venom. The UV-spectrum of the peptide fraction was quite similar to that of tryptophan. After removing protein contaminants by gel-filtration on a column (7.0 · 3.6 cm) of Sephadex G-25, the eluted peptide fraction was applied to a column (5 · 19 cm) of DEAE Sephadex A-25 (acetate form), and absorbed materials were eluted by 0.5 *M* pyridine acetate buffer at pH 5.0. Final purification was achieved by rechromatography of the partially purified peptide fraction on DEAE cellulose and DEAE Sephadex A-25 and 160 mg dry weight of purified material were obtained.

At the beginning of this study, it was considered that only one peptide, which stained with Ehrlich's reagent, was present in this peptide fraction, for only single spots were seen on paper and thin layer chromatography and paper electrophoresis. Acid hydrolysis of the sample in constant boiling HCl in a sealed tube, in vacuo, yielded aspartic and glutamic acids, ammonia and traces of tryptophan. Sanger's technique failed to reveal a free N-terminal residue, and C-terminal analysis by hydrazinolysis gave only tryptophan. Pyroglutamic acid was identified in partial hydrolysates prepared with *N* NaOH or 0.1 *N* HCl. After digestion with carboxypeptidase A, tryptophan and two fragments, namely pyroglutamylasparagine and pyroglutamylglutamine, were separated from the reaction mixture, and these dipeptides were separated from each other by high voltage electrophoresis at pH 3.5 at 3000 V per 60 cm for 120 min. They were identified by comparison with synthetic samples[5].

The above results suggest that there are two peptides of similar electrophoretic mobility in the peptide fraction. Two components, peptide A and peptide B, were separated from the peptide fraction by high voltage electrophoresis at pH 3.5 at 4000 V per 60 cm for 240 min. Using ninhydrin and microbiological methods, the molar ratio of amino acids in the acid hydrolysate of peptide A was established as L-Try:L-Glu = 1:2 and that of peptide B as L-Try:L-Glu:L-Asp = 1:1:1. Thus the sequence in peptide A was deduced to be Pyro-Glu(NH_2)-Try and that of peptide B to be Pyro-Asp(NH_2)-Try.

Presence of peptide A and peptide B in other snake venoms. The venoms of *Crotalus adamanteus, Bothrops jararaca* and *Trimeresurus flavoviridis* contained peptide A and peptide B, and the venom of *Vipera russelli* contained only peptide B. However, in the venom of *Naja naja atra* neither peptide A nor peptide B was found. The significance of peptide A and peptide B in the salivary gland of poisonous snakes is obscure. It is tempting to speculate that peptide A and peptide B originate from precursors of enzymes or biologically active peptides, which are present in especially high concentrations in *Crotalidae and Viperidae* venoms, during activating processes.

Zusammenfassung. Die Strukturaufklärung von zwei neuen tryptophanhaltigen Peptiden im Schlangengift von *Agkistrodon halys blomhoffii* ergeben: L-Pyroglutamyl-L-Glutaminyl-L-Tryptophan und L-Pyroglutamyl-L-Asparaginyl-L-Tryptophan. Diese Peptide sind in Schlangengiften der Viperidae- und Crotalidae-Arten verbreitet.

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[5] We wish to thank Dr. T. SHIBA (Faculty of Science, Osaka University, Japan) for supplying synthetic pyroglutamylglutamine. Pyroglutamylasparagine was prepared by the action of carboxypeptidase A on synthetic pyroglutamylasparaginyltryptophan, which was synthesized by Dr. S. SAKAKIBARA in our Institute.

Stimulation of Hemin Synthesis in Ehrlich Ascites Tumor Cells by Mouse Liver RNA

It has been reported that treatment of ascites tumor cells with RNA prepared from mouse, rat and calf livers will induce the synthesis of the liver-specific proteins, serum albumin, tryptophan pyrrolase and glucose-6-phosphatase[1,2]. Ehrlich ascites tumor cells utilize Fe^{59} for heme formation[3] and the purpose of the present investigations was to ascertain if the levels of hemin biosynthesis of Ehrlich ascites tumor cells could be altered by incubation with various types of RNA preparations.

Ribonucleic acid was prepared by cold phenol extraction[4] and RNA concentration determined with orcinol[5]. The incubations were performed by culturing Ehrlich ascites tumor cells at a concentration of 8 · 10^6 in 1 ml aliquots of 57% ascites cell-free fluid obtained by centrifugation at 27,000 *g* and 43% Hanks balanced salt solution buffered at pH 7.4. This medium was supplemented with polyvinylsulfate (10 μg/ml) to prevent RNase action and protamine sulfate (100 μg/ml) to aid entry of the RNA into the cells[6]. Carbon-14 labeled δ-aminolevulinic acid (1 μc/ml) was added as a hemin precursor in order to estimate the synthesis of labeled hemin and $FeSO_4$ (140 μg/ml) was present as a co-factor for hemin synthesis. Preparations of RNA from various tissues and tissue-fractions were added to the experimental cultures usually at concentrations of 1 and 2 mg/ml. These cultures were incubated for 1 h at 6°C and then for 6 h at 37°C in an incubator flushed with 95% air-5% CO_2 under humid conditions. At the conclusion of the incubation period the cells were collected by centrifugation at 1000 *g* and washed five times with 0.9% NaCl and then homogenized. The method of LABBE and NISHIDA[7] was used to isolate hemin; 2.5 mg of crystalline hemin was added as carrier. $1^1/_2$ mg of isolated hemin from each sample were suspended in amyl alcohol and plated on a millipore filter

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(0.45 μ pore size) by filtration under partial vacuum. These filters were attached to aluminum planchets, dried and counted with a thin-window gas flow counter and corrected for background activity.

