original glycerylphosphorylinositol phosphate was found in the inorganic phosphate fraction. In a control experiment with boiled enzyme no radioactive inorganic phosphate was liberated. This indicates that Reaction 1 was taking place in the mitochondria.

$$\text{Phosphatidylinositol} + \text{ATP} \rightarrow \text{diphosphoinositide} + \text{ADP} \qquad (1)$$

Experiments in which oxidative phosphorylation was inhibited indicated that the labelling of diphosphoinositide was dependent upon ATP production. Results are given in Table I.

It seems unlikely that diphosphoinositide could be an intermediate in oxidative phosphorylation[1]. Moreover, its structure contains no high-energy phosphate bonds. However, the rapid and transient labelling may be explained by assuming that the lipid is acting as a carrier in cation transport. During the early stages of incubation there would be a greater need for active transport, *e.g.* to restore mitochondrial potassium lost during the preparation of the mitochondria.

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Received April 29th, 1963

Biochim. Biophys. Acta, 70 (1963) 479–481

PN 1277

Tightly-bound proteolipid phospholipid in bovine brain white matter

In 1961 WEBSTER AND FOLCH-PI[1] demonstrated that aqueous tripotassium citrate when added to chloroform–methanol solutions of crude proteolipid rendered 80% of it insoluble. These authors suggested that tripotassium citrate split lipid–protein bonds, liberating lipid and precipitating protein. The present communication, as well as confirming the above results, will show that there is still tightly-bound phospholipid associated with the insoluble protein residue after citrate splitting.

Crude proteolipid isolated from bovine brain white matter was purified[2] and dissolved in chloroform–methanol (2:1) containing 5% water. This was treated with tripotassium citrate as above and the insoluble material, collecting at the interface, was removed, washed with chloroform–methanol (1:1) and extracted 3 times with chloroform–methanol (1:1) containing 0.5% conc. HCl. These acid chloroform–methanol extracts were pooled and the acid removed by equilibration with water[3]. Table I shows the distribution of phosphorus among the various fractions of tripotassium citrate-treated proteolipid.

TABLE I

DISTRIBUTION OF PHOSPHORUS AMONG FRACTIONS AFTER SPLITTING WITH TRIPOTASSIUM CITRATE

Fraction	*Percent of total phosphorus**	*Lipids present*
Upper phase	1.46 ± 0.97	None
Lower phase	81.6 ± 3.9	Phosphatidylserine, phosphatidylethanolamine, glycolipid, cholesterol
Acid chloroform–methanol extract	11.3 ± 5.4	Phosphatidylserine, inositide
Residue after acid chloroform–methanol extraction	3.0 ± 1.0	Not examined

* Mean ± standard error.

Analysis of the lower phase which contains lipid split from proteolipid protein showed the presence of glycolipid, cholesterol and phospholipid. 60–70 % of the phospholipid was phosphatidylserine. It seems that these lipids are responsible for, and essential to, the solubility of 80 % of the proteolipid in chloroform–methanol.

It was noted that about 13 % of the total proteolipid phosphorus remained with insoluble protein after citrate splitting, the majority of which was extractable with acid chloroform–methanol. After equilibration with water to remove the acid only a very small amount (1–2 %) of inorganic phosphorus was found in the upper aqueous phase indicating that there was very little extensive destruction of the material by exposure to acid for a short time. In thin-layer chromatography on silica gel G with several solvent systems[4] the material did not move from the point of application. This suggested a highly polar lipid molecule. Acid hydrolysis and examination by paper chromatography according to DAWSON AND DITTMER[5] showed the presence of inositol phosphate. This was confirmed by three other procedures: Diethylaminoethyl cellulose[6] and silicic acid[7] column chromatography and separation of the products of alkaline hydrolysis on Dowex anion-exchange resin[8] with ammonium formate in formic acid.

The work of GAITONDE[9] with rat brain and WOLMAN[10] with human brain have suggested the presence of several types of proteolipid in nervous tissue. In this laboratory Dr. M. MATSUMOTO has succeeded in effecting a separation of purified proteolipid into several fractions by the use of hydrated silicic acid columns with gradually increasing concentrations of methanol–water in chloroform. The largest fraction containing 30–40 % of the protein was eluted last from the column and then only by the use of chloroform–methanol–water (50:50:10) containing HCl. The lipids of this fraction exhibited remarkable similarity to those of the acid chloroform–methanol extract of the insoluble residue after citrate splitting.

These findings strongly suggest that an inositol lipid is associated with a particular lipid–protein complex under the extraction conditions employed here and it is possible that this represents a particular tissue component *in situ*.

We are indebted to Dr. M. LEES for her interest and assistance. This investigation was supported in part by grants from the National Multiple Sclerosis Society (U.S.A.)

and grant No. NB 00130, National Institute of Neurological Diseases, National Institutes of Health.

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Received April 29th, 1963

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PN 1276

Fractionation of wax D, a peptidoglycolipid of *Mycobacterium tuberculosis*

Wax D is a macromolecular glycolipid extractable from *Mycobacterium tuberculosis*[1]. WHITE *et al.*[2] found that wax D of human type *M. tuberculosis* which contains amino acids has a characteristic biological activity, an adjuvant effect, while wax D from the other strains of *M. tuberculosis* lacking amino acid has no such activity. Very recently, JOLLÈS, SAMOUR AND LEDERER[3] reported further fractionation of wax D of human type *M. tuberculosis* by ultracentrifugation into a supernatant fraction designated as Ds (6–10 %) which is free from amino acids, and five other fractions, all of which are sedimentable and contain meso-DAP, Glu, Ala and sometimes Gly.

In the present study, chromatography of wax D on a column of silicic acid or magnesium trisilicate repeatedly proved unsatisfactory. But in the course of chemical modifications of wax D, it was found that acetylated wax D (AD) of human type *M. tuberculosis* ($H_{37}Ra$) could be easily separated by chromatography and thus the heterogeneity of it was confirmed. Further findings were: (1) fractions free from amino acids amounted to 30 %; (2) another 30 % covered fractions containing Glu, Ala and DAP; (3) a fraction of 14 % contained at least six amino acids including two amino acids which have not hitherto been recognized in wax D.

Crude wax D was kindly supplied by Dr. LEDERER of the University of Paris. It was further purified by exhaustive extraction with boiling acetone (15 times, 10 h for each extraction) until the amount of a soluble material was negligible. 2 g of

Abbreviation: DAP, diaminopimelic acid.