DISCUSSION

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Discussion

PART I. CRITERIA FOR THE ASSESSMENT OF DRUG ACTIVITY

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Dr Browne
Our first theme for discussion is the criteria for the assessment of drug activity. Later we shall be dealing with other aspects, in particular clinical trials and the practical problems of chemotherapy. I would remind you of a sentence in one of the letters sent to you earlier; the aim of the meeting will be to compare the results of the relatively large number of studies recently undertaken, to evaluate these studies, and to make recommendations and suggestions for future work.

What are the criteria that we should observe as we attempt to assess the activity of a drug? We will start with the clinical criteria, go on to bacteriological criteria and then compare animal studies, footpad activity for instance, with clinical response. Yesterday we heard that sometimes these two facets of investigation are not "superposable" (to use a very good French word). We should also examine both clinically and bacteriologically the importance of "persisters", viable persisters, in different organs because this will determine the duration of treatment, the declaration of non-infectivity, and of the reduction to the minimum of the risk of relapse. I hope we shall have time to think of the activity of the various groups of drugs that we have been considering yesterday and today, i.e. particularly the sulphones, clofazimine, rifampicin and Transfer Factor. At the end of this, we shall discuss "Combined therapy", the pros and cons, whether, as we look on the world as a whole, we can recommend combined therapy, or whether, wearing blinkers and with partial blindness, we will persist in the advocacy of monotherapy in a chronic mycobacterial disease in which we wholeheartedly invite the emergence of numerous cases of drug resistance in the future. Now we will begin with the criteria for the assessment of drug activity.

I.1. Clinical Criteria

Dr Krenzien
Yesterday Professor Pattyn showed in his controlled trial an objective criterion concerning the clinical observation. I should like to ask him if he will be so kind as to explain what is involved in this objective control criterion.

Prof. Pattyn
This is purely an evaluation of the degree of skin involvement in terms of infiltration and extent of skin lesions. This criterion is indeed difficult to measure very precisely, but it was noted as such by the clinicians.

Dr Browne
We remember the Leonard Wood Memorial trials of olden days and how they lumped together so many clinical criteria, some of which were due directly to the drug, some to the body reaction to the drug or even to the development of cell-mediated immunity, and some to the results of fibrosis. Now I'd very much like Dr Davey and Dr Leiker to give us some ideas of their assessment of drug activity from the clinical standpoint.

Dr Davey
Without any doubt I would look first in the patients' noses, because I am quite convinced that if we are beginning to study a new drug in a person who has untreated lepromatous leprosy, the first signs of improvement are going to be in relation to his nasal discharge and the state of his nasal mucosa. We should expect to see a change there within three weeks to a month at any rate and with rifampicin probably before that. This is my experience in India. After that I would expect to see a steady reduction in lepromatous infiltration obvious first in the most recent lesions, last in the most fibrotic lesions. One would look for this, of course, on the face where it is readily seen, and also especially on the extensor surfaces of the limbs. At the same time one would turn one's attention to the nervous side; there should be no extension of anaesthesia, one would hope before very long to see the beginning of improvement in nerve enlargement, etc. The suggestion that in the long term, absence of relapse should come into our assessment is important I think. Another valuable criterion is the regrowth of hair.

Dr Leiker
Those who are familiar with the old literature undoubtedly know that even in the period of chaulmoogra oil the clinical appearance of the patient changed remarkably, often the patients were considered as cured. Now I had the privilege of working for some time in Indonesia in an area where leprosy treatment had not been introduced. After the surveys, it was not possible to introduce leprosy treatment immediately. I came back a few years later and I noticed marked changes in many patients for the better. I regard the clinical criteria as most unreliable. Here we have seen an impressive series of photographs with marked clinical improvement, on the other hand, I have also seen many patients who have to be classified as borderline or borderline-lepromatous. In these patients spontaneous clinical changes are very common; marked skin lesions may disappear spontaneously and nearly completely.

Dr Davey
I would like also to comment on that: in the last 24 hours I have seen a large number of photographs of borderline cases and I would not consider these at all suitable for pilot drug trials.

Dr Pearson
The major problem to me in assessing clinical improvement is how to quantify it. It is very easy to look at slides before and after and say the patient is improving well or satisfactorily and get a rough idea whether one drug is better than another, but it is almost impossible to put this improvement into figures. I think that the only way in which this can be done is by Dr Ridley's technique of examining serial biopsies, taken from the same lesion or comparable lesions and

assessing the area, the proportion of the biopsy, affected by the infiltrate or granuloma.

