The Hepatic Uptake of Bacterial Endotoxin
I. The Influence of Humoral and Cellular Factors[1] (34217)

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Gram-negative bacillary endotoxin has been shown to localize in the liver and spleen after injection into the bloodstream of experimental animals (1). Enhanced clearance of endotoxin from the circulation by the reticuloendothelial system has been suggested as at least one of the mechanisms responsible for tolerance; *i.e.*, the state of partial refractoriness to the pyrogenic and other toxic effects of endotoxin induced by a series of injections of this material (1, 2). However, the factors affecting the reticuloendothelial removal of endotoxin have not yet been defined. The present studies, utilizing an isolated perfused rabbit liver model, were designed to characterize the cellular and/or humoral factors involved in the reticuloendothelial uptake of bacterial endotoxin, especially in the endotoxin-tolerant state.

Materials and Methods. Experimental animals. Albino New Zealand male rabbits weighing approximately 3 kg were used in all experiments.

Radioisotopic tagging of endotoxin. E. coli 0127:B8 lipopolysaccharide (Boivin) was obtained from Difco Laboratories and used in all experiments. This material was labeled by a modification of the method of Braude (3). Ten to 20 mg of endotoxin were suspended in distilled water and incubated at 37° for 48 hr with 200 to 275 μCi of ^{51}Cr–sodium chromate in a total volume of 10 ml. This mixture was then dialyzed against distilled water until the dialysate was essentially free of radioactivity (usually 7 days). Labeled endotoxin was stored at 4° and used within 1 week. The ^{51}Cr label was shown to be stable and tagged endotoxin was found to possess pyrogenicity and ability to produce a localized Schwartzman reaction equivalent to that of the untagged lipopolysaccharide.

Production of tolerance. Endotoxin (3 μg/kg) was injected intravenously into rabbits daily for 7 days. These animals usually exhibited maximal pyrogenic tolerance to the seventh endotoxin injection. Animals were bled by cardiac puncture on Day 8; serum from these rabbits was pooled and designated "tolerant serum." Serum was similarly obtained and stored from rabbits which had not received bacterial endotoxin and is referred to as "normal serum." Other groups of rabbits were bled on the second, third, and fourth days after beginning the series of endotoxin injections. These animals, while not maximally tolerant, manifested a diminished pyrogenic response to a test dose of endotoxin compared to untreated animals.

Isolated liver perfusions. The liver circulation of normal or endotoxin-tolerant rabbits was isolated by cannulation of the portal vein and inferior vena cava as previously described (4). Krebs–Henseleit buffer solution containing 100 mg heparin and 1 g dextrose per liter was used as the perfusion fluid. Test perfusates of 200 ml containing a final concentration of 5% rabbit serum and 500 μg tagged endotoxin were routinely employed unless otherwise specified. Perfusates were used within 2 min of adding the tagged endotoxin. After perfusing the liver with 1 liter of buffer, freshly prepared test perfusates were added to the system. After 1 min of perfusion to allow equilibration, four simultaneous samples were drawn from the inflow

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and outflow catheters at intervals of 30 sec. Five hundred milliliters of plain Krebs–Henseleit buffer was used to flush the system between perfusates. Three to four perfusates were put through a single liver and their order was varied from experiment to experiment. Perfusates containing 5×10^8 radiolabeled staphylococci (^{32}P) per ml were occasionally interjected in order to test the effect of endotoxin on the known phagocytic capacity of this experimetal model (5). A complete series of studies in a given liver took less than 45 min. The percentage of hepatic uptake of ^{51}Cr–endotoxin was calculated from the difference between inflow and outflow counts per minute. For each experiment, a single result was recorded which represented the average of four paired samples.

During the isolated liver perfusions, inflow pressures (11–12 cm H_2O) and effluent flow rates (60–75 ml/min) remained remarkably constant. Specifically, there was no alteration in flow rate during or after perfusion of samples containing endotoxin. In addition, whenever tested, the phagocytic activity of this experimental model for ^{32}P labeled coagulase positive staphylococci was the same as that already reported using an endotoxin-free system (5).

Antibody testing. Serum from normal and endotoxin-tolerant rabbits was tested for the presence of agglutinating antibodies by adding a suspension of 1.25×10^8 killed *E. coli* 0127:B8 to serial twofold dilutions of the test serum. These were then incubated at 37° for 4 hr and left overnight at 4° before reading the agglutination titer.

