# Ability of *Endamoeba histolytica* to Phagocytose Red Blood Cells.* (20773)

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The ability of *Endamoeba histolytica* to ingest red blood cells is a well-recognized characteristic. The following experiments were designed to study this property quantitatively and to determine what the effect of certain drugs and of antiserum might be on this phagocytic tendency.

*Materials and methods.* It was found that reproducible phagocytic indices could be obtained in the following manner: Three-tenths ml of a one in 10 dilution of defibrinated rabbit blood was added to 48-hour cultures of the 200† strain of *Endamoeba histolytica* in the Shaffer-Frye medium(1). Only cultures showing 4+ amoebae were used. The tubes were gently agitated to distribute the RBC and the petrolatum seal restored. These amoeba-RBC mixtures were then incubated at 37°C for 15 minutes. At the end of this period, most of the culture fluid was removed and discarded. Ten ml of distilled water was then gently added. This hemolysed all unphagocytosed RBC, but did not disrupt the amoebae and left the phagocytosed RBC intact. Samples were removed from the bottom of the tube and the number of RBC in 25 amoebae counted and averaged. The resulting phagocytic indices were constant to within ±1 RBC over a series of similar tubes run in a given experiment. It was found that after 30 minutes in the distilled water the RBC began to appear distorted, thus, it was important to set up the tubes individually allowing an interval between each succeeding culture to provide time for counting. The rabbit blood was collected by cardiac puncture and defibrinated by shaking with glass beads. Blood kept more than 5 or 6 days was found undesirable. Hank's balanced salt solution (Microbiological Associates, Bethesda, Md.) was used as a diluent for all reagents. All materials were brought to 37°C before addition to the cultures.

A series of experiments was next designed to study the influence of certain compounds on the ability of the 200 strain of *Endamoeba histolytica* to phagocytose rabbit RBC. Aureomycin HCl, terramycin HCl, ilotycin, fumagillin and emetine HCl were used. The first 4 compounds were the pure crystalline substances, while the fumagillin was in the form of 10 mg capsules (Abbott). The crystalline compounds were weighed on an analytical balance and dissolved to appropriate concentration in the balanced salt solution, while the fumagillin was made up to the desired volume and thoroughly emulsified from the capsule. Twelve 48-hour cultures of *Endamoeba histolytica* were used in each experiment. A series of 5 drug solutions was made to contain appropriately decreasing amounts, so that when 0.2 ml was added to a culture, the desired final concentration was reached. Each of the 5 solutions was added through the petrolatum seal to a pair of amoeba cultures and 0.2 ml of Hank's solution was added to an additional pair of cultures as controls. All were gently mixed and re-sealed. One of each pair of cultures was incubated at 37°C for 2 hours and the other member was incubated for 20 hours. At the end of the incubation period, rabbit RBC were added and the phagocytic index determined. The results of the experiments were recorded as the phagocytic index for each drug concentration and the control, and graphs made, with the drug concentrations on the ordinates and the phagocytic indices on the abscissas. In figuring the phagocytic index, only those amoebae containing RBC were included in the experiments here described. The numbers of trophozoites which did not phagocyte RBC varied greatly between similar cultures and appeared to be unrelated to drug con-

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† The 200 strain of *Endamoeba histolytica* was obtained from Mr. M. C. McCowen of The Lilly Research Laboratories. He originally obtained the culture from Dr. C. W. Rees of the National Institutes of Health.

centrations or other measurable factors. Further investigation of this is necessary.

