
C.M.S. School, Ife	Rural 2	448
C.S.M. School, Abiriba ...	Rural 3	661
C.S.M. School, Nkporo ...	Rural 4	516
C.S.M. School, Ndi Oji ...	Rural 5	180
	Total	2,601

Results

(A) TUBERCULIN SENSITIVITY

In Figure I tuberculin positive rates at each school are presented graphically as a percentage of the total examined in each three year age group. The figures are analysed in Table I. At Umuahia, the urban school, the tuberculin positive rate is high, decidedly higher than was found for corresponding age groups in rural England in the M.R.C. Tuberculin survey (1953), as is evident from the following comparison.

	Age Groups			
	5	6—8	9—11	12—14
Umuahia, Nigeria	11.8	23.0	45.2	50.0
Rural England	15.0	19.1	27.6	34.6

For a fair comparison, the M.R.C. figures are here adjusted to cover a 10 TU dose only. In that survey a reaction of 5 mm. diameter was regarded as positive. The Umuahia figures are based on a minimum reading of 6 mm., so the difference is a little larger than the figures indicate. It is larger still if the Umuahia findings are compared with those in urban England.

In rural areas in Nigeria tuberculin sensitivity is at a lower level, and in general becomes lower as contact with townships decreases. We are here not concerned with bovine tuberculosis. Cattle are very scarce, and no fresh milk whatever is drunk, the very idea being abhorrent to the people. The tuberculin positive levels found appear to give a fair indication of the degree to which human tuberculosis has penetrated into the localities concerned, and do in fact reflect not only the geographical position of each locality but also the mode of life of the people. Ife, Ndi Oji and Abiriba are predominantly agricultural communities. Akwete is located on an important river, and has many contacts with the outside world. Nkporo is a very interesting case. Here the people, traditionally farmers, have progressively abandoned agriculture for trade since a road to the area was opened 10 years ago, and now have many contacts with Umuahia, where tuberculosis is known to be rife. In all cases the incidence of positive reactions in the two sexes was closely parallel.

These findings form a pattern which is reasonable and in line with local conditions. In comparison with the M.R.C. figures the level of sensitivity is rather higher than was expected. It is, however, in line with the findings of earlier workers, Lowe and McFadzean with a 5 TU dose obtaining at Uzuakoli figures rather lower than those found here at Umahia, while Lowe and McNulty's findings were well within the limits recorded here.

(B) LEPROMIN SENSITIVITY

(a) *Mitsuda Reaction*

Positive rates for the Mitsuda reaction are presented for each school and age group in Figure 2. They are analysed in Table I. It is evident that in these localities sensitivity to the late lepromin reaction varies widely, high in the urban area and one rural area, and lower in other rural areas. The findings of Lowe and McNulty and Lowe and McFadzean all fall within the range seen here.

(b) *Fernandez Reaction*

In all cases sensitivity to the early (Fernandez) reaction was much less than that shown to the Mitsuda reaction. The lepromin used is itself less sensitive to the early reaction than it is to the late, but this does not account for the findings in this work. A review of considerable numbers of leprosy patients shows that with this lepromin, among adults who are Mitsuda positive, 55% may be expected to give a positive Fernandez reaction. Among children the corresponding figure is 25%. In the healthy schoolchildren examined in this work, the level was much less than this, so low in fact that its presentation in the form of a graph is impracticable. The percentage of children giving positive Fernandez reactions at each school is compared with the corresponding Mitsuda figure as follows:—

School	Positive Mitsuda %	Positive Fernandez %
Urban	58	3·7
Rural 1	29	4.0
Rural 2	25	3.0
Rural 3	78	2.4
Rural 4	29	0.8
Rural 5	40	nil

This contrast between the findings in the two reactions among healthy children has also been found by Guinto and his colleagues (1955) in the Philippines.

The question arises how far these findings are related to the incidence of leprosy in the localities concerned. A considerable amount of information is available on this point. Leprosy was formerly exceedingly rife in three of these localities, but has declined very markedly during recent years. At Nkporo three leprosy surveys

have been undertaken, and indicated an incidence of 60 per mille in 1937, 57 per mille in 1939, and 9 per mille in 1956. At Ndi Oji, an incidence of 121 per mille in 1941 had become 76 per mille by 1947, and in 1955 had fallen to 17 per mille. In both these localities vigorous anti-leprosy measures have been in operation throughout the period of these surveys with excellent co-operation from the people, and for at least 10 years no open case of leprosy has been known to be living in the community. Abiriba comes under the same category. Although no repeated survey results are available, the locality was surveyed for leprosy purposes in 1943-4, all leprosy cases found enrolled for treatment, and open cases isolated at a segregation village three miles away. The virtual disappearance of fresh cases of clinical leprosy during recent years in all three localities is sufficient evidence of the effectiveness of the control measures being maintained there. It is difficult to see how infection with *M. leprae* can be held responsible for the Mitsuda positive levels found among young children in these localities, 69% at Abiriba, 41% at Ndi Oji and 39% at Nkporo in the 6—8 age group.

The same is true of Umuahia. This growing township is located near to the area of former high leprosy incidence, though in nearby villages an incidence of only 14 per mille was encountered in 1938. Although 61 patients are still attending a nearby leprosy clinic, very few come from the township itself, none of them an apen case. It is difficult to see how 47% of children by the age of 5 can have had sufficient contact with *M. leprae* to have been sensitised to it.

At Akwete and Ife the incidence of leprosy has never been high.

In comparing the levels of sensitivity between one locality and another, the possibility of variations induced by season and states of nutrition must not be ignored. The school at Abiriba was studied in the cool wet season (June), the other schools in the hot dry season. Diet is least satisfactory during the wet season, particularly where the vitamon B complex is concerned. Seasonal influences on the lepromin reaction were studied by Dharmendra, Lowe and Mukherji (1942), who noticed in leprosy patients an enhancement of the reaction in summer. We think such influences cannot explain the difference in level found between different localities in this work.

Thus although higher levels of sensitivity to the Mitsuda reaction are found in localities where leprosy has been rife in the past, it is very unlikely that here or elsewhere infection with *M. leprae* is alone responsible for the actual levels found among children at the present time. Neither can it be said that comparing results in one locality with those in another, the Fernandez positive rates reflect the known incidence of leprosy.

A curious finding in three localities is a tendency for the levels of lepromin sensitivity actually to fall in the years around puberty. No explanation can be offered for this. In the second part of this work also, large groups at these ages displayed a tendency for a slowing down in the progress of lepromin sensitivity.

(C) THE INFLUENCE OF TUBERCULIN SENSITIVITY ON LEPROMIN SENSITIVITY

The tuberculin and Mitsuda findings are brought together in Table I, which shows for each school the percentage of children in each age group giving positive or negative reactions to the two tests. Actual numbers examined in each age group are also given.

TABLE I

Tuberculin and lepromin positive rates

To—Tuberculin negative % age group. Lo—Lepromin negative % age group. T+—Tuberculin positive % age group. L+—Lepromin positive % age group.

Place		Age Groups 5		6—8		9—11		12—14		15—16	
		To	T+	To	T+	To	T+	To	T+	To	T+
Urban	Lo	47	6	43	5	31	9	19	15		
	L+	41	6	34	18	24	36	31	35		
Numbers tested		17		248		135		55			
Rural 1	Lo			60	10	69	10	57	17	35	31
(Akwete)	L+			18	12	12	19	11	15	19	15
Numbers tested				169		58		88		26	
Rural 2	Lo	82	9	65	7	60	11	60	15	39	35
(Ife)	L+	15	3	22	6	22	7	14	11	23	3
Numbers tested		39		106		46		126		31	
Rural 3	Lo			31	1	17	1	11	0	11	11
(Abiriba)	L+			59	9	71	11	76	13	64	14
Numbers tested				277		188		168		28	
Rural 4	Lo			56	6	54	12	51	29	27	47
(Nkporo)	L+			21	17	18	16	9	11	3	23
Numbers tested				68		304		114		30	
Rural 5	Lo			77	3	65	0	40	10	40	3
(Ndi Oji)	L+			17	3	18	17	39	11	33	24
Numbers tested				49		47		54		30	
Total tested		56		917		878		605		145	

At every school these findings indicate the existence of a relationship between tuberculin and lepromin sensitivity. If individual age groups are examined it is found that with two exceptions, both in the 15—16 age group, the proportion of lepromin positive reactors is higher among tuberculin positive reactors than it is in the age group as a whole. The converse is also true, that tuberculin positive reactors are relatively more numerous among lepromin positive reactors than they are in the age group as a whole. This association does indeed extend further than is indicated here. The proportion of strongly positive lepromin reactors is higher among strongly positive tuberculin reactors than it is among weak reactors. The converse also applies.

That is as far as the examination of individual age groups can take us. It demonstrates the existence of a relationship between the two sensitivities, but gives no clue to its significance. There are at least three possibilities here. Both leprosy and tuberculosis flourish in the same conditions of overcrowding. It is possible therefore that both infections have a bias towards the same groups in the community, and this could explain their association. There is also the familiar possibility that tuberculosis is inducing lepromin positivity. There still remains the possibility that other sensitising agents are entering into the picture and confusing it. Some light on these questions begins to appear when we compare the relationship between the two sensitivities in different localities, and follow their changes through succeeding age groups. If one is influencing the other, there should appear a constant element in their relationship which such a study should reveal.

We may begin by observing that in the urban area the proportion of children exhibiting sensitivity both to tuberculin and lepromin is decidedly higher than elsewhere, 36% between the ages of 9 and 11 and 35% between the ages of 12 and 14. Any relationship that exists between the two sensitivities is neverthless very far from perfect. Even if we could assume that tuberculosis infection was responsible for all the lepromin positive reactors in this group, there would still remain important minorities among positive tuberculin reactors in whom no lepromin conversion had occurred, 20% of them in the 1—11 age group and 30% in the 12—14 age group. At its best, therefore, the relationship is defective and tends to grow less as the level of tuberculin sensitivity rises with age.

In rural areas the proportion of children positive to both tests is decidedly lower, and varies between 7% and 19%. The influence of tuberculosis on the lepromin reaction is best examined by considering the proportion of tuberculin positive reactors who are also lepromin positive. In Table II this proportion is given as a percentage of tuberculin positive reactors in each age group. Consideration is given only to age groups between the extremes of 6 and 14 years, including thereby in the analysis only age groups containing considerable numbers of children. The average tuberculin and lepromin levels over the same period are also given.

TABLE II

Percentage of positive tuberculin reactors who are positive to lepromin

	AGE GROUP				Average rate %	
	6—8	9—11	12—14	Average	Tuberculin	Lepromin
Urban	78	80	70	77	33	56
Rural 1	55	60	47	53	37	41
Rural 2	47	39	42	42	19	27
Rural 3	90	92	100	97	11	79
Rural 4	74	57	28	50	31	31
Rural 5	50	100	52	71	15	42

The wide variation in these figures between one place and another, and the irregularity of their movement through succeeding age groups make it hard to believe that a common influence is at work in their production. We may very well ask how tuberculosis could induce 97% lepromin conversions in one place and only 42% in another. Furthermore, the percentage of positive tuberculin reactors who are positive to lepromin does not bear any direct relation to the level of tuberculin sensitivity itself. The differences in level from place to place are indeed related not to the tuberculin positive rate but to the lepromin positive rate. Where the lepromin rate is high, a high proportion of positive tuberculin reactors are lepromin positive. When it is low, the proportion is low. It is also noticeable that in four of the six localities, as the number of positive tuberculin reactors increases with age, the proportion of them who are lepromin positive actually falls. It is impossible to reconcile these findings with the theory that tuberculosis is inducing lepromin postivity to any significant degree. The same result is obtained if the findings are examined from other angles.

f the lepromin positive levels in rural areas lack any significant component contributed by tuberculosis, their further examination is of interest. It has already been stated that infection with *M. leprae* is not likely to be responsible for the high levels found among young children in certain localities. What then is responsible? It is impossible at present to answer this question. It is discussed further later.

