Table IV. Purification of nucleoside analogues by column chromatography on ion-exchange resins

Compound	Resin type	Column dimensions	Elution with water at flow rate (ml/h)	Major fraction emerged between (ml)
III	DE 52	32 × 2.0	12	63– 85
IX (picrate) [a]	DE 52	15 × 1.0	15	70– 91
V	AG 1 × 8 (Formate) 200–400 mesh	58 × 3.5	33	116–164
VI	,,	19 × 1.5	48	246–317 [b]
VIII	,,	45 × 3.5	45	324–404
X	,,	16.5 × 3.0	33	146–252
XI	,,	32 × 3.5	33	175–294

[a] a crystalline picrate was formed by adding aqueous picric acid, this was collected, washed, dissolved in water and applied to the column; [b] washed initially with water (112 ml) then eluted with M-formic acid.

TON-MARSHALL test[6] indicative of aminoimidazoles. The compound quickly darkened in dilute acid to produce a black solid (mp > 350 °C) which did not move from the origin on t.l.c. plate and is presumably a polymer.

For in vitro studies nucleotides are often preferred to nucleosides but the acid sensitivity of compound (III) precluded phosphorylation directly. Therefore, the related methyl 2-altropyranoside derivative (V) was synthesized from methyl 2-amino-2-*deoxy*-D-altropyranoside[7] and imidate (IV). This glycoside was much more stable to acid and alkali and could be purified on an ion-exchange column (Table IV) and phosphorylated by the method of TENER[8]. Despite the fact that the 2′-and 4′-hydroxyl groups were not protected during the phosphorylation reaction the 6′-monophosphate (VI) was the only major product, after purification by ion-exchange chromatography it had a similar electrophoretic mobility to AMP and GMP, contained 1 mole equivalent of phosphate, consumed 1 mole equivalent of periodate[9], gave a purple colour in the BRATTON-MARSHALL test and showed the characteristic ultraviolet spectrum of an aminoimidazole.

A number of other 2′-substituted analogues were synthesized from the appropriate amino sugar. Both the mannose derivative (VII) and arabinose derivative (VIII) have a simliar stereochemistry to (II). The galactose compound (IX) which is related to the α-anomer[10] of (II) was also prepared. All these compounds mutarotate in water and are unstable in acid although they decompose at a slower rate than compound (III).

The method was also applied to the synthesis of 3′-(amino-imidazole carboxamide) derivatives from the methyl glycosides of 3-amino-3-*deoxy*-D-glucose and 3-amino-3-*deoxy*-D-altrose[11]; as expected, the products (X and XI) did not consume periodate but were otherwise similar to the isomeric 2′-examples (Tables II and III).

Experimental procedures. The 2-amino-2-*deoxy*-D-monosaccharide hydrochloride (270 mg) (or methyl glycoside, etc.) dissolved in a minimum volume of water was adjusted to pH 8.0 with solid potassium bicarbonate and then diluted to 8 ml with methanol. Ethyl *N*-[carbamoyl-(cyano)methyl] formimidate (350 mg) was added and the mixture kept at room temperature for 24 to 48 h. The mixture was evaporated to dryness in a rotary evaporator at 38 °C. The residue was dissolved in methanol and again evaporated then redissolved in water and purified by column chromatography (Table IV). In one case (compound VII) the product crystallized out when the reaction mixture was concentrated and chromatographic purification was unnecessary. Most of the methods used in characterising the products we have described elsewhere[4,10]. Electrophoresis was carried out on a Shandon flat bed apparatus at 10 °C using Whatman 3 mm paper at 2 volts per cm at *pH 1.85* [acetic acid (15)-formic acid (10)-water (255) (v/v)], *pH 9.4* [in NaH CO_3 (56.8) (N · Na_2CO_3 (14.4)-water (929) (v/v)], and *pH 9.1* (1% sodium tetraborate in water); products were detected using a) the BRATTON-MARSHALL spray reagents[12] and b) ammonium molybdate spray reagents for phosphate[13].

