XXXV. THE CHEMOTHERAPY OF DERIVATIVES OF HARMINE AND HARMALINE. II.

By CHARLES EDWARD COULTHARD.

*From the Bacteriological Laboratory, Boots Pure Drug Co., Ltd.,
Nottingham.*

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IN the first paper of this series, Coulthard *et al.* [1933] described the preparation of a long series of *O*-alkyl derivatives of harmol and recorded their bactericidal properties. It was shown that the peak compound of the normal alkyl series differed with the test organism, being situated at the butyl compound for *Bacillus typhosus* and at the amyl for *Staphylococcus aureus*, whilst it was recorded briefly that the most effective of the *n*-alkylharmols towards *Entamoeba histolytica* was *O*-*n*-nonylharmol. The experimental results upon which the latter statement was based are described in the present communication.

The organism chosen for our tests was *Entamoeba histolytica*, two strains of which, D.K.B. and N.R.S., were kindly supplied by Mr C. Dobell. Since a survey of the literature appeared to indicate that emetine was the most efficient lethal agent for this protozoon *in vitro*, this alkaloid was used as a control, but in addition tests were made upon some other substances used, or recommended for use, in the treatment of amoebic dysentery.

Laidlaw *et al.* [1928] described a method of testing emetine in which the test organism, *Entamoeba histolytica*, is cultivated in a medium consisting of Ringer's solution with horse-serum and a little rice starch, disodium hydrogen phosphate being added as a buffer. In this medium they found that the amoebae were destroyed in four days by emetine 1 in 5,000,000 provided that the medium did not become too acid. When the phosphate buffer was omitted, however, the end-point was not always sharp but varied from strain to strain and even with the same strain, the tubes which became most acid in culture being most likely to contain living amoebae. In these experiments the lethal concentration varied from 1 in 300,000 to 1 in 750,000.

In order to carry out our tests in such a manner that the minimum lethal concentration of our emetine control was relatively level from test to test, the medium buffered with phosphate was used in a series of determinations of the amoebicidal efficiency of the hydrochlorides of these harmine and harmaline derivatives. The results of this series of tests are given in Table I.

This table shows the gradual increase of the minimum lethal concentration of the *O*-*n*-alkylharmols from 1 in 80,000 (*O*-*n*-ethylharmol hydrochloride) to 1 in 200,000 to 1 in 500,000 for the *O*-*n*-nonyl derivative with subsequent decline for higher members of the series. The corresponding derivatives of harmalol are less efficient as are the *iso*-alkyl ethers of harmol.

The figure obtained for emetine is in good agreement with that (1 in 5,000,000) found by Laidlaw *et al.* [1928] using the same medium. By the courtesy of Dr T. A. Henry we obtained samples of *O*-methylpsychotrine sulphate and conessine acid oxalate, and brief reference to our results with these two salts may

Table I.

Compound	Minimum concentration lethal to <i>Entamoeba histolytica</i>
Hydrochlorides of:	
Harmol	1 in 40,000 to 1 in 80,000
<i>O</i> -Ethylharmol	1 in 80,000
<i>O</i> - <i>n</i> -Propylharmol	1 in 80,000 to 1 in 120,000
<i>O</i> - <i>n</i> -Butylharmol	1 in 20,000 to 1 in 80,000
<i>O</i> - <i>n</i> -Amylharmol	1 in 100,000 to 1 in 200,000
<i>O</i> - <i>n</i> -Hexylharmol	1 in 100,000 to 1 in 200,000
<i>O</i> - <i>n</i> -Heptylharmol	1 in 200,000
<i>O</i> - <i>n</i> -Octylharmol	1 in 200,000 to 1 in 300,000
<i>O</i> - <i>n</i> -Nonylharmol	1 in 200,000 to 1 in 500,000
<i>O</i> - <i>n</i> -Decylharmol	1 in 100,000
<i>O</i> - <i>n</i> -Dodecylharmol	1 in 100,000 not lethal
<i>O</i> -isoPropylharmol	1 in 80,000 not lethal
<i>O</i> -sec-Octylharmol	Less efficient than <i>O</i> - <i>n</i> -octylharmol
<i>O</i> -Allylharmol	1 in 80,000
<i>O</i> -Benzylharmol	1 in 80,000
Emetine hydrochloride	1 in 2,000,000 to 1 in 10,000,000
Conessine acid oxalate	1 in 80,000 to 1 in 200,000
<i>O</i> -Methylpsychotrine sulphate	1 in 80,000 not lethal

be made in passing. The very low result obtained with methylpsychotrine bears out the clinical results of Jepps and Meakins [1917] and the laboratory work of Dobell and Laidlaw [1926]. On the other hand, the figure obtained for conessine appears surprisingly low in view of the work of Brown [1922] and Henry and Brown [1923] on the action of conessine on free living protozoa, and the experiments of Chopra *et al.* [1927] on *Entamoeba histolytica* from kitten's faeces. A number of clinical successes have been reported with kurchi but it is interesting to note that conessine is regarded by some workers as less efficient than kurchi itself. [See, for example, Caius and Mhaskar, 1928; Knowles, 1928; Henry and Brown (quoting Willmore), 1928.] In support of our figure it may be noted that Knowles [1928] as the result of a clinical investigation suggested that the injection of conessine or total kurchi alkaloids was less efficient than kurchi orally, and Leake [1932] also considers kurchi ineffective, quoting the result of a clinical investigation by Reed using kurchi bismuth iodide.

