THE LEPROSY BACILLUS AND THE HOST REACTION TO IT*

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[We have had three days of discussion on the tubercle bacillus and there are indications that it could have gone on for three weeks or three months; the scope of work has been great and workers numerous; and much work has been done and knowledge gained though much remains to be done.

I appear as a student of the leprosy bacillus, and I feel rather like a poor relation at a large family gathering.

Our scope is limited, and our workers have been few; definite knowledge based on direct study is limited. I don't think we could spend even three days on our subject.

When I was asked to present a paper on the leprosy bacillus and the host reaction to it, I wondered whether I had enough ideas to make a paper; but, as is quite common with subjects about which we know very little, there is a great deal that may be said. And as it does appear possible that findings of studies of the tubercle bacillus may be applicable to the leprosy bacillus, and also vice versa, the subject is not perhaps without its interest, to students of tuberculosis—as well as of leprosy.

I will try to summarize what is known of my subject; but I shall not touch on questions of life, growth and metabolism of the leprosy bacillus, for these matters are, I believe, to be dealt with by others. I should, moreover, make it clear that I am not a chemist—nor a bacteriologist, but a clinician and a research worker from the clinical angle, to whom, as I expect you will detect, chemistry and much of bacteriology is a closed book. I hope that you will make a full allowance for this.]

INTRODUCTORY REMARKS.

Since we have no culture and no experimental animal, the only source of bacilli is the tissues of man with lepromatous leprosy, in which bacilli are numerous. For many years it was impossible to separate the bacilli from the tissue, but now two methods are available, those of Dharmendra (1942 and 1942a) and of Fernandez and Castro (1941).

Although Hanks (1945) recorded bacillary counts of over one thousand million per cubic centimetre of tissue, Dharmendra (1942a) found that, in such tissue, the bacilli formed about 0.4% by weight of the tissue, so that from 1 gramme of tissue not many milligrammes of bacilli can be obtained, and that by excision of lepromatous skin, of which supplies are limited. Thus, bacillary material has been scarce, and now with chemotherapy of leprosy being widely practised, it is becoming much scarcer. Some useful work has been done, however, and I will try to summarise it.

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THE SEPARATION OF THE BACILLI.

Dharmendra's method is briefly as follows:

Excised lepromatous tissue is autoclaved, cut in small pieces, and ground in a glass mortar in chloroform, the chloroform being pipetted off; this process is repeated until few bacilli remain. The chloroform is allowed to evaporate, and the fatty residue is suspended in ether, which dissolves the tissue fats, and the suspension is then centrifugalised at high speed to deposit the bacilli, and the ether is pipetted off. More ether is added and the centrifugalising is repeated, and the ether again pipetted off. The residue, consisting of bacilli only, is dried and weighed. Lepromin for routine testing is made up at 1 milligramme in 10 c.c. and 0.1 c.c. is injected intradermally as in the tuberculin test.

The method of Fernandez and Castro consists of centrifugalising ground suspensions of leprous nodules in salines of different specific gravities to separate the bacilli from the tissue. The yield of bacilli is lower than with Dharmendra's method, which is now more widely used.

It is possible that Dharmendra's method may denature the bacilli to some extent, but this has not been proved; on the whole it is much the most simple and economical method of getting leprosy bacilli free from tissue.

CHEMISTRY OF THE LEPROSY BACILLUS.

By getting large amounts of nodular material and extracting the bacilli from it, Dharmendra (1942a) got enough bacilli to work with. He ground them in a ball mill for many hours, and from the ground bacilli by simple methods he prepared the following fractions: three protein fractions, a polysaccharide fraction, glyceride and phosphatide fractions, waxes, and final residue.

This work was done about thirteen years ago by methods then available to Dharmendra, and no doubt modern methods would be better. Further, he made these fractions for use in skin testing in a study of the lepromin reaction, and not for a study of chemistry. But as far as I know, no later work of this nature has been done, and what little we know about the chemistry of the leprosy bacillus is what he found out. These fractions are obtainable from the leprosy bacillus in about the same proportion as from the tubercle bacillus, and they show a close resemblance to the fractions of the tubercle bacillus.

BIOLOGICAL REACTIONS TO THE LEPROSY BACILLUS AND ITS FRACTIONS.

From 1916, the Mitsuda test, (Mitsuda 1916), named after its Japanese originator, has been increasingly used and studied, frequently under the name " lepromin test ". Lepromin is the name given to a suspension of lepromatous tissue rich in bacilli. The classical test is positive when, at the site of the intradermic injection of 0.1 c.c. of this suspension, there develops a small nodule, usually

appearing in 1-2 weeks, reaching its maximum size at 3-4 weeks, and then slowly subsiding over a period sometimes lasting many weeks.

