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AMOEBICIDAL ACTIVITY OF DICHLORO-*N*-2-HYDROXY-ETHYL-*N*-*p*-METHYLSULPHONYLBENZYLACETAMIDE AND SOME RELATED COMPOUNDS

BY

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Compounds structurally related to known amoebicidal dichloroacetamides were examined for activity against *Entamoeba histolytica* by an *in vivo*/*in vitro* method and an experimental caecal infection in weanling rats. Dichloro-*N*-2-hydroxyethyl-*N*-*p*-methylsulphonylbenzylacetamide (M&B 4321) was the first compound of this series to show high amoebicidal activity against experimental *E. histolytica* infections in young rats. Some subsequent compounds, particularly several closely related to M&B 4321, had a similar effect. Comparative experiments on both activity and toxicity were carried out on M&B 4321 and diloxanide, since the latter compound had already been administered safely to man and had shown good activity against non-dysenteric amoebiasis. The two compounds were found to be generally similar in properties. Other compounds which were of the same order of activity were discovered later, but as none had much more activity than M&B 4321 they were not studied in detail. A small trial against dysenteric amoebiasis showed that M&B 4321 was amoebicidal in man but that the cure rate was disappointing.

In spite of the introduction during recent years of several new amoebicidal substances, the treatment of amoebiasis, which has been the subject of many reviews (Anderson, Bostick & Johnstone, 1953; Porter, 1953; Schofield, 1956; Adams & Maegraith, 1960; Anderson, 1960; Bell, 1960), still depends to a large extent on a range of older drugs. The number of these and of newer drugs, together with the frequent necessity for combined therapy, demonstrates the need for a single new compound to deal effectively with all forms of the disease without unduly toxic side-effects.

Compounds of various chemical structures have been examined in these laboratories for activity against *Entamoeba histolytica*, particularly those that contain neither arsenic nor iodine, and the present paper is concerned with the evaluation of some dichloroacetamides.

Dennis & Berberian (1954) found that dichloro-*N*-2,4-dichlorobenzyl-*N*-2-hydroxyethylacetamide (chlorbetamide) was active against natural *Entamoeba criceti* infections in hamsters, *E. coli* in monkeys and *E. histolytica* in man. In these laboratories, however, it was not possible to demonstrate high amoebicidal activity for this substance against experimental *E. histolytica* in young rats. The most

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active compound examined against these rat infections was dichloro-*N*-2-hydroxyethyl-*N*-*p*-methylsulphonylbenzylacetamide (M&B 4321).

Bristow, Oxley, Williams & Woolfe (1956) subsequently reported the high activity in rats of dichloro-*p*-hydroxy-*N*-methylacetanilide (diloxanide). Its activity was confirmed in our experiments with rats, and since this compound was reported to be well tolerated in man (Woodruff, Bell & Schofield, 1956 ; Foll, 1957) it was used in our tests for comparison.

METHODS

Preliminary determination of amoebicidal activity. The initial screening of compounds was carried out by an *in vivo/in vitro* method based on that described by Woolfe (1957).

Single high doses of the test compound were given orally to groups of six to ten albino rats weighing between 25 g and 50 g. Five hours later the animals were killed and their caeca were removed. The caecal contents were dropped individually into centrifuge tubes in which they were mixed with 0.5 ml. of distilled water. After immersion in boiling water for 5 to 10 min to kill the flagellates and most of the bacteria, the tubes were centrifuged at 3,000 rev/min for 5 min. 0.2 ml. of the supernatant was then mixed with an equal volume of a 48-hr culture of *E. histolytica*. This culture was grown in Jones's (1946) medium and the number of parasites was adjusted before use to between 100 and 300 thousand per ml. A small quantity of each mixture of culture and caecal extract was drawn up into two-inch lengths of melting-point capillary tubing by means of a capillator bulb. The ends of the tubes were then plugged with modelling clay and the tube was incubated horizontally at 37° C for 24 hr, after which the contents were examined by means of a phase-contrast microscope.

Earlier experiments showed that to record only the proportion of tubes cleared of parasites could give a misleading impression and that it was better to record the reduction in the number of amoebae as well. This showed activity at a lower level, which would be missed if only the number of tubes completely cleared was recorded. Consequently, in addition to the proportion of tubes cleared, an arbitrary indication of the number of parasites present is given in the tables as the average Parasite Index.

