usually narrow (300 Å diameter) and the striations are rather weak although the fibrils appear clean. Narrow fibrils of polymeric collagen resembling these have been noted elsewhere[6,10] and in the present case there is evidence of the partial unravelling of fibrils to show the 'stocking' type structure of protofibrils noted by STEVEN in intestinal submucosal polymeric collagen[6].

Amino acid compositions of invertebrate collagens are more varied than those of vertebrate materials. That of the squid collagen isolated here is broadly typical (Table) of invertebrate collagens although the glycine content is lower than might be expected of contaminant-free collagen. The hydroxyproline, proline and hydroxylysine values are within the ranges found in other invertebrate collagens[11]. No 3-hydroxyproline was detected. The hydroxyproline content estimated colorimetrically is rather low (5.35% of the weight) which is suprising in view of the clean electron microscopic appearance of the material and the low hexosamine content (below).

The squid collagen contains 4.42% neutral sugar and 0.032% hexosamine. The neutral sugar is mainly glucose and galactose in 1:1 molar ratio with small amounts of mannose and fucose and a trace of xylose. The quantity of neutral carbohydrate present is greater than in most vertebrate collagens but is lower than in many invertebrate collagens[11]. The presence of glucose and galactose or galactose alone, as predominant monosaccharides, is a characteristic feature of vertebrate and invertebrate collagens[12,13].

The reasons for the indistinctness of the banding pattern in the collagen fibres is not clear; this may be connected with the moderately elevated carbohydrate content as may be the thinness of the fibrils[9]. It is of interest to note that thin polymeric collagen fibres closely resembling the present ones can be isolated from vertebrate cornea[10]; both cornea and squid skin contain the unusual unsulphated mucopolysaccharide chondroitin[14,15]. The lack of strong banding may however indicate conformational abnormality of the tropocollagen; the IR-spectrum shows that the N-H stretching mode has its peak at the unusually low value of 3290 cm^{-1} in contrast to 3330 cm^{-1} for most collagens. This observation would suggest that the stabilizing hydrogen bonds are shorter than normal[16].

The Nishihara method can thus be equally applied to preparation of insoluble collagen in improved yields from vertebrate and invertebrate tissues.

Amino acid composition of squid mantle wall polymeric collagen

Residue	Composition	Residue	Composition
Hydroxyproline	90	Isoleucine	17
Aspartic acid	68	Leucine	38
Threonine	33	Tyrosine	10
Serine	72	Phenylalanine	18
Glutamic acid	88	Hydroxylysine	16
Proline	78	Lysine	21
Glycine	298	Histidine	7
Alanine	75	Arginine	48
Valine	23		

Amino acids in residues per 1000 total residues. Serine, threonine and tyrosine corrected for hydrolytic losses.

Résumé. On a isolé du collagène naturel et polymère du tissu du manteau du calmar *Loligo peallii* par la méthode de Nishihara, les fibres présentant au microscope électronique une périodicité de 680 Å. Le collagène a une composition typique d'amino acides et contient l'hydrate de carbone dans une proportion restreinte; principalement sous forme de glucose et de galactose.

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Structure of Bradykinin-Potentiating Peptide Containing Tryptophan from the Venom of *Agkistrodon halys blomhoffii*

In a previous paper[1], we have reported on the isolation of 5 bradykinin-potentiating peptides (potentiators A, B, C, D and E) and an examination of their amino acid composition. These peptides potentiated the bradykinin action on guinea-pig ileum in vitro. Out of these five peptides, the amino acid sequence of the potentiator B has been determined to be as follows[2]:

Pyr-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro

This paper describes the amino acid sequence of the potentiator E, which contains tryptophan and has an amino acid composition which is dissimilar to that of the other potentiators A, B, C and D.

The N-terminal amino acid of the potentiator E was not detected by Edman degradation, but a C-terminal amino acid was found, by hydrazinolysis, to be proline. From the tryptic hydrolysate of potentiator E, 2 peptide

fragments, E-T-1 and E-T-2, were separated by paper electrophoresis at pH 3.5. Their amino acid compositions are shown in the Table. E-T-2 was a dipeptide containing 1 mole of glutamic acid and 1 mole of lysine. Carboxypeptidase B digested E-T-2 to free lysine and pyroglutamic acid, which were identified by paper electrophoresis at pH 3.5. From this, the amino acid sequence of E-T-2 was deduced to be Pyr-Lys.

