concentration was measured spectrophotometrically according to Rasanen et al.[15] who gave $E^{1\%}_{280nm} = 11.4$ and $M_r = 126{,}000$ (tetramer). Kinetic parameters (K_m and K_i) were calculated by linear regression analysis.

Results. Results show that these lectins inhibit the enterokinase activity on duodenal brush border membranes. Lineweaver-Burk plots are summarized in figure 1; graphs derived from these data, i.e. V_m^{-1} vs lectins concentrations, are shown in figure 2. From these results, the 3 lectins appeared to act as fast-binding reversible inhibitors of rat enterokinase activity. Inhibitions were purely noncompetitive; Con A and soybean lectins appeared to be weak inhibitors (K_i respectively equal to 10^{-5} and $0.64 \cdot 10^{-5}$ M) whereas PHA appeared 20–30 times more potent ($K_i = 3.2 \cdot 10^{-7}$ M).

Discussion. Only human, bovine and pig enterokinases have been reported to be glycoproteins[10,11]. Little is known about the structure of their oligosaccharide moieties[16]; our results may thus provide indirect evidence that rat enterokinase is also a glycoprotein, a point that has not been verified yet. The K_i values found for Con A and soybean lectin fit with the K_d values for the binding of Con A with monomeric saccharides[17] and serum glycoproteins[18]. The K_i value found for PHA is 20 times greater than K_d values reported for its interaction with lymphocytes[18]. These K_i may thus reflect the apparent dissociation constant for lectin-enterokinase interaction. The non-competitive model of inhibition due to these 3 lectins from leguminous seeds is consistent with a mechanism of steric hindrance of the active site of rat enzyme.

In conclusion, we assume that the inhibition of rat enterokinase we observe in vitro may also occur in vivo; this could explain the etiology of the enterokinase deficiency syndrome previously described[19]. Thus, the lectins from leguminous seeds may contribute to the impairment of proteolysis in the digestive tract.

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Creatine phosphate inhibition of heart 5′-nucleotidase appears due to contaminants

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Summary. Creatine phosphate does not inhibit 5′-nucleotidase preparations from rat, dog or guinea-pig hearts. Previously reported inhibitory effects must have been due to contaminants present in some commercial preparations of creatine phosphate.

Inhibition of cardiac ecto-5′-nucleotidase (E.C. 3.1.3.5) by ATP or ADP has been observed over a decade ago, and speculations regarding the possible physiological importance of these effects with respect to the regulation of coronary dilatation and adenosine formation have been made[2-4].

More recently it has also been reported that creatine phosphate inhibits 5′-nucleotidase from rat heart and, in view of the key role played by this compound in myocardial energy transport, it was suggested that this effect may be of importance in the physiological control of 5′-nucleotidase activity and adenosine formation[5]. However, in the present study we have been unable to observe inhibition of cardiac 5′-nucleotidases by creatine phosphate, suggesting that observations to the contrary[5] were the results of contaminants known to be present in some commercial preparations of this phosphate ester[6-8].

Materials and methods. Hearts were excised from decapitated Sprague-Dawley rats (180–250 g) and guinea-pigs (300–400 g) of both sexes and then washed free of blood by retrograde perfusion via the aorta with ice-cold saline solution. Dog hearts were removed from mongrel dogs (13–16 kg) of both sexes, anesthetized with sodium pentobarbital (32 mg/kg) and washed as above. Ventricles were cut out, blotted, minced, and microsomal fractions were obtained by differential centrifugation according to the procedure of Dutta and Mustafa[9] with minor modifications. Thus polytron homogenization was performed in 0.25 M sucrose and the resultant microsomal 100,000 g pellet was suspended in 50 mM Tris-HCl buffer, pH 7.5. Final protein concentration was approximately 1–1.5 mg/ml. Fresh or stored (at −20 °C) microsomes were used in assays.

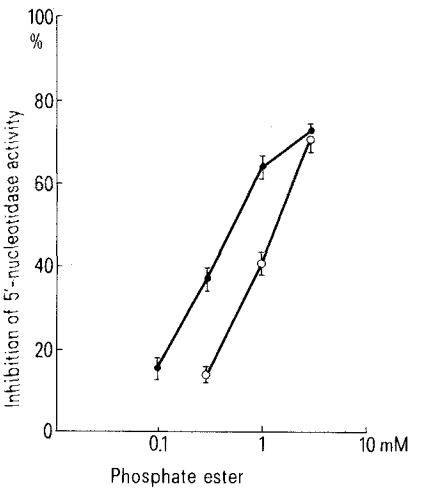
To solubilize 5′-nucleotidase, rat heart microsomes (1 ml) were suspended in 50 mM Tris-HCl buffer, pH 7.5, containing 1% deoxycholate (DOC), 1% Triton X-100 or 1% Zwittergent 3-14 (SB-14). After 4 h at 4 °C the suspension was dialyzed against the same buffer containing 0.05% of the respective detergent. A partially purified enzyme preparation from rat heart acetone powder was obtained ac-

