

non-cancerous individuals still holds true. The p value, as calculated by the statistical method of Fisher(12), of the normal, of the non-cancerous, and of patients after removal of a malignant tumor showed no difference, but was statistically significant when compared with that of the cancer group.

No specific reaction for adenosinetriphosphatase could be demonstrated. Yeast adenylic acid substrate gave the most consistent results, it is a stable compound, not subject to spontaneous hydrolysis in acid solutions and has a much lower S.I. than that of the other substrates. These factors might be of some value in the differential diagnosis of neoplastic malignant disease. Under our experimental conditions the dephosphorylation of the β -glycerolphosphate was lower than in the usual Bodansky procedure(13).

12. Fisher, R. S., *Statistical Methods for Research Workers*, Edinburgh, 9th Edition, 122, 1944.

13. Bodansky, A., *J. Biol. Chem.*, 1933, v101, 93.

No specificity or differentiation of serum alkaline phosphatase could be established, since the same serum gave similar dephosphorylation regardless of the substrate employed. The relationship of phosphorus release between cancerous and non-cancerous individuals was not influenced by substrate used.

Summary. The sera of normal and non-cancerous individuals have the same magnitude of dephosphorylation. In patients having a malignant tumor, phosphorus release of the serum was generally higher than the normal and non-cancerous group. The highest values were found in patients with tumors of the liver, pancreas and genito-urinary system. The sera of patients from whom a malignant tumor had been previously removed showed a phosphorus release which was identical with the normal and non-cancerous group.

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Histamine as Possible Chemical Mediator for Cutaneous Pain. Dual Pain Response to Histamine. (17842)

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This is the eighth in a series of studies that support the theory of histamine or a histamine-like substance as the chemical mediator for cutaneous pain, be it direct or referred(1-7). It has been demonstrated(7) that as little as 54 molecules of histamine (0.01 ml of 10^{-18} base) introduced into the

superficial layers of the skin will cause painful responses, and that the intensity of the response is directly proportional to the concentration of histamine. The sensations considered previously were of the latent type only. In the present study both the immediate and latent responses to histamine in human subjects are considered.

Methods. Care of materiel. Scrupulous care was taken in the cleaning of the glassware, needles, etc. All glassware was cleaned in sulphuric acid potassium dichromate solution. The needles were placed in running water for 4 to 6 hours and then rinsed by forcing 10 cc of distilled water through them. After sterilization and before use, needles

1. Rosenthal, S. R., and Minard, D., *J. Exp. Med.*, 1939, v70, 415.

2. Lambert, E. H., and Rosenthal, S. R., *Proc. Soc. Exp. Biol. and Med.*, 1940, v44, 235.

3. Minard, D., and Rosenthal, S. R., *Proc. Soc. Exp. Biol. and Med.*, 1940, v44, 237.

4. Rosenthal, S. R., Minard, D., and Lambert, E., *Proc. Soc. Exp. Biol. and Med.*, 1943, v52, 317.

5. Lambert, E., and Rosenthal, S. R., *Proc. Soc. Exp. Biol. and Med.*, 1943, v52, 302.

6. Rosenthal, S. R., and Lambert, E., in publication.

7. Rosenthal, S. R., and Sonnenschein, R. R., *Am. J. Physiol.*, 1948, v155, 186.

TABLE I.
Immediate Painful Response on Intradermal Injection of Histamine in Human Subjects.

Conc.	No. subjects	No. samples	Pain on injection				
			0	+	++	+++	% pos.
Saline	40	48	32	14	2	0	33
10-15	26	37	6	25	6	0	84
10-7	36	28	4	9	11	4	86
10-5	33	30	0	6	9	15	100

TABLE II.
Delayed Painful Response to Intradermal Injection of Histamine in Human Subjects.

Conc.	No. subjects	No. samples	Delayed pain response					
			No. +	% +	Time onset, seconds*	Duration, min.†	No. with incr.	% with incr.
Saline	40	76	44	58	32	0.56	2	3
10-15	26	61	52	85	33	1.6	8	13
10-7	36	49	46	94	27	1.8	14	29
10-5	33	44	42	95	16	3.5	34	79‡

* Considering only those in which there was a delayed response.

† Considering all samples.

‡ Considering 34 of 43 samples.

and syringes are rinsed in sterile saline 3 times. Histamine hydrochloride or phosphate as base was diluted in sterile saline made with chemically pure water.

Procedure for testing. The subjects were drawn mainly from the medical faculty of the University of Illinois and supplemented by second year medical students. The subjects were not told of the nature of the experiment other than that they would receive intradermal injections and that they were expected to describe any local effects sustained. The subjects were made comfortable in a reclining chair with foot stool. All external noises or interruptions were minimized. The subjects were told not to look at the injection site. Using a 27 gauge needle and a 2 cc syringe, the needle was gently inserted as superficially as possible, just burying the needle aperture. When it was ascertained that the pain from this insertion was over, about 0.01 ml was injected very slowly, producing a 2 to 3 mm wheal. No prompting by the operator was given with the following exception: in the low concentrations, many of the subjects would fail to voice the result during the time of injection. At this point the subject would be asked if he noted anything during injection. Com-

parisons with previous injections were also solicited. The results of each injection were timed by stop watch for a minimum of three minutes and recorded in the words of the subject.

Results. Forty subjects received a total of 230 injections. Both the immediate and latent sensations were studied.

