Ocular histopathology in animals experimentally infected with *Mycobacterium leprae* and *M. lepraemurium*

1. *Mycobacterium leprae* and *M. lepraemurium* infections in the mouse

H. E. HOBBS, D. J. HARMAN, R. J. W. REES, AND A. C. McDougall

2. *Mycobacterium leprae* infections in the 9-banded armadillo (*Dasypus novemcinctus L.*)

A. C. McDougall AND R. J. W. Rees

From the Leprosy Study Centre, London, the National Institute for Medical Research, London, and the Slade Hospital, Headington, Oxford

SUMMARY At varying periods of time following the successful establishment of systemic infections with *Mycobacterium leprae* or *M. lepraemurium* in the mouse and the nine-banded armadillo eyes were examined by light microscopy. Inoculation of bacilli was by the intravenous or intraperitoneal route or directly into the hind footpads; eyes were not directly inoculated in this study. During periods of up to 3 years under laboratory conditions no animal showed evidence of impaired vision or blindness, and the external appearance of both eyes was normal. The ocular histopathology and the sites of accumulation of bacilli are described. In immunologically normal mice infected with *M. lepraemurium* bacilli were much commoner in extraorbital tissues, but they were, nevertheless, found in various tissues within the orbit, including the ciliary body and sclera. In immunologically normal mice (and one rat) injected with *M. leprae* of human origin no bacilli were found in the eye, but in mice immunologically depressed by thymectomy and total body irradiation considerable numbers of bacilli were present in the iris and ciliary body and also in the limbal cornea. In the armadillo bacilli were found in large numbers in virtually all tissues except the lens, retina, optic nerve, and aqueous and vitreous humours, but the uveal tract was heavily involved.

Findings are discussed in relation to the great frequency of ocular involvement and the importance of immune-complex disease in patients with lepromatous leprosy, and to factors which may favour the localisation and multiplication of *Mycobacterium leprae* in the eye.

In lepromatous leprosy it is now well established (Drutz et al., 1972; Shankara Manja et al., 1972) that there is a continuous bacteraemia, and it is common knowledge to the histopathologist familiar with this form of the disease that bacilli can be found in a wide variety of host cells and in many different tissues. It is therefore not surprising that the eye is affected, but it is perhaps not generally recognised that there is in leprosy a 'higher percentage of ocular involvement than in any other systemic infection' (de Barros, 1946). In a large proportion of cases of lepromatous leprosy—and more often in Asians and Chinese than in Africans—this may lead to blindness, which, for reasons still unknown, arises almost invariably from disease in the anterior segment of the eye—the cornea, sclera, iris, and ciliary body. Involvement of the optic nerve, choroid, or retina is so rare as to be of academic interest. Many authorities consider that the

Address for reprints: Dr A. C. McDougall, Slade Hospital, Headington, Oxford OX3 7JH
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occasional reports of leprosy in the posterior parts of the eye may have been due to syphilis, tuberculosis, or ophthalmia.

Although Armauer Hansen established *Mycobacterium leprae* as the cause of leprosy in man in 1873, it is still impossible to grow this organism *in vitro*. This has given rise to the development of various experimental animal models, the most important being that devised by Shepard (1960), in which he demonstrated a localised growth in the footpads of normal mice. Enhanced growth and a generalised infection were later achieved in thymectomised and irradiated mice (Rees, 1966) and in thymectomised rats (Fieldsteel and McIntosh, 1971). The concept that low body temperature might be conducive to the growth of the leprosy bacillus in the experimental animal led to the discovery (Kirchheimer and Storr, 1971) that the nine-banded armadillo (*Dasypus novemcinctus L.*.) is highly susceptible to infection with the leprosy bacillus and capable of producing quantities of bacilli in the tissues in the order of 10^9 to 10^12/g, yielding up to about 3-5 g from 1 animal.

