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Liberation of a Histamine-Like Substance on Stimulation of Sympathetic Nerves.

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Several attempts have been made to find a nervous mechanism for the release of histamine from body tissues,[1, 2] but thus far the only evidence that such may exist has been indirect.[3] The present study deals with the release of histamine by the skin upon stimulation of sympathetic nerves. The epidermal layer of the skin contains relatively large quantities of histamine (24 γ/g).

Experiments were carried out on rabbits, unanesthetized or anesthetized lightly with urethane or ether. The cervical sympathetic trunk and its superior ganglion were prepared for stimulation with bipolar electrodes (4 to 60 sec, primary 3 volts, secondary coil at 10 cm). Blood samples obtained from the great auricular vein by venipuncture before and after nerve stimulation were compared for their ability to contract an isolated segment of guinea pig ileum according to the method of Schultz and Dale. Atropinized Ringer-Locke solution was used in the muscle bath in every case. Standard histamine solutions were used for comparison of contractions.

In early experiments the blood samples were allowed to clot; the serum obtained was diluted immediately with Ringer's solution or buffer solution of pH 7.15 and tested on the guinea pig ileum. In these experiments (10 animals) serum obtained 15 to 60 seconds following nerve stimulation almost invariably produced a greater contraction of the guinea pig ileum than the control serum (15 to 50% greater). This difference was present after and often increased by heating the diluted sera in a water bath at 60° to 70°C for 30 minutes, provided the pH of the sera was not above 7.15. The entire contractor effect of both control and stimulation sera could be abolished by previous addition of 0.5 γ of thymoxyethyldiethylamine to the muscle bath.

Rabbit blood has already a high content of histamine which is almost entirely stored in the cellular elements (platelets, Minard[4]),

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1 MacGregor, R. R., and Peat, S., *J. Physiol.*, 1931, **71**, 31.

2 Bulbring, E., and Burn, J. H., *J. Physiol.*, 1935, **83**, 483.

3 Lewis, T., and Marvin, H. M., *Heart*, 1927, **14**, 27.

4 Minard, D., *Am. J. Physiol.*, 1937, **119**, 375.

but is liberated into the serum after clotting.[5] Since only that histamine found free in the blood plasma is active *in vivo*, an attempt was made to determine whether there was an increase in plasma histamine after nerve stimulation (5 animals). Whole blood (0.1 to 0.2 cc) was drawn directly into a syringe containing an equal quantity of heparinized Ringer's solution. These were drawn, mixed, and added at once to the muscle bath. It was found that control samples of unclotted blood contained no demonstrable contractor substance. However, following nerve stimulation, ability to contract the muscle appeared in ½ to 3 minutes and was usually indetectable again after 8 to 15 minutes. This property of the whole blood could be abolished by previous addition of thymoxyethyldiethylamine to the muscle bath. The experiment could be repeated more than once using the same rabbit ear.

Two experiments with cats, testing blood serum, gave results similar to those in the rabbit. Blood was drawn from the external jugular vein following stimulation of the superior cervical sympathetic ganglion.

Adrenalin which inhibits to some extent the muscle contraction produced by histamine is liberated on stimulation of sympathetic nerves to the rabbit's ear.[6] Frequently, in our own experiments, blood drawn immediately following stimulation inhibited the effect of standard histamine solutions added to the bath more than did control samples. Our results may have been made less apparent by this antagonism.

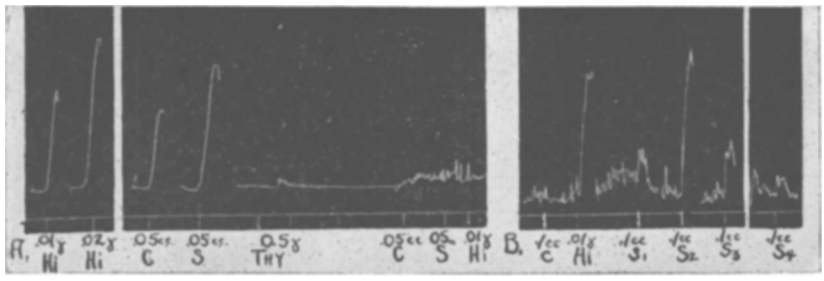

FIG. 1.

A. Effect of thymoxyethyldiethylamine on C, control serum, on S, serum obtained after stimulation of the superior ganglion (4 sec), and on Hi, standard histamine.

B. Effect on the ileum of C, control heparinized, whole blood; S_1, obtained immediately after stimulation of ganglion (10 sec); S_2, 2 min after stimulation; S_3, 4 min after stimulation; S_4, 13 min after stimulation.

5 Code, C. F., *J. Physiol.*, 1937, **90**, 349.

6 Gaddum, J. H., Jang, C. S., and Kwiatkowski, H., *J. Physiol.*, 1939, **96**, 104.

The identification of the contractor substance is aided by the use of thymoxyethyldiethylamine. This drug is specific in counteracting the effect of histamine on the guinea pig ileum.[7] It has no comparable effect upon contractions produced by KCl, $NaHCO_3$, acetylcholine, or the contraction produced in rare instances by adrenaline. Acetylcholine produces no contraction of an atropinized muscle. The slight changes in pH of the blood following nerve stimulation were not sufficient to affect the activity of the muscle strip and most sera were diluted with a buffer solution. On the other hand, the contractor substance obtained in the blood from the rabbit's ear following nerve stimulation was heat stable, active in an atropinized bath, but inactive after addition of thymoxyethyldiethylamine. In these respects it is "histamine-like". The source of this substance in the rabbit's ear has not been determined.

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Thymoxyethyldiethylamine Antagonism to Circulatory Effects of Histamine in Anesthetized and Nonanesthetized Dogs.

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Although thymoxyethyldiethylamine (Thym.) has been found to antagonize histamine (Hi) effects on isolated smooth muscle[1-4] and to exert a protective action in guinea pigs against anaphylactic and Hi shock,[2, 3, 5] little work has been done regarding its action in carnivorous animals in which the circulatory effects of Hi are most striking. We have undertaken to investigate Thym. antagonism to the hypotension and hemoconcentration resulting from Hi administration in anesthetized and nonanesthetized dogs, and to observe the effects of Thym. alone.

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7 Rosenthal, S. R., and Minard, D., *J. Exp. Med.*, 1939, **70**, 415.

1 Bovet, D., and Staub, A. M., *C. R. Soc. Biol.*, 1937, **124**, 547.

2 Staub, A., and Bovet, D., *C. R. Soc. Biol.*, 1937, **125**, 818.

3 Staub, A. M., *Ann. Inst. Pasteur*, 1939, **63**, 400, 485.

4 Rosenthal, S. R., and Minard, D., *J. Exp. Med.*, 1939, **70**, 415.

5 Rosenthal, S. R., and Brown, M. L., *J. Immunol.*, in press.