does not serve as an adequate stimulus to increased erythropoietin production. A turpentine abscess was chosen as an easily standardized method of producing an inflammatory lesion with the realization that inflammatory lesions from bacterial(13), antigenic(14) or other agents may give quite different results.

Summary and conclusions. Rats with a turpentine abscess developed characteristic mild anemia. Such rats showed an erythropoietic response comparable to that of normal controls following treatment with human urinary erythropoietin or exposure to hypoxia. Therefore, the rat having a turpentine abscess is capable of producing erythropoietin and the marrow is capable of responding when stimulated, suggesting that the mild anemia which accompanies a turpentine abscess does not serve as an adequate stimulus for increased red cell production.

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Effect of Endotoxins on Phagocytic Activity of the Reticuloendothelial System of the Rat. (27955)

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Intravenous or intraperitoneal injection of lipopolysaccharides from gram-negative bacteria into mice and rabbits results in an early injury to the phagocytic cells of the liver and spleen(1,2). These injured cells show a deficiency in ability to clear and metabolize injected colloidal material from the blood. These effects were dose-dependent, and were followed after recovery by a period of increased phagocytic activity of the Reticuloendothelial System (RES) which might have been due to new cell formation.

Interest in the RES in our laboratory has been focused on its effects on iron metabolism, particularly iron transport in the rat(3, 4,5). Blocking of the RES in both dog and rabbit has been shown to result in an increase of plasma iron concentration and to prevent the decrease in plasma iron which normally follows production of a sterile inflammation (6,7). Our inability to demonstrate a similar increase in plasma iron concentration after attempted blockade of the RES in the rat (8) has led us to postulate that the response of the RES to injected materials may be different in this species. The present experiments on phagocytic activity of the RES after injections of endotoxin would tend to

confirm this idea.

Materials and methods. Female Holtzman rats, 50 to 70 days old, weighing 170-200 g and female C3H mice, bred in our laboratories, were used in these experiments. Lipopolysaccharides from *Escherichia coli, Serratia marcescens* and *Staphylococcus aureus* were obtained from Difco Laboratories, Detroit, Mich.

The activity of the RES was determined by slight modification of the method of Biozzi *et al.*(9). A suspension of carbon (C 11/143 a)* was injected (16 mg/100 g body weight) into the femoral vein. The animals were kept under light sodium seconal anesthesia during administration of carbon and sampling of blood. At appropriate intervals blood was collected from the tail vein of the rat and the retro-orbital venous plexus of the mouse. A 0.02 ml sample of blood was pipetted into 5 ml of hemoglobin diluent (1 g $NaHCO_3$, 50 mg KCN and 200 mg $K_3Fe(CN)_6$ diluted to 1 liter). The concentration of carbon was determined by its absorption at 540 mμ in a Beckman DU spectrophotometer. The blanks were samples of blood obtained prior to injection of carbon. Blood carbon concentration decreased according to an exponential function of time, expressed as follows:

$$\log C_1 - \log C_2/T_2 - T_1 = K$$

C_1 and C_2 were the concentrations of carbon in the blood at times T_1 and T_2 respectively. K, which expressed the phagocytic activity of the RES for the injected dose, was called the phagocytic index.

It has been shown that the phagocytic index depends on the weights of the liver and spleen(10). This allows the use of a corrected phagocytic index (α) expressed as:

$$\alpha = \frac{W}{WLS}\sqrt[3]{K}.$$

W is body weight and WLS the sum of weights of liver and spleen.

Results. The effect of lipopolysaccharides from *Escherichia coli* on the phagocytic index of rats is shown in Table I. There was an increase in both the K and α values 2 hours after injection of the endotoxin. The phagocytic index declined through the next 6 hours but once again showed a slight increase 2 days after administration of endotoxin. Lipopolysaccharide prepared from *Serratia marcescens* and *Staphylococcus aureus* gave K values 2 hours after injection into the rat of .111, .121, and α values of 11 and 11 respectively.

TABLE I. Effect of 100 μg of Lipopolysaccharide from *E. coli* on Phagocytic Activity of Reticuloendothelial System of the Rat.

Time after inj (hr)	No. of rats	K	α
0	8	.007 ±.001*	4.1 ±.3
1	6	.006 ±.001	4.4 ±.3
2	10	.094 ±.018	10.0 ±.8
4	6	.041 ±.016	7.7 ±.5
6	"	.031 ±.012	7.2 ±.4
8	"	.017 ±.004	6.5 ±.4
12	"	.016 ±.002	6.5 ±.3
24	5	.016 ±.001	6.2 ±.3
48	6	.026 ±.004	6.6 ±.3
72	"	.014 ±.002	5.9 ±.2

* Mean ± S.E.

The effect of varying the dose of endotoxin is shown in Table II. None of the doses used produced an inhibition of phagocytic activity. The lethal dose of lipopolysaccharide from *E. coli* normally falls within the range of 4 to 10 mg for the rats used in these experiments.

Repeated daily injections of the endotoxin from *E. coli* enhanced the 2 hour phagocytic index when 1 μg was used (Table III).

TABLE II. Effect on Phagocytic Index 2 Hours after Injection of *E. coli* Lipopolysaccharide.

Amt of endotoxin inj	No. of trials	K	α
1 μg	6	.020 ±.006*	4.6 ±.4
100 μg	10	.094 ±.018	10.0 ±.8
1 mg	4	.109 ±.024	9.2 ±.6

* Mean ± S.E.

TABLE III. Effect on Phagocytic Index of Repeated Daily Injection of *E. coli* Lipopolysaccharide.

No. of daily inj	Dose of endotoxin: 1 μg K	1 μg α	100 μg K	100 μg α
1	.020	4.6	.094	10.0
3	.024	6.3	.051	6.6
5	.039	6.7	.049	6.5

* John Henschel & Co., Inc., New York City.

