carboxylic acid and imidazole were utilized. In the case of histamine and 5(4)-amino-4(5)-imidazolecarboxamide, if utilized, the stimulatory effect must be due to amounts less than could be detected by the micromethods described above. Whether or not these effects have bearing on purine metabolism, or possess a vitamine-like function are under investigation.

Summary. Histamine and certain derivatives of imidazole nucleus have been found in trace amounts to exercise stimulatory effects on the growth of *K. pneumoniae.*

Received June 7, 1949. P.S.E.B.M., 1949, **71.**

17192. Mechanism of Inhibition of Glycogen Synthesis by Endotoxins of *Salmonella aertrycke* and Type I Meningococcus.*

ERNEST KUN† AND L. G. ABOOD.

From the Departments of Pharmacology and Medicine, University of Chicago.

Endotoxins of *Salmonella aertrycke* and meningococcus were found to have a marked effect on carbohydrate metabolism as measured *in vivo*[1,2] and *in vitro*.[2,3] These observations indicated that the endotoxins act on both anaerobic and aerobic phases of carbohydrate metabolism. The present paper describes experiments, dealing with the effect of endotoxins *in vivo* and *in vitro* on the oxidation of pyruvate as related to glycogen synthesis.

Experimental. The inhibitory effect of crude Salmonella endotoxin on the synthesis of liver glycogen was studied in detail. The experimental conditions were similar to those previously described,[2] but the time of *in vivo* glycogen synthesis was extended to a period of 5 hours. Twelve rats were divided into 4 groups: the first group received no glucose and no toxin; the second group was given glucose by stomach tube but no toxin; while the third group received Salmonella endotoxin but no glucose; and the fourth received both glucose and endotoxin. The amount of Salmonella endotoxin was 9 mg per 100 g body weight, a dose which was lethal for starving rats in 18 to 36 hours. Glucose was given 1 hour after the intraperitoneal injection of the endotoxin, and all animals were sacrificed 5 hours after glucose administration. The method of removal of liver samples and detailed analytical procedures for metabolic intermediates have been reported elsewhere.[4] Analytical results in each group of rats agreed from animal to animal within ± 15% for triosephosphates, phosphopyruvate, hexose mono- and diphosphates and pyrophosphates, and within ± 10% for the rest of the intermediates. Results are summarized in Table I, where the analytical data are expressed in terms of micrograms of intermediates per gram of liver wet weight.

These analytical results confirmed previous findings[2] since even small doses of the endotoxin completely inhibited glycogen deposition. The lactic acid content of the livers of rats, injected with Salmonella endotoxin, was markedly increased, an effect which was similar to that observed in rabbits after the injection of endotoxins.[1] This finding itself indicated tissue anoxia, which, on the other hand, resulted in an inhibition of the function of the adenylic system. The inhibition of carbohydrate synthesis at the level of phosphopyruvate was suggested by the absence of

* This investigation was supported by the U. S. Navy, Office of Naval Research, and the University of Chicago.

† Present address: Tulane U. School of Medicine, New Orleans, La.

1 Kun, E., and Miller, C. P., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 221.

2 Kun, E., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 496.

3 Kun, E., *J. Biol. Chem.*, 1948, **761**, 174.

4 Kun, E., and Abood, L. G., *Arch. Internat. Pharmocodyn. et Ther.*, (Gand, Belgium), 1949, **80**, 51.

TABLE I.
Effect of Salmonella Endotoxin on Intermediate Metabolites of the Rat Liver During Glycogen Synthesis. (Dose of endotoxin = 9 mg/100 g rat).

Metabolite	Normal starving	Normal fed glucose	Starving, inj. with endotoxin	Fed glucose and inj. with endotoxin
Glycogen	440	41,500	0	0
Glucose-1-PO_4	86	68	15	10
Glucose-6-PO_4	1,980	1,350	220	240
Fructose-6-PO_4	200	80	10	10
Fructose-1-6-diphosphate	187	180	220	230
Pentose-PO_4	130	128	74	30
Triose-PO_4-phosphorus*	17	31	50	75
Phosphopyruvic acid	68	15	0	0
Pyruvic acid	9.5	9.5	18	12
Lactic acid	37	148	310	390
Pyrophosphate phosphorus†	46	10	6.0	29
Phosphocreatine	90	1,470	500	800
Insoluble nucleic acid phosphorus‡	1,100	830	760	550

* Values for triose phosphate phosphorus express the alkali labile phosphorus of the Ba soluble, alcohol-precipitable fraction.

† Values for pyrophosphate phosphorus represent the 7 minute phosphorus, obtained by hydrolysis of the Ba insoluble fraction in 1 N HCl.

‡ The nucleic acid phosphorus was determined by the trichloroacetic acid procedure of Schneider in the insoluble protein residue.[4]

this intermediate. On this basis the inhibition of glycogen synthesis could at least partially be explained by an inhibition of biological oxidation. This was already suggested by previous observations, which showed the decreased succinoxidase capacity of the tissues of rabbits injected with endotoxins.[1] The significance of the decrease of the insoluble nucleic acid phosphorus cannot be evaluated at present.

Since it is generally accepted that the main pathway of biological oxidation is by way of pyruvate oxidation,[5] it appeared to be of special interest to demonstrate pyruvic oxidase inhibition *in vitro*, which inhibition was suggested by the results obtained in animal tissues.

