Effects of Bacteria Endotoxin on Plasma Iron. (27463)

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It was recently reported from our laboratory that very small doses of lipopolysaccharides from bacteria would markedly decrease the plasma iron concentration when injected into normal rats(1). This effect on plasma iron resembles the many other host responses which have been shown to be produced by endotoxins(2-5). These effects such as: fever, Shwartzman reaction, tumor necrosis, leucocytosis etc. have been extensively studied. These studies, especially on pyrogenicity, indicate development of a tolerance to repeated injections and formation of an endogenous pyrogen(4,5). The demonstration of similar effects by lipopolysaccharides during the lowering of plasma iron concentration would aid in understanding the dearrangement of the hosts iron metabolism during infection and cancer.

Methods. Female Holtzman rats, 50 to 70 days old and weighing 170-200 g were used in these experiments. Lipopolysaccharide from *Escherichia coli* 055:B5, obtained from Difco Laboratories, Detroit, Mich., was dispersed in distilled water, and a uniform volume of 1 ml was injected intraperitoneally into each rat. Plasma iron concentration was measured by the 2, 2′, 2″-terpyridine method as described by Schade *et al.*(6).

In the studies on development of tolerance, 6 groups with 18 rats in each group, were given either a sham injection of water or 1 μg of lipopolysaccharide. The injections were given daily and plasma iron was determined at the following times after the last injection: 4, 8, 12, 16, 24 and 48 hours.

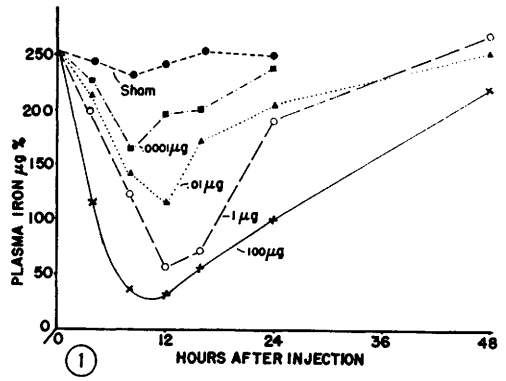
To demonstrate the presence of an endogenous factor, 10 μg of lipopolysaccharide was administered to groups of normal rats and the activity present in the plasma was measured at frequent intervals. Plasma activity was checked at 15, 30, 45, 60, 90, 120, 150, 180 and 240 minutes by injecting 0.5 ml of the plasma into other groups of normal rats and measuring plasma iron concentration after 16 hours. Further to demonstrate a difference in the plasma activities at 30 and 120 minutes the plasma was heated at 90°C for ½ hour and then injected.

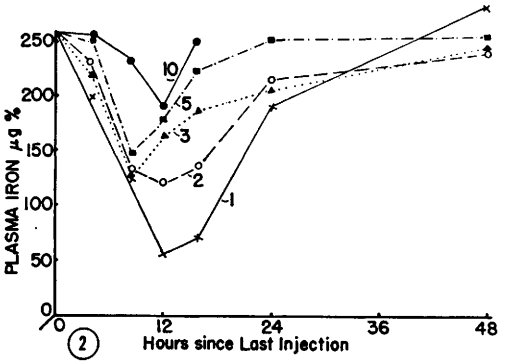
Results. The response of the plasma iron concentration to various doses of lipopolysaccharide is shown in Fig. 1. The maximum decrease for all of these doses was found to occur between the eighth and sixteenth hour after injection. When large amounts of lipopolysaccharide were administered, plasma iron concentration remained at the minimum for a longer period. The smallest amount of endotoxin tested, 0.0001 μg, produced a significant decrease in plasma iron concentration.

The development of tolerance to daily injections of 1 μg of lipopolysaccharide from *E. coli* is shown in Fig. 2. A decreased response was observed after the second injection and still further decreases were found after 3, 5 and 10 daily injections. The response of the tolerant animals resembles the curves obtained with single injections of much smaller doses of lipopolysaccharide.

It is possible to calculate the area under these curves and obtain a plasma iron index similar to the fever index used in calculating the pyrogenic activity(7). The plasma iron index of various amounts of lipopolysaccharide administered as a single injection or 1 μg given daily for several days is shown in Table I. After the second daily injection the 1 μg dose was less effective than 0.01 μg as a single injection. Five daily injections of the 1 μg dose did not lower the plasma iron concentration as much as 0.0001 μg given as a single injection.

Some indication of the formation of an endogenous factor, which will depress plasma iron concentration, is shown in Fig. 3. The initial decrease in plasma iron was attributed to the endotoxin which had not been completely cleared from the plasma. The second decrease at 120 minutes was believed to be due to formation of a new endogenous factor. Additional support for the formation of an





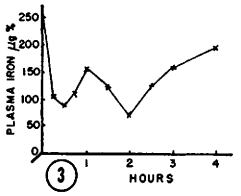


FIG. 1. Effects of varying doses of *E. coli* lipopolysaccharide on plasma iron concentration.