TABLE IV. Effect of *E. coli* Lipopolysaccharide Administered 2 Hours or 48 Hours Prior to *Salmonella typhimurium* on Mean Death Time of Rats.

No. of bacteria inj. I.P.	No. of rats	Mean death time (days) of rats		
		Control	100 μg of endotoxin at these times prior to giving bacteria	
			2 hr	48 hr
1×10^9	14	5.7 ±.8	4.6 ±.7	13.5 ±2.7
5×10^9	14	2.0 ±.1	2.1 ±.2	3.4 ± .2

With the 100 μg dose a slight inhibition of the 2 hour phagocytic index was observed.

The results obtained with these endotoxins in mice were similar to those reported(1,2). The effect of the increased phagocytic activity upon survival time of rats injected with *Salmonella typhimurium* is shown in Table IV. Endotoxin administered 2 hours before the bacteria failed to protect the animal, even though it did markedly increase the phagocytic activity of the RES. Although the phagocytic index was lower in the rats 48 hours after the injection of endotoxin than at 2 hours, the former were more capable of surviving the infection with *S. typhimurium*.

Discussion. The pronounced increase in the phagocytic index of the rat at 2 hours after an intravenous injection of endotoxin is in strong contrast to the early depression observed with mice and rabbits(1,2). The effects noted in the rat apparently were not due to our technics or the endotoxins used. Our results with mice also indicated an early depression followed by a stimulation of the RES at 2 or 3 days after injection. Treatment with cortisone has been shown to have an inhibitory effect on the phagocytic function in the mouse(10), while the same treatment has no effect in rats(11).

There is not only an early decrease in the phagocytic index but also an increased susceptibility of mice to infection following injection of large doses of lipopolysaccharide (12,13,14). A similar relationship of phagocytic activity and susceptibility was not found in the rat, Table IV. It has been suggested by Jenkin and Palmer(15) that the decrease in rate of removal of bacteria shortly after endotoxin injection in the mouse may result indirectly from circulatory changes due to shock conditions produced by the endotoxin. This, however, would not explain the situation in the rat where there was increased uptake of carbon but no increase in protection to infection. It is possible that failure to provide increased protection to *S. typhimurium* was due, not to phagocytosis, but to failure to destroy these cells which are capable of multiplying in the RES(16). It is also possible that a high titer of serum opsonin is present for carbon but not for *S. typhimurium* in the rat(15). If we accept the suggestion that the initial depression of RES function after endotoxin in the mouse is due, at least in part, to the exhaustion of the serum opsonins, then the marked early elevation found in the rat might help explain the difficulty of obtaining a block of the RES in this animal(17).

The inability to correlate RES activity and susceptibility to *S. typhimurium* is paralleled by similar attempts to relate the RES and resistance to endotoxin(18,19,20).

Summary. Lipopolysaccharides from gram-negative bacteria produced a marked increase in the phagocytic activity of the reticuloendothelial system in the rat 2 hours after injection. This increased phagocytic activity failed to provide additional protection to infections with *Salmonella typhimurium*.

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Studies on Inhibition of 5-Hydroxy Tryptophan Decarboxylase by Phenylalanine Metabolites.* (27956)

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The conversion of 5-hydroxy tryptophan (5 HTP) to 5-hydroxy tryptamin (5 HTA) is catalyzed by the enzyme 5 HTP decarboxylase(1) and requires pyridoxal phosphate as a cofactor(2,3). Davison and Sandler(4) have shown by *in vitro* studies that phenylpyruvic acid, phenyllactic acid, and phenylacetic acid inhibit 5 HTP decarboxylase.

The present communication describes experiments carried out to determine the mechanism of inhibition of 5 HTP decarboxylase by phenylalanine metabolites.

Materials and methods. For the *in vivo* studies, weanling rats of Sprague-Dawley strain were made phenylketonuric using high phenylalanine diets described by Wang and Waisman(5). Two series of experiments were carried out. In the first, 5 HTP decarboxylase was determined weekly in groups of 10 control and experimental rats fed 3.75% each of DL-phenylalanine and L-tyrosine for a period of 2 months. In the second, the enzyme was assayed at 4 weeks of age in groups of control and experimental rats fed on 2 dietary regimes: 1) 4% L-phenylalanine and 4% DL-phenylalanine, and 2) 7% L-phenylalanine alone.

Serum phenylalanine was determined by the spectrofluorimetric method of McCaman and Robins(6) and urine phenylpyruvate by the procedure of Berry and Woolf(7). All the animals were killed by a sharp blow on the head and bled from the neck. The kidneys were removed and decarboxylation carried out in Warburg flasks using the technic previously described(8). Studies were carried out both with and without supplementation with pyridoxal phosphate in the incubation mixture.

The *in vitro* inhibition studies were carried out using the same procedure as described above. For each inhibitor, littermates of albino rats weighing about 200 g on a normal diet were used. The inhibitor was dissolved in the buffer and incubation carried out under 3 sets of conditions: 1) without pyridoxal phosphate, 2) with 0.1 μM pyridoxal phosphate added at the start, and 3) with 0.5 μM pyridoxal phosphate added from the side arm of the Warburg flask one hour after incubation and permitting the incubation to continue for another hour. For the kinetic studies 0.1 μM pyridoxal phosphate was added in all experiments, and at least 2, and more usually 5 or more runs were made for each substrate concentration. The mean values were plotted and eye-fitted curves were drawn for Lineweaver-Burk analysis(9).

Results. The weekly values of 5 HTP decarboxylase of rats fed 3.75% each of DL-phenylalanine and L-tyrosine are given in Fig. 1. When the incubation mixture was

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