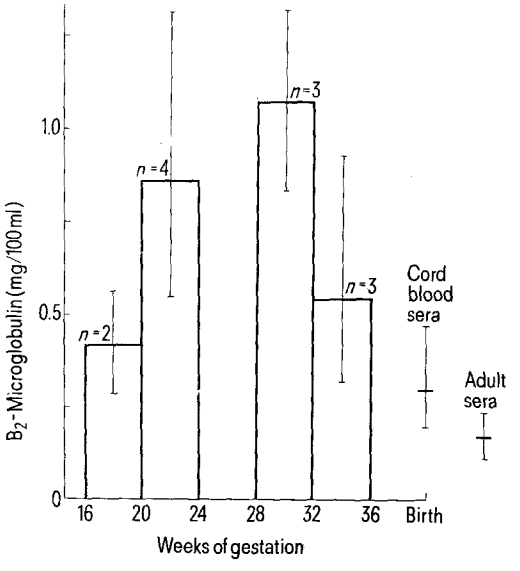
globulin concentration ranged from 0.18 to 0.55 mg/100 ml. In 37 samples of amniotic fluids from pregnant women of different gestational ages the concentration of the protein ranged from 0.12 to 1.80 mg/100 ml.

β_2-Microglobulin present in fetal sera may be of fetal as well as of maternal origin. A possible transplacental passage of β_2-microglobulin from the maternal to the fetal circulation was not investigated in this study; however, β_2-microglobulin concentration in sera of 84 pregnant women of different durations of pregnancy was found to be within the range of normal adult levels. On the other hand, there is strong evidence that the human fetus is capable of autonomous synthesis of β_2-microglobulin; we found that kidney, liver, thymus and testis tissues of a 16-week-old fetus incorporated ^{14}C-labelled amino acids into β_2-microglobulin in tissue cultures. In addition, our experiments with established cell lines from fetal lung, skin and from cord showed that the fetal cells are able to release β_2-microglobulin into culture medium. Whatever the source of the protein in fetal sera, its concentration changes during the fetal life present β_2-microglobulin as a protein with feto-specific features.



Dependence of fetal serum β_2-microglobulin on gestational age.

Feto-specific proteins are known to occur at increased concentrations in sera of patients with certain malignant diseases. β_2-Microglobulin seems to present similar features: in a large group of patients studied by Evrin and Wibell[10] the majority of patients with increased β_2-microglobulin concentration suffered from malignant diseases. Also in our survey of patients with malignant disorders a high percentage of these patients showed increased β_2-microglobulin levels as compared to patients with non-malignant diseases. This increase was particularly pronounced in multiple myeloma (80% of patients), carcinoma of stomach (57%), cervix and uterus (54%) and carcinoma of breast, lung and colon (40%). Since the biological function of β_2-microglobulin is not known at the present time, we can only speculate on the relationship between its feto-specific features and increased concentration in some neoplastic diseases.

Zusammenfassung. Im fötalen Blut erreicht die Konzentration von β_2-Mikroglobulin in der 30. Schwangerschaftswoche ihr Maximum. Im mütterlichen Blut tritt während der Schwangerschaft kein Konzentrationsanstieg auf. Hingegen wurden bei Erwachsenen mit verschiedenen malignen Tumoren erhöhte Serumkonzentrationen beobachtet.

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[10] P. E. Evrin and L. Wibell, Clin. chim. Acta *43*, 183 (1972).

A New Pyroglutamylpeptide (Pyr-Lys-Ser) Isolated from the Venom of *Agkistrodon halys blomhoffii*

Five bradykinin-potentiating peptides (potentiators A, B, C, D and E), which potentiate specifically the actions of bradykinin, were isolated from the venom of *Agkistrodon halys blomhoffii*, and the amino acid sequences of three of them were determined successfully with the combined method of Edman degradation and mass spectrometry[1,2].