Another set of experiments was done to test the effect of antiserum on the ability of the amoebae to phagocytose RBC. Anti-amoebic serum was produced in 2 rabbits by giving a series of 8 bi-weekly intravenous injections of concentrated suspensions of *E. histolytica,* strain 200. Each inoculation consisted of 2 to 3 million *E. histolytica* trophozoites prepared from 48-hour cultures in S-F medium by washing once with saline (0.85% NaCl) and concentrating in a volume of one to 2 ml. Blood was collected from the rabbits by cardiac puncture before the series of inoculations was started and 13 days after the final inoculation. The serum was stored in the deepfreeze. The technic was modified when testing the effect of serum on the phagocytic activity of the *E. histolytica* cultures in order to conserve serum and was as follows: Forty-eight-hour cultures of the amoebae were used as before. Just prior to addition of the serum, most of the culture fluid was removed leaving a previously determined volume so that a desired final concentration of serum could be obtained. Thus, if the effect of serum 1:8 was to be determined, 1.75 ml of culture was left and 0.25 ml of serum added. Other desired dilutions could be reached in a similar manner, keeping the final volume of fluid constant in the tubes. After addition of the serum, the cultures were sealed with vaseline and incubated at 37°C for 2 hours. At the end of this incubation period, rabbit RBC were added and phagocytic indices calculated as previously described.

*Results.* The results of the experiments on drugs are illustrated graphically in Fig. 1-3. Each of the graphs is a composite of 4 or 5 individual experiments with one of the compounds studied. The starred circles represent the phagocytic indices obtained after 2 hours exposure to the drug and the solid black circles represent the phagocytic indices obtained after 20 hours exposure to the drug. The broken line connects the midpoints of the 2-hour indices and the dotted line connects the midpoints of the 20-hour indices.

Fig. 1 shows the results obtained with

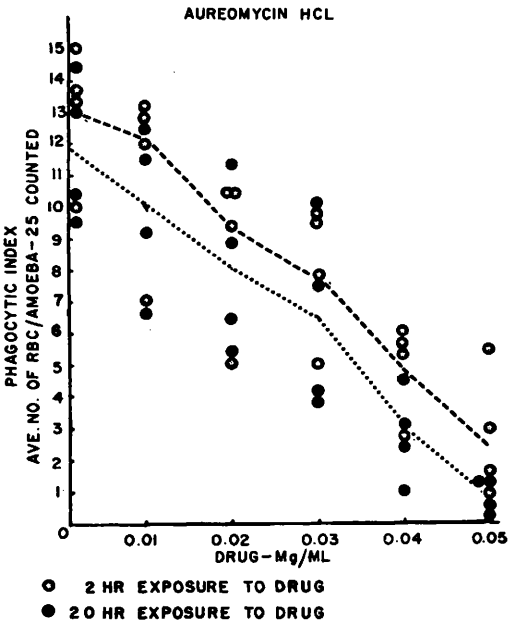


FIG. 1.

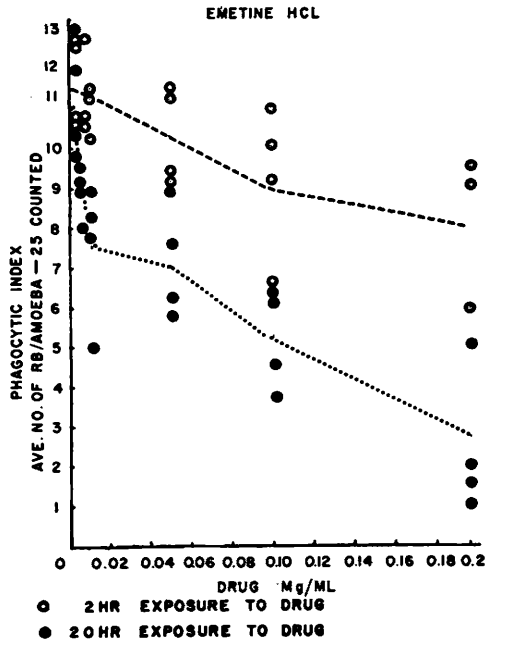


FIG. 2.

aureomycin HCl. It will be seen that, as the concentration of drug was increased, the phagocytic index showed a steady decline. Essentially similar decreases occurred whether there was 2- or 20-hour exposure to the drug.