In this paper we report ocular findings in (1) immunologically depressed (thymectomised-irradiated) mice following infection with *Mycobacterium leprae* of human origin, (2) immunologically normal mice infected with *M. lepraemurium*, and (3) the immunologically normal armadillo infected with *M. leprae* of human origin, and we discuss them in relation to the frequent and often grave involvement of the eye in lepromatous leprosy in man.

1. *Mycobacterium leprae* and *M. lepraemurium* infections in normal and immunologically suppressed rodents

**Infections with M. leprae**

**MATERIALS AND METHODS**

The experimental animals prepared by one of us (R.J.W.R.) consisted of 25 mice (20 CBA and 5 Parkes albino) and 1 albino rat. Inoculation of *M. leprae* in differing dosages was intraperitoneal, intravenous, or subcutaneous in both footpads, as shown in Table 1. The mice were killed at intervals of 64 to 141 weeks after inoculation and the rat after 22 weeks. Fifteen of the mice had been subjected to thymectomy (T) and total body irradiation (900r) by methods already described (Rees, 1966). The remainder were immunologically normal and served as controls. The 1 rat was also immunologically normal. After the animals were killed both eyes were enucleated, together with a few adnexae, and fixed in formol-Zenker, with transfer to 70% alcohol 15 to 24 hours later. They were blocked in paraffin wax, cut at 5 μm, and stained with a trichome and Fite-Faraco modification of Ziehl-Neelsen—TRIFF (Wheeler et al., 1965)—thus showing tissues, infiltrating cells, and bacilli simultaneously. Approximately 20 sections of both eyes from each animal were examined by two of us (H.E.H. and D.J.H.) independently.

**RESULTS**

In the majority of infected eyes organisms were confined to the ciliary body and iris root. *Light and moderate* infections were characterised by mainly intracellular organisms lying within the heavily pigmented cells of the iris and ciliary body (Figs. 1 and 2). In the heaviest infections they were confined to the cells forming the walls of the juxtascleral blood vessels, which, in the absence of muscle from the rodent ciliary body, are a very prominent feature of this tissue. In *heavily infected* eyes bacilli were also seen to involve the limbal cornea from the ciliary body or to extend slightly posteriorly into the choroid. Most organisms were in the cytoplasm of macrophages, but extracellular organisms were profuse. In some eyes involvement was confined to one side, a condition comparable to the limbal ‘nodule’ of human leprosy; in others, both sides were

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Inoculation of mice (and 1 rat) with Mycobacterium leprae of human origin</th>
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</thead>
<tbody>
<tr>
<td>Group</td>
<td>Number of animals</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3*</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
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<tr>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
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<tr>
<td>9*</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Total 26</td>
<td></td>
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</tbody>
</table>

1-P: intraperitoneal; I-V: intravenous; FP: both footpads; *: each of these groups included 1 Parkes-albino mouse.
uniformly involved. Bacilli were not found in the optic nerve, lens, retina, or aqueous or vitreous humours.

These observations, recorded in Table 2, are shown in relation to the immunological state of the animals, where it can be seen that bacilli were found only in those animals immunologically depressed. They were totally absent from the immunologically normal mice and from the rat.

In this study we did not use special techniques for the examination of immune complexes or lymphocytes, but no tissue showed any disturbance of structure, and there was no evidence of vasculitis or of infiltration by polymorph neutrophils, eosinophils, plasma cells, or lymphocytes. In other words no indication was found of either a cell-mediated immune response or the changes which are known to occur in the immune-complex syndrome in human lepromatous leprosy, of which erythema nodosum leprosum (ENL) may be a feature. A high percentage of free-standing bacilli were solid-staining along their entire length on Fite-Faraco staining (i.e., not fragmented or granular), and thus presumably viable (Rees and Valentine, 1962).

