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# THE THERAPEUTIC ACTION OF SOME KNOWN AMOEBICIDES IN RATS

BY

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Three main types of drug are used in the treatment of human amoebiasis: firstly, drugs of the emetine type, e.g. emetine hydrochloride, emetine bismuth iodide, and auremetine; secondly, arsenical drugs, e.g. carbarsone and stovarsol; and thirdly, halogenated hydroxyquinolines, e.g. chiniofon, vioform, and diodoquin. Opinions differ concerning the respective merits of these three types; indeed the general opinion seems to be that no one type is entirely satisfactory in itself, and that the best clinical results are obtained by the judicious use of all three. As we now have a technique for evaluating the anti-amoebic properties of drugs, using experimentally infected rats (Jones, 1946), it was considered of interest to make a close comparison of the most commonly used of the above types. Such a comparison would also serve to supply standards against which any newly discovered anti-amoebic drug could be compared.

The drugs selected for the comparison were emetine hydrochloride, chiniofon, stovarsol, carbarsone, and diodoquin. They were compared when given as a single dose, and also when given according to a multiple-dose schedule.

## Experimental Work

Several experiments were carried out, each involving the use of 144 recently weaned rats weighing approximately 20–33 g. In each experiment the rats were separated into six groups of matched weights. They were then injected intracaecally, after laparotomy, with 0.25 c.c. of a suspension of *Entamoeba histolytica* in 5 per cent gastric mucin. The amoebae were cultivated in the enriched serum-buffered saline medium described previously (Jones, 1946).

Two different dosage schedules were employed: in the first a single dose was given 24 hours after the operation, and in the second doses were given 24, 30, 48, 54, and 72 hours after the operation. All doses were given orally, by means of a metal catheter. Emetine and chiniofon were given as solutions in water, and stovarsol, carbarsone, and diodoquin as finely dispersed suspensions made by ball-milling for several hours with a suitable dispersing agent (1 per cent Dispersol O.G., I.C.I.).

In order to assess the therapeutic effect of the test drug the rats were killed six days after the operation. After this time the infection in the control group is maximal, and any therapeutic effect in treated groups is therefore most readily detectable. Careful post-mortem examinations were made of each rat in the experiment, and according to the degree of infection found a score was allocated. The six standard degrees of infection are shown in Fig. 1.

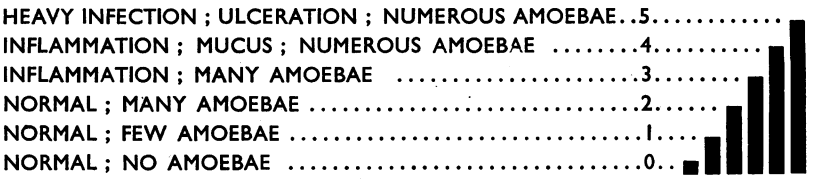


FIG. 1.—The degree of amoebic infection found in the caecum of experimentally infected rats.

An average degree of infection (ADI) was calculated for each group by finding the average of the individual scores.

The statistical significance of a treatment effect may be assessed according to the following formula:

$$z = \frac{x - y}{\sqrt{\frac{\sigma^2 x}{m} + \frac{\sigma^2 y}{n}}}$$

where $x$ and $y$ are the ADIs for the control and treated groups respectively.

$\sigma x$ and $\sigma y$ are the standard deviations for $x$ and $y$ respectively, and $m$ and $n$ are the numbers of rats in the control and treated group respectively.

The values of $\sigma x$ and $\sigma y$ used in the above formula were read from a curve derived from the results of a large number of control and treated groups (Jones, 1946).

In this series of experiments most of the drugs, when given at the highest dose, showed a therapeutic effect of high statistical significance ($P<0.01$). This value ($P<0.01$) was therefore taken as the standard representing definite positive therapeutic effect (+). Therapeutic effects of significance $P=0.05$ to $P=0.01$ were regarded as indicating slight though definite therapeutic effect (±); whilst effects of $P>0.05$ were regarded as indicating insignificant therapeutic effect (−).