Summarising the findings so far it may be said that in both urban and rural areas tuberculin and lepromin sensitivity tend to be associated in the same individuals. In the urban areas it is possible that tuberculosis infection is responsible for this to a small extent, but in rural areas where tuberculosis is at a lower level it is impossible to find any evidence that it is having any appreciable influence on lepromin sensitivity. In some localities it is very doubtful that *M. leprae* could itself be responsible for the level of lepromin sensitivity seen in young children and here at least it is probable that geographical and constitutional factors are influencing the situation.

In view of the findings so far in this study it was considered desirable to expand the investigation in one of the localities concerned to cover as many of the population as possible, and follow the levels of tuberculin and lepromin sensitivity through successive age groups over the whole span of life. Such an investigation is described in Part 2 of this paper.

PART II
TUBERCULIN AND LEPROMIN REACTIONS IN ENTIRE COMMUNITIES

For the second part of this work a group of four associated villages was chosen, having a combined population of almost exactly 2,500. It was from these villages that the children attending Ndi Oji school were mainly drawn. Leprosy was formerly very rife in this locality, and as repeated leprosy surveys have been undertaken during the past 15 years, the people could be relied upon to co-operate in the rather strenuous demands made upon them in the present work. There would also be the important advantage that lepromin and tuberculin sensitivity could be considered against a background of accurate knowledge of the incidence and trend of leprosy during recent years.

Tuberculin and lepromin tests were carried out on the entire resident population of these villages, from infants in arms to the oldest inhabitant, and although some wastage was inevitable, 2,221 out of a total of 2,491 inoculated, were actually present and examined on both the occasions when the reactions were read.

Results

The percentage of tuberculin and lepromin positive reactors in each age group is shown in Figure 3, which is based on the following figures.

	AGE GROUP							
	5-9	10-14	15-19	20-29	30-39	40-49	50-59	60+
Tuberculin positive %	7.1	16.0	28.1	34.2	51.6	64.9	62.0	64.3	65.8
Lepromin positive %								
(a) Mitsuda	13.2	28.0	43.2	44.4	49.9	62.0	65.6	59.4	68.4
(b) Fernandez	1.1	1.5	2.5	0.9	4.0	7.8	4.8	5.6	8.9
Number tested	450	482	248	108	327	204	163	160	79

Differences in the development of tuberculin and lepromin sensitivity noticed among schoolchildren are again evident in the large groups tested in these villages. Signs of sensitivity to lepromin began to be evident in the first year of life and developed rapidly up to the age of twelve. Sensitivity to tuberculin tended to appear later and develop more slowly, but more steadily. From the age of twelve onwards there was a noticeable slowing down in the rate of increase of lepromin sensitivity, with the result that the difference between the two sensitivities diminished during adolescence, and early in adult life both levels met. From the age of about 35, when approximately 64% of the population had become sensitised to both reactions the level of each remained remarkably steady and in close approximation to the other.

The extremely low level of sensitivity to the Fernandez

reaction is again noticeable. It tends slowly to increase with age, and is not considered further.

During childhood there were no appreciable differences between the two sexes in sensitivity to either reaction. During adult life distinct differences became perceptible. The respective levels are given below and drawn in Figure 4.

	AGE GROUPS								
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	60+
Tuberculin positive rate %									
Males	9	16	26	52	67	80	74	72	72
Females	9	16	33	32	48	57	53	48	61
Lepromin positive rate % (Mitsuda)									
Males	12	33	40	70	60	77	74	74	74
Females	11	26	49	35	48	56	60	50	67

The influence of tuberculin sensitivity on lepromin sensitivity

A quite remarkable similarity between the two sensitivities is evident in this community. Although the actual level of tuberculin sensitivity is related to the 10 TU dose used, and higher levels would doubtless have resulted from the use of a larger dose, it is impossible to deny the existence of a relationship between the two sensitivities so plain in their behaviour through succeeding age groups and between the sexes. When analysed in detail, however, the picture is once again confused, and it is impossible to find real evidence that the lepromin level is in any way dependent on the tuberculin level. The percentage of each age group giving negative, weak or strong reactions to each of the two tests is as follows:—

TABLE III

AGE GROUP

	0-4			5-9			10-14			15-19		
	To	Tw	Ts	To	Tw	Ts	To	Tw	Ts	To	Tw	Ts
Lo	81.6	3.5	1.7	64.9	4.3	2.8	47.2	6.5	3.1	41.7	7.4	6.5
Lw	9.7	0.4	0.9	15.9	2.9	3.9	20.6	8.1	4.1	15.8	7.4	0.9
Ls	1.6	0.4	0.2	3.2	1.0	1.1	4.1	3.2	3.1	8.3	6.5	5.5
Numbers tested		450			482			248			108	

	20-29			30-39			40-49			50-59			60+		
	To	Tw	Ts	To	Tw	Ts	To	Tw	Ts	To	Tw	Ts	To	Tw	Ts
Lo	31.5	12.8	5.8	19.7	10.9	7.4	16.6	9.8	8.0	19.4	13.1	8.1	12.7	8.8	10.1
Lw	12.6	11.9	8.6	8.8	11.2	9.8	12.2	9.8	10.4	9.4	6.2	12.5	15.2	8.8	19.0
Ls	4.3	5.2	7.3	6.6	12.8	12.8	9.2	7.4	16.6	6.9	8.8	15.6	6.3	7.8	11.3
Numbers tested		327			204			163			160			79	

To. Tuberculin negative %

Tw. Tuberculin positive weak %

Ts. Tuberculin positive strong %

Lo. Lepromin negative %

Lw. Lepromin positive weak %

Ls. Lepromin positive strong %

These findings have been examined statistically for us by Mrs. A. Foster who has also prepared the accompanying histograms which show the relation of each sensitivity to the other at negative, weak and strong levels of response. They illustrate the conclusion, that neither reaction is directly dependent upon the other to any significant extent.

Applying the method of analysis followed in Part I of this paper, the percentage of those positive to one test who are also positive to the other is as follows:—

	AGE GROUPS								
	0-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	60+
% of T plus who are also L plus	27	56	66	59	62	72	71	67	71
% of L plus who are also T plus	14	32	43	46	66	75	67	73	69

In both cases we notice that these figures are dependent on the actual level of the other sensitivity in the age group concerned. When in adult life a stable situation is attained, the proportion of tuberculin positive reactors who are lepromin positive is only slightly larger than it is in the general population. The same is true of the proportion of lepromin positive reactors who are tuberculin positive. Both sensitivities behave in a manner independent of the other, but both are related in some way.

There remains one line of inquiry open to us. During recent years leprosy has declined very markedly at Ndi Oji, from an incidence of 120 per mille in 1941 to 17 per mille in 1955. It is of interest to compare the tuberculin and lepromin levels among present and past patients with those encountered in the general population. The population tested in this work include 144 patients and ex-patients, most of whom are distributed fairly evenly among the adult age groups. Among them the tuberculin and lepromin positive rates are as follows:—

	AGE GROUPS				
	20-29	30-39	40-49	50-59	60+
T+% ...	56	69	60	57	61
L+%	50	69	41	61	79

The tuberculin levels in every case fall between the levels for the two sexes in the general population. Of the lepromin levels, three fall between the limits found in the general population, one below them, and one above them. There is thus no evidence that clinical leprosy has been associated with a lower level of tuberculin sensitivity than prevails in the general population.

Discussion

The findings obtained in this work may be summarised as follows. Among large groups of children in urban and rural areas in Nigeria and in equally large groups of adults in one rural area, sensitivity to lepromin and to tuberculin tends to be associated in the same individuals, and each occurs more frequently among those

sensitive to the other than among those insensitive to it. In the urban area the possibility cannot be ruled out that tuberculosis is exerting some influence on lepromin sensitivity. There is, however, no real evidence that in rural areas sensitivity to tuberculin has any capacity to induce sensitivity to lepromin to any appreciable extent. There is certainly no simple direct relationship between them.

The low level of sensitivity to the Fernandez reaction encountered in both schoolchildren and adults is of interest. Tuberculosis infection has clearly had no tangible influence on this. In this work healthy people exhibited decidedly less sensitivity to the Fernandez reaction than is encountered among leprosy patients.

These findings immediately present an important problem. It is an established fact that BCG inoculation can and usually does produce lepromin conversion in healthy negative reactors. There is strong evidence that in many parts of the world infection with tuberculosis can do the same. Here in rural Nigeria it is failing to do so, in spite of the existence of an obvious relationship between the two sensitivities. It is impossible at present to offer any satisfactory explanation for this phenomenon. A few observations can, however, be made.

In the first place, although tuberculosis is certainly rife in Umuahia township, all the indications are that in rural areas it is as yet a minor problem. No case of clinical tuberculosis was encountered at Ndi Oji among the 2,500 people seen, and although in the absence of radiography accurate information is lacking, it can at least be said that tuberculosis is not an important public health problem in that locality. With active tuberculosis at a low level exposure to *M. tuberculosis* is likely to be casual and infrequent. BCG inoculation administers a dose of antigen of a totally different order from that likely to be encountered in these circumstances.

A most interesting finding at Ndi Oji was the steady level at about 65% maintained by both sensitivities from the age of about 30 onwards. Continuing contact with the sensitising agents concerned must be assumed at all ages, but in later decades was not associated with any increase in sensitivity to either. This finding can only suggest that saturation point had been reached in the capacity of that community to react positively in the conditions which applied, and that geographical and constitutional factors were therefore exerting a significant influence upon both sensitivities.

What are these factors One geographical factor may very well relate to the frequency and extent of exposure to the sensitising agents concerned. The habits of the people should be noted here. It is a general custom for youths to leave home and see the world, seeking a living for a number of years in one or other of the towns

or cosmopolitan areas favoured by them. Here, living often in overcrowded conditions, contact with *M. tuberculosis* is almost inevitable. At around the age of 30, they tend to return home and settle down, subsequently having only occasional contacts with townships. Women do not travel in this way, their movements being mainly confined to frequent visits to markets in nearby villages. These facts doubtless have some bearing on the levels of tuberculin sensitivity in various age groups. Once tuberculin sensitivity has been produced, exposure appears to be sufficient to maintain it at a relatively steady level during adult life, but the reinforcement needed to raise it is lacking. It is not surprising that the stimulus needed to produce lepromin conversion is also lacking.

In the same conditions, sensitivity to lepromin is also limited during adult life, although the likelihood of contact with *M. leprae* in rural localities is greater than the likelihood of contact with *M. tuberculosis*. We note that sensitivity to lepromin tends to be very persistent, and the marked reduction in exposure to *M. leprae* which has undoubtedly taken place during the past 15 years may have a bearing on the matter. At the same time, as has already been pointed out, it is difficult to implicate *M. leprae* alone as the agent responsible for the lepromin levels observed among young children, or, when every allowance has been made for seasonal and nutritional factors, for the variation in level between one place and another. With *M. tuberculosis* largely excluded from this role we are faced with the possibility that other as yet unidentified sensitising agents may be inducing some degree of sensitivity in these cases. Lepromin sensitivity is not specific, though a lack of it may be. We have to admit the possibility that just as non-specific factors may influence tuberculin sensitivity, so they may also influence lepromin sensitivity, and may account for the association found between the two sensitivities in conditions where tuberculosis does not appear to be responsible for it.

Summary

In order to study further the relations between tuberculin and lepromin sensitivity in Eastern Nigeria, simultaneous tuberculin and lepromin tests were undertaken (a) on 3,000 schoolchildren in six localities, one urban, the others rural, (b) on the complete population of a group of four villages comprising just under 2,500 individuals. In the variations of each sensitivity between one locality and another, between the sexes and through succeeding age groups, it was hoped to obtain fresh information regarding their relationship. By choosing localities where the incidence and trend of clinical leprosy were known, the findings could also be related to this.

Tuberculin sensitivity to a 10 TU dose was high in an urban

area, lower in rural areas. Late lepromin sensitivity (Mitsuda) also showed considerable variation but during the years of childhood followed a different pattern. Although it is possible that in the urban area where tuberculosis was rife, tuberculosis infection was contributing to lepromin sensitivity, no evidence could be found in rural areas, where tuberculosis was at a lower level, that tuberculin sensitivity was having any appreciable influence on lepromin sensitivity during the years of childhood, in spite of the fact that both senstivities tended to be associated, both in their occurrence in the same individuals and in their intensity.

