at 15,000 × g for 10 min at 4 °C and this process repeated times 3. The pellet was recovered and incubated in a total volume of about 8 ml of Na_2HPO_4 (0.083 M), pH 7.5 for 20 min at 26 °C to release CK from the mitochondria. Following centrifugation (50,000 × g) the supernatant was recovered and applied to sephacryl S-200 column (1.5 × 90 cm, Pharmacia) with a bed volume of 159 ml and a flow rate of 30–34 ml/h. Elution was performed with Tris barbital (0.050 M, pH 7.8) containing mercaptoethanol (0.005 M) in fractions of 2 ml. The fractions containing activity were further fractionated on CM sephadex cation exchange column using a Glenco column (2.6 × 30 cm) with a bed volume of about 40 ml and a flow rate of 1 ml/min. Elution was performed with Tris barbital (0.050 M), pH 7