The result is negative in most healthy young children, and in lepromatous (anergic) cases of leprosy; it is positive in many healthy adults and in tuberculoid (allergic) cases of leprosy; in other healthy persons, and in other types of leprosy, the results are variable.

This strange phenomenon is different from any other biological skin test that I have heard of.

Fernandez (1950) reported that a positive Mitsuda test at 2-4 weeks is almost invariably preceded by a 24-48 hour reaction of the " tuberculin " type. Lowe and Dharmendra (1941) soon confirmed this, and others also, and the Fernandez phenomenon as it is called is widely recognised.

Thus we have these two phenomena produced by the intradermic injection of lepromin in sensitive persons, the Fernandez reaction at 24-48 hours, and the Mitsuda reaction at 2-4 weeks. We (Lowe and Dharmendra, 1941) found that by grinding the bacilli to break down the bacilli, the Fernandez reaction was increased and the Mitsuda reaction was diminished, and that with complete grinding of the bacilli the Fernandez reaction was increased still more, and the Mitsuda reaction was abolished. Dharmendra (1942a) studied the mechanism of these reactions by the use of the fractions which he had isolated, and he found that the Fernandez reaction was due entirely to the protein fractions. None of the fractions given alone produced the Mitsuda reaction; only the intact bacilli did this. We concluded that the early (Fernandez) reaction was caused by sensitivity to free protein, and that the late (Mitsuda) reaction was caused by the slow liberation, over several or more weeks, of minute amounts of protein from the slowly disintegrating bacilli at the site of the injection. It should be remembered that intact bacilli can be detected at the site of the injection for several weeks.

How do these findings compare with findings made with the tubercle bacillus? The finding that the 24-48 hours reaction is caused entirely by the allergic reaction to the bacillary protein is exactly paralleled in the tuberculin test, I believe. The late Mitsuda reaction has no parallel in skin testing in tuberculosis; the only parallel is in histopathology. The nodule produced in the Mitsuda test is histologically a tubercle, with quite typical epithelioid cells focally arranged, with multinucleated giant cells, and sometimes caseation.

In tuberculosis, the attempt to attribute tubercle formation to a particular chemical fraction of the tubercle bacillus has not been very successful; according to Lederer (1951), the phosphatide

fraction of Anderson, with its phthioic acid content, branched chain fatty acids, lipopolysaccharides, and other fractions have been cited by different workers. I have been much struck by Lederer's comments on this matter, quoted from Rich; he states that a single tubercle bacillus can stimulate giant cell formation; that several bacilli can cause the formation of a typical tubercle; that the intact bacilus is a minute foreign particle possessing a far greater power of evoking giant cell and tubercle formation than has been shown to be possessed by any or all of the lipids exactracted from it.

In leprosy the situation seems to be similar; the tubercle formation seen in the Mitsuda phenomenon seems to depend on the presence of intact bacterial cells, and not on the action of any one constituent. Further, it is possible that the explanation of the Mitsuda reaction advanced by myself and Dharmendra, and quoted above, is wrong. Fernandez (1953) reports that in a lepromin positive person, one can, by repeated injections of the leprosy bacillus protein, desensitize to the protein and abolish the early reaction to lepromin yet leave the late reaction (the Mitsuda phenomenon with nodule formation) unchanged.

Fernandez has abandoned his earlier view that the early and late reactions to lepromin are of the same significance. He regards the early reaction as indicating sensitivity to protein, and the late reaction as indicating resistance to the leprosy bacillus, these ideas of course having parallels in tuberculosis. These views have much to commend them.

There is little more that I can say about direct observations of the leprosy bacillus and its components, and of cellular activity induced by them. I would here mention and emphasize one most striking feature of leprosy, the complete cellular inactivity to the enormous numbers of leprosy bacilli seen in the typical lepromatous case; this I believe has no real parallel in tuberculosis unless it be in the tuberculin negativity of acute miliary tuberculosis, or of advanced generalised tuberculosis.

THE USE OF ANTIGENS PREPARED FROM THE TUBERCLE BACILLUS FOR DETECTING ANTIBODIES IN LEPROSY.

For over forty years, workers have tried various preparations of the tubercle bacillus as antigens for the detection of antibodies in cases of leprosy.

(a) Complement fixation tests.

Numerous such preparations have been used in complement-fixation tests. The matter was reviewed by Lowe and Greval (1939). They used the preparation of Witebsky, Klingenstein, and Kuhn (1931), (WKK antigent), which was prepared by taking

up in benzine the residue left after extracting tubercle bacilli with alcohol, pyridin, and acetone. It was originally used for complement-fixation tests in tuberculosis, with inconstant results. When used in leprosy it gave strongly positive results in practically every bacteriologically-positive case, and weaker positive results in some of the others.

