

Effect of Meningococcal Endotoxin on Histamine Content of Blood and Tissues of Rabbits.*

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The following studies were undertaken as part of an investigation of the biochemical changes which occur in the animal body after the intravenous injection of meningococcal endotoxin. The pathological condition produced in this way is known as toxemia and since different bacterial toxins seem to elicit changes in metabolism which have certain characteristics in common,¹ the results of these studies may be of some general interest.

The important role of histamine in the pathology of infectious diseases, as well as in conditions closely related to them, such as allergy and anaphylaxis, is well recognized; and has been reviewed by Best² and by Dragstedt.^{3,4} Feldberg and Keogh⁵ and Feldberg and Kellaway⁶ reported experiments which showed that an increase in histamine liberation from isolated organs occurred when the perfusion fluid contained staphylococcal endotoxin. Kellaway, Trethewie and Turner⁷ showed that the toxin of *Clostridium welchii* increased the histamine output of isolated

organs of the cat and the rabbit. These results suggest that bacterial toxins interfere in some way with histamine metabolism.

The present study is concerned with the changes in the histamine content of the blood and tissues of rabbits injected intravenously with meningococcal endotoxin.

Materials and Methods. The meningococcal endotoxin, as prepared by Boor and Miller, is a mixture of nucleoproteins, globulins, albumins, and a highly toxic glycolipid.^{8,9,10} Endotoxin was prepared from type I meningococcus grown for 18 to 20 hours on casein-digest agar. The microorganisms were washed thrice in saline, resuspended in water, brought to pH 8.2 to 8.5 and kept at a temperature of about 27°C for 2 or 3 hours and in the refrigerator overnight. The suspension was then neutralized and sterilized by heating 2 or 3 times at 60° C for 25 minutes. Sterility was always proved by culture. This toxin mixture was heat stable,¹¹ and could be kept in the icebox for several months without much change in toxicity. Since the chemical nature of meningococcal endotoxin is not yet known, it seemed best to use the original protein-glycolipid suspension. The rabbits were injected intravenously with a dose sufficient to cause death within a few hours and this dose is recorded in ml per kg body weight. The solid content of the toxin was 1.14% as determined by Boor. These experimental conditions can be considered as acute meningococcal endotoxin poisoning.

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¹ Holmes, E., *Physiol. Rev.*, 1939, **19**, 439.

² Best, C. H., *Physiol. Rev.*, 1931, **11**, 371.

³ Dragstedt, C. A., *The Significance of Histamine in Anaphylaxis and Allergy*, 1943.

⁴ Dragstedt, C. A., *The Role of Histamine in Various Pathological Conditions and the Methods Controlling Its Effects*, 1945.

⁵ Feldberg, W., and Keogh, V., *J. Physiol.*, 1937, **90**, 280.

⁶ Feldberg, W., and Kellaway, C. H., *Australian J. Exp. Biol. Med. Sci.*, 1938, **16**, 219.

⁷ Kellaway, C. H., Trethewie, E. R., and Turner, W., *Australian J. Exp. Biol. Med. Sci.*, 1940, **18**, 253.

⁸ Boor, A. K., and Miller, C. P., *J. Exp. Med.*, 1934, **59**, 63.

⁹ Boor, A. K., and Miller, C. P., *Arch. Path.*, 1941, **29**, 724.

¹⁰ Boor, A. K., and Miller, C. P., *J. Inf. Diseases*, 1944, **75**, 47.

