calculi was, undoubtedly, carbonatoapatite, $Ca_{10}(PO_4)_6CO_3$ and in all such cases the qualitative chemical tests showed presence of calcium, phosphate and carbonate ions. In some cases, however, the characteristic carbonate bands (at about 1470 and 1423 $cm^{-1}$) were absent from the spectra of the calculi and the chemical tests failed to reveal presence of carbonate ions. Since, obviously, the calcium phosphate in these cases could not be carbonatoapatite, proof was sought to determine whether it could then be hydroxyapatite, whitlockite or brushite, i.e. any of the calcium phosphates usually reported, besides carbonatoapatite, as stone-forming compounds. The possibility, mentioned without much emphasis only recently by LONSDALE[2] of octacalcium phosphate, $Ca_8H_2(PO_4)\cdot 5H_2O$ being a constituent of urinary calculi, was also investigated.

The comparison of the spectra of the urolithic material with those reported for whitlockite[6] and brushite[4,6,7] showed that these 2 compounds have numerous absorption bands not present in the spectra of the uroliths in question. Hydroxyapatite also shows a characteristic band (at around 630 $cm^{-1}$ and attributable[6] to OH libration) not present in the spectra of the calculi. As seen from the Figure (a) this band is clearly visible in the spectra of the artificial hydroxyapatite – calcium oxalate mixtures even when the intensity of the 514 $cm^{-1}$ calcium oxalate band (and hence the relative concentration of the latter) was much higher than in the spectra of the uroliths which, however showed no trace of the 630 $cm^{-1}$ band (Figure d). On the other hand, the spectra of the octacalcium phosphate – calcium oxalate mixtures (Figure c) were strikingly similar to the spectra of the uroliths under consideration, especially in the 750–500 $cm^{-1}$ region where the agreement was well within the absolute accuracy with which the frequencies of the bands were measured.

It could thus be concluded that in the uroliths investigated by us carbonatoapatite was by far the most common of the calcium phosphate constituents, that octacalcium phosphate could be a constituent in some cases and that hydroxyapatite, whitlockite and brushite were not found.

*Résumé.* Parmi les phosphates de calcium, la carbonatapatite est le plus fréquent constituant de quelque 120 calculs urinaires provenant de la Macédoine (Yougoslavie). Le phosphate octacalcique pourrait en être aussi, mais on n'y a pas constaté la présence d'hydroxyapatite, de whitlockite et de brushite.

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## Bradykinin-Potentiating Peptides from the Venom of *Agkistrodon halys blomhoffii*[1]

In 1965, FERREIRA[2] found that the bradykinin-potentiating factors in the venom of *Bothrops jararaca* seem to be peptide-like substances which are dialyzable and heat-stable. We isolated similar factors from the venom of the Japanese snake, *Agkistrodon halys blomhoffii* (trivial name, Mamushi), and confirmed that the factors which show bradykinin potentiation on isolated guinea-pig ileum are quite similar to those demonstrated in *Bothrops jararaca* venom[3]. This report describes the isolation and characterization of bradykinin-potentiating factors in the venom of *Agkistrodon halys blomhoffii*.

Five grams of lyophilized venom were dissolved in 20 ml of 0.01 *M* phosphate buffer, pH 8.0, and immediately applied to a column (4.5 × 135 cm) of Sephadex G-100 equilibrated with the same phosphate buffer in a cold room. Elution with the same buffer was performed at a flow rate of 30 ml/h. Bradykinin-potentiating activity was found in a low molecular weight fraction while large amounts of venom proteins and enzymes were removed from the column in the void volume. The pyroglutamyl peptides, which were found in this venom and characterized as Pyroglu-Asn-Try and Pyroglu-Gln-Try[4], were eluted from the column later than the potentiating factors. The fractions containing potentiating factors were lyophilized and the residue was dissolved in 10 ml of distilled water and gelfiltered through a column (3 × 90 cm) of Sephadex G-25. The potentiating factors (144 mg) were purified further by column chromatography on CM-Sephadex C-50 (1.5 × 92 cm), equilibrated with 0.005 *M* sodium acetate buffer, pH 5.0. The column was eluted at a flow rate of 10 ml/h first with 240 ml of the equilibration buffer and then with the same buffer at a

Amino acid compositions of bradykinin potentiating peptides, A, B, C, D and E from the venom of *Agkistrodon halys blomhoffii*

| | Amino acid residues per mole | | | | |
|---|---|---|---|---|---|
| | A-peptide | B-peptide | C-peptide | D-peptide | E-peptide |
| Lys | – | 0.9 | – | – | 0.7 |
| Arg | 1 | 1 | – | 1 | – |
| Glu | 1 | 1 | 1 | 1 | 1 |
| Pro | 3.8 | 4 | 4 | 3.7 | 4 |
| Gly | 1.7 | 1 | 1.2 | 1.6 | – |
| Ile | 1 | 0.7 | 0.7 | 1 | – |
| Leu | – | 0.64 | 0.7 | 1 | – |
| Asp | – | – | – | – | 0.8 |
| Ser | – | – | – | – | 0.7 |
| Val | – | – | – | – | 1 |
| Try | – | – | – | – | 0.8 |
| Ammonia | (1.0) | (0.5) | (0.3) | (1.0) | (0.5) |
| Total residues | 9 | 10 | 8 | 10 | 10 |

Amino acids were analyzed in a JEOL-3BC 'Auto Analyser'. Hydrolysis was carried out in constant boiling HCl at 110 °C for 12, 24 and 48 h. The tryptophane content was determined spectrophotometrically by the method of GOODWIN and MORTON[6].

