[CONTRIBUTION FROM THE INSTITUTE OF EXPERIMENTAL BIOLOGY, UNIVERSITY OF CALIFORNIA]

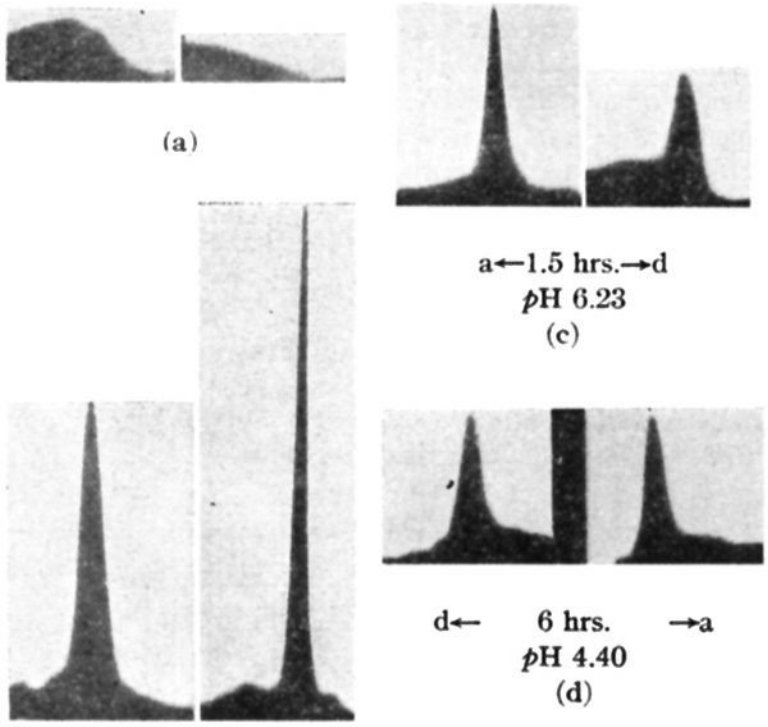
Electrophoresis of Crotoxin*

BY CHOH HAO LI AND H. FRAENKEL-CONRAT

The crystalline protein (crotoxin), isolated by Slotta and Fraenkel-Conrat[1] from rattlesnake venom, has been shown to behave as a homogeneous substance in ultracentrifuge and diffusion studies.[2] The electrophoretic behavior of this beautifully crystalline substance will now be reported.

Experimental

Approximately 150 mg. of crystalline crotoxin was prepared from the crude venom[2a] and crystallized twice according to the method described previously.[1] The electrophoretic experiments were carried out in the Tiselius apparatus[3] at 1.5°.



(a)

a←1.5 hrs.→d
*p*H 6.23
(c)

d← 6 hrs. →a
*p*H 4.40
(d)

d←2 hrs.→a
*p*H 7.20
(b)

Fig. 1.—Electrophoretic patterns of crotoxin solutions in buffers of different *p*H and ionic strength 0.10 at 1.5° after the current has been put on for 2.0, 1.5 or 6 hours: (a) is the base line for the electrophoretic patterns.

The electrophoretic patterns were recorded by the method described by Longsworth.[4] Buffers were prepared according to Clark[5] and brought to ionic strength 0.10 by the addition of sodium chloride. The *p*H of the solution was obtained with the aid of a glass electrode–vacuum tube assembly at room temperature. No corrections were made for 1.5°. The conductance was measured with the usual Wheatstone bridge type of circuit and a Washburn conductivity cell at 1.5°. The mobility was calculated from the descending boundary as recommended by Longsworth and MacInnes[6] and was determined in the manner described in a previous paper.[7]

Results

The first experiment was conducted in a phosphate buffer of *p*H 7.20 with 1.0% crotoxin solution. The boundaries appeared very sharp and there was no indication of the appearance of other components after the current had been on for two hours in a field gradient of 6.40 volts per cm. (see Fig. 1b). Studies in solutions of other *p*H also indicate that the crystalline crotoxin is an electrophoretically homogeneous protein. Fig. 1[8] presents a few typical patterns obtained in *p*H 7.20, 7.00, 6.23 and 4.40 buffer solutions. Each experiment was made in a field gradient approximately 6 volts per cm.

