

Hypoferremia in Rats Following Injection of Bacterial Endotoxin. (26504)

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The anemia of infection has been shown to be associated with or accompanied by a hypoferremia(1). Both the anemia and hypoferremia were produced by several different bacteria and also by turpentine, but endotoxins from bacteria were found to be relatively ineffective(2). Ho and Kass, however, showed that a mild hemolytic anemia could be produced in rats by injections of bacterial endotoxins(3). In the experiments by Cartwright *et al.*(2) daily injections of *Staphylococcus* toxin for several days produced only a moderate lowering of plasma iron and typhoid vaccine was without effect. It has also been shown that daily injections for several days will result in development of a tolerance to several other effects of bacterial endotoxins(4).

The experiments reported here demonstrated that a single injection of a small amount of bacterial endotoxin caused a marked lowering of plasma iron, and that a tolerance was developed with daily injections.

Methods. Female Holtzman rats 50 to 70 days old and weighing 170-200 g were used in these experiments. Lipopolysaccharides from *Escherichia coli* 055:B5, *Serratia marcescens* and *Staphylococcus aureus* were obtained from Difco Laboratories, Detroit, Mich. These lipopolysaccharides were dispersed in pyrogen-free distilled water, and 1 ml was injected intraperitoneally into each rat. Plasma iron was measured by the method of Schade *et al.*(5).

Results. Plasma iron was markedly decreased by all 3 lipopolysaccharides with a maximum depression occurring approximately 16 hours after a single injection. The plasma iron concentration of rats receiving 3 different doses of these endotoxins is shown in Table I. The lipopolysaccharide from *E. coli* caused the greatest lowering of plasma iron concentration, but all produced a significant hypoferremia in doses as low as 0.1 μ g per rat.

Two days after administration of 1 μ g of endotoxin plasma iron returned to the normal value (Table II). Determination of plasma iron 16 hours after last injection, in a series of 4 daily injections, indicated development of a tolerance with repeated injections.

Discussion. Studies by Janoff, Zweifach and Shapiro(6) on the levels of plasma iron in various types of experimental shock indicated a decreased concentration after administration of lethal doses of *E. coli* endotoxin. The degree of hypoferremia due to endotoxin was difficult to evaluate due to the decrease of plasma iron in control animals under their experimental conditions.

It appears quite likely that failure of Cartwright *et al.*(2) to observe a marked hypoferremia after administration of endotoxins was due to the length of time between injection and measurement of plasma iron, as well as development of a tolerance after daily injection.

A similar observation of a decrease in plasma iron after a single injection, and development of a tolerance after daily injection has been observed with extracts prepared from tumor tissues(7). It was found possible to overcome this tolerance by frequent administration of small amounts of material

TABLE I. Plasma Iron Concentration in the Rat 16 Hours after Intraperitoneal Injection of Endotoxin.

Source	Dose (μ g/rat)	No. of rats	Bound plasma iron (μ g %)
Sham	0	39	238 $\pm$ 6*
<i>Escherichia coli</i> 055:B5†	10	9	50 $\pm$ 8
	1	15	82 $\pm$ 7
	.1	9	116 $\pm$ 12
<i>Serratia marcescens</i> †	10	6	103 $\pm$ 5
	1	6	131 $\pm$ 9
	.1	6	156 $\pm$ 13
<i>Staphylococcus aureus</i> †	10	6	91 $\pm$ 6
	1	6	150 $\pm$ 10
	.1	6	179 $\pm$ 14

* Mean $\pm$ stand. error.

† Obtained from Difco Labs, Detroit, Mich.

TABLE II. Comparison of Effects of Single and Repeated Injections of 1 μ g of *E. coli* Lipopolysaccharide on Plasma Iron in the Rat.

No. and frequency of injections	Time since last inj. (hr)	No. of rats	Bound plasma iron (μ g %)
1 Sham*	16	39	238 $\pm$ 6†
1	16	15	82 $\pm$ 7
1	48	3	280 $\pm$ 46
1	72	3	217 $\pm$ 18
4 daily	16	6	235 $\pm$ 15

* One ml of pyrogen-free distilled water.

† Mean $\pm$ stand. error.

at injection sites where it would be absorbed slowly (8).

Summary. A single injection of as little as 0.1 μ g of lipopolysaccharide from bacteria produced a marked hypoferremia in the rat. A tolerance was developed with daily administration of the endotoxin.

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Received February 23, 1961. P.S.E.B.M., 1961, v106.

Effects of Exposure to Continuous Light on the Eye of the Growing Chick.* (26505)

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A peculiar eye abnormality observed in chicks exposed to continuous light during the first 6 weeks of life has been briefly described (1). This observation has been confirmed repeatedly, using several different breeds of chicks (2). Further work has been conducted on this abnormality to obtain information on the histological changes associated with the eye enlargement.

Day-old, male chicks were randomly placed in floor pens from which natural light was excluded. Incandescent bulbs were used to provide approximately 3-foot-candle intensity measured at bird height. The chicks were brooded under shielded electric brooders in the normal manner. Feed and water were supplied *ad libitum*.

One group of chicks was exposed to 12 hours light per day (controls) and another group received 24 hours light per day. The

birds exposed to 24 hours light per day invariably developed elongated eyelids, or a peculiar "slant eyed" condition. The cornea appeared to be less convex than in controls, with the iris closely associated with the inner surface of the cornea. Fig. 1 illustrates these effects.

Four birds, 2 from each treatment, were sacrificed at 10 weeks of age. The eyeballs were removed, trimmed of external musculature and weighed (Table I). The average weights agree with previous reports based on a much larger number of birds (1,2). The eyeballs were then fixed in Bouin's solution for 24 hours, cut in half with a razor blade and kept in fixative for 24 hours more. A comparison between 2 such longitudinally sectioned eyes (Fig. 2), shows flattening of the cornea and reduction in thickness of the eye wall. The most striking difference appears to be in size of the vitreous body. The hemispheres were embedded in paraffin, sec-

* Scientific Paper No. 2087, Washington Agri. Exp. Stations, Pullman. Project No. 1444.