Infections with *M. lepraemurium*

MATERIALS AND METHODS

Twenty immunologically normal albino mice were inoculated intravenously with 10⁹ *M. lepraemurium*...
Table 2  Intensity of infection in the eyes of mice (and 1 rat) following inoculation by various routes with Mycobacterium leprae of human origin

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunological state</th>
<th>Light infection</th>
<th>Moderate infection</th>
<th>Heavy infection</th>
<th>Uninfected</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T/900r</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>T/900r</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>T/900r</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>T/900r</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>T/900r</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<td>9</td>
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<td>11</td>
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<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>17 (26)</td>
</tr>
</tbody>
</table>

(R.J.W.R., National Institute for Medical Research) and killed at intervals of 16 to 20 weeks. All animals showed the usual gross involvement of liver and spleen, and before their death blood from the tail vein showed a bacteremia of about $10^7$ organisms/ml of blood. This figure is some 100-fold higher than that recorded in the peripheral venous blood of patients with untreated lepromatous leprosy. Both eyes were enucleated, processed, cut, and stained as described above.

RESULTS

Of the 20 mice in this series all but 1 were positive for bacilli. In 15 of them associated structures such as eyelid were included, and bacillary accumulations were much more marked in this area than within the eye itself (Fig. 5). In 4 mice the optic nerve was well shown and found to be completely free of bacilli, though they lay in great numbers mainly in near-by macrophages (Fig. 6). Branches of the ciliary nerves were also negative for bacilli and none was found in endothelial lining cells of blood or lymphatic vessels in any of 210 sections examined. Within the globe bacilli were most consistently seen in the limbal area, lying superficial to or within the substance of the sclera or, frequently, at the base of the ciliary body. In only 1 animal were they seen in the ciliary processes; in 2 they extended posteriorly into the choroid; but in none were they seen in the cornea. They were not present in the aqueous or vitreous humours, lens, or retina. No cataract was seen. Apart from macrophages other types of infiltrating cell were conspicuous by their absence. As in the experiment above, large numbers of bacilli were solid-staining in these sections.

CLINICAL FINDINGS

Detailed slit-lamp microscope examination of these mice infected with either M. leprae or M. leprae muri-um was not attempted, but their external eye appearances in life were entirely normal, and observations of their cage movements at dusk, during the night, and by day gave no indication of impaired vision or blindness. Our experience on several thousand similarly infected rodents under the same conditions gave no indication of significantly impaired vision, blindness, or disease of the anterior eye with the obvious exception of those damaged or lost in fighting. Furthermore, the histopathological examination of the eyes in this series disclosed nothing which might have produced impaired vision, still less blindness.

2. Mycobacterium leprae infection in the 9-banded armadillo (Dasypus novemcinctus L.)

MATERIALS AND METHODS

A colony of 19 armadillos was established in the United Kingdom late in 1974, and in December each was inoculated (R.J.W.R.) with pooled M. leprae of human origin (Ethiopia and Malaysia), $10^6$ intravenously and $8.9 \times 10^6$ subcutaneously into the right ear. During the next 7 months there were 3 deaths due to incidental infection or failure to adapt to feeding in captivity, leaving a total of 16, of which 9 ($56\%$) showed gross infection within 11 months of inoculation. The full experimental and histopathological data from these animals are to be published separately, and for the purposes of this report we have selected 1 animal (No. 19) in which 'lepromatous' changes were particularly marked in all organs examined, including the eyes. Enucleation, processing, and staining were as described above for the mice. Professor Norman Ashton (Institute of Ophthalmology, London) very kindly reported independently on these sections before and after bleaching, and the main findings were as follows.

Bacilli in varying numbers were found in every ocular tissue with the notable exception of the lens, retina, aqueous, and vitreous humours. They were most numerous in the iris, ciliary body, and choroid, but also present in considerable numbers in the cornea (Fig. 3) and corneoscleral junction, in the eyelids and in the palpebral and bulbar conjunctivae (Fig. 4). They were found in the endoneural zones...
of small branches of external ocular nerves.

In the choroid numerous bacilli were seen both within and outside the pigmented cells; there was no special predilection for melanocytes. In the cornea bacilli were seen just below the epithelium (there being no Bowman's membrane in the armadillo) and internal to this in the substantia propria (Fig. 3) as far forward as the apex, to which point the armadillo eye is normally vascularised (Duke-Elder, 1958).

