Comparative Amoebacidal Activity of Some Compounds Related to Emetine.*† (19738)

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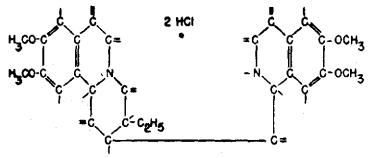
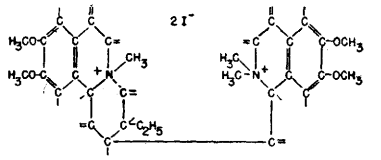
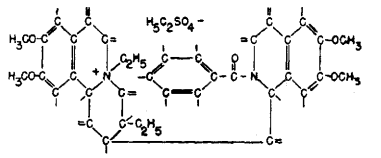
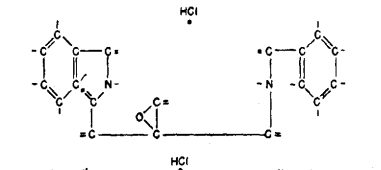
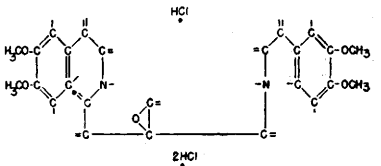
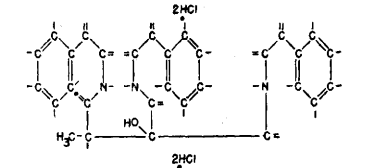
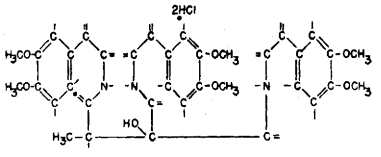
Although emetine, in its various pharmaceutical preparations, was early used in the treatment of amoebiasis(1), has retained its position among the most modern drugs in the therapy of this disease. Although its usefulness as an amoebacidal agent is seriously limited by its cumulative properties, cardiotoxic effects, and narrow margin of safety(2), many workers believe that somewhere in the structure of emetine(3-5) lies the answer to a useful amoebacidal drug.

Several attempts have been made to obtain compounds structurally related to the formula of emetine, eliminating the undesirable side-effects while retaining or enhancing the amoebacidal action of the alkaloid. Pioneer research was done by Pyman and his coworkers (6). Goodson and his associates(7-9), and Sugasawa's group(10) have helped clarify the pharmacophore of amoebacidal agents; recent contributions were made by Osbond(11). A detailed treatment of this topic is available elsewhere(12).

The present report originates from an investigation of a synthesis of amoebacidal agents structurally related to emetine, directed by Dr. George L. Webster. During the chemical study a series of phenylalkylamides of phenylalkylaminoisovaleric acids was prepared(13). A different approach to the problem is represented by quaternary emetine derivatives, whose chemistry and pharmacology have been reported(14).

The object of the present report was to de-

TABLE I. Structural Formulae of Emetine and Related Compounds.

Designation	Formula*
Emetine · 2HCl	
E–1	
E–2	
V–1	
V–2	
V–3	
V–4	

* Structure as established by Pailer and Porschinski(3), Battersby and Openshaw(4), and Evstigneeva, *et al.*(5).

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TABLE II. Relative Ranking of Selected Compounds Related to Emetine, in Terms of Amoebacidal and Bactericidal Activity, and General Toxicity.

Chemical agent	Amoebacidal endpoint (μg/ml)	Bacterial growth ($\times 10^6$/ml)	pH at end of test	Approx. LD_{50} (mg/kg) in mice, i.v.
V–3	200	485	7.8	60–70
V–1	500	120	7.8	120–125
V–4	2000	130	7.7	160–180
V–2	4000	90	7.2	160–200
E–2	500	130	7.7	16*
E–1	5000	100	7.8	9*
Emetine·2HCl	1	540	7.8	16
Control		400	7.5	

* Lasslo and Kimura(14).

termine the relative activity *in vitro* of these compounds, and to draw any pertinent conclusions relating chemical constitution to amoebacidal activity.