The stimulation of hemin synthesis in cultured Ehrlich ascites tumor cells by the total RNA extract of mouse livers, as well as by RNA fractions isolated from the nuclear, ribosomal and soluble fractions is given in the Table. The greatest stimulation resulted from addition of the soluble RNA fraction. Stimulation of hemin synthesis of minced frog embryos by adding RNA isolated from frog livers has been described[8]. Mouse liver RNA did not stimulate hemin synthesis of frog embryos and, in the present study, bovine liver soluble RNA did not affect the rates of hemin synthesis of Ehrlich ascites tumor cells (Table). The levels of hemin synthesis were stimulated by a mixture of the four ribonucleosides, but not as much as by the RNA preparations. An RNA-core diffusate (Worthington), which contained dialyzable mono-, di-, tri- and tetranucleotides did not stimulate the incorporation of δ-aminolevulinic acid C^{14} into hemin.

These experiments indicate that the addition of liver RNA to intact ascites cells can stimulate hemin synthesis. This is not an induction of a new biosynthetic pathway but probably is the esculation of synthesis of enzymes of an established pathway[9].

The stimulation of hemin synthesis of Ehrlich ascites tumor cells by mouse liver RNA isolated from the residue from a 15,000 *g* centrifugation (nuclear fraction), the residue from ultracentrifugation for 2 h at 105,000 *g* (ribosomal fraction), the ultracentrifugal supernatant (soluble fraction) and the RNA of entire livers (total RNA)

Incubation	Isotopic activity of hemin-C.P.M. (average)
Control	2695 ± 325
Total RNA, 1 mg/ml	3385 ± 5
2 mg/ml	4630 ± 260
Nuclear fraction, 1 mg/ml	3045 ± 45
2 mg/ml	3955 ± 205
Ribosomal fraction, 1 mg/ml	3230 ± 160
2 mg/ml	4005 ± 315
Soluble fraction, 1 mg/ml	3965 ± 185
2 mg/ml	5193 ± 127
Four ribonucleosides (each at 0.001 *M*)	3490 ± 430
Ribonucleic acid core diffusate, 1 mg/ml	2420 ± 60
(Worthington Biochemical Corp.) 2 mg/ml	2335 ± 25
Bovine liver soluble RNA, 1 mg/ml	2215 ± 95
(Nutritional Biochemical Corp.) 2 mg/ml	1731 ± 87

Résumé. Les cellules ascites de la tumeur Ehrlich couvaient in vitro avec le précurseur radioactif de l'hémine δ-aminolévulinic acid-4-C^{14}. Les préparations de ARN du foie de la souris stimulaient le niveau de synthèse de l'hémine.

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The Responses of Units in the Superior Colliculus of the Cat to a Moving Visual Stimulus[1]

Experiments were conducted in 28 cats which had been subjected to complete transection of the brain-stem at a midpontine level under deep ether anaesthesia[2]. To prevent ocular movements, the cats were curarized and artificially respired. The pupils were dilated with atropine. The visual stimulation was produced by manually sliding a white object (7 · 2 cm) along a rail 1 m in length. The rail could be rotated around its centre in a vertical plane and its position could be fixed in any selected meridial plane. The axis of rotation of the system was made to coincide with the visual axis of the eye by means of optical collimation (the visual axis intersects the retina in the centre of the area centralis, e.g. *visual pole* of Bishop et al.[3]. The minimal distance between the plane of rotation and the eye for which no accommodation was required was 1 m[4]. This distance was kept constant throughout the experiment. The distance over which shifting the object excited collicular units could be read in cm directly from the rail, and then converted to angular values. Whenever the effective trajectory of the object did not coincide with the meridial plane, a rectangular screen of matt plastic replaced the system described above, and was centred in a similar fashion. A small light spot (the actual visual object) of 50 Lux over a background of 10 Lux was focused on the screen from the reverse side. The position of the trace of the light spot, moved manually and effective in eliciting responses from collicular units, was then marked on the screen and its amplitude measured. Activity from superior collicular units was recorded by using stainless steel microelectrodes with a tip diameter of 3–5 μ and a resistance of 100 K Ohm measured on an A.C. bridge.

Two groups of units were found which could be activated by a moving stimulus. The first group (23 units) was activated by angular movements of 6–12°, while 30–45° were required by the second group (18 units)

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