process[17]. We now report the adverse effect of fluoride on in vivo nitrate reduction in rice leaves, caused by inhibition of NADH supply.

Methods. Rice seedlings (variety CV P-2-21) were grown in pots under sunlight. Seedlings were periodically irrigated with 15 mM KNO_3. The method of Sawhney et al.[18] was followed for the determination of in vivo nitrate reductase activity. Sodium fluoride was added to the assay mixture as indicated in the table. In vitro nitrate reductase activity in leaf extracts was measured in the presence of excess NADH as described[19].

Results and discussion. Sodium fluoride even at 100 mM concentration had no effect on the in vitro nitrate reductase activity of the rice leaf extract. In vitro nitrate reductase activity of soybean leaf extract was also found to be unaffected by fluoride[20]. However, in vivo activity was strongly inhibited as shown in the table. The extent of inhibition was proportional to the fluoride concentration used, and at 100 mM as much as 90% inhibition in 1 h was observed. Excess exogenous NADH is supplied in the in vitro assay, while the in vivo assay depends on endogenous generation of NADH within the plant tissue. Since fluoride has no effect on the nitrate reductase enzyme as such, it is clear that NADH supply is blocked by fluoride.

Klepper et al.[21] proposed that glycolytic triose-phosphate dehydrogenase is an important source of NADH for in vivo nitrate reduction. This enzyme is not affected by fluoride, which inhibits glycolysis at enolase step[12]. It has also been suggested that citric acid cycle dehydrogenases provide NADH for in vivo nitrate reduction[18,22]. Fluoride has been shown to inhibit some citric acid cycle enzymes[14,15]. Fluoride has also been found to disrupt mitochondrial membranes[15,23-25], the structural integrity of which is probably essential for in vivo NADH generation and hence nitrate reduction[26].

Thus fluoride seems to inhibit in vivo nitrate reduction indirectly, by blocking the physiological NADH supply.

Effect of fluoride on in vivo and in vitro activities of nitrate reductase in rice leaves

Sodium-fluoride (mM)	In vivo activity: µmoles NO_2^- formed per g tissue in 30 min	60 min	In vitro activity: µmoles of NO_2^- formed/h/g tissue
0	2.9	5.8	4.2
10	1.7	2.2	4.4
50	0.9	1.1	4.3
100	0.7	0.7	4.4

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Age-related differences in the urinary excretion of prostaglandin $F_{2\alpha}$ catabolites in the rat

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Summary. Tritium-labelled $PGF_{2\alpha}$ was administered i.v. into rats of varying ages (2, 4, 6 weeks and adult). Urine was collected and assayed for radioactive products by thin-layer-chromatography. Results showed a distinctly different urinary profile between the 2-week-old and the adult rat. While the urinary pattern from the 2-week-old rat gave a single less polar product than $PGF_{2\alpha}$, the pattern from the adult rat gave products more polar than $PGF_{2\alpha}$. Urine from the 4- and 6-week-old rats gave a mixture of these types of products. These results indicate that some prostaglandin catabolic pathway (likely the ω-oxidative system) is activated in vivo within the 4-6-week postnatal period in the rat.

Several years ago we embarked on a program to investigate whether the activity of the prostaglandin system (biosynthesis and catabolism) was affected by the developmental status of the animal. While we could find little change in the biosynthetic system, great differences in the activity of the various prostaglandin catabolic enzymes were observed[3]. Although all these studies were carried out in vitro with homogenates of tissues taken from animals (rat, lamb) of various ages (fetal, neonatal and adult) we speculated that the changes in enzyme activity reflected changes in enzyme content since the prostaglandin catabolic enzymes are short lived[4].

In order to investigate whether the previously observed in vitro differences in prostaglandin catabolic activity (type and capacity) are evident in vivo we infused tritium-labelled $PGF_{2\alpha}$ into rats of varying ages and analyzed the

tritiated urinary metabolites excreted. This report deals with the results of that study.