The C-terminal amino acid of E-T-1 was found by hydrazinolysis to be proline. The amino terminal part of E-T-1 was confirmed by direct Edman degradation to be

Trp-Asp-Pro-Pro-Pro-Val-

E-T-1 was hydrolyzed with a protease from *Streptomyces griseus*, and from the hydrolysate free tryptophan, valine and 2 peptide fragments, E-T-1a and E-T-1b, were separated by paper chromatography (*n*-butanol : pyridine : acetic acid : water = 15:10:3:12). The N-terminal amino acid of E-T-1a was found by direct Edman degradation to be aspartic acid and that of E-T-1b was found by subtractive Edman degradation to be serine. Consideration of these results and the amino acid compositions of E-T-1a and E-T-1b (Table), led to the conclusion that the amino acid sequence of E-T-1a and E-T-1b must be Asp-Pro-Pro-Pro and Ser-Pro-Pro, respectively.

The full structure of the potentiator must therefore be Pyr-Lys-Trp-Asp-Pro-Pro-Pro-Val-Ser-Pro-Pro.

Another bradykinin-potentiating peptide which contains tryptophan has been isolated from the venom of *Bothrops jararaca* and found by FERREIRA et al.[3] to have the structure Pyr-Lys-Trp-Ala-Pro (BPF 5a). The N-terminal part of the potentiator E is similar to that of the peptide BPF 5a. Although BPF 5a had a strong potentiating activity on guinea-pig ileum[4], the potentiator E had a weak activity in the same experiment. As against this, however, the potentiator E had a potent bradykinin-potentiating activity on the rat uterus. 0.75 µmole of potentiator E had a twofold potentiating effect on the bradykinin action on the guinea-pig ileum and 0.015 µmole of potentiator E had the same effect on the rat uterus. The potentiators B and C had, however, potent bradykinin-potentiating activities on the guinea-pig ileum, and weak activities on the rat uterus. These results suggest that the mechanism of the biological activities of bradykinin-potentiating peptides should be made the subject of further studies.

Amino acid composition of potentiator E and its fragments

Amino acid	E	E-T-1	E-T-2	E-T-1a	E-T-1b
Trp	0.8[a] (1)	N.D. (1)	–	–	–
Lys	0.7 (1)	–	0.9 (1)	–	–
Asp	0.8 (1)	1.2 (1)	–	1.0 (1)	–
Ser	0.7 (1)	0.9 (1)	–	–	0.8 (1)
Glu	1.0 (1)	–	1.0 (1)	–	–
Pro	4.1 (5)	5.1 (5)	–	3.0 (3)	2.0 (2)
Val	1.1 (1)	1.0 (1)	–	–	–

N.D., not determined. [a] Determined spectrophotometrically by the method of GOODWIN and MORTON[5].

Zusammenfassung. Es wird über die Strukturaufklärung eines Bradykinin-potenzierenden Peptids aus dem Gift von *Agkistrodon halys blomhoffii* berichtet.

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The Effect of Guanidinosuccinic Acid on in-vitro Carbohydrate Metabolism

Glucose intolerance is well-documented in chronic uremia[1]. Although the early response of insulin to various stimuli may be blunted, the eventual attainment of normal to increased levels has suggested that insulin antagonism may play a role in this condition[1]. Guanidinosuccinic acid (GSA), undetectable in normal individuals (< 0.15 mg/100 ml) accumulates in the serum of patients with renal insufficiency (mean — 2.53 mg/100 ml)[2]. It has been postulated that the 'defect in cellular glucose uptake in uremia' is secondary to the presence of this compound[3]. Accordingly, the effect of GSA on the in vitro response of the rat diaphragm to glucose and insulin was studied.

Methods. The rat diaphragm assay for assessment of indulin antagonism has been described previously[4]. Briefly, it involves the serial incubation of paired hemidiaphragms, first in 2.0 ml of buffered glucose (2.0 mg/ml) alone and then in media containing added insulin (500 µU/ml). One of each pair of hemidiaphragms was exposed to GSA (0.03 mg/ml). Thus, basal and insulin-stimulated glucose uptakes in the presence and absence of GSA were obtained on the tissues from each animal which avoids the marked variation observed with hemidiaphragms from separate rats[1,4].

Results. The Table shows that GSA did not affect basal or insulin-stimulated glucose uptake of paired rat hemidiaphragms in vitro. The insulin effect (I–B) did not differ when the *t*-test for differences between paired observations was used to compare the data but was significantly increased ($p < 0.05$) in the presence of GSA if the *t*-test for differences between means was utilized.

Discussion. The data presented here do not support the hypothesis that GSA accumulation in uremia is

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