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)

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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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Organisms were grown in 400 ml of a synthetic glucose-containing media(9) to which 0.2 mc of uniformly labeled C^{14}-glucose (New England Nuclear Corp., Boston, Mass.) was added. After 24 hours of incubation at 37°C with shaking, the cells were harvested, washed with 70% EtOH, 95% EtOH, and acetone, and dried at 37°C for 3 days. A yield of 126 mg of dried cells was obtained. Unlabeled *E. coli* was added to the labeled cells in a proportion of 7:1, and the bacteria dispersed in 5 ml of water. This suspension was shaken with 4 ml of Ballotini no. 12 glass beads in a high speed reciprocal shaker of the type described by Nossal(10) at −10°C for 2 10-second periods. The thick syrupy suspension was refrigerated overnight and on the following morning 5 ml of ice cold 0.25 M trichloracetic acid were added. The mixture was again disrupted in the Nossal shaker under the same conditions, and lipopolysaccharide was subsequently isolated according to the method of Braude(9). The dried endotoxin weighed 65.2 mg (yield of 6.5% from dry cells). A weighed portion was dissolved in distilled water and small aliquots were plated on thin aluminum planchettes, dried and counted in a Nuclear Chicago gas counter equipped with a D-47 Geiger tube and Micromil window. The endotoxin had an activity of 5.5×10^4 cpm/mg. (The results of biuret and ninhydrin tests of the preparation were negative. The absorption spectra in the 258 to 260 mμ region indicated the material was essentially free of nucleic acid.) The results of elemental microanalysis were as follows: C, 43.38%; H, 5.95%; P, Trace; N, 3.62%; and Ash (as sulfate), 9.48%. The integrity of the biological activity of the endotoxin was demonstrated by its ability to prepare for and precipitate the Shwartzman phenomenon in rabbits.

Leukocytes. Male rabbits, weighing 3.5 to 4.5 kg, were given 200 ml of 7.2% sterile caseate solution intraperitoneally. Sixteen hours later, 500 ml of isotonic phosphate buffer, pH 7.4, was injected into the peritoneal cavity. The exudate was removed through a 15-gauge needle and collected in 250 ml siliconized centrifuge cups. After centrifugation, the cells were washed with phosphate buffer, recentrifuged and suspended in Hanks' medium buffered at pH 7.4 or in physiologic saline solution at a concentration of 30,000 cells/mm^3, (94 to 98% were polymorphonuclear leukocytes and the remainder were mononuclear cells).

Erythrocytes. Blood was obtained by cardiac puncture from the same rabbits from which exudate was obtained. After defibrination, cellular elements were pipetted from around the fibrin clot, diluted with physiologic saline, centrifuged and suspended in saline solution at a concentration of 4.4×10^6 cells/mm^3.

Serum. After defibrination of blood obtained from rabbits by cardiac puncture, cellular elements and the fibrin clot were removed by centrifugation in the cold. The pH of the serum was adjusted to either 7.0 or 7.4 with 1 N HCl.

Electrophoresis. Starch block zone electrophoresis of 4 ml of rabbit serum was carried out according to the method of Kunkel and Slater(11) in barbital-lactate buffer, pH 8.6. At the end of the run (16 hours at 375 volts at 4°C) the block was cut in segments 1 cm wide. Each strip was shaken in 4 ml of saline solution and centrifuged, and the concentration of protein in the supernatant was determined by measurement of the optical density at 284 mμ in an ultra-violet spectrophotometer (Beckman Instruments, Fullerton, Calif.).

Incubation. The incubation mixture contained the following components: 0.5 ml of the described suspension of white blood cells or red blood cells; 1.0 ml of serum (pH 7.0 or 7.4) or 1.0 ml of one of the serum fractions obtained by electrophoresis; 0.25 ml of a solution of Cr^{51}- or C^{14}-labeled endotoxin (0.25 mg) in saline or Hanks' buffer and 0.25 ml of saline or buffer solution. In the control studies 1.0 ml of buffer or saline solution was substituted for the serum or serum fractions. The mixture was incubated in small tubes with shaking at 37°C, for various periods. At the end of the incubation periods, the tubes were centrifuged, the supernatant decanted, and the cellular sediment washed 3 times with saline or buffer solution. Finally, 5 ml of distilled water was added to the sedi-