Materials and methods. Male Wistar rats (2, 4, 6 weeks and adult) were anaesthetized with Inactin (100 mg/kg). The trachea was cannulated to facilitate breathing and polyethylene cannulae (PE-10 or PE-50 depending on the size of the rat) were inserted into the jugular vein and bladder. Prostaglandin $F_{2\alpha}$ (NEN, sp. act. 11.1 Ci/mmole) was diluted with authentic unlabelled product (gift of Drs U. Axen and J.E. Pike, the Upjohn Co., Kalamazoo) to a sp. act. of 220,000 cpm/µg and injected in 200 µl saline at a dose of 0.17 µg/g b.wt. After the bolus injection of the labelled product normal saline (200 µl) was given by the same route. Urine was collected every h for 4 h. Workup consisted of dilution of urine with methanol (5 vol.) and evaporation to complete dryness in vacuo. A small aliquot was assayed by TLC (Silica gel G: chloroform-methanol-acetic acid-water 90/9/1/0.65 v/v) which directly revealed the effectiveness of catabolism as well as the type of catabolites formed. In some experiments, the crude urinary extract was diluted with water, acidified to pH 3 with N HCl and applied to a column of XAD-2 resin (Rohm and Hass). After loading the column with sample, the column was subsequently eluted with 5 vol. of water, then 2 vol. of acetone. The latter fraction was evaporated to complete dryness and methylated with diazomethane in diethyl ether. The methyl esters were subsequently converted into trimethylsilyl ethers with TRI SIL Z (Pierce Chemical Co.) and analyzed by radio gas chromatography. For this purpose a Hewlett-Packard Model 5700A series was used in which the effluent from the column was split between a flame ionization detector and a Packard Model 891 proportional counter (ratio 1:5).

Results. The urinary pattern of labelled metabolites found after i.v. infusion of tritiated $PGF_{2\alpha}$ into rats aged 2, 4, 6 weeks and adult is shown in the thin layer chromatograms in figure 1. A distinctly different pattern occurs between the products excreted by the 2-week-old rat and the adult. Based on reference standards, the less polar product observed in the 2-week-old rat is likely a product of 15-hydroxydehydrogenase and Δ-13-reductase. These enzymes are known to be abundantly present in the lungs and are responsible for the inactivation of $PGF_{2\alpha}$ which might enter the circulation[5,6]. The radioactive peak migrating more polar than $PGF_{2\alpha}$ excreted by the adult is a mixture of products resulting from additional β and ω-oxidation reactions. This is likely composed of the mixture of products reported earlier including the major urinary product observed in the human, i.e. 5α,7α-dihydroxy-11-keto-tetranorprostane-1,16-dioic acid[7-12]. In order to obtain more information on the structures of these products, we analyzed the samples by radio gas chromatography. The radioactive patterns obtained are shown in figure 2. In confirmation of our TLC data, the urine from the 2-week-