FIG. 2. Development of tolerance to 1 μg of *E. coli* lipopolysaccharide by daily intraper. inj. The figures by the curves indicate number of inj.

FIG. 3. Effect of plasma inj., from animals which had at various previous times received 10 μg of *E. coli* lipopolysaccharide, on plasma iron of normal rats. Plasma iron concentration was determined 16 hr after inj. of 0.5 ml of plasma.

endogenous factor is presented in Table II. The activity in the 30-minute plasma was retained after heating at 90°C for 30 minutes. The lipopolysaccharides also retain their activity after similar treatment. The activity of the 120-minute plasma was destroyed by this treatment, indicating the formation of an endogenous factor which was probably a protein.

Discussion. The plasma iron concentration of the normal rat is very sensitive to alteration by small doses of lipopolysaccharide.

TABLE I. Plasma Iron Index after Single and Multiple Injections of Lipopolysaccharide.

Dose of lipopolysaccharide (μg)	No. of daily inj.	Plasma iron index*
100	1	2058
10	1	1463
1	1	1076
.1	1	841
.01	1	724
.001	1	530
.0001	1	325
1	2	691
1	3	605
1	5	258
1	10	84

* Area under the curve between 0 and 48 hr after inj. This area is determined by the decrease below the normal value of 258 μg % found for 60 normal rats.

The tolerance developed to a decrease in plasma iron resembles the development of tolerance to fever by repeated injections of endotoxin. The tolerance to a decrease in plasma iron after injections of endotoxin develops rapidly, so that the effectiveness of a 1 μg dose is decreased over 10,000-fold after 5 daily injections (Table I).

The tolerance which develops to fever production after repeated injections of endotoxin can be markedly diminished by blocking the reticulo-endothelial system (RES)(8). A similar reversal of tolerance for the effects on plasma iron concentration is difficult to demonstrate due to the active role of the RES in normal iron metabolism(9). Blocking of the RES has been shown to prevent the decrease in plasma iron concentration which normally follows the production of a sterile inflamma-

TABLE II. Effect of Heating upon Activity of Plasma at Various Times after Injecting 10 μg of Lipopolysaccharide.*

Group	Time after lipopolysaccharide (hr)	Treatment	No. of trials	Plasma iron (μg %)
C	0	None	8	205 ± 15†
CH	0	Heat‡	8	213 ± 20
1/2	½	None	8	101 ± 9
1/2H	½	Heat	8	120 ± 13
2	2	None	12	83 ± 12
2H	2	Heat	12	195 ± 16

* Activity of the plasma was checked after the treatment by inj. ½ ml intraper. into normal rats and determining plasma iron 16 hr later.

† Mean ± stand. error.

‡ Plasma was heated at 90°C for ½ hr.

tion(10,11). A single injection of 1.25 ml/kg of Thorotrast (colloidal thorium dioxide, a RES blocking agent) to rabbits has also been shown to produce a rise in plasma iron 3 hours after injection(12). Blocking of the RES may, therefore, retard removal of both the endotoxin and the iron from the plasma. This difference in the tolerance produced by lipopolysaccharide for fever and plasma iron can only be answered by further experimentation on the effects of RES blocking on the rate of disappearance of both the endotoxin and plasma iron. Our own experiments in this area have been hampered by an inability to duplicate in rats the increased plasma iron or longer T½'s for plasma iron after injections of either Thorotrast or trypan blue which have been shown in the rabbit and dog (10-12).

The rapid decrease in plasma iron concentration after injections of endotoxin may be due to either a faster removal of the iron from the plasma or an inhibition of the release of iron from storage. Increased incorporation of serum-bound Fe^{59} into liver and spleen ferritins has been shown after injection of endotoxin(13). The increased amount and speed of incorporation of serum-bound iron into iron stores after fairly large injections of lipopolysaccharide, however, do not seem to be sufficient to account for the marked and rapid decrease in plasma iron concentration which occurs after administration of a small amount of endotoxin. Some evidence for an impaired reutilization of iron from senescent red blood cells has recently been reported in dogs with sterile inflammation(14).

The formation of the endogenous factor, which will decrease the plasma iron of a normal rat, resembles both in time of formation and in its destruction by heat the endogenous pyrogen(15,16). An endogenous factor might help to explain the lowering of plasma iron concentration in certain types of shock and during sterile inflammation(12,14).

Summary. A single injection of as little as 0.0001 μg of lipopolysaccharide from *Escherichia coli* produced a hypoferremia in the rat. The maximum decrease in plasma iron occurred 8 to 16 hours after injection. A tolerance to this endotoxin was produced by repeated daily injections. An endogenous, heat labile factor, which would decrease plasma iron, was formed in the plasma, 2 hours after injection of endotoxin.

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