In the complete communities examined this association was also found, though when both sensitivities attained stable levels in adult life, it largely disappeared. The association operated in both directions and no satisfactory evidence could be found that tuberculin sensitivity was influencing lepromin sensitivity to any appreciable extent.

In all localties sensitivity to the early lepromin reaction (Fernandez) was at a very low level.

It is very doubtful that contact with *M. leprae* could alone be held responsible for the levels of lepromin sensitivity found and their variation from place to place. In this and in the steady levels maintained by lepromin sensitivity during adult life, non-specific geographical and constitutional factors appear to be operative, and the possibility arises that they are responsible for the association found between tuberculin and lepromin sensitivity in these areas.

Acknowledgments

Grateful thanks are due to Mrs. A. Foster for the statistical examination of the Ndi Oji figures, also to our colleagues, Mr. G. O. Okezie, Mr. A. Eden and Dr. G. Moneta, to whom reference has been made in the text, also to Dr. A. Kissaun. Thanks are also due to Dr. A. Zahra, acting Leprosy Adviser, and to the Director of Medical Services, E. Region, Nigeria, for permission to publish this work.

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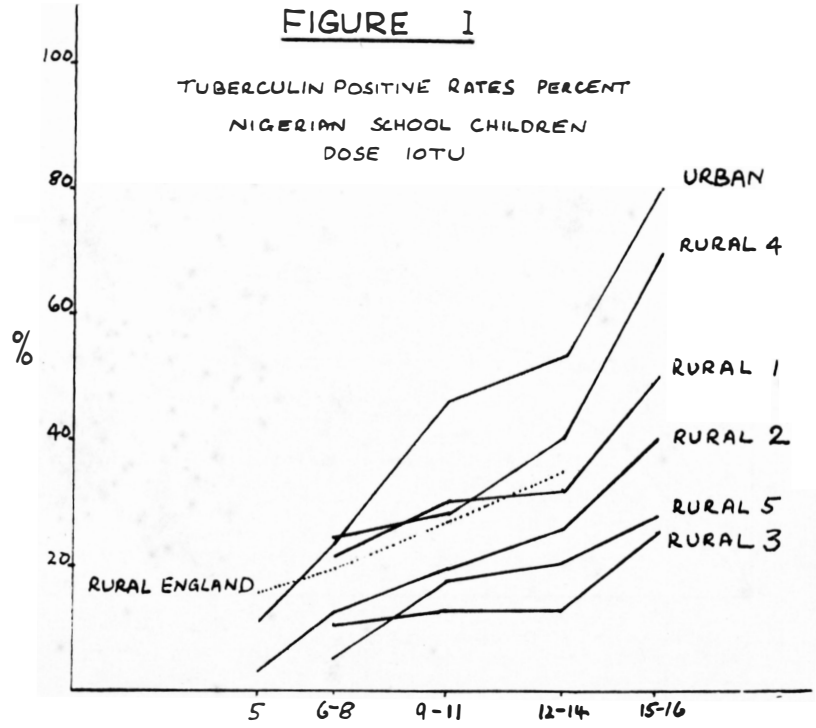
FIGURE 1



GURE 2

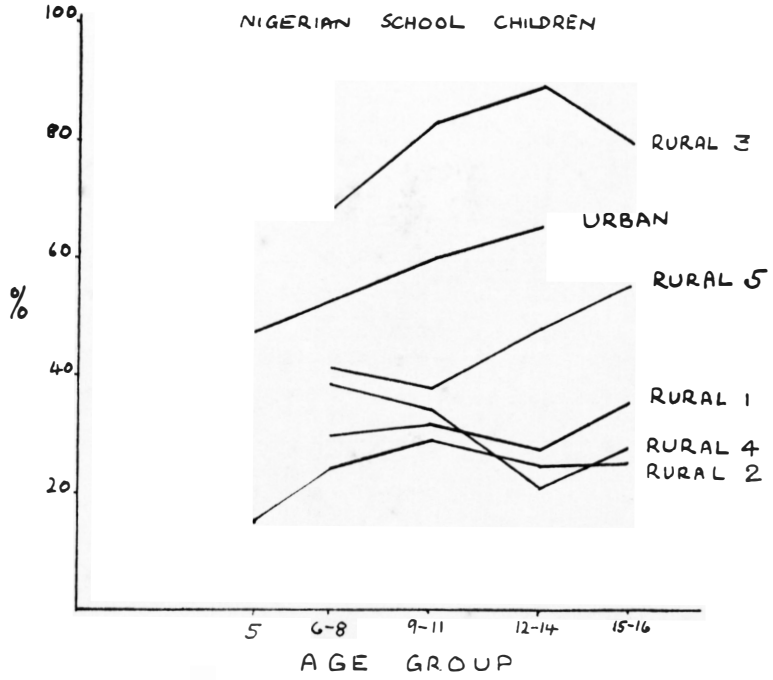


FIGURE 3

TUBERCULIN AND LEPROMIN SENSITIVITY
NDI OJI

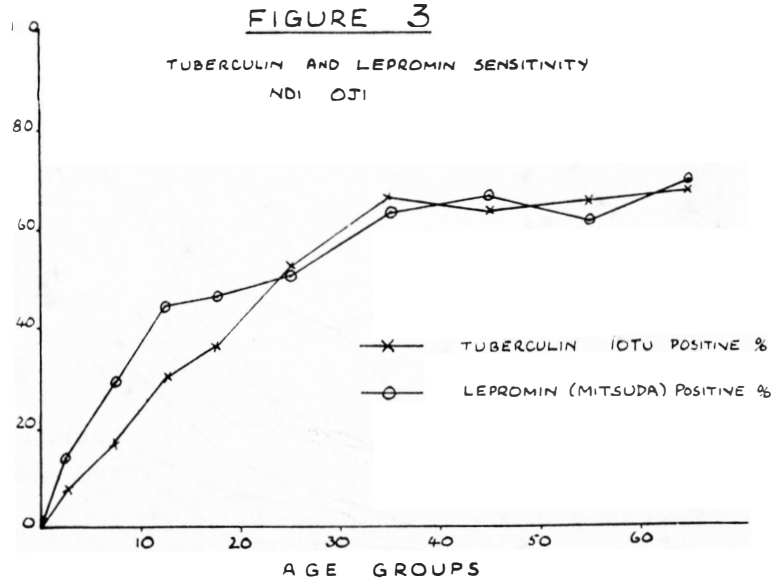
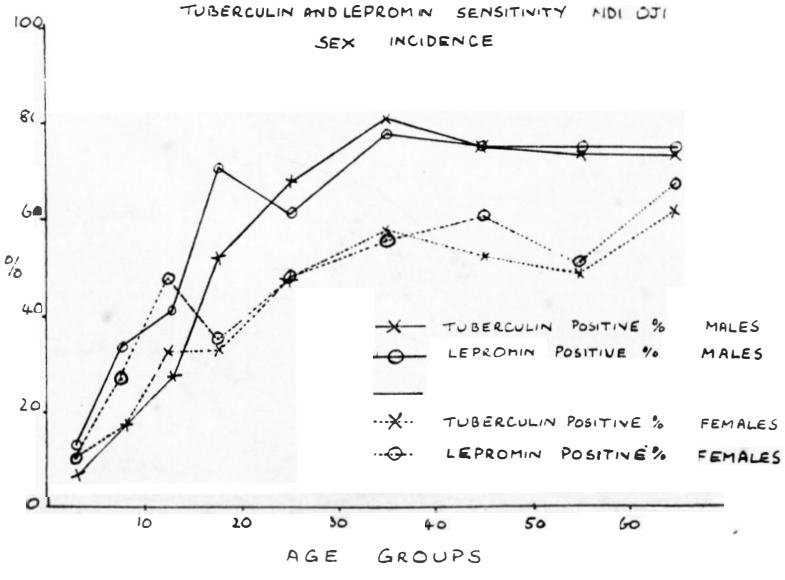


FIGURE 4

TUBERCULIN AND LEPROMIN SENSITIVITY NDI OJI
SEX INCIDENCE



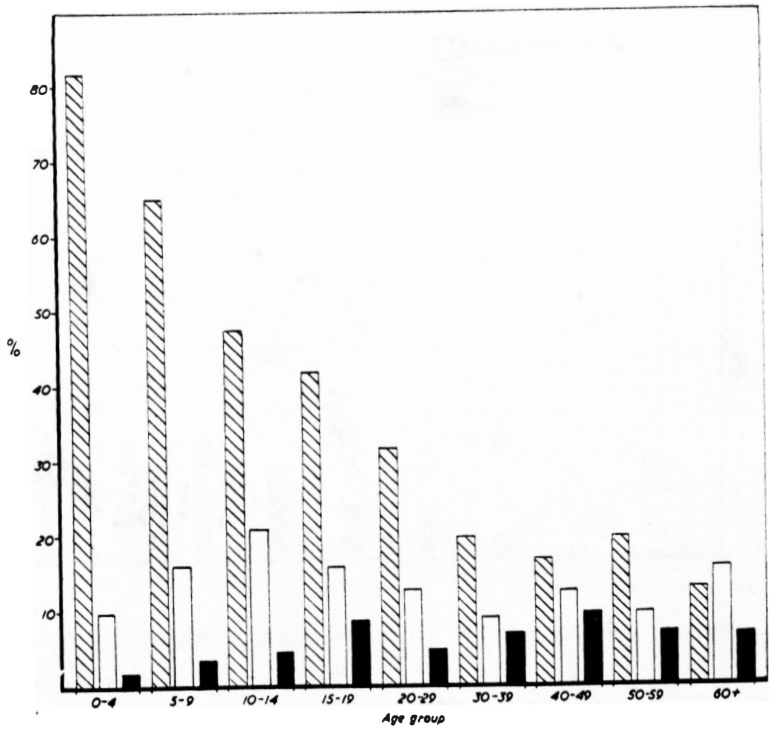


Fig. 5a. Lepromin sensitivity among negative tuburculin reactors.

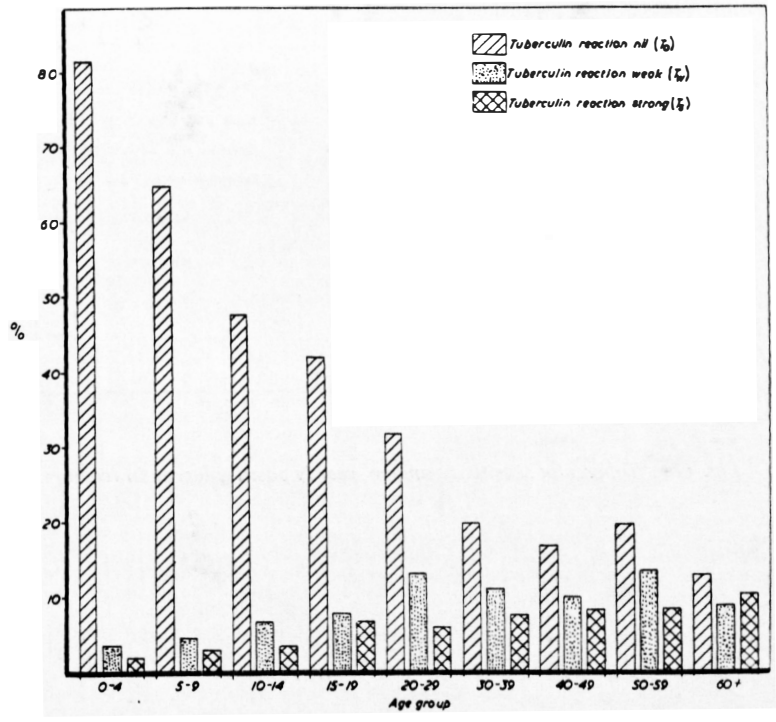


Fig. 5b. Tuberculin sensitivity among negative lepromin reactors.