cording to the procedure of Baer et al.[2] using DOC for enzyme solubilization, this being the same preparation used by Rubio et al.[5]. 5′-Nucleotidase activity was determined by measuring the conversion of [^{14}C]-AMP to [^{14}C]-adenosine according to the procedure of Avruch and Wallach[10]. The assay was performed at 37 °C in 100 µl (final volume) of 50 mM Tris-HCl buffer, pH 8.5 or 7.5, containing 0.1 µM [^{14}C]-5′-AMP, 2 mM Na p-nitrophenylphosphate and inhibitors at various concentrations. Mg^{2+} (5 mM) was employed in some experiments. After a 4-min preincubation period reactions were initiated by addition of enzyme whose concentration was adjusted to obtain 50% substrate conversion in the absence of potential inhibitors (control rate). After 10 min of incubation, reactions were stopped by adding 0.25 ml each of 0.25 M $Ba(OH)_2$ and 0.25 M $ZnSO_4$. After centrifugation the supernatants were counted for [^{14}]-adenosine in a liquid scintillation spectrometer using Aquasol as scintillation medium. In blanks no enzyme was added. Protein was determined by the method of Lowry et al.[11]. To express the potency of inhibitory compounds, plots of percent inhibition of control rate against the logarithm of inhibitor concentration were obtained, and concentrations causing 50% of inhibition of control rates (IC_{50}) interpolated.

[^{14}C]-5′-AMP (471 mCi/mmole) and Aquasol were purchased from New England Nuclear (Boston, Mass.), creatine phosphate from Boehringer-Mannheim Canada Ltd (Dorval, Quebec), DOC and Triton X-100 from Sigma Chemical Company (St. Louis, MO), and SB-14 from Calbiochem-Behring Corp. (La Jolla, CA).

Results and discussion. Two different lots of creatine phosphate did not show any inhibition of rat, guinea-pig and dog heart 5′-nucleotidase when testing concentrations ranging from 2 to 16 mM. Instead, slight stimulatory effects were observed at 8 mM and 16 mM creatine phosphate when enzyme activity was determined at pH 8.5. After microsomal enzyme solubilization with DOC and SB-14, but not with Triton X-100, weak stimulation of activity by creatine phosphate occurred both at pH 7.5 and 8.5. Similarly no inhibitory effect of creatine phosphate was observed when a partially purified 5′-nucleotidase preparation from rat heart acetone powder was used. Magnesium (5 mM) did not influence the lack of an inhibitory effect of creatine phosphate on microsomal or solubilized 5′-nucleotidase.

The effects of potential contaminants of creatine phosphate on 5′-nucleotidase activity were studied with guinea-pig microsomal enzyme. Triphosphate was shown to be the most effective inhibitor with an IC_{50}-value of 0.55 mM followed by diphosphate with an IC_{50}-value of 1.5 mM (fig.). Neither 1 mM oxalate nor monophosphate inhibited microsomal 5′-nucleotidase. Magnesium ion (5 mM) reversed the inhibition of 5′-nucleotidase by either 3 mM diphosphate or triphosphate from about 75% to 20%. It is noteworthy that the previously reported inhibitory effect of creatine phosphate on 5′-nucleotidase was completely reversed by Mg^{2+} concentrations in the range of 1–10 mM[5]. Creatine phosphate has been reported to inhibit different muscle and heart enzymes such as lactate dehydrogenase[12], pyruvate kinase[13] and 5′-adenylic acid aminohydrolase at high concentrations (2.5–20 mM)[14,15]. More detailed studies revealed that creatine phosphate purified by ion-exchange column chromatography did not inhibit any of these enzymes and that the observed effects were due to various contaminants identified as oxalate in the case of lactate dehydrogenase and pyruvate kinase[8], and as inorganic phosphates, particularly diphosphate and triphosphate, in the case of adenylic acid aminohydrolase[7]. Contamination levels in creatine phosphate preparations varied between suppliers and batches. For example, 0.3 and 1.3 M% oxalate have been found in 2 preparations, sufficient to account for the observed inhibition of lactate dehydrogenase and pyruvate kinase[8]. Likewise, contamination with about 1.5 M% diphosphate[6] and a range of 0.1–0.9 M% diphosphate[7] for different preparations could account for inhibitory effects on nucleoside triphosphatase and adenylic acid aminohydrolase, respectively. These levels of contamination could account for the findings of Rubio et al.[5], who used creatine phosphate up to 10 mM, only if rat heart 5′-nucleotidase was about 10-fold more sensitive to inhibition by di- and triphosphate than enzyme from guinea-pig heart (cf. the fig.); in any case, our conclusion is that their results could not be due to creatine phosphate since various enzyme preparations trom 3 species, including rat, were not inhibited in the present study.



Inhibition of guinea-pig microsomal 5′-nucleotidase with inorganic triphosphate (●) and diphosphate (○) (Tris-HCl buffer, pH 7.5). Values given are the means ± SEM of 5 experiments.

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