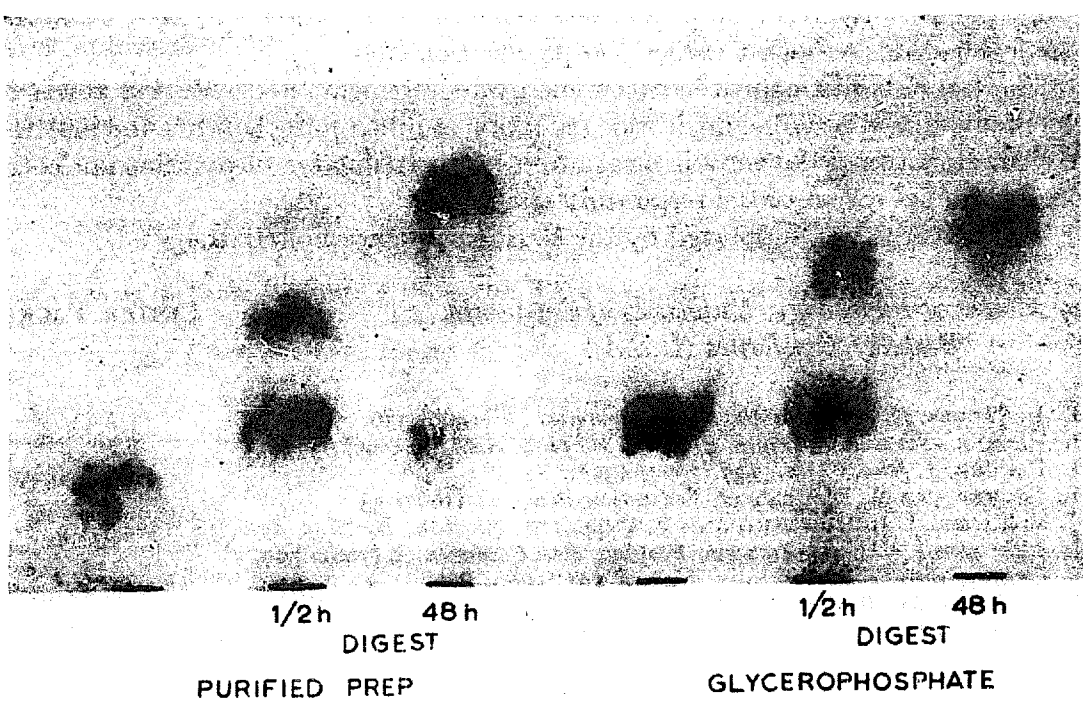
SC 2262

Isolation and identification of glycerylphosphorylethanolamine from aqueous extracts of mitochondria

This report describes the purification and identification of glycerylphosphorylethanolamine as isolated from water or saline extracts of washed liver mitochondria.

The mitochondria were isolated from rabbit liver in sucrose (0.25 M) and were washed twice with cold NaCl (0.15 M). Fresh preparations were extracted at 23°, either with water for 15 min or with NaCl (0.15 M) for 60 min. The mitochondria were removed by centrifugation, and the supernatant was treated with 3 % trichloroacetic acid. The deproteinized fluid was passed through an anion-exchange resin (3 ml Dowex-1 formate) which retained approx. 80 % of the total phosphate. This portion includes the adenine nucleotides[1]. The effluent was passed through a cation-exchange resin (3 ml Dowex-50), and the second effluent, containing approx. 16 % of the original acid-soluble phosphate, was evaporated to dryness at 35° to remove formic acid. As the final step, the material was purified with paper chromatography with 80 % *tert.*-butanol as the solvent. The paper was removed from the jar after 16 h. The phosphate band was identified with the HANES AND ISHERWOOD spray[2] and eluted with water. This step served to separate the organic phosphate (R_F 0.13) from material (R_F 0.23) which reacted with TREVELYAN's silver stain[3].

