the interstitial spaces (moving openings or channels) between the large carbohydrate molecules, chiefly filled with the small water molecules. That these interstitial spaces very probably fluctuate as a result of diffusion of the water molecules and of the heat vibration of carbohydrate molecules which are partially bound together, does not in any way invalidate this sieve principle. At the same time this might explain why no clear coupling has been observed between water vapour transport and aroma transport through such selective polymeric membranes[10,11].

Zusammenfassung. Der Einfluss des Wassergehaltes auf die beim Trocknen pflanzlicher Säfte und Extrakte an der Flüssigkeitsoberfläche sich bildende, nahezu trockene, hydrophile Haut wurde untersucht.

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Unilever Research Laboratory Duiven, Zevenaar (The Netherlands), 28th March 1967.

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[11] The authors are indebted to Prof. H. A. C. THIJSSEN of the Technical University Eindhoven for his helpful suggestions and to Mr. G. K. KOCH of Unilever Research Laboratory Vlaardingen for his help with the radioactive measurements.

Estimation of Free Glutamic Acid in the Differentiating Central Nervous System of the Chick

Glutamic acid is known to play an important role in morphogenesis[1] and metabolism[2] of the vertebrate central nervous system. For this reason, a quantitative survey of free glutamic acid concentrations in the 4 principal sectors (viz. fore-, mid-, hindbrain and the spinal cord) of the differentiating central nervous system of White Leghorn chick embryos at 4–20 days of incubation was made.

The estimation of the amino acid from the carefully weighed fresh tissue was made by the method of maximum colour density[3] resulting from the interactions of ninhydrin and the amino acid on a two-dimensional paper chromatogram (Whatman paper No. 1), with water-saturated phenol and the butanol-acetic acid-water (4:1:1) as the first and second solvent respectively at a constant temperature of 21 °C. The colour density was measured in a photovolt densitometer (Photovolt Corp., N.Y., USA; Model No. 501 A). The quantity of glutamic acid was found by comparing the readings of the measured quantity of the unknown samples with the standard curve prepared from a pure sample of glutamic acid by an identical method.

Observations and discussions. The concentrations of the glutamic acid in the tissue are shown in the Table. Though we had little success in tracing the appearance of adult-like amino acid patterns in many tissues, the embryonic brain of some amphibians[4] and of rats[5] shows high concentrations of glutamine and glutamic acid like those of the adult. Similarly, the varying concentrations of glutamic acid, as evidenced by the present study, may be correlated to a great extent with the epigenesis of the central nervous system in the chick. It may be observed from the Table that the first period of high glutamic acid concentrations occurs between the 4th and 8th day, and on the 10th day there is a decline in the quantity. In the chick, the differentiation of the central nervous system begins first in the spinal cord and last in the forebrain region[6], and the process is primarily complete mostly after 9 or 10 days' incubation[7]. During this time there is extensive cell proliferation, migration, degeneration and histogenesis. In this process, apart from playing a structural role, glutamic acid contributes much to (1) the supply of energy[8], (2) the mediation of the entrance of ammonia into the amino acid pool and also (3) into the transamination system[9]. It thus maintains an equilibrium between the concentrations of amino acids and proteins of the nervous system – the synthesis of which has much to do with morphogenesis[10]. Early differentiation of the spinal cord explains the higher concentrations of glutamic acid in this area than that in the 4-day-old embryos. In the case of mid- and also hindbrain regions of 12-day-old embryos, further differentiations[7] (viz. appearance of some finer strata in the optic lobes) start from the 11th and subsequent days, while in the case of hindbrains differentiations of 'folia' begin on the 12th day of incubation when the quantity of glutamic acid also becomes increased in these 2 sectors. The enhancement of glutamic acid concentrations in the 18- and 20-day-old embryos'

Amount of free glutamic acid (μg/100 mg of tissue) in the differentiating central nervous system of chick

Type of tissue	Age of embryos, in days							
	4[a]	6	8	10	12	15	18	20
Forebrain[a]	21.44	31.33	29.07	18.13	5.89	5.40	23.21	30.19
Midbrain		36.49	31.72	2.33	4.94	4.10	28.86	22.15
Hindbrain		30.40	51.94	8.13	16.56	3.29	21.96	6.43
Spinal cord	26.87	30.97	43.33	35.84	18.69	10.04	18.87	39.24

[a] Entire brain was considered.

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central nervous systems may be accounted for by the high activity of 2 enzymes which are closely associated with glutamic acid metabolism (viz. glutamotransferase and glutamine synthetase) in the later stages of development of the nervous system. But it is difficult to explain the low value of glutamic acid content in the hindbrain compared to that of the other sectors of the central nervous system of the 20-day-old embryo.