An alcohol precipitable fraction of meningococcal and Salmonella endotoxin proved to be a powerful inhibitor of pyruvate oxidation *in vitro*. The preparation of the inhibitory substance was carried out following a procedure used by Boivin and Mesrobeanu for extracting an antigenic carbohydrate-lipid complex from Gram negative bacteria (bibliography cited in [6]). The crude endotoxins were centrifuged at 4000 R.P.M. for 60 minutes at 0°. The supernatant mixture of soluble proteins was precipitated by the addition of equal volumes of ice cold 10% trichloracetic acid. The precipitate was sedimented by centrifugation and the supernatant neutralized to pH 7.0. This neutralized supernatant was dialyzed against running tap water for 18 hours. The final step was the addition of 8 to 10 volumes of cold 95% alcohol. A light flocculent precipitate settled down when the mixture was kept for 24 to 48 hours at 0°C. This alcohol precipitable fraction was centrifuged and after several washings with 95% alcohol was dried under a stream of warm air. The yield of this fraction was estimated to be 25 to 30 mg per liter of crude meningococcal endotoxin containing 1% solid material, and the yield from Salmonella endotoxin was 6 to 8 times greater. These materials could be resuspended in isotonic salt solution, yielding a slightly opalescent suspension.

Pyruvic oxidase was prepared by a method similar to that employed by Lehninger and Green.[7,8] Three grams of rat or rabbit liver were homogenized in 15 ml ice cold potassium

[5] Vennesland, B., *Ann. Rev. Biochem.*, 1948, Vol. XVII, 227.

[6] Boor, A. K., and Miller, C. P., *J. Infect. Dis.*, 1944, **47**, 75.

[7] Lehninger, A. L., *J. Biol. Chem.*, 1945, **437**, 161.

[8] Green, D. E., Loomis, W. F., and Auerbach, V. H., *J. Biol. Chem.*, 1948, **389**, 172.

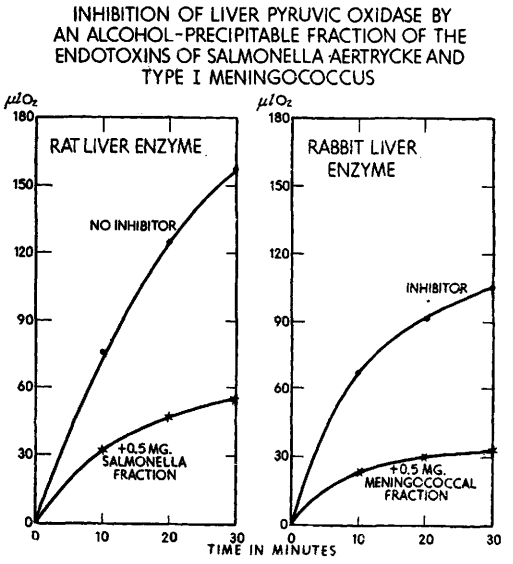

FIG. 1.

Each Warburg flask contained 300 mg enzyme, 0.5 ml 0.01 M adenosinetriphosphate, 10 mg sodium pyruvate as substrate, and 0.2 ml 20% sodium hydroxide in the center. The endotoxin fraction (0.5 mg) was suspended in 1 ml potassium medium. The final volume in each flask was made up to 3 ml. Oxygen consumption was measured at 37°C. The gas phase was air.

medium (containing 100 parts of 0.9% potassium chloride, + 21 parts 1.3% potassium bicarbonate, + 1 part 3.82% magnesium sulfate heptahydrate; to 120 ml of this mixture 1 ml of 2.1% potassium acid phosphate was added immediately before use). The homogenate was centrifuged and washed 3 times in the cold as described by Green[8] and finally resuspended to a volume, which contained 300 mg of original liver homogenate per ml. After the addition of adenosinetriphosphate and sodium pyruvate, oxygen consumption was measured with and without the addition of 0.5 mg of the endotoxin alcohol-precipitable fraction. In the presence of this fraction the oxygen consumption, due to the addition of pyruvate, was invariably depressed.

It was of interest that the alcohol precipitable fraction of meningococcal endotoxin was very toxic to rabbits (0.5 to 1.0 mg injected intravenously produced death in 24 hours) while rats and mice were susceptible to the Salmonella fraction (70 to 150 μg injected intravenously were lethal to mice and rats in 16 to 24 hours).

A similar difference was observed *in vitro*: the pyruvic oxidase of the rabbit liver was inhibited by the meningococcal endotoxin fraction, while the rat liver enzyme was sensitive to the Salmonella endotoxin preparation.

Summary. *In vivo* and *in vitro* experiments indicated that the inhibition of pyruvate oxidation occurred in animals injected with bacterial endotoxins. The inhibitory factor was probably identical with the toxic "antigene complet" of Boivin and Mesrobeanu. It is suggested that the inhibition of pyruvate oxidation plays an important role in the toxic effect of endotoxins. The exact mechanism of the inhibition of pyruvate oxidation and the identification of the inhibitor as well as the biochemical nature of other components of the endotoxins remains to be further investigated.

The authors wish to thank Dr. C. Phillip Miller for the crude endotoxins prepared in his laboratory and for valuable criticism of this paper.

Received April 20, 1949. P.S.E.B.M., 1949, **71.**