Zusammenfassung. Es wird eine Reihe von Imidazolglykosiden und deren Synthese beschrieben. Diese eignen sich als Analoga der natürlichen Nukleoside für biologische Versuche.

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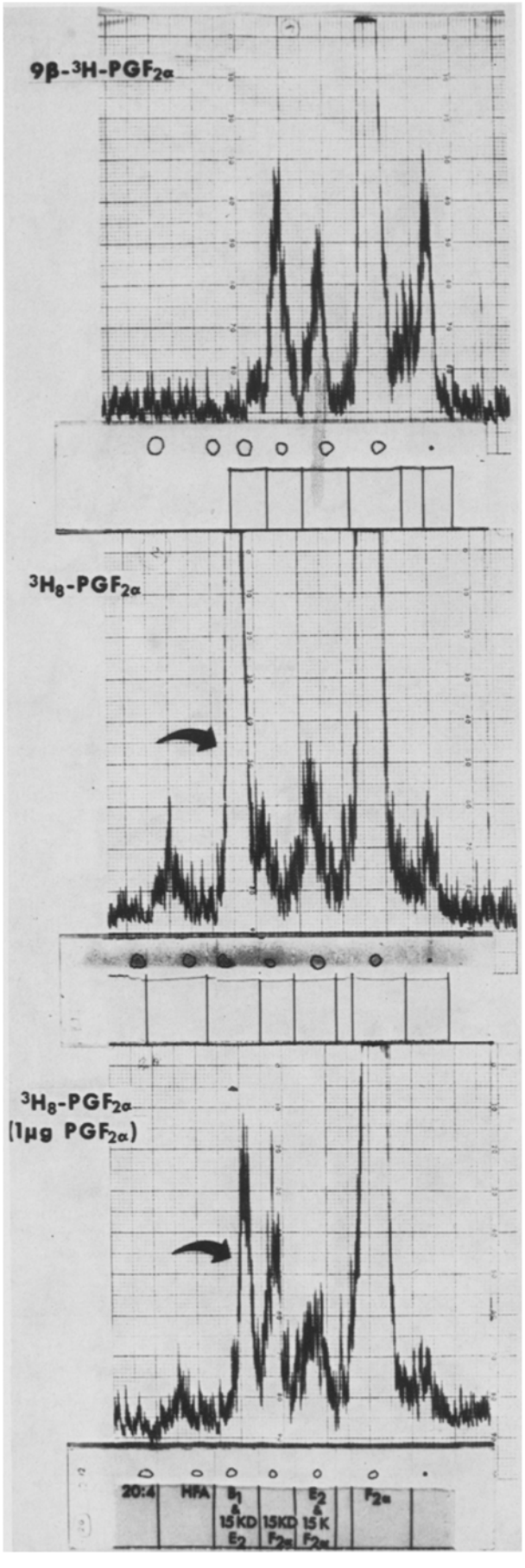
Prostaglandins During Development. II. Identification of Prostaglandin 9-Hydroxy Dehydrogenase Activity in Adult Rat Kidney Homogenates

An earlier investigation of the major prostaglandin-inactivating enzymes, prostaglandin 15-hydroxy dehydrogenase (15-PGDH) and Δ-13 reductase (13-PGR), in rat lungs revealed age-related changes in the activity of these enzymes. The high 13-PGR activity observed in foetal and early postnatal vs. adult rats suggested to us that an efficient inactivation of the prostaglandins and primary metabolites might constitute an important feature of cell



function in the developing animal[1]. While screening various rat tissues for their PG-metabolizing activities during ontogeny, we discovered a novel enzyme, 9-hydroxyprostaglandin dehydrogenase, in adult rat kidneys capable of oxidizing the 9-hydroxyl group of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) into an E_2-type catabolite, viz 15-keto-13, 14-dihydroprostaglandin E_2 (15KD-PGE_2). This enzyme appears to be characteristic of adult kidneys since it is absent in tissues derived from early newborn rats[2].