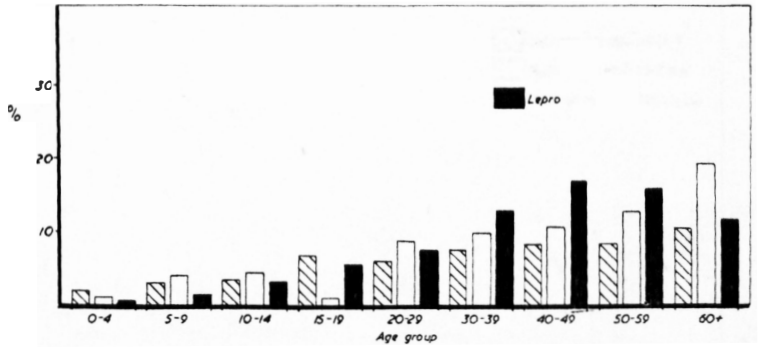


Fig. 6a. Lepromin sensitivity among weakly positive tuberculin reactors.

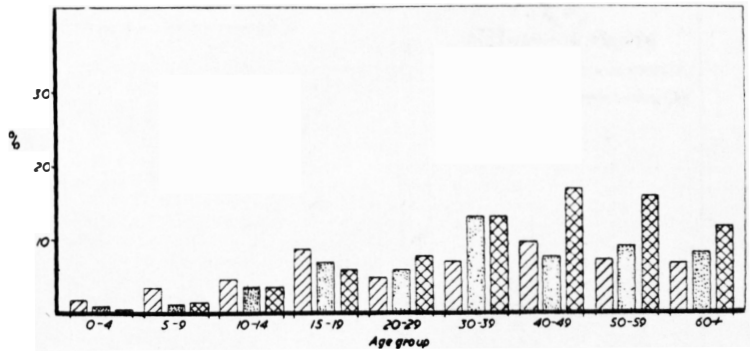


Fig. 6b. Tuberculin sensitivity among weakly positive lepromin reactors.

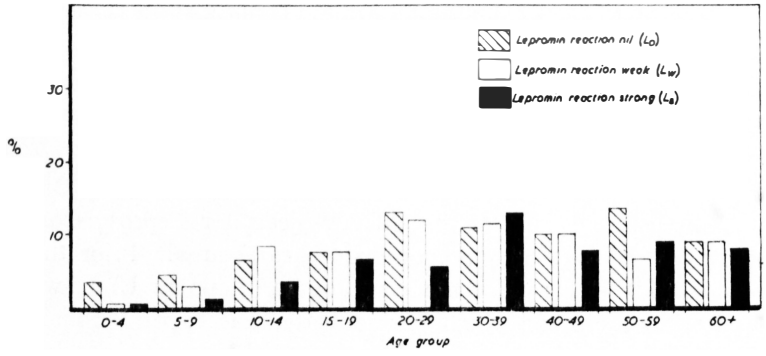


Fig. 7a. Lepromin sensitivity among strongly positive tuberculin reactors.

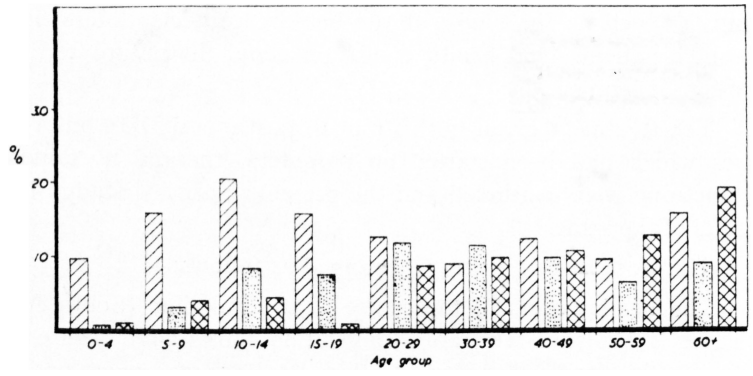


Fig. 7b. Tuberculin sensitivity among strongly positive lepromin reactors.

SIX YEARS AQUEOUS SULPHETRONE THERAPY IN A RURAL AREA

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Introduction

The sulphones have become firmly established as an effective method of treatment of leprosy and any more literature on this subject may appear superfluous but the apology for this paper is that it deals with the results of aqueous sulphetrone therapy in outpatient clinics in a rural area where the patients differ from those in the urban areas and institutions.

This centre is one of the Pilot Projects for Leprosy Control established in 1955 in a predominantly rural area. Prior to the opening of this centre, there was another Leprosy Unit, which was working here and which was converted and expanded into the present one. As the present centre has taken over the working of the same area, there has been uninterrupted observations of the effects of the therapy. (See map of the area of this centre.)

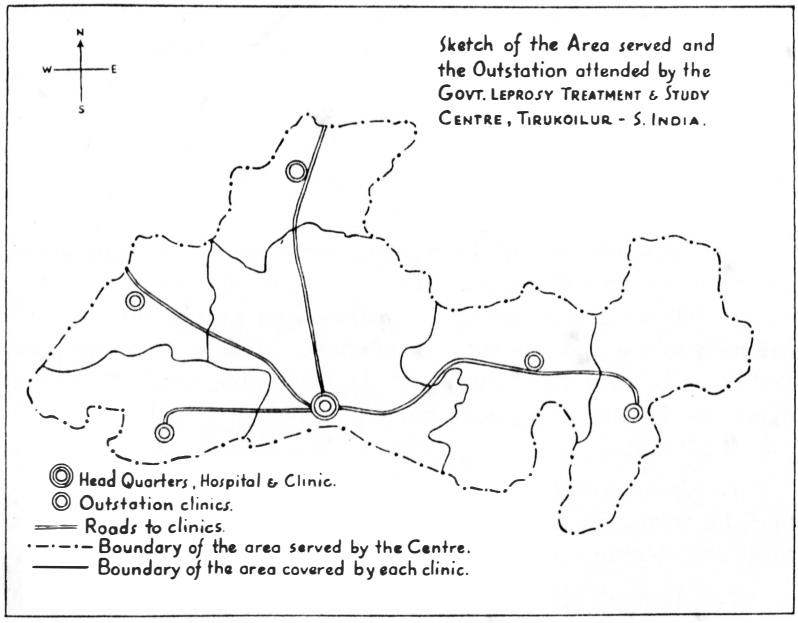
This centre is unique of its kind in this country as it consists of the Medical and Public Health wings working in co-ordination for the control of leprosy. The Medical Unit undertakes the curative part of the work and the Public Health section does intensive survey on the incidence of leprosy in the area chosen for the intensive work, as well as the study of the healthy contacts, follow-up of absentee patients, and health education about the nature and prevention of leprosy.

The treatment given in this unit is mostly oral DDS but a few cases which had been started on parenteral therapy by aqueous sulphetrone were continued and the present paper is a study of the results of the same.

The work of this centre is arranged as follows:—

The headquarters of the centre is at Tirukoilur (South Arcot District of Madras State) and 5 outstation clinics have been established in the various zones of the Pilot Project area, where patients get treatment once a week.

Each clinic is worked once a week at each of these zones so that the treatment is taken to the nearest possible point to the patients' homes. The patients are examined and treated and their progress noted in the case sheets with records of bacteriological examinations. This being a rural area, the clinics are held under the shade of trees, as buildings are not available except at two places. Also, no facilities are available for biopsy or any other



GOVERNMENT
LEPROSY TREATMENT & STUDY CENTRE TIRUKOILUR

Work begun: 28.4.55.

No. of villages served by the Centre: 54.

Total population of these villages: 65,290.

Total No. of out-station clinics: 6.

Ser. No.	Name of Clinic	Day open	Distance from Headquarters	No. of villages served
1.	Tirukoilur	Saturday	———	18
2.	Velandai & Sangiam	Monday	8 miles	7
3.	Veerapandi	Wednesday	6 miles	7
4.	Attipakkam	Thursday	6 miles	4
5.	Mugaiyur	Tuesday	9 miles	13
6.	Ayandur	Friday	11 miles	5

All these clinics operate once a day on one day of the week at each place.

Total number of cases under treatment on 31.12.57:

Lepromatous	536
Non-lepromatous	1,352
TOTAL	1,888

complicated laboratory investigations, and the only routine investigations that are performed are the smear examinations for leprosy bacilli as well as the ordinary haemotological examinations. Every patient is given treatment as an outpatient and attends the clinic once a week (in the case of aqueous sulphetrone therapy once at the outstation clinic in the respective zone and once at the headquarters clinic).

The patients are all from rural areas, most of them being agriculturists and illiterates. They are normal members of the village following their usual occupations and having superstitions common to the illiterate rural population. Most of them are poor and have to earn their livelihood daily as labourers. There are many special difficulties encountered in such a population which are as follows:—

1. As many of them are agriculturists, regularity of attendance for treatment is seasonal, depending on the agricultural work; most absenteeism occurs during the agricultural season.

2. As most of them are illiterate, they have to be instructed repeatedly about the necessity for regularity in treatment and to report complications at the earliest moment.

3. Superstitions and religious obligations cause absenteeism for many.

4. They are usually hired labourers and so it is difficult to get them to be regular every week for treatment.

5. There is a well equipped hospital in this centre for the hospitalization of any intercurrent complications, but because of illiteracy, poverty, and superstitions, it is difficult to get them to come to hospital for the treatment of any complication.

Aqueous sulphetrone therapy for 6 years in this centre is assessed as follows:—

The Number and Types of Cases Chosen and Previous Treatment

All the 34 cases chosen for this therapy were lepromatous, and except for 6 cases have had hydnocarpus oil treatment for varying periods as shown in Table 1. These were started on the sulphetrone therapy because they did not derive much benefit from hydnocarpus oil. It happens that this was the first sulphone to be used in this centre.

Initially 22 cases began treatment in July 1951 and 12 cases were added up to the end of the year 1951. The present report is based on the findings made in the 15 patients who have been on continuous treatment since 1951 to date from among the 34 initial cases. Reasons for the stoppage of treatment in the other 19 cases are given below.

No. of cases	Reasons for stopping treatment
3	Died due to intercurrent illness not connected with leprosy.
8	Migrated to other areas.
5	Changed to oral DDS therapy as they could not come twice a week for sulphetrone injections.
3	Discontinued treatment for any reason.
19	Total.

The types of cases who have been on continuous treatment are as follows:—

No. of cases	Types of cases
6	Macular lepromatous (L1).
6	Diffuse lepromatous (L2).
3	Nodular lepromatous (L3).
15	Total.

As regards the previous treatment of these cases, all of them except one case have had treatment with hydnocarpus oil for varying periods as shown in Table II.

Initial Examination

This comprised examination of the leprotic lesions and general physical examination: also bacteriological examination using the " slit-scrape " method. At least 6 smears were taken of each patient and the bacterial index calculated, taking the maximum positivity as 6, as described in Practical Textbook of Leprosy by R. G. Cochrane, page 71. Also there were routine haemotological examinations, i.e. haemoglobin percentage and RBC count were done for the first year as a routine every three months but these were given up later as unnecessary, except in cases where there was clinically recognised anaemia.

Periodical Examinations

These comprised clinical and bacteriological examination once in six months to assess the progress of the cases; and clinical photography of a few cases where there were lepromatous nodules; biopsy or lepromin test could not be done due to lack of facilities.

Records

Complete and detailed records were maintained, with diagrammatic representations of the lesions and photographs when necessary. Notes were recorded about any serious complication

or intercurrent illness affecting any patient during the course of therapy.

The Treatment

(a) *Basis for Aqueous Sulphetrone Therapy:* As the Madras Leprosy Workers regarded DDS as too toxic for mass treatment (Cochrane and Ramanujam—1945) during this period and as aqueous sulphetrone was considered a cheap, effective and safe therapy, this therapy was introduced here. (Ref. Ramanujam " A cheap, safe and effective sulphone " at the Third All India Leprosy Workers' Conference at Madras in 1950.)

(b) *The Drug:* The drug used for this has been the sulphetrone granules manufactured by Messrs. Burroughs Wellcome and Company Limited.