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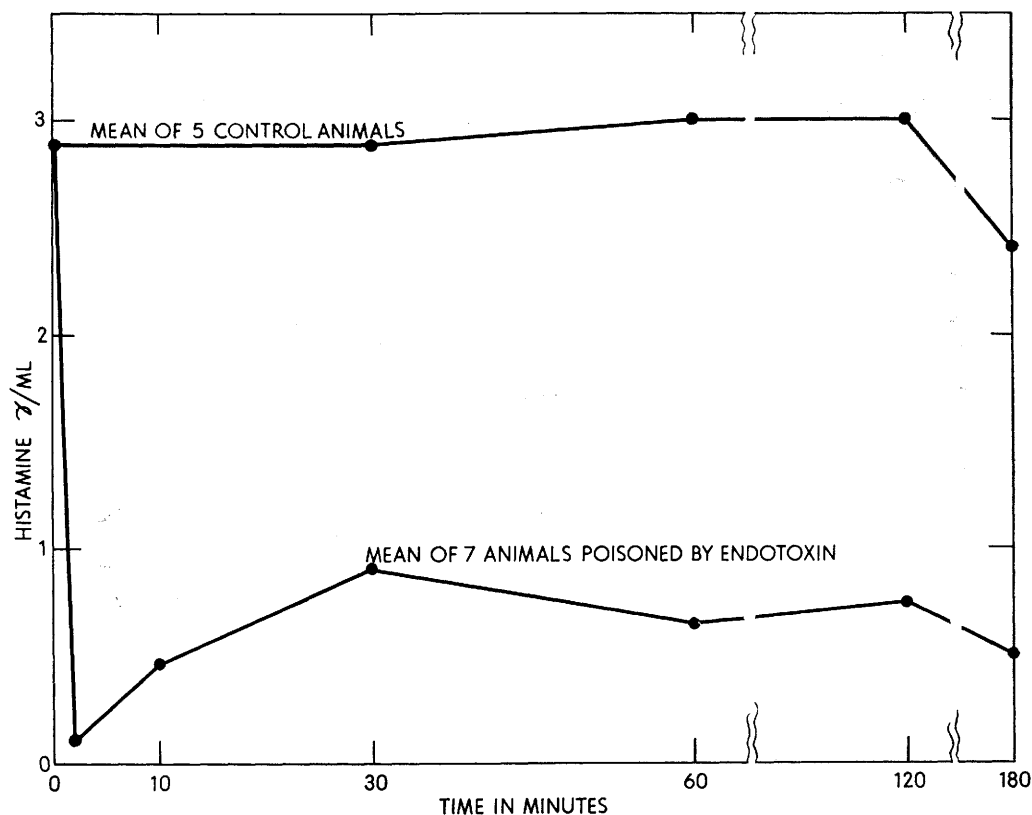
EFFECT OF MENINGOCOCCAL ENDOTOXIN ON THE BLOOD HISTAMINE
OF RABBITS

Fig. 1.

Thirty-eight rabbits, weighing from 2.4 to 3.7 kg were used as experimental animals. No anesthesia was used, because it was found that narcosis itself caused significant changes in the blood histamine content of the rabbit.[†] Blood was removed by cardiac puncture and immediately delivered into a weighed amount of 10% trichloroacetic acid. The extraction was made shortly afterwards. Blood histamine was determined according to the method of Barsoum and Gaddum,¹² using the modification suggested by Code.¹³ The histamine content of the tissues was determined on weighed samples ground in a glass tissue homogenizer¹⁴

in 10% HCl. Lipoids were removed from the tissue homogenates by ether extraction. The isolated ileum of the guinea pig, suspended in atropinized Tyrode at 37°C, was used as test object and the height of contraction was read directly on a scale. Each figure given in the tables and graphs is the mean of 5 parallel measurements (the S.D. did not exceed $\pm 15\%$). In all measurements, the effect of known amounts of histamine phosphate was compared with the tissue extracts, and the results were expressed in terms of histamine base.

Results. Control Observations. In 5 normal rabbits the histamine content of the blood was determined at 0, 30, 60, 120, and 180 minutes. There was no significant variation in the blood histamine level during the course of

[†] Unpublished experiments.

¹² Barsoum, G. S., and Gaddum, G. H., *J. Physiol.*, 1938, **85**, 1.

¹³ Code, C. F., *J. Physiol.*, 1937, **89**, 257.

