Contraction of Frog Stomach Muscle and Heart in Non-Ionic Solutions

Frog stomach muscle frequently washed with half-isotonic (0.112*M*) solution of sucrose, continues to contract spontaneously for 24 h at 25–30°C[1–3]; these contractions are accompanied by conducted action potentials[4–6]. Frog heart perfused with half-isotonic solution of sucrose contracts spontaneously for about 2–7 h at 18–20°C and shows conducted action potentials[7–13]; at a higher temperature it passes into a contracture. In the present research, experiments have been performed to study the relationship between the spontaneous contractions of frog stomach muscle and heart and their sodium content.

Methods. These experiments were performed on the stomach muscle and heart of the frog *Rana tigrina*. Frogs weighing about 300 g were pithed and their stomachs removed. The stomach was cut longitudinally at the greater curvature and the mucous membrane removed. It was first washed with Ringer solution, and then suspended in 100 ml of half-isotonic (0.112*M*) solution of sucrose, which was renewed every 15 min. The mechanical activity was recorded with an isotonic lever. The heart after being taken out was perfused continuously with half-isotonic solution of sucrose using a Symes cannula. Its contractions were recorded with an isotonic lever.

The tissues were removed, dried on filter paper, weighed and ashed at 580°C. Sodium and potassium were estimated by flame photometer.

Results. When washed frequently, or when perfused with half-isotonic solution of sucrose, both frog stomach muscle and heart lose 90% of their sodium in the first 15 min, and are sodium-free in 30–60 min (Table I, II). BOZLER[3] also found that, in the frog stomach muscle, sodium is completely washed out by isotonic solution of sucrose in 1 h.

Spontaneous contractions in the frog heart, perfused with half-isotonic solution of sucrose, develop when it contains no sodium and stop when the heart is again perfused with isotonic or half-isotonic sodium chloride or Ringer solution, thus increasing its sodium content (Fig. 1). The spontaneous contractions of frog stomach muscle also develop when it is free of sodium, and are bigger than in Ringer solution; they stop when the muscle is re-immersed in Ringer solution and its sodium content is increased (Fig. 2).

Table I. Effect of perfusion with 0.112*M* sucrose on the sodium and potassium content of frog heart

Time of perfusion (min)	No. of hearts perfused	Sodium		Potassium	
		m*M*/kg of wet muscle	% of that in fresh heart	m*M*/kg of wet muscle	% of that in fresh heart
0	6	24.8 ± 0.76	100	72.1 ± 3.19	100
15	6	2.8 ± 0.16	11	28.5 ± 1.1	40
30	6	0.06 ± 0.0018	0.026	21.5 ± 0.44	30
45	6	Not calculable, less than 0.05	–	21.2 ± 0.46	29
60	6	Not calculable, less than 0.05	–	16.1 ± 0.39	22
120	6	Not calculable, less than 0.05	–	17.1 ± 0.45	24

Table II. Effect of washing every 15 min with 0.112*M* sucrose solution on the sodium and potassium content of frog stomach muscle

Time of washing (min)	No. of experiments	Sodium		Potassium	
		m*M*/kg of wet tissue	% of that in fresh tissue	m*M*/kg of wet tissue	% of that in fresh tissue
0	6	53.5 ± 1.4	100	69.8 ± 1.9	100
15	6	5.8 ± 0.26	10.9	53.7 ± 2.0	76.9
30	6	1.3 ± 0.12	2.4	38.9 ± 1.4	55.6
45	6	0.17 ± 0.28	0.3	36.1 ± 2.1	51.6
60	6	Not calculable, less than 0.05	–	35.4 ± 1.9	50.6
90	6	Not calculable, less than 0.05	–	33.8 ± 1.4	49.3
120	6	Not calculable, less than 0.05	–	30.1 ± 1.8	43.0