The organic phosphate eluted from the paper was found to react with ninhydrin. On paper electrophoresis, it appeared as a single compound that remained at the origin between pH 2 and pH 8. When the material was heated in 2 N HCl at 100° for 30 min, ninhydrin-positive and phosphate components were separated, and these were found to migrate to the cathode and to the anode, respectively. With more thorough digestion (2 N HCl, 125° for 48 h), compounds were obtained which with paper chromatography had migration and staining characteristics of ethanolamine, inorganic phosphate and of glycerol[2,3]. A solvent containing 80 % *tert.*-butanol and 5 % formic acid was found to be convenient, and a chromatogram obtained with



this system and stained for phosphate is shown in the photograph. This chromatogram describes the migration of the unknown and of glycerophosphate and of products obtained after digestion in 2 N HCl for 30 min at 100°, and for 48 h at 125°. The R_F of the unknown was 0.08 compared to 0.17 for glycerophosphate. Double spots appeared after the 30-min digestion. The slower of the two, in each case, is presumably unaltered glycerophosphate, while the faster may represent formation of cyclic intermediates[4]. Material reacting after 48 h of digestion had the migration and staining characteristics of inorganic phosphate. Table I describes the recovery of glycerol and of phosphate after each of the three preparative steps.

Ethanolamine has been identified by electrophoresis as a ninhydrin-positive product of digestion which, at pH 6.5, migrated towards the cathode at a rate approx. twice as fast as the basic amino acids. At pH 8.9, in 2.5% $(NH_4)_2CO_3$ buffer, this component and ethanolamine moved together at a rate distinguishable from other organic amines (ethylamine, *n*-propylamine, *n*-butylamine, 1-amino-2-propanol, 3-amino-1-propanol). One preparation (analyzed by Dr. A. G. OSLER) was found to contain 4.7 μmoles of phosphate and 5.0 μatoms of N (microkjeldahl). Digested samples have not contained significant quantities of ether-soluble titrable acid.

In summary, aqueous extracts of washed mitochondria contain an organic phosphate substance that has the chemical and physical characteristics of glycerylphosphorylethanolamine. This substance has been found to be retained and to be released by mitochondria under conditions that result in retention or release of mitochondrial potassium[6]. Isolation of glycerylphosphorylethanolamine from whole tissue extracts with use of column chromatography has been described by SCHIMASSEK, KOHL AND BÜCHER[7].

TABLE I

COMPARISONS OF THE AMOUNTS OF TOTAL PHOSPHATE AND GLYCEROL RECOVERED DURING A TYPICAL PREPARATIVE PROCEDURE

The samples were digested for 48 h at 125° in 2 N HCl prior to analysis. Glycerol was determined by measurement of formaldehyde release after periodate oxidation as described by RENKONEN[5].

	Phosphate (μmoles)	*Glycerol (μmoles)*
Dowex-1 effluent	5.5	4.7
Dowex-50 effluent	4.3	4.3
Paper elution	3.6	3.8

This work was done during the tenure of an established investigatorship of the American Heart Association and it was supported by grant number A-1775 from the National Institutes of Health, Bethesda, Md. (U.S.A.). The author is indebted to Miss J. MAZUR for technical assistance.

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[1] P. SIEKEVITZ AND VAN R. POTTER, *J. Biol. Chem.*, 215 (1955) 221.
[2] C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
[3] W. E. TREVELYAN, D. P. PROCTOR AND J. S. HARRISON, *Nature*, 166 (1950) 444.
[4] P. E. VERKADE, J. C. STOPPELENBURG AND W. D. COHEN, *Rec. Trav. Chim. Pays-Bas*, 59 (1940) 886.
[5] O. RENKONEN, *Biochim. Biophys. Acta*, 56 (1962) 367.
[6] J. L. GAMBLE, Jr., *Am. J. Physiol.*, 203 (1962) 886.
[7] H. SCHIMASSEK, D. KOHL AND TH. BÜCHER, *Biochem. Z.*, 331 (1959) 87.

Received January 22nd, 1963