Prof. Saerens
I have the impression that photography is being used for the evaluation of clinical assessment. Why can't we standardize photography as has been done in X-ray photographs for instance? Why can't we learn in this respect from biometricians?

Prof. Freerksen
The effect of a therapy should not solely be judged according to a patient's general clinical improvement, since the latter cannot be standardized. In this respect I quite agree with Professor Saerens and Dr Pearson. The clinical improvement is, however, very important for the patient and his position in society. In our experience, clinical improvement means a relationship to what you can find with other objective methods. This should also be a matter of discussion. A therapy which does not cause any clinical improvement is, in my opinion, of no real value.

In the case of leprosy an impartial and independent registration of phenomena appearing in the clinical picture can only be achieved by means of photographs. However, only outward changes can be registered by photographs. Any changes taking place inside the body cannot be demonstrated by this method. A series of photographs must be taken, one every month, for instance, or every three months. As far as the exterior pathological phenomena of the skin are concerned, the importance of serial photos resembles that of X-ray exposures in the case of pulmonary tuberculosis. Here again the pathological phenomena can only be registered and compared, but not standardized.

Dr Browne
Dr Karat—you have been engaged in drug trials in South India.

Dr Karat
I find it extremely difficult to quantify clinical improvement in patients. Flattening of evidently raised lesions is obviously like the changes in the quantity of nasal discharge that Dr Davey referred to. But I believe that these are very superficial and inadequate criteria for the assessment of therapeutic response, because they do not bear a constant relationship to the change in the bacteriological status of the patient. For the same reason we have also found that the assessment of enlargement of nerves is a very unreliable index, because there is no correlation between the size of the peripheral nerve and its function. We have assessed this using new techniques of electro-myography. More often we have been surprised to find perfectly normally functioning nerves despite gross thickening. That is why I think that clinical assessments can only demonstrate whether a patient looks better, the same or worse, but cannot quantify into terms which can be used by doctors working in different areas of the world.

Dr Ramanujam
We are familiar with the numerical evaluation of clinical progress from time to time. We still follow the traditional method of charting cases, that is representing the clinical condition of the patients on a suitable proforma. We divide the human body into seven areas; the head, the trunk, the two upper extremities, the

buttocks and the two lower extremities. Depending on the predominant type of skin lesion present in these areas, we give them "clinical scores". If there are essentially macular lesions we give them the score of one, if there are areas of diffuse infiltrations we give the clinical score of two, if in addition to diffuse infiltration we find nodules we give a clinical score of three. If the nodules are predominant manifestations we give a clinical score of four. Patients are assessed clinically in our triple trials once in three months and this assessment of the clinical condition on the proforma is done without reference to the previous condition of the patients. In addition to this giving of clinical scores, we note on the chart of these patients, the condition of the peripheral nerves not only regarding their size but also with reference to the presence of tenderness and otherwise, anaesthesia in the peripheral part of the limbs, and also deformities present, if any. We find this way of recording clinical assessment quite useful. This is, of course, only a qualitative assessment. We know that in patients who show very good clinical improvement, the bacteriological changes may not be in conformity with it, but, however, by comparing the chart every three months it gives us an idea whether the patient is progressing satisfactorily or not.

Dr Rees

Can I add to what Dr Ramanujam has just said? By and large, in the trials with which the Medical Research Council are concerned, our policy has been very similar to his, and includes what other leprologists have said here, but there is one important addition which must be mentioned, namely that in all our trials clinical progress is judged by an independent assessor. It is very important to have an independent clinical assessor who is not seeing the patient from day to day, as only in this way is maximum objectivity assured. The assessor examines the patient at the beginning. On each subsequent occasion he first examines the patient, and having made notes, he is given successive photographs, together with his previous notes. His guidance that the patient is improving or deteriorating is of course qualitative, but his independence is important not only from the standpoint of the total pattern to which Professor Freerksen has referred, but also because it enables the group responsible for the patient from day to day to be sure that they are not missing deterioration, an aspect obviously important from the ethical point of view.

Dr Browne

There are only about four centres in the world where competent independent observers can be found within an easy geographical range. This state of affairs is perhaps a reflection on our lack of enthusiasm in leprosy.

