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.

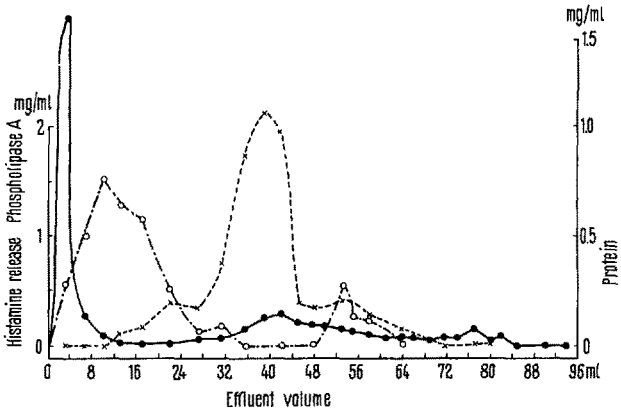
The Figure shows that histamine releasing activity emerged from the column shortly after the appearance of a large peak of unadsorbed protein material. According to HABERMANN[4], crotactin, the neurotoxic component of rattlesnake venom is contained in this fraction; it seems, therefore, that crotactin 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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release histamine from mast cells pre-treated with 2–4 dinitrophenol (DNP) in the absence of glucose, places this factor in a group of histamine releasing agents like antigen[5,6], compound 48/80[6,7], chymotrypsin[8,9] and dextran[10], whose action on rat mast cells is also inhibited by DNP in the absence of glucose.

The lack of an in vitro, direct histamine releasing action of rattlesnake venom phospholipase A agrees with reports showing the absence of a mast cell degranulating or histamine releasing effect of the phospholipase A present in bee venom[11–15].

Zusammenfassung. Die chromatographische Auftrennung von Klapperschlangen- (*Crotalus durissus terrificus*) Gift ermöglicht, den Unterschied zwischen Phospholipase A und einer Histamin freisetzenden Komponente dieses Giftes zu bestimmen. Letztere ist ein hitzelabiles, nicht dialysierbares Protein (enzymatisch), leicht von Crotamin unterscheidbar. Phospholipase A ist nicht fähig, Histamin aus isolierten Ratten-Mastzellen freizusetzen. Der hier isolierte Histaminliberator wirkt nicht auf mit 2, 4-Dinitrophenol vorbehandelte Mastzellen.

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Effect of Some Substances on the Mitochondrial Swelling Induced by Diphtheria Toxin in Chicken Embryo Heart Cell Cultures

Recently it has been observed that diphtheria toxin induces a remarkable mitochondrial swelling in chicken embryo heart cell cultures and in other primary cell cultures, whereas any swelling effect is detectable in the mitochondria of established cell lines as HeLa cells and RC 37 cells. Crude, highly purified and crystalline diphtheria toxin exhibit the same effect[1,2].

In this paper we have studied the effect of some well-known inhibitors of the mitochondrial swelling, as the blocking agents for the respiratory chain on the swelling induced by diphtheria toxin and the effect of ATP and seroalbumin which are related to mitochondrial contraction.

Materials and methods. Cell cultures. Chicken embryo heart cell cultures were obtained from 6-day-old chick embryos as reported elsewhere[1,2]. The cells were cultured in the ROSE's chambers[3]. The culture medium did not contain antibiotics.

Diphtheria toxin and other reagents. Highly purified diphtheria toxin (Lilly, containing 1120 Lf/ml, Lot. L 00087) and crystalline diphtheria toxin (Wellcome, Lot. RX 7238, containing after dilution with 5 ml of Hanks' BSS, 4.300 Lf/ml) were used. Diphtheria toxin was diluted in Hanks' BSS to the following concentrations: 0.5, 1.0, 5.0, 10 and 20 Lf/ml. The following reagents of the highest purity were employed: Amytal (5-ethyl-5-isoamyl-barbituric acid) (Lilly), KCN (B.D.H.), sodium azide (Merck), Rotenone (B.D.H.), ATP (adenosine triphosphate) (Sigma), seroalbumin (Sigma). All these substances were dissolved, immediately before use, in Hanks' BSS to obtain the final concentrations indicated in the Tables I and II.

Experimental. About 20 h after cell establishment, the nutrient medium was eliminated and substituted with diphtheria toxin-test substance mixture. The ROSE's chambers were then placed under a Leitz Ortholux phase-contrast microscope equipped with a thermoregulated box (37°C) and continuously observed during a period of 30 min. For testing the action of ATP and Seroalbumin the

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Table I. Mitochondrial swelling in diphtheria toxin treated cells (chicken embryo heart cells) in presence of some electron transport blocking agents. +, thickened mitochondria; ++, swollen mitochondria; +++, swollen mitochondria with differences in optical density.

	KCN			Sodium Azide			Amytal			Rotenone		
Diphtheria toxin	1 μM	10 μM	1 mM	1 μM	10 μM	1 mM	1 μM	10 μM	1 mM	1 μM	10 μM	1 mM
0.5 Lf/ml	—	—	—	—	—	—	—	—	—	++	++	+–
1.0 Lf/ml	—	—	—	—	—	—	—	—	—	+++	+++	+
5.0 Lf/ml	—	—	—	—	—	—	—	—	—	+++	+++	+++
10 Lf/ml	—	—	—	++	++	+–	—	—	—	+++	+++	+++
20 Lf/ml	—	—	—	+++	+++	++	++	+	+	+++	+++	+++