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Letters to the Editor

On the Mode of Transmission of *Mycobacterium leprae*

I was most interested to read Dr Leiker's article on this important subject (*Leprosy Review* **48** Number 1, March 1977, 9–16) and its publication prompts me to ask if you could find space for me to record a request concerning nipple biopsies in untreated lepromatous patients who are actively lactating?

It is perhaps a printing or typing error, but on page 10 of the above article, Dr Leiker refers to the presence of large numbers of bacilli in the milk glands of lactating lepromatous mothers, but in fact the article, as correctly referenced at the top of p. 10, referred to a *non*-lactating patient.

This route of transmission is obviously difficult to investigate, particularly as regards entry of bacilli into the susceptible child. However the exit of bacilli from the lactating breast and nipple is worth further study, as it has proved to be with the nose, and the purpose of this letter is to record my interest in receiving such biopsies from any leprologist who may have the opportunity to obtain them.

I fully realise that it would be unethical to attempt such a biopsy in an actively lactating mother with a living child. If however, there is a neonatal death, or for some other reason, breast feeding is to be stopped, I would be grateful for the opportunity to study the histopathology of tissues from this site. Formol-Zenker, with subsequent transfer to 70% alcohol, as described in previous articles in the *Leprosy Review,* would be the ideal fixative.

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The Hypothesis of Skin to Skin Transmission

May I be allowed to make some comments on Dr Leiker's paper entitled: "On the mode of transmission of *M. leprae*" (*Leprosy Review* (1977) **48**, 9–16). In the paragraph headed: "Transmission via the skin", he says:

> ". . . the number of bacilli reaching the surface of the skin is . . . . sufficiently high for the transmission of *M. leprae*".

I do not know if Dr Leiker has actually attempted to *count* the number of bacilli reaching the surface of the skin, as I reported so doing, in the paper (Pedley, 1970*b*) to which he refers. My purpose in making a prolonged search of the skin surface of lepromatous patients was primarily to challenge the age-old hypothesis accepted by generations of leprosy workers. The hypothesis was this: that *M. leprae* emerged on to the surface of the skin in "great", "large",

"enormous", "innumerable" numbers (to quote some of the adjectives used in the literature). From the above quotation, Dr Leiker appears to have much the same idea. His reasons for it are given as follows:

(1) The probability that what looks like intact skin is "seldom unbroken, minor scratches . . . usually being present."
(2) The presence of *M. leprae* found in the epidermis at various levels.
(3) The presence of *M. leprae* in sweat-ducts.

When I reported my search, in the paper to which Dr Leiker refers, I, too, was aware of these possible sites of discharge of *M. leprae*; also of another which Dr Leiker does not mention, namely hair-follicles. Using a method which I called the Composite Skin Contact Smear (CSCS) method (Pedley, 1970*a*), in which every field searched was actually 10 fields, I examined ONE MILLION consecutive microscopic fields of very infiltrated and highly bacilliferous intact (to the naked eye) skin of 28 untreated advanced lepromatous cases. This search was a long and tedious one. It took 70 h of microscope work—spread over a period of 14 months. I would emphasize that in order to insure a uniform standard of technique and work on which I could rely, the preparation, staining, and examination of the slides was done entirely by myself. I claim that by the CSCS method, a scientific attempt was made to collect bacilli from the orifices of COUNTLESS sweat-gland ducts, hair-follicle openings, and MINOR SKIN ABRASIONS (not easily visible to the naked eye). In this extensive search I found only 52 acid-fast bacilli. As the presence of these 52 AFB was associated with nose-blows, heavily infected with *M. leprae,* and were found on skin READILY ACCESSIBLE TO THE FINGERS, I concluded that, in all likelihood, they had been transferred from the nose to the skin. That so few bacilli were found, appeared to me to be clear evidence that *M. leprae* are seldom, if ever, discharged from (what appears to be) intact skin.

In another part of his paper, Dr Leiker writes: "Pedley (1970) has shown that the number of bacilli present on the surface of the skin of highly bacilliferous patients is *relatively low* as compared with the large numbers of bacilli released by the nasal mucosa" (underlining—J.C.P's). This is *not* so. Actually what I did show was this: the number of bacilli reaching the surface of the skin of highly bacilliferous patients is practically NIL as compared with the large numbers discharged from the nose.

Thus, I am unable to agree with Dr Leiker when he says: "The transmission of *M. leprae* via the skin remains a definite possibility". My findings lead me to believe that the transmission of leprosy by skin to skin contact is unlikely to occur.

I would also like to comment on Dr Leiker's concluding remark to his paper, which reads:

> "Until such time . . . . that conclusive evidence is found that leprosy can be spread by droplet infection from one person to another . . . . great caution is needed in publicizing this hypothesis".

I submit, however, that the following findings do provide strong evidence for this hypothesis:

(1) Millions of bacilli can be discharged even in ONE nose-blow of an untreated advanced lepromatous case.
(2) *M. leprae* can remain viable for days outside the human body (Davey and Rees, 1974).

(3) The presence of *M. leprae* in *multitudes* of droplets discharged by advanced untreated lepromatous patients was observed by Schaffer (1898), and confirmed (in sneezing) and photomicrographed by Pedley and Geater (1976).

In my view, these findings do make the conclusion stated negatively in the Summary of Dr Leiker's paper: ". . . that droplet infection via the respiratory tract is not a common mode of transmission", a highly debatable point. Nevertheless, I am still of the opinion that: ". . . the present trend of abandoning segregation and other restrictive measures against leprosy patients should not be reversed".