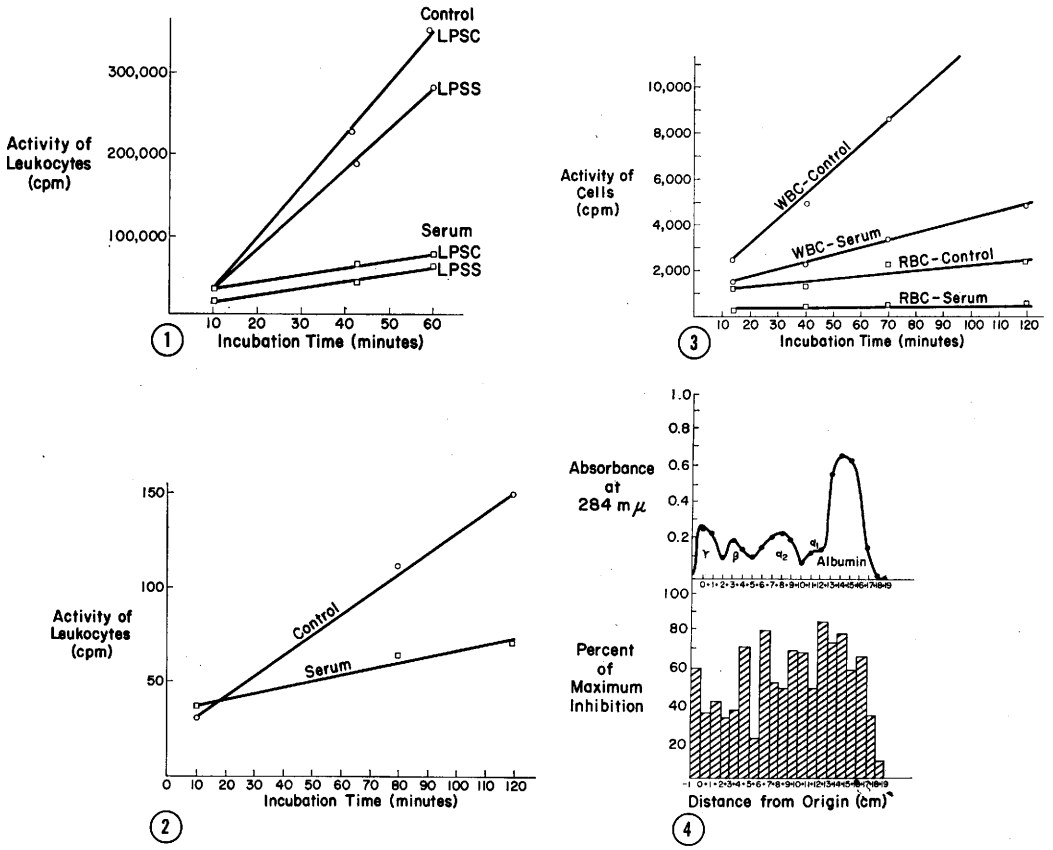

FIG. 1. Inhibition by serum Cr^{51}-*E. coli* and *S. typhosa* lipopolysaccharide (LPSC and LPSS) uptake by leukocytes; control is Hanks' buffer.

FIG. 2. Inhibition by serum of C^{14}-*E. coli* lipopolysaccharide uptake by leukocytes; control is Hanks' buffer.

FIG. 3. Inhibition by serum of Cr^{51}-*E. coli* lipopolysaccharide uptake by leukocytes and erythrocytes; control is saline solution (physiologic).

FIG. 4. Inhibition by serum electrophoretic fractions of Cr^{51}-*E. coli* lipopolysaccharide uptake by leukocytes. Plotted as percent of maximum inhibition exhibited by whole serum over saline control after 2 hr of incubation.

ment and aliquots were removed and radioactivity of the sample was determined as described.

Results. Fig. 1 shows (in a typical experiment) the rate of uptake by leukocytes of Cr^{51}-labeled lipopolysaccharide from *E. coli* and *S. typhosa* when incubated in the presence of serum or Hanks' buffer. The results clearly demonstrate a reduction in the rate of uptake of endotoxin by leukocytes in the presence of serum. Since the measure of uptake of endotoxin in this experiment was based on determination of Cr^{51}-radioactivity, it seemed important to exclude the possibility that the endotoxin-Cr^{51} complex was unstable. For this reason C^{14}-labeled lipopolysaccharide in which radioactivity was an integral part of the molecular structure was used in identical experiments. The results obtained in (Fig. 2) exclude the possibility that the data from the Cr^{51}-experiments represent an artifact due to an unstable tag.

In vivo studies show that very little lipopolysaccharide is taken up by red blood cells in comparison to the buffy coat(12). To test the validity of the *in vitro* technique and to confirm the inhibitory effect of serum on erythrocyte endotoxin uptake(4), parallel incubations with erythrocytes and leukocytes were carried out. The results (Fig. 3) show that erythrocytes take up relatively little lipopolysaccharide and that the uptake is al-

most completely inhibited by serum. The results also show that uptake of endotoxin in the control studies is increased when either saline or Hanks' buffer solution is used as the suspending medium.

In an attempt to determine whether or not serum inhibition of cellular uptake of lipopolysaccharide could be ascribed to a certain serum component, 20 serum protein fractions were obtained by starch block electrophoresis and tested for inhibition. The results (Fig. 4) indicate that all serum fractions demonstrate significant inhibition of endotoxin uptake.