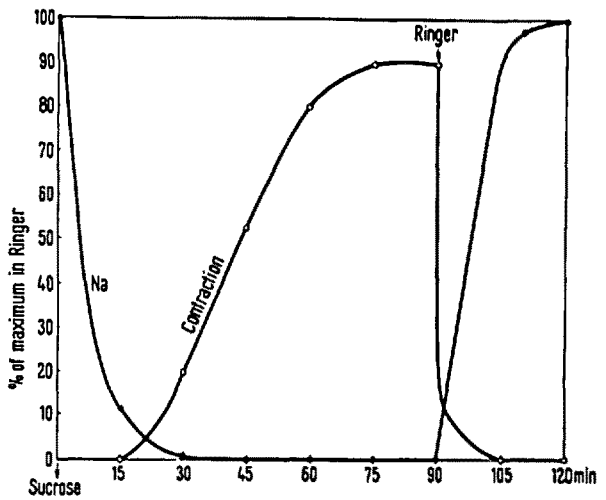

Fig. 1. Effect of perfusion with half-isotonic, 0.112*M*, solution of sucrose on the sodium content and magnitude of the spontaneous contractions of frog heart.

[1] S. I. SINGH and I. SINGH, Proc. Indian Acad. Sci. *18*, 56 (1943).
[2] I. SINGH, Current Sci. (India) *13*, 231 (1944).
[3] E. BOZLER, Am. J. Physiol. *199*, 299 (1960).
[4] I. SINGH and J. BHATT, Proc. Indian Acad. Sci. *46*, 211 (1957).
[5] I. SINGH and A. K. ACHARYA, Proc. Indian Acad. Sci. *46*, 285 (1957).
[6] R. L. KOLODNEY and W. G. VAN DER KLOOT, Nature *190*, 786 (1961).
[7] I. SINGH, K. B. SEHRA, and S. I. SINGH, Current Sci. (India) *14*, 152 (1945).
[8] I. SINGH, K. B. SEHRA, and S. I. SINGH, Proc. Indian Acad. Sci. *21*, 259 (1945).
[9] W. G. VAN DER KLOOT and N. S. RUBIN, J. gen. Physiol. *46*, 35 (1962).
[10] I. SINGH, Am. J. Physiol. *203*, 422 (1962).
[11] I. SINGH, Arch. int. Physiol. *71*, 361 (1963).
[12] I. SINGH, Exper. *20*, 33 (1964).
[13] I. SINGH, Arch. int. Physiol. *72*, 378 (1964).

Discussion. It has been argued by BRADY[14] that the electrical and mechanical activity of frog heart in sucrose solutions is due to retention of about 10% sodium in interspaces. This argument is untenable as there is no correlation between mechanical activity of frog stomach muscle and its sodium content. Electrical and mechanical activity in these tissues continue when they contain no sodium; in fact the more thoroughly the sodium is washed out, the better its mechanical activity. Thus the electrical and mechanical activity of frog stomach and heart could not be due to retention of sodium in the interspace; sodium is actually deleterious to such activity after these tissues have become acclimatized to sucrose. The ionic hypothesis of HODGKIN and HUXLEY[15] is thus not applicable to these tissues[16].

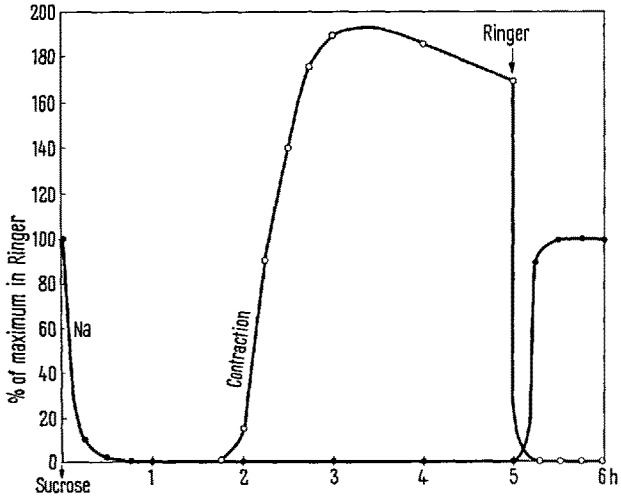

Fig. 2. Effect of washing every 15 min with half-isotonic, 0.112 *M*, solution of sucrose on the sodium content and magnitude of the spontaneous contractions of frog stomach muscle.

Zusammenfassung. Herz und Magenmuskulatur des Frosches verlieren in halb-isotoner Rohrzuckerlösung innert 1 h alles Natrium. Trotzdem bleibt die spontane Kontraktilität erhalten. Natrium ist also für die Erregbarkeit dieser Muskeln nicht Voraussetzung.