Apart from host macrophages no ocular tissues showed any evidence of cellular infiltration (such as to suggest a cell-mediated immune response), nor was there any evidence of vasculitis or neutrophil polymorph infiltration such as might be seen in erythema nodosum leprosum. The picture on light

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**Fig. 5** M. lepraemurium in the mouse. Anterior to the tarsal plate (TP) there is a large mass of bacilli (arrowed) in the substance of the eyelid. TRIFF stain. (Original magnification × 250)

**Fig. 6** M. lepraemurium in the mouse. There is a dense accumulation of bacilli (arrowed, left) in the juxta-scleral tissue. They are completely absent in the optic nerve and in the endothelial lining cells of small vessels (arrowed, centre) here and in all other tissues examined. TRIFF stain. (Original magnification × 250)

**Fig. 7** M. lepraemurium in the mouse. Bacillary masses are seen in the substance of the sclera (arrowed, left) and in the cornea (arrowed, centre). R = anterior end of retina, displaced medially in cutting. TRIFF stain. (Original magnification × 250)

**Fig. 8** M. lepraemurium in the mouse. Bacilli (arrowed) lie in the deeper vascular and pigmented layers of a ciliary process. TRIFF stain. (Original magnification × 1000)
microscopy was essentially one of intense invasion by bacilli, sometimes in very large numbers, without alteration of host tissues or of cellular invasion apart from macrophages.

**Clinical Findings**

As with the mice noted above, no special ophthalmological examination was attempted in life. However, neither in this animal nor in others in the same colony, nor in a second colony established in this country in 1977, do we have any reason to suspect that bacillary invasion of the eyes causes impaired vision or night blindness,* and these clinical defects have not been described from experimental armadillo colonies of larger size in the USA.

**Discussion**

*M. lepraemurium Infections in Immunologically Normal Mice*

The eyes of these animals were examined during the course of a larger study of various tissues infected with *M. lepraemurium* to see if bacilli could be found in endothelial lining cells of blood or lymph vessels, or in nasal mucus or the mucous membrane of the nasal cavity. Our results were negative in all instances. Bacilli did not invade parenchymal cells of any type (even in very heavy infections), in striking contrast to the situation in immunologically depressed mice with a generalised infection with *M. leprae* and in the untreated lepromatous patient. *M. lepraemurium* does not affect man, but the findings are included in this paper because the organism has some features in common with *M. leprae* and has been extensively used in laboratory studies. Furthermore, although the finding of large numbers of *M. lepraemurium* in external ocular muscles and in various tissues adjacent to the eyeball was entirely in keeping with the heavy bacillary growth in other tissues, the finding of bacilli within the orbit was at variance with the observations of several authors (Uchida, 1935; Lowe, 1937; Meissner and Schmiedel, 1967) who have stated that *M. lepraemurium* is not to be found in this situation. In direct intraocular inoculation experiments, however, Guillen and Montestruc (1933) found slight lesions in the ciliary body, and growth was also observed by Hanks and Backerman (1950) in the anterior chamber of the eye, although of an order which in no way approached that of the growth in testis in the same series, and which produced the 'astronomical concentration' of 700 × 10⁶ bacilli/g of tissue. Although there is some evidence (Closs and Haugen, 1974) that certain strains of mice infected locally with *M. lepraemurium* may produce varying cell-mediated responses, including lymphocytic infiltration, this has not been our experience with intravenous inoculations in ordinary laboratory strains over a period of many years, and it is not shown in the present tissues; indeed, the total lack of infiltrating cell, apart from the macrophage, is striking.