It is to be noted that here, as in the case of other proteins, the ascending boundary is always sharper than the descending one. This anomaly is currently attributed to variations of the electric field strength in the boundary.[9] When the current is reversed and the descending boundary becomes a rising one, this boundary should gradually become sharper. This is the case for the crotoxin solutions. Figure 2A shows a series of photographs taken after electrolysis for one and two hours at 6.26 volts per cm. with a 0.5% crotoxin solution

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(1) Slotta and Fraenkel-Conrat, *Ber.*, **71**, 1076 (1938); *Nature*, **142**, 213 (1938).

(2) Gralen and Svedberg, *Biochem. J.*, **32**, 1375 (1938).

(2a) We are greatly indebted to Professor K. H. Slotta for his interest in this investigation as well as for his coöperation in drying and preserving the crude rattlesnake venom kindly put at our disposal by Dr. Cavalcanti of the Instituto Butantan, São Paulo. Our thanks are also due to Professor H. M. Evans for enabling us to undertake this investigation.

(3) Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(4) Longsworth, THIS JOURNAL, **61**, 529 (1939).

(5) Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, Md., 1922.

(6) Longsworth and MacInnes, THIS JOURNAL, **62**, 705 (1940).

(7) Li, Lyons and Evans, *J. Gen. Physiol.*, **23**, 433 (1940).

(8) It may be noted that the base lines of these patterns are very irregular. This is generally the case using a poor Schlieren lens. For the interpretation of these patterns, the distorted base line is given in Fig. 1.

(9) Longsworth and MacInnes, THIS JOURNAL, **62**, 705 (1940).

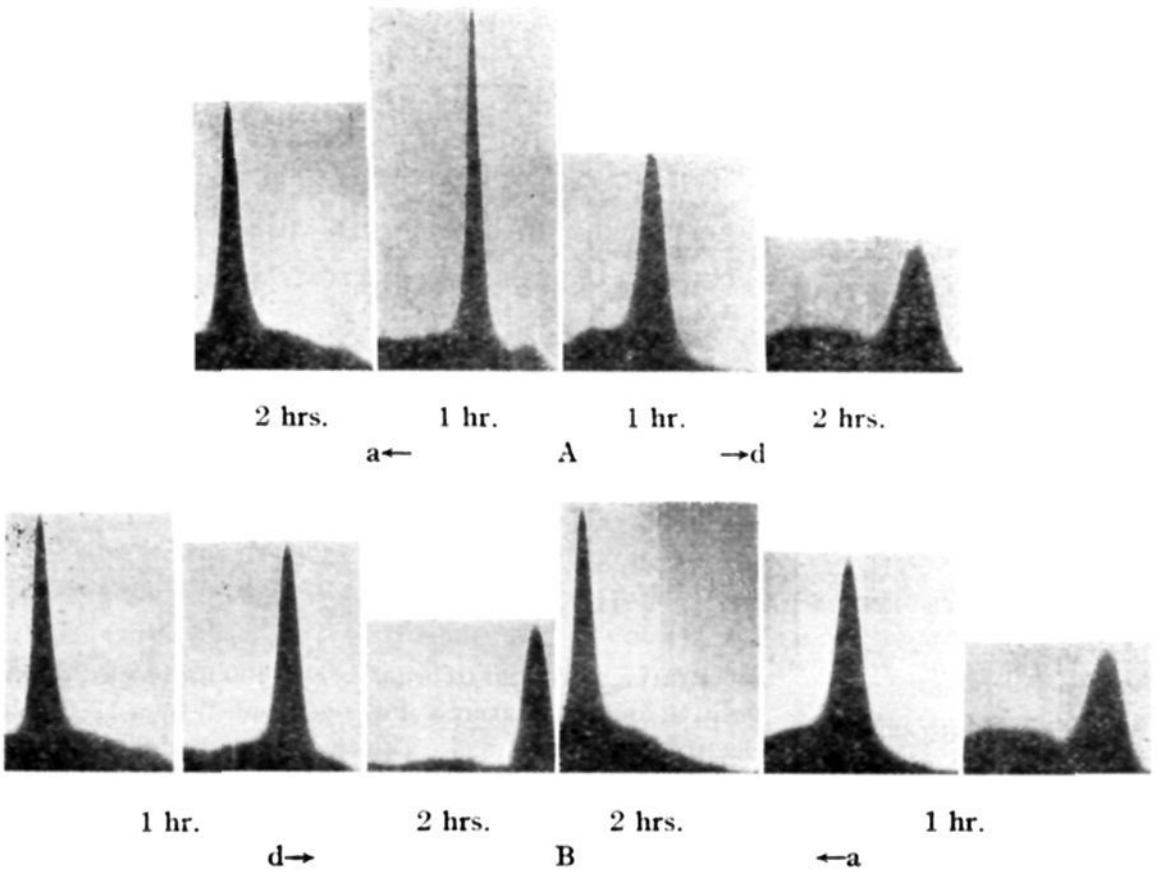

Fig. 2.—Electrophoretic patterns of a crotoxin solution illustrating no "reversible boundary spreading" phenomena in *p*H 7.00 phosphate buffer of ionic strength 0.10 at 1.5°.