The results of the comparisons are recorded in detail in Figs. 2 and 3, and summarized in the Table.

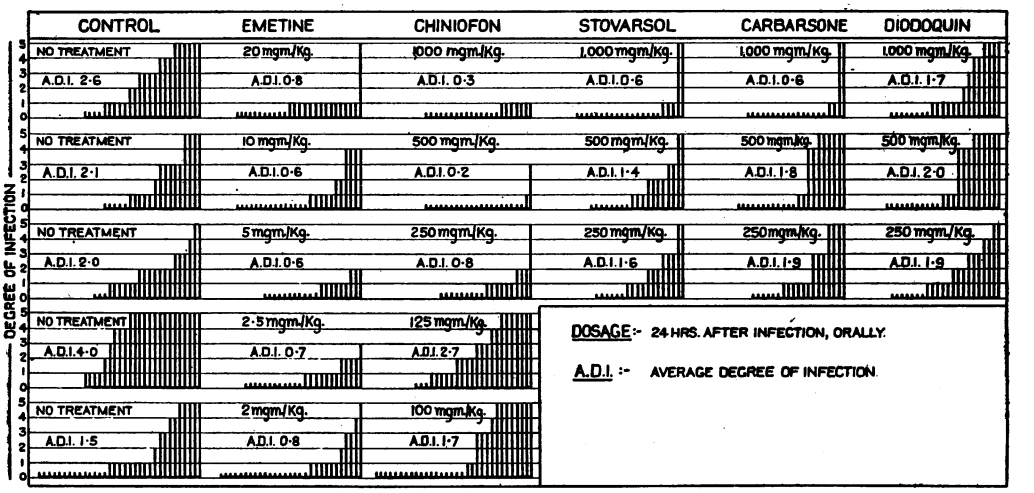


FIG. 2.—The effect of drugs on experimental amoebiasis in rats. Single-dose therapy.

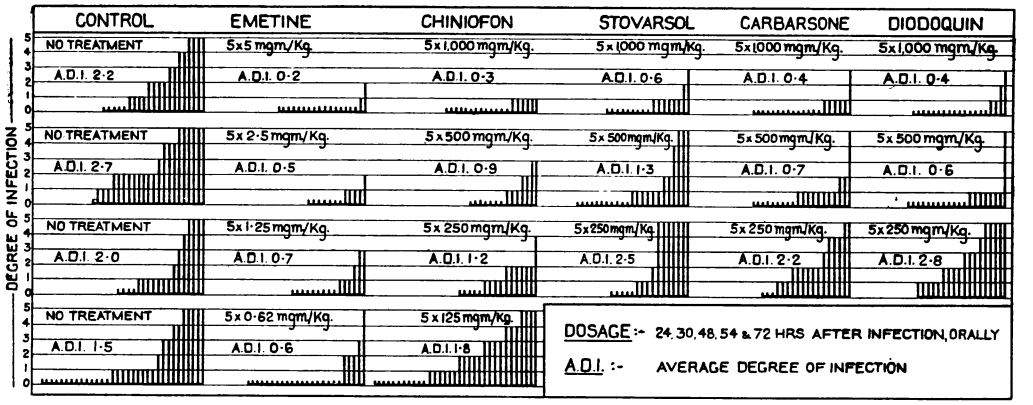


FIG. 3.—The effect of drugs on experimental amoebiasis in rats. Multiple-dose therapy.

