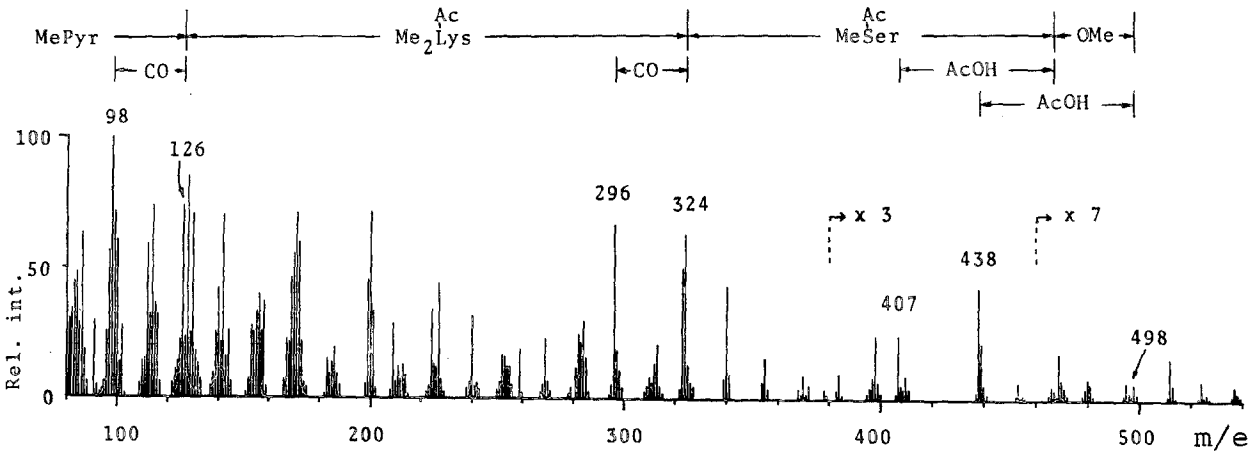

Mass spectrum of permethylated acetyl derivative of (I).

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

Effect of sodium chlorphenoxyisobutyrate on the binding of vitamin K antagonists to human albumin at 4 °C

Drug	Concentration (μg/ml)	Binding percentage	
		Alone	With sodium chlorophenoxyisobutyrate
Warfarin	3.0	78.8 ± 2.9	68.2 ± 3.7[a]
Ethylbiscoumacetate	3.2	88.2 ± 5.0	67.8 ± 15.6[b]
Acenocoumarin	1.2	81.0 ± 3.5	63.5 ± 8.0[b]
Phenindione	20.0	77.7 ± 2.4	71.6 ± 4.3
Fluorophenindione	20.0	87.3 ± 1.7	88.5 ± 1.0

Each result is the mean (± SD) of 5 determinations. The concentration of CPIB is 100 μg/ml; its binding percentage remains constant, 21.6 ± 6.9 in all determinations; [a] and [b] indicate significant changes: [a] $P < 0.001$; [b] $P < 0.01$.

SELLERS and KOCH-WESER[9]. The amounts of drugs were measured by isotopic dilution using labelled compounds: warfarin ^{14}C (7,1 Ci/*M*, Amersham), acenocoumarin ^{14}C (3,5 Ci/*M*, Ciba-Geigy laboratories) and sodium chlorophenoxyisobutyrate ^{14}C (2,0 Ci/*M*, ICI laboratories) and by spectrophotometry for ethylbiscoumacetate[10] and phenindione derivates[11]. The binding percentage was determined with each anticoagulant drug, with CPIB, and then with each anticoagulant drug associated with CPIB.

The results are shown in the Table. CPIB inhibits the binding of coumarinic drugs but does not alter the binding of phenindione derivates. Similarly, the latter do not change the binding of CPIB: it therefore seems that CPIB and phenindione derivates are bound to different sites of the albumin molecule.

But it has been proved that CPIB, phenylbutazone, coumarinic derivates, free fatty acids and likely bilirubin, are bound to the same sites of human albumin[3,5,8,12]. It can be thought that next to that first type of sites, there is another one that binds only phenindione derivates. This hypothesis rests on the fact that phenylbutazone, like CPIB, inhibits the binding of coumarinic drugs but does not alter the binding of phenindione derivates[3].