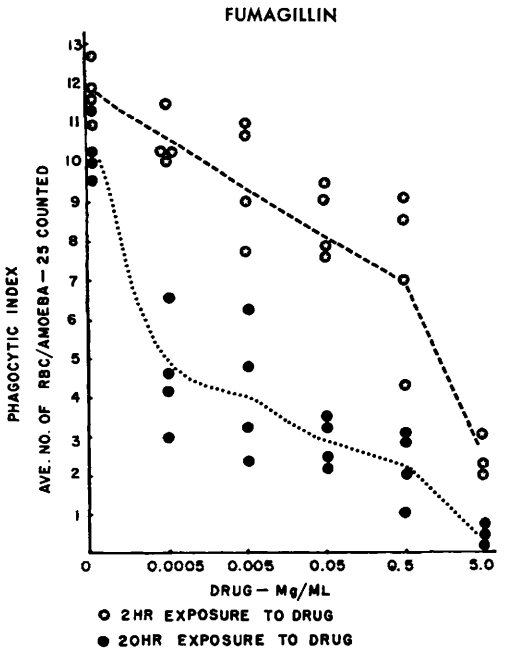


FIG. 3.

Fig. 2 shows the results obtained with emetine HCl. With this drug there was a difference between the 2- and 20-hour phagocytic indices. It was found in preliminary experiments that the range of concentrations showing little or no activity and that showing marked activity, especially at 20 hours, was rather narrow. Thus, it was desirable to use closely graded concentrations in the lower range. An examination of Fig. 2 reveals that a relatively greater reduction of phagocytic index occurred after 20 hours than after only 2 hours exposure to the various concentrations of emetine HCl.

Fig. 3 shows the results obtained with fumagillin. It will be seen that there is an even greater difference between the 2- and 20-hour results with fumagillin than observed in the case of emetine HCl. As was also true with aureomycin HCl and emetine HCl, increases in the concentration of fumagillin resulted in decreased phagocytic indices. It will be noted, however, that the range of drug concentrations exhibiting minimal and maximal activity was much greater with fumagillin than with either aureomycin HCl or emetine HCl.

Results obtained with terramycin HCl were of some interest since the general pattern fell between that of aureomycin HCl and emetine HCl. There were differences between the results on 2- and 20-hour exposure, but not as marked as those seen with emetine HCl as shown in Fig. 2. The range of this activity with terramycin HCl fell between 0.05 and 0.3 mg/ml, a relatively narrow range.

Ilotycin was found to have a pattern similar to that of aureomycin HCl, with little difference in the phagocytic indices after 2- and 20-hour exposure of the amoebae to the various concentrations. The range of this activity with ilotycin was from 0.25 to 1.25 mg/ml.

The results of the studies made using the normal and anti-*E. histolytica* (strain 200) rabbit serum with 4 different strains of *E. histolytica* are shown in Table I. These results reveal that incubation of the 4 strains of *E. histolytica* for 2 hours in the presence of the rabbit antiserum diluted 1:8 resulted in a definite reduction in the phagocytic index. The normal serum of rabbit No. 1 exerted little, if any, effect, while the normal serum of rabbit No. 2 reduced the phagocytic index moderately. Thus, the inoculation of these rabbits with suspensions of strain 200 *E. histolytica* resulted in increased ability of their serum to inhibit phagocytosis of RBC by not only strain 200, but also strains 103, Cook and K-14, *E. histolytica*. In further experiments it was found that exposure of the amoebae to the antisera diluted 1:16 had little effect on the phagocytic index. Results in a limited number of experiments with normal and "immune" serum, 1:4, were inconsistent.