TABLE

THE EFFECT OF DRUGS ON EXPERIMENTAL AMOEBIASIS IN RATS

| | Dose, mg./kg. orally 24 hr. after operation | | Significance of treatment + = P < 0·01. ± = P, 0·01–0·05. − = P > 0·05 | | | | |
|---|---|---|---|---|---|---|---|
| | Emetine | Chiniofon, etc. | Emetine | Chiniofon | Stovarsol | Carbarsone | Diodoquin |
| Single dose therapy | 20 | 1000 | + | + | + | + | ± |
| | 10 | 500 | + | + | − | − | − |
| | 5 | 250 | + | ± | − | − | − |
| | 2.5 | 125 | + | ± | .. | .. | .. |
| | 2.0 | 100 | − | − | .. | .. | .. |
| Multiple dose therapy | orally 24, 30, 48, 54 and 72 hr. after operation | | | | | | |
| | 5 | 1000 | + | + | + | + | + |
| | 2.5 | 500 | + | + | ± | + | + |
| | 1.25 | 250 | ± | − | − | − | − |
| | 0.62 | 125 | ± | − | .. | − | .. |

## DISCUSSION

In comparing the results of our experiments in rats with the results obtained in human amoebiasis with the same drugs, we must consider certain important differences between the infections in the two species. Whereas in rats the infection is an acute one and is reasonably standardized, in human amoebiasis there is not only the acute disease but also a chronic phase of widely varying symptomatology. In the latter form of the disease we may have also to deal with the cystic form of the parasite, against which there is as yet no suitable means of testing drugs experimentally. If we compare our results with the results usually obtained in acute amoebic dysentery in man we find reasonable agreement. Thus emetine and chiniofon appear to be the most effective, with stovarsol,

carbarsone, and diodoquin definitely inferior. If, however, we consider the respective merits of these drugs in chronic amoebiasis, we find them at variance with our experimental results, for against this form of the disease, carbarsone, stovarsol, and diodoquin are undoubtedly of value. The minimal effective therapeutic dose of this type of compound in rat and man differs considerably if the comparison is made on a mg./kg. basis. Thus a dose of 1,000 mg./kg., or 5 doses of 500 mg./kg., is required to produce an effect in rats, whereas the dosage used in humans, namely 4 gr. twice daily for 10 days (Manson-Bahr, 1945), corresponds to a total dosage of only 80 mg./kg. The validity of such a comparison, however, is questionable, and it is perhaps more reasonable to consider the relationship of the minimal effective therapeutic dose to the toxic dose in the two species. If this is done, the relationship is found to be approximately the same.

It is of interest to note the behaviour of diodoquin in our tests. When it was given as a single dose, its therapeutic effect was barely significant, and compared unfavourably with carbarsone and stovarsol. When it was given repeatedly, however, its activity was better demonstrated. The fact that it is poorly absorbed was no doubt responsible for this difference. This compound has been introduced comparatively recently (Hummel, 1939). It does not appear to be much more effective than the other amoebicides (Morton, 1945).

The strain of *E. histolytica* used in this series of experiments (isolated in culture from material kindly supplied by Dr. A. R. D. Adams) was one which produced infections susceptible to treatment with emetine. Not all strains do so, as has been mentioned in a previous paper (Jones, 1946). It was decided to use an emetine-susceptible strain for comparison as there was no evidence that the other drugs showed similar differences in effectiveness against different strains. The comparison was accordingly carried out under conditions equally favourable to all the test drugs.

## SUMMARY

A study has been made of the therapeutic action of emetine, chiniofon, stovarsol, carbarsone, and diodoquin against experimental amoebiasis in rats. Emetine and chiniofon appeared to have the widest range of activity. Stovarsol, carbarsone, and diodoquin were effective when given in large doses.

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## REFERENCES

Hummel, H. G. (1939). *Amer. J. digest. Dis.*, **6**, 27.
Jones, W. R. (1946). *Ann. trop. Med. and Parasitol.*, **40**, 130.
Manson-Bahr, P. H. (1945). *Manson's Tropical Diseases*, 13th edit., London: Cassell and Co., Ltd.
Morton, T. C. St. C. (1945). *Brit. med. J.*, **1**, 831.