Dr Krenzien

Within my relatively short experience in clinical leprosy it is quite obvious to me that there always exists a delay between clinical improvement and the bacteriological response of the host. I think that this might be different under treatment with different drugs. For instance rifampicin causes a quick response of the host within four weeks. Clinical improvement takes a much longer time under standard therapy with dapsone only. This is why we should always note precisely the duration of time for clinical improvement to appear. With some drugs, such as Lamprene, rifampicin and combined therapy, it is typical that clinical improvement will appear within a very short period of time. Of course, the bacteriological response, the decrease of the BI, takes much longer.

Dr Browne
I would like us to return to those peripheral nerves which in the past have been gauged as an important indication of improvement. A lot would depend naturally upon the precise form of leprosy, whether pure lepromatous or subpolar, and the precise duration of leprosy as well as the degree of fibrosis within the nerve, which would account for the persistence of tenderness on palpation. The increase in the extent of cutaneous anaesthesia and paralysis may coincide with clinical improvement in other areas. I would like Dr Davey to take up this aspect which is really an extension of one of his earlier remarks.

Dr Davey
I do not think changes in nerve enlargement are an important criterion of progress really, because you can get extension of anaesthesia during a successful course of treatment, but I do believe that diminution in nerve tenderness in the early stages may be more helpful. My own feeling is of course that we are seriously limited where the assessment of clinical improvement is concerned and can really only say that the patient is improving or getting worse.

Prof. Azulay
It is very difficult to demonstrate clinical improvement by photograph. For us leprologists the clinical improvement is of value, but we have other and better criteria; for the patients themselves the clinical criterion is the most important one, the reason why he comes to the doctor. There may be drugs that act against the bacilli and of course improve the clinical appearance, but there are also drugs that act against the bacilli and against the infiltration, and this fact is of significance.

Dr Pearson
We need to remember that when we are measuring clinical improvement in a long term trial, i.e. one to three years, the clinical improvement is not basically a measure of the continuing effect of the drug; it is a measure of the effect of the host in clearing the results of the infection. It measures the continued action of the drug on the small number of persisting bacilli. Improvement after the initial period is primarily a measure of what the body is doing and not of what the drug is doing.

Dr Jopling
May I just revert for a minute to the question of nerve thickening? The subsiding of nerve thickening can be of very great value in assessing the progress of a borderline or tuberculoid case. Here we have granulomatous involvement of the nerve; the granuloma is absorbed as the result of treatment, the nerve very definitely subsides and can give a very useful assessment of the response to treatment. But I do agree, as Dr Davey says, in a lepromatous case this does not apply; in a lepromatous case, even successfully responding to treatment, there may be steady increase in nerve thickening as well as increase in areas of anaesthesia and in muscle paralysis.

Dr Browne
I would like to take up Dr Pearson's point because we have not yet explained why dapsone is remarkably efficacious in some cases of tuberculoid leprosy and the

sulphonamides, too, as Dr Languillon pointed out. We are dependent not upon the bacteriostatic activity of the drug but on something else. When we are assessing clinical improvement under treatment, what are our criteria then, Dr Pearson?

Dr Pearson
I think I'd query whether even in tuberculoid leprosy all the bacilli *are* fragmented. If one looks in the right places, that is, in particular, nerves which are liable to be enlarged and damaged in three months' time, but are not now, or dermal nerves in the area just outside tuberculoid lesions, one will find occasionally small numbers of viable bacilli. After all, much tuberculoid leprosy is not usually self-limiting because the patches enlarge, and I still think that even tuberculoid leprosy is caused basically by the presence of leprosy bacilli. Killing the bacilli or stopping their multiplying by any drug is the prerequisite for curing the disease.

I.2. Bacteriological Assessment of Drug Activity

Dr Browne
Since the clinicians seem to be limited, I think we had better go along to the bacteriologists now and to those who are concerned with the bacteriological assessment of drug activity in leprosy. We have heard a lot about MIs and BIs, and one of our participants went so far as to suggest that the MI should be abolished. Perhaps he would like to take up that assertion.

Dr Karat
In my experience there has been no consistent relationship between the number of solidly staining organisms present in a given biopsy homogenate and the cultivation of those bacilli in the footpads of mice. I believe that, while there is a change in the appearance of bacilli under treatment, interpretation of the change as indicating non-viability is premature.

