APPARENT RESISTANCE OF *M. LEPRAE* TO "B 663"

By S. G. BROWNE, M.D., F.R.C.P., F.R.C.S., D.T.M.
and
L. M. HOGERZEIL, MED. DRS., LEYDEN
Leprosy Service Research Unit, Uzuakoli, Eastern Nigeria

One incidental and unexpected finding during our treatment of five cases of severe lepromatous leprosy for twelve months with B 663 (J. R. GEIGY S.A.) alone (BROWNE and HOGERZEIL, 1962 (*a*) and (*b*)), appears to shed some light on the controversial question of drug resistance in leprosy.

Following our preliminary report in this pilot trial (BROWNE and HOGERZEIL, 1962 (*a*)), *clinical* progress (marked in some and moderate in others) continued to be satisfactory in all 5 patients for the whole period of twelve months during which B 663 was given alone, and for the three months subsequently, when standard dapsone treatment was given. (BROWNE and HOGERZEIL, 1962 (*b*)).

Bacteriological improvement continued to be satisfactory, as shown by progressive reduction in the Bacterial Index calculated from smears taken every fortnight from eight comparable sites (four from skin; two from ear lobes; two from nasal mucosa). The degeneration of the individual *M. leprae* was also satisfactorily progressive, normal forms having almost completely disappeared from all eight sites in all five patients: only five sites showed a few (about 10%) of normal bacilli in the 24th and 25th series of smears (i.e., a total of 80 sites) made towards the close of the trial period on 5th and 19th January, 1962.

The features of bacteriological relapse during treatment with B 663

With no clinical indication that anything untoward had occurred, however, in the next series of smears taken a fortnight later, viz., on 2nd February, all patients showed a raised Bacterial Index, the rise being attributable to an increase in the Index at the majority of the sites smeared in each patient. The last dose of B 663 was due to be given, and was actually given, on 8th February. Thereafter, dapsone was given according to the following dosage scale: 100 mgm. twice weekly for two weeks; 200 mgm. twice weekly for four weeks; and 300 mgm. twice weekly subsequently.

Similarly, in the same smears (taken on 2nd February) normal bacilli reappeared in all the patients; of the total of 40 sites smeared (i.e., eight in each of the five patients), 31 contained normal bacilli The number of sites showing normal bacilli at successive subsequent

BACTERIAL INDEX :

Serial No.	*Initial Index*	*Index at end of:*					12*th month*		13*th month*		14*th month*		15*th month*	
		2nd month	*4th month*	*6th month*	*8th month*	*10th month*	*25th smears*	*26th smears*	*27th smears*	*28th smears*	*29th smears*	*30th smears*	*31st smears*	*32nd smears*
7	4·0	3·4	3·1	2·9	2·8	2·7	2·6	3·1	3·1	3·1	2·75	2·9	2·7	2·25
8	3·1	2·5	2·25	2·1	2·1	1·6	1·4	2·2	2·1	2·25	2·25	2·25	1·9	1·5
9	3·2	2·25	2·5	2·25	1·4	2·0	1·6	2·1	1·9	1·75	2·0	1·9	1·4	1·2
10	3·2	2·6	2·1	1·9	2·0	1·9	2·1	2·3	2·0	1·6	2·0	1·9	1·6	1·3
11	3·5	3·25	3·1	2·9	2·6	2·5	2·0	2·4	2·4	2·6	2·6	2·4	2·25	2·5
Aver-age	3·4	2·80	2·61	2·41	2·18	2·14	1·94	2·42	2·30	2·26	2·32	2·27	1·97	1·75

fortnightly examinations was: 26, 27, 34, 24, 30 and 22 (out of a total of 40 sites).

Four of the five patients were affected to a similar degree, having a large proportion of sites containing normal bacilli; thus, patient No. 7 had 50 sites with normal bacilli (out of 56 sites smeared); No. 8 had 46; No. 9 had 39; No. 10 had 19; and No. 11 had 45. No explanation is offered for the fact that the smears of patient No. 10 had a low proportion of normal bacilli.