(c) *Method of preparation:* The solution used is of a 50% strength and is made up as follows:—

50 gm. of sulphetrone granules are dissolved in hot distilled water and the final volume made up to 100 ml. No preservative is used. This solution is then placed for 10 minutes in a boiling water-bath as a substitute for autoclaving, as no facilities exist in this centre for autoclaving. This solution is acid in reaction and gives rise to excessive burning sensation at the site of the injection, hence it is neutralized by the addition of sodium carbonate (about 1.5 gm. of sodium carbonate to 1 litre of the solution). The solution is then transferred to a sterilized transfusion bottle from which it is drawn into a syringe for injections.

To avoid deterioration, only about 8 ozs. (327.44 ml.) of the solution are prepared at a time so that the solution is not used for more than two weeks before a fresh solution is made.

Injection Method and Dosage

The injections are given intramuscularly twice a week. The starting dose is ½ ml. twice a week increased by ½ ml. every week till a maximum of 3 ml. twice a week is given (3 gm. of sulphetrone). No abscesses or any other complications have been met with from these injections except at one period, when there was a series of injection abscesses from the injections given during one week. On investigation the defect was found to be due to the carelessness of the injectors, which was immediately rectified. Except for this very brief period the injections have been free of such complication.

Duration of Treatment

The maximum period of treatment of the 15 cases who have been on continuous treatment has been 72 months and the minimum period has been almost the same, but the actual period of treatment of each has been much less due to the irregularity of the patients.

The total duration of treatment in weeks and the actual period of treatment for each patient is given in Table II.

Complications

The complications during treatment have been very few and not of such a serious nature as to stop treatment permanently. The commonest complication has been the lepra reaction which necessitated temporary hospitalization and temporary cessation of treatment. The details are as follows:—

1. *Lepra reaction*
   8 cases—maximum number of days of absence due to this was 30.
2. *Anaemia*
   Very few cases have shown any serious degree of anaemia such as to warrant any special attention or hospitalization.
3. *Neuritis*
   Nil.
4. *Eye complications*
   Only one patient developed iritis of both eyes which later on developed into iridocyclitis. But in a later series of cases started in 1952, there were a few cases of iritis for which I cannot account properly. The patients who manifested such eye complications had an allergic diathesis, as evidenced by eczema in many of them.

There was one patient who showed a peculiar allergic manifestation immediately after the injection which consisted of severe itching and urticarial rashes but which did not occur when the dose of sulphetrone was kept below 2 ml.

Results of Therapy

Among the fifteen cases treated, six are completely negative, bacteriologically and clinically, five of them for one year and one since July 1957. Of these six cases, three cases are L2 and three cases L1. All of them have been very regular in treatment, the attendance ranging from 75% to 92% of the total treatment days which can be considered extraordinarily good in a rural area in India. It is also noteworthy that none of these patients had any complication except a very minor attack of lepra reaction occasionally so that their treatment has been almost continuous.

Among the remaining nine cases, three cases have improved very greatly, their BI being very small, i.e., less than one. They have also become almost free of clinical signs and their regularity of attendance has been high, ranging from 66% to 90.1%. Of these three cases, one is L3, one is L2, and one is L1. One L1 case in this group had become bacteriologically negative in 1954

but after a period of absence from treatment he was found to have relapsed into mild positivity on reporting for routine check in July 1957, i.e., three years after the cessation of treatment.

As regards the remaining six cases, one patient who has been very irregular (the regularity of his attendance being only 54% of the total clinic days) and who was treated for iritis by quacks, has shown slight clinical improvement and moderate bacteriological improvement. The remaining five cases have still clinical lesions of a very low intensity, i.e., slight infiltration of skin or slight thickening of ears (some of them are also free of signs and their bacteriological index is 1). Except for the one patient who had an iritis and who had absented himself from treatment, all the other patients have shown very good improvement, clinically and bacteriologically.

Conclusions

The advantage observed for therapy by intramuscular injection of an aqueous solution of sulphetrone are:—

1. Because of its low toxicity, it can be administered even to anaemic patients and those with low general standard of health. (The general standard health of these patients is poor.)

2. Bacteriological negativity was attained in 40% over the period of five years, which is quite encouraging.

3. Serious complications are uncommon.

Disadvantages

This therapy is not suitable for mass treatment for the following reasons:—

1. This treatment involves bi-weekly injections which are difficult and cumbersome for the field workers; facilities for injection in field conditions are deficient.

2. Patients in a rural area are not able to attend the clinics regularly even once a week due to their occupations and hence it is very difficult to get them to attend twice a week for this therapy.

3. The period required for bacteriological negativity is rather long (five years being the minimum).

4. The cost of the treatment is definitely higher than that of DDS without the results of the therapy being any better.

5. In an endemic area like this, where a large area has to be covered by this centre under field conditions, a therapy which involves bi-weekly injections and also which costs more without proportionately better results than oral DDS is definitely not suitable for mass treatment.

My thanks are due to Sri. T. R. Krishnamachari, Secretary, Thakkar Baba Kushta Nivaran Sangh, Tirukoilur, for having permitted me to treat the cases in the clinics of the Thakkar Baba Kushta Nivaran Sangh, Tirukoilur.

I also thank Dr. U. Maruthi Rao, B.Sc., M.B.B.S., D.L.O., former District Medical Officer South Arcot District, Cuddalore, for having given me guidance and encouragement in my work.

TABLE I

Analysis of the Series of Cases on Sulphetrone

Total No.	34
Clinical Type	6 of L1, 22 of L2, 6 of L3
Previous Treatment with Hydnocarpus oil	1 for 2—3 years or more nil for 2 years or more 27 for ½—1 year or more 6 new cases
Stopped treatment	3 died 8 migrated 5 were transferred to oral DDS 3 lapsed from treatment
Continued treatment for at least 32.4 weeks	15

TABLE II

Analysis of the 15 cases persisting on Sulphetrone

Case 1. Clinical type L2 N1. Treated over 329 weeks with 90% effective attendance, and a total dose of 771 gm. Previous treatment by other methods was for 1 year 3 months. The Bact. Index came down from 4 to greatly improved, and he became symptom free.

Case 2. Clinical type L3 N1. Treated over 329 weeks with 90.1% effective attendance, and a total dose of 761 gm. Previous treatment was for 2 months. The Bact. Index came down from 6 to 0.5, or very greatly improved, and he became symptom free.

Case 3. Clinical type L2 N1. Treated over 329 weeks with 91% attendance, and a total dose of 709 gm. Previous treatment was for 1 year 4 months. The Bact. Index came down from 4 to negative on 13.6.56, and he became symptom free.

Case 4. Clinical type L2 N1. Treated over 329 weeks, with 75% effective attendance, and a total dose of 719 gm. Previous treatment was for 1 year 5 months. The Bact. Index came down from 4 to negative in June 1956, and he became symptom free.

Case 5. Clinical type L1 N2. Treated over 329 weeks, with 78% effective attendance, and a total dose of 471 gm. Previous treatment was nil. The Bact. Index came down from 2 to 0.75, or very greatly improved. The ears remained thickened and erythematous. He was negative bacteriologically in 1954, and did not attend for nearly a year after that: he has now returned with relapse.

Case 6. Clinical type L1 N2. Treated over 329 weeks with 92% effective attendance and a total dose of 604 gm. Previous treatment was for 1 year. The Bact. Index came down from 2 and has been negative since June 1956, and he became symptom free. Operation for gynaecomastia was performed in the Centre.

Case 7. Clinical type L3. Treated over 329 weeks, with 92% effective attendance and a total dose of 690 gm. Previous treatment was for 1 year. The Bact. Index came down from 6 to 1, and there is very slight infiltration of the skin.

Case 8. Clinical type L1. Treated over 328 weeks with 89% effective attendance, and a total dose of 440 gm. Previous treatment was for 1 year. The Bact. Index came down from 3, and was negative since June 1956: symptom free.

Case 9. Clinical type L1. Treated over 329 weeks, with 85% effective attendance, and a total dose of 722 gm. Previous treatment was for 1 year 6 months. The Bact. Index descended from 4 to negative since June 1956: symptom free.

Case 10. Clinical type L3. Treated over 328 weeks, with 88% effective attendance, and a total dose of 769 gm. Previous treatment was for 6 months. The Bact. Index came down from 5 to 1, which is very good. There remains slight thickening of the ears.

Case 11. Clinical type L1 N1. Treated over 327 weeks, with 54% effective attendance, and a total dose of 345 gm. Previous treatment was for 3 months. The Bact. Index came down from 3 to 1.5. Skin infiltration is still present but nodules have gone. Old age and poor vision have caused his irregularity of attendance.

Case 12. Clinical type L1. Treated over 324 weeks, with 84% effective attendance, and a total dose of 543 gm. Previous treatment was for 1 year 3 months. Bact. Index came down from 4 to 1, and there are no lesions now.

Case 13. Clinical type L2. Treated over 324 weeks, with 84% effective attendance, and a total dose of 627 gm. Previous treatment was for 1 year 3 months. Bact. Index came down from 3 to negative since 1956. Slight thickening of ears remains.

Case 14. Clinical type L2. Treated over 323 weeks, with 81% effective attendance, and a total dose of 472 gm. Previous treatment was for 1 year. Bact. Index came down from 5 to 1. Slight thickening of ears, and slight infiltration of skin remains.

Case 15. Clinical type L2. Treated over 324 weeks, with 66% effective attendance, and a total dose of 409 gm. Previous treatment was for 1 year. Bact. Index came down from 5 to 0.3. Very slight infiltration of skin remains.

THE ANTI-PROTOZOAL ACTION OF THE SULPHONES AND THE ANTI-MALARIAL ACTION IN PARTICULAR

DR. G. TARABINI

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In a brief note, Leiker gives his observations on the possible antimalarial action of the sulphones in leprosy patients. He records that in a malarial endemic zone some years ago he noted that 10 leprosy inpatients and 25 outpatients did not show attacks of malaria, and throughout one year in the Miei leprosarium, which is also in a malarial area, 164 inpatients had no attacks of malaria, nor were there malarial parasites on examination of thick drop blood films. More than this, a patient who came in suffering from malaria became cured of it a short time after beginning to take a small dose of DDS. Leiker ends by saying " it is permissible to conclude that DDS has a suppressive action against malária ' . He had made his remarks as the result of those of the Editor of " Leprosy Review " (**26**, 1, 1955, pp. 3-4) on the activity of the sulphone drugs against one protozoon at least, namely toxoplasma.

The argument is of great importance, especially for the areas in which these two grave diseases coincide, and where the treatment and prevention of leprosy are carried on by a country-wide and regional plan (a system such as in India, according to our conversations with Dr. Wardekar on the occasion of his visit to Fontilles) and where it would result in the treatment and prevention of malaria

In 1937 Diaz de León reported in the Boletin Sanat. Panamer. that the sulphamides which a short time before had appeared in the therapeutic arsenal had an evident antimalarial action in man. Niven in 1938 in England reported, though with less enthusiasm, that prontosil was more effective against *Plasmodium falciparum* than *Plasmodium vivax;* but Menk and Mohr in 1939 in the Institute for Tropical Diseases at Hamburg had less encouraging results with 10 patients, inasmuch as they confined themselves to saying that prontosil could not in any way be a substitute for quinine or the atebrine class of compound.

The second wave of these studies arose in America in 1941 with the work of Coggeshall, Mayer, and Best, who used promine in daily doses of 10g. or more and obtained very good results in human and experimental malaria. This study deserves special attention because it was carried out at a time when the sulphone derivatives were being investigated and it was being shown that 4-4'-diamino-diphenyl-sulphone which was previously abandoned was not only less toxic and more soluble than the glucoside derivatives but had a

very efficacious action; on the other hand the glucoside derivatives of sulphapyridine were not so efficient because they did not break down fast enough in the blood, (Taylor *et al.*).

Patrono (1943) using a product of the promine type in dosage of 36 g. in 4 days and of 108 g. in 6 days obtained good results in human malaria. He noted the disappearance of the fever in 1 or 2 days in the cases with *Plasmodium falciparum* infection and in 5 to 6 days in those with *Plasmodium vivax*.

After the Congress of Dermatology and Syphilology held in Padua in 1943, where promine and tibatin were discussed, Messrs. Recordati in Italy manufactured the product called *reconan* which differed from promine (the sodium disulphonate of the diglucoside of DDS) in being the dextrose diglucoside of DDS, and thus being similar to tibatin which is the digalactoside of DDS.

