with the decreased levels of reduced glutathione observed in older erythrocytes of rabbits with acetylphenylhydrazine-induced anemia(11,12), of fava bean-sensitive subjects (13) and of a group of hematologically normal adults(14). The finding that erythrocyte pyrophosphatase activity of normal newborn infants does not differ significantly from that of adults is compatible with the observations that there is no significant difference in glutathione content between the red cells of newborns and of adults(15).

Summary. Erythrocyte inorganic pyrophosphatase activity was measured on 90 samples of human blood. The enzyme activity was found to be similar in normal newborn infants and normal adults. Erythrocyte pyrophosphatase activity was increased in infants affected with Rh and ABO hemolytic disease. When erythrocytes were separated into light and heavy cell fractions, pyrophosphatase activity was increased in the lighter fraction with higher reticulocyte content.

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Effects of Amebicides on Growth of *Acanthamoeba* sp. (30824)

MADGE A. PFAFFMAN* AND RICHARD L. KLEIN†

Department of Pharmacology, University of Mississippi Medical Center, Jackson

The effects of 3 chemically distinct types of amebicides: carbarsone, a pentavalent arsenical; emetine, an alkaloid of *Ipecacuanha*; and glaucarubin, derived from the fruit of *Simarouba glauca*, were tested during the initial 14-day period of growth of *Acanthamoeba* sp. under axenic conditions. This organism is a small free-living, usually non-pathogenic, soil amoeba which can be easily grown in large numbers in a relatively well-defined medium. Certain strains of *Acanthamoeba* sp. which have similar appearance under the light microscope are known pathogens and can cause death in rodents and monkeys(1, 2). This genus has also been isolated from routine throat cultures of humans.

For these reasons, a study of the growth inhibiting effects of amebicides on this amoeba seemed warranted as a first step to future comparisons of the biochemical and ultrastructural differences which may exist between the free-living and pathogenic forms of this genus. There is some morphological evidence that a fundamental relationship does exist between parasitic and free-living forms of amoebae(3).

* Present address: Dept. of Physiology, Duke Univ., Durham, N. C.

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TABLE I. Comparison of the Effects of 3 Amebicides on *Acanthamoeba* sp.; Data Are an Average of at Least 8 Separate Experiments with Each Drug. S.D. = standard deviation.

Drug	Dose causing 50% reduction in total cells at 4 days	% increase in MGT at previous dose	*In vitro* estimated LD_{50} for *Acanthamoeba*	*In vitro* reported amoeba endpoint for *E. histolytica*
		(range)	(S.D.)	
Carbarsone	.28 mM	23 (11.7-38.9)	.44 mM .19	.38 mM
Emetine	.60 mM	54 (32.7-76.3)	1.31 mM .36	.01 mM
Glaucarubin	2.16 mM	22 (14.5-31.3)	2.61 mM .60	3.73 mM

Methods. Amoebae were cultured in 250 ml Erlenmeyer flasks, which had previously been treated with Dow-Corning high vacuum grease, a silicone lubricant, which was essential to prevent the cells from sticking to the sides of the containers. The liquid growth medium was of the following composition: 1.5% proteose peptone (Difco Certified), 1.5% dextrose, 120 mg NaCl, 3 mg each of $MgCl_2 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$ and $CaCl_2$ anhydrous, 0.002 M KH_2PO_4-K_2HPO_4 at pH 5.5 (adjusted with HCl), and distilled water to one liter(4). Five ml of prepared inoculum were added to 25 ml of sterile growth medium, with and without drug, so that each ml contained a final concentration of approximately 25,000 cells per ml. Drug solutions were sterilized by filtration through an ultrafine presterilized bacteriological vacuum filter. Flasks were incubated at 30-32°C and sampled at various times during growth by removing one ml of medium for counting. The use of single flasks from which multiple samples were taken or separate flasks for each sample gave no difference in growth curves. Counts were made using a Levy chamber with French-Rosenthal rulings. Five mm^2 areas were counted in each ruling, and this was performed in quadruplicate for each ml sample. In addition all experiments were performed at least in duplicate. Growth curves were corrected for an average of 1.5% evaporation per day as determined from volume control flasks.

Glaucarubin from Messengill Co. and carbarsone and emetine from Eli Lilly Co. were kindly donated.

