Our serum folate levels do not preclude a more subtle effect on folate activity, such as inhibition of the enzyme tetrahydrofolate formylase (Bertino *et al.*, 1965), and this must also be considered as a possible pathogenesis. If, however, the finding of Gitlin (1969) is confirmed, then a specific toxic effect of alcohol may have been uncovered. Gitlin described transient porphyrinuria in an alcoholic, which had disappeared seven days after cessation of alcohol. This porphyrin might be of hepatic or erythroblastic origin—if the latter, then an abnormality of haem synthesis differing from that found in folate or vitamin-B_{12} deficiency is likely, probably affecting an earlier stage in the build-up of haemoglobin.

We feel that a direct toxic effect of alcohol an haem synthesis is the most likely explanation of our findings. Evidence for a direct effect of alcohol on the liver has been brought forward in recent years (Lieber and Rubin, 1968), and a transient suppression of platelets (Lindenbaum and Hargrove, 1968; Post and Desforges, 1968) and leucocytes (McFarland and Libre, 1963) is probably also a direct effect.

Experimental ethanol feeding of dogs results in leucopenia (Beard *et al.*, 1963) and there is evidence from in-vitro experiments that ethanol suppresses some hepatic enzymes (Rubin and Lieber, 1968). Our findings may well indicate a similar action on erythropoietic cells, but acute experiments have produced no alterations in the levels of serum iron. A single three-hour bout of alcohol intake in 10 volunteer students had no effect on serum iron readings despite alcohol levels of up to 240 mg./100 ml. Our patients usually had a single three-hour bout of alcohol intake in 10 volunteer students had no effect on serum iron readings despite alcohol on erythropoiesis is first manifested.

The incidence of haemosiderosis in chronic alcoholics has been reported to be 50% of patients with alcoholic cirrhosis. The cause has been variously recorded as due to excess iron in alcoholic liquors (MacDonald, 1963) or to increased iron absorption as a consequence of pancreatic damage (Davis and Badenoch, 1962). It may be that a further factor leading to excess accumulation of iron in the liver and other tissues in chronic alcoholic patients is defective utilization of iron by the bone marrow during prolonged alcohol consumption.

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Preliminary Communications

Experimental and Clinical Studies on Rifampicin in Treatment of Leprosy

British Medical Journal, 1970, **1**, 89-92

Summary: Rifampicin showed high activity against experimental leprosy, inhibiting the multiplication of dapsone-sensitive and dapsone-resistant strains of Mycobacterium leprae in mice fed 5 mg./kg. body weight. In a formal pilot-type trial on six previously untreated patients with active lepromatous leprosy, rifampicin (600 mg. daily by mouth) was as effective as standard treatment with dapsone. Myco. leprae, however, appeared to be killed more rapidly by rifampicin than by dapsone or other antileprosy drugs so far studied. This was confirmed on a further 10 patients, including two with dapsone resistance, and from the infectivity in mice of bacilli recovered from patients during treatment with rifampicin or dapsone. These results are consistent with the bactericidal activity of rifampicin against other micro-organisms, which could be important to the chemotherapy of leprosy, since all antileprosy drugs in current use are bacteriostatic.

INTRODUCTION

Until recently all drugs for the treatment of leprosy were chosen empirically or on the basis of their efficacy against tuberculosis and then had to be submitted to clinical trials in man. No preliminary screening of potential antileprosy drugs in the laboratory was possible, because the causative organism, *Mycobacterium leprae*, could not be grown in vitro or in vivo. Since 1960, however, as a result of the successful transmission of leprosy to experimental animals (Shepard, 1960; Rees, 1964), it has been shown that antileprosy drugs effective in

man inhibit the growth of *Myco. leprae* in the mouse foot-pad infection (see review, Rees, 1969). Moreover, this experimental infection has been used successfully to determine the sensitivity of strains of *Myco. leprae* to dapsone (4,4'-diaminodiphenylsulphone) and to demonstrate the emergence of dapsone-resistant mutants in patients who relapsed despite prolonged treatment with dapsone (Pettit *et al.*, 1966; Rees, 1967). These results were of the greatest importance, because they established the reliability of the foot-pad infection for laboratory studies on the chemotherapy of leprosy.