Results. The effect of serum from normal and endotoxin-tolerant rabbits on the uptake of ^{51}Cr–endotoxin by normal rabbit livers is shown in Fig. 1 by the open circles. Both plain perfusion fluid (Krebs–Hensleit buffer) and 5% native rabbit serum produced low uptakes of bacterial endotoxin. However, the mean uptake in 5% serum was significantly greater than that observed in the control ($p<.001$). Five per cent fresh tolerant serum, on the other hand, strikingly enhanced the hepatic removal of endotoxin when compared to normal serum ($p<.001$). In a given liver tolerant serum promoted hepatic uptake of endotoxin 5 to 30 times more than that observed with normal serum.

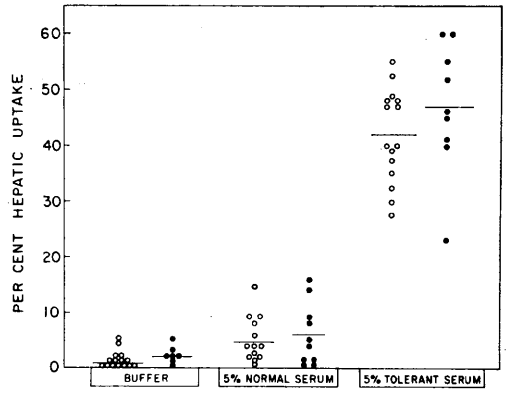

FIG. 1. The influence of normal rabbit serum and serum from endotoxin-tolerant rabbits on the hepatic uptake of ^{51}Cr–endotoxin. The open circles refer to experiments performed with normal rabbit livers; the solid circles refer to experiments carried out with livers from rabbits made tolerant to bacterial endotoxin. The horizontal lines indicate the mean hepatic uptakes.

A series of experiments utilizing livers from rabbits rendered tolerant to endotoxin are depicted in Fig. 1 by the solid circles. These studies were designed to assay possible cellular effects of endotoxin tolerance on the hepatic uptake of endotoxin. Although there appeared to be a slightly higher mean uptake of endotoxin for all three perfusates in livers from tolerant animals, there was no statistically significant difference between these uptakes and those obtained using normal rabbit livers ($p<.5$).

In order to test the effect of heat-labile serum factors on the hepatic uptake of endotoxin, aliquots of normal and tolerant rabbit serum were heated to 56° for 30 min. This procedure resulted in the complete loss of hemolytic complement activity. The results of liver perfusions carried out with heated sera are shown in Fig. 2. Hepatic removal of endotoxin from perfusates containing both fresh and heated normal or tolerant rabbit serum was essentially identical. In an additional series of experiments, heating tolerant serum to 65° for 60 min did not significantly diminish the enhanced hepatic uptake of endotoxin observed with this serum.

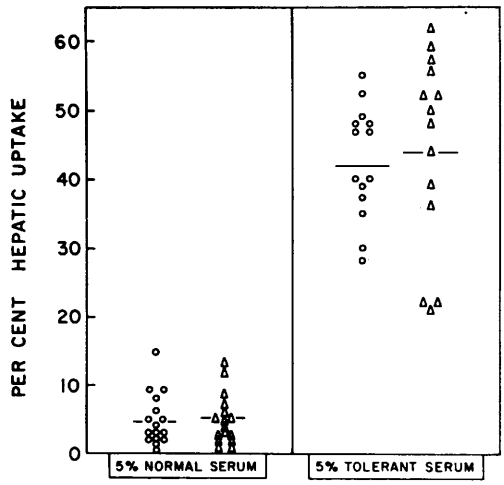

FIG. 2. The influence of heat on the ability of normal rabbit serum and serum from endotoxin-tolerant rabbits to promote hepatic uptake of ^{51}Cr–endotoxin. The circles refer to fresh serum; the triangles refer to serum heated to 56° for 30 min. Mean hepatic uptakes are shown by the horizontal lines.

Liver perfusions identical to those described above were carried out using 50 μg and 2000 μg of endotoxin as the test dose. The results were similar in every way to those cited utilizing 500 μg of endotoxin in the perfusates.

The "O" agglutinin titers to *E. coli* 0127:B8 of sera obtained from normal rabbits in the present experiments ranged from 1:8 to 1:64. Serum from rabbits rendered tolerant to endotoxin had "O" agglutinin titers from 1:8192 to 1:16,384. Serum obtained from rabbits which had received one, two, and three daily injections of endotoxin had agglutinin titers higher than those observed with normal serum. These sera, however, were not effective in enhancing the hepatic uptake of endotoxin. Figure 3 shows a typical perfusion carried out in a normal rabbit liver employing sera from partially tolerant rabbits. Note that serum obtained after three daily doses of endotoxin, which had an agglutinin titer as high as 1:2054, did not increase the uptake of endotoxin in comparison to normal serum.

Discussion. The results of the present studies show that serum enhances the removal of bacterial endotoxin by isolated perfused livers from normal or endotoxin-tolerant rabbits. Serum from tolerant rabbits strikingly promotes hepatic uptake of the homologous endotoxin. This serum property was shown to be heat-stable, withstanding temperatures as high as 65° for 1 hr, and most likely represents antibody.