Complement fixation tests are notoriously non-specific. There is the Wasserman test itself, which, as well as the Kahn and other such tests, is usually positive in severe lepromatous leprosy. The WKK antigen gave positive results not only in tuberculosis and leprosy but also sometimes in syphilis, and always in kala-azar, in the early diagnosis of which the test is very useful. Further, the same fraction of some non-pathogenic acid-fast bacilli gives identical results (Dharmendra; personal communication). In general, in cases of leprosy, if this test is negative, ordinary bacteriological examination gives negative results, and also the lepromin test is positive; if the complement-fixation test is positive, ordinary bacteriological examination, which is much easier, shows leprosy bacilli, and the lepromin test is negative. The test is therefore of little practical value. It is of theoretical interest in that it shows that in lepromatous leprosy there is in the serum something which acts as an antibody to a fraction common to mycobacteria.

(*b*) *Precipitin tests.*

Certain polysaccharide fractions of the tubercle bacillus have given positive precipitin tests, sometimes with the sera from persons with tuberculosis, and often in very high dilution with anti-tuberculous sera of animals. (Siebert, Stacey, and Kent, 1949; Iland, 1951; Haworth, Kent and Stacey, 1948 and 1948a; Aselineau and Lederer, 1950; Choucroun, 1949). With the same fraction, Choucroun obtained positive results in lepromatous leprosy. This matter appears to have been little studied.

(*c*) *Agglutination tests.*

The heat-stable component present in the polysaccharide fraction of the tubercle bacillus, isolated by Middlebrook and Dubos (1949) and used by them to sensitize sheep's red cells to agglutinins present in tuberculous sera, has, with the modification of Scott and Smith (1950) been used in studies of sera of leprosy cases, mainly by French workers, with striking results, [Gernez-Rieux and Tacquet (1950); Gernez-Rieux, Montestruc and Tacquet (1951 and 1952); Montestruc (1952); Montestruc, Gernez-Rieux and Tacquet (1953); Floch and Sohier (1950); Levine (1950 and 1951)]. Over 80% of lepromatous cases have given positive results, usually in far higher titre than in tuberculosis; the titre may be as

high as 1 in 2000. In non-lepromatous cases the positives are fewer and the titres lower.

Basset and Bougna (1953) have used the lipo-polysaccharide fraction of Choucroun (1946 and 1947) in the same way, with similar results.

Thus, here again is evidence that in lepromatous leprosy there are circulating antibodies to a component of the tubercle bacillus. But here again, if there is a positive agglutination test, there is usually a negative lepromin test.

(*d*) *The tuberculin test.*

The tuberculin test in leprosy, with either old tuberculin or purified protein derivative, has been much studied in leprosy, both in lepromatous (lepromin-negative) cases and in tuberculoid (lepromin-positive) cases. The matter has recently been reviewed by Wade (1950) and by Lowe and McNulty (1953). The tuberculin test is little influenced by the presence or absence of leprosy, or by the form of leprosy; the findings are about the same as in healthy people in the same environment.

LEPROSY BACILLUS ANTIGENS IN TUBERCULOSIS.

Many workers in different countries, including countries in which there is practically no leprosy, have done lepromin tests in persons suffering from tuberculosis, frequently with positive results. Moreover, when lepromin and tuberculin tests are done in healthy persons in such countries, the two tests agree too often for the agreement to be caused by chance. There is strong evidence that tuberculous infection can make a person lepromin positive. Moreover B.C.G. vaccination has the same effect; it can and usually does produce lepromin conversion. This whole matter has recently been reviewed and studied by Lowe and McNulty (1953 and 1953a).

These facts mean that in a person who is tuberculin positive, the tissues can react to the leprosy bacillus by tubercle formation (for that is what a positive late lepromin reaction means) and this is a more definite indication of immunity than mere protein sensitivity. This finding, backed up by clinical observations that lepromin-positive persons rarely develop leprosy, and that if they do, it is usually mild, provides the basis for the advocacy, particularly by French and South American workers, of the use of B.C.G. to immunize contacts or potential contacts of open cases of leprosy,

There is thus some evidence that tuberculous infection and B.C.G. vaccination can sensitize a person to, and possibly produce a degree of immunity to, the leprosy bacillus. (It may here be interpolated that the evidence that leprous infection can produce

sensitization and immunity to tuberculous infection is scanty and not strong.)

DISCUSSION AND CONCLUSIONS.

I want first to stress the point that most cases of leprosy belong to either of two quite markedly contrasting types, and that changes from one type to the other are rare, or, in the opinion of some workers, impossible. (This generalization is not invalidated by the fact that there are certain cases that do not fit well into either of the two main types, and that in these atypical cases marked variations may be seen.) The differences between these two main types are striking, and are summarised later, but they seem to indicate sensitization and immunity on the one hand, and complete lack of these on the other.

