That the chromatographic mobility of this new peak is different from 15-keto-13,14-dihydro-$PGF_{2\alpha}$ can be seen from the lower scan where a resolution between the new peak (arrow) and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ is observed. The chromatographic mobility of this product (arrow) coincided with 15-keto-13,14-dihydro-PGE_2. This compound is also formed when $^3H_7$-PGE_2 is incubated with rat lung or rat kidney homogenates (PACE-ASCIAK, unpublished observations).

These findings demonstrate the occurrence of a 9-hydroxydehydrogenase activity in kidneys from adult rats. Such activity is absent in newborn rats. The main product from incubation of $PGF_{2\alpha}$ is 15-keto-13,14-dihydro-PGE_2 (Figure, middle scan). Further studies are in progress to determine whether the crossover from PGF to PGE type takes place at the primary prostaglandin level or at any of the subsequent PGF metabolic stages. Also under investigation is the stage in kidney development that this enzyme activity first appears.

Time course of 9-hydroxydehydrogenase activity in the rat kidney

Incubation time (min)	Recovery of counts (%) Adult	Newborn (day 6)
0	100	100
1	98; 98	98; 100
5	82; 83	102; 103
10	59; 72	100; 101
20	45; 59	100; 102

Time-related loss of radioactivity from 9β-$^3H_1$-$PGF_{2\alpha}$ during incubation.

Résumé. Dans le rein du rat le $PGF_{2\alpha}$ est transformé en 15-kéto-13,14-dihydro-PGE_2 par des homogénats du rat, ce qui atteste pour la première fois l'activité de la prostaglandine 9-hydroxy déshydrogenase dans cet organe. Cet enzyme était présent chez le rat adulte, mais non pas chez le nouveau-né.

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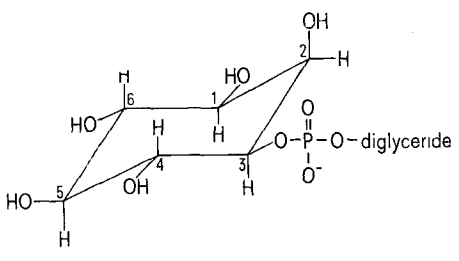
Structure-Function Relationships in Phosphoinositides

Schizosaccharomyces pombe, a fission yeast and *Neurospora crassa* (inositolless), a mycelial fungus, are dependent for their growth on exogenous *myo*-inositol. It has been shown by SCHOPFER and POSTERNAK[1] that a number of inositol derivatives inhibit the growth of these organisms. One of the potent substances is isomytilitol (2-C-methyl-*myo*-inositol). The inhibition caused by this compound is accompanied in *S. pombe* by extensive morphological changes, which might be due to the effect of abnormal phospholipids resulting from the incorporation of isomytilitol in positions normally occupied by *myo*-inositol[2,3]. In this paper, the effects of isomytilitol on *S. pombe* and on *N. crassa* are examined, and a hypothesis for the physiological activity of the compound is proposed.

Material and methods. Schizosaccharomyces pombe Lindner CBS 1042 was grown as described[2], and conidia of *Neurospora crassa* (inositolless) CBS 259.47 were obtained from 5 days culture on solid medium[4] supplemented with 10 mg/l of *myo*-inositol. Conidia were separated from hyphal material by suspending the scraped cultures in sterile medium, stirring for 30 min and filtering on a triple layer of nylon cloth. Germination was reached within 4 to 6 h in the liquid medium without *myo*-inositol.



Structure of the *myo*-inositol phosphate moiety of the phosphatidyl-inositol

Cell-free extracts were prepared, using the method of WHITE and HAWTHORNE[5] with 4% glycerol and 200 mg/l of dithiothreitol. Unbroken cells and cell debris were removed by centrifugation at $800 \times g$ for 5 min. Since the system involved in the phosphoinositides formation is very sensitive to freezing in the presence of mineral salts, cells were broken in the absence of buffer.

The phospholipid biosynthesis assays were performed in a final volume of 1.6 ml containing 4.5 µmoles $MnSO_4$, 100 µmoles α-glycerophosphate, 2 µmoles of the labelled cyclitol and 5 mg protein of the cell-free preparation. After 30 min incubation at 30 °C, the reaction mixture was extracted twice with 8 ml of a mixture $CHCl_3$, CH_3OH, HCl conc. (100:50:2). The combined organic phases were chromatographed on Whatman SG 81 paper in $CHCl_3$, CH_3OH, water (65:25:4). In this system the free cyclitols stay near the origin. The chromatograms were cut and the strips were placed in the scintillation fluid for counting.

The transport assays were performed as previously described[6]. Cells of *S. pombe* were from 44-h culture. Germinated conidia of *N. crassa* were used.

Myo-inositol phosphate was detected by electrophoresis of the aqueous phase of the phospholipid biosynthesis assays on Whatman No. 3MM paper, in ammonium acetate buffer pH 5.3, 0.1 *M*, at 20 V/cm.

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(2-^{3}H)*myo*-inositol was purchased from NEN (Dreieichenhain, Germany) and (^{3}H)*scyllo*-inositol from the Radiochemical Center (Amersham, U.K.). Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland) supplied the other chemicals.

Results. *Scyllo*-inositol has no influence on the growth of *S. pombe*. While maximal growth is obtained with 1 mg/l of *myo*-inositol, there is no growth when the same concentration of *scyllo*-inositol is used. The growth observed at a concentration of 50 mg/l of *scyllo*-inositol is due to a 0.8% contamination with the *myo*-isomer.