Dr Ridley
I think that any index which depends on staining bacteria is notoriously open to difficulties and to change from one set of conditions in one laboratory to another, due to slight differences in technique. I was particularly aware of this myself when I found that indexing, which I have found to be extraordinarily constant, suddenly showed an increase of five times in the apparent rate of progress of lepromas; I traced this to a change in the alteration of the maximal Bacterial Index in sections from six to six and a half.

While I am speaking, could I also mention the question of the granuloma which two colleagues have referred to and which Dr Pearson said he thought was a good index of clinical improvement, with which I agree, because after all it is infiltration in nodules, which for the most part the clinician is looking at, unless there is a reaction present. This can be quite accurately estimated. Prof. Azulay said that he thought that some drugs acted on the granuloma, others on the bacilli. I am not sure if that is true, but it is interesting that in the trial of clofazimine versus dapsone which I referred to yesterday, the Granuloma Index was the only one which showed any difference between the two drugs.

Dr Browne
Dr Ridley, may I ask you kindly to amplify that phrase, "certain slight differences in technique"? I think it would be most helpful to all of us if Dr Ridley would give us a résumé of the slight differences in technique to which he refers.

Dr Ridley
Well, this goes back mainly to the questions of staining. The differences are just too numerous; we have written papers about this. Differences in the technique of making smears, the way they are spread, the way they are fixed, the temperature at which they are stained, the time of differentiation, all these things have a most definite effect on any sort of bacterial index. There is after all no absolute point at which a bacillus is converted from acid-fast to non-fast, and it is obviously difficult to determine the exact point at which it ceases to be viable. Mere acid-fastness is useful, but after that, bacilli are still present for a long time when they are no longer acid-fast at all; if you use a silver stain you can see bacilli, even solid stained bacilli, when there is no acid fastness whatever.

Prof. Freerksen
When talking about the so-called "effect" of chemotherapy, we have to consider another point which is often neglected. Strictly speaking we are using chemical substances capable of inhibiting in macro-organisms the growth of micro-organisms, or even killing them. The activity of such substances is, however, not directed against the disease itself, nor are the substances able to eliminate dead bacteria from the body. This is one of the organisms' properties and cannot be influenced by means of chemotherapeutic substances. The disappearance of dead bacteria from the body during treatment therefore indicates only indirectly the therapeutic activity, but is nevertheless of remarkable importance. It is apparently very difficult for the organism to disintegrate mycobacteria. Disintegration may perhaps be easier with living than with dead bacteria, since the body can react more strongly with the former than with the latter. Chemotherapeutic substances therefore cannot be compared with "true" pharmaceutical substances, neither be submitted to the same test methods.

When finding acid-fast bacteria in homogeneous material in biopsies and sections we cannot distinguish whether these bacteria were already dead or still alive in the tissue. It is typical for all mycobacteria that by alteration of the medium they undergo considerable morphological changes interpreted as phenomena of adaptation. When chemotherapeutic substances are applied to an organism, the "tissue medium" is rendered unfavourable for the growth of leprosy bacilli. The same thing happens when bacteria are transferred from one culture to another or from one experimental animal to another. This phenomenon is not restricted to *Myco. leprae*; it is common to all mycobacterial species we have studied with regard to this property.

Another symptom of adaptation to a new medium can be the loss of acid-fastness. This means that living mycobacteria may be present even if we do not find any acid-fast bacteria.

As to the double or multiple effect of medicaments several points have to be considered. Apart from their antibacterial activity all antibacterial substances possess a pharmacological and toxicological effect. A classical example of this is the orally administered antidiabetic substances which were not found by research in the glucose field, but rather through chemotherapeutic research (sulphona-

mides). They were only discovered because quite apart from the tests regarding the antibacterial activity of these substances, their biological aspects were also taken into consideration. In such tests the antibacterial aspect may prove to be of no interest at all.

Another aspect of this multiple activity is indicated by the fact that an antibacterial substance may have a very *specific* inhibitory effect, as for example, isoniazid, which is highly active only against *Myco. tuberculosis,* but hardly effective against any other mycobacterial species, while on the other hand rifampicin is a broad range antibiotic used almost universally in antibacterial therapy. I especially noticed this yesterday when the effect upon nasal discharge was mentioned. Nasal discharges are always induced by mixed infection, i.e. they are not purely "leprosy". This also applies to many ulcers. The broad range antibiotic rifampicin covers leprosy as well as tuberculosis, but also many "unspecific" diseases. Furthermore, it must be considered that certain antibacterial substances mutually intensify their action. Here again we are dealing with a "multiple activity".