Materials and methods. The compounds in question were tested for amoebacidal activity *in vitro,* in comparison to emetine hydrochloride (Table I); the isovaleric acid derivatives are drawn so as to emphasize their constitutional relationship to emetine. A known antibacterial agent, aureomycin hydrochloride, was added to the series for comparison with any antibacterial compounds, in the event of uncertainty about direct versus indirect amoebacidal action of such agents.

Aqueous stock solutions were prepared of all compounds, or were weighed directly into culture tubes in those cases requiring high concentrations. The test medium was liver-egg yolk infusion (pH 7.4) plus rice starch(15). Tests were conducted with the *UC* strain of *Entamoeba histolytica* growing with 2 strains of *Escherichia coli.* The definitive experiments involved 5 ml volumes in 16 x 150 mm Pyrex tubes: 4.25 ml sterile medium + 0.5 ml drug in distilled water + 0.25 ml inoculum of pooled amoebae and bacteria. Amoebic inocula exceeded 100,000 per tube. Experimental runs extended 48 hours, record being made of amoebacidal endpoints along with bacterial populations, pH, and oxidation-reduction (O-R) potentials at those critical concentrations. In all, 9 series of experiments were conducted in determining specific endpoints.

To determine the advisability of possible further experiments *in vivo,* the comparative acute toxicity of these compounds was determined in cooperation with Miss Elizabeth H. Jenney of the Department of Pharmacology, University of Illinois. The relative acute toxicity was determined on the Harlan strain of Swiss mice, by intravenous injection into the tail vein of the animals (avg wt. 20-25 g), of 0.1-0.2 ml of solution during a period of 20-25 seconds. Owing to the limited quantities available of each compound, the small number of animals used (15 to 20 per compound) permitted only approximate estimation of LD_{50} values.

Results. The activities of the several compounds are summarized in Table II. It is clear that emetine ranks alone in its amoebacidal activity, in agreement with most *in vitro* findings(16,17). The relative standings within the 2 groups of compounds are important. *V-3* and *E-2* were distinctly most active in their respective groups. It may be noted that vioform, an accepted therapeutic agent, yielded endpoints of 100-200 μg per ml under similar testing conditions(18). Under the aerobic conditions of incubation used in fixing endpoints, the pH values remained at low alkaline levels throughout the experiments. Some antibacterial effect was exhibited above levels of 200 μg per ml, but our experience over a period of several years had indicated that minimal bacterial populations of the order of 10^7-10^8 per ml were suitable for amoebic growth.

The case of aureomycin, on the other hand, discloses the difficulty of interpretation raised by strongly antibacterial agents. At the apparent amoebacidal endpoint of 17 μg per ml under aerobic conditions, and with pH values similar to those found with other agents, bac-