A *myo*-inositol active transport system (K_m: 0.26 mM; V: 0.6 nmole/mg per min) has been described in *S. pombe*[6]. In identical experiments it could be shown that *scyllo*-inositol is also transported actively with an apparent K_m of 0.6 mM and a V of 1.0 nmole/mg per min. However, no evidence for an active transport system for cyclitols was found in germinated conidia of *N. crassa*, since the internal concentration of the cyclitol after the uptake was always lower than the concentration in the medium.

Cell-free preparations of both organisms were found to catalyse the incorporation of free cyclitols into the phospholipid fraction. Since 2.9 mM Mn^{++} causes a 10-fold enhancement of *myo*-inositol incorporation, it was used routinely in all incubations. Neither triphosphonucleosides (ATP, UTP, GTP, TTP, 2×10^{-4} M) nor CDP-diglyceride had any effect on *myo*-inositol incorporation into the phospholipid fraction; however, α-glycerophosphate at an optimal concentration of 60 mM enhanced the total incorporation of the cyclitol by 500%. β-glycerophosphate was not an activator.

Isomytilitol (2-C-methyl-*myo*-inositol), which is a growth-inhibitor of *S. pombe* and *N. crassa*, was found to be incorporated in vivo into the phospholipid fraction of both organisms[7,8]. It is also incorporated into the lipids in vitro at a similar rate as *myo*-inositol (0.9 μmole/mg protein/30 min), and as *scyllo*-inositol. In *N. crassa* the radioactive peaks from chromatography of the phospholipids containing *scyllo*-inositol are found at the same place as those for the lipids containing *myo*-inositol: mono-, di-, and triphosphoinositides.

When *myo*-inositol is used with cell-free preparation of *S. pombe*, the radioactivity is concentrated mainly in one peak which migrates near the solvent front; with *scyllo*-inositol the radioactivity is also found in 2 other peaks. These were similar in mobility to the di-, and triphosphoinositides found in *N. crassa*; they were not further investigated.

Discussion. WHITE and HAWTHORNE[5] first showed that the enzymatic system which synthesizes phosphoinositides in *S. pombe* is membrane-bound, and they established that the synthesis does not follow the scheme of PAULUS and KENNEDY[9] in which the direct precursor is CDP-diglyceride. This view is consistent with our finding that in *N. crassa* no activation of the synthesis was observed upon the addition of CDP-diglyceride. Of the numerous substances tested on the extracts of both organisms, only α-glycerophosphate enhanced incorporation. These data suggest that the direct precursor of phosphoinositides in yeast and fungi is a lipid, probably phosphatidyl-glycerophosphate; the phosphoinositide could be formed by direct exchange with free *myo*-inositol (the formation of *myo*-inositol phosphate has not been detected) in a reaction similar to that involved in the synthesis of phosphatidyl-serine[10].

The enzymes which synthesize phosphoinositides in *N. crassa* and in *S. pombe* do not seem to be specific. *Myo*-, *scyllo*-inositol and isomytilitol are incorporated at similar rates. As other labelled cyclitols were not available, their eventual incorporation has not yet been studied. All incorporations studied are activated by Mn^{++} so far and α-glycerophosphate, suggesting a common mechanism for the synthesis of the three types of lipids.

It has been shown that, in vivo, isomytilitol is incorporated into phospholipids of *N. crassa*[7] and *S. pombe*[8] with concomitant growth-inhibition and spectacular morphological modifications in *S. pombe*[2,3]. In vitro, *scyllo*-inositol, which cannot replace *myo*-inositol as a growth-factor, is also incorporated into the phopholipids.

On the basis of the results presented in this paper, it is proposed that, to function as a growth factor, a cyclitol must have at least the following structural feature: 1. three *cis* hydroxyl groups of which the medial is axial[11], 2. an equatorial hydrogen in position 2 (Figure).

When inside the cell, *myo*-inositol is incorporated into the phospholipids and the latter becomes an integral part of the membrane structure.

Isomytilitol, which differs only from *myo*-inositol by the replacement of the equatorial hydrogen at C-2 by a methyl group, can replace *myo*-inositol in the phospholipids of both *N. crassa* and *S. pombe*; however in *S. pombe* it cannot impart the unique function in the cell division process which occurs with the normal growth-factor. Hence the function of the membranes in promoting division, is in some way disturbed and separation of the daughter cells does not take place in the usual way, leading to bizarre structure.

Scyllo-inositol differs more markedly from *myo*-inositol than isomytilitol, since it not only lacks the equatorial H at C-2, but also does not have the requisite 3 *cis* hydroxyls. Therefore although the transport and lipid incorporation systems are sufficiently unspecific to permit incorporation of the compound into the phospholipids, these are so different from *myo*-inositol containing phospholipids by the absence of the axial hydroxyl group that they do not interfere with normal membrane function. This explains the lack of growth-inhibition exibited by *scyllo*-inositol.

From these observations, one can conclude that, to be able to fulfil the biological functions, the phospholipids containing cyclitols must have a highly definite structure of the polyalcohol moiety, which is that of *myo*-inositol.

Résumé. Les relations entre la structure et la fonction des phosphoinositides a été précisée en comparant la spécificité de la perméabilité et de la biosynthèse des phospholipides chez *S. pombe* et *N. crassa*. Les exigences stériques de la position C-2 ont été définies.

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