BLOOD HISTAMINE OF RABBITS AFTER INTRAVENOUS INJECTION OF HISTAMINE

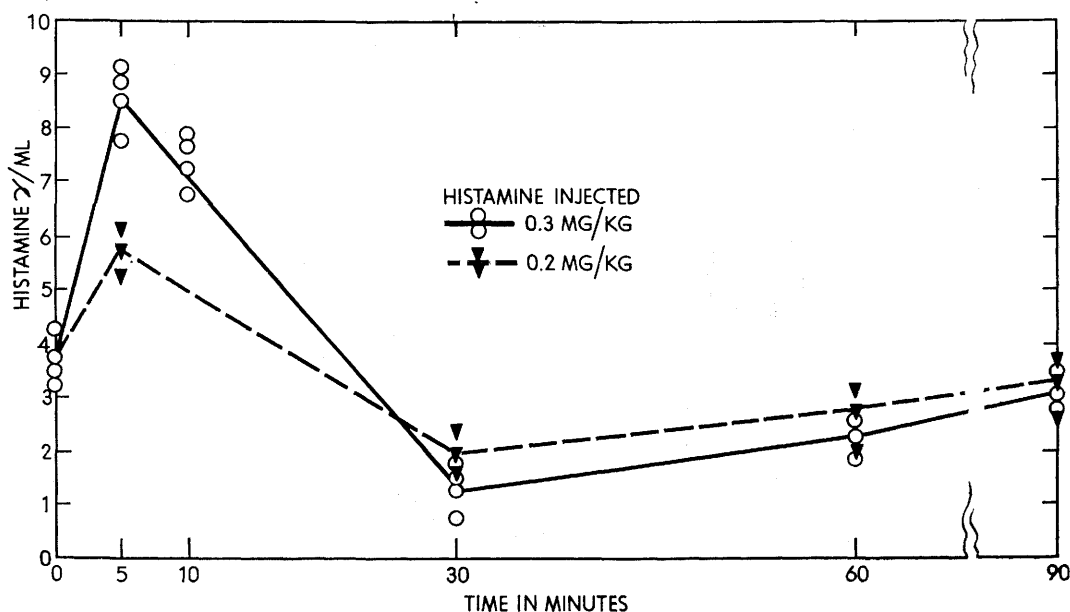


Fig. 2.

the experiment.

In the first series of experiments the concentration of histamine was studied in the blood of 7 rabbits, following the intravenous injection of 3.6 ml of endotoxin per kg body weight.

In all experiments the histamine content of the blood fell sharply immediately after the intravenous injection of endotoxin and remained well below its normal level as long as the animal lived—a period of 2-3 hours. (Fig. 1)

The mechanism involved in the disappearance of the blood histamine seems to be rapid. Since the endotoxin was the only foreign substance introduced into the animal body in these experiments, it might be supposed that endotoxin inactivates histamine. It was found, however, that when various amounts of histamine phosphate were added to the toxin, they could be recovered without significant loss by the same extraction method used for the blood analyses.

In the second series of experiments, the effects of simultaneous injections of histamine and endotoxin were studied in 7 rabbits. In

each experiment a control observation was first made by injecting histamine alone. When 0.3 mg histamine per kg body weight, in the form of histamine phosphate, was injected intravenously, the blood histamine content rose in 5 minutes to a level 2 to 3 times the normal. A 50% rise in blood histamine level was caused by an injection of 0.2 mg histamine per kg. (Fig. 2)

When meningococcal endotoxin (3.6 or 1.8 ml per kg) was injected simultaneously with a dose of histamine, which varied from 0.3 to 0.1 mg per kg no increase in blood histamine occurred. In each instance it fell to a significant degree. (Fig. 3)

The simultaneous injection of meningococcal endotoxin plus histamine markedly shortened the survival time of the rabbits. A dose of endotoxin, which usually caused death in 7-10 hours (1.8 ml toxin per kg), when injected simultaneously with 0.3-0.15 mg histamine per kg killed the animal in 5-10 minutes. These amounts of histamine alone injected intravenously were never fatal.

In the third series of experiments, determinations were made of the histamine con-

BLOOD HISTAMINE AFTER SIMULTANEOUS INJECTION OF HISTAMINE + MENINGOCOCCAL ENDOTOXIN

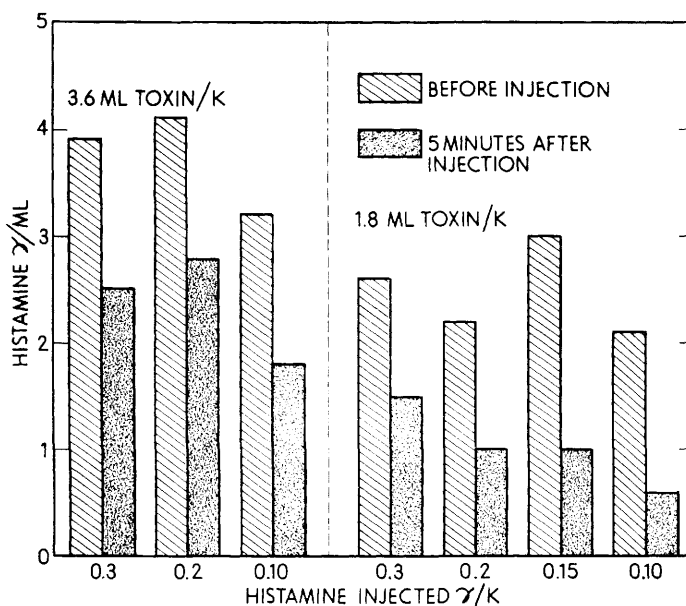


FIG. 3.

tent of the tissues of rabbits poisoned by meningococcal endotoxin. Seven animals were injected intravenously with 3.6 ml meningococcal endotoxin per kg, and their tissues analyzed immediately after death. The results are given in Fig. 4, which includes, for comparison, the histamine content of the organs of 5 normal rabbits. A significant increase in muscle and liver histamine was observed. The histamine content of the liver, as well as that of the diaphragm, showed about a 4-fold increase over the normal, while the skeletal muscle showed an average increase of 2.4 times the normal.