The Parasite Index for the contents of each microtube was obtained by counting the number of parasites in 20 microscope fields (× 160 magnification) and allotting an arbitrary score:

No. of parasites in 20 fields	Index
None	0
1–3	1
4–9	2
10–20	3
More than 20	4

From the individual scores, the Parasite Index was calculated for each group. Groups of undosed controls and of animals receiving a reference amoebicide were included in each experiment. Any compound which reduced the Parasite Index to half that of the controls was examined further *in vivo*. If the index in the controls was less than 3.5 the experiment was discarded.

Further examination of active compounds. Compounds found to be active in the preliminary test were compared for activity against experimental caecal infections of *E. histolytica* in weanling rats with a standard amoebicide and later with M&B 4321. The method used was based on that of Jones (1946). The drugs were administered orally through a metal stomach tube, the total dose being given in five divided doses 24, 30, 48, 54 and 72 hr after infection. The animals were killed and examined on the sixth day after infection. The system of caecal scoring described by Goodwin, Hoare & Sharp (1948) was used, and the final criterion of cure was the absence of parasites in a smear of the caecal contents.

An invasive strain of *E. histolytica* was used in all the *in vivo* work. The earlier experiments were carried out with strain "G" (obtained from the Liverpool School of Tropical Medicine), but when it had been cultured *in vitro* for about three years it gradually lost its power of ulcerating the caecal wall in young rats. However, it was found that the strain ulcerated young guinea-pig caeca, and after 32 serial passages in guinea-pigs a culture free from other protozoa was established *in vitro*. This new strain ("G.32") was found to be highly invasive to the young rat caecum and was used for subsequent *in vivo* experiments.

Dose response curves were not drawn for all the active compounds, since repeated experiments showed that there was frequently considerable variation of response, and shallow curves were common. The mean caecal scores were used as a guide to activity, and when the control group contained uninfected animals the percentage of treated animals which had been cleared of amoebae was corrected by Abbott's formula (Finney, 1947); by this method the number of uninfected controls is, in effect, reduced to zero.

In order to record the *in vivo* activity related to M&B 4321 in each experiment without overburdening the tables, the percentage of animals cleared by M&B 4321 was expressed as 100 and a "therapeutic-equivalent" in proportion to this was calculated for each active compound. The corrected values were used for this calculation, but they can be regarded only as a general guide to relative activity.

Therapeutic experiments with M&B 4321 and diloxanide. Experiments were carried out to compare the activity of these compounds against *E. histolytica* in young rats. By the *in vivo/in vitro* method, the activity of M&B 4321 was also determined at a partly effective dose against three strains of *E. histolytica* (two of which did not ulcerate the caeca of young rats).

Antibacterial experiments (by Mr D. J. N. Hossack). Simple tests were carried out in order to discover whether diloxanide and M&B 4321 had a direct action on amoebae or if they influenced amoebic growth indirectly by modifying the bacterial flora. The drugs were made up in serial dilutions on agar plates, which were inoculated with the bacterial cultures consisting of a range of Gram-negative and Gram-positive bacteria, including those of intestinal origin.

Toxicity experiments. These experiments, carried out with the assistance of Mr K. Rivett and Mr A. D. Speed, were designed to compare the oral toxicity of M&B 4321 directly with that of diloxanide.

(a) *Oral toxicity after a single dose.* Single-dose LD50 figures were determined by oral administration to albino mice, and rats (50 to 60 g).

(b) *Oral toxicity after repeated doses.*

(i) *Short term.* Groups of 10 rats, equal numbers of each sex between 30 g and 40 g in weight, were given five daily doses each week for four weeks. Three doses (0.1 g/kg, 0.4 g/kg and 1.6 g/kg) were chosen to represent the approximate daily therapeutic dose, four times and sixteen times this amount, respectively. Each compound was administered orally by stomach tube and the growth rates were plotted from daily weight records.

(ii) *Long term.* In this experiment rats of a similar weight to those in the short-term experiment were given a quantity of drug mixed with ground diet so calculated as to give the same daily doses as before. The weight of each rat was recorded three times each week for three months while drug was present in the diet and a further two weeks after the drug was withheld. The quantity of drug/diet consumed was checked twice weekly and the % of the intended dose which was actually received was calculated for each group of rats. This procedure allowed for the effect of palatability or toxicity on the amount consumed.