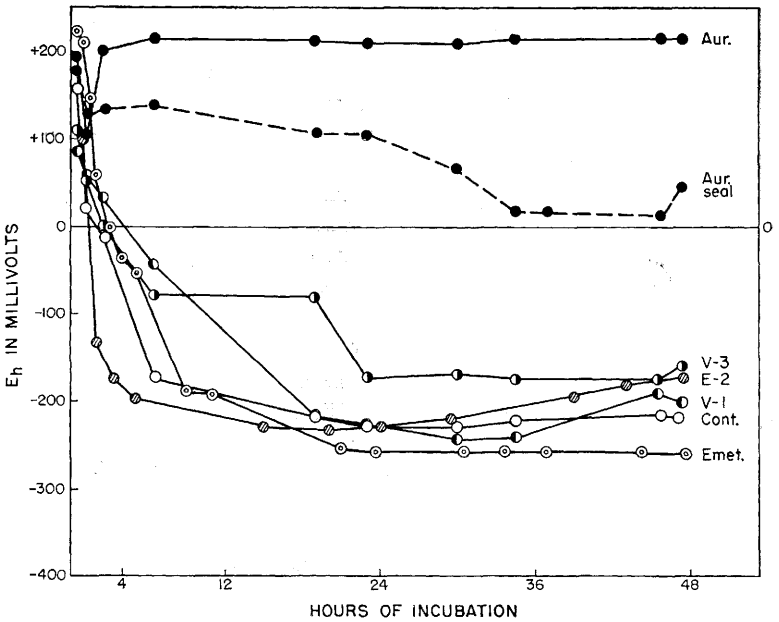

FIG. 1. Oxidation-reduction potentials (E_h) in cultures of *Entamoeba histolytica* containing amoebacidal concentrations of the following agents (experiments conducted in liver-egg yolk infusion under aerobic incubation unless otherwise specified): Cont.—control; Emet.—emetine 1 μg/ml; Aur.—aureomycin 100 μg/ml; Aur. seal—same, with petrolatum seal; E-2—500 μg/ml; V-1—500 μg/ml; V-3—500 μg/ml.

terial populations reached less than one-hundredth of the average of other agents tested. Under these circumstances it becomes impossible to separate direct from indirect action of such an agent against the amoebae, since the amoebae require the presence of metabolizing bacteria in cultures of this kind. A further complication arises from the difference in susceptibility of different floras to antibacterial agents. For example, we have shown (summarized in 17) that the *NRS* strain of *E. histolytica,* in the presence of different floras from the one used here, survived in concentrations of aureomycin as great as 200 μg per ml.

It is well known that O-R potentials, as expressed by E_h, provide a good measure of the reducing activity of microbial cultures(19), and that low potentials (*i.e.,* negative values of the order of —100 to —300 mv) form one requirement for good growth of amoebic cultures(20,21). Time-potential curves were traced for amoebacidal concentrations of emetine and the 3 most active compounds of the series (Fig. 1). These data are in agreement with those for bacterial survival, since they indicate that at amoebacidal endpoints the O-R potentials reach levels compatible with good amoebic growth.

The present experiments afforded an opportunity to note the comparative effects of aerobic and anaerobic incubation upon amoebacidal endpoints. Bradin and Hansen(21) have reported that melted-petrolatum seals greatly increase the "apparent amoebacidal endpoint"‖ of cultures containing antibacterial agents. Thus, in their experiments the amoebacidal endpoints for aureomycin in cotton-stoppered and petrolatum-sealed cultures respectively were 2 μg per ml and 50 μg per ml. This protective influence was attributed to preventing entrance of oxygen into a test medium of already lessened reducing activity, and O-R potentials were distinctly more negative under anaerobic incubation. On the other hand, as might be expected with a non-antibacterial agent, in their experiments emetine displayed a constant endpoint (10 μg per ml)

‖ *Amoebacidal endpoint* is that concentration of agent at which no viable amoebae can be recovered, either by direct examination or upon subculture into sterile culture medium.

with both kinds of incubation.

The bearing of this factor on the present results was investigated. In all cases anaerobic incubation caused a shift in amoebacidal endpoint to higher concentrations by a 2- to 5-fold factor. Whereas Bradin and Hansen had reported this effect only with antibacterial agents, we observed the same effect with compounds which were essentially not antibacterial (Table II); this also held true for emetine. A possible explanation of this discrepancy will be offered below. Time-potential curves of anaerobic cultures resembled those of Bradin and Hansen, in disclosing somewhat more negative potentials for emetine-like compounds than of parallel aerobic cultures. This effect was most striking with aureomycin (Fig. 1), where extreme bacterial inhibition exaggerated differences in reducing activity. The endpoint for aureomycin under anaerobic conditions was 100 μg per ml (*i.e.*, about 6 times greater than under the aerobic conditions reported above), despite a surviving flora of only 0.17 x 10^6 per ml (compared to 0.7 x 10^6 per ml at the same concentration under aerobic conditions) and relatively constant pH values between 7.05 and 7.30 in all aureomycin-treated cultures. It seems clear that aureomycin inhibited bacterial metabolism so drastically that bacterial populations were inadequate to maintain the amoebae.