In those years I had a fair number of malaria patients in the Tropical Diseases Clinic at Modena and I wished to try reconan to see if the simple dextrose diglucoside of DDS was as equally effective as promine which was a more complex product. I reported the results in February 1945 to the Medico-Surgical Society of Modena, together with Dr. Enzo Secreto. We adopted the dosage of 16 g. during 9 days and repeated the treatment after 10 days of rest. A definite fall in the fever occurred on the second day of treatment, and there were only 2 cases of relapse in the 12 patients treated with this dosage. In 10 other cases who were given only the first period of treatment, success was obtained, but somewhat less striking. Almost all our patients were parasitized by *Plasmodium falciparum:* we had no cases with the other plasmodia. It was noted that there was a much greater action against the schizonts than the gametocytes. In 2 patients we made check studies and found that the activity of the drug was due exclusively to the DDS.

It was also noted that a patient with diabetes complication had a striking definite cure in two days, notwithstanding an application of treatment for only 6 days; in this patient the degree of urinary elimination of the drug was less than in the others.

From what has been said, it can be concluded that the sulphones are very active against human malaria, and much more so than the sulphamides.

Other studies also carried out in Modena enabled us to note an ineffectiveness of reconan against the intestinal protozoa: we had some informative cases of colitis due to *Entamoeba histolytica.* However we now think that the sulphones given orally and for a long time can have a certain activity; for in the years I spent at Fontilles we often found intestinal protozoa and never *Entamoeba histolytica,* and hence there is some validity in the idea. In regard to cutaneous leishmaniasis we do not have enough data, but the case

which we studied (Contreras *et al.*) in a leprosy patient treated for some years with oral DDS in whom oriental sore developed, makes us think that this drug neither impedes nor cures infection by *Leishmania tropica.*

In short we can say that the observations of Leiker arouse interest in the use of the sulphones as antimalarials and there is confirmation in the studies of the authors quoted, and they suggest the use of this therapy at the one time for malaria and leprosy in the areas where these two diseases exist in association, and also as a prophylaxis for both diseases when DDS is given for a long time to the whole population of areas where these two great scourges exist.

Summary

Emphasis is given to the observations of Leiker on the anti-malarial action of the sulphones, and the studies are recalled of Coggeshall *et al,* of Patrono, of Tarabini and Secreto in the years 1941-45 carried out on the glucoside derivatives of DDS (promine and reconan) and which show a marked antimalarial activity.

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THE DRY PIT LATRINE

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Some 32 years ago an article was published in the British Medical Journal in which the use of a dry closet was advocated, both on hygienic grounds and with a view to restoring to the earth rich fertilizer which would otherwise be wasted.

With this in the back of my mind—for I had lost two collections of Medical books and Journals in the political disturbances of recent time in China—I worked out details for the construction of dry pit latrines as soon as the development of the Hay Ling Chau Leprosarium, (The Isle of Happy Healing) Hong Kong had reached a stage which made such an innovation possible.

Suggestions from friends have been incorporated and no credit is claimed for any originality; the end result is a latrine which has proved simple and effective, free from offensive smells, and from the menace of flies, providing a rich soil, which for greater safety is composted for a further 12 weeks before being returned to the vegetable gardens; as such it may be of interest to those faced with similar problems.

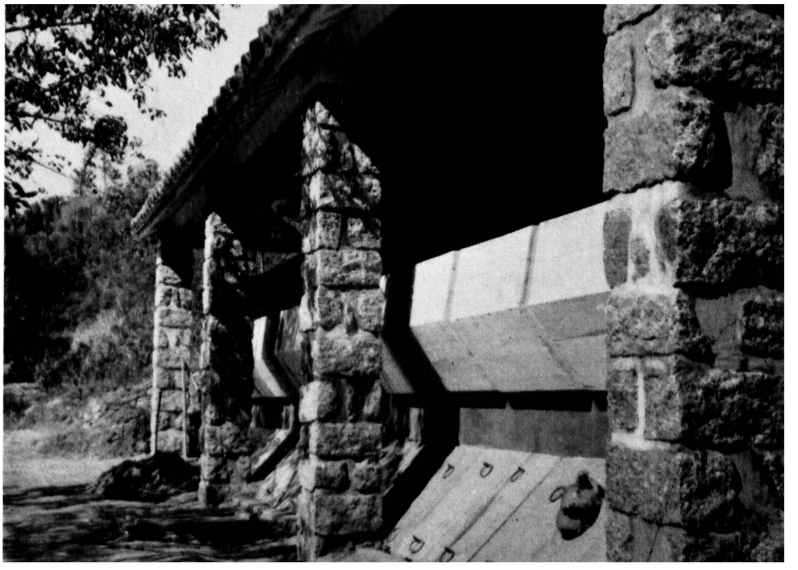
Two pits have been prepared for use by each of the cottages in which 24-28 patients live together; one pit is used first by all the patients; and they have been instructed to add a shovel full of garden soil each time they use it; when filled, which takes about 2 months, the pit is closed and the second pit comes into use. When the second pit is nearly full the contents of the first are dug out and are removed to the compost pits alongside our pig-sties.

By this time the contents are free from all objectionable smells and offensive matter, through decomposition due to bacterial action.

Some details of the construction of the latrines may be of interest. Each pit is approximately 3ft. deep, 10ft. long and 5 ft. wide (about 0.9 m. deep, 3 m. long and 1.5 m. wide).

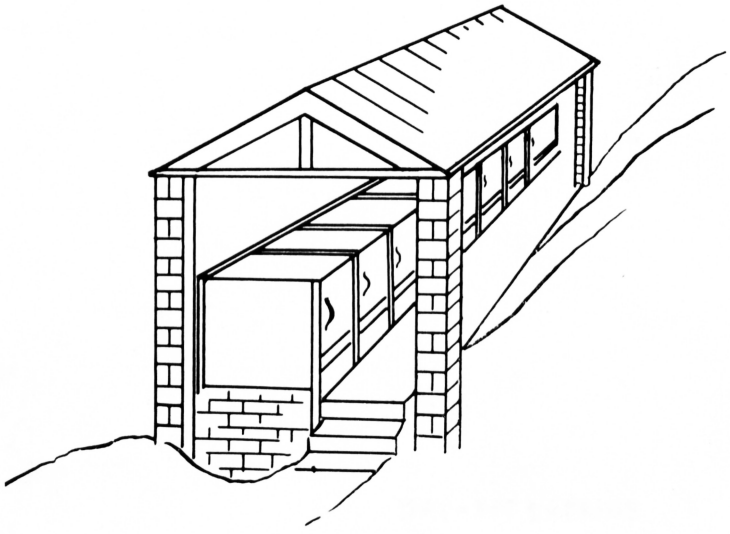
Access, from one side, is to 3 cubicles 3ft. x 3ft. 3in. (0.9 m. x 1.0 m.) with squatting plates, which rest on a beam running the length of the trench. Hinged lids cover the holes in the plates. Access from the other side, which should if possible be some 1½ ft. (0.45 m) lower, is to the fly-proof covers of the pit which can be lifted, making removal of the contents easy.

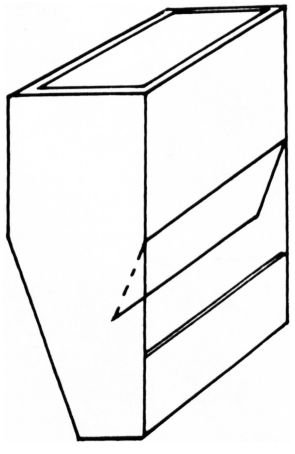
Between the two pits a smaller pit can be used as a urinal; if this is filled with saw-dust an enormous quantity of urine can be absorbed; all offensive smell is removed and the ammonia concentrated; the Chinese vegetable gardeners however are so keen to use the urine, diluted, to water vegetables that buckets are usually found in this section, and such a " compromise " may be allowed.



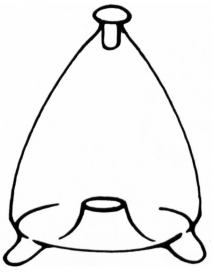
Showing earth-boxes, drainage pit, and covers to pit.

This dry-pit latrine is for 50 patients. The heap of earth is for filling the boxes.





EARTH BOX



GLASS FLY-TRAP

Flies, in spite of care in seeing that lids are closed after use, inevitably gain access to the pits and breed there. Traps however can easily be fitted to the covers, to catch all the flies that emerge, and this is probably simpler and more effective than expensive screening which invelves regular maintenance, or automatic mechanical devices to close the lids which inevitably get out of adjustment sooner or later or are deliberately wedged open.

It should be noted that the latrines are built immediately adjacent to the cottages, so that the first to suffer from flies or objectionable smells will be patients living there; should they complain it can easily be demonstrated to them that the cause is their own neglect of the simple rules which they have been instructed to carry out.

Success however depends on the addition of a shovel full of garden soil whenever the latrine is used, and so both soil and shovels should be provided. Boxes have been built into the back of the cubicles which can be filled from the outer sides and from which a shovel full at a time can be removed from the inner side.

The pits should be drained and should be protected from flooding by heavy rain. If on a slope a drain can be led to a sump—the contents of which can be used for watering flowers or vegetables.

If on level ground a deeper pit or well should be dug into which excess liquid can be drained which again can be removed daily for use in the gardens.

LETTER TO THE EDITOR

Jordan Hospital,
Earlswood,
Surrey.
17th February, 1958.

The Editor,
"Leprosy Review."
Sir,

I feel sure your readers will be interested in the enclosed Summary of a paper which, I believe, is the first one in which the term "erythema nodosum leprosum" appears. The paper was written by Dr. M. Murata of Tokyo, and was published in *Japanische Zeitschrift für Dermatologie u. Urologie* as long ago as 1912 (vol. 12, p. 1013), and the Summary was sent to me by Dr. Yoshinobu Hayashi of National Leprosarium Tama Zensho-en, Tokyo, as a result of an enquiry of mine.

The remarkable thing is that so much has been written about *erythema nodosum leprosum* in recent years yet no reference has been made to this excellent paper, a regrettable fact which demonstrates how far-reaching can be the effect of the language barrier in impeding the spread of knowledge about leprosy. In this respect I would like to congratulate you, Sir, on your work in translating current papers from South America and the Soviet Union and bringing them to the notice of your readers in the form of Abstracts (Leprosy Review, October 1957, pp. 164-173).

I am, etc.,

W. H. JOPLING.

[The Summary as made by Dr. Hayashi and sent by Dr. Jopling is given below, slightly compressed and rephrased for reasons of space. We thank Dr. Jopling for eliciting this early and valuable contribution by Dr. Murata on erythema nodosum leprosum, and share his admiration for the clinical wisdom and acute observation of the author. As regards the language barrier, we wish that all medical writers would adopt an international scientific language. There is one now available, called Interlingua, which seems quite practical, and even easy. To illustrate the point, these remarks are here repeated in Interlingua . . . *Editor*. "Le summario como facite de dr. Hayashi e inviate de dr. Jopling es date infra, poco compresse e rephrasate a causa de rationes de spatio. Nos regratia dr. Jopling re extraher iste contribution prompte e importante de dr. Murata re erythema nodosum leprosum, e prenda parte in su admiration del sagessa clinic e observation acute del autor. Re le barriera linguistic, nos desira que omne scriptores medical adoptarea un lingua scientific e international. Il ha ora uno usabile, nominate Interlingua, que sembla toto practic e mesmo facile. Pro illustrar le puncto, iste observationes es hic repetite in Interlingua . *Redactor*."]

UEBER ERYTHEMA NODOSUM LEPROSUM
(On Erythema Nodosum Leprosum)

M. MURATA

As a result of clinical and histopathological studies of nodular (lepromatous) cases suffering from erythema nodosum leprosum (e.n.l.) the author summarises as follows:

1. E.n.l. may be found only in cases with nodular (lepromatous) leprosy, in one-third or one-fourth of such cases.
2. The eruption is more often infiltrative than nodular, in a ratio of 2 to 1.
3. Sex incidence of e.n.l. follows that of nodular (lepromatous) leprosy.
4. E.n.l. is not an initial event: it breaks out about 6 years after a certain initial symptom was found.
5. E.n.l. occurs mostly in adolescence: initial symptoms appearing at puberty often transform into nodular (lepromatous) leprosy in adolescence.
6. The clinical features include those typical of common eruptive febrile diseases.