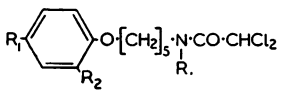
(iii) *Blood picture.* Each drug (0.2 g/kg) was given to adult male albino guinea-pigs by stomach tube daily, five times a week for four weeks. A complete blood picture examination was made twice weekly.

(iv) *Histology.* Three rats of each sex were examined histologically after receiving M&B 4321 (0.4 g/kg) in the diet for two weeks.

RESULTS

Screening experiments

Compounds of general formula



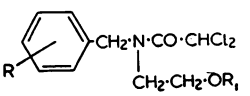
Four compounds in which R = H, $R_1 = NO_2$ or NH_2, and $R_2 = H$ or OCH_3 were inactive, but dichloro-*N*-2-hydroxyethyl-*N*-5-(2-methoxy-4-nitrophenoxy)pentylacetamide ($R = CH_2.CH_2OH$; $R_1 = NO_2$; $R_2 = OCH_3$) was active *in vivo/in vitro*, at 2.0 g/kg. *In vivo*, this last compound (M&B 4842) at a total dose of 1.0 g/kg showed about one-third the activity of M&B 4321.

Compounds of general formula

Results for this series, which includes M&B 4321, are shown in Table 1.

TABLE 1

N-BENZYLDICHLORACETAMIDES

Group formula: 

M&B no.	R	R_1	Single dose LD50 (g/kg)	*In vivo/in vitro* expts. Single dose (g/kg)	Av. parasite index (max.= 4·0)	Proportion of tubes clear of amoebae	*In vivo* expts. Activity (M&B 4321 = 100)
3844	2,4-Cl_2	H	>8·0	2·0	0·4	5/8	25
5540	3,4-$(CH_3O)_2$	H	>4·0	2·0	0·1	7/8	11
5297	*p*-CH_3	H	3·0	1·0	2·8	2/8	Inactive
5317	*p*-$CH_3.S$	H	2·5	1·0	0·6	5/8	Inactive
6437	*p*-$C_2H_5.SO_2$	$CO.CH_3$	>4·0	2·0	0·3	6/8	58
6421	*o*-$CH_3.SO_2$	H	2·0	2·0	2·8	0/8	No test
4321	*p*-$CH_3.SO_2$	H	3·8	0·5	0	10/10	100
5343	*p*-$CH_3.SO_2$	$CO.CH_3$	3·5	1·0	0	8/8	110
5344	*p*-$CH_3.SO_2$	$CO.C_3H_7$	>4·0	1·0	0	8/8	93
5472	*p*-$CH_3.SO_2$	$CO.CHCl_2$	4·0	2·0	0·1	7/8	99
6163	*p*-$CH_3.SO_2$	$CO.CH_2Br$	3·8	1·0	0	10/10	84
5682	*p*-$CH_3.SO_2$	$CO.[CH_2]_2.CO_2H$	>4·0	2·0	0	8/8	88
5624	*p*-$CH_3.SO_2$	$CO.C_6H_5$	>4·0	1·0	0·4	5/7	75
5363	*p*-$CH_3.SO_2$	C_2H_5	>4·0	1·0	0	8/8	82
6111	*p*-$CH_3.SO_2$	$CH_2.CH(CH_3)_2$	>4·0	0·5	0	10/10	70

Other amides derived from p-*methylsulphonylbenzylamine*

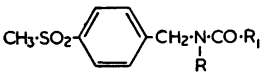
Results for six compounds of this type are shown in Table 2.

Analogues of diloxanide

A series of compounds in which changes in the amide function and two in which *p*-methylsulphonylphenyl groups were used to modify the phenolic substituent had varying degrees of activity, summarized in Table 3. Two further analogues in which $R = p\text{-}CH_3.SO_2$; $R_1 = CH_3$, and $R_2 = CO.CH_3$ or $SO_2.CH_3$, respectively, were inactive by both tests. When $R = p\text{-}CH_3.SO_2$; $R_1 = CH_3$, and $R_2 = CO.CHCl_2$, moderate *in vivo/in vitro* activity was obtained but no activity *in vivo*.