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Schaffer (1898). On the spread of leprosy bacilli from the upper parts of the respiratory tract. *Arch. Derm. Syph. XLIV,* 159. Reprints in English available from the Leprosy Study Centre, London. Also extensive quotations given in the next reference:

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Clofazamine From Broken Bottles

I have been interested to read the reports of gastro-intestinal side-effects of clofazamine. In my experience this is a real problem, but also rare. It is interesting to note also, that symptoms may be intermittent even on a constant dose of the drug.

I have wondered how much these symptoms can be related to the ingestion of capsules from broken bottles. Sometimes the jars are broken in transit, the glass becomes powdered and firmly adherent to the capsules, hardly sparing 1 in 1000. I am grateful to Dr M. F. R. Waters who has described to me a method of cleaning the capsules by washing them in spirit, and then cleaning each individually with a spirit soaked swab. Unfortunately this is a very time consuming process demanding the ultimate in patience and good eyesight to do the job properly.

I am concerned that some centres are probably using the capsules from broken bottles without taking proper care to see they are properly cleaned of glass pieces. In these circumstances the risk of serious gastro-intestinal side-effects are significantly increased. I would appeal to all leprosy workers to be very careful in the use of capsules from broken bottles, and to the drug company and agencies

supplying clofazamine to please pack the capsules in unbreakable containers and thus eliminate this risk.

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Evidence for the Occurrence of Tissue Inhibitors of *o*-Diphenoloxidase in *Mycobacterium leprae* Obtained From Infected Armadillos

We routinely test *Mycobacterium leprae* separated from armadillo tissues for *o*-diphenoloxidase, using D-dopa as substrate. The bacilli convert D-dopa to pigment; and when the bacterial preparations are heated. the activity is lost, indicating that dopa oxidation by the organisms is an enzymatic process. Except for *Mycobacterium leprae* (obtained from different sources), no other mycobacteria have so far been found to possess this metabolic property. Of course, it is well-known that dopa undergoes auto-oxidation under alkaline pH, and that metal ions stimulate the conversion of dopa to pigment. However, this is not the same as the enzymatic oxidation of the substrate by *o*-diphenoloxidase.

Recently, some preparations of *Mycobacterium leprae* from armadillo tissues showed extremely low levels of the enzyme or no activity at all. We diluted the bacterial suspensions and compared the oxidation of dopa by the concentrated and the diluted preparations. All the diluted samples oxidized dopa, and surprisingly, these samples had higher activity than the concentrated suspensions. This observation suggests that the *Mycobacterium leprae* preparations contain inhibitors of *o*-diphenoloxidase, derived from the armadillo tissues.

The bacilli were separated from the infected tissues by differential and density-gradient centrifugation in solutions of sucrose and KC1. For routine testing of *o*-diphenoloxidase, these preparations are used without further purification, because mammalian tyrosinase does not act on D-dopa. (Greater purification is achieved by treating the bacterial suspensions with trypsin, NaOH, acetone and ether.) In the experiments reported here, the bacilli were incubated with D-dopa at pH 6.8 for 60 min. The reaction mixture was centrifuged at 25 000 g for 30 min and the spectrum of the supernatant fraction was determined. After centrifugation, the bacterial residue also was examined. A good proportion of the pigment formed from dopa sediments with the bacteria.

If the reaction is positive, the sediment of the sample containing bacilli and dopa would be black; bacilli alone or heated bacilli plus dopa would show little colour development. When the bacterial preparations have low enzyme activity, the spectrum of the supernatant fraction reveals no well-defined peak, since sufficient amounts of quinones do not accumulate in the reaction mixture. In the data presented in Table 1, absorbance values for 400 nm or 480 nm are given, as an indication of the amount of pigment contained in the supernatant fraction.

TABLE 1
Comparison of o-diphenoloxidase in concentrated and diluted suspensions of Mycobacterium leprae *from armadillos*

Experiment number	Concentrated suspension		Dilute suspension	
	Colour of sediment	Absorbance of supernatant	Colour of sediment	Absorbance of supernatant
1	Light black	0.022	Deep black	0.036
2	Light black	0.035	Deep black	0.045
3	Brown	0.008	Black	0.025
4	Brown	0	Black	0.022
5	Light black	0.022	Deep black	0.035

The readings for the different samples vary, because the concentration of inhibitory material present in them would not be the same.

The undiluted bacterial suspension contained over 10^{10} bacilli/ml. These were diluted 5 times or 10 times. If the preparations had no inhibitory substances, readings for the concentrated samples would have been 5 to 10 times higher than those for the diluted suspensions. The presence of higher amounts of inhibitory material in the concentrated preparations appears to be responsible for the lower activity shown by them. When the bacterial suspensions are too dilute, very little colour development would be observed, because of the insufficient number of organisms to oxidize the substrate. Another significant finding was regarding samples 3 and 4; one would have concluded that they contained no dopa oxidase activity, if the diluted samples were not tested. In purified preparations of *Mycobacterium leprae* from human sources, such marked inhibition of *o*-diphenol-oxidase has not been detected.

At the time of sacrificing the animals (from which the bacterial suspensions used in the study were derived), they were subjected to cardiac exsanguination under anaesthesia. Further studies on the tissue inhibitors of *o*-diphenoloxidase of *Mycobacterium leprae* are in progress.

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