EXPERIMENTS ON HISTAMINE AS THE CHEMICAL MEDIATOR FOR CUTANEOUS PAIN*

BY SOL ROY ROSENTHAL, M.D., AND DAVID MINARD, Ph. D.

(From the Tice Laboratories of the Chicago Municipal Tuberculosis Sanitarium, and the Departments of Pathology, Bacteriology and Public Health, and Physiology, University of Illinois College of Medicine, Chicago)

(Received for publication, June 24, 1939)

It has long been known that a histamine-like substance is extractable from practically every organ in the body but especially from the liver, lungs and skin (1). Normally this substance has to do with regulation of circulation (2), but during damage to the tissues by chemical, mechanical or thermal means or through anaphylactic shock, varying quantities are liberated which act directly on the capillaries, causing them to dilate and increase in permeability (1-8). All the evidence presented has been indirect, and some authors therefore do not accept the hypothesis that the substance in question is histamine (9).

The results of the investigation here reported seem to indicate that histamine is liberated by the skin and the cornea in response to non-injurious as well as injurious stimuli, that it acts directly on the sensory nerve endings and that it may be the chemical mediator for pain, much as acetylcholine and sympathin are the mediators in the case of the autonomic nervous system.

Liberation of Histamine by the Superficial Layers of the Skin

By means of a sharp razor, tissue paper thicknesses of skin in which the epidermis and varying amounts of the cutis were included were obtained from human beings (student volunteers), dogs, cats, guinea pigs and rabbits. No anesthetic was used. The skin from the human beings was from the anterior aspect of the arm or thigh, while that from the animals was from the abdomen, chest or back. The tissue was placed over one of the open ends of a glass tube 7.5 mm. in its inside diameter and 1½ inches long, with the cut surface adjacent to the cavity of the tube. The skin was secured by rubber bands to the glass to make that end water tight. By this method the epidermis was made easily available for stimulation and the products thereof could be gathered by allowing them to diffuse into the Ringer-Locke's solution within the tube.

All diffusates were tested on an intestinal strip of the guinea pig suspended in a 4 cc. capacity tube. The method followed was that of Dale and Schultz. The cut surface in contact with the cavity of the tube was first washed, and a control sample

* Aided partially by a grant from Abbott Laboratories.
was obtained by allowing 0.25 cc. of solution to remain in contact with the cut surface of the skin for an average of 1½ minutes. The fluid was pipetted off and another 0.25 cc. was placed in the tube and promptly recovered; the mixture was added to the muscle bath. This procedure was repeated until practically no contraction of the muscle ensued. (Small amounts of histamine are probably liberated as a result of the cutting.) The epidermis was then stimulated with a tetanizing current by bipolar electrodes from a Harvard inductorium (three dry cells in primary circuit). The distance between the primary and secondary coil varied, beginning at the threshold value for the intact skin (7 cm.) or slightly below it. Usually from 6 to 10 areas were stimulated for 10 seconds each and the fluid was recovered and tested by the method mentioned, constant conditions being maintained for the control and the experimental samples. Diffusates procured after pinching (from 10 to 20 times), pricking (from 10 to 20 times) and burning were also tested.

The results obtained with 10 human beings (12 pieces of skin), 11 dogs (36 pieces of skin), 4 cats (10 pieces of skin), 2 guinea pigs (4 pieces of skin) and 2 rabbits (4 pieces of skin) were uniform, in that the amount of presumptive histamine (for identification see below) liberated was directly proportional to the degree of stimulation (Fig. 1). There appeared to be differences depending on the site from which the skin was derived and the species of the subject. It is significant that stimuli at threshold levels (7 to 6 cm.) liberated histamine and that skin subjected to such stimuli showed no gross or microscopic alterations. Under the conditions of the experiment the equivalent of 0.001 gamma of histamine was liberated at the threshold level of 7 cm., 0.0015 gamma at 6 cm. and so on. The skin to be tested must be obtained immediately before stimulation. The skin of dead animals gave negative results except with intense stimuli.

An in vivo experiment in which the rabbit’s cornea, which supposedly contains only sensory nerves for pain, was utilized for electrical stimulation and the diffusate was collected from the corresponding anterior chamber of the eye revealed that here too the amount of histamine liberated was as the intensity of the stimulus (Fig. 2). 23 animals were used, one or both eyes being tested. Sodium amytal anesthesia was employed. Because of the thickness and density of the cornea, stimuli somewhat higher (10 cm.) than threshold stimuli (12 cm.) were used. There was no gross evidence of tissue damage. To rule out the possibility of neutralizing substances, the aqueous humor was replaced by Ringer-Locke’s solution.

