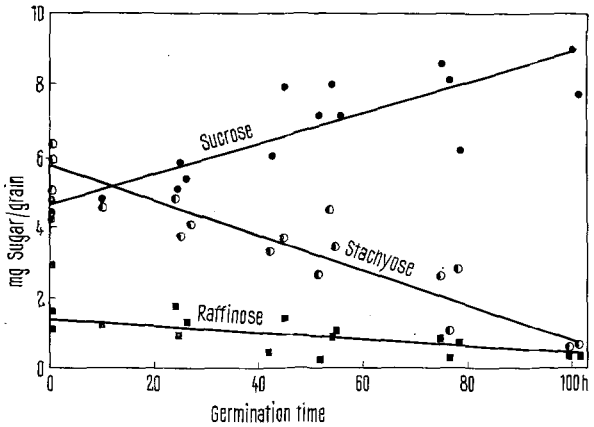
other metabolites. It is interesting to note that only galactose liberated from the reserve oligosaccharides actively participated in the metabolic processes of germinating pea seeds, while sucrose did not. On the other hand, sucrose is reported to be utilized rapidly during germination process of soybeans[10].

These results indicate that α-galactosidase is the major active enzyme system in pea seeds in the process of utilization of reserve oligosaccharides at the early stage of germination, and, therefore, it caused the oligosaccharides of the raffinose family to decrease in concentration, and free sucrose concentration is to increase.



Changes in concentrations of pea seed oligosaccharides during germination.

Zusammenfassung. Bei biochemischen Untersuchungen während der Keimung von *Pisum sativum* wurden Veränderungen im Gehalt von freien Zuckern beobachtet. α-Galaktosidase ist das aktivste Enzym unter den verschiedenen Enzymsystemen der Erbsen, die im Anfangsstadium der Keimung Reservezucker verwerten, wobei die Konzentration der Oligosaccharide abnimmt, während diejenige von Saccharose eine Steigerung erfährt.

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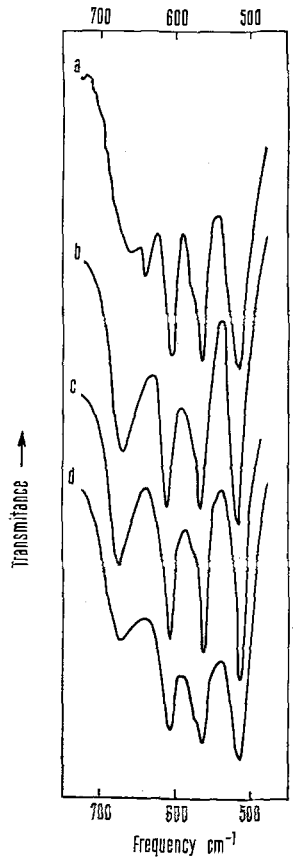
On the Nature of Calcium Phosphates in Urinary Calculi

Among the substances occurring most commonly in urinary calculi, calcium phosphates are, along with calcium oxalates, the ones found most frequently (thus, in our recent study of some 120 human uroliths from Macedonia (Yugoslavia)[1], we have found calcium phosphates in almost half of the investigated stones). Despite this fact, however, the true chemical nature of the calcium phosphate constituents is difficult to determine, mainly because they appear to be poorly crystallized and are, moreover, found almost always in an intimate mixture with calcium oxalates (apparently as a result of epitaxial overgrowth[2]). Having a long-standing interest in the IR-spectroscopy of calcium phosphates[3–5], we employed this technique in an attempt to solve, at least partly, this interesting problem.

Materials and method. The IR-spectra (recorded of KBr pressed discs on a Perkin-Elmer 521 Infrared Spectrophotometer) of the calculi were compared with the spectra (recorded by us or published) of the common stone-forming compounds. Artificial mixtures of some calcium phosphates (carbonatoapatite, hydroxyapatite, octacalcium phosphate) with calcium oxalates were prepared and their spectra also recorded. Particular attention was paid to the 750–500 cm^{-1} region in which the absorption bands are relatively sharp (cf. Figure) and their frequency can thus be measured with a rather high accuracy. Qualitative chemical tests were also performed.

Results and discussion. The analysis of the spectra showed that the calcium phosphate constituent of the



IR-spectra of (a) an artificial hydroxyapatite – Ca oxalate mixture; (b) an artificial carbonate-apatite – Ca oxalate mixture; (c) an artificial octacalcium phosphate – Ca oxalate mixture; (d) a calculus which does *not* contain carbonate-apatite.

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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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[8] Acknowledgment. The financial support by the State Foundation for Scientific Research of Macedonia (Yugoslavia) is gratefully acknowledged.

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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