Investigations with two 'irreversible' inhibitors—DFP as a preferential inhibitor of pseudo-ChE and tri-o-cresyl phosphate (TOCP) as a preferential inhibitor of ali-esterases[16]—showed that these compounds, unlike eserine, cannot be used as a general means of distinguishing pseudo-ChE and ali-esterase activities: certain ali-esterases, *e.g.*, the ali-esterase of rat plasma hydrolysing ECIA, exhibit abnormally high sensitivity to DFP, while others, *e.g.*, the ali-esterase of rat brain hydrolysing tributyrin, proved to be quite insensitive to TOCP. In other words, the portion of the total activity inhibited by low concentrations of DFP may not always be a reliable index of pseudo-ChE activity and similarly the portion of the total activity inhibited by TOCP may not always be a reliable criterion of ali-esterase activity. However, in all cases tested as yet the ali-esterases have proved to be much less sensitive to eserine than the cholinesterases of the same tissue. In fact, so far as is known, a concentration of 2×10^{-6}M eserine can be used to inhibit completely or almost completely the cholinesterase activities in mammalian tissue preparations while leaving those of the ali-esterases unaffected.

Summary. Recent observations by McNaughton and Zeller on the high 'eserine-insensitive' activity displayed towards ethyl chloroacetate by various crude preparations of pseudo-ChE have been confirmed but the experimental results reported here fail to substantiate their conclusion that the activity of pseudo-ChE towards ECIA is insensitive to eserine. The evidence presented here has, in fact, indicated that the activity of any of the cholinesterases towards ethyl chloroacetate is inhibited by low concentrations of eserine, the remaining eserine-insensitive activity being due to ali-esterases. The value of eserine for distinguishing that portion of the hydrolysis of aliphatic esters which is due to the cholinesterases from that due to ali-esterases in crude tissue preparations has been re-affirmed. It would seem that of the three inhibitors tested (eserine, diisopropyl fluorophosphonate, tri-o-cresyl phosphate), only eserine can be relied upon to give a clear cut distinction between cholinesterase and ali-esterase activities in all cases.

[16] Mendel, B., and Mortimer, E. M., Report to National Research Council of Canada, October, 1944.

Received June 2, 1949. P.S.E.B.M., 1949, **71**.

17191. Histamine and Other Imidazole Compounds as Bacterial Growth Stimulators.*

W. R. STRAUGHN† AND M. G. SEVAG.

From the Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

In the course of a study it was observed that small amounts of histamine markedly stimulated the growth of certain bacteria. This report represents typical results of a comparative study of this effect with histamine and other derivatives of the imidazole nucleus.

* This investigation was supported, in part, by a research grant from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

† Abbott Fellow in the Department of Bacteriology, 1948-1949.

Materials and methods. The culture medium was composed of NaCl (0.5%), $MgSO_4 .7H_2O$ (0.02%), $(NH_4)H_2PO_4$ 0.1%, K_2HPO_4 (2.4%), KH_2PO_4 (0.6%), glucose (0.2%) and distilled water. The reaction of the medium was pH 7.0. It was passed through a sintered glass filter to sterilize. Solutions of the test substances in the salt-glucose medium were also sterilized by filtration. No significant drop in pH occurred during growth with this high phosphate buffer concentration. A stock strain of *Klebsiella pneumoniae* was

TABLE I.
Effects of Imidazole Derivatives on the Growth of *K. pneumoniae.*

Substance tested	Concentration × 10^{-5} M	Turbidity readings, time in hr					
		14	16	18	20	22	24
Histamine • 2HCl	50	9	34	96	130	130	130
	10	7	22	70	130	130	130
l (+) Histidine • HCl	50	50	104	130	130	130	130
	10	19	62	116	130	130	130
5-(4)-amino-4(5)-imidazolecarboxamide • HCl*	50	0	14	40	110	122	122
	10	0	4	15	60	116	126
Imidazole-4,5-dicarboxylic acid*	50	21	74	120	123	123	123
	10	6	38	100	130	130	130
Imidazole*	50	0	2	10	37	98	130
	10	0	1	6	25	86	128
p-Aminobenzoic acid 20 γ/ml†		0	0	3	12	53	109
Control		0	0	4	19	66	112

* 5(4)-Amino-4(5)-imidazolecarboxamide was obtained from Dr. John M. Buchanan. The other compounds were from Eastman Kodak Co.

† With 0.1 and 1.0 γ/ml growth was of similar magnitude.

used in this study. The inocula, derived from 24-hour salt-glucose cultures, were washed once with and resuspended in phosphate buffer and diluted so that after inoculation each system contained approximately 50 viable cells per ml. All cultures were incubated at 37°C. The turbidity readings (filter No. 54, Klett-Summerson photoelectric colorimeter) represent averages of triplicate determinations.

Micro-determination of Histamine and Histidine. This method is an adaptation of the method of Macpherson[1] and was carried out in Klett tubes. The final volume of the reaction system was 6 ml and consisted of from 2 to 20 γ of histamine or histidine per ml, 3 ml of water, 0.4 ml of sulfanilic acid in 10% hydrochloric acid solution, 0.4 ml of 5% sodium nitrite, and 1.2 ml of 30% sodium carbonate added after 30 minutes standing at room temperature. Colorimetric readings were made immediately with the use of the No. 54 filter. This permitted the determination of 2 γ of either compound.

5(4)-Amino-4(5)-imidazolecarboxamide.[2] This substance was determined according to the method of Bratton and Marshall.[3] This method carried out in Klett tubes permits the determination of 2 γ of the compound.

Results. The results given in Table I clearly indicate the stimulatory effects of histidine, histamine, imidazole-4, 5-dicarboxylic acid, and 5(4)-amino-4(5)-imidazolecarboxamide. Histidine concentration of 2 x 10^{-5}M, and histamine, 5 x 10^{-5}M, also exercised definite stimulatory effects. Imidazole has but slight effect and p-aminobenzoic acid has no effect. Maximal growth and the logarithmic growth rates are approximately equal for all systems. At the times of maximal growths in the accelerated systems (18 to 20 hours), the turbidities were 5 to 10 times greater than that of the controls. The observed stimulatory effect is that of shortening the lag phase, and is not as apparent when large inocula are used.

During growth there was no measurable utilization of histamine or of 5(4)-amino-4(5)-imidazolecarboxamide. Histidine was utilized in proportion to growth. Histidine can serve as a sole source of carbon for growth in the absence of glucose while histamine cannot. For lack of a quantitative method, it was not determined whether or not imidazole-4,5-di-

[1] Macpherson, H. T., *Biochem. J.*, 1942, **36**, 59.

[2] Shive, W., Ackermann, W. W., Bordon, M., Getzendaner, M. E., and Eakin, M. E., *J. Am. Chem. Soc.*, 1947, **69**, 725.

[3] Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, 1939, **128**, 537.

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.