

was then developed with acidic-ninhydrin reagent (2).

Under the conditions described, a separation of 5.5 cm has been obtained between glycine and trifluoroacetyl glycine, the acetylated derivative migrating to the anode and the glycine remaining almost stationary. The use of the same technique afforded a separation of 4.5 cm between alanine and trifluoroacetyl alanine.

It is conceivable that the technique described may be of use in evaluating (i) the hydrolysis of trifluoroacetyl amino acids by enzymes (3), (ii) the solubilizing effect of trifluoroacetic acid on proteins (4), and (iii) the cleavage of peptides after trifluoroacetylation of the peptide bond (5). In addition, the purity of trifluoroacetyl amino acids may be determined by separating large quantities of these compounds electrophoretically and then checking for ninhydrin-positive compounds (the free amino acids are ninhydrin-positive without basic hydrolysis) (6).

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Occurrence of Plasmalogens in Lipids of Green Peas

The plasmalogens (1), a group of glycerophosphatides which contain a higher fatty aldehyde, have been reported to be constituents of various animal phosphatides (2). Recently Lovern (3) has shown that plasmalogens also occur in vegetable lipids. Commercial samples of the total phosphatide fractions of soybeans and peanuts possessed an aldehyde content equivalent to about 7 percent of palmitaldehyde. Plasmalogens are readily cleaved by acid and mercuric chloride, and the liberated aldehyde may be determined with fuchsin-sulfite solution (Feulgen reagent) (4).

The lipids of green peas (*Pisum sativum* L.) have been under investigation because of their probable role in the development of off-flavors during frozen storage of raw peas (5). Crude lipid

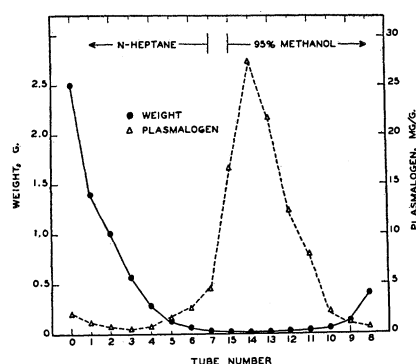


Fig. 1. Countercurrent distribution of acetone-soluble pea lipids between *n*-heptane and 95 percent methanol, showing distribution of weight (circles) and plasmalogens (triangles).

material was extracted from lyophilized raw peas, Perfected Freezer variety, with chloroform-methanol, 2:1. The lipid components of the mixture were then separated by means of a solvent fractionation procedure (6). The various fractions were then analyzed for their plasmalogen content with Feulgen reagent. A reaction time of 4 hours at room temperature was employed. The color which developed was extracted from the aqueous reaction mixture with 4 ml of isoamyl alcohol and read at 572 mμ in a Beckman spectrophotometer, model DU, against reagent blanks. The amounts of plasmalogen were determined from a standard curve obtained with known amounts of palmitaldehyde treated as described above.

Crude pea lipids were separated into acetone-soluble and acetone-insoluble fractions, the latter primarily a mixture of phosphatides. Phosphatides may be classified on the basis of their solubility in glacial acetic acid and in 95 percent ethanol (7). Lecithin (phosphatidyl choline) and cephalin (phosphatidyl serine and phosphatidyl ethanolamine) are soluble in glacial acetic acid, whereas phosphatidyl inositol is not (8). Furthermore, lecithin is soluble in alcohol, and cephalin is not. The pea phosphatides (acetone insoluble lipids) were fractionated with glacial acetic acid and with 95 percent ethanol. The plasmalogen content of the phosphatide fractions (expressed as milligrams per gram of lipid) was as follows: alcohol soluble (lecithin), 3.9, alcohol insoluble (cephalin) 7.3, acetic acid insoluble (phosphatidyl inositol) 29.4. Thus the greatest concentration of plasmalogens would appear to be associated with the more complex inositol phosphatides which normally are insoluble in glacial acetic acid.

The acetone-soluble pea lipids were subjected to countercurrent distribution between *n*-heptane and 95 percent methanol in a seven-transfer system with single withdrawal (6) at a concentration

of 13 percent with respect to each solvent (Fig. 1). It was anticipated that the bulk of the material would consist of triglycerides and that it would, therefore, be concentrated in the nonpolar solvent. In fact, over 75 percent of the total weight of lipids was found in the first three heptane fractions (tubes 0, 1, 2).

The plasmalogens were found to be distributed in the methanol fractions in the region of minimum weight distribution. The maximum amount was 27.5 mg/g in tube 14, which contained but 0.26 percent of the total weight. The tubes on either side contained lesser amounts of the plasmalogens, and only small amounts were present in the heptane fractions which comprised nearly all of the weight. Although phosphatides are normally considered to be insoluble in acetone, their presence in the acetone-soluble portion of a lipid mixture may be attributed to the well-known solubilizing powers of triglycerides.

Inasmuch as the separation of individual lipids from a naturally occurring mixture is always a difficult task, the apparent enrichment of plasmalogens by countercurrent distribution may provide a basis for the isolation and characterization of these unusual phosphatides in vegetable lipids. The lipids of green peas would appear to provide a convenient source of plant plasmalogens for such studies (9).

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Sex Chromatin and Sex Differentiation in Human Embryos

In the past, embryologists studying sex differentiation in man paid little attention to the fact that *chromosomally* there exists no indifferent stage because every fertilized egg is either XY-male or XX-female. Chromosome analysis was