The bacterial flora used in the present experiments were distinctly aerobic;§ thus, petrolatum-sealed control tubes contained only about one-fourth as many bacteria as aerobic controls; this was the case with all agents tested. This created the paradoxical situation of the same condition apparently both inhibiting the bacterial flora and protecting the amoebae. However, one important variable not studied critically by Bradin and Hansen could be shown to operate. In all petrolatum-sealed cultures—excepting the aureomycin series—the pH was lowered by one unit or more, presumably owing at least partly to retention of gaseous metabolites. This effect of pH on reducing the amoebacidal activity of emetine was discovered by Dobell and his colleagues(16), but frequently has been neglected since then.¶ Our data demonstrate that both experimental series of compounds behave like emetine. The indifferent effect of anaerobic incubation in the emetine tests of Bradin and Hansen may have been due to the relatively low pH existing in both kinds of incubation with their test medium and flora, and this in turn would explain the relatively high amoebacidal endpoint reported by them.

Summary and conclusions. 1. Members of 2 different series of compounds were tested against cultures of *E. histolytica* grown with *E. coli*. 2. *E-2* proved the more effective of the 2 quaternary emetine derivatives, exhibiting an amoebacidal endpoint of 500 μg per ml; this was 10 times as effective as *E-1*. 3. Of the series of phenylalkylamides of phenylalkylaminoisovaleric acids, *V-3* and *V-1* proved approximately 10 times more effective than the corresponding methoxy-substituted moieties. *V-3* was the most amoebacidal of all the above compounds, with an amoebacidal endpoint of 200 μg per ml, while the endpoint for *V-1* was 500 μg per ml. 4. The amoebacidal activity of the compounds was shown to be independent of effects upon the associated bacterial flora, as measured both by surviving bacterial populations and oxidation-reduction potentials of the cultures. This activity was definitely influenced, however, by the pH during tests, more alkaline conditions being correlated with greater effectiveness. 5. Parallel tests with aureomycin, included to reveal effects of a distinctly antibacterial agent, supported the conclusion that, unlike aureomycin, the present series of compounds had acted directly against the amoebae.

Some concluding observations on structure-activity relationship. Quaternization of both nitrogens in the emetine molecule (*E-1*)

§ On the other hand, the flora used by Bradin and Hansen consisted of a single *Clostridium*-like anaerobe (organism *t*) with relatively fastidious nutritional requirements.

¶ At present it is not possible to separate the biological and chemical phases of this effect of pH. The influence of pH on emetine itself in media is not entirely clear. On a physico-chemical level the hypothesis might be advanced that the solvation, and therefore the relative effectiveness, of emetine varies directly with the hydrogen-ion concentration of its medium,

yielded weak amoebacidal action. *E-2,* with only one quaternized nitrogen, proved a considerably stronger amoebacidal agent. It might be concluded that quaternization of the nitrogen atoms in the emetine molecule decreases amoebacidal activity, the number of quaternized nitrogen atoms being inversely proportional to amoebacidal action. Correlation of the pharmacologic studies on the quaternary emetine derivatives(14) with the amoebacidal tests indicates that, although quaternization of both nitrogens produced a compound lacking the cumulative and cardiotoxic effects of the original alkaloid, this derivative exhibited a strong curare-like action but only weak amoebacidal properties. Under these circumstances, a promising lead might be offered by a partially quaternized emetine derivative, which could reach a therapeutically active level in the host's tissues without causing a curare-like paralysis of the neuromyal junctions of the striated musculature.

In the other series of compounds, our data indicate that 3,4-dimethoxy substitution of phenyl radicals decreased substantially the amoebacidal action (*V-2, V-4*). Furthermore, the phenylalkylamides of the monophenylalkylaminoisovaleric acids (*V-1, V-2*) were less amoebacidal than the corresponding phenylalkylamides of the bis-substituted ones (*V-3, V-4*). It should be noted, however, that the influence of the epoxy group and the corresponding hydroxy function is not known and cannot be determined from this series of compounds alone.

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