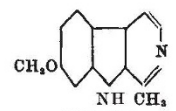
Chemotherapy of Amœbicides*

By Dr. F. L. Pyman, F.R.S.

RESEARCH on amœbicides was greatly facilitated by the technique developed by Dobell and Laidlaw (1926), and Laidlaw, Dobell and Bishop (1928) for testing amœbicides *in vitro*. Emetine has for long been the principal drug used in the treatment of amœbic dysentery, but it has some undesirable by-effects, amongst others a nauseating effect. In a search for substances having the amœbicidal action of emetine without its nauseating effect, a number of alkaloids very closely related to emetine in chemical structure were made at an earlier period. When tested by Dale and Dobell (1917), by an early laboratory method, several of them, *O*-methylpsychotrine (a substance which differs from emetine structurally only in containing two hydrogen atoms fewer) and *N*-methylemetine, for example, were found to be more toxic to *Entamœba histolytica* than emetine itself. Clinical trials of *O*-methylpsychotrine (Jepps and Meakins, 1917) and *N*-methylemetine, however (Low, 1915; Wenyon and O'Connor, 1917), showed them to be of little or no value in the treatment of amœbic dysentery.

The method of Dobell and Laidlaw, however, depending on the cultivation of amœbæ in a medium consisting partly of solid (inspissated fresh horse serum) and partly of liquid (egg-white diluted with Ringer's fluid) with a little starch, gave results which fell into line with the clinical results. Emetine was found to be fifty times as toxic to amœbæ *in vitro* as *N*-methylemetine, *iso*-emetine, and *O*-methylpsychotrine, which are clinically inactive. Later, Laidlaw, Dobell and Bishop described a simpler medium, consisting of 1 part of sterile horse serum, 8 parts of Ringer's fluid with a small quantity of sterile solid rice-starch, disodium hydrogen phosphate being added as a buffer. In this medium they found that the amœbæ were destroyed in four days by emetine 1 in 5,000,000, provided that the medium did not become too acid. We have made use of this method in the work which I am about to describe.

A homologous series of normal alkylharmols, from methylharmol (harmine) up to dodecylharmol, was examined, and it was found that both bactericidal and amœbicidal activity increased, on ascending the homologous series, up to a point and then started to fall. Peaks of bactericidal activity were reached at butyl for *B. typhosus* and at amyl for *S. aureus*, whilst the peak of amœbicidal activity was reached at *O*-*n*-nonylharmol.



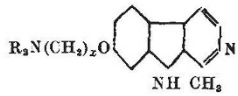
Harmine

Compound.	R.W. Coefficients. *B. typhosus.*	*S. aureus.*	Minimum concentration lethal to *Entamœba histolytica.*
Harmol	1	—	1 in 40,000 to 1 in 80,000
Harmine	<1	—	
O-ethylharmol	25	—	1 in 80,000
O-*n*-propylharmo	225	75	1 in 80,000 to 1 in 120,000
O-*n*-butylharmol	350–400	150	1 in 20,000 to 1 in 80,000
O-*n*-amylharmol	350	250–300	1 in 100,000 to 1 in 200,000
O-*n*-hexylharmol	50	45–50	1 in 100,000 to 1 in 200,000
O-*n*-heptylharmol	30–35	45–50	1 in 200,000
O-*n*-octylharmol	15	35–40	1 in 200,000 to 1 in 300,000
O-*n*-nonylharmol	10–15	15	1 in 200,000 to 1 in 500,000
O-*n*-decylharmol	10	—	1 in 100,000
O-*n*-dodecylharmol	5	—	1 in 100,000 not lethal

The salts of this and other high members of the series were very sparingly soluble in water, and consequently a further series of compounds was prepared, with the hope of obtaining more readily soluble compounds.

The method adopted was to add a further salt-forming group to the molecule in the form of a terminal dialkylamino-group, such as is employed in the antimalarials, plasmoquine and atebrin.

In this way there was made a series of derivatives of harmol having the general formula given below, the salts of which proved, as had been expected, to be readily soluble in water.



The size of both R (the N-alkyl groups) and x the number of carbon atoms in the chain separating N from O was varied, and the results may be illustrated by reference to a series in which the decyl group ($x = 10$) was a common factor, whilst the dialkylamino group was varied.