Immediate response. In Table I the immediate responses to 143 injections are recorded. This number is less than the total number of injections because at the beginning of the study many individuals failed to voice the effect produced during the injection of the solutions. They stated that after the experiment that they expected some sort of sensation on injection, and therefore did not mention it. For this reason, the procedure of direct questioning of the subject immediately after injection was adopted. The intensity of the immediate painful response was judged by comparing the responses to various solutions injected in each individual subject. The grading was from 0 (no response) to 3+ (reserved for some exclamation of pain). The table thus represents the summation of individual experiments (0 to 3+) as well as a consideration of the responses as a whole (in percentages). From

this table it is evident that the degree of response was directly related to the concentration of histamine. It is also noted that the highest number responding on injection (100%) was to the most concentrated solution of histamine (10^{-5}). The nature of the immediate response varied from a pricking, tingling sensation to acute pain, depending upon the concentration of histamine. Some described it as "being stuck with a needle" or "being burned" or "being stung by a bee or a nettle".

Latent response. The time of onset after injection, duration and the augmentation of the latent response is recorded in Table II. A little better than half (58%) gave a latent response following saline. When this occurred, it was relatively late after the injection (mean = 32 seconds) and was of very short duration (mean = 34 seconds). Only 2% or 3% showed an augmentation of this sensation. Following histamine injections practically all (94% and 95%) responded to the higher concentrations of histamine. What is equally significant is that the mean duration of this response was directly related to the concentration of histamine (1.8 minutes for 10^{-7} and 3.5 minutes for 10^{-5} dilution). Similarly, in the higher concentrations a greater percentage of subjects indicated an augmentation of the painful response. The time of onset of this latent response was shortened appreciably in the 10^{-5} dilution (mean = 16 seconds). In some instances the immediate and latent responses merged. The character of this sensation was described as pricking, stinging, tingling, burning, dull and sharp pain. Itching was noted with the higher concentrations(7). Sensations of cold, warmth or pressure were occasionally reported, but were not included in the tabulation of painful responses.

Considering the presence or absence of the latent painful sensation only, and combining results of this study with those of a previous one, 67 subjects are represented upon whom 408 injections were performed. These subjects represent both sexes, Negro, white and Asiatic races, and age ranges from 20 to 45 years (Table III). The percentage of subjects yielding a latent painful response is di-

TABLE III.
The Presence or Absence of the Delayed Painful Response in Human Subjects Following Histamine Injection.

	No. subjects	10^{-5}			10^{-6} to 10^{-8}			10^{-5} to 10^{-15}			Saline		
		No. samples			No. samples			No. samples			No. samples		
		No. +	% +		No. +	% +		No. +	% +		No. +	% +	
Exp. 1	27	10	100		64	58	91	44	31	70	60	21	35
" 2	40	44	95		49	46	94	61	52	85	76	44	58
Total	67	54	96		113	104	92	105	83	79	136	65	48
Value of p		<.001			<.001			<.001					

rectly proportional to the concentration of histamine. Comparing the latent response of histamine to that of saline, the value of p in each concentration was less than .001, indicating its significance statistically.

Discussion. Under the conditions of this experiment, histamine injected into the superficial layers of the skin caused immediate painful sensations which usually disappeared promptly, but reoccurred after a lapse of time (latent or secondary responses). The intensity of the painful sensation and its duration was directly proportional to the concentration of histamine, while the time lapse between the primary and secondary response varied inversely with the concentration. Histamine when injected into the deeper layers of the skin fails to produce pain, but will cause a wheal and hyperemia. In two subjects having pronounced dermatographia or skin allergies, the histamine pain response was diminished. The immediate response, especially in the lower concentrations, may not be elicited at a given point of inoculation, but may be noted at a site adjacent to it. This phenomenon is possibly related to the proximity of the injected histamine to the specialized pain receptor points. The latent response is most likely due to the summation of subthreshold impulses initiated by the diffusion of histamine. The relatively prolonged length of time for the appearance of this response excludes the time of conduction through nerves of varying diameter as a factor.

The high percentage of individuals reacting to saline injection, especially for the latent response, can in part be due to the fact that upon insertion of the needle, a certain amount of histamine is liberated. The fact that 58% of the subjects in this study as compared to 35% of the subjects in a pre-

vious study experienced delayed responses to saline may be explained by the fact that in this study the majority of the subjects were older and engaged in research and thus were more perceptive, whereas in the former study the majority were medical students. The failure of Lewis(8) and more recently Emmelin and Feldberg(9) to note pain after histamine injection is probably due to the method of pricking the histamine into the skin, and the concentration of histamine. By injecting histamine by means of needle and syringe, larger quantities are more easily delivered to the site of the specialized receptors of the skin and the fibers of the sensory nerves about them. The concentration of histamine in the skin is considered to be about 1:50,000(8). This is a sufficient concentration to cause all gradations of cutaneous pain. There is no histaminase in the skin(10).

Summary. 1. Histamine introduced into the superficial layers of the skin of human subjects causes an immediate as well as a latent painful sensation. 2. The intensity and the duration of the response are directly proportional to the concentration of histamine. 3. The lapse of time between the primary and the latent secondary response is inversely proportional to the concentration of histamine. 4. These findings give further support to the theory that histamine or a histamine-like substance may be the chemical mediator for cutaneous pain.

8. Lewis, T., *Pain*, Macmillan Co., New York, 1942.

9. Emmelin, M., and Feldberg, W., *J. Physiol.*, 1947, v106, 440.

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