**M. lepraemurium Infections in T/900r Mice and in the Immunologically Normal Armadillo**

The present study of *M. leprae* infections in the eyes of immunologically depressed mice arose originally from our interest in the location of bacilli in plain and striated muscle fibres, since it was thought that the ciliary body and the external ocular muscles would provide a good contrast, separated by only a few millimetres, in the same section. In practice it was found difficult to identify bacilli within plain muscle fibres in the animal ciliary body on light microscopy, but the study was given further impetus from the reading of a paper at a meeting of the Royal Society of Tropical Medicine and Hygiene in London on the transmission of human leprosy to the mouse and its clinical implications (Rees and Weddell, 1970). The ophthalmic interest centred on the importance of uveitis in the production of blindness in man and the uncertainty of the route of infection—blood stream, nerve, or direct invasion. The present findings indicate that the uveal tissues are indeed heavily affected in the experimental mouse, and suggest that blood stream spread to the similarly vascular uveal tract in man may be involved in the production of leprotic iritis.

In *M. lepraemurium* infections bacillary masses may achieve astonishing size, and in the armadillo infected with *M. leprae* bacilli are both numerous and widespread, but the concentration of bacilli in the ciliary body and iris of the T/900r mouse (Figs. 1 and 2) is perhaps one of the most remarkable for any tissue in this study. In the case of armadillos successfully infected with *M. leprae* bacilli are distributed so widely (and in tissues such as brain and lung, where they are virtually unrecorded in the human lepromatous patient), that it is clearly necessary to interpret the intraocular invasion of bacilli with care. Although we did not carry out bacillary counts on the eyes, it was clear from the microscopic appearances that even the most heavily infected intraocular tissues were certainly not more heavily infected than many others, including skin, nerve, spleen, and lymph node. However, both in the armadillo and the T/900r mouse 3 observations

*The armadillo retina consists entirely of rods, and these impressions are based on observations at dusk and by night.
are of particular significance: (1) Appreciable numbers of bacilli are seen in the anterior parts of the eyes, especially iris, ciliary body, and to a lesser extent in the cornea; (2) although in smaller numbers, bacilli were found in the choroid, a site in which they have never been found in human eyes (Choyce, 1959); and (3) in neither model did we observe infiltrating cells, vascular changes, or alteration of tissue structure such as to suggest immunological disturbances of either cell-mediated or humoral type.

Some of the earlier publications on clinical leprosy in the human eye (Bull and Hansen, 1873; Jeanselme and Morax, 1898) were remarkably comprehensive, and it was in fact not until a good many years after the evolution of the slit-lamp microscope in about 1911 that important advances were made. Valuable clinical or clinicopathological observations include those of Fuchs (1937), Viallefont and Fuentes (1938), Shionuma (1938), de Barros (1938, 1940, 1946), Valle (1946), Harley (1946), Elliott (1951), Lowe (1954), Kirwan (1955), Choyce (1959, 1964), Allen and Byers (1960), and Allen (1966). On the blinding lesions of leprosy and involvement of the uveal tract, clinical and therapeutic aspects have been described by Choyce (1955), Hobbs (1963, 1972), and Hobbs and Choyce (1971). Lepromatous changes in the iris as seen on electron microscopy have been published by Hashizume and Shionuma (1965) and these authors noted the presence of *M. leprae* within smooth muscle cells. Most of these observers draw attention to the extraordinarily quiet state of the lepromatous eye unless it is disturbed during the course of an immune-complex reaction, in which case a severe plastic iritis of disastrous severity may occur. Apart from this, involvement of the eye, particularly the iris and cornea, may completely escape the attention of both patient (owing to lack of pain) and physician—a situation which is in marked contrast to the early symptomatology of, for instance, tuberculosis in the eye. This remarkably low level of response to such enormous numbers of bacilli has been considered doubly unfortunate, since it not only allows the disease to advance without recognition, but also deprives the physician of a valuable microcosm of the disease in the skin and mucous membranes (Muir, 1948). The factors which change this quiet unresponsive situation into one of adverse reaction in the patient are still far from clear. It has generally been thought that lepromatous immune-complex reaction, which may include erythema nodosum leprosum of the skin, usually occurs some months or a year or so after the start of antileprosy drug treatment, when increasing quantities of bacillary antigen may be released into the circulation and tissues. However, this is by no means invariable, and many cases have now been reported with ENL as the first manifestation of the disease (Rea and Levan, 1975). The findings in the animals in this series may correspond with those in the untreated and totally unreactive lepromatous patient, though histopathological data from such patients are virtually undocumented.

