[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY AND RESEARCH BACTERIOLOGY, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

A Spectroscopic Study and Assay of Histamine

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Work in progress in this Laboratory on the physiological importance of histamine made it advisable to study the possibility of assaying small quantities of this base. There are three different methods by which this may be accomplished: (1) physiological—making use of smooth muscle preparations, the stimulation of gastric secretion, certain skin tests, etc., (2) a combined chemical and physical—based upon colorimeter methods, and (3) physical—spectroscopic methods, a possibility not heretofore realized.

Ellinger[1] in 1928 reported a difference in the absorption curves of histamine and histidine, histidine showing a maximum at about 280 mμ, while histamine gave only an end absorption. Evidence that both compounds give only end absorption is contained in the publications of Bourdillon, Gaddum and Jenkins,[2] Becker,[3] and data in the "International Critical Tables."[4] The possibility, however, of Ellinger being correct led us to make some preliminary determination of the absorption spectra of histidine and histamine, while determining the sensitivity of a possible spectroscopic assay of the base.

A medium size Hilger quartz spectroscope, having a dispersion of 10 cm. between the wave lengths 210–700 mμ was used. The photographic plates used were Eastman D. C. Ortho and Eastman Commercial Panchromatic. The latter were found to be more satisfactory in the ultraviolet region. For the preliminary tests the sources of light were: (1) a hydrogen tube provided with a quartz window and operating at 4 mm. pressure, and (2) a water-cooled mercury arc. For later work we employed a water quenched discharge between two brass electrodes 4 mm. apart. The electrodes were connected to a Tesla coil and apparatus similar to that described in the Bureau of Standards Technical Papers, No. 148 (appendix). A Hilger rotating sector was inserted between the source of light and the spectroscope. The length of exposure was twenty-five seconds for the assay of the base, which through the sector is approximately 12.5 seconds of continuous exposure.

The plates were developed with Eastman Formula D-61a for seven minutes at 18° and fixed with acid hypo formula F-1.

For the preliminary studies the solution was contained in a quartz cell of 5.5 mm. thickness, while a cell of 2 cm. thickness was employed with the sectorphotometer.

(1) Ellinger, *Arch. Exp. Path. Pharm.*, **136**, 129 (1928).
(2) Bourdillon, Gaddum and Jenkins, *Proc. Roy. Soc.* (London), **B106**, 388 (1930).
(3) Becker, *Arch. ges. physiol.* (*Pflügers*), **288**, 755 (1931).
(4) "International Critical Tables," Vol. V, p. 373.

As to the question of the similarity or dissimilarity of the absorption curves of histamine and histidine, our rather preliminary determinations showed but an end absorption in each case. No maximum point in the absorption curve for histidine, as reported by Ellinger, was observed.

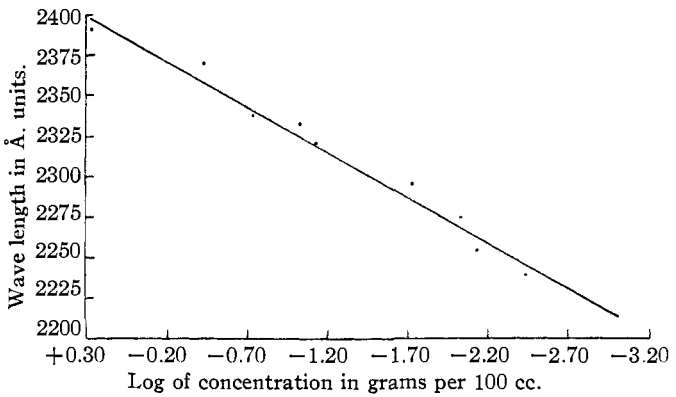

Fig. 1.

Å.	Concn. g./100 cc.	log	Å.	Concn. g./100 cc.	log
2390	1.82	0.260	2305	0.0367	−1.435
2370	0.367	−0.435	2295	0.0182	−1.740
2338	0.182	−0.740	2274	0.0091	−2.041
2333	0.091	−1.041	2255	0.0073	−2.136
2320	0.073	−1.136	2240	0.00367	−2.435

This end absorption was seen to shift functionally toward the shorter wave lengths with increasing dilutions of both histidine and histamine. It is upon this phenomenon that the following table is based. This table shows the relationship between concentration and wave length of the end absorption of histamine, and the lowest concentration of histamine to be determined by the method and apparatus referred to above.

If a comparison is made of the sensitivity of physiological, chemical and physical tests for histamine, the following figures will approximate the facts.

Physiological methods	
Guinea pig intestine	0.0004 mg./100 cc.[6]
Guinea pig uterus	.004–0.0004 mg./100 cc.[6]
Gastric secretion	.0033 mg. per kilo dog weight[7]
Wheal test	.02 mg./100 cc.[8]
Combined chemical and physical methods	
Colorimetric	.0125 mg./100 cc.[9]
Physical methods	
Spectroscope	3.67 mg./100 cc.

(5) Guggenheim and Löffler, *Biochem. Z.*, **72**, 303 (1916).
(6) Burn, "Methods of Biological Assay," Oxford University Press, 1928.
(7) Popielsky, *Pflügers Arch.*, **178**, 214 (1920).
(8) Lewis and Grant, *Heart*, **11**, 209 (1924).
(9) Hanke and Koessler, *J. Biol. Chem.*, **39**, 497 (1919).

In this connection mention should be made of attempts chemically to alter histamine and histidine in the hope of obtaining substances that would permit a differentiation between the base and the amino acid. The histamine–formaldehyde complex, histidine–formaldehyde complex, and the corresponding condensation products with diazotized sulfanilic acid were chosen to represent colorless and colored derivatives, respectively, with the additional expectation of intensifying absorption by the added double bonds ($R—N=CH_2$)($R—N=N—R$). It appeared that no decisive advantage was forthcoming from these experiments. However, both the histamine-formaldehyde complex and the condensation product of the base with diazotized sulfanilic acid seemed to absorb slightly more than the corresponding histidine derivatives of equal concentration. The reverse was observed in certain concentrations with the parent substances (hydrogen tube and mercury arc).

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Summary

1. Histamine may be estimated spectroscopically under the conditions described between the concentrations 1.82–0.00367 g./100 cc.

2. The lowest quantity of histamine detectable was found to be 3.67 mg./100 cc.

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