Is such a phenomenon seen in tuberculosis? I do not think so. In most cases of tuberculosis you have an interplay of findings, some indicating the invasive powers of the infection, and some indicating sensitization and resistance of the host tissues.

In leprosy it is usually quite different.

On the one hand there is the lepromatous case, with extensive lesions and abundant bacilli but no cellular reaction to them, with circulating antibodies easily demonstrated, but no sensitization and no cellular antibodies revealed by the lepromin test, and no resistance to the infection.

On the other hand there is the tuberculoid case, with only local lesions, which contain very few bacilli, but which show intense cellular reaction to them in the form of tubercle formation; circulating antibodies are difficult to demonstrate, but the lepromin test is strongly positive, and there is apparently a high degree of sensitization and immunity to the infection.

There is this curious dichotomy in the manifestations of leprous infection. How can it be explained?

It cannot be that in lepromatous cases the bacilli are not antigenic, for it is from these cases that we get our lepromin. A few workers have thought that in lepromatous cases there is an inherent constitutional factor preventing any effective host response to leprous infection. This theory might explain some facts but not others.

One could surmise that with the marked infiltration of the reticulo-endothelial system which is characteristic of lepromatous leprosy, the normal production of protective antibodies by this system might be upset; such findings have been recorded in other affections of this system, for example in Hodgkins disease and in sarcoidosis, in which tuberculin sensitivity may be suppressed

(Hoyle, Dewson and Mather, 1954). But in lepromatous leprosy, there is little or no suppression of tuberculin sensitivity or of other forms of sensitivity or immunity, although lepromin sensitivity is completely absent. No, the anergy, insensitivity, lack of effective response, or whatever name one likes to give to this strange phenomenon, is specific for the leprous bacillus and its antigens. It might be that in lepromatous leprosy, the protective antibodies to the bacillus are produced, but that they are blocked or inactivated in some way, probably by some mechanism associated with the infection. An attempt to study this idea seems worth while.

The other apparent anomaly in leprosy cases, the frequent absence of, or the low titre of, the circulating antibodies in tuberculoid leprosy in which immunity is high, presents no great difficulty. It is apparently associated with the low level of infection. In such cases, temporary phases of increased activity of the disease, with an increase of the number of bacilli in the lesions, are sometimes seen, and, during these phases, circulating antibodies as shown by complement-fixation tests and Middlebrook-Dubos tests increase.

This dichotomy of leprosy, as I have called it, is a most interesting and baffling phenomenon, which appears to have no real parallel in tuberculosis. It does seem to me that a study of this matter might illuminate the question of immunity in leprosy and possibly in other mycobacterial diseases.

There is some factor or group of factors operating in the tuberculoid case which is absent or inactivated in the lepromatous case, and this absence renders the patient susceptible and the disease progressive.

Observations of the host reaction to the leprosy bacillus as seen in the two main types of leprosy suggest the following points:

1. Resistance to leprous infection bears no relation to the presence of circulating antibodies to polysaccharide or lipid fractions of mycobacteria.
2. Resistance to infection is accompanied by evidence of sensitization of the host cells to the leprosy bacillus as a whole, and to its protein components.
3. Protein desensitization may be effected without impairing cellular response to the whole bacillus.
4. Thus cellular response to the whole bacillus appears to be the main factor in immunity.
5. Sensitization of the tissues to whole bacilli can be induced only by whole bacilli, living or possibly killed, and not by any component of the bacilli.

6. The tubercle bacillus as well as the leprosy bacillus can induce this sensitization of the cells to the bacilli, which accompanies resistance to the infection and which appears to constitute the main factor in resistance to leprosy.

Have these ideas any bearing on the question of resistance to tuberculosis? I leave that question to the tuberculosis worker.

SOME CLOSING REMARKS.

We students of leprosy can learn much from students of tuberculosis. For example, our chemotherapy of leprosy is built up on studies of possible treatments for tuberculosis, and in fact, on agents, mainly the sulphones, which tuberculosis workers seem to have discarded.

I wonder if I might suggest that tuberculosis workers might gain from, as well as contribute to, a study of leprosy; that a study of mycobacteria and of mycobacterial disease as a whole is worth while; and that we should all do well to broaden our horizons.

I spoke to begin with, of leprosy research being a poor relation of tuberculosis research, and in some ways we do seem to benefit from the crumbs which fall from the rich man's table.

But I think that I should express the relationship much more truly if I said that tuberculosis research is the benevolent rich uncle to whom leprosy research is very grateful, for all he has done, and, we hope, will do for us; and that we hope that the day will soon come when we shall be able to do something for him.

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