in a *p*H 7.00 phosphate buffer of ionic strength 0.10. After the current was reversed, the descending pattern became much sharper while the rising one showed spreading as indicated in Fig. 2B. These observations are particularly interesting, for certain proteins always give rise to a phenomenon of "reversible boundary spreading" which, as first discovered by Tiselius and Horsfall,[10] indicates that "an initial sharp boundary will become diffuse as the electrolysis proceeds but if the direction of the amount is reversed will progressively recover most of its initial sharpness."[11] Some authors[11] believe that this phenomenon is due to the inhomogeneity of the protein and others[12] express the view that it is a property of pure proteins. Here, we have a case which does not show the phenomenon of "reversible boundary spreading" as illustrated in Fig. 2, nor was it observed in any other experiment with crotoxin.

Table I summarizes the mobility determinations in different *p*H solutions of ionic strength 0.10. A plot of *p*H against mobility is found in Fig. 3. The isoelectric point is located from the straight line which is drawn through the points made in acetate buffers of *p*H 3.91, 4.23, 4.40 and 4.90. This seems to be justified, for phosphate ions have been shown[13] to alter the electrophoretic mobility of a protein as compared with that made in a monovalent salt buffer. Thus, the isoelectric point of crotoxin is found to be 4.71 and the $d\mu/dpH_0$ value is 4.65×10^{-5} in a solution of ionic strength 0.10.

TABLE I

ELECTROPHORETIC MOBILITY OF CROTOXIN IN DIFFERENT *p*H OF IONIC STRENGTH 0.10 AT 1.5°

*p*H	Buffer salt	Protein concn.	μ_d (10^5)
7.20	Phosphate	1.0	−4.97
7.00	Phosphate	0.4	−4.51
6.23	Phosphate	.5	−2.80
4.90	Acetate	.3	−0.57
4.40	Acetate	.4	+1.19
4.23	Acetate	.3	+2.28
3.91	Acetate	.5	+3.71

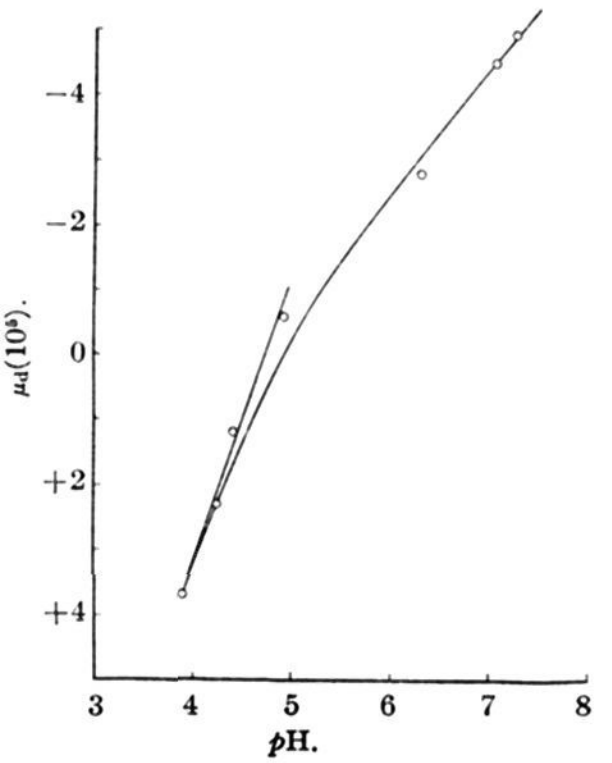

Fig. 3.—The electrophoretic mobility of crotoxin as a function of the *p*H.

(10) Tiselius and Horsfall, *Ark. Kemi. Mineral Geol.*, **13A**, No. 18 (1939).

(11) Longsworth, Cannan and MacInnes, THIS JOURNAL, **62**, 2586 (1940).