Discussion. These experiments show that the intravenous injection of meningococcal endotoxin into rabbits is followed by a rapid decrease in the histamine content of the blood and a considerable increase in the histamine content of the liver and muscle. The decrease in histamine content of the blood amounted to 2.75 histamine per ml of blood. If one assumes the total blood volume of a 3 kg rabbit to be 7% of its body weight¹⁴ the total quantity of histamine which disappeared from

the blood was 0.77 mg; *i.e.*, 0.26 mg per kg. Based on the results of Levin and co-workers¹⁵ who determined the average weight of rabbit organs, the amount of histamine accumulated in the tissues was about 5 mg. (1.7 mg histamine per kg in a 3 kg rabbit.) Since the amount of histamine which disappeared from the blood is about 1/7 the amount found in the tissues of poisoned animals, it is probable that most of the increased tissue histamine did not originate from the blood but was formed in the tissues themselves. The tissues of the poisoned rabbits contained about 3 times more histamine than the lethal intravenous dose of this substance¹⁶ which circumstance suggests that histamine may be responsible for the death of the animals. These experiments, however, do not provide con-

¹⁴ Potter, V. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1936, **116**, 495.

¹⁵ Levine, C. J., Mann, W., Hodge, H. C., Ariel, I., and DuPont, O., *Proc. Soc. Exp. Biol. and Med.*, 1941, **47**, 318.

¹⁶ Sollmann, T., and Hanzlik, P. J., *Introd. to Exp. Pharmacol.*, 1928, p. 284.

HISTAMINE CONTENT OF TISSUES OF RABBITS POISONED BY ENDOTOXIN

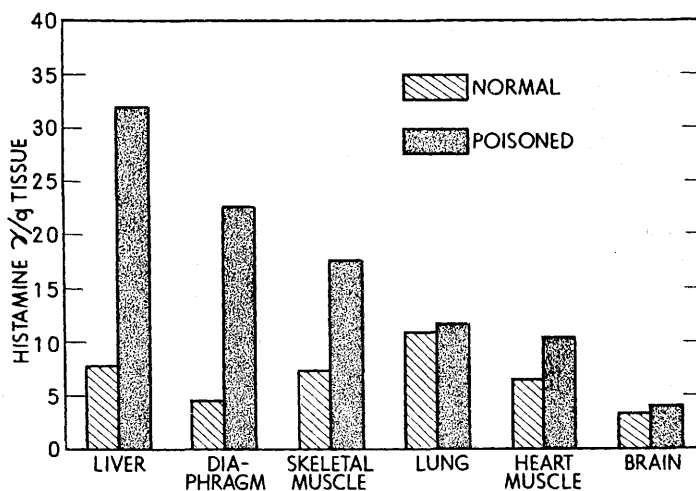


FIG. 4.

clusive proof of this supposition.

Summary. Meningococcal endotoxin injected intravenously into rabbits caused a rapid decrease in the histamine content of the blood and an increase in the histamine content of liver and muscle.

The decrease in histamine in the blood was not prevented by the intravenous injection of histamine along with the endotoxin.

The survival time of rabbits injected intravenously with meningococcal endotoxin was markedly shortened by a simultaneous injection of a small dose of histamine.

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Effect of Oral Streptomycin on the Intestinal Flora.

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The majority of evidence seems to indicate that *E. coli* plays a predominant role in the pathogenesis of peritonitis following large bowel surgery.¹ Among the other microorganisms most frequently encountered are the fecal streptococci (Lancefield Group D strep-

tococci), and *Clostridium welchii*.¹ With these facts in mind, it seems logical to suppose that the incidence of post-operative peritonitis would be reduced if the *E. coli* present in the bowel were eliminated. The less readily absorbable sulfonamides such as sulfaguanadine, sulfasuxidine and sulfathaladine have been used in an endeavour to accomplish this.

¹ Meleney, F. L., Harney, H. D., and Jern, H. Q., *Arch. Surg.*, 1931, **22**, 1.