Since tolerant serum invariably possessed a high titer of "O" agglutinin, it is tempting to speculate that the effect of this serum on hepatic uptake was due to agglutination of endotoxin particles into larger, more easily phagocytable aggregates. However, this would not appear to be the only mechanism operative. Serum obtained from animals after three injections of endotoxin had an "O" agglutination titer up to 200-fold that of normal serum, yet was no more active than normal serum in promoting hepatic uptake. Similarly Benacerraf *et al.* (6) who demonstrated a marked enhancement of bloodstream clearance of labeled *E. coli* by endotoxin-immune serum were unable to correlate this effect with the agglutinin titer of the serum. Interestingly, passive transfer of pyrogenic tolerance with serum from endotoxin-tolerant animals also does not correlate with serum "O" agglutinin titers (7). Furthermore, there is evidence that endotoxin-reactive antibodies distinct from "O" agglutinins are

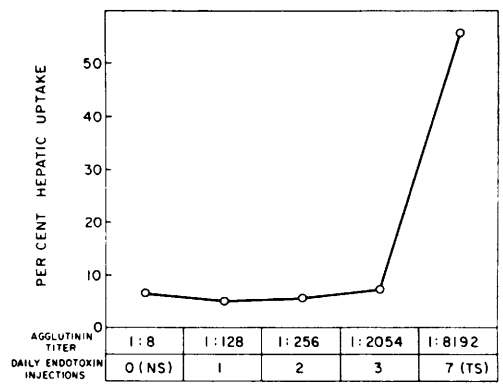

FIG. 3. The effect of serum from normal (NS), partially tolerant, and tolerant rabbits (TS) on the hepatic uptake of ^{51}Cr–endotoxin. Indicated are the number of daily endotoxin injections preceding collection of each serum sample perfused and the corresponding "O" agglutinin titer.

present in the IgG and IgM fractions of serum from endotoxin-tolerant animals (1, 7). The relationship of these antibodies to endotoxin tolerance or to reticuloendothelial clearance of endotoxin has not been accurately defined. Serum chromatographic studies are presently being carried out in our laboratory in order to determine which antibody fractions of tolerant serum are active in promoting hepatic uptake of endotoxin.

Bacterial endotoxin has been shown to interact with serum-complement components, and it has been suggested that complement promotes the reticuloendothelial clearance of endotoxin (1). Our data, however, indicate that the hepatic removal of endotoxin is not affected by the destruction of hemolytic complement. Opsonic requirements for hepatic removal of endotoxin may thus be different than for live *E. coli*. Uptake of the latter in an isolated perfused liver model has been shown to be enhanced by heat-labile serum components (8).

Bacterial endotoxin has been reported to have variable effects on reticuloendothelial phagocytic function in laboratory animals depending on the time of its administration. Depression of phagocytic activity occurs as an early effect of endotoxin (9). Such depression has been attributed by some investigators to a diminution in hepatic blood flow secondary to an endotoxin-mediated fall in cardiac output (10). Other workers have described a direct vascular effect of endotoxin on the isolated perfused rat liver (11). In the present experiments multiple boluses of endotoxin in doses from 50 to 2,000 μg had no measurable effect on perfusate flow rates through isolated rabbit livers over a 1-hr observation period. Also notable is the finding that large amounts of endotoxin perfused through isolated normal rabbit livers did not impede the uptake of subsequent endotoxin boluses or of test doses of ^{32}P-labeled coagulase-positive staphylocci.

Reticuloendothelial hyperplasia with enhanced phagocytosis of test colloids has been found to occur in animals given repeated daily injections of endotoxin and has been suggested as one of the determinants of endotoxin-mediated tolerance and resistance to infection (9). Although hepatic tissues were not examined for evidence of hyperplasia in the present experiments, livers from endotoxin-tolerant animals appeared no more avid than livers from normal animals in removing endotoxin from the perfusates. We are not able to comment on the possibility that livers from tolerant animals may be have been able to detoxify endotoxin more effectively than normal livers.

Summary. An isolated perfused rabbit liver model was utilized to study the hepatic uptake of ^{51}Cr–endotoxin. Perfusates containing 5% normal rabbit serum produced relatively low uptake of endotoxin compared to the striking enhancement of uptake caused by 5% serum from endotoxin-tolerant rabbits. The serum factor (or factors) responsible for hepatic uptake of endotoxin was heat-stable. There was not a close correlation between the "O" agglutinin titer of serum samples and their effect on hepatic uptake. There was no significant difference between endotoxin uptake recorded in perfused livers from normal rabbits and those from endotoxin-tolerant rabbits.

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