Dr Walter

To have a redefinition of our criteria for therapeutic trials, one could for instance introduce two phases. The first one would be a relatively short "anti-bacterial" phase in which the morphological changes in the bacilli due to the treatment could be measured. The clinical side would not be so very important. This phase could last from 20 to 48 weeks. It would be followed by the "therapeutic" phase, in which the BI would be measured and the clinical improvement assessed. Even if we don't know exactly the final meaning of the morphological changes, we can be quite sure that the activity of the drugs introduced is definitely shown by the changes in the Morphological Index. This would give us some quite valuable measurement for a short time-period. If the first phase shows clearly that there is a significant change as compared with the established value of standard dapsone treatment we would perhaps not need control cases in many instances. We could measure the effect of a new drug by comparison with dapsone therapy. This is why we should distinguish between a short, "antibacterial phase", and the therapeutic phase which might last two years or longer.

Prof. Azulay

I had one female patient in whom I made the diagnosis of borderline leprosy. At that time I was using thiacetazone (TBI) in leprosy treatment. The patient was improving, but the supply of TB1 came to an end, so I gave her dapsone and she improved much more than with TB1. I found out that the patient did not suffer from leprosy, but from premycosis fungoides which developed into mycosis fungoides. From that mistake I learned that dapsone was helping much more than TB1 and is likely to have a cytostatic effect, too. Furthermore I discovered that several cytostatic drugs have some similarity to dapsone. In clinical use all cases of premycosis fungoides showed that dapsone stops the course of the mycosis fungoides or decreases the speed of the evolution of this disease. We also administered dapsone to patients with psoriasis, as some people will give nitrotrexate, and obtained good results. This is why I am quite sure that dapsone has a cytostatic effect, too.

Prof. Pattyn

Concerning the MI. It has been stated frequently that we are handicapped in leprosy by the fact that we cannot cultivate the bacillus and I would stress that the discussion on the MI is a very nice illustration of this. What do the tuberculosis doctors do in their controlled trials? Their main parameter for assessment is cultivation of the bacilli during treatment, not X-ray photographs, because these are not sufficiently reliable. In leprosy unfortunately we cannot do this. We can isolate bacilli in the mouse footpad, but the technique is much more complicated than is the case of cultivation of tubercle bacilli. This is why we confine our attention to something less good, namely, the MI. It is less good, because it is a morphological assessment. As has been stressed by Dr Ridley, all morphological assessments are extremely dependent on very small details in technique. The MI in my eyes is the second step down from the optimum, the optimum being the cultivation of the bacillus. We are in need of some sort of biochemical reaction that we would undertake on say a cryostatic slide of leprous tissue in order to have some more biochemical way to determine viability. But up till now we do not have these means, so we have to try everything else we have and we are very well aware of the fact that everything else other than the mouse footpad is less good.

Dr Rees

May I first of all say that Professor Pattyn has stated very well the relative importance, and limitations, of the MI as an indirect morphological method for assessing the viability of *Myco. leprae.* However, in spite of the technical difficulties in interpretation and staining associated with the MI, it is based on sound bacteriological criteria which seem always to be ignored when the subject is discussed. Since in this Colloquium the same basic principles of the MI are being challenged, I would like to clarify the scientific evidence and assess the practical value of the MI. When *Myco. leprae* from patients are examined by electron microscopy, as whole or sectioned preparations, bacillary forms are seen with intact cell walls containing either a complete and well structured or a disorganized and variably deficient cytoplasm distributed irregularly within the cell wall. Since the latter picture of all other species of bacteria was seen only in degenerating and non-viable organisms, it was concluded that such changes were incompatible with viability of *Myco. leprae.* Having identified individual organisms with these various morphological features in the electron microscope, the same preparation was stained with carbon fuchsin and re-examined in the electron microscope. These studies showed that bacilli with homogeneous and well structured cytoplasms stained uniformly and "solidly" with carbol fuchsin, whereas, organisms showing degenerative changes in their cytoplasm with only partial cytoplasmic residues, stained irregularly with carbol fuchsin. Thus these detailed comparisons on the morphological appearances of individual organisms at the level of electron microscopy provided irrefutable evidence that bacilli showing irregular staining identified those organisms that, in their pre-stained condition, showed degenerative changes incompatible with viability. Incidentally, these direct comparative studies of stained and unstained bacilli established that the acid-fastness of *Myco. leprae,* and for that matter other mycobacteria, applied only to the cytoplasmic moiety and that the cell walls of mycobacteria are not acid-fast, since organisms which had degenerated and lost all their cytoplasmic content were no longer acid-fast. On the basis of our original studies at the level