It is evident from various papers, for example, that of Laidlaw *et al.* [1928] and that of Henry and Brown [1923], that the exceedingly high efficiency of emetine *in vitro* of the order of 1 : 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 amoebicide in the treatment of amoebic 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 amoebae.

We have been unable to find any reference to the actual hydrogen ion concentration in the amoebic ulcer, but Knowles *et al.* [1923] found that the p_H of a number of stools containing motile amoebae 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, 1, 2],

and Chambers *et al.* [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 suggests that in any comparisons of amoebicides with emetine *in vitro* the effect of acidity should be studied, particularly when the amoebicides are to be administered orally, and that tests should be carried out at a p_H value of 6.2 or 6.3.

We have carried out a number of tests to study the efficiency of emetine at various hydrogen ion concentrations. In the course of this work we noted that the amount of rice starch added to the tubes of culture medium might also considerably influence the results. Table II summarises the results of three tests,

Table II.

Dilutions of emetine hydrochloride	Test A Medium without 0.2 % phosphate		Test B Medium without 0.2 % phosphate R.S. 0.02 g.		Test C Medium with 0.2 % phosphate R.S. 0.02 g.		
	R.S. 0.02 g. p_H 7.7	R.S. 0.08 g. p_H 7.57	p_H 7.15	p_H 6.2 about	p_H 6.65	p_H 6.22	p_H 6.12
1-1,000,000	O	AA	O	AA	AA	.	AA
1-750,000	.	.	.	.	AA	AA	AA
1-600,000	O	A	O	AA	.	.	.
1-500,000	.	.	.	.	AA (6.57)	AA	AA
1-400,000	O	O	.	AA	.	.	.
1-300,000	.	.	.	AA	O (6.89)	AA (6.53)	AA
1-200,000	O	O	.	AA (5.57)	O (6.57)	O (6.60)	AA (5.97)
1-100,000	.	.	.	AA (5.83)	O (6.46)	O (6.73)	AA (6.33)
1-50,000	.	.	.	.	.	O (6.71)	O (6.49)

Medium = Horse-serum + Ringer's solution.
R.S. = Rice starch.
AA = Living amoebae in original culture.
A = Living amoebae in subculture only.
O = No amoebae observed in original culture or in subculture.

two in medium without phosphate showing the effect of increased acidity and of increased rice starch, and one in medium with buffer showing the effect of increased acidity. Approximately equal amounts of rice starch were added to each tube in a series by preparing a suspension of sterile starch in sterile Ringer's solution and adding equal volumes of this aseptically. The p_H value of this culture medium varies with the methods of determination. The figures quoted were obtained by Mr E. A. Harvey, using the potentiometer and the quinhydrone electrode, those at the top of each column refer to the unsown medium and those inserted in the column to the sown medium after incubation.

If we now proceeded to compare our peak compound, *O-n-nonylharmol*, with emetine under those conditions of acidity which it is suggested may represent conditions *in vivo* more nearly, the ratio of efficiencies showed a considerable alteration (Table III).

Table III.

Culture medium	Starch (per tube) g.	p_H before sowing	p_H after incubation	Lethal concentration	
				<i>O-n-Nonyl-</i> harmol hydro- chloride	Emetine hydro- chloride
With phosphate 0.2 %	0.02	6.18	6.2	1-300,000	1-400,000
"	0.02	6.65	6.46 to 6.89	1-200,000	1-300,000
"	0.02	6.22	6.53 to 6.73	1-100,000	1-200,000
"	0.02	6.12	5.97 to 6.49	1-100,000	1-50,000
No phosphate	0.02	6.5	—	1-200,000	1-100,000
"	0.08	7.4	—	1-100,000	1-100,000

After incubation the hydrogen ion concentration of several tubes was determined, and the limits between which these tubes fell are given in column four. Similar results are obtained if unbuffered medium be used.

It has thus been demonstrated that the amoebicidal value of *O-n*-nonylharmol hydrochloride, although considerably less than that of emetine under optimum conditions for this compound, may nevertheless equal, or even surpass, that of the latter if these conditions are varied slightly, particularly if the reaction of the medium is allowed to become more acid. It has also been pointed out that the literature indicates the possibility that the hydrogen ion concentration in the gut may be in the neighbourhood of p_H 6.2 in cases of amoebic dysentery. It therefore appeared desirable that clinical comparison of *O-n*-nonylharmol hydrochloride with emetine should be carried out, and this substance has therefore been submitted to the Therapeutic Trials Committee of the Medical Research Council with this end in view.

It may also be noted that additional confirmation of the amoebicidal value of *O-n*-nonylharmol is given by Gunn [1932]. Experimenting with *Amoeba proteus*, he found it lethal in 24 hours at a dilution of 1 in 1,000,000 or even 1 in 2,000,000.

SUMMARY.

1. The members of a long series of *O*-alkyl derivatives of harmol have been tested for amoebicidal efficiency *in vitro* in comparison with emetine by the method of Laidlaw, Dobell and Bishop.

2. The most efficient derivative, *O-n*-nonylharmol, has been compared with emetine under different conditions. The results indicate that *O-n*-nonylharmol may be equal to, or even better than, emetine as an amoebicide under appropriate conditions.

I have great pleasure in thanking Mr C. Dobell for his advice when we were about to commence this work and for providing strains of amoebae, Dr F. L. Pyman for general direction of the investigation, Dr T. A. Henry for supplying the *O*-methylopsychotrine sulphate and conessine acid oxalate, and Miss D. Wainman for much careful work at the bench.

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