Nevertheless, it still remained to be demonstrated that an entirely new type of antileprosy drug, first shown to have antileprosy activity in the mouse foot-pad infection, would be similarly active against leprosy in man, though a repository type of sulphone closely related to dapsone, DADDS (4,4'-diacetyldiaminodiphenylsulphone), proved to be active in human leprosy (Shepard *et al.*, 1968b) after preliminary tests in the mouse (Shepard, 1967). This opportunity presented in 1967 following on the studies showing that rifampicin (3-(4-methyl-1-piperazinyl)iminomethyl-rifamycin SV) (Maggi *et al.*, 1966), a semisynthetic derivative of the antibiotic rifamycin (Sensi *et al.*, 1959), was active against tuberculosis in man (*Lancet*, 1969). Rifampicin would be of particular interest if it was shown to be active against leprosy, because all drugs currently in general use in the treatment of leprosy are bacteriostatic against *Myco. tuberculosis*, whereas rifampicin has been shown to be bactericidal (Grumbach and Rist, 1967). Therefore rifampicin presented a possible first opportunity for testing a known bactericidal antimycobacterial drug against leprosy and to check the assumption that such a drug would be more efficacious than a bacteriostatic one.

In this paper we present results of experimental and clinical studies which show that rifampicin is an effective antileprosy drug and kills *Myco. leprae* more rapidly than dapsone or any other antileprosy drugs in current use.

Materials and Methods

Experimental

All the experiments were based on the mouse foot-pad infection, methods previously described for the preparation of skin biopsies and the inoculation and harvesting of *Myco. leprae* from the foot pads being used (Rees, 1964). The skin biopsies were dispatched to London on wet ice and within 72 hours were homogenized and a standardized suspension of bacilli prepared for inoculation of both hind foot pads of female CBA mice. The sensitivity of *Myco. leprae* to rifampicin was determined on five strains—three from previously untreated patients and two from patients with proved dapsone resistance (Pettit *et al.*, 1966). The foot-pad inoculum contained 10^4 acid-fast bacilli, and batches of six mice each were used for the untreated control and the rifampicin-treated groups; the drug was administered mixed with the food at three concentrations (0·01, 0·005, and 0·0025%) throughout the experiment, starting on the day of infection.

All the mice from each group were killed six to eight months later and the number of acid-fast bacilli in homogenates from each foot pad was counted. To determine the rate of killing of *Myco. leprae* by dapsone or rifampicin in leprosy patients biopsies of skin were taken at 0, 3, 10, 17, 24, and 69 days after beginning treatment, and a standardized suspension of bacilli was prepared from each biopsy for the inoculation of foot pads. Viability, assessed as infectivity, of the bacilli was determined from the yield of organisms in homogenates of foot pads harvested from individual mice killed at monthly intervals from the sixth month of inoculation. From each homogenate 10^4 bacilli were inoculated into six normal mice and 10^5 into six thymectomized-irradiated (Rees, 1966) CBA mice. The latter mice were included because of their possible increased susceptibility to infection with *Myco. leprae*.

Clinical

The main part of this study was based on a "pilot-trial" protocol from which it had previously been established that the initial effect of an antileprosy drug can be accurately assessed on six carefully selected patients receiving treatment for a period of only 4·5 months (Pettit and Rees, 1967; Waters *et al.*, 1967). The six patients selected were suffering clinically and histologically from pure lepromatous leprosy (LL, Ridley and Jopling, 1966), but were otherwise healthy and had no history of previous treatment (including no detectable sulphones in their urine at the time of admission). The clinical, bacteriological, and histological assessments were made by independent assessors unaware of the treatment the patients were receiving. The clinical and histological assessments were made at the beginning and end of the trial; the bacteriological assessments comprised the bacterial index (bacillary concentration) and morphological index (percentage of solid staining bacilli) and were average results of skin smears taken from the ear lobes and at least four other selected skin sites made at 0, 1·5, 3, and 4·5 months of treatment. No patients were admitted with a morphological index of less than 20.