Although some authorities have maintained that infection of the eye in leprosy occurs through direct nerve spread, a review of the literature shows that there may be some confusion on the type of leprosy (i.e., lepromatous, borderline, tuberculoid, etc.) which is being described. The intraocular localisation of bacilli in our animals suggest that, in the case of the uveal tract and the limbus, bacilli have almost certainly arrived by the blood stream. However, in the case of the cornea, although it is vascularised to the apex in the armadillo, the presence of bacilli in the substantia propria in considerable numbers is difficult to explain. On the light microscopic appearances alone they could have either gained access by direct neural infection (and thus into the abundant free nerve endings of the cornea) or emerged from limbal vessels in association with the ‘wandering’ lymphoid cells which are normally present in the corneal stroma.

The presence of large numbers of bacilli in crucially important tissues and the possibility that antileprosy drugs may in some cases precipitate immune-complex reactions raise a problem for the clinician in treating patients with lepromatous leprosy. This is further complicated by the fact that some authorities hold that immune-complex reaction (which may include ENL on the skin) occurs in eyes which are ‘clinically unaffected by leprosy’ (Choyce, 1959), and that circulating immune complexes may be deposited in the eye (particularly the iris and ciliary body) even when there are no bacilli present in these tissues (Bryceson and Pfaltzgraf, 1973).

Apart from the fact that the eye is heavily invaded in lepromatous leprosy the curious predilection for the tissues of the anterior third has frequently been observed but never explained. It is certainly confirmed in the animals of this study infected with *M. leprae*, and, in view of the findings from one research group (Prabhakaran and Kircheimer, 1966) on the importance of DOPA in the metabolism of the leprosy bacillus, it may not be without interest that in the armadillo so many bacilli were seen within melanocytes of the uveal tract. The anterior third of the rabbit eye, on examination with thermistor probes, has been shown (Schwarty, 1965), to be cool and the factor of low temperature has been considered important to the growth of *M. leprae* in the mouse footpad model (Shepard, 1965).
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and in accounting for the distribution of lesions in patients with leprosy, in whom the cooler tissues, such as skin, superficially placed peripheral nerves, testes, and anterior eyes, are mainly affected. It was in fact the apparent importance of low temperature which led to the use of the armadillo (Kirchheimer and Storrs, 1971) as an experimental animal, on the basis of its body temperature (30 to 35°C). But whether the extraordinary success of this animal is concerned with the temperature optimum of M. leprae or with other factors such as depression of lymphocyte transformation by cool temperature (Purtillo et al., 1974) is still not clear. Present evidence suggests that the pattern of tissue localisation of the leprosy bacillus—not only in the eye but also in other organs—will have to take account of numerous factors, which may include DOPA, temperature, pH, oxygen and carbon dioxide tensions, hyaluronic acid, and the microanatomy and density of nerves and vessels.

For obvious reasons biopsies of ocular tissues from leprosy patients at any stage of their disease are rare, and it is even less common for the histopathologist to be presented with an eye from a lepromatous patient who is either untreated, or in the early stages of treatment, or in immune-complex reaction. Animals, particularly those which are highly susceptible, may therefore be of continuing value. Among other studies with direct application to leprosy in man it should now be possible to follow the clinical progress of leprosy in the eye of the experimentally infected animal by slit-lamp microscopy and other procedures, and both to produce and to suppress cell-mediated and immune-complex reactions. We may thus learn more, not only about the mode of action of antileprosy drugs and immunosuppressants, but also about the element which probably matters at least as much as the presence of bacilli in the eye of the lepromatous patient, namely, the immune response.

We are indebted to Dr S. G. Browne, director of the Leprosy Study Centre in London, where the mouse tissues were processed and interpreted, and to Professor Norman Ashton, at the Institute of Ophthalmology, who kindly reported independently on the findings in the armadillo eye. A. C. McDougall is supported by the British Leprosy Relief Association (LEPRA).

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