Dietary cholic acid, deoxycholic acid, or cholesterol did not alter liver structure. Removal of lithocholic acid from the diet resulted in regression of the reaction.

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Some Effects of Endotoxin upon Plasma Iron Turnover in the Rat. (28301)

RALPH F. KAMPSCHMIDT AND MARY I. ARREDONDO
Biomedical Division, Samuel Roberts Noble Foundation, Inc., Ardmore, Okla.

Previous investigations in our laboratory indicated that injection of very small amounts of endotoxin would cause a pronounced decrease in the plasma iron concentration of normal rats(1,2). These lipopolysaccharides from bacteria may be responsible for the decreased plasma iron concentration frequently shown during infection(3,4). The plasma iron in patients or animals with infection disappears rapidly(5,6), and has been shown to be diverted to the liver and spleen instead of the bone marrow(7).

Adrenalectomy or hypophysectomy caused a decrease in plasma iron, but these effects were not directly related to the hypoferremia of infection(8,9). Freireich *et al.*(10) have suggested that the decreased plasma iron during inflammation was due to a decreased return of iron from recently destroyed erythrocytes to the plasma. Other investigators have suggested decreased synthesis(11) or increased catabolism(12) of the iron-binding protein of the plasma. Considerable evidence has been obtained to indicate that the reticuloendothelial system (RES) may be involved in the decrease of plasma iron during infection(10,13,14).

Endotoxins should be useful in studies on the mechanisms of plasma iron lowering, since a rapid decrease and a return to normal can be obtained within a very few hours(2). In the present investigation plasma iron turnover was studied during the period in which plasma iron was decreasing rapidly after an injection of endotoxin.

Methods. Female Holtzman rats weighing 200 to 250 g were used. Each experimental animal received 100 μg of lipopolysaccharide from *Escherichia coli* 055.B5 obtained from Difco Laboratories, Detroit, Mich. Plasma iron concentration was measured by the 2, 2′, 2″-terpyridine method described by Schade *et al.*(15).

Plasma pooled from several rats was incubated with Fe^{59} (specific activity 1-2 μc/μg). One-half milliliter of plasma containing 0.5 to 2 μc of radioiron was injected intravenously into each rat. Blood samples (0.5 ml) were obtained by cardiac puncture at 30, 60, 90, and 120 minutes after injecting radioiron. The effect of the removal of this volume of blood was checked by sampling groups of 3 normal rats at only one of these 4 intervals. The activities obtained in this manner cor-

TABLE I. Effect of Endotoxin on Plasma Iron Turnover in the Rat.*

Interval after endotoxin (hr)	No. of trials	Plasma iron (μg %)	Fraction of plasma iron removed/hr	Plasma iron removed (μg/100 g B.W./hr)
Control	22	250 ± 9†	.36 ± .02	3.1 ± .1
4	3	160 ± 11	.47 ± .02	2.7 ± .2
6	9	158 ± 8	.45 ± .07	2.6 ± .2
8	7	125 ± 7	.54 ± .04	2.8 ± .2
10	7	88 ± 6	.74 ± .07	2.4 ± .2
12	8	77 ± 8	.80 ± .06	2.5 ± .3
14	6	83 ± 6	1.03 ± .05	2.9 ± .2

* 100 μg of *E. coli* lipopolysaccharide was administered intraperitoneally to each rat.
† Mean ± standard error.

responded closely to those obtained from animals sampled every 30 minutes. Plasma volume was calculated by extrapolating the curve of plasma clearance to zero time. The formula of Huff and associates was used in determining plasma iron turnover(16).

The distribution of radioactive iron in the tissues was examined 2 hours after injection of Fe^{59}. The method of determining the distribution has been described by Garcia(17). At the time of killing, the rats were anesthetized, the abdomen opened, and as much blood as possible was drawn into a heparinized syringe from the heart. The blood was centrifuged, plasma removed, and red blood cells were washed in saline. Total bone marrow was calculated from the assumption that it would weigh approximately 0.8 g/100 g of body weight(17).

Results. Shortly after injection of endotoxin, the plasma iron was found to decrease very rapidly. Plasma volume increased slightly from an average normal value of 4.9 ml/100 g body weight to 5.2 ml/100 g body weight. Rate of plasma iron removal during this period is shown in Table I. Plasma iron concentration decreased rapidly, reaching a minimum 12 hours after injection of endotoxin. The T/2 of the injected Fe^{59} decreased progressively so that at 14 hours after administering endotoxin, the fraction of the plasma iron removed per hour was increased almost 3-fold. Because of the decreased concentration of plasma iron, the actual amount of iron being removed from the plasma was less in the endotoxin treated animals than in the normal ones, indicating that lowering of plasma iron concentration after injections of endotoxin was due to less iron entering the plasma rather than to more rapid removal.

The distribution of radioiron 2 hours after an intravenous injection is shown in Table II. As indicated in the previous table, a higher percentage of the iron was removed from the plasma at the longer time intervals after endotoxin. The injections of endotoxin seemed to cause little change in distribution of iron to any of the tissues. There was, however, an increased proportion of the injected dose found in the bone marrow and the red blood cells after administration of endotoxin.

Discussion. Freireich *et al.*(10) have

TABLE II. Effect of Endotoxin on Distribution of Fe^{59} 2 Hours After an Intravenous Injection.