Discussion. Neter and coworkers(4,13) demonstrated that incubation of lipopolysaccharides from Gram-negative bacteria with certain lipids, proteins and sera results in a loss of the affinity of the endotoxin for the surface of red blood cells. These investigators observed the ability of lipopolysaccharide to form stable complexes with protein and lipid and showed the existence of a relationship between the tendency of endotoxin to form complexes and to react with the cell surface. It was, therefore, assumed that the receptors of the cell were probably lipid and/or protein in nature.

The results of the studies reported herein similarly demonstrate that incubation of lipopolysaccharide with serum results in a marked loss of endotoxin affinity for leukocytes. Furthermore, each of the fractions of serum (albumin and α_1-, α_2-, β-, γ-globulins) inhibits lipopolysaccharide uptake by leukocytes. It is conceivable that each of the serum proteins may be capable of reacting with and altering the active group(s) of the lipopolysaccharide (presumably the "lipid A" component(14)) in such a way that its affinity for the surface of the leukocyte cell is reduced. It is also possible that these components react with the active receptor sites of the cell surface and thus inhibit lipopolysaccharide uptake.

Incubation of enterobacterial lipopolysaccharide with leukocytes in the presence of serum has been shown to retard the rate of release of "leukocytic pyrogen" in comparison to leukocyte incubated with endotoxin in the presence of saline(8). An explanation for this observation can be proposed on the basis of the results reported herein and on the current concepts of the pathogenesis of endotoxin fever. Wood(15) has proposed the following sequence of events in the development of fever following the injection of pyrogen:

Injected pyrogen (exogenous) → Injury of cells (leukocytes) → Release of endogenous pyrogen → Stimulation of thermoregulatory centers of brain → Fever.

Further studies(16) indicate, however, that the production of leukocytic pyrogen may involve metabolic reactions of the cell rather than a mere "leakage" of a preformed intracellular constituent through an altered cell membrane. In view of these studies, as well as our own, it is conceivable that serum in inhibiting leukocyte uptake of endotoxin retards the release of "leukocytic pyrogen" either by a) interfering with physical alterations of the cell membrane caused by endotoxin and hence retarding the liberation of endogenous pyrogen, and/or by b) interfering with biochemical leukocyte-endotoxin interactions leading to the production of pyrogen. Studies are now in progress to examine further the nature of these enzymatic reactions.

Summary. Chromium^{51}-labeled enterobacterial lipopolysaccharides were incubated with leukocytes or erythrocytes in the presence or absence of serum (or serum protein components). Leukocytes exhibit a much greater affinity for endotoxin than erythrocytes. Serum inhibits the rate of uptake of lipopolysaccharide by the blood cells. Each of the serum fractions (albumin, and α_1-, α_2-, β-, γ-globulins) interfere in the uptake of endotoxin by leukocytes.

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Action of Gastrin on the Isolated Gastric Mucosa of the Bullfrog.* (30826)

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Gregory and Tracy isolated from hog antral mucosa 2 polypeptides, gastrins I and II, which proved to be the most potent of all known stimuli of gastric secretion(1). They and their colleagues later reported the complete characterization of the 2 heptadecapeptides and the total synthesis of both (2,3). Gastrins I and II are, on a molecular basis, about 500 times more potent gastric stimulants than histamine in the dog (denervated fundic pouch)(1).

Because the secretion of acid in the whole animal is subject to complex neural and humoral influences, it was desirable to attempt the study of the action of gastrin on isolated tissue.

Materials and methods. The bullfrog (*Rana catesbiana*) was chosen for study because the gastric mucosa is separated readily from the muscular layers as an intact sheet; also, the thin mucosal layer is easily oxygenated(4). The studies were carried out during June, July and August. Bullfrogs were stored in a dilute saline solution at 4°C until 24 hours prior to use. They were then fasted at room temperature until they were killed by decapitation and pithing. The mucosa was immediately mounted between 2 chambers containing the following solutions: a) *nutrient* or submucosal side—sodium chloride, 85.3 mM/l; potassium chloride 3.4 mM/l; calcium chloride 1.8 mM/l; magnesium sulfate 0.8 mM/l; potassium dihydrogen phosphate 0.8 mM/l; sodium bicarbonate 17.8 mM/l; and glucose 11.0 mM/l; b) *secretory* side—a similar solution except that the sodium bicarbonate, magnesium sulfate and potassium dihydrogen phosphate were omitted(5). A mixture of 95% O_2 and 5% CO_2 was bubbled through the nutrient solution, while 100% O_2 was passed through the fluid on the secretory side(6). All incubations were carried out at room temperature (22-24°C). The exposed area of mucosa was 4.5 cm^2. The acid formed on the secretory side was titrated automatically and continuously to pH 5.5 with 0.1 N NaOH using a Radiometer pH meter and automatic titrator(6).

Gastrin was prepared by the method of

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