(12) Shedlovsky, *et al.*, *Science*, **92**, 198 (1940).

(13) Longsworth, *Ann. N. Y. Acad. Sci.*, **XLI**, 267 (1941).

Summary[14]

1. Crotoxin, the crystalline neurotoxin isolated from rattlesnake venom, has been shown to be a homogeneous substance in electrophoresis experiments.

2. Crotoxin solutions do not exhibit the phenomenon of "reversible boundary spreading" to a detectable extent, in contradistinction to all other proteins studied.

3. The isoelectric point of crotoxin has been determined and the $d\mu/dpH_0$ value.

(14) The authors are indebted to Dr. L. G. Longsworth for his suggestions during the preparation of the manuscript.

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Ionization and Dissociation by Electron Impact: Normal Butane, Isobutane, and Ethane

BY D. P. STEVENSON[1] AND J. A. HIPPLE, JR.

Introduction

Mass spectrometer studies of the dissociation products resulting from electron bombardment of gases at low pressures have provided many interesting and instructive results. In particular, the work on the lower hydrocarbons, methane,[2] ethane,[3] ethylene,[4,5] propane,[6] propylene[6] and allene[6] has yielded considerable data on energies and unimolecular reactions of the ions CH_4^+, $C_2H_6^+$, etc., not obtainable in any other way. It was felt that the extension of such studies to one of the simplest isomeric pairs, *n*- and *i*-butane, would be helpful in deciding or at least defining questions of interpretation raised by the work on the simpler molecules. Furthermore, the continually growing interest in the application of the mass spectrometer to problems in the analysis of hydrocarbon mixtures makes necessary a knowledge of the complete mass spectra, so that the limitations of this analytical tool can be discussed. With this latter point in mind we have extended the results of the previously published investigation of ethane.[3]

Experimental

Inasmuch as the apparatus is to be described in detail by one of the authors (J. A. H.) in a separate publication, we will give but a cursory discussion of the pertinent details here.

The 180° tube (~16 cm. radius) and accessories are supported within a water-cooled spherical solenoid. The strength of the magnetic field used throughout this investigation was ~1000 Oersteds, corresponding to 130 volts accelerating potential to bring $m/e = 100$ into focus. The positive ion accelerating voltage is supplied by a 1200 volt electronic power source. The electrons are obtained from an oxide coated platinum cathode, their accelerating potential being controlled by means of a wire wound drum potentiometer. An electrometer tube amplifier and sensitive galvanometer are used to measure the positive ion current. The resolving power of the tube with the wide slits used is 1:150 as indicated by the extent to which the mercury isotopes are resolved.

The gases are admitted to the ionization chamber through a glass capillary leak. A separate pumping lead to the arm of the tube containing the cathode chamber assures the complete removal of any pyrolysis products formed on the filament.

The samples of the two butanes were obtained from the Gulf Research Laboratories, while the ethane was taken from a sample given to us by the Standard Oil Co. of Indiana.

Preliminary examinations of the mass spectra of the butanes were made using an automatic recorder. The peaks corresponding to the various masses were all quite symmetrical, and were spaced in exact accord with the inverse relationship between m/e and accelerating voltage. No satellites or shoulders were observed for any of the masses. The measurements reported in this paper were all manually recorded. The symmetry of the peaks indicated that the current at the top of the maxima could be taken as a measure of the total current due to the corresponding ion. The linear variations of the positive ion current with the density of the electron beam and with the pressure in the ionization chamber indicate that only the products of primary reactions were observed. The total electron emission from the cathode ran from 5 to 15 μ amp., while the intensity of the bombarding beams lay between 0.1 and 3 μ amp.

Results

The results of this investigation may be divided, for convenience, into two parts: (A) the variation of the mass spectra of the molecules with the electron energy, V^-, for V^- large with respect to the critical potentials; and (B) the determination

(1) Westinghouse Research Fellow.
(2) L. G. Smith, *Phys. Rev.*, **51**, 263 (1937).
(3) J. A. Hipple, *ibid.*, **53**, 530 (1938).
(4) P. Kusch, A. Hustrulid and J. T. Tate, *ibid.*, **52**, 843 (1937).
(5) J. Delfosse and J. A. Hipple, *ibid.*, **54**, 1060 (1938).
(6) J. Delfosse and W. Bleakney, *ibid.*, **56**, 256 (1939).