   The latent period is inconstant and mostly uncertain. The eruption appears with an attack of fever accompanied by rigor, but sometimes there is non-febrile e.n.l. The eruption attains the size of a lentil or walnut, and is scarlet in colour, solid and elastic in consistence, elevated, and hypersensitive. There are solitary and confluent forms of e.n.l. At the beginning the eruption appears mostly on the face, but later in the region where leproma occurs, as well as on the flexor surface of the limbs where leproma usually does not occur. The distribution of the eruptions corresponds to that of veins. E.n.l. occurs chiefly in spring and autumn, especially at the change of season. Colour changes during disappearance of the eruption are from dark red, to bluish green, then bluish brown. After disappearance of the eruption the residual findings are of dryness and creases of the skin with bluish pigmentation, desquamation of the epidermis, softening of the skin, or there may be no trace of the eruption left at all. Softening of the eruption during its clinical course may be associated with the detection of suppurative germs, in which case yellow liquid pus may be formed: in the absence of suppuration a thick glutinous orange-coloured clot of pus may be discharged. Neuralgia as a result of nerve enlargement is prominent among the general symptoms. Arthralgia as a result of synovitis is the next in prominence. There seem to be no marked changes in the urine, nor in the internal organs. Leucocytosis occurs in the blood.

7. The clinical course may be acute, subacute, or chronic: it is most often acute.
8. The essential pathological structure is of an inflammatory formation within fatty tissue, of infiltrating colonies of polymorphonuclear leucocytes.
9. In the diagnosis it is important to differentiate erysipelas which may accompany nodular (lepromatous) leprosy.
10. Prognosis is generally benign.
11. There is no specific remedy for e.n.l. so far, though calcium preparations, quinine hydrochloride, and laxatives have a favourable action.

Finally, the author recommends the designation of *erythema nodosum leprosum*, because the condition clinically resembles erythema nodosum and is a characteristic syndrome appearing in the course of leprosy, especially nodular (lepromatous) leprosy. The term erythema nodosum is already approved as a clinical entity, and there is one form due to potassimu iodide observed by the late Dr. Shitachi, so *erythema nodosum leprosum* is a term which reasonably may be adopted for this singular and independent clinical syndrome which also has its own histological features.

ABSTRACTS

A Survey of Leprosy amongst the Lovale Tribe in the Upper Zambesi Basin, Northern Rhodesia, **J. T. Worsfield,** Central African J. of Med., III, Nos. 9 and 10, Sept. and Oct. 1957, pp. 359-365 and 401-406 resp.

This paper is in two parts, with 4 illustrations in the first and 7 in the second. The Lovale tribal area comprises about 8,000 sq. miles (20,720 sq. kilom.) with a population density of 2 per sq. mile (0.8 per sq. kilom.). It is largely a sandy dry country inundated for 3 months of the year by the Zambesi. Nearly all the villages lie along the banks of this river, and contain 20 to 50 people living a primitive life. A child sleeps with its mother until weaned, then is taken over by other female relatives. There is no fear of leprosy. The diet is scanty, deficient in protein, and based on cassava. In a survey of 5 months duration Worsfold visited every village and examined each individual, to a total of 20,148 people. The general leprosy prevalance was 11.85 per thousand, and 65% of all cases admitted to having a near relative with leprosy. The lepromatous rate was 23.75%. There was a low childhood rate, and a steadily increasing prevalence with the advancing age groups. The author supports the view that transmission is mainly through the skin, either intact skin or skin abrasion. He gives a clear account of the clinical features of leprosy in this region, and emphasizes the symmetry of lesions in lepromatous cases. The generally excellent result of oral DDS treatment are described, which has been in use in the area since 1950.

The Significance of Experimental Murine Leprosy and Screening Test in Studies of Chemotherapeutic Agents for Leprosy, **S. Nishimura,** Med. J. of Osaka Univ. VII No. 4, March 1957, pp. 735-776, 9 figs., 5 Tables.

He has studied the chemotherapeutics of murine leprosy since 1942. In the beginning it was thought that agents effective against murine leprosy would also act against human leprosy. Recently it has become clear that this is not necessarily so, and biological studies of the 2 diseases have revealed many points of difference. To draw analogies for human leprosy from experiments with murine leprosy is mistaken. The first step in a true advance would be to find an agent which would be effective both in murine and human leprosy, first collecting as many agents as possible which have an effect in murine leprosy. New agents with the double action have not yet been found and this may be only a mirage. However there may be some common point in the metabolic processes of the human and murine leprosy bacilli, and another hopeful line is to carry on with the screening test that he has devised. The results of chemotherapeutic tests in murine leprosy are given by the author,

both for his own department and for other workers, on hydnocarpus oil, cepharanthin, promin, DDS, etc., and Table 2 gives a comparative study of the therapeutic action in tuberculosis, leprosy, and murine leprosy.

Nuevos Aspectos y Problemas Actuales de la Lucha Contra la Lepra: *(New features and present-day problems of the leprosy campaign).* **Félix Contreras,** Revista de Leprología, Fontilles IV, No. 3, June 1957, pp. 103-122.

Great advances in leprosy control have been made in the last 15 years, not least in Spain, but Dr. Contreras notes a certain inquietude, in that our highest hopes have not been fulfilled. In Spain they share this inquietude, and intend to revise and rethink their work, and press on to attain perfection. He describes the origin and foundation of the Fontilles work by Padre Ferrís, and the collaboration of Guillén and La Portilla. The results have been highly satisfactory. Though it is too soon to proclaim the decline of prevalence, lepromatous cases grow less in proportion to the non-lepromatous, more and more child cases are found and less and less adults, patients attend at an earlier stage of their disease, and the advanced deformities are less common. The number of cases in Spain was estimated in 1943-47 as between 4,000 and 5,000, and they think this about correct for the present time. Improvement in clinical condition of the majority of patients has been very striking, such as in eye and throat complications, and secondary ulcers of the extremities, and deformities, and the psychic state of the patient is much calmer and co-operative. The humane and friendly attitude of the doctors and attendants has played a great part in the psychic change, as well as the expectation of cure by the sulphone drugs.

The Review ' Fontilles ' was' founded in 1944 and full contact has been kept with the world of leprosy. New leprosaria were founded in Trillo in 1942, and in other places. Sainz de Aja in 1943 enunciated the principles of leprosy control in Spain, and was hopeful of winning the battle against the leprosy endemia in 2 or 3 generations. Contreras in later works amplified these and introduced points for emphasis. In 1947 promin was first used in Fontilles: it and the later sulphones had a good effect. In 1953 the VI International Congress of Leprosy was held in Madrid, when many visitors studied the Spanish leprosy campaign and commented favourably on it.

New features. The medical, public health, and social aspects of leprosy have changed completely. The early curable forms of the disease are more readily diagnosed, and classification of all forms is better understood. Early and even later cases respond to the sulphones and the disease is arrested. Today we know that

leprosy is less contagious than tuberculosis and contagion requires a long period of living contact, and is less marked towards adults. The lepromatous cases are the most infectious, but even in them the infection can be terminated in time. In effect, leprosy today is a disease which is one of the most easily avoidable infections. Social conditions have also improved greatly, both for the patients and their dependants, and rehabilitation of former patients into civic life now receives a great deal of attention. Children of leprosy patients and child contacts are also cared for.

Presend-day problems. The solution of ancient problems and the new orientation have led to new problems. It has become clearer that *dispensaries* form the chief element in the leprosy campaign. These may be exclusively for leprosy in countries of high leprosy incidence, or polyvalent in countries of least incidence. In countries like Spain, with median incidence, use can be made of the Skin Dispensaries. For all dispensaries, however, one must not count on the patients to come; they must be sought out in their homes. Each dispensary must have transport, and in areas of heavier incidence must have a *mobile team*. The salaries for workers in the Skin and Venereal Diseases dispensaries which now undertake leprosy work should be augmented. *Sanatoria or leprosaria* of the ancient pattern, in which segregation was the only good obtained, are not necessarily to be condemned, for some countries did seem to attain a decrease in the endemic when they had them. Nowadays prophylaxis by means of treatment and by mass campaigns threatens to extinguish the leprosaria. If we understand leprosaria as dumps of patients segregated by force, Contreras thinks we should certainly close them. If we understand them as sanatoria, we should certainly continue these, because they are most valuable for the proper study of the disease and for skilled treatment and the prevention of complications. Admission should be voluntary, and open to all lepromatous cases, and all other bacilliferous cases, such as indeterminate and borderline and reactive cases. The home conditions of many patients are poor, and sanatoria offer definite advantages to such cases, as well as a sure way of removing infection from the family. All restrictive legislation should be abolished. In Spain legislation makes no difference between leprosy and other diseases. The less infectious types of leprosy can be treated at home, but they should not be barred from the sanatoria if other reasons exist, such as bad treatment by the neighbours, or membership of a family where other cases exist. Separate sanatoria for males and females are not advisable just now, when treatment takes a long time, but when in future the duration of treatment is shortened, it would be worth while. Discharge of patients from sanatoria on reaching arrest from their disease should

not be too hasty; a period of " consolidation " should intervene, and later when they go, a long period of supervision should be arranged. Propaganda is necessary to ensure a happy return to civic life. A talk between the leprologist and the employer is very useful, and also a talk to the work-mates. Previous plastic and reconstructive surgery is a great help in securing rehabilitation. It is most important in cases where the nose has been destroyed. *Preventoria* still remain of great value, for leprosy is a familial infection very largely, and child contacts must be cared for and given all the protection against the disease that can be given. Some of these children can be cared for by relatives or foster-parents, but preventoria still remain necessary for many. There has been a swing of opinion against preventoria, but it all depends on the national conditions. In Spain they still need them because there prejudice has not decayed enough to allow of all children being cared for by relatives or ordinary colleges. Those like Chapinería have saved many hundreds of children from becoming victims ot leprosy, and at the same time have fitted them for civic life. Children with early lesions and children with low resistance to leprosy have been rendered safe. Some children free from the disease from the beginning are later transferred to ordinary colleges or relatives outside the focus of disease. By the time of puberty all types are ready to resume normal life. *Treatment of leprosy* in Spain is based on the use of DDS, both orally, and by I.M. injection in oily suspension. As the latter is given weekly the author thinks it would be suitable for mass campaigns in countries of high endemia. The search for other treatments still goes on. *Work* is important to all patients according to their clinical state; it is beneficial both psychically and in helping towards cure; this importance of work should not be forgotten for out-patients. *Social assistance* to patients and relatives is also important, and is available in Spain. The climate of opinion towards discharged patients still hinders rehabilitation for some; in difficult cases where patients cannot make headway at home, they make place for them in the sanatoria again, perhaps in a separate section. *Marriage of patients* is a subject for much thought. The general trend is not to interfere with the marriage bond or contraction of it, though delay may be advised because of the probability of the arrest of the disease in a fairly limited time, but all cases are to be considered on their merits. All cases should have explained to them the full implications of the disease, as far as our present knowledge goes. *Propaganda* to explain the nature of leprosy to the people must continue, and a special campaign to the cultured classes is of value, so that they also can act as propagandists. Writers in particular need a special approach to persuade them not to use the word

leprosy in its horror context.

The Chemotherapeutic Activity of Triton WR 1339 and Macrocyclon in Murine Leprosy. **R. J. W. Rees,** Amer. Review of Tuberc. and Pulmonary Diseases. **76** No. 5, Nov. 1957, 915-916. (Letter to Editor).