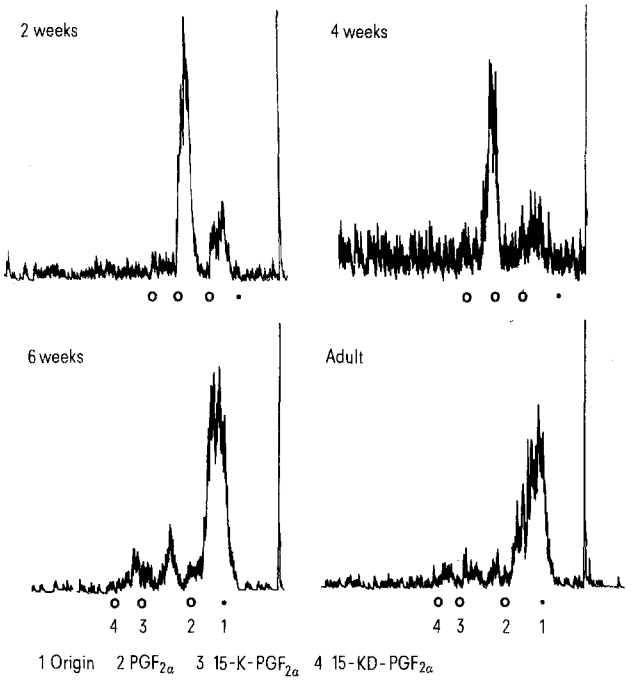

Fig. 1. Thin layer radioactivity profiles of methanol extracts of urine collected from rats at 2, 4, 6 weeks of age and adult into which was injected tritium-labelled $PGF_{2\alpha}$ i.v. Migration of standards shown are: 4 = 15-keto-13,14-dihydro $PGF_{2\alpha}$, 3 = 15-keto $PGF_{2\alpha}$, 2 = $PGF_{2\alpha}$. Samples were spotted at 1.

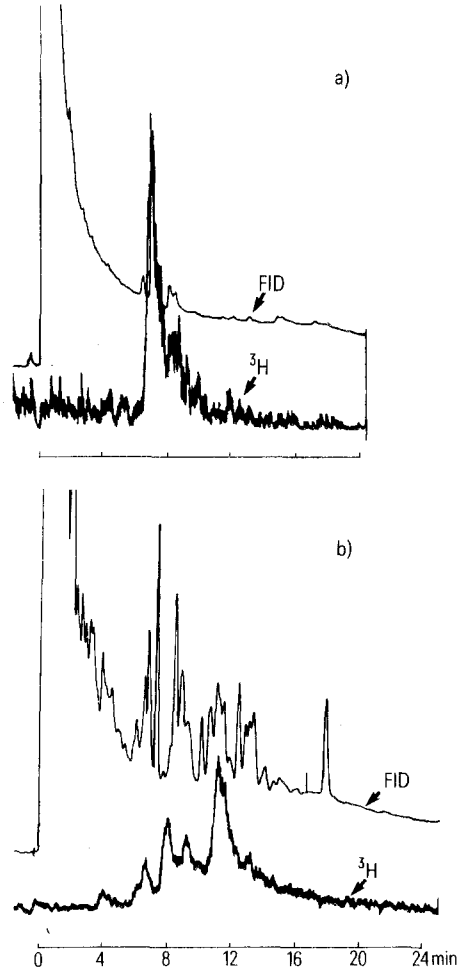

Fig. 2. Gas-liquid radioactivity profiles of urinary extracts from a 2-week-old (A) and adult (B) rat. The adult urine was only purified on an XAD-2 column while that from the 2-week-old rat was additionally subjected to high pressure liquid chromatography from which the only radioactive product present was collected. Samples were analyzed as methyl esters and trimethyl silyl ethers on a column packed with 3% SE-30 on Gas Chrom Q. The column temperature was set at 200 °C for sample injection and programmed at 4 °C/min up to 280 °C. The radioactivity detector was operated at a setting of 1 K in A and 10 K in B and a time constant of 10.

old rat contained essentially 1 radioactive product (figure 2, A) while a considerable mixture of products occurred in the adult (figure 2, B). In order to determine whether the product obtained from the 2-week-old rats was an intermediate in the metabolic sequence found in the adult, it was purified and injected into an adult rat. The resulting urine contained tritium-labelled products identical to those observed from $PGF_{2\alpha}$ indicating that the urinary metabolite from the 2-week-old rat is a normal intermediate in the adult pathway and that its accumulation in the neonate is a result of the absence of certain enzymes in the newborn rat or enzymes which have not yet become active at 2–4 weeks of age.

Our findings reveal that some enzyme pathways in the prostaglandin catabolic sequence are age-dependant in vivo and that although certain enzymes are active at an early age (fetal or early neonatal) others in the catabolic sequence (likely the ω-oxidative system) are induced at a later stage. In the rat these latter enzymes become active around 4–6 weeks postnatally.