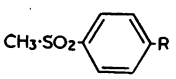
TABLE 2

OTHER AMIDES DERIVED FROM *p*-METHYLSULPHONYLBENZYLAMINE

Group formula: 

M&B no.	R	R_1	Single dose LD50 (g/kg)	*In vivo/in vitro* experiments: Single dose (g/kg)	Av. parasite index (max.= 4·0)	Proportion of tubes clear of amoebae	*In vivo* expts. Activity (M&B 4321= 100)
5613	$CH(CH_3)_2$	$CHCl_2$	>4·0	1·0	0	8/8	85
5612	$CH(CH_3)_2$	CH_3	2·0	1·0	4·0	0/8	No test
6225	$[CH_2]_3.O.CH_3$	$CHCl_2$	>4·0	1·0	0	10/10	42
6212	$CH_2.CH_2Cl$	$CHCl_2$	>4·0	1·0	0·1	9/10	30
5473	$CH_2.CH_2OH$	CH_2CN	>4·0	1·0	1·8	2/8	Inactive
5697	$CH_2.CH_2OH$	CH_2Cl	1·4	0·5	2·8	1/8	No test

Miscellaneous p-*methylsulphonylphenyl compounds*



When R was $CH_2.NH.CH_2.CH_2OH$, $CH_2.NH.CO.O.CH_2.CH_2Cl$, CH:N.OH, $CH_2.S.C(\overset{+}{N}H_2)$: NH_2 or $CH_2N(CH_2)_2O$, no activity was found. The rhodanine, R = CH:C.S.CS.NH.CO, showed activity *in vivo/in vitro* but not *in vivo*.

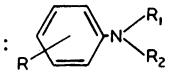
Therapeutic experiments with M&B 4321 and diloxanide. Since there was some variation in the results obtained from the *in vivo* experiments in young rats, the experiments with these compounds were repeated many times so that a more accurate impression of their efficacy could be gained.

An average number of 13 animals (range 9 to 16) was used in each group for both compounds. In five experiments with diloxanide at an oral dose of 0.05 g/kg given 5 times over 3 days the mean % cleared was 66 (range 31 to 88); at a dose of 0.1 g/kg given on the same schedule the mean % from six experiments was 67 (range 10 to 100). In 14 experiments with M&B 4321 at an oral dose of 0.05 g/kg given 5 times over 3 days the mean % cleared was 57 (range 23 to 80); at 0.1 g/kg the mean % cleared from 16 experiments was 83 (range 60 to 100). All these % have been corrected by Abbott's formula to reduce the controls to a common denominator, but in no instance were results included where the controls were less than 70% infected.

Table 4 shows the *in vivo* effect of diloxanide and M&B 4321 against strain "G.32" (which was established after animal passage) and strain "G," while a comparison of *in vivo/in vitro* activity of M&B 4321 against G.32 and two other strains is given in Table 5.

Antibacterial experiments. Both M&B 4321 and diloxanide were found to be inactive against *Pasteurella pseudotuberculosis, Neisseria catarrhalis, Escherichia coli, Shigella sonnei, Salmonella typhi-murium, Pseudomonas pyocyanea* and *Vibrio*

TABLE 3
ANALOGUES OF DILOXANIDE

Group formula: 

M&B no.	R	R_1	R_2	Single dose LD50 (g/kg)	*In vivo/in vitro* experiments: Single dose (g/kg)	Av. parasite index (max.= 4·0)	Proportion of tubes clear of amoebae	*In vivo* expts. Activity (M&B 4321 = 100)
4453 (diloxanide)	*p*-OH	CH_3	$CO.CHCl_2$	2·0	1·0	0	10/10	72
4470	*p*-OH	CH_3	$SO_2.CH_3$	2·0	0·5	3·3	0/8	44
5623	*p*-OH	CH_3	$CO.CH_2.N(C_2H_5)_2$	2·5	1·0	4·0	0/8	No test
4935	*p*-OH	$CH_2.CH_2OH$	$CO.CHCl_2$	1·5	1·0	0·3	4/6	74
4897	*o*-OH	$CH_2.CH_2OH$	$CO.CHCl_2$	2·0	1·0	4·0	0/6	No test
6226	*p*-$CH_3.SO_2.C_6H_4.CH_2.O$	CH_3	$CO.CHCl_2$	>4·0	1·0	0	9/9	43
6233	*p*-$CH_3.SO_2.C_6H_4.CO.O$	CH_3	$CO.CHCl_2$	4·0	1·0	0	10/10	73