**Production of Pain by Histamine When Applied to Skin Denuded of Epidermis and Varying Amounts of Cutis or to the Cornea or When Injected Intradermally**

From ½ to 1 hour after the superficial layers of the skin were removed, varying dilutions of histamine, acetylcholine, histidine and dextrose (the last named having the
Fig. 1. Dog skin (abdomen). 6 areas stimulated, 10 seconds each. Inside diameter tube 7 mm.
This and the following figures represent contractions of the guinea pig small intestine contained in a 4 cc. bath. No = no stimulation; Hi = histamine in gamma; cm. = centimeters on the Harvard inductorium.

Fig. 2. Rabbit cornea. 9 areas stimulated, 10 seconds each. Fluid tested from anterior chamber.
Fig. 3. The action of histaminase and heat on the diffusate of burned skin.

Fig. 4. On the specificity of thymoxyethylidethylamine (Ac, NaSCN, KCl).
same molecular weight as histamine) were applied to the denuded areas by means of a capillary pipette. The solutions, applied in the order mentioned, were sterile and kept at 37°C until used. They were made up in physiologic salt solution (pH 5.8 to 6). 11 human beings (11 areas), 6 dogs (22 areas), 3 cats (6 areas), 2 guinea pigs (12 areas) and 1 rabbit (3 areas) were tested.

The results in human beings were the most striking and constant. The threshold of pain for histamine was at dilutions of 1:60,000 to 1:40,000, with which a slight tingling, pricking or burning sensation was experienced. At a 1:4,000 dilution there was a marked burning or sticking sensation with acute pain. Acetylcholine (the other most likely substance to be found in the skin that causes smooth muscle contraction) gave a slight to moderate burning sensation in a dilution of 1:4,000, but on reapplication even after many washings caused no response in a concentration as high as 1:1,000. The response to histamine in a 1:4,000 dilution was reversible and without much loss in intensity. The threshold for histidine was at dilutions of 1:8,000 to 1:4,000, and dextrose gave no response in a 1:1,000 dilution.

In the cat and the dog the threshold of pain for histamine was difficult to determine, although in many instances dilatation of the pupil occurred with a dilution of 1:20,000. Acute pain responses were obtained at concentrations of 1:2,000 to 1:1,000. For guinea pigs the threshold of pain was determined by first depressing the higher centers with a subanesthetic dose of pentobarbital sodium (25 mg. per kilo of body weight administered intraperitoneally). The animals thus treated were quiet and when irritated responded by exaggerated muscular movements (spinal reflex). The threshold of muscular contractions was at a dilution of 1:2,000 for histamine. When the animals responded to acetylcholine (1:1,000 dilution) or histidine (1:1,000 dilution) it was only on the first application.

The rabbit is a poor experimental animal for testing the production of pain by the application of histamine to the denuded skin. The threshold for acute pain, as noted by a general response, seemed to be at dilutions of 1:1,000 to 1:500 when applied to the skin devoid of the upper layers. Injecting histamine intradermally in dilutions of 1:50,000 to 1:4,000 into 19 human beings gave constant results. At a dilution of 1:50,000 there was a slight tingling or burning sensation after a delay of a few seconds. At a dilution of 1:4,000 there was a definite stinging or sharp sticking pain. At a dilution of 1:1,000 the pain was usually very sharp and severe, lasting as long as 1½ minutes. 2

1 Buffered phosphate solutions of histamine were also used as is discussed later.
2 Histamine made up in a buffered phosphate solution with a pH of 7.0 gave sharper and more lasting painful sensations than the same concentration contained in physiologic solution of sodium chloride (pH 5.8). The buffered solution was without effect.
saline solution gave no reactions. Histamine injected subcutaneously in concentrations as high as 1:1,000 usually failed to cause painful sensations. Topically applied to the cornea of 6 human beings, histamine produced a sensation of warmth and very slight irritation in a dilution of 1:50,000. When a dilution of 1:20,000 or 1:10,000 was instilled into the eye or applied directly to the cornea there was a sharp burning sensation after a delay of a few seconds. A higher concentration (1:1,000) caused a sharp burning to sticking pain immediately. In all instances the painful responses lasted several minutes. Histamine in a 1:1,000 concentration applied to the tongue or mucous membrane of the mouth was without effect.

Evidence Indicating That the Substance in Question Is Histamine

1. The other substance in the skin that would be most likely to cause contraction of the intestinal strip of the guinea pig is acetylcholine. That the substance in question was not acetylcholine was demonstrated when the diffusate obtained by electrical stimulation of the skin or by burning continued to cause contraction of the muscle strip after atropine was added to the bath. (Either 1 gamma of atropine was added to the 4 cc. capacity bath or the Ringer-Locke’s solution was made up to a 1:2,000,000 solution of atropine.)