Materials and methods. Wistar rats (male, 250–300 g) were killed by a blow to the neck and decapitated. Kidneys (approx. 1.40–1.50 g each) were removed, isolated from capsular tissue and fat, cut open and washed thoroughly with ice-cold 0.05 *M* KH_2PO_4-NaOH (pH 7.4). Kidneys from 6-day-old rats were removed from decapitated rats. Tissue was homogenized in buffer (20 vols) and aliquots (0.5 ml) were incubated with $PGF_{2\alpha}$ (9β-$^3H_1$-$PGF_{2\alpha}$, S.A. 7.5 Ci/mmole or $^3H_8$-$PGF_{2\alpha}$, S.A. 120 Ci/mmole; 1×10^6 cpm for each experiment) in the presence of 4 m*M* NAD for variable times (see Table) at 30 °C. Incubations were terminated by the addition of 5 vols of dry ethanol and after centrifugation of the protein precipitate, the supernatant was taken to dryness in vacuo. Aliquots of the residue were assayed for total radioactivity in a Beckman LS-255 scintillation counter and for radioactive products by radio thin layer chromatography as reported previously[1].

Results. Data from 2 sets of experiments are presented in the Table showing a time-dependent loss of total radioactivity when 9β-$^3H_1$-$PGF_{2\alpha}$ is incubated with a homogenate of adult rat kidneys. In the second column is shown data obtained from parallel experiments with newborn rats in which no losses are encountered. Since tritium label is present in only the 9-position of the $PGF_{2\alpha}$ used in these studies, we thought that a specific enzymatic removal of the 9β-tritium atom had likely taken place during incubation with adult kidney homogenates especially since no losses were encountered in parallel incubations with newborn rat kidneys. To confirm this possibility and to determine the type of product resulting from oxidation of the 9-hydroxyl group in these studies we performed additional experiments with $PGF_{2\alpha}$ containing tritium atoms at positions 5, 6, 8 9, 11, 12, 14, 15 thereby permitting measurement by radio thin layer chromatography of the product resulting from a 9β-tritium loss. A radio thin layer chromatogram of the products obtained after incubating 9β-$^3H_1$-$PGF_{2\alpha}$ and $^3H_8$-$PGF_{2\alpha}$ for 10 min at 30 °C with an adult rat kidney homogenate is shown in the Figure. Radioactive peaks due to 15-keto-$PGF_{2\alpha}$ and 15-keto-13, 14-dihydro-$PGF_{2\alpha}$ can be seen (upper scan). However, when $^3H_8$-$PGF_{2\alpha}$ is used as substrate (middle and lower scans) an additional peak, not seen when 9β-$^3H_1$-$PGF_{2\alpha}$ is used, appeared (see arrow in Figure).

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Radio thin layer chromatogram of products derived from incubation of 9β-$^3H_1$-$PGF_{2\alpha}$ (upper scan) and $^3H_8$-$PGF_{2\alpha}$ (middle and lower scan) with adult rat kidney homogenates for 10 min at 30 °C in presence of 4 m*M* NAD. 20:4, arachidonic acid; HFA, hydroxy fatty acid; B_1, PGB_1; 15KD-E_2, 15-keto-13,14-dihydro-PGE_2; 15KD-$F_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$; E_2, PGE_2; 15K-$F_{2\alpha}$, 15-keto-$PGF_{2\alpha}$; $F_{2\alpha}$, $PGF_{2\alpha}$; origin at dot.

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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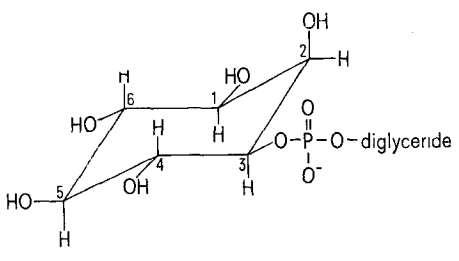
Structure-Function Relationships in Phosphoinositides

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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