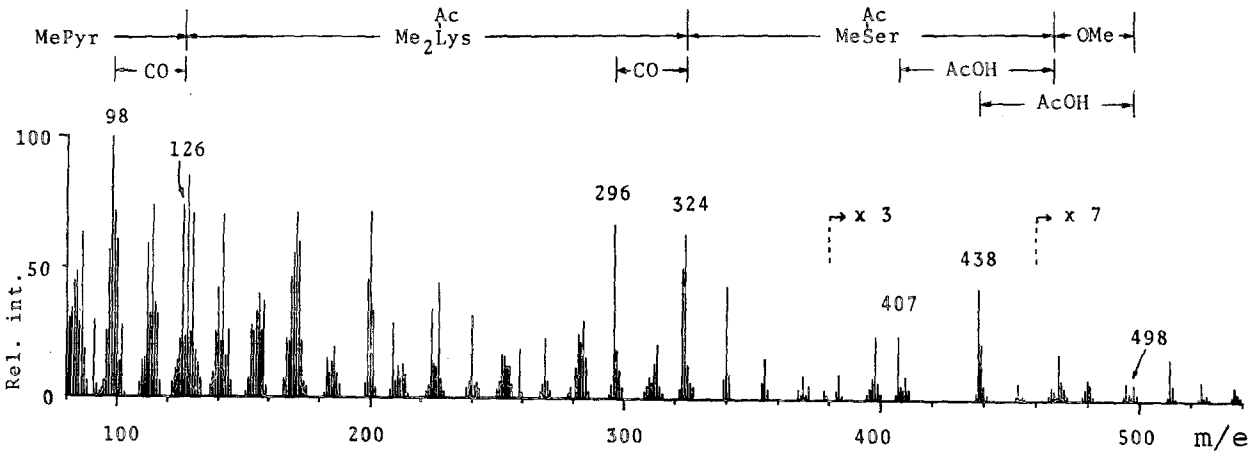
During the course of the mass spectrometric investigation on the structures of these peptides, it was found that some preparations were contaminated with a small amount of unknown peptides[2]. Further purification was achieved by passing the preparations through a Dowex 50×2 column instead of CM-Sephadex C-50 column previously employed, and potentiator A was separated from the contaminating peptides and determined to be Pyr-Gly-Arg-Pro-Pro-Gly-Pro-Pro-Ile-Pro[3].

The present paper describes the chromatographic isolation of a new peptide (I) and its amino acid sequence, which was determined by mass spectrometry and confirmed by chemical synthesis.

A pool of the minor peptide mixture, which was eluted from a Sephadex G-25 column later than the potentiating peptides[1], was applied on a Dowex 50×2 column equilibrated with 0.05 *M* pyridine-formic acid buffer, pH 3.1. (I) was eluted between potentiators A and E with the same buffer, and about 7 mg of the material were obtained from 10 g of the venom.

Acid hydrolysis of the sample by constantly boiling HCl yielded equimolar amounts of glutamic acid, serine and lysine (Glu 1.0, Ser 0.8, Lys 0.7), and it had no free N-terminal residue, as in the cases of potentiators A, B, C and E, when detected by Edman degradation. (I) was

[1] H. Kato and T. Suzuki, Experientia *25*, 694 (1969); Biochemistry *10*, 972 (1971).
[2] K. Okada, T. Uyehara, M. Hiramoto, H. Kato and T. Suzuki, Chem. Pharm. Bull. *21*, 2217 (1973).
[3] H. Kato, T. Suzuki, K. Okada, T. Kimura and S. Sakakibara, Experientia *29*, 574 (1973).
[4] D. W. Thomas, Biochem. biophys. Res. Commun. *33*, 49 (1966).



