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].

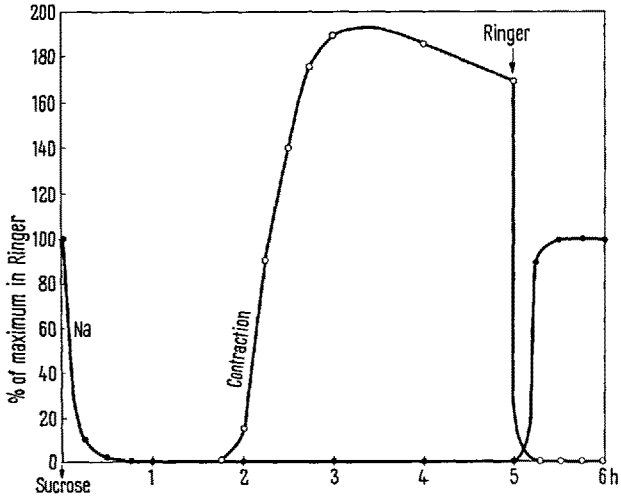

Fig. 2. Effect of washing every 15 min with half-isotonic, 0.112 *M*, solution of sucrose on the sodium content and magnitude of the spontaneous contractions of frog stomach muscle.

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.

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

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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.
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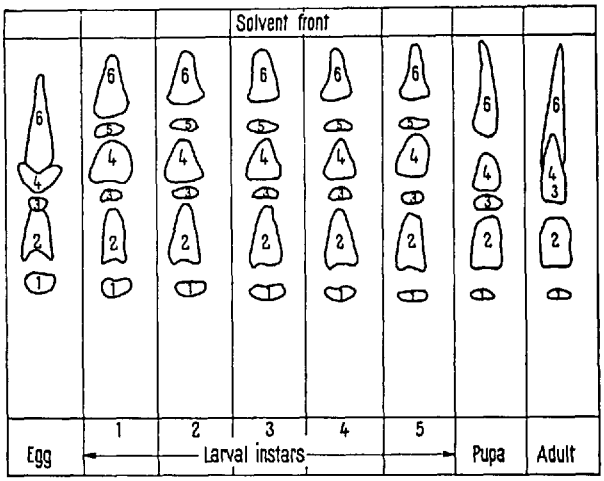
Molar ratios of phospholipid constituents in the peaks from silicic acid columns. (Phosphorus, ester, and aldehyde determinations were carried out on the intact phospholipids and ethanolamine, serine, choline, and the unknown ninhydrin positive compound were carried out on acid hydrolysates)

	% of lipid P	Ester: P	Aldehyde: P	Ethanol-amine: P	Serine: P	Choline: P	Unknown: P
Larval peak I	42	2.61	0.07	0.73	0.05	0.01	0.16
Adult peak I	62	2.56	0.14	0.41	0.03	0.11	0.06
Larval peak Ia	8	1.41	0.04	0.27	0.06	0.01	–
Adult peak Ia	14	2.60	0.02	0.10	0.28	0.91	0.10
Larval peak II	44	1.95	0.01	0.09	0.08	0.53	0.05
Adult peak II	24	2.0[a]	0.03	0.07	0.08	0.97[a]	0.12
Larval peak IIa	6	0.31	0.04	0.11	0.03	0.30	–

[a] Due to an error in some of the phosphorus determinations these two figures are less accurate.

water; ethanol-ammonia-water; methanol-ammonia-water; butanol-acetic acid-water. None of the other 21 compounds tested (including all the common amino acids) gave the same result. The dinitrophenyl derivative of this compound corresponds to DNP-alanine when chromatographed on paper in butanol-butyl acetate:ammonia.

That this compound is not part of a lipid molecule seems unlikely in view of the following. Following chromatography on silicic acid impregnated paper[17] in chloroform-methanol (2:1), free amino compounds remain on the origin, and the ninhydrin positive phospholipids form a single spot near the solvent front. Lipid extract from fifth instar hornworms was chromatographed as a band, the area behind the solvent front eluted, hydrolyzed in $2N$ H_2SO_4 at 110°C, and examined for ninhydrin-positive compounds. The unknown compound and ethanolamine were present in the same ratio before and after chromatography. Passing chloroform solutions of the phospholipids through columns of cellulose caused no change in the ratios of these two compounds, and, when free C^{14}-L-alanine was added during the extraction process it could not be detected in the phospholipid peaks from the silicic acid column.



Chromatography of *Protoparce sexta* phospholipids on silicic acid treated paper. The reactions of the spots marked were as follows: (1) rhodamine 6G positive, weak positive reaction with phosphomolybdic acid and potassium permanganate; (2) rhodamine 6G positive, weak positive with potassium permanganate and a strong positive reaction to Dragendorff's reagent and phosphomolybdic acid. Phosphatidyl choline; (3) rhodamine 6G positive, weak response to phosphomolybdic acid and a strong positive response to ninhydrin; (4) rhodamine 6G positive, weak positive response to potassium permanganate and phosphomolybdic acid and a strong positive response to ninhydrin. Phosphatidylethanolamine; (5) rhodamine 6G positive; (6) mixture of pigments apparently without any phospholipid constituents.

The phospholipids of eggs, the larval instars, pupae and moths of the hornworm were chromatographed on silicic acid impregnated paper in diisobutyl ketone, acetic acid and water (MARINETTI[17]). The Figure was obtained by spraying identical chromatograms with the following color reagents: rhodamine 6G, ninhydrin, phosphomolybdic acid, potassium permanganate, Dragendorff's reagent, 2,4-dinitrophenyl hydrazine. Two of the five spots were particularly prominent. One of these contained choline and the other ethanolamine, confirming the results from silicic acid chromatography. Plasmalogens were not apparent. There are few qualitative differences between developmental stages although one minor component (5) present in the larva is absent from the egg, pupa and adult. All components separated on silicic acid treated paper were unsaturated.

These investigations show that the principal phospholipids of the tobacco hornworm are phosphatidylethanolamine and phosphatidylcholine but a significant minor component contains a compound tentatively identified as alanine. This minor component may contain both ethanolamine and alanine. Plasmalogens are a minor constituent of the adult, and even less is present in the larva while spingomyelin and lipids containing inositol could not be detected[18].

Zusammenfassung. Es wird gezeigt, dass es sich bei den Phospholipiden von *Protoparce sexta* vorwiegend um Phosphatidyläthanolamin und Phosphatidylcholin handelt, dass aber ein wichtiger kleiner Bestandteil eine Verbindung enthält, die zunächst als Alanin identifiziert wurde.

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Department of Entomology, University of North Carolina, Raleigh (USA), June 22, 1964.

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[18] The skilled technical assistance of Mrs. A. SKABORN and Miss A. HAWKINS and the generosity of Dr. R. L. RAUN in providing the *Protoparce sexta* is gratefully acknowledged.