TABLE I. Phagocytic Indices Obtained Following Exposure of 48-Hr Cultures of Four Strains of *E. histolytica* to Anti-Amoebic Rabbit Serum for 2 Hours at 37°C.

| Rabbit serum (anti-200) (1 in 8 dilution) | Phagocytic index* *E. histolytica* strain | | | |
|---|---|---|---|---|
| | 200 | 103 | Cook | K-14 |
| #1 Normal | 12 | 10 | 11 | 11 |
| "Immune" | 3 | 3 | 2 | 4 |
| #2 Normal | 9 | 9 | 9 | 9 |
| "Immune" | 3 | 4 | 3 | 3 |
| Control (no serum) | 13 | 10 | 14 | 10 |

* Avg No. of RBC/amoebae in a total of 25 amoebae.

Since the antisera were produced by inoculation of *E. histolytica* grown in the S-F medium which depends on the presence of bacterial cells of a gram negative anaerobic streptobacillus, it was desirable to adsorb the sera with concentrated suspensions of these bacteria to see if this affected the ability of the serum to reduce the phagocytic index. The adsorbed "immune" and normal sera exhibited the same activity as similar unadsorbed sera. So far, no tests have been made using anti-streptobacillus serum.

*Discussion.* The significance of the observations recorded in Fig. 1-3 is not yet known. It seems likely that the results reflect a variation in the behavior of the compounds involved. This variation may be in terms of fundamental differences in the point of attack on the amoebae, selective effects on the most actively phagocytic cells by aureomycin HCl, but not emetine HCl and fumagillin, or any one of a number of other possibilities. Further experimental and statistical examinations are necessary before definite conclusions can be reached.

Tabulation of the distribution of RBC per amoeba in the individual experiments has revealed some interesting data. The number of RBC per trophozoite varies from none to as high as 30. Plotting of these figures indicates a normal distribution with a low peak at the level of the median which has in all calculated cases been within one of the mean or phagocytic index. Consequently, the standard deviation is likely to be high. The reasons for the variation in numbers of phagocytosed RBC in individual amoebae are not known. It may be that this is a reflection of the metabolic or developmental state of the individual organisms. The irregularity of phagocytic activity exhibited by older cultures may present evidence favoring this viewpoint.

Insufficient experiments have been performed with anti-amoebic rabbit serum to determine the nature of this activity. It would seem likely that an antibody to some surface component of the cell is present in the serum as a result of the inoculation of the rabbits. The results shown in Table I show that all 4 strains of *E. histolytica* tested were affected by the serum. The technic is not yet standardized to the point where it can be determined whether or not there are antigenic differences between the strains. Further experiments, designed to produce higher titered sera and to further standardize the procedure, are in progress.

Cole and Kent(2) have recently published the results of amoeba-immobilization tests in which they tested the effect of anti-amoebic rabbit serum and the serum of human patients with amoebiasis for ability to cause *E. histolytica* to round up. It would seem that immobilization might be reflected directly in loss of ability to ingest particles, thus the two methods appear likely to be testing similar phenomena.

It is felt that the methods described above may offer an objective approach in studying certain fundamental characteristics of *E. histolytica* and in understanding the mode of action of anti-amoebic drugs and of antiserum. The technic itself needs further study and standardization as indicated before. It may be that the phagocytic index is not the most desirable unit to use, but this can only be determined by future experiments.

*Summary.* 1. A method for determining phagocytic indices for *E. histolytica* with rabbit red blood cells has been described. 2. The effect of several drugs on the ability of *E. histolytica* to phagocytose rabbit RBC has been tested and recorded. 3. All drugs tested were found capable of reducing the ability of the amoebae to phagocytose rabbit RBC. A range for this activity was established under the conditions used. 4. Certain differences in the effect of the different compounds were observed. 5. The effect of anti-amoebic serum on the phagocytic indices of 4 strains of *E. histolytica* was tested. It was found that definite reductions could be obtained.

1. Shaffer, J. G., *Am. J. Hyg.*, 1952, v56, 119.

2. Cole, B. A., and Kent, J. F., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 811.

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