In order to widen our experience with and knowledge of rifampicin in the treatment of leprosy 10 additional patients were studied. Three had relapsed despite previous treatment with dapsone; two of these had proved dapsone resistance, and the third had received only a short course of treatment, which was then stopped. The remaining seven patients comprised a mixed group; four were suffering from near-lepromatous leprosy (BL, Ridley and Jopling, 1966) and were therefore unsuitable for a formal drug trial (Waters *et al.*, 1967). Two of the remainder failed to complete the full period of study, being discharged after two to three months because of social problems, and the last patient was rejected from the formal trial because of a low biopsy index, indicating a mild infection. There is no difference in the rate of fall of the morphological index between BL and LL patients, and the results from the group of seven "non-trial" patients are confined to the figures for the morphological index in the first six weeks of treatment, which were in a number of cases determined additionally at two and four weeks. All patients were males, and they received rifampicin daily in a single dose given before breakfast. The usual dose was 600 mg.; patients weighing less than 35 kg. received 450 mg.

Results and Comments

Sensitivity of Myco. leprae to Rifampicin in Mouse.—Rifampicin at the three levels tested inhibited the multiplication of both dapsone-sensitive and dapsone-resistant strains of *Myco. leprae* in the mouse foot pad (yields of $<5 \times 10^4$ acid-fast bacilli/foot pad). Therefore, though the doses chosen were not low enough to establish the minimal inhibitory concentration of rifampicin, the smallest dose of 0·0025% in the diet (equivalent to a daily dose of 5 mg./kg. body weight) was indicative of high activity for any antibacterial agent and is the lower concentration of rifampicin active against experimental tuberculosis in the mouse (Grumbach and Rist, 1967). It was on the basis of these promising results with rifampicin against experimental leprosy in mice that we undertook pilot chemotherapeutic trials in man.

Chemotherapeutic Trials

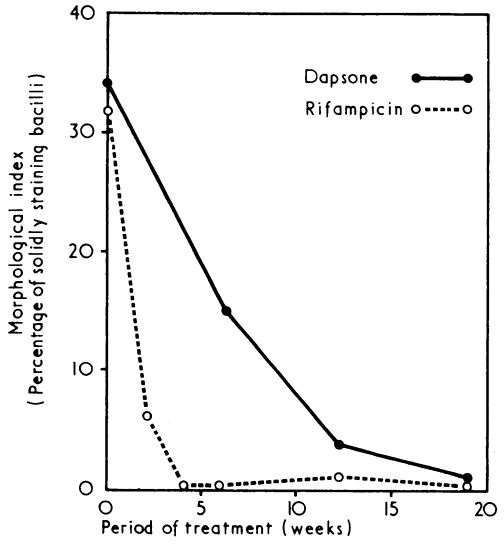
The main part of this study was undertaken in a pilot-type trial designed to detect antileprosy activity within a period of

TABLE I.—*Bacteriological, Histological and Clinical Response of 6 Patients in Formal Pilot-Type Trial Treated for 4·5 Months with Rifampicin (600 mg. daily)*

Case No.	Bacterial Index		Morphological Index			Logarithmic Biopsy Index		Clinical Improvement
	Start	4·5 Months	Start	1·5 Months	4·5 Months	Start	4·5 Months	
1	4·7	4·2	33	0	0	5·35	4·5	None
2	4·7	4·0	48	1	1	4·8	5·2	Moderate
3	4·7	4·5	33	0	0	4·9	4·7	Slight/moderate
4	4·8	4·2	27	0	0	4·9	4·65	" "
5	4·9	4·6	30	0	0	5·7	6·15	Moderate
6	4·3	4·2	23	0	0	4·85	4·45	Marked
Average	4·7	4·3	32	0	0	5·25	4·49	

4·5 months on only six carefully selected patients with previously untreated lepromatous leprosy. The results in this trial of the basic bacteriological (bacterial index and morphological index), clinical, and histological assessments are given in Table I. They show by all these criteria that rifampicin at 600 mg./day is therapeutically active against leprosy, and within this limited period it appears at least as effective as standard treatment with dapsone (Waters, 1963; Waters and Pettit, 1965). The assessments on the morphological index at standard periods of 1·5, 3, and 4·5 months, however, indicated more rapid falls than we had ever experienced with dapsone.

