The experimental results described are in accord with the hypothesis that potatoes contain a high molecular weight, nondialyzable ampholyte which can act as an inhibitor of potato invertase[3] and that the invertase exhibits double pH optima because only one ionic species of the inhibitor (whose pK values lie within the pH range of activity of the uninhibited enzyme) can combine with the enzyme to form an inactive enzyme-inhibitor complex inactive[4]. It appears that the inhibitor accompanies the enzyme in the preparation of the protein fraction. The different K_m values which were observed would thus be expected in this case if a competitive inhibitor were present and active at pH 4.0 but not at pH 6.0.

Applying this theory to the data of the Table, one can calculate that the inhibition is greatest near the pH minimum, pH 4.7, but inhibition is negligible at pH 6, near one of the pH optima. It will also be noted in accordance with theory, that the calculated activity (as a function of pH) in absence of inhibitor, as well as that found at the lowest enzyme concentration investigated, shows no minimum. This suggests that the true optimum of the enzyme lies in the region of pH 3.5. The final proof of this hypothesis will be dependent upon the separation of the inhibitor from the preparation of potato protein.

It should be mentioned that protein preparations from potatoes stored at 2°C contained about four times more invertase activity than similar preparations from potatoes stored at room temperature, as measured at pH 6.0 by the method described for Figure 1.

Effect of enzyme concentration on rate of hydrolysis of sucrose by potato invertase

Enzyme concentration, mg of protein nitrogen per ml of reaction mixture[a]						
pH	0.035	0.07	0.035	0.07	0.035	0.07
	Observed rate[b]		Rate in absence of inhibitor[c]		% Inhibition[c]	
4	41	54	87	174	53	69
4.5	30	38	72	143	58	74
5.0	24	34	42	83	42	59
6.2	20	40	20	40	0	0

[a] Conditions same as for Figure 1.
[b] µg reducing sugar produced per h per ml of reaction mixture.
[c] Calculated on assumption of the presence of an endogenous inhibitor in the enzyme preparation. The derivation of the equation for this calculation has been described earlier[3].

Zusammenfassung. Kartoffelinvertase scheint zwei pH-Optima zu haben; ein Optimum bei pH 6 und das andere bei oder unterhalb pH 3,5. Es scheint evident, dass das Phänomen nicht mit dem Vorhandensein von zwei Enzymen zusammenhängt. Untersuchungen über die Kinetik des Enzyms lassen daran denken, dass das doppelte pH-Optimum mit einer amphoteren Hemmungssubstanz der Invertase in Beziehung steht.

E. S. Rorem and S. Schwimmer

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Gel Filtration of Protease Inhibitors from Potatoes

The inhibitor of proteases (IPP), isolated from potatoes[1], possesses pronounced anti-inflammatory activity[2,3]. By high-voltage electrophoresis, IPP was found to be composed of 5 protein fractions. By isolation procedures several preparations were obtained showing different degrees of purity and activity. It was of special interest to study whether some of the macromolecular fractions mixed with the anti-inflammatory fraction possess other characteristic activities and whether by their separation the anti-inflammatory activity remains unaltered. For this purpose, an isolation was carried out on an analytical scale such that the amount of fractions obtained sufficed for biological tests.

As the most suitable method, the column chromatography ('gel filtration') on Sephadex was suggested for preliminary information because of a large amount of inorganic material (mostly NaCl) present in the initial preparation and because of the lack of any information about the molecular weight of the individual fractions.

In experiments with Sephadex G-50 and G-25 (Pharmacia, Uppsala, Sweden) the presence of three maxima was found at 280 mµ. Figure 1 shows the chromatography of 1.5 g of lyophilized material dissolved in 30 ml of 0.01*M* acetic acid on Sephadex column G-25, water regain of 2.5 g/g of dry weight (3.2 × 200 cm) in equilibrium with 0.01*M* acetic acid. The elution was carried out by the same solution at room temperature. The localization of the maximum No. 3 (representing the anti-inflammatory activity) indicates a molecular weight of less than 3000. As the separation on Sephadex need not be complete, as was suggested in fact by the character of the maximum No. 3, a more detailed separation was realized before the lyophilization following the scheme in Figure 1 (fractions 1–3/3). Repeated chromatography of the main fractions (No. 2 and No. 3) gives identical pattern of separation, eliminating possible artefacts. The corresponding freeze-dried fractions obtained in the four individual chromatographic experiments were finally combined and evaluated biologically for irritant, haemagglutinating and anti-inflammatory activity.

As the preparation possess an inhibitory activity against a series of proteolytic enzymes, the inhibitions of trypsin, chymotrypsin, plasmin and plasmin-activator of the individual fractions were determined, and a possible correlation between the antiphlogistic and antiproteolytic activity was investigated. The results revealed (Figure 2 and Figure 3) that whereas fraction 2 is the main carrier of the antitrypsin, possibly antichymotrypsin activity and

