

rat intestine as in those from the rabbit. The relatively low incorporation obtained in the rat with  $\alpha$ -glycerophosphate might therefore be due to the more rapid splitting of this compound to inorganic phosphate and glycerol. The glycerol cannot then be used for glyceride synthesis<sup>6</sup>.  $\alpha$ -Monostearin, when supplemented with adenosine triphosphate, coenzyme A, glutathione and magnesium chloride, stimulated the incorporation of labelled palmitate to about the same extent as in the rabbit.

A more detailed report of this work will be published elsewhere.

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### Presence of Sialic Acid in Connective Tissues

SIALIC ACID was first demonstrated in the sub-maxillary gland by Blix<sup>1</sup> and afterwards in many secretions of higher animals—in liver, ovomucin, meconium, colostrum, erythrocytes, nervous tissue and micro-organisms. Comprehensive reviews on sialic acids have been published by Zilliken and Whitehouse<sup>2</sup> and by Blix<sup>3</sup>.

The presence of sialic acid in connective tissue has not so far been reported.

In the course of our research on chemical composition of epiphyseal cartilage<sup>4</sup> and particularly on the mucopolysaccharidic components<sup>5</sup>, the presence of this acid in epiphyseal cartilage of young rabbits was demonstrated<sup>6</sup>. We have extended this study to other tissues in order to see whether a polysaccharide containing sialic acid was present in the ground-substance of all connective tissues.

The tissues were hydrolysed with 0.1 *N* sulphuric acid at 80° C. for 1 hr.; the hydrolysate was filtered on a sintered glass filter and chromatographed on 'Dowex 2 × 8' by Svennerholm's method<sup>7</sup>; after elution with 1 *M* acetate buffer pH 4.6 the eluate was tested by the resorcinol reaction<sup>7</sup> and sialic acid was identified by the absorption spectrum of the chromatic reaction.

To identify and determine quantitatively the sialic acid, we used as standard compound *N*-acetylneuramic acid isolated from sheep N-acetylneuramic acid and kindly supplied to us by Prof. G. Blix.

The presence of sialic acid was demonstrated in the following tissues: epiphyseal cartilage of young pigs (1.6 mgm. per gm. fresh tissue), rib cartilage of young rabbits (0.6 mgm./gm.), bovine cornea (0.4 mgm./gm.), bovine aorta (0.6 mgm./gm.) and bovine dental pulp

(0.6 mgm./gm.); smaller quantities are also present in bone and dentin.

From these results we may conclude that sialic acid is a diffuse constituent of the polysaccharidic fraction of ground substance in connective tissues.

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### Configuration of Inositol Phosphate in Liver Phosphatidyl Inositol

THE hydrolysis of synthetic glycerol 1-(inositol 2-phosphate)<sup>1,2</sup> has recently been compared with that of the glycerylphosphoryl inositol isolated from hydrolysates of liver phosphatidyl inositol<sup>3</sup>. The results suggest that the phosphate group in the original lipid is attached to the 1- or 2-hydroxyl of the *myo*inositol. Inositol 1-phosphate should be optically active, while inositol 2-phosphate should not.

Previous work<sup>4,5</sup> had suggested that the inositol monophosphate produced on hydrolysis of ground-nut or soya phosphoinositides was optically inactive. It was therefore concluded<sup>6</sup> that phosphatidyl inositol has the 2-phosphate structure. However, Pizer and Ballou<sup>7</sup> have now provided evidence that the inositol monophosphate from soya phosphoinositide has a small but definite optical activity ( $[\alpha]_{589}^{25} + 3.4^\circ$  for the *cyclohexylamine* salt,  $-9.8^\circ$  for the free acid). This called for a re-investigation of the earlier results.

One of the problems in determining the rotation of inositol monophosphate from soya lipids is that there are contaminating sugar phosphates which are strongly dextro-rotatory. A small amount of such an impurity could easily mask the low inositol monophosphate rotation. The soya inositol monophosphate, prepared as before<sup>5</sup>, has therefore been purified by passage through three successive 'Dowex 1' columns, two using the formic acid-ammonium formate system and one using the sodium borate-ammonium formate system<sup>3</sup>. It was finally crystallized as a *cyclohexylamine* salt and shown to be optically active. Since the rotation was quite small, determinations were made at several different wave-lengths. The activity increased considerably at lower wave-lengths. Molecular rotation was as follows:  $+12^\circ$  (600  $m\mu$ ),  $+18^\circ$  (500  $m\mu$ ),  $-32^\circ$  (400  $m\mu$ ),  $-54^\circ$  (350  $m\mu$ ),  $-89^\circ$  (300  $m\mu$ ). Calculations are based on the *dicyclohexylamine* salt. Using a similar salt of inositol monophosphate produced by enzymic degradation of phytic acid and believed to be the 2-phosphate<sup>8</sup>, values of zero (within the error of the instrument) were obtained at all the above wave-lengths. Soya inositol mono-