TABLE 4

EFFECT OF M&B 4321 AND DILOXANIDE AGAINST *E. HISTOLYTICA* IN RATS

Dose given 5 times over 3 days

Strain	Compound	Oral dose (g/kg)	Proportion clear of infection	Percentage clear and corrected percentage (Abbott's formula)	Mean caecal score (maximum infection=8·0)
" G "	4321	0·025	5/10	50 (42)	2·8
		0·05	8/10	80 (77)	1·1
		0·1	10/10	100 (100)	0·5
	Controls	—	1/7	14 (0)	4·3
" G "	Diloxanide	0·125	7/9	78 (73)	2·0
		0·25	9/10	90 (88)	0·3
		0·5	9/10	90 (88)	1·2
	Controls	—	2/10	20 (0)	3·2
" G.32 "	4321	0·125	5/11	46 (33)	4·6
		0·25	6/10	60 (50)	3·7
		0·5	7/9	78 (73)	3·6
	Diloxanide	0·125	0/9	0 (0)	6·1
		0·25	4/8	50 (38)	3·4
		0·5	6/10	60 (50)	2·6
	Controls	—	2/10	20 (0)	5·6

TABLE 5

IN VIVO/IN VITRO ACTIVITY OF M&B 4321 AGAINST 3 STRAINS OF *E. HISTOLYTICA*

	E. histolytica strain					
	A.68	Control	MAL	Control	G.32	Control
Single oral dose of M&B 4321 (g/kg)	0·25	—	0·25	—	0·25	—
Average parasite index (maximum=4·0)	1·3	3·6	1·4	4·0	1·4	3·9
Proportion of microtubes cleared of amoebae	4/9	0/9	5/9	0/10	3/11	0/12

metchnikovi. *Streptococcus pyogenes* was inhibited by 40 μg/ml. of diloxanide and by 200 μg/ml. of M&B 4321.

Toxicity experiments with M&B 4321 and diloxanide

Oral dosage. The single-dose LD50 of M&B 4321 in mice was 3.8 g/kg, compared with 2.0 g/kg for diloxanide. In rats, the LD50 of M&B 4321 was 12.5 g/kg, whereas diloxanide was not toxic at this dose.

In the experiments using daily repeated doses of 0.1 to 1.6 g/kg in rats, no deaths were caused by either compound. In the one-month experiment in which the compounds were given by stomach tube, there was some depression of the growth rate compared with a control group at the highest dose of each compound; the depression was greater in the group receiving diloxanide than in that given M&B 4321. In the three-month drug/diet experiments, the depression of growth rate was more marked: at the end of the period, diloxanide produced a 47% depression compared with 16% for M&B 4321 on an intended dose of 1.6 g/kg (on food con-

sumption records the actual dose received varied for M&B 4321 between 2.0 g/kg and 2.6 g/kg and for diloxanide between 3.0 g/kg and 3.5 g/kg). The lower doses, 0.1 g/kg and 0.4 g/kg, of diloxanide had much less effect and those of M&B 4321 depressed the growth hardly at all. The blood picture and histological experiments revealed no abnormalities.

DISCUSSION

Structure-activity relationships

The most interesting series of compounds is that shown in Table 1. The introduction of a *p*-methylsulphonyl group into the nucleus (M&B 4321) effected a considerable increase in activity over 2,4-dichloro-substitution (chlorbetamide), and the position of this group is evidently highly specific, since the analogous *o*-methylsulphonyl compound (M&B 6421) was completely inactive.

The nature of the amide function was equally specific, since the monochloroacetamide (M&B 5697, Table 2) corresponding to M&B 4321 was virtually inactive, an observation which is parallel with the optimal activity of the di- as compared with the mono- and tri-chloroacetamide-group against *E. criceti* of the hamster in a related series (Surrey & Rukwid, 1955). Comparison of the *N*-acetamido- with the *N*-dichloroacetamido-derivative of *N*-isopropyl-*p*-methylsulphonylbenzylamine (M&B 5612, M&B 5613, respectively) provides further evidence of the importance of halogenation of the amide function in this type of compound.

Replacement or modification of the *N*-2-hydroxyethyl group of M&B 4321 had a less marked effect on the activity, which was retained within certain limits. Thus, esterification of the hydroxyl group by aliphatic acids (Table 1) caused very little, if any, loss of activity, though benzoylation produced a more significant drop (M&B 5624). Two aliphatic ethers (M&B 5363, M&B 6111) showed about 75% of the *in vivo* activity of M&B 4321. The methyl ether of the *N*-3-hydroxypropyl compound (M&B 6225, Table 2), however, had still less *in vivo* activity, which appears to indicate that a longer alkyl chain than hydroxyethyl is undesirable. Similarly, replacement of the hydroxyethyl group by isopropyl group (M&B 5613, Table 2), while not affecting the high *in vivo*/*in vitro* clearance of M&B 4321, reduced its *in vivo* level of activity. The loss of activity on removing the hydroxyethyl group from this type of structure is revealed in the small series of dichloro-*N*-5-phenoxypentylacetamides, where activity was introduced only by *N*-hydroxyethylation of an otherwise inactive amide.

