Failure of Two Strains of *Endamoeba histolytica* to Develop Resistance To Amoebacidal Agents *In Vitro*. (19523)

JAMES G. SHAFFER AND JAMES E. WASHINGTON.

From the Department of Microbiology, University of Louisville, School of Medicine, Louisville, Ky.

A point of critical importance in the use of antibiotics and other compounds in the treatment of infectious diseases is the ability of the infectious organism to develop resistance to the therapeutic agent. The tendency of certain susceptible bacterial strains to become resistant to streptomycin, sulfonamides, and certain other commonly used drugs is well known. So far, little is known about the ability of *Endamoeba histolytica* to develop such resistance.

This brief report will describe attempts to demonstrate the development of resistance to aureomycin, terramycin and emetine HCl by two strains of *Endamoeba histolytica*.

Material and methods. The Luna† and 200‡ strains of *Endamoeba histolytica* were used in the experiments to be described and the substrate was the Shaffer-Frye (S-F) medium(1). The *S-F medium* was made up in the usual manner and sufficient aureomycin, terramycin, or emetine HCl added to bring the final concentration to the proper level. It was desirable to use a concentration sufficiently high to markedly inhibit propagation of the amoebae, but still allow enough propagation to be able to make serial transplants. The amounts used were arrived at by a series of trials in which serial transplants were attempted in the presence of varying concentrations of the drugs to be used. It was found that the desirable concentrations were, aureomycin 0.013 mg/ml, terramycin 0.04 mg/ml, and emetine HCl 0.0072 mg/ml of completed medium. These amounts allowed but minimal propagation of the amoebae, but it was possible to maintain serial 48- to 72-hour transplants of the two strains. In terms of actual numbers, the amoebae propagated to 1/8 to 1/4 the numbers usually seen in S-F medium without drugs. After 10, 20, 30 and 38 transplants, drug sensitivity tests were done on the two strains being studied using the method of Biegeleisen and Shaffer(2). Simultaneous tests were done on the normal Luna and 200 strains and the results compared. In preparation for the *sensitivity tests*, the amoebae were transplanted from the drug-containing medium to routine S-F medium for one transplant. From this transplant inoculations were made into 7 tubes of S-F medium and these cultures were incubated at 37°C. A similar series of tubes was inoculated with "normal" amoebae. At the end of 48 hours the cultures were examined for amoebae and the readings recorded. Serial 2-fold dilutions of the drugs were then added in a volume of 1 ml to the first 6 tubes, and 1 ml of saline (0.85% NaCl) was added to the seventh tube as a control. The cultures were reincubated at 37°C and examined after 24 and 48 hours. The relative numbers of amoebae remaining at the time of examination were recorded.

Periodically, during the course of the 38 transplants, attempts were made to increase the concentrations of the drugs. This was done by adding extra culture tubes containing more drug and attempting to make serial transplants. In no case was this successful, even small increases in the drug concentrations resulted in loss of the transplants after 1 or 2 transfers.

Results. The results of the drug sensitivity tests done on the experimental and "normal" *E. histolytica* of the Luna and 200 strains are shown in Table I.

Examination of these results reveals that

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† The Luna strain of *Endamoeba histolytica* was isolated by J. G. Shaffer in 1948 from a patient with acute amoebic dysentery directly from feces into S-F medium. It has never been propagated in any other medium.

‡ The 200 strain of *Endamoeba histolytica* was obtained in 1950 from Mr. M. C. McCowen of the Lilly Research Laboratories. It was obtained by Mr. McCowen from Dr. C. W. Rees of the National Institute of Health.

TABLE I. Results of Drug Sensitivity Tests on the Luna and 200 Strains of *E. histolytica* Transplanted in Presence of Low Concentrations of Aureomycin, Terramycin, and Emetine HCl Compared with Sensitivity of Unexposed Amoebae of the Same Strains.

	Sensitivity											
	Luna strain						200 strain					
Transplant	Aureomycin* (mg/ml)		Terramycin (mg/ml)		Emetine HCl (mg/ml)		Aureomycin (mg/ml)		Terramycin (mg/ml)		Emetine HCl (mg/ml)	
	N.†	E.‡	N.	E.	N.	E.	N.	E.	N.	E.	N.	E.
10	.0332	.0332	.1329	.1329	.0216	.0216	.0332	.0332	.1329	.1329	.0216	.0432
20	.0332	.0166	.2658	.2658	.0432	.0432	.0166	.0332	.1329	.1329	.0216	.0216
30	.0664	.0332	.1329	.1329	.0108	.0108	.0332	.0166	.0664	.0329	.0108	.0216
38	.0332	.0332	.2658	.2658	.0216	.0216	.0166	.0166	.1329	.1329	.0216	.0432

* Concentrations in these columns resulted in a decrease of at least 75% in the number of amoebae seen in cultures after 48 hr.

† "Normal" *E. histolytica* not exposed to the drugs.

‡ Experimental *E. histolytica* transplanted in the presence of partially effective concentrations of the drugs.

in no case was there more than a 2-fold difference in the concentration of a compound found capable of destroying 75% or more of the "normal" or experimental cultures. In 5 instances the concentration found effective in the experimental cultures was twice that found effective in the "normal" cultures, in 3 instances the reverse was true, and in 15 instances the concentrations were identical. Thus, the differences observed were probably due to experimental error and no evidence of the development of resistance by either strain of *E. histolytica* was observed. The inability to maintain transplants in the presence of drug concentrations somewhat above those routinely used, as mentioned previously, is also interpreted as evidence of failure to develop resistance.

Discussion. The method used in these experiments for testing the drug sensitivity of *E. histolytica* has the disadvantage that there are still a certain number of viable bacterial cells in the cultures at the time the compounds are added(2). However, evidence has been obtained(3) which indicates that the effects observed using this technic are the result of activity of the drugs on the amoebae rather than the bacteria. If this be true, then the results shown in the table are suggestive that *E. histolytica* does not readily develop resistance to aureomycin, terramycin, or emetine HCl *in vitro*.

Summary. Two strains of *E. histolytica* failed to show evidence of the development of resistance to aureomycin, terramycin or emetine HCl after 38 serial transfers in S-F medium containing partially effective concentrations of the agents.

1. Shaffer, J. G., Ryden, F. W., and Frye, W. W., *Am. J. Hyg.*, 1949, v49, 127.

2. Biegeleisen, J. Z., and Shaffer, J. G., *Am. J. Hyg.*, 1951, v53, 146.

3. Unpublished data.

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Viability and Dispersion of BCG Inoculated Subcutaneously in Guinea Pigs. (19524)

KONRAD BIRKHAUG, DORIS MCGLYNN, AND MARY E. CLARK.

From the Division of Laboratories and Research, New York State Department of Health, Albany.

Studies on the fate of BCG in guinea pigs inoculated by the intradermal route(1), multiple puncture(2,3), and by scarification(4,5) have shown that BCG were dispersed to regional lymph nodes within one week and to the spleen and distant lymph nodes in 2 to 4 weeks and that they may remain viable in the latter organs up to 6 months. Van Deinse(6)