I. SINGH and N. V. RAJU

Department of Physiology, Medical College, Agra (India), October 10, 1964.

[14] A. J. BRADY, Ann. Rev. Physiol. *26*, 341 (1964).
[15] A. L. HODGKIN and A. F. HUXLEY, J. Physiol. (London) *117*, 500 (1951).
[16] We wish to thank the Indian Council of Medical Research for defraying part of the expenses of this research.

The Phospholipids of the Tobacco Hornworm, *Protoparce sexta* Johan. (Lepidoptera; Sphingidae)[1,2]

Studies[3,4] of the phospholipids of lepidopterous insects have shown phosphorylcholine and phosphorylethanolamine to be incorporated into the phospholipids of *Celerio euphorbiae*. In *Arctia caia* moths[5] the principal phospholipids are phosphatidylcholine and phosphatidylethanol amine with only small amounts of other phospholipids.

In this study of the hornworm, *Protoparce sexta*, the phospholipids of fifth instar larvae and adults were chromatographed on silicic acid as described previously[6]. Phosphorus determinations[7] showed two asymmetrical peaks. The fractions comprising the peaks (I and II) and the trailing areas behind them (Ia and IIa) were pooled and the following analyses carried out: esterified fatty acid[8], plasmalogens[9], ethanolamine[10], serine[10], choline[11], and an unknown amino compound[10]. Qualitative tests for inositol[12] and spingosine[13,14] were carried out (Table). These results indicate that both peaks are mixtures of phospholipids. The major component of the first peak is phosphatidylethanolamine and the second, phosphatidylcholine. Serine containing phospholipids are present in only small amounts, and neither inositol nor spingosine could be detected in acid hydrolysates. Plasmalogens form only a small part of the phospholipids of the adults (peak I) and are almost absent from the larva.

The unknown amino compound is seen on paper chromatograms of phospholipid hydrolysis products. In view of the high level of free amino acids in insects[15] and the ability of phospholipids to solubilize water soluble compounds in lipid solvents[16], it appeared necessary to identify this compound and to establish that it was part of a lipid molecule. The compound corresponds to alanine when chromatographed on paper in the following solvents: methyl ethyl ketone-methyl cellosolve-acetic acid-

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[2] Contribution from the Entomology Department, North Carolina Agricultural Experimental Station, Raleigh. Published with approval of the Director of Research, Paper No. 1795 of the Journal Series.
[3] T. CHOJNACKI and M. J. PIECHOWSKA, Acta biochim. pol. *8*, 157 (1961).
[4] T. CHOJNACKI, Acta biochim. pol. *8*, 167 (1961).
[5] T. CHOJNACKI and T. KORZYBSKI, Acta biochim. pol. *9*, 95 (1962).
[6] L. L. BIEBER, E. HODGSON, V. H. CHELDELIN, V. J. BROOKES, and R. W. NEWBURGH, J. biol. Chem. *236*, 2590 (1961).
[7] R. J. L. ALLEN, Biochem. J. *34*, 858 (1940).
[8] D. M. MORGAN and K. J. KINGSBURY, Analyst *84*, 409 (1959).
[9] M. M. RAPPORT and N. ALONZO, J. biol. Chem. *217*, 199 (1955).
[10] W. L. MAGEE, R. W. K. BAKER, and R. H. S. THOMPSON, Biochim. biophys. Acta *40*, 118 (1960).
[11] J. S. WALL, D. D. CHRISTIANSON, R. J. DIMIER, and F. R. SENTI, Anal. Chem. *32*, 870 (1960).
[12] F. FEIGEL and V. GENTIL, Mikrochem. Acta *M 53*, 1004 (1955).
[13] H. SAITO, J. Biochem. (Tokyo) *47*, 573 (1960).
[14] C. J. LAUTER and E. G. TRAMS, J. Lipid Res. *3*, 136 (1962).
[15] M. FLORKIN, Proc. 4th Internat. Congr. Biochem., Vienna *12*, 63 (1958).
[16] R. M. C. DAWSON, Biol. Rev. *32*, 188 (1957).