Compound.	Minimum concentration lethal to *Entamœba histolytica.*
O-*κ*-dimethylaminodecylharmol	1 in 300,000 to 1 in 500,000
O-*κ*-diethylaminodecylharmol	1 in 200,000 to 1 in 500,000
O-*κ*-di-*n*-butylaminodecylharmol	1 in 750,000 to 1 in 2,000,000
O-*κ*-di-*n*-amylaminodecylharmol	1 in 750,000 to 1 in 3,000,000
O-*λ*-di-*n*-butylaminoundecylharmol	1 in 750,000 to 1 in 4,000,000
O-*n*-nonylharmol	1 in 200,000 to 1 in 500,000
Emetine hydrochloride	1 in 2,000,000 to 1 in 10,000,000

It was thus found that the activity of members at the peak of the series, such as *O*-λ-di-*n*-butylaminoundecylharmol, was many times that of

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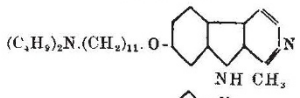
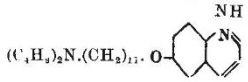
O-n-nonylharmol, and this fact led us to suspect that the harmol residue might not be an important contributor to the amœbicidal properties of the molecule.

A number of compounds were then prepared in which dibutylaminodecyl (or undecyl) groups were introduced into molecules of varying structures. The last columns in the following tables show the limits of the range of the minimum concentration found lethal to *Entamœba histolytica* in three days, under the conditions laid down by Laidlaw, Dobell and Bishop (*loc. cit.*).

Compound.	Minimum concentration lethal to *Entamœba histolytica.*
	1 in 750,000 to 1 in 4,000,000
	1 in 100,000
$(C_4H_9)_2N.(CH_2)_{10}O.CO.CH_3$	1 in 100,000
$(C_4H_9)_2N.(CH_2)_{10}O.CO.C_6H_5$	1 in 100,000
$(C_4H_9)_2N.(CH_2)_{10}N.(C_4H_9)_2$	1 in 2,000,000

It was thus shown that the attachment of the group $(C_4H_9)_2N.(CH_2)_{10}$ to a simple substituted amino group gave very high efficiency.

A long series of tetraalkyldiamino paraffins of the general formula $NRR'.(CH_2)_n.NRR'$ was then prepared, and the minimum amœbicidal concentration under the optimum conditions for emetine determined.

In the first place, derivatives of heptane and decane were examined; of the heptane series the tetraethyldiamino and tetra-*n*-butyldiamino compounds were prepared and tested. The tetrabutyl member of the series was superior as an amœbicide to the tetraethyl one, but neither showed more than a fraction of the efficiency of the best harmol derivative. More promising results were obtained with the corresponding decane derivatives, and ultimately the efficiency of dibutylaminoundecylharmol was equalled or even, in some of our tests, surpassed.

The following table shows the results of a test in which a number of decane derivatives of the general formula, $R_2N.(CH_2)_{10}NR_2$, were examined simultaneously, so that the 'peak' of the series could be ascertained. This was found at αx-tetra-*n*-amyldiaminodecane, which was used as a standard of comparison in later work. For brevity, it is referred to below as T.A.D.D.

Compound.	Minimum concentration lethal to *E. histolytica.*
αx-Decanes	
Tetra-*n*-propyldiamino	1 in 250,000 *not* lethal
Tetra-*n*-butyldiamino	1 in 1,500,000
Tetra-*n*-amyldiamino	1 in 3,000,000 (or less)
Tetra-*n*-hexyldiamino	1 in 1,000,000
Tetra-*n*-heptyldiamino	1 in 250,000 *not* lethal

A similar test indicated that the corresponding series of undecane derivatives also showed the peak with the tetraamyldiamino member.

Next, keeping a tetrabutyl or tetraamyl group constant, the hydrocarbon residue was varied. The following table shows the results of two tests on these series of compounds.

Compound.	Minimum concentration lethal to *E. histolytica.*
	Test 1.
αω-Tetra-*n*-butyldiamino-	
nonane	1 in 800,000
decane	1 in 1,000,000
undecane	1 in 2,000,000
dodecane	1 in 1,500,000
tridecane	1 in 1,000,000
	Test 2.
αω-Tetra-*n*-amyldiamino-	
octane	1 in 400,000
nonane	1 in 1,000,000
decane	1 in 2,000,000
undecane	1 in 1,500,000
dodecane	1 in 200,000

As the results of the foregoing experiments, αx-tetra-*n*-amyldiamino-*n*-decane (T.A.D.D.) was selected for further study. The conditions of all the amœbicidal tests described above were those most favourable for emetine, that is, in a faintly alkaline medium. It is well known (Laidlaw and others; Henry and Brown, 1923) that the exceedingly high efficiency of emetine *in vitro*, of the order of 1 in 5,000,000, is only found in alkaline, neutral or only very faintly acid media. Our results afford abundant confirmation of this fact. When endeavouring to assess the value of an amœbicide in the treatment of amœbic dysentery by comparison with emetine *in vitro*, it appears therefore necessary to consider carefully the hydrogen ion concentration likely to be met with in the areas infested with amœbæ.

