

Study of Skin Histamine (With Some Results of Splanchnic Nerve Stimulation).*

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Rosenthal and Minard^{1,2} devised a direct method of detecting diffusible histamine in the skin. The object of this paper is to present: 1. A modification of this method whereby extraneous substances which interfere with the assay of histamine can be avoided. 2. Preliminary experiments using this method to determine whether any alteration of the histamine content of the abdominal skin occurs following stimulation of the splanchnic nerve.

Method. Uniform pieces of skin about one centimeter in diameter and comparable in thickness to a Thiersch graft were placed between anode and cathode chambers for electro dialysis (Fig. 1). The raw surface of the skin was placed against a cellophane membrane cemented on the end of a glass tube, 8 mm inside diameter. The skin was held securely over the end of the tube by a rubber band. A rubber dam with a minute perforation was stretched so that the edge of the perforation reinforced the rubber band and protected the cut edges of the skin from the anode fluid. Three-tenths milliampere of current was passed through the skin for a period of 3 minutes. The cathode fluid, consisting originally of 0.2 ml of Ringer's solution, was removed and heated for 30 minutes at 100°C with 0.05 ml of normal hydrochloric acid. It was then evaporated to dryness to remove the excess hydrochloric acid and made up to 0.2 ml volume with distilled water. The pH was adjusted to that of Ringer-Locke solution with neutral red indicator. The samples thus obtained were assayed on a strip of guinea pig

ileum in a 3.5 ml bath of atropinized (0.5 γ per cc) Ringer-Locke solution, using histamine hydrochloride as a standard.

Results. Samples of normal skin from the abdomen and chest of dogs, when treated as described, yielded an equivalent of 0.000 to 0.005 γ of histamine. The amount in each of several pieces of skin from the same animal was relatively constant.

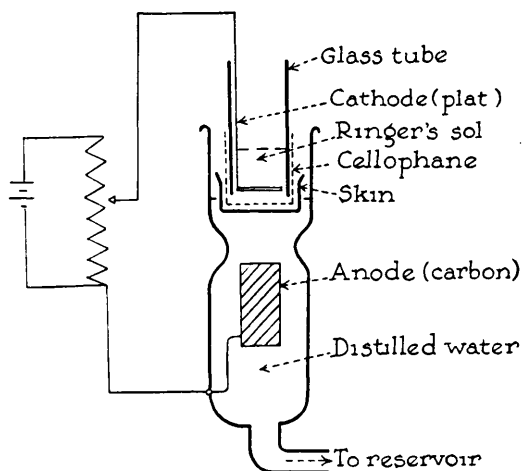


FIG. 1.

Apparatus for Electro dialysis of Skin Samples.

Small amounts of histamine (1:5,000,000 solution) injected into the skin increased the histamine equivalent of the cathode fluid. Burning of the skin resulted in a large increase of the histamine equivalent. Experiments in which turpentine, xylol or chloroform were applied to 15 samples of skin (intact or removed from body) for from 5 to 10-minute periods, indicated that these chemical irritants may also increase the histamine equivalent of the cathode fluid.

That the active substance in the cathode fluid is most probably histamine is indicated by the following facts: 1. It causes contraction

* Aided partially by a grant from Abbott Laboratories.

¹ Rosenthal, S. R., and Minard, D., *J. Exp. Med.*, 1939, **70**, 415.

² Emmelin, N., Kahlson, G. S., and Lindström, K., *Acta Physiol. Scand.*, 1941, **2**, 78.

of the atropinized guinea pig ileum. 2. This activity is heat stable in acid. 3. It is heat labile in alkaline solution. 4. It is inhibited by thymoxyethyldiethylamine.

These observations demonstrate that, using the method described, very little histamine can be obtained from untreated dog skin. However, histamine injected into the skin or liberated in the skin by burning or chemical irritation can be readily detected.

2. The above technic for the study of skin histamine was devised originally to determine whether any alteration of the histamine content of the skin occurred in association with referred pain. Rosenthal and Minard¹ have presented evidence indicating that histamine is the chemical mediator of cutaneous pain. Pollack and Davis³ and others have suggested that referred pain may be due to the action of metabolites released in the skin by viscerocutaneous reflex activity.

A preliminary study has been carried out with dogs to determine whether histamine is liberated in the skin of the abdomen following stimulation of the splanchnic nerve. Control samples of skin were taken from the right side of the upper abdomen and lower chest of dogs. Electrodes were then applied to the left splanchnic nerve under procaine or light ether anesthesia. Additional samples of skin were taken from the left side following the operation and after electrical stimulation of the left splanchnic nerve (Harvard inductorium, using 2 batteries; secondary coil at 10 cm or less). Both the control samples and those obtained following electrical stimulation were taken from scattered sites on the skin area mentioned above, since there was no evidence to indicate where maximal viscerocutaneous reflex activity might occur. Immediately after it was obtained, each sample of skin was subjected to the method of extraction described above and, later, was tested on the guinea pig ileum. The effect of electrical stimulation was tested from 4 to 10 times in each animal.

Fifteen experiments were carried out according to this procedure. Table I shows the results in an experiment which is typical of 3

of these. Fig. 2 is a portion of the assay from another of these. There was an increase in the histamine equivalent of the skin dialysate following the operation and, in each case, following stimulation of the splanchnic nerve.

TABLE I.
Histamine Equivalents (in γ) Obtained from the Skin following Splanchnic Stimulation.

Sample No.	Heated in acid	Not heated in acid	Controls
1, 2	.000	.000	
3, 4	.000	.000	
5, 6	.000	.001	
7, 8	.000	.000	
9, 10	.001	.000	Operation
11, 12	.000	.000	
12, 14	.0025	.001	Stimulation
15, 16	.002	.000	"
17	.0075		
18	.000		"
19, 20	.0035	.000	"
21, 22	.0035	.000	
23	.000		
24	.000		
25	.000		

It is to be noted, however, that each sample of skin was taken in duplicate and that the dialysates of one set were not heated in acid as prescribed above. No change in the histamine equivalent was seen in the latter set. This suggests that there is in untreated dialysates either a diffusible substance which is activated by heating in acid, or a heat labile substance which inhibits the action of histamine on the guinea pig ileum. The fact that untreated dialysates of skin when added to the bath in which the gut is suspended frequently depress the activity of added standard histamine solutions favors the latter explanation.

Six of the remaining experiments were not as clear cut as that just described, but showed an increase in histamine equivalent in only occasional skin samples after splanchnic nerve stimulation. Six experiments showed no appreciable change in the histamine equivalent. Three control experiments were carried out in which there was no attempt to apply electrodes or stimulate the splanchnic nerve. No change in the histamine equivalent of the skin samples

³ Davis, Loyal, and Pollack, Lewis J., *Arch. Neur. and Psychiatr.*, 1932, **27**, 282.

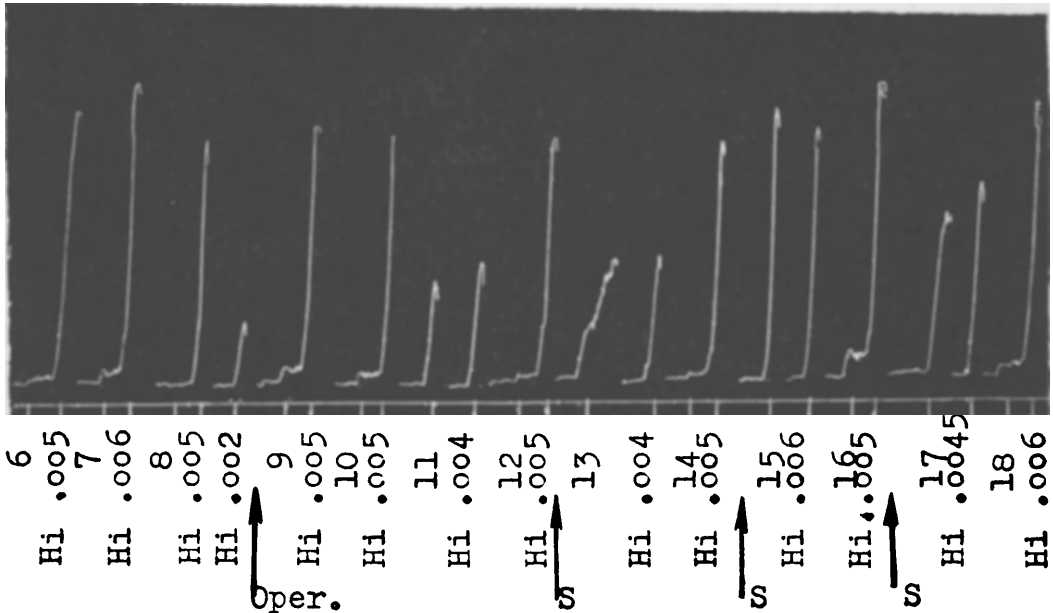


FIG. 2.

Effect on the ileum of standard histamine solutions (Hi., in γ) and of skin dialysates (samples 6-18). Samples 7 and 8, 9 and 10, etc., are duplicates. Odd numbered samples were heated in acid; even numbered samples, untreated. Oper., application of electrodes; S, stimulation.

occurred. However, a slight increase in the histamine equivalent of the skin samples occurs following application of the electrodes to the splanchnic nerve, without there having been an electrical stimulation. This may be due to irritation or mechanical stimulation of the nerve by the application of the electrodes.

Summary. A method for the study of skin histamine has been described. The histamine equivalent of skin extracts obtained by this

method has been shown to increase following injection of histamine into the skin and following burning or chemically irritating the skin. That the substance studied is closely related to histamine has been shown by several tests. Using the method described, experiments have given suggestive evidence that histamine may be liberated in the abdominal skin upon the application of electrodes to and stimulation of the splanchnic nerve.

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The Neurenteric Canal in the Frog, *Rana pipiens*.

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Even the temporary appearance of a tubular connection in the embryo between the neurocoel and the hindgut, known as the neurenteric canal, has considerable phylogenetic significance if its presence can be demonstrated and the parts homologized in such a variety of forms as Amphioxus, Fish, Amphibia, Reptiles,

Mammals and possibly even in birds (duck, goose, Sterna., Lillie¹). It is the purpose of this paper to add another link in the chain of evidence of the embryological existence of such a canal, using the Anuran, *Rana pipiens*.

¹ Lillie, F. R., *The Development of the Chick*, Henry Holt Co., 1919.