Zusammenfassung. Es wird der Gehalt an Glutaminsäure in den embryonalen Entwicklungsstadien des Hühnergehirns festgestellt.

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Chromatographic Separation of Phospholipase A from a Histamine Releasing Component of Brazilian Rattlesnake Venom (*Crotalus durissus terrificus*)

The histamine-releasing action of rattlesnake venom, extensively studied by FELDBERG and KELLAWAY[1], has been associated with phospholipase A[2] or with crotamine[3], a low molecular weight, thermo-stable basic protein found in some crotalic venoms. The present report describes the chromatographic separation and some properties of a component of the venom from the Brazilian rattlesnake, *Crotalus durissus terrificus* which, while clearly not phospholipase A nor crotamine, is nevertheless highly active as a releaser of the histamine of rat isolated peritoneal fluid mast cells.

Rattlesnake venom, naturally free of crotamine according to a paper-electrophoretic criterion of analysis[3], was obtained through the courtesy of Prof. J. MOURA GONÇALVES from the Department of Biochemistry of the Faculty of Medicine of Ribeirão Preto, Brazil. It was submitted to ion-exchange chromatography on an Amberlite CG-50 (XE-64) resin prepared and used as described by HABERMANN[4]. The effluent fractions were assayed for histamine releasing activity using rat isolated peritoneal fluid cells as sources of the amine, for phospholipase A activity by the egg-yolk coagulation test[4] and for protein content by light absorptimetry at 280 nm with a PMQ II Zeiss spectrophotometer.

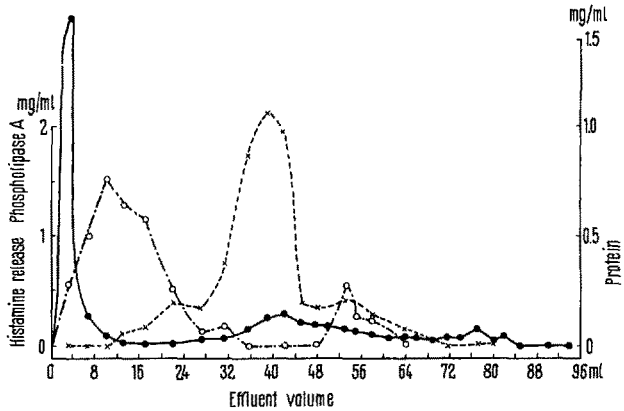
The Figure shows that histamine releasing activity emerged from the column shortly after the appearance of a large peak of unadsorbed protein material. According to HABERMANN[4], crotactin, the neurotoxic component of rattlesnake venom is contained in this fraction; it seems, therefore, that crotactin has no histamine releasing ability. Phospholipase A activity appeared in the eluates after the major portion of the histamine releasing component; the Figure shows clearly that the 2 components are not identical. It will be noted that the total phospholipase A activity recovered after chromatography was well above 100%; a similar result obtained by HABERMANN[4] has been interpreted as being due to the retention of phospholipase A inhibitors by the chromatography column.

The 3 fractions containing the major part of the histamine releasing component were pooled and further examined. This material was highly active: 0.20 µg/ml of it caused maximal release of histamine from rat isolated peritoneal fluid cell suspensions, and on a weight basis, it was more active than compound 48/80. The data on the Table, indicating thermal instability, resistance to dialysis and sensitivity to digestion by a proteolytic enzyme, suggest that this histamine releasing factor is a protein, possibly of enzymatic nature. Lack of capacity to

Properties of the histamine releasing component (HRC) isolated by ion-exchange chromatography from rattlesnake venom

Treatment of HRC	% reduction of the histamine releasing capacity[a]
Heating to 100 °C for 5 min	75
Dialysis for 2 · 20 h at 4 °C against 100 volumes of saline	0
Incubation for 10 min at 37 °C with acetyl-trypsin, 100 µg/ml	88
Action of HRC on mast cells pre-treated for 15 min at 37 °C with DNP, 0.1 m*M*	78
Idem on mast cells pre-treated with 0.1 m*M* DNP in the presence of 4.5 m*M* glucose	0

[a] Determined by incubating a rat peritoneal fluid cell suspension in Krebs-Ringer phosphate buffer, pH 7.4 with 0.25 µg/ml of HRC for 10 min at 37 °C (see ROTHSCHILD[13]).



Chromatographic fractionation of crotalic venom on Amberlite CG-50 (XE-64) ion-exchange resin. Histamine releasing (HR) and phospholipase A (Ph.A.) activities as well as protein contents of the chromatographic fractions are expressed in terms of crude venom after reference to standardization curves. Total recoveries in the effluent fractions were: HR, 73%; Ph.A, 320%; protein, 85%. ●——● protein, ×----× phospholipase A, o——o histamine release.

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