Rees refers to the report of Kátó and Gözsy (Am. Rev. Tuberc. 1957, **75,** 684) confirming the powerful antituberculous activity of Triton A-20, which is a 25% aqueous solution of the solute Triton WR 1339, in the mouse and guineapig. This had been reported earlier by Rees and others (Cornforth, J. W. *et al.* Nature, London, 1951, **168**, 150; Rees, R. J. W. Proc. Roy, Soc. Med. 1953, **46,** 581: Solotorovsky, M. and Gregory, F. J., Am. Rev. Tuberc., 1952, **65,** 718). Favourable results agains murine leprosy in the rat or mouse on the part of the Triton type of surface-active agents can now be reported by Rees.

A partially purified suspension of *M. lepraemurium* obtained from homogenized rat lepromata was used by intravenous injection to infect mice. This method produces a fairly standard type of systemic infection bearing most on the liver, spleen, skin, and heart. To assess the progress of the infection macro- and microscopic examination of the various tissues was carried out, also bacillary counts of stainable acid-fast bacilli on homogenates from the liver and spleen. From the day after infection, twice weekly subcutaneous injections were given of Triton WR 1339 and Macrocyclon; the latter is less toxic though chemically similar. They showed high therapeutic activity in doses of 5 mg. for 20 to 27 weeks.

Rees mentions that Kátó and Gözsy failed to show any activity against murine leprosy infection in the rat or mouse. They used larger doses but assessed the result after only 6 to 8 weeks of treatment by the subcutaneous route in rats and intraperitoneal route in mice. The apparently contradictory results may be due possibly to the different routes of infection, but Rees thinks it much more likely that the favourable results in his work are due to the longer course of treatment.

Hence it seems that other mycobacteria than *M. tuberculosis* can share in the therapeutic activity of surface-active agents of the Triton type. Present study also suggests that the monocyte is the site of action of these agents *in vivo,* since murine leprosy is caused by an obligate intracellular organism infecting cells of the reticuloendothelial system.

The Antituberculous Activiity of Ethyl Thiolesters, by **G. E. Davies** and **G. W. Driver,** Brit. J. of Pharm. and Chemotherapy, **12,** No. 4, Dec. 1957, p. 434-437.

The authors refer particularly to diethyl dithiol*iso*phthalate

among the thiol esters. The thiol esters have been the most promising of the antituberculous derivatives of ethyl mercaptan, as reported by Davies, Driver *et al.* in 1956, and Solotorovsky *et al.* later in 1956.

They selected diethyl dithiol*iso*phthalate (ETIP) for study in mice because it seemed the most suitable of the series for later clinical trial in man. In the experiments now reported they found that ETIP in mice by *subcutaneous injection* of 500 mg/kg. was as active against tuberculosis as the best of the previously tried derivatives of ethyl mercaptan (diethyl dithiolcarbonate), and it had the advantage of being almost odourless (unlike diethyl dithiolcarbonate). Further, as it was noted that the application of ETIP to the intact human skin resulted in an odour of ethyl mercaptan in the breath, clearly indicating *percutaneous absorption,* experiments on infected mice were by this method carried out, and showed a high effectiveness of ETIP. The compound was also found highly effective against intracorneal tuberculous infection in mice, as shown also by Naguib and Robson (1956) for murine leprosy intracorneal infection.

ETIP action against an established infection was studied and found to be high. *Weekly doses* of ETIP were also studied; a marked antituberculous effect was obtained with a single subcutaneous dose given on the day of infection and still larger effects were obtained by giving one or more further doses at weekly intervals.

Combined treatment with INH proved much better than ETIP or INH alone, and a single dose of ETIP markedly prolonged the life of infected mice that had relapsed and were about to die after treatment with INH. There is no cross-resistance between INH and ETIP. When in the experiments ETIP-resistant strains were produced and used for infections, INH was found to be fully effective against them. The effect of ETIP on a streptomycin resistant strain was not tested, since earlier it had been found that other ethyl mercaptan derivatives were effective against infections caused by such strains.

A possible *mode of action* of ethyl mercaptan is that it interferes with a biological methylating or thiomethylating system. Some support was found for this hypothesis. The development of drug-resistance suggests that at least part of the action is exerted directly on the bacteria and not solely through the defence mechanisms of the host. It may be that a hitherto unknown biological system is involved.

Limited Multiplication of M. lepraemurium in Tissue Culture. **R. J. W. Rees** and **P. C. Wong.** Nature, **181,** No. 4605, 1st Feb. 1958, pp. 359-360.

Rees had previously shown that the generation time *in vivo* of the organism of rat leprosy was 13 days, and the authors devised a method for counting the total number of bacilli per culture before and after cultivation. The first attempt at culture they made on macrophages, but found no multiplication after 80 days, though bacilli recovered from some of these cultures were still infective. Experiments were now directed to tissue cultures on spleen explants obtained from previously infected animals. A mouse was infected intravenously with *M. lepraemurium* and 8 weeks later the spleen was removed and cut into uniform explants of 1 mm. The explants were put out, 6 per culture tube lined with plasma clot to which was added 1 ml. of medium containing 20% horse serum, 5% chick embryo extract, and 75% Hanks' balanced salt solution. Random groups of culture tubes were formed in two categories, those with no added antileprosy drugs and those to which streptomycin and INH were added, and the tubes were incubated at 37°C in roller drums and the medium changed twice weekly. By a modified Breed technique a count was made of equivalent standard samples of homogenates from each culture at 24 hours and at 15 and 29 days, after dispersal of the bacilli by ultrasonic vibration in each case. The results showed a highly significant increase in the number of bacilli in cultures free of streptomycin or INH, by the 15th day of incubation; after a further 14 days there was no increase. There was no increase in cultures which contained high concentrations of the drugs.

In 5 experiments by these methods limited multiplication *in vitro* of *M. lepraemurium* has been demonstrated. The rate of multiplication *in vitro* corresponds closely to that *in vivo* in the mouse. Hanks and colleagues have recently obtained much the same results. The confining of the multiplication to the initial period of cultivation seems to be due to release of the bacilli in increasing numbers from the infected cells as they degenerate, thus exposing the bacilli to relatively high concentrations of serum; (it has been shown by Hanks and Gray that this inhibits their endogenous metabolism). It looks as if success in continued culture will require a regular supply of healthy cells in a medium with low concentrations of serum. The authors continue work on methods to provide these culture conditions.

Prophylaxie et Thérapeutique de la Lèpre by **R. Chaussinand,** 1958, is a booklet of 98 pages published by Bibliotheque de Thérapeutique Médicale, Paris (G. Doin et Cie., 8 Place de l'Odéon, Paris VI).

This is a clear and concise work of great practical value, with useful bibliography and index. Chaussinand writes in beautiful, limpid, and unequivocal French, which brings an added pleasure

to the reading of this booklet. He gives a balanced account of all the modern aspects of prevention and treatment of leprosy, and there is a section on bacteriological methods and the lepromin test. Every section will be read with interest and profit. Because there is a special interest nowadays in BCG, the section dealing with *BCG vaccination in the prevention of leprosy* (Section D, pp. 25-27) may be summarized as follows:—

Because BCG vaccination produces a sensitivity to lepromin, it is tempting to assign to it a considerable influence in the prevention of leprosy, but many years of trial of BCG are needed before proof is available. Chaussinand thinks, however, that it should be widely used in tropical and subtropical countries; even if it turns out to be of little value in the prevention of leprosy, it will not have failed to be valuable against tuberculosis. The Brazilian method of BCG vaccination by the oral route (a single dose of 200 mgm. or 3 doses of 200 mgm. at intervals of a week) has the advantage that a tuberculin test is not necessary; but in a mass campaign large amounts will be required and the giving of 3 doses at weekly intervals might cause difficulty. The intradermal route is more precise and economical; however a preliminary tuberculin test is necessary, and a small chronic ulceration may develop in the site of the injection of BCG. The method of vaccination by skin scarification through 2 to 4 drops of the vaccine containing 75 mgm. of BCG per cc. is less precise than the intradermal method and also requires a preliminary tuberculin test. The simplest control experiment on the value of BCG would be to give it to 50% of the newborn of each sex, leaving the others unvaccinated. Also one could vaccinate 50% of the whole population, or 50% of the children, in an area of high prevalence of leprosy, leaving the rest as controls. One would have to remember for the two groups that leprosy might appear more than 3 years after the vaccination, and take care to eliminate those who were already incubating leprosy at the time of vaccination; annual examinations would need to be made of all subjects in both groups. The amount of protection against leprosy given by BCG cannot be other than relative. On the other hand it is highly probable that subjects of infection by the leprosy bacillus or virulent tuberculosis bacillus better resist the leprosy infection than those merely vaccinated by BCG. The attenuated bovine type bacillus of BCG can only maintain itself a relatively short time in the vaccinated subject. It would not be wise to believe that a subject of BCG vaccination is incapable of contracting leprosy, but at least it is reasonable to believe that there is an increase in resistance.

REPORTS

Annual Report, 1957, Leprosy Control, Onitsha Area of E. Nigeria

The Area Superintendent, Dr. A. S. Garrett, reports that the new features of the year at Oji River were physiotherapy on an organized basis, the beginning of the building of a School for the Blind, and the beginning of a new orthopaedic block. Restorative surgery for deformities of hands and feet has been carried on by Dr. Garrett, and Dr. Price, a new orthopaedic surgeon, was expected in December, so this work is likely to grow greatly. In collaboration with Dr. Davey of Uzuakoli, trials of new drugs have been pursued, chiefly of DPT, which has many good points. Discharges of patients on arrest of their disease have remained at an appreciable level. In buildings, more staff houses are needed, and repairs to patients' houses and waterworks. Electric power is available not far away and still needs the connection. In staff Dr. K. M. Ellis has been lost on her marriage and a new medical officer, Dr. W. F. Ross has arrived. Training continues of Leprosy Inspectors and Medical Field Unit Assistants. Mr. C. O. Onyia, the laboratory technician, has received a course of training in BCG inoculation, and the use of this is now possible. Of Dispensary Attendants 13 have been trained in simple leprosy treatment. Mr. Savory and Miss Smith have conducted a physiotherapy course. In the *District Work* there has been a large and rapid drop in the total of patients in the south of the province, but the north lags behind, in the Nsukka division particularly. In an effort to remedy this more treatment centres are being opened in Nsukka area and also the Niger flood area. There is a scattered population and poor communications and patients hang back from treatment. Much persuasion and explanation are still needed. Even in the south, one case was found who must have avoided treatment for 8 years. As Rural Health Centres are further developed more existing geographical gaps will be covered.

Rajah Sir Charles Brooke Memorial Settlement, Kuching, Sarawak

By kind permission of Dr. W. Glyn Evans, Director of Medical Services, Mr. Hamish Macgregor has forwarded an excellent series of statistical tables on the work of this leprosarium in 1957 and before. During the years 1950 to 1956 admissions were 449, or an average intake rate of 64 p.a. Discharges were 385, or an average rate of 55 p.a. Deaths were 91 or an average death rate of 13 p.a. Of the 867 patients resident in the leprosarium during the period, 44·5% have been discharged, 10.5% have died, and 2% have left of their own accord. Types of leprosy were, L 62%, I 24%, and T 14%. Malays and Chinese had a higher lepromatous rate and Land Dayaks a lower lepromatous rate than the average for

the area. The child rate is 19% of all cases, and again there are racial group variations, with the Malays showing the highest child rate of 28%. The sex rate is 77% for males; children have a 73% male sex rate. A study of causal contacts of 400 cases showed close or very close contact in 125 and intermittent contact in 67: for the remaining 208 cases there was no certainty. Of the known contacts members of family were the most incriminated.

Leprosy in Cyprus

Dr. J. H. C. Clarke, Director of Medical Services, has provided the following information. Restrictive legislation was repealed in 1957. Outpatient treatment had been introduced in 1952 and voluntary admission to the St. Charalambos Home, which has a beautiful situation and is provided with a hospital, treatment clinic, bath houses, club, store, and bungalow. This Home now has 42 male and 21 female inpatients, who keep active and cheerful. Notifications of leprosy were 11 in 1953, 9 in 1954, 10 in 1955, 1 in 1956, 9 in 1957. Besides the 63 inpatients there are now 67 outpatients, and 53 patients discharged under observation. Child cases have not been seen for many years, and it seems that leprosy is dying out. The existing patients are cooperative and the community at large has now a more enlightened attitude towards leprosy.