As soon as this important trend became apparent we increased and broadened the criteria for study *and* performed additional bacteriological assessments at two and four weeks. The data on the morphological index in the different groups of patients at more frequent intervals are given in Table II, and the data from the specially selected pilot-trial patients are plotted in the Chart. From the superimposed curve of the



Effect of treatment with rifampicin (600 mg. daily) compared with dapsone (50 mg. twice weekly) for 18 weeks on the morphological index in six previously untreated lepromatous patients.

morphological index figures accumulated from similar patients in a previous trial with dapsone (Pettit and Rees, 1967) it can be seen that the fall in the morphological index in patients on rifampicin was very significantly more rapid than that obtained with dapsone. Moreover, all patients studied, including the three with relapsing infection, responded similarly (Table II). Liver function tests and urinary and haematological examinations undertaken throughout the trial period showed no evidence of toxicity. The only possible toxicity was in one patient who developed mild and transitory nausea and epigastric pain.

TABLE II.—*Decrease in Morphological Index in 16 Patients During First 6 weeks of Treatment with Rifampicin (600 mg. daily)*

Group	No. of Patients	Average Morphological Index			
		Weeks of Treatment			
		0	2	4	6
Pilot trial	6	32	5*	1†	0
Relapse	3	24	8	2	1
"Non-trial"	7	32	4†	0†	1
Average—All groups		30	6	1	1

*5 patients only. †4 patients only.

To date three of the patients have been maintained on rifampicin at 600 mg./day for a year and their clinical, bacteriological, and histological responses have been satisfactorily maintained. Erythema nodosum leprosum, however, developed in two of the pilot-trial patients with lepromatous leprosy between the fourth and sixth months of treatment, and mild erythema nodosum leprosum at the time of entry in one of the dapsone-resistant patients persisted unchanged throughout the 12 months of treatment with rifampicin. One patient in the "non-trial" group developed "reversal reaction" (Ridley, 1969) and two others showed mild neuritis during treatment.

RATE OF KILLING OF MYCO. LEPRAE

The much more rapid fall in the morphological index in patients receiving rifampicin compared with those receiving dapsone is consistent with a more rapid killing action by rifampicin. The mouse foot-pad infection provided a direct method for assessing the viability (infectivity) of *Myco. leprae* after treatment with rifampicin or dapsone. The results from the first two suitable volunteer patients prepared to have a series of skin biopsies are given in Table III. A previously

TABLE III.—*Infectivity (Viability) as Assessed in the Mouse Foot Pad of* Myco. leprae *Obtained from Biopsies of Skin from a Patient During a 69-Day Course of Treatment with Rifampicin (600 mg./day) or Dapsone (100mg./day)*

Skin Biopsy. Day of Treatment	Rifampicin Patient		Dapsone Patient	
	Inoculum M.I.	Proportion of Foot Pads* in which Bacilli Multiplied†	Inoculum M.I.	Proportion of Foot Pads* in which Bacilli Multiplied†
0	19	17/22	22	17/22
3	13	0/24	18	16/24
10	6	2/24	14	18/24
17	2	7/24	13	13/22
24	4	0/22	12	12/22
69	1	0/24	13	3/24

M.I. = Morphological Index.
*Includes results from normal and thymectomized-irradiated mice, as the proportion of "takes" in the 2 groups was similar.
† $>5 \times 10^4$ acid-fast bacilli/foot pad.

untreated case of borderline lepromatous leprosy was given the maximum standard dose of 100 mg. of dapsone by mouth daily and compared with a relapsed patient, also with borderline lepromatous leprosy, given 600 mg. of rifampicin daily

by mouth. The results clearly show that the infectivity of *Myco. leprae* in the mouse is reduced much more rapidly in the patient receiving rifampicin than in the patient receiving dapsone. In particular, with minor fluctuations, infectivity is significantly reduced by rifampicin within a period of only 3 to 24 days, whereas a comparable loss of infectivity with dapsone is not reached until 69 days. In these studies the thymectomized-irradiated mice proved to be no more sensitive than normal animals for determining the infectivity of *Myco. leprae*.