Interval after 100 μg endotoxin (hr)	% recovery of injected dose				
	Liver	Spleen	Bone marrow	Erythrocytes	Plasma
Control	19 ± 1*	2 ± 1	11 ± 1	2 ± 1	29 ± 2
4	21 ± 2	1 ± 1	18 ± 2	3 ± 1	20 ± 4
6	19 ± 2	3 ± 1	16 ± 3	4 ± 1	21 ± 5
8	16 ± 3	2 ± 1	17 ± 2	3 ± 1	17 ± 3
10	22 ± 1	2 ± 1	18 ± 2	11 ± 2	8 ± 2
12	25 ± 2	2 ± 1	16 ± 2	8 ± 2	7 ± 1
14	18 ± 3	2 ± 1	14 ± 2	8 ± 2	5 ± 1

* Mean ± standard error.

shown that during inflammation the utilization of transferrin bound plasma iron for red cell production was not impaired. There was, however, a diminished reutilization of iron from senescent erythrocytes for red cell production. This defect in release of iron from senescent erythrocytes to the plasma would result in hypoferremia. A similar situation was found during the initial period after injecting endotoxin as shown in Tables I and II. The iron from the plasma was entering the bone marrow and erythrocytes at its usual rate, but plasma iron concentration was decreasing. The decrease was not due to a faster removal, and therefore must be attributed to a decrease in iron coming to the plasma from storage pools or recently destroyed erythrocytes.

Mazur *et al.*(18) have shown that tissue hypoxia will favor the movement of iron from ferritin to the plasma, while aerobic conditions favor the movement of plasma iron to liver and tissue ferritins. They further state that any interpretation of observed alterations in plasma iron content or in rate of plasma iron clearance must take these factors into account. However, they show an increased incorporation of Fe^{59} into ferritin 24 hours after injection of endotoxin(18), and at this time the plasma iron is also increasing(2). Thus tissue hypoxia or aerobic conditions cannot be the entire explanation for the movement of iron during infection or inflammation. Another possible explanation would be that shortly after injection of endotoxin the functional activity of the RES was depressed(19), and less iron from recently destroyed red cells was being returned to either the ferritin or plasma. Approximately 12 to 18 hours after endotoxin, depending upon the dose employed, the RES would be stimulated(19), thus once again releasing iron from the destroyed red cells to both the ferritin and plasma iron.

A better understanding of the nature of the damage to the RES by endotoxins will be necessary to explain iron metabolism during infection. Blocking of the RES by Thorotrast or other colloidal materials in mice and rabbits(13,14) prevented the decrease in plasma iron ordinarily produced during turpentine inflammation. Plasma iron also remained low during chronic infection(3,4) when RES was shown to be stimulated(20). Thus plasma iron concentration may be affected by other functions of the RES in addition to phagocytosis of colloidal particles. The methods for measuring the phagocytosis by the RES usually measure only the activity of the liver and spleen, while the bone marrow, which has been shown to be an important site for reutilization of recently destroyed erythrocytes(21), has not been extensively studied. Extended knowledge of red cell catabolism and iron release or storage in a labile pool(22) may be more important than the phagocytic function of the RES for a thorough understanding of iron metabolism during infection.

Summary. Injections of endotoxin will produce a rapid decrease in plasma iron concentration of normal rats. During this interval the fraction of the total plasma iron which was removed per hour was markedly increased, but the actual amount of iron removed per hour was decreased. There was no unusual distribution of the iron which was removed. This would suggest that endotoxin was blocking the iron entering the plasma from recently destroyed erythrocytes.

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The Age of Dependency of Enzymes in Reactive Glia.* (28302)

KENNETH A. OSTERBERG AND LEE W. WATTENBERG
Department of Pathology, University of Minnesota School of Medicine, Minneapolis

Morphologic histochemical studies have indicated that a marked and sustained increase in activity of a number of oxidative enzymes occurs in reactive glia(1-3). Since this phenomenon resembles adaptive enzyme synthesis in bacteria(4) and inductive enzyme synthesis in mammalian tissues(5,6), a search for possible inducing factors responsible for this increase in enzyme activity appeared warranted. Previous work has shown that during central Wallerian degeneration gliosis occurs despite an unaltered blood-spinal cord barrier, and suggested that substances inducing gliosis or their precursors may pre-exist in normal central nervous system tissue(7). Because major alterations in the composition of central nervous system tissue are known to occur during development(8), examination of glial reaction at various stages of development appeared profitable. This study will demonstrate that reactive gliosis is strongly dependent on factors associated with maturation and is indeed absent in the newborn animal.

Methods. Holtzman Sprague-Dawley rats were employed. Animals were divided into 2 experimental groups:

Group A. Animals 0, 5, 10, 15, 20, 25, 30, 40, 50 and 70 days old were utilized as well as a control group of adult animals 150-180 days old. A total of 132 animals were employed. In each age group 6 animals were sacrificed and served as normal controls, while in 6 other animals the spinal cord was transected immediately above the lumbar enlargement. Operated animals were killed 96 hours following cord transection and the cervical cord was removed for examination. Experimental animals and their controls were of the identical age at time of autopsy.

Group B. A total of 66 animals of the same ages as in Group A were employed. In each age group 6 animals received intracerebral implants of paraffin introduced by trocar. Animals were killed 96 hours after operation and the brain removed for examination. In each case the undamaged cerebral hemisphere served as a control.

Following removal, specimens were placed on block holders and quick frozen in pulverized dry ice. Sections were cut at 16 μ in a cryostat, mounted on coverslips, thawed at room temperature and air dried. The enzymatic histochemical procedure for demonstration of triphosphopyridine nucleotide

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