In view of the apparent importance of the 2-hydroxyethyl, or closely related, group in the *N*-benzyldichloroacetamide series, it is remarkable that the introduction of such a group (M&B 4935, Table 3) into diloxanide, a dichloroacetanilide, in place of the *N*-methyl group did not significantly increase amoebicidal activity *in vivo*. The two compounds M&B 6226 and M&B 6233 (Table 3), representing an attempt to combine features of both diloxanide and M&B 4321, showed very good *in vivo*/*in vitro* results. The first of these showed only moderate activity *in vivo*, while the second proved comparable with diloxanide and inferior to M&B 4321 in this test.

The conclusion reached from the screening results was that variation of the original M&B 4321 structure produced a few derivatives of equal activity but none with any advantage.

M&B 4321 and diloxanide

M&B 4321 was the first compound of the series to show high activity and it was therefore selected for comparative study with diloxanide.

Evidence that different strains of amoebae were not influenced by the drugs to the same extent was afforded by the experiments with strain G, which had been subcultured *in vitro* many times, and with strain G.32, which had recently been established *in vitro* from animal passage and which caused a higher degree of caecal ulceration in rats. Both diloxanide and M&B 4321 were less effective against strain G.32 (Table 4).

However, M&B 4321 showed a similar effect against strains G.32, A.68 and MAL. Strain A.68 (isolated at the Amoebiasis Research Unit, Durban) and Strain MAL (obtained from Dr R. A. Neal, Wellcome Laboratories of Tropical Medicine, London) gave poor infections in rats and did not ulcerate the caecum, so the *in vivo/in vitro* procedure was used to compare the action of a partially effective dose of M&B 4321 against these three strains.

The antibacterial experiments showed that neither M&B 4321 nor diloxanide possessed any marked activity against Gram-positive or Gram-negative bacteria, and it was concluded that the amoebicidal effect was principally a direct one.

It was decided that more definite information on the therapeutic properties of the compound could be obtained only from a clinical trial. This was arranged after subsequent toxicological studies (see below) had shown M&B 4321 to be no more toxic than diloxanide, which was known to be safe in man.

Toxicity. After the preliminary short-term experiments had shown M&B 4321 to be rather less toxic than diloxanide in mice and not markedly more toxic in rats, the one-month repeated oral dose experiment confirmed the impression that the two compounds were generally similar and could be given without ill effect in amounts many times greater than the therapeutic dose. In the three-month drug/diet experiments the growth rate was depressed at the 1.6 g/kg rate more by diloxanide than by M&B 4321, but the animals consumed about twice the calculated quantity of diloxanide throughout the three months whereas M&B 4321 was consumed at about one and a half times the calculated rate. Similarly, at 0.4 g/kg, although the growth rate for M&B 4321 was similar to the controls and that for diloxanide was depressed, the animals received in the first and third months about one and a half times the intended dose of the latter compound. The animals all rapidly gained weight after the drugs were withheld and appeared normal at autopsy.

It was concluded from these and the blood picture experiments that the two compounds were similar in properties and virtually non-toxic by mouth. It was reported that no toxic effects occurred in man after the early trials of diloxanide, so a small clinical trial of M&B 4321 was arranged.

Clinical trial. An initial evaluation in man was carried out at the Amoebiasis Research Unit, Durban, South Africa, where 10 patients suffering from the typical

dysenteric amoebiasis found in this area were treated with M&B 4321 5 mg/kg three times a day for 10 days. The assessment of results at this Unit has been clearly described (Elsdon-Dew, 1955).

Five out of ten patients were clear at 27 days. Three cases showed healed ulcers but parasites were still present at the end of the treatment. Two cases were absolute failures. Of the five clear at 27 days one produced cysts at one month and another at two months after treatment (Elsdon-Dew, personal communication). These results, while demonstrating considerable amoebicidal activity, were disappointing in comparison with those obtained at this Unit with other amoebicidal drugs (Elsdon-Dew, Wilmot & Powell, 1960).

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