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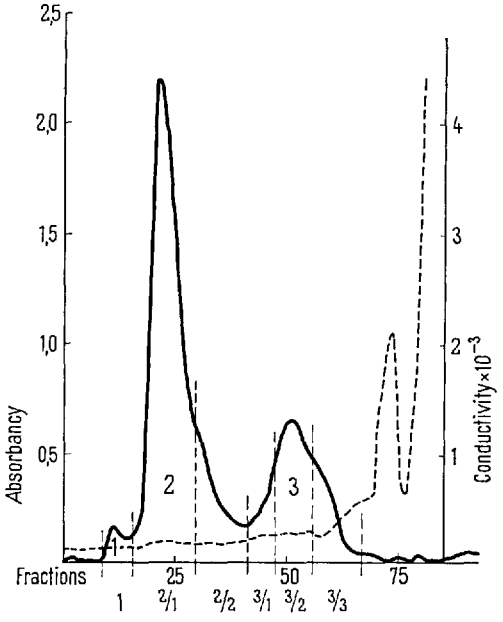

Fig. 1. Gel filtration of 1.5 g/30 ml of inhibitors (IPP) through Sephadex G-25 column (3.2 × 200 cm) equilibrated and eluted with 0.01 *M* acetic acid. Fractions (15 ml) collected at a rate of 60 ml/h. Solid line: absorbancy at 280 mμ, broken line: specific conductivity.

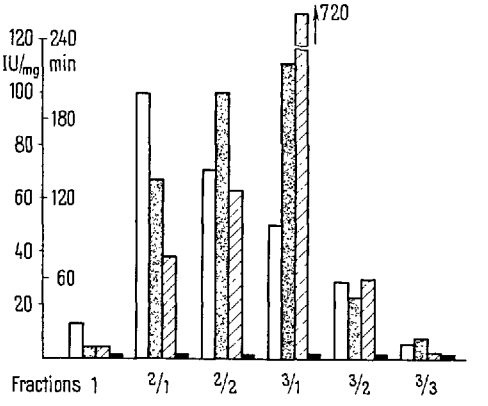

Fig. 2. Inhibitory activity of individual Sephadex-fractions of IPP against some proteolytic enzymes. Equal weights of individual freeze-dried taken into experiment. Trypsin □ and chymotrypsin ⁞⁞⁞: inhibitory activity is relation of the total amount of enzyme to the total amount of inhibitor which decreases its activity to 50%. Plasmin /// and plasmin-activator ●: lysis-time of a standard coagulum in min (control time 5 min).

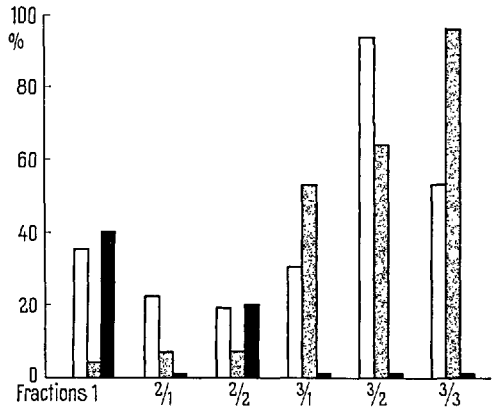

Fig. 3. Some biological properties of individual Sephadex-fractions of IPP. Anti-inflammatory activity □: inhibition of the kaolin inflammation of paw in rats in %. Irritant activity ⁞⁞⁞: mg of material in 0.1 ml of saline causing 3 times area of blueing after saline alone. Haemagglutinating activity ● :amount of the material in mg in the system with 0.05 ml of rabbit blood causing 50% agglutination.

fractions 3/2 and 3/3 of antiphlogistic activity, fraction 3/1 showed an antichymotrypsin and particularly antiplasmin activity. The high-molecular fraction 1 provokes a marked agglutination of erythrocytes. Its antiphlogistic activity is then probably the result of pronounced toxicity. Fractions 2 and 3, on the other hand, are relatively non-toxic. Inhibition of the plasmin-activator did not attain estimable values. More detailed fractionation studies are in progress[4].

Zusammenfassung. Vorläufige Fraktionierung an Dextran-Gel Sephadex G-25 (Pharmacia, Uppsala) ergab eine Trennung des Proteaseinhibitors aus Kartoffeln in die einzelnen Komponenten, die neben der eigentlichen entzündungshemmenden Aktivität noch Antitrypsin- bzw. Antichymotrypsin-, Antiplasmin- und ausgeprägte Hämagglutinierungsaktivität aufwiesen.

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Research Institute for Pharmacy and Biochemistry, Praha (Czechoslovakia), November 2, 1962.

[4] We wish to express our thanks to Mr. D. KAŠÍK and Mrs. L. ŠEBKOVÁ for helpful technical assistance. Biological tests were carried out by Dr. J. HLADOVEC and Dr. Z. HORÁKOVÁ of our Institute; plasmin-activity was determined by Ing. M. RYBÁK from Institute for Haematology and Blood Transfusion.

On the Mechanism of Spore Germination in *B. subtilis*. Permeability and Germination by Pyruvate

L-alanine induces germination in spores of *B. subtilis* incubated at 37° in phosphate buffer at neutral pH[1]. Undoubtedly *L*-alanine acts through a 'substrate' mechanism[2,3], i.e. germination depends upon the breakdown of the aminoacid. The first step which occurs in spores is deamination of alanine by the action of an *L*-alanine oxidase[4] or a DPN-linked *L*-alanine dehydrogenase[5–7]. It has been demonstrated that the production of pyruvate and ammonia does not imply loss of heat resistance and that pyruvate must be further metabolized to have germination[3,4].

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