Results. Average data (16 exp.) from control cultures initially inoculated to 25,000 cells per ml showed an increase of 96,000 cells per day and a mean generation time (MGT) of 26-28 hours during the midlogarithmic phase of growth at about 4 days after incubation. The growth inhibiting effects and estimated LD_{50} of each drug on *Acanthamoeba* were compared with the endpoint concentrations (100% lethal) on *Entamoeba histolytica* as reported in the literature(5,6). The data are shown in Table I.

All the drugs were found to cause an initial lethal effect followed by an inhibition of growth rate of the surviving cells. Growth inhibiting effects were calculated on the basis of changes in MGT at 4 days of incubation. Growth curves were nearly linear up to this time in control and in drug flasks, except for the initial lethal effect of the drugs which occurred between 4 and 12 hours after inoculation. Therefore, one could calculate not only the increase in MGT from the slope of the growth curve, but also what fraction of the total decrease in cell numbers was due to initial lethal effects, and thereby, estimate an LD_{50}.

Discussion. Kaushiva(5,6) reported that arsenical derivatives of certain compounds have shown a high correlation between the sensitivities of *E. histolytica* and certain free-living amoeba. Our results support this contention using carbarsone. The present data also agree with previous reports that free-living amoebae are much more tolerant to emetine than is *E. histolytica*. With *Acanthamoeba* a dose at least 2 orders of magnitude higher is needed to produce comparable lethal effects. As reported for *E. histolytica* glaucarubin is also least effective against *Acanthamoeba* on a molar basis; however, this drug is the most effective of the 3 on *Acanthamoeba* when compared to the amebicidal endpoint for *E. histolytica*.

Summary. Three well-known amebicides,

carbarsone, emetine and glaucarubin were tested against *Acanthamoeba* sp., a small free-living soil amoeba. The drugs have an initial lethal effect followed by a lesser inhibition of growth rate. On a molar basis, carbarsone is the most effective agent against *Acanthamoeba*. The results support the contention that emetine is more potent against the pathogenic *E. histolytica* than against free-living amoebae, whereas, carbarsone has similar inhibitory effects on the two types. Glaucarubin, although the least effective against *Acanthamoeba* on a molar basis is most effective when compared to the amebicidal endpoint doses on pathogenic amoebae.

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In vitro Inhibition of Leukocyte Uptake of Radioactive Endotoxin by Components of Normal Serum.* (30825)

RUDOLF E. NOBLE† AND DIETER KOCH-WESER‡
Department of Medicine, Western Reserve University, Cleveland, Ohio

Neter and co-workers(1-3) have shown that enterobacterial endotoxins are readily adsorbed by red blood cells and render these modified erythrocytes specifically agglutinable by homologous bacterial antibodies. These investigators have reported that normal serum interferes with the adsorption and inhibits hemagglutination(4).

Several investigators(5-7) have supplied evidence that leukocytes also may take up soluble bacterial toxins. Collins and Wood (8) have shown that incubation of *Shigella flexneri* endotoxin and rabbit leukocytes in saline causes rapid discharge of "leukocytic pyrogen," but that the release of such pyrogen is much slower in the presence of normal serum. These results represent further evidence that serum may interfere in leukocyte-endotoxin interaction.

The present study describes the inhibition by normal serum of leukocyte (and erythrocyte) uptake of Cr^{51}- and C^{14}-labeled enterobacterial endotoxin. Explanation of the inhibitory effect of serum on the release of "leukocytic pyrogen" is proposed on the basis of the observed results and in terms of current concepts of the pathogenesis of endotoxin fever.

Materials and methods. Cr^{51}-labeled lipopolysaccharide Escherichia coli and *Salmonella typhosa* lipopolysaccharides were purchased from the Difco Laboratories (Detroit, Mich.) and tagged with $Na_2Cr^{51}O_4$ (Abbott Laboratories, Chicago, Ill.) according to the method of Braude *et al*(9). In the *S. typhosa* preparation it was necessary to precipitate the endotoxin after incubation with $Na_2Cr^{51}O_4$ by adding ethyl alcohol to a concentration of 90% (instead of the recommended 68%). The *E. coli* preparation had an activity of approximately 3.0×10^6 cpm/mg and the *S. typhosa* preparation about 2.5×10^6 cpm/mg. Activity was determined in a Picker auto-well scintillation counter.

C^{14}-labeled lipopolysaccharide. In order to obtain endotoxin containing radioactivity as an integral component of its molecular structure Carbon^{14}-labeled lipopolysaccharide was prepared. A strain of *E. coli* was used which had been isolated from a human infection.

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† Present address: Dept. of Medicine, Univ. of California, San Francisco.

‡ Harvard School of Public Health, Boston, Mass.