We have been unable to find any reference to the actual hydrogen ion concentration in the amœbic ulcer, but Knowles and others (1923) found that the *p*H of a number of stools containing motile amœbæ averaged 6·22. They also reported the results of experiments on kittens artificially infected with *E. histolytica* in which the colon and rectum of the animals were minced in saline and the hydrogen ion concentration of the suspension determined. The average *p*H value obtained in these experiments was 6·33, and the livers when similarly treated showed an average *p*H value of 6·34.

Furthermore, a considerable amount of work has been carried out upon the reaction of living, dead and diseased body cells, and the work of Rohde (1927) and Chambers and others (1927) suggests that the contents of the ulcers may have a hydrogen ion concentration more acid than *p*H 7·0.

A consideration of these papers suggested that in any comparisons of amœbicides with emetine

in vitro the effect of acidity should be studied, particularly when the amœbicides are to be administered orally, and that tests should be carried out at a *p*H value of 6·2 or 6·3.

Under these conditions T.A.D.D. is three to five times as efficient as emetine. Moreover, when blood is added to the medium even at *p*H values otherwise favouring emetine, T.A.D.D. and emetine are of very similar amœbicidal value, the former at times showing a definite superiority.

The toxicity of T.A.D.D. to mice has been compared with that of emetine, with the following results :

	Median Lethal Dose (mgm./gm.)		
	Oral.	Subcut.	Intraven.
αx-Tetra-*n*-amyldiaminodecane dihydrochloride . .	0·45	0·35	0·04
Emetine dihydrochloride .	0·04	0·06	0·013

It has thus only one tenth of the toxicity of emetine when administered orally to mice and one sixth on subcutaneous injection. Its therapeutic index is therefore much more favourable than that of emetine, and it appeared to be an exceptionally promising compound for clinical trial in conditions of ill-health due to infestation with *Entamœba histolytica*. At this point, it was recommended to and accepted by the Therapeutic Trials Committee of the Medical Research Council for clinical trial. It was tried clinically by Prof. Warrington Yorke, who has kindly allowed me to state his results. He finds that T.A.D.D. has some action in amœbic dysentery, when administered orally, but is not sufficiently active to be of any real value. Unfortunately, it cannot be given intramuscularly, subcutaneously or intravenously, as it is intensely irritating.

It appears, therefore, that the comparison of the amœbicidal values of emetine and T.A.D.D. with a faintly alkaline medium gives a better indication of their relative clinical value than the comparison in a slightly acid medium. This knowledge will be of value in further work on the subject.

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A New Conception of Supraconductivity*

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5. According to these conceptions, *there cannot exist any magnetic flux 'frozen' in the interior of pure supraconductors* ; a permanent flux should only be found confined to the *hollows of supraconducting rings*. The topological connectivity of a supraconductor, therefore, is a property extremely characteristic of its behaviour : the multiplicity of its connectivity, diminished by one, immediately indicates the number of independent conservative quantities, that is, of independent invariant magnetic fluxes.

Actually, however, in the classical experiments of Kamerlingh Onnes, already there have been found magnetic fields 'frozen' in even simply connected supraconductors. It was these permanent fluxes which seemed at that time directly to indicate the elementary phenomenon : an *infinite conductivity*. We, on the contrary, do not consider these experiments as representing the elementary case of the phenomenon, but rather as a relatively complicated affair which can be reduced to a still more elementary phenomenon.

According to our conceptions, we interpret these magnetic fluxes 'frozen' in the interior of the supra-

*Continued from page 796.

conductors as follows[6] : One knows that the presence of a magnetic field exceeding a certain critical value H_T (depending on the temperature T) destroys the supraconductivity. Now it can happen that some magnetic fluxes are confined in certain regions of the metal in such a manner that the critical magnetic field is there exceeded, whereas in the supraconducting regions the supraconductivity is maintained. Thus the appearance of the permanent fluxes should be conditioned by the formation of a complicated structure of the supraconducting and the normal phases in the metal in such a way that the supraconducting regions constitute rings embracing the magnetic fluxes in their non-supraconducting hollows.

6. It is easy to see that, even in very simple experiments, such a *mixed structure of the two phases* must automatically arise. This can be shown by considering, for example, a supraconducting sphere which is brought into a homogeneous magnetic field.

The sphere pushes back the magnetic lines of force and compresses them in the region near the equator. An elementary calculation shows that the intensity of the field immediately on the