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concentration of 1.0 *M*. Applying the ninhydrin reaction after alkaline hydrolysis at 100 °C for 2.5 h, 5 peptide peaks, A, B, C, D and E, were found in the eluate. The yields of peptides isolated were as follows; A, 10 mg; B, 37.5 mg; C, 9 mg; D, 4 mg; and E, 13 mg. On high voltage paper electrophoresis at pH 3.5, each peptide gave a single spot staining with the peptide reagent *tert*-butylhypochlorite-*o*-tolidine-KI[5]. Peptide E gave a positive Ehrlich's reaction and peptides A, B and D gave positive reactions with Sakaguchi's reagent. Moreover, peptides A, C and D did not react with ninhydrin reagent, suggesting the absence of free amino groups in their N-terminals. The action of bradykinin on isolated guinea-pig ileum was potentiated twofold by 5–20 µg of any of these peptides. The Table shows the amino acid compositions of the peptides. All contained 1 mole of glutamic acid and 4 moles of proline and a total of 8–10 amino acid residues. From their amino acid compositions, it is assumed that parts of the amino acid sequences of peptides A, B, C and D are homologous. Investigations on the amino acid sequences of these peptides are now in progress. One characteristic of these materials is their high proline content. Bradykinin also has a high proline content, therefore its structural relationship with bradykinin potentiating peptides seems interesting.

*Zusammenfassung.* Aus dem Gift von *Agkistrodon halys blomhoffii* konnten durch Säulenchromatographie an Sephadex G-100, G-25 und CM-Sephadex C-50 fünf Peptidkomponenten isoliert werden, welche die kontrahierende Wirkung von Bradykinin auf den isolierten Meerschweinchendarm signifikant erhöhen.

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## Inhibition of Reduction in Female Mouse Meiosis*

The mitotic spindle inhibition effect of Colchicine and Vincristine is well known[1,2], as is the effect in mammalian embryonic heteroploidy[3] and pronuclear formation[4]. Earlier stages in female oogenesis have not been studied, although experiments with mammalian spermatogenesis[5] and annelid oogenesis exist[6–8]. Since inhibited reduction of meiosis could lead to pentaploidy in zygotes[9], the effects of spindle inhibition on early mouse oogenesis in vivo and in vitro have been studied in the experiments described herein.

*Materials and methods.* Groups of twelve 21-day-old Strong-A derived female mice weighing 10–12 g were injected s.c. with doses of the colchicine derivative Colcemid from 0.1–50 µg/g body wt. or Vincristine from 0.01 to 5.0 µg/g body wt. at the same time that 2.5 IU of pregnant mare serum (PMS) were given and on the subsequent 2 days, or the total minimum effective dose (MED) of Colcemid was given with the human chorionic gonadotrophin (HCG) and Vincristine with PMS or up to 10 h after HCG. 48 h after PMS, 1.0 IU of HCG were given and 14–16 h thereafter the oviducts were examined for ova. Ova thus recovered were prepared for cytogenetic examination by a modified TARKOWSKI method[10] and assessed as to number and morphology of chromosomes in the second metaphase ($M^{II}$) and first polar body ($PB^{I}$). If ovulation had not occurred, it was considered that the agent had blocked spindle formation and intrafollicular ova were prepared in a similar fashion. The presence of a first meiotic metaphase ($M^{I}$) configuration in these ova was considered evidence that the agent had inhibited spindle formation. In vitro studies were carried out with ova from adult dioestrous females. Approximately 20 ova were added to each culture flask which contained $4 \times 10^{-1}$ to $10^{-8}$ (stepwise) µg of Colcemid or $4 \times 10^{-1}$ to $10^{-9}$ (stepwise) µg of Vincristine/ml of media. These were harvested after 14–16 h of incubation and prepared for cytogenetic examination. Criteria for spindle block were the same as in vivo.

In vivo recovery was studied, by giving a single dose of the total MED of Vincristine coincident with the PMS or Colcemid with HCG to groups of 12 female mice and checking at hourly intervals for ovulation beginning 12 h after the HCG, the time when ovulation normally occurs. When it had occurred, half of the recovered ova were observed with phase microscopy for spindle constitution, particularly for bi- and tripolar orientations and preparations were made for chromosome analysis to determine distribution in $M^{II}$ with $PB^{I}$. Actinomycin D (AMD), freshly prepared in saline, was given in a dose of 1.0 µg/g body wt. s.c. with the HCG or at 1, 3, 4, 5, etc. hours after HCG. This dose was selected on the basis of preliminary experiments as the maximum dose which did not produce any microscopically observable effects on the chromosome configurations of ova. Observations for recovery were made as above.

For study of in vitro recovery, eggs were incubated in standard fashion using the same concentrations as in the inhibition studies. After 14 h of incubation the ova

* Part of this data was presented in abstract in Clinical Research, vol. XV, No. 2, April 1967, p. 268.

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