Inositol Phosphatide in Peas

SOME properties and analytical data concerning an inositol phosphatide of peas have been reported previously^{1,2}. The availability of accurate methods for the determination of inositol in lipids, both by microbiological³ and chemical means^{4,5}, has made it possible to conduct further experiments which are reported here.

The quantity of inositol phosphatide fraction obtained by Wagenknecht's modification of Carter's procedure⁶ has been reported to increase during storage of raw peas (*Pisum sativum*) at -17.8°C . for periods of several days to several months². Since this inositol phosphatide fraction is known to contain impurities it was thought desirable to determine whether the percentage of inositol in the fractions was constant with time over the entire period of frozen storage. Aliquots of the inositol phosphatide fractions obtained from peas of Pluperfect variety were subjected to hydrolysis with 4*N* hydrochloric acid in sealed tubes at 120°C . for 16 hr. A modification of the periodate oxidation methods of Agranoff *et al.*⁵ and Le Baron *et al.*⁴ was used to determine inositol content of the hydrolysates. The results (Table 1) indicate that the percentage of inositol in these samples varies with time at -17.8°C ., but the total weight of inositol in the fractions is relatively invariant over the period from two days to four months of storage.

Table 1. INOSITOL CONTENT OF PEA INOSITOL PHOSPHATIDE FRACTIONS

Duration of frozen storage	Inositol* (per cent)	Inositol phosphatide (mgm.)	Inositol content (mgm.)
0 days	6.4	23	1.5
2 days	1.8	133	2.5
2 months	1.0	229	2.3
4 months	1.0	241	2.4
6 months	1.1	169	1.8

* Determined by modified periodate oxidation procedure (refs. 2, 4 and 5).

An enzymatic liberation of components of the inositol phosphatide fraction had previously been postulated, based on the observation that the inositol phosphatide content of raw (but not of heat-treated) peas increased with time². On the basis of the results presented here it would appear that this increase is caused by the enzymatic liberation, during frozen storage, of inositol-free lipid materials the solubility properties of which are sufficiently similar to those of phosphatidyl inositol to allow them to be extracted with the inositol phosphatide fraction.

Although several components were known to be present in the inositol phosphatide fraction, as was shown by chromatographic methods², previous paper chromatographic results gave no evidence for the presence of free inositol. As a further check on the absence of free inositol a sample of the water-soluble intact potassium form, which had been prepared from the calcium-magnesium mixed salt as described in a previous publication¹, was analysed for inositol by the excellent microbiological assay of McKibbin³, utilizing *Saccharomyces carlsbergensis* strain 4238. The purified potassium salt of the inositol phosphatide fraction was shown to contain less than 1 μgm . of free inositol per milligram.

Paper chromatographic evidence from another laboratory (Benson, A. A., personal communication) indicated that the calcium-magnesium mixed salts of pea inositol phosphatide contained, besides monophosphoinositide, components possessing the

R_F values of glycerophosphorylglycerol, phosphatidyl ethanolamine, phosphatidic acid, and some free inositol. The results presented here confirm that the purified potassium salt of the inositol phosphatide fraction contains inositol only in the bound form.

L. M. LEWIN*

A. C. WAGENKNECHT†

New York State Agricultural Experiment Station,
Cornell University, Geneva, New York.

* Present address: Division of Biochemistry, Department of Biology, Massachusetts Institute of Technology.

† Present address: Fundamental Food Research Dept., General Mills, Inc., Minneapolis, Minn.

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5-Deoxycytidylic Acid-deaminating Activity in Human Gastric Mucosa and Other Adult Mammalian Tissues

THE enzymatic deamination of deoxycytidylic acid to deoxyuridylic acid in sea urchin eggs and embryos has been demonstrated by Scarano¹ and Scarano and Maggio². Moreover, later results obtained by Scarano and Tallarico³ on the presence of deoxycytidylic acid-deaminase in livers of rat, chicken, rabbit in various stages of their embryonic development may indicate that this enzymatic activity, in developing or regenerating tissues, is proportional to their growth-rate. This latter view was confirmed in experiments by Maley and Maley⁴ on the determination of this enzymatic activity in embryonic tissues, in some spontaneous and experimental tumours and in regenerating livers.

In order to ascertain whether deoxycytidylic acid-deaminase is only characteristic of tissues in evolution or is common to tissues in continuous physiological regeneration, we examined several adult mammalian tissues for this enzyme activity. The tissues and organs investigated have been chosen in view of their active cellular proliferation.

The experimental procedure was essentially that proposed by Scarano¹. The homogenates were prepared by scraping of the mucosa surface. The specific activity is referred to as μmoles of deoxycytidylic acid deaminated in 10 min. per 10 mgm. nitrogen of the homogenate (the deamination of 1 μmole of deoxycytidylic acid giving a ΔA_{250} of -0.344). The human tissues used for the enzymatic assays were collected immediately from surgical operations. Histological examinations were carried out on each of the specimens used.

The results obtained for the human gastro-enteric mucosa and for other mammalian tissues are reported in Table 1.

The results indicate that the rabbit gastric mucosa has a deoxycytidylic acid-deaminating activity although in a lesser amount as compared to the human. This enzymatic activity is not found in other segments of rabbit gastro-enteric mucosa and in testicle, being scarcely detectable in bone marrow. Cat and ox gastric mucosa have also been examined, and a very low deoxycytidylic acid-deaminating activity was found.