Mass spectrum of permethylated acetyl derivative of (I).

acetylated and then permethylated by the method of HAKOMORI[4] to convert into the permethylated acetyl-peptide, which was then subjected to mass spectrometry. The derivative underwent the normal fragmentation of amino acid type giving a molecular ion (*m/e* 498) as well as a set of sequence ions at *m/e* 98, 126, 296 and 324 which are attributable to the tripeptide derivative MePyr-Me_2Lys-(Ac)-MeSer(Ac)-OMe (Figure). Besides these, the spectrum also exhibited intense ions at *m/e* 407 and 438, which are due to the characteristic loss of acetic acid from *O*-acetyl serine containing fragments. The presence of N-terminal pyroglutamic acid was clearly indicated by the intense *m/e* 98 (N-methyl pyrrolidone ion) and 126 peaks (N-methyl pyrrolidone carbonyl ion). The results of the accurate mass measurements of the principal peaks were also in full accord with the above interpretation[5]. From these results and the amino acid composition, the structure of (I) was deduced to be Pyr-Lys-Ser.

The assigned structure was also ascertained by chemical synthesis as follows[6]: BOC-Lys(Z)-Ser-OMe, prepared from BOC-Lys(Z)-OTCP and H-Ser-OMe, was subjected to saponification followed by acidolysis with trifluoroacetic acid and the resulting H-Lys(Z)-Ser-OH was coupled with Z-Pyr-OSu to give Z-Pyr-Lys(Z)-Ser-OH. Z-groups from the protected tripeptide were removed by catalytic hydrogenation to give the desired tripeptide (I) (mp 154–7°, $[\alpha]_D^{22}$-30.3° (c = 1, H_2O)), which was homogeneous on TLC (*n*-BuOH:AcOH:H_2O = 5:1:5, v/v), paper chromatography (*n*-BuOH:pyridine:AcOH:H_2O = 15:10:3:12, v/v) and paper electrophoresis (pH 3.7, at 28 v/cm for 3 h), and was shown to be identical with the natural (I) in Rf values on the chromatograms and electrophoretic mobility.

The new peptide (I), as well as 2 analogous peptides (Pyr-Asn-Trp and Pyr-Gln-Trp), previously isolated from several kinds of snake venoms by KATO et al.[7], is still obscure in its biological significance in the submaxillary gland of the poisonous snake. It is tempting to assume that it originates from enzymes, biological active peptides or their precursors, which are present in the venoms of snakes of several species, during activating or metabolic processes[8].

Zusammenfassung. Es wird über die Strukturklärung eines neuen pyroglutaminsäurehaltigen Peptids aus dem Gift von *Agkistrodon halys blomhoffii* berichtet.

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[5] The spectrum (Figure) showed prominent peaks at *m/e* 453, 481 and 512. The sequence of Pyr-Lys-Thr, in which the C-terminal serine of (I) is replaced by threonine, seemed to account for these peaks; the acid hydrolysate of (I) gave about 0.07 residue of threonine. These observations may suggest that the preparation of (I) was contaminated by an analogous peptide Pyr-Lys-Thr which was not separated from (I) on paper electrophoresis and paper chromatography.

[6] The abbreviations used are: Pyr, pyroglutamyl; BOC, *tert.*-butyloxycarbonyl; Z, benzyloxycarbonyl; OSu, N-hydroxy-succinimide ester; OTCP, 2,4,5-trichlorophenyl ester. Satisfactory elementary analyses and chromatographic data were obtained for all the compounds described.

[7] H. KATO, S. IWANAGA and T. SUZUKI, Experientia *22*, 49 (1966).

[8] Acknowledgment. We wish to thank Professor T. SUZUKI of Osaka University for his interest and encouragement and also Miss T. TSUJI of Kanazawa University for her assistance in mass spectrometric analyses.

Effect of Sodium Chlorophenoxyisobutyrate on the Binding of Vitamin K Antagonists to human Albumin in vitro[1,2]

It has recently been observed that, in vitro, phenylbutazone partly inhibits the binding of coumarinic drugs to human albumin but has no effect on the binding of phenindione derivates[3]. We wanted to know whether sodium chlorophenoxyisobutyrate (CPIB), the active form of clofibrate, has the same effect, since it is bound to human albumin[4] on the same sites as phenylbutazone[5] and since it can also induce haemorrhagic accidents in patients treated with vitamin K antagonists[6–8].

The binding percentages of vitamin K antagonists have been determined at 4 °C, with human albumin (Sigma), using the method of equilibrium dialysis as described by