of electron microscopy we extrapolate the criteria of irregular staining to light microscopy as a degenerative percentage on the basis that organisms in this category were incapable of life. By this definition we were well aware that of the organisms that still stained uniformly with carbol fuchsin some were not necessarily "viable", since they might have died more recently without having lost sufficient cytoplasmic content to stain irregularly. Therefore our original "degenerate index" represented a maximal index of viable organisms. On the other hand, clinicians chose to present the index in the opposite direction—i.e. the Morphological Index (MI), as the proportion of solidly staining organisms, and equating this to the proportion of viable bacilli. For the reasons given, therefore, the MI, however carefully assessed, could over-estimate the proportion of living *Myco. leprae.* However, on the basis of our original, and entirely morphological indices, it has since been established with the mouse footpad infection that under well-defined criteria there is a very good correlation between the MI of a suspension of *Myco. leprae* and the ability of the organisms to multiply in the mouse. Therefore, as the mouse infection is the only means of culturing *Myco. leprae* it must at present be accepted as strong supporting evidence for the reliability of the MI as a measure of *Myco. leprae* viability. However, Professor Pattyn has quite rightly stated that the MI is only second best to *in vivo* assessment by mouse inoculation. Since there are few laboratories throughout the world that can undertake the mouse footpad infection, the MI provided an apparently simple indirect method for determining the viability of *Myco. leprae.* Unfortunately, as has already been clearly stated by Dr Ridley, the MI has so many technical difficulties that by and large it cannot be easily accomplished or standardized as a routine procedure. For a proper assessment the MI can only be assessed by high-class microscopy and, moreover, unless well standardized preparations of carbol fuchsin are available, the methods of staining and fixation of the smears can influence considerably the interpretation of solid and irregular staining. These variabilities unfortunately exclude the use of the MI for routine field studies. However, I believe that the MI still has an important place in centres concerned with chemotherapeutic trials. For while such centres may not be in a position to standardize their methods of staining with other centres and, therefore, with untreated patients they may well have very variable figures for their initial MIs, whatever criteria they use there are no technical details that I know of which would fail to show a very significant fall in the Morphological Index to approximately 0 following a six month treatment with dapsone or an equivalent active drug.

Dr Leiker

I have encountered the same difficulties which Dr Ridley has mentioned concerning the MI. This is the reason why I switched over from the MI to the GI for the time being. It is impossible to standardize the technique to such an extent that we can definitely distinguish between completely solidly stained bacilli and those which are slightly fragmented. Nobody can tell whether the slightly fragmented ones are alive or not, but it is possible after some training to recognize granular bacilli with reasonable certainty. For this reason I believe that this is basically the same principle, a more convenient way of expressing the changes in the morphology, just a practical thing. Secondly, concerning the Granuloma Index quantification of the degree of infiltration, we again encountered difficulties. In the biopsy specimen it is quite possible to measure the degree of granuloma. This

offers fairly constant results in pure lepromatous cases. But in borderline-lepromatous cases it becomes more difficult and in borderline ones even more so. In these types of leprosy the clinical lesions are different, it is not an entirely generalized disease, it is to some extent localized. Under the microscope the granuloma, too, is not generalized but localized. When taking successive biopsies, we may take the first biopsy in large part from the infiltrated area in the corium, the next biopsy we may take just a little bit outside the infiltrated part and it seems that there is some improvement, because there is less infiltration as compared with the first biopsy. This is why I think that in clinical trials it is extremely important to separate two groups; to assess (a) the purely lepromatous cases including all the criteria such as granuloma index, and (b) the borderline-lepromatous cases which I believe have a place in clinical trials.