If these two types of sites seem different, they do not seem totally independent. In fact, phenindione and fluorophenindione inhibit the binding of warfarin[3], according to a non-competitive mechanism. The lack of competitive phenomenon favours the hypothesis of the existence of two types of binding sites of vitamin K antagonists, but suggests that the binding to the second type of sites can alter the binding of other drugs to the first type of sites.

Further experiments will be necessary to explain this interaction. The administration of CPIB can induce haemorrhagic accidents both in patients treated with warfarin or with phenindione[6]. In the first case, these accidents can be explained by a plasma displacement of warfarin. The mechanism is then identical to the one described for the association phenylbutazone – warfarin[3,8,13]. However, it is less powerful with clofibrate in vitro as well as in vivo, since it does not induce a noticeable increase in the plasma concentration of free warfarin[14] while this one occurs with phenylbutazone[13].

The results obtained show that this mechanism does not apply to phenindione derivates. However, since haemorrhagic accidents can occur when phenindione and clofibrate are associated, one must admit that these are due to other phenomena, particularly to the effects of CPIB on the coagulation factors. O'REILLY et al.[14] have, in fact, shown that the administration of CPIB to man induces a faster decrease of factors II and X and a decrease of thrombocytes adhesivity.

The results obtained show that on human albumin, coumarinic derivates are bound to sites different from those of phenindione derivates. CPIB inhibits the binding of the first ones, but is without effect on the binding of the second ones. Haemorrhagic accidents observed when a vitamin K antagonist and clofibrate are associated can be explained by a plasma displacement of the bound form of the anticoagulant when it is a coumarinic derivate. But this mechanism cannot be applied to phenindione derivates.

Résumé. In vitro, le chlorophénoxyisobutyrate de sodium (CPIB) s'oppose à la fixation sur l'albumine humaine des dérivés de la coumarine mais ne modifie pas celle des phénindiones. Les races accidents hémorragiques observés lors de l'emploi simultané de CPIB et d'un dérivé de la phénindione ne peuvent s'expliquer par une défixation, due au CPIB, de l'anticoagulant fixé sur l'albumine plamatique.

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[2] We are grateful to Imperial Chemical Industries and Ciba-Geigy laboratories for their generous gift of labelled compounds.

[3] J. P. TILLEMENT, R. ZINI, C. MATTEI and E. SINGLAS, Eur. J. clin. Pharmac. *6*, 15 (1973).
[4] J. M. THORP, Lancet *1962*, 1323.
[5] H. M. SOLOMON, J. J. SCHROGIE and D. WILLIAMS, Biochem. Pharmac. *17*, 143 (1968).
[6] A. S. ROGEN and J. C. FERGUSON, J. Atheroscl. Res. *3*, 671 (1963).
[7] M. F. OLIVER, S. D. ROBERTS, D. HAYES, J. F. PANTRIDGE, M. M. SUZMANN and I. BERSOHN, Lancet, *1963*, 143.
[8] J. J. SCHROGIE and H. M. SOLOMON, Clin. Pharmac. Ther. *8*, 70 (1967).
[9] E. M. SELLERS and J. KOCH-WESER, Clin. Pharmac. Ther. *11*, 524 (1970).
[10] M. VERSTRAETE and C. VERMYLEN, Rev. Hémat. *14*, 212 (1959).
[11] B. C. BOSE and R. VIJAYVARGIYA, J. Pharm. Pharmac. *14*, 58 (1962).
[12] G. CHAN, D. SCHIFF and L. STERN, Clin. Biochem. *4*, 208 (1971).
[13] R. A. O'REILLY and P. M. AGGELER, Proc. Soc. exp. Biol. Med. *128*, 1080 (1968).
[14] R. A. O'REILLY, M. A. SAHUD and A. J. ROBINSON, Thromb. Diath. haemorrh. *27*, 309 (1972).