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PURIFICATION AND ANALYSIS OF PHYSIOLOGICALLY ACTIVE LIPIDS

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Abstract—Modern methods for the isolation and identification of small quantities of physiologically active lipids are discussed.

THE methods which will be described are designed to overcome the two major difficulties confronting workers in the field of physiologically active lipids. The first difficulty is that methods for the separation and purification of any complex lipids are still quite primitive in comparison with the techniques available for protein or carbohydrate work. The second is that the tissues to be studied may contain only microgram quantities of the active lipids. Since they show physiological activity at extremely low concentrations this will be the method of choice for their detection. Chemical or enzymatic procedures which modify or destroy biological activity may then be used to gain information about the chemical structure of the active lipids.

SEPARATION OF COMPLEX LIPIDS

The problem here is that the physical properties of the lipids, on which separation methods depend, are greatly influenced by the fatty acid composition of the lipids. To take a simple example, dipalmityl lecithin will be insoluble in ether, while most natural lecithins are freely soluble. In most tissues each lipid such as phosphatidic acid is really a family of closely related lipids, which differ only in fatty acid composition and which therefore have finely graded differences in physical properties. Methods are not yet adequate for the separation of one family from another (e.g. phosphatidyl ethanolamine from phosphatidyl serine), while separations within a family are hardly even thought of.

(*a*) *Extraction from tissues*

Extraction of the wet tissue with roughly ten times its volume of 2:1 v/v chloroform–methanol will remove lipids very efficiently. The extract will contain non-lipid impurities which can be removed by shaking with 0·2 vols. of 0·01M $CaCl_2$ or 0·01M KCl. This washing procedure may remove some of the acidic phospholipids. An alternative is evaporation to dryness *in vacuo*, followed by re-extraction with dry chloroform. If saline extracts of tissues form the starting materials, these may be extracted directly with chloroform to remove lipid material. In some cases acidification

may improve extraction, but speed and a low temperature are necessary to avoid hydrolysis of the active lipid.

(*b*) *Separation on silicic acid*

In the form of columns, impregnated papers or as a thin layer on glass plates, silicic acid is widely used for lipid separations. Separations are rarely complete, however, and other methods should be used to check the identity and purity of fractions from silicic acid. The column technique has been reviewed by Wren.[1] Lipids are generally applied as solutions in chloroform and eluted with more polar solvents such as methanol–chloroform mixtures containing increasing amounts of methanol. Silica-impregnated papers have been widely used by Marinetti.[2] They require much less lipid. For microgram quantities the thin-layer technique can be used.[3] This is also much more rapid, separation being obtained in about an hour.

(*c*) *Separations after hydrolysis of the lipids*

The fatty acid can be readily removed from most phospholipids, leaving water–soluble phosphate esters. These characterize the lipids in a mixture and may be separated by paper chromatography[4] or on ion-exchange columns.[5] These methods are useful in checking the identity of fractions from silicic acid. Further information may be obtained by more vigorous hydrolysis and paper chromatography of the hydrolysate. Reagents suitable for the detection of glycerol, inositol, choline, amino acids, carbohydrates, etc., may then be applied to the papers.

(*d*) *Solvent separations*

In some cases, chromatography is ineffective. Partial separations may then be made by extraction and precipitation with suitable organic solvents. Sometimes a chemical treatment may help, e.g. sphingomyelin, unlike the other phospholipids, is alkali-stable, so that the latter can be removed by mild alkaline hydrolysis. Counter-current distribution also provides a useful alternative to chromatography. Olley[6] has reviewed some applications of this method to lipids.

IDENTIFICATION OF ACTIVE LIPIDS

It should be emphasized that lipid solutions in organic solvents often contain traces of non-lipid contaminants, even after chromatographic purifications. Such contaminants may be responsible for biological activity. To exclude this possibility, the biological assay should be correlated with a chemical assay specific for the lipid suspected, e.g. if it is a phosphatidic acid, biological activity in different preparations should run parallel with phosphate, glycerol and ester content of the extracts.

Where too little is available for such estimations, attempts can sometimes be made to elucidate the structure of the unknown by degradation studies. In some cases, e.g. certain phospholipids, enzymes which hydrolyse the lipid will be available. Where a carbohydrate or other poly-alcohol group is suspected, periodate oxidation should destroy biological activity. Mild alkaline hydrolysis will remove esterified fatty acids from glycerides or phospholipids. If the active principle is alkali-stable the field is narrowed considerably. Infra-red analysis may also give information about characteristic molecular groupings in a lipid.

When some chemical information has been obtained, the biological activity of known lipids with the same chemical properties can be compared with that of the unknown. In all cases the final identification should be confirmed by synthesis of the compound or comparison with a natural product whose structure has been completely worked out. Collaboration with a group of chemists is desirable here, since the chemical synthesis of complex lipids is extremely difficult.

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