Dr Ridley
Could I just say that I agree with all that Dr Rees said? I did say that acid-fast stain had proved useful in showing a correlation with viability and when I went on to mention silver stains I should like to make it clear that I was talking about staining in a general sense and that the loss of acid-fastness did not mean that bacilli had disappeared altogether, and in fact that there is no sudden end point to bacterial treatment.

Prof. Freerksen
I should briefly like to support Dr Rees' opinion and slightly extend what he said in one particular point: We are not dealing with single bacteria, but rather with a whole bacterial population in the diseased body. With or without treatment this population contains all forms of bacteria in different ratios. During an effective treatment the number of solid forms gradually decreases. It is futile to discuss the question whether they are alive or dead, because nobody knows it. Of course we have to base our judgement on bacteriological data in the case of leprosy, since we are dealing with an infectious disease. But tuberculosis is also a mycobacterial disease and also in populations of *Myco. tuberculosis* all forms are to be seen. If the curative effect would solely be judged by the disappearance of bacteria from the tissues, we would never have cured any patient.

Dr Browne
I think we all agree that the MI is a useful indication of the efficacy of a drug, but that there must be impeccable standardization of technique, if possible between different laboratories and certainly within the same laboratory by the same competent and trustworthy technician. I don't think that many of us would have any doubts as to the usefulness of the BI not as an indication of the efficacy of a drug, but rather of the competence of the body in clearing bacilli, which does not depend directly upon the activity of the drug itself. In this connection, the subject of animal studies should be mentioned and whether there is a definite correlation or not between footpad activity in the mouse and the clinical response. This does affect us tremendously in our practical application of the results of investigations of the activity of different drugs in human leprosy.

I.3. Animal Models

Dr Browne
We should say a word or two about animal models and their use in the assessment of drug activity in leprosy. We have heard a lot about the mouse footpad and

something about the armadillo, but there are other animal models, some of which should be re-examined, some of which are coming into their own for the first time.

Dr Walter
Perhaps one could make a distinction between established animal models for leprosy and those which are potential animal models. We take the mouse as an established animal model—with limitations—and the armadillo with a question mark. The range of susceptibility in the mouse is about 99 or 98%, in the armadillo between 40-60%. Other animals may finally become suitable models for leprosy, such as Dr Convit's hamster, the chipmunk in Korea and others. In this context, I would like to ask Prof. Freerksen to say a few words about the hedgehog as a laboratory animal, because it is very important to find an animal model which can easily be kept in captivity and which provides sufficient amount of *Myco. leprae.*

Prof. Freerksen
There is no doubt that we need experimental animals in chemotherapeutic research, but it is very difficult to choose the right type of experimental animal for leprosy studies. Moreover we do not possess the large quantities of animals necessary for such field experiments. A method which has proved to be very useful and even indispensable is the mouse footpad test employed by Shepard and Rees. In mice, however, there will be no generalization, and the clinical picture we obtain is not the one we call "leprosy" in human beings. This may perhaps be not quite so important. Robert Koch's claim that pathogenic bacteria must induce exactly the same disease in experimental animals as in humans is ideal, yet can hardly ever be realized. Everybody dealing with experimental animals knows that, for instance, the tuberculosis induced in mice has only a slight similarity to the one observed in human beings. The tuberculosis of the guinea-pig differs just as much from that of the rabbit. In leprosy there are no decisive criteria for the choice of experimental animals. Just as in trying to grow the bacteria on culture media, we have to rely here on the hazard selection of possible animals. There is almost no type of animal which has not been used in such trials. Here the conviction plays a part that leprosy bacteria prefer low temperatures. This was mentioned yesterday, but I should like to repeat it today believing personally that this is wrong. This opinion is based upon the fact that leprosy bacteria preferably manifest themselves in the nerves and skin layers situated at the periphery of the body. But they may also appear within the body, in the bone marrow, for instance, as was demonstrated again today by Dr Karat. The temperatures there are certainly not low. We have made the experience that leprosy bacteria multiply in the hedgehog. This animal, too, is only a model, but it is an accessible and cheap living medium suitable for animal trials on a large scale.

Dr Walter
Prof. Freerksen, can you give us some specific data concerning multiplication time, generation time, number of hedgehogs under test, etc.?

Prof. Freerksen
Up to now we have only infected 26 hedgehogs, this is why I would not like to give any further comment. The information I have given should only be seen as an initiative to the study of the hedgehog as a laboratory animal.