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Synthesis of L-[3-^{2}H,^{18}O]glycerol and its incorporation into the 4-methyl-5-hydroxyethyl thiazole moiety of thiamine by *Escherichia coli*

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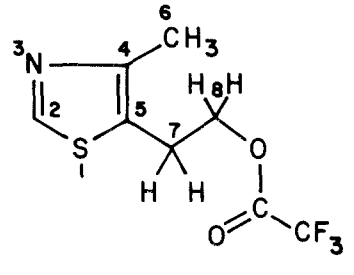
Summary. L-[3-^{2}H,^{18}O]glycerol was prepared and fed to *Escherichia coli* in order to determine the origin of the oxygen atom in the biosynthesized thiazole moiety of thiamine. Measurement by GC-MS of the isotope incorporation into the thiazole from this substrate confirmed that the 2 hydrogens and the oxygen on the C-3 carbon of glycerol are incorporated directly into the thiazole.

Recent work has established that the 5 contiguous carbon atoms in the 4-methyl-5-hydroxyethyl thiazole moiety of thiamine are derived in *E. coli* from pyruvate and a 3-carbon unit originating from glucose[1]. Pyruvate was shown to provide the C-4 and 6 carbons of the thiazole and the C-4, 5 and 6 carbons of glucose were shown to provide the C-5, 7, and 8 carbons of the thiazole, respectively. It was proposed that the 3-carbon unit was incorporated via a triose-P during the biosynthesis. If this theory is correct, then the oxygen atom of the thiazole would also have its origin from a triose-P. This paper reports the further confirmation of this theory by the observation of the incorporation of ^{18}O from stereospecifically labeled glycerol into the thiazole. Furthermore, by using glycerol labeled at C-3 with both ^{2}H and ^{18}O, it was shown that both hydrogens and the oxygen on the C-3 of the glycerol are incorporated into the thiazole with no exchange.

Materials and methods. E. coli B was grown on 100 ml of a defined medium containing 400 mg of labeled glycerol and casamino acids as previously described[2]. The labeled glycerol was prepared as follows: 1,2-O-isopropylidene-D-glyceraldehyde[3] (340 mg) was dissolved in 3.2 ml of tetrahydrofuran containing 200 µl of $H_2{}^{18}O$ (90 atom% ^{18}O) and 120 µl of piperidine. [Under these conditions the aldehyde oxygen was found to exchange with the labeled water with a half life of ~2.3 min at 24 °C. In contrast to this rapid oxygen exchange, no change was observed in the optical rotation of control solutions (using nonlabeled water) for periods up to 6 h, the longest tested. This indicates that epimerization at C-2 of the glyceraldehyde did not occur under these reaction conditions.] After 2 h at 24 °C, 60 mg of sodium borodeuteride (98 atom% ^{2}H) was added to the reaction mixture with constant stirring. After 3 h, 3 ml of saturated sodium bicarbonate solution was added and the resulting solution extracted 2 times with methylene chloride. Evaporation of the combined extracts gave 300 mg of crude 1,2-O-isopropylidene-D-[3-^{2}H,^{18}O]glycerol which was purified by column chromatography to give a pure oil. Mass spectral analysis of this product showed 1.3% of the molecules had no label, 22% had 1 ^{2}H, 3.2% had 1 ^{18}O and 73.3% had both an ^{18}O and ^{2}H. This material was subsequently deprotected with dilute acid and the resulting free glycerol was mixed with nonlabeled glycerol. The final glycerol had 4.1% of its molecules with 1 ^{2}H, 0.61% with 1 ^{18}O and 14.1% with both an ^{18}O and ^{2}H.

This labeled glycerol was subsequently fed to *E. coli* and the isotopes' incorporation into the thiazole and the glycerol-P in the cells were measured by GC-MS[1].

Results and discussion. The isolated thiazole, as the trifluoroacetate ester, showed the following isotopic distribution as measured from the molecular ion at m/e 239: 79.2% no label, 11.4% 1 ^{2}H, 1.4% 2 ^{2}H or 1 ^{18}O, 6.9% 1 ^{2}H and 1 ^{18}O and 0.95% 2 ^{2}H and 1 ^{18}O. The m/e 112 ion in the MS of



4-methyl-5-hydroxymethyl thiazole trifluoroacetate.