All sites smeared were affected at some time, but not indiscriminately. Thus, the ear-lobes contained normal bacilli 55 times out of 70, the skin (obviously lepromatous skin, and apparently normal skin in equal proportions) 114 times out of 140, and nasal mucosa 37 times out of 70.

The proportion of bacilli that were judged to be morphologically normal in each successive fortnightly series of smears was as follows: 16%, 15%, 21%, 14%, 13%, 13% and 4%.

After twelve weeks' treatment with dapsone, the temporarily raised B.I. (i.e. the average of the five patients) returned to the level it had fallen to at the beginning of the twelfth month of B 663 treatment.

To complete the picture, it should be added that apparent resistance did not occur in the 10 patients who received dapsone together with B663, nor in the three patients who received dapsone after taking B 663 alone for 6 months. However, in three of five patients who had received B 663 for 6 months and ditophal for the first three months in addition (and standard dapsone alone after six months) (BROWNE and HOGERZEIL, 1962 (*a*)), normal bacilli appeared in the smears; and in two of them the Bacterial Index rose—both phenomena occurring at the same time as in the group who had received B 663 alone for twelve months. The magnitude of the rise in B.I. and the proportion of normal bacilli appearing in the smears, were correlated. These three patients were those whose clinical and bacteriological states showed improvement at the end of the first six months of treatment and subsequently while receiving dapsone alone.

Discussion

The findings are not in dispute, for circumstances exclude the explanations that might ordinarily be invoked, viz., the technical hazards of smearing, staining and microscopic examination; observer error; different technicians or different techniques; climatic or other extraneous factors.

The bacteriological relapse here recorded occurred when the patients were all under the full therapeutic influence of B 663; the prescribed doses of B 663 had been regularly taken, and, to judge from the degree of ruddiness of the soft tissues and the excretion of red pigment in the urine, absorption from the gut was proceeding as

before. In view of the long generation time of *M. leprae*, it is permissible to suppose that the factor determining the appearance of morphologically normal forms in all patients at the same time, had been operative for some weeks before the cessation of B 663 therapy.

The occurrence of this "resistance" may be associated with the likelihood that B 663 exerts a true bactericidal action on *M. leprae*.

It would seem that the majority of "resistant" bacilli, presumably mutants, were sensitive to dapsone (in the sense of "sensitivity" usually accepted when discussing drug therapy in leprosy), since they became progressively fewer, both proportionately and absolutely, in the fortnightly smears during the three months after dapsone treatment was begun.

It would thus seem legitimate to conclude that the phenomenon observed indicates either the development of resistance in the bacteriological sense, or the action of some undisclosed factor that stimulates multiplication of morphologically normal forms of *M. leprae*.

In this connection, it is interesting to note that in a recent report by D'ARCY HART *et al.* (1962), a similar phenomenon developed in mice treated for prolonged periods with Isoniazid after infection with *M. lepraemurium*. The proportion of degenerate bacilli recovered from the livers of such mice decreased progressively with the duration of treatment.

Summary

All five patients who had made satisfactory clinical and bacteriological progress while receiving B 663 (J. R. GEIGY S.A.) alone and uninterruptedly, suddenly showed a raised Bacterial Index in the fortnightly smears made six days before the end of the twelfth month of B 663 therapy. In the same smears, normal bacilli reappeared at the majority of sites smeared in skin, ear lobes and nasal mucosa. At the end of the full twelve months of the trial with B 663 alone, dapsone was substituted, and by the time the patients had been receiving dapsone for three months, the Bacterial Index returned to the level previous to the sudden rise, but a reduced proportion of morphologically normal bacilli persisted in the majority of the sites smeared in all the five patients.

It is considered that the most probable explanation is that resistance to B 663 had developed.

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