Discussion

The present studies demonstrate, for the first time, the high activity of rifampicin against leprosy in mice and man, and as the activity was found first with the mouse foot-pad infection it again underlines the importance and reliability of this experimental model. Rifampicin has already been used to treat many patients with advanced pulmonary tuberculosis and found to be highly effective. Moreover, studies on its activity against *Myco. tuberculosis* in vitro and in experimental animals suggest that it is the most active antituberculosis drug discovered since isoniazid (Canetti *et al.*, 1968; Grumbach, 1969). In particular, rifampicin was shown to have bactericidal activity against *Myco. tuberculosis*, and therefore if it inhibited the growth of *Myco. leprae* it was likely also to be bactericidal, and this might be advantageous in the chemotherapy of leprosy since, hitherto, all antileprosy drugs were bacteriostatic against other mycobacteria.

The efficacy of rifampicin from the present clinical trials based on clinical, histological, and bacteriological assessments established not only the antileprosy activity of rifampicin but that the rapid fall of the morphological index was consistent with a bactericidal activity of the drug. The period of treatment with rifampicin was largely free of complications, though it is clear that erythema nodosum leprosum, reversal reactions, and neuritis can all occur, as they do during the course of treatment with other antileprosy drugs. Despite the very rapid bactericidal action of rifampicin, however, there was no evidence of a Herxheimer-like reaction, as had been feared from the use of such a drug and as can occur when mice with a heavy injection of *Myco. lepraemurium* receive treatment with the bactericidal drug isoniazid (Hart *et al.*, 1962).

The mode of action of rifampicin has been shown to be due to the inhibition of bacterial R.N.A. synthesis by interacting with D.N.A.-dependent R.N.A. polymerase (Hartmann *et al.*, 1967), and therefore is entirely different from that of dapsone or, for that matter, any other known antileprosy drugs. Rifampicin would therefore be expected to retain full activity against strains of *Myco. leprae* resistant to any other antileprosy drugs. Again these results and observations are in line with our experimental and clinical studies that rifampicin inhibited the growth of dapsone-resistant strains of *Myco. leprae*, and that the patients with known dapsone resistance responded as favourably as those with previously untreated leprosy. The most important finding, however, was the rapid rate at which the leprosy bacilli became degenerate (fall in the morphological index) after treatment with rifampicin, indicating that it was a bactericidal drug. Therefore it was essential to substantiate further this important observation, making use, again, of the mouse foot-pad infection as an experimental model for determining the viability of *Myco. leprae*.

In the two patients used for this special study, one receiving rifampicin and the other dapsone, the findings fully substantiated that the bacilli isolated from the patients at regular intervals up to 69 days after the beginning of treatment were killed in greater numbers and more rapidly by rifampicin than by dapsone. Though, to date, direct evidence for the more rapid killing of *Myco. leprae* by rifampicin compared with dapsone based on animal inoculation has been made on only a pair of patients, and therefore must await confirmation from a larger series, the much slower rate of killing of bacilli by dapsone in our patient is similar to that obtained by Shepard *et al.* (1968a) using identical methods on six lepromatous patients treated with dapsone.

The present preliminary experimental and clinical studies on rifampicin indicate beyond doubt the high activity of rifampicin for the short-term treatment of leprosy. The final place of rifampicin alone or in combination with other antileprosy drugs in the treatment of human leprosy must await more knowledge gained from larger and long-term studies and, in particular, confirmation of its probable bactericidal activity and whether the emergence of drug resistance is a common occurrence.

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