2. The diffusate obtained from electrically stimulated or burned dog skin by the glass tube method described had the following properties:

   (a) Heat Stability.—The diffusates were heated in a water bath for from 3 to 30 minutes. Assaying the heated fluid on the muscle strip as compared with the unheated sample (Fig. 3) showed from little to no loss of activity.

   (b) Diffusibility through a Cellophane Membrane.—0.2 cc. of the diffusate was placed in a glass tube, one end of which was sealed with a cellophane membrane. This membrane was submerged in 0.2 cc. of 0.9 per cent saline solution contained in a test tube. After 1½ hours the concentrations of the fluids on the two sides of the membrane were equal as judged by their ability to contract the smooth muscle strip.

   (c) Neutralization by Histaminase.—Fresh dog kidneys were ground, dried and extracted with acetone and ether by the method of Best and McHenry (10). An extract of the kidney powder made with 20 parts of a buffered phosphate solution (pH 7.0) and then diluted with saline solution from 2 to 4 times was able to neutralize 0.006 gamma of histamine when incubated at 37°C. for from 15 to 30 minutes. The same extract when incubated with a diffusate containing the equivalent of 0.006 gamma of histamine neutralized the active principle in exactly the same degree as it did histamine (Fig. 3).

3. Thymoxyethylidihethylamine, a phenol ether, has been shown by Bovet
and Staub (11) to neutralize the action of histamine \textit{in vitro} as well as \textit{in vivo}. This has been verified by one of us. Further studies indicate that when as little as 0.5 gamma of the substance is added to a 4 cc. muscle-containing bath and from 3 to 5 minutes allowed to elapse, as much as from 0.02 to 0.04 gamma of histamine fails to cause a contraction of the muscle. On the other hand, when acetylcholine, sodium sulfocyanide, barium chloride, sodium bicarbonate, potassium chloride or tyramine in isotonic concentration was added, contraction of smooth muscle continued in the same or in only slightly less magnitude than before thymoxyethylidethylamine was added to the bath (Fig. 4).

This phenol ether neutralized the action of diffusates from electrically stimulated or burned skin in the same degree as it neutralized the action of histamine and appears to be a specific antagonist of histamine.

\textbf{Abolishment of Cutaneous Pain by Thymoxyethylidethylamine Administered Parenterally or Enteraly}

Thymoxyethylidethylamine, when injected intracutaneously or applied locally to the denuded skin in 0.5 per cent concentrations, produces local anesthesia to the same extent as 1 per cent novocaine but is of longer duration. With dogs (80 animals), when injected subcutaneously in amounts of from 20 to 40 mg. per kilo of body weight or administered rectally in amounts of from 50 to 100 mg. per kilo, it abolished pain responses to pinching, pricking or cutting generally, and raised the electrical threshold from a normal of 7 cm. to from 4 to 0 cm. on the Harvard inductorium. (Dilatation to the pupil was found to be the most sensitive indicator of a painful stimulus.) The electrical threshold for somatic sensory nerves (saphenous) remained unchanged. There was no loss of consciousness, there was slight or no ataxia and there was no loss of knee, pupillary or abdominal reflexes. Stroking the mucous membranes of the nose and lips with cotton elicited retraction of the head. Similar results have been obtained in monkeys (5 animals) and guinea pigs (40 animals); with the latter only pinch and prick were tested. With the monkeys subanesthetic doses of pentobarbital sodium (from 20 to 30 mg. per kilo) were given intraperitoneally from 20 to 45 minutes before the thymoxyethylidethylamine was injected. This was necessary because of the marked excitability of these animals (Sooty Mangabeys). The pentobarbital sodium removed the central inhibition but did not alter appreciably the mechanical or electrical threshold of the skin. An animal thus treated reacted violently to stimulus with a clonic type of movement. From 5 to 10 minutes after the subcutaneous administration of thymoxyethylidethylamine to the monkeys, the
electrical threshold of the skin rose from a normal of 7 cm. to less than 2 cm. on the Harvard inductorium.

COMMENT

That the chemical substances liberated after tissue damage may play a part in the production of pain was hypothesized by Lewis. He at first (6) believed that of the double pain response to a single stimulus, the first component was due to a physical or physical-chemical action on the nerve endings. The histamine liberated produced erythema and wheals like those from firm stroking. The second component was the result of some metabolite other than histamine. Later (8) he explained the second response according to the interpretation of Erlanger and Gasser (12), namely that pain-conducting fibers of different diameters exist and have varying rates of conduction, the smaller fibers accounting for the delay in response and thus the second component. Lewis, in describing his “noci-fensor” system of nerves of the skin, postulated that after gross tissue injury an “H” substance was liberated which stimulated this special system of nerves to liberate more “H” substance at some distance from the site of damage. By this mechanism he explained the hyperesthesia surrounding wounds. His evidence is by inference and not by direct demonstration of chemical substances. Bickford (13), a pupil of Lewis, explained itching on a similar basis. In his late publications, Lewis (8) failed to mention a relation between metabolites liberated after tissue injury and pain.

In this study direct evidence is put forth to establish that skin subjected to electrical stimuli at the threshold of pain liberates histamine. With more intense stimuli, a corresponding increase in the liberation of histamine was noted; burning yielded the largest amount of this substance. The evidence offered by the glass tube method is the most direct yet presented that histamine is liberated when the skin is burned. The data previously reported have been indirect, in that after burning or trauma samples of blood from the general circulation or that draining the injured part were usually collected and examined. The lymph or tissue fluid of the affected part was not tested directly (14–19).

The difference in the type of sensations caused by varying concentrations of histamine is a matter only of degree. Heinbecker, Bishop and O’Leary (20) have described a non-painful pricking touch sensation as the threshold for pain. With an increase of intensity and frequency this gives way to a painful sensation as an adequate number of impulses reach the central nervous system. The delay of the response after the application of histamine in a low concentration speaks for stimulation of the nerve endings
of slowly conducting fibers, whereas more concentrated solutions stimulate nerve endings of more rapidly conducting fibers.

Our evidence indicates that histamine was actually liberated when the skin or cornea was irritated. The possibility that its action was vascular had to be considered. It is well known that direct application of histamine to capillaries causes them to dilate, but this action for an interval is irreversible. The capillaries seem to be paralyzed and will not respond to epinephrine or pituitrin (21). However, in our experiments histamine applied to the denuded skin caused pain on repeated applications and of the same intensity. (The skin was washed with saline solution between each test.) On the other hand, acetylcholine, which produces dilatation of the arterioles and small arteries, when applied in concentrations of 1:4,000, caused some pain but the action was irreversible. After numerous washings and with an increase in the concentration to 1:1,000, acetylcholine still failed to cause pain; histamine continued to cause pain in the same degree.

The mode of action of thymoxyethyldiethylamine is not clearly understood. It does not neutralize histamine directly, because mixing the two and incubating for as long as 4 hours at 37°C. and then adding the mixture to the muscle bath caused an initial contraction of the muscle followed by relaxation, after which the effect of histamine could not be elicited. That its action in the body is peripheral was shown by the fact that after its administration the threshold of electrical stimulation of the skin would often be less than 1 cm. on the Harvard inductorium but the somatic sensory nerves showed no elevation of their thresholds. This action differs from that of procaine hydrochloride, which, as is well known, acts on the nerves directly. The action of this drug also differed when applied to the muscle strip. Thus, as shown in Fig. 6, procaine hydrochloride failed to neutralize the action of histamine. Assayed on the guinea pig’s small intestine, the action of thymoxyethyldiethylamine differs from that of epinephrine (Fig. 5), which also causes smooth muscle relaxation but does not hinder the action of histamine under the conditions of the experiment.

The relaxation of the muscle strip after the addition of the phenol ether does not explain the failure of histamine contraction, for if the phenol ether is added to the bath when the muscle first reaches its maximum relaxed state (in from 1 to 2 minutes) a histamine contraction will ensue. From 3 to 5 minutes of action of the drug is minimal to abolish the histamine response. The muscle tonus is then restored (Fig. 6).

The disposition of the histamine after it is liberated in the skin cannot be told. That a painful sensation, once initiated, usually is lasting speaks against neutralization in the skin. It is well known that histaminase is not present in the skin (10).
SUMMARY

Experimental evidence shows that histamine is liberated when the upper layers of the skin are stimulated in the threshold range although no gross or microscopic evidence of tissue damage is demonstrable. A histamine-like substance is recoverable from the anterior chamber of the rabbit's eye on electrical stimulation of the cornea. This substance is liberated in direct proportion to the intensity of the stimulus. Histamine when injected
intradermally or applied to the denuded skin (less epidermis and some cutis) or cornea causes pain. That the substance liberated is most likely histamine was shown by its action on the intestinal strip of the guinea pig, which action was not effaced by adding atropine to the bath; by its heat stability, its neutralization by histaminase and its dialysability through cellophane membranes, and by the fact that thymoxyethyldiethylamine, which appears to be a specific antagonist to histamine, neutralizes the action of the diffusates of stimulated skin and when injected subcutaneously or rectally abolishes generally the pain responses to pinching, pricking and cutting, and lowers the electrical threshold of the skin markedly without affecting the somatic sensory nerve trunks.

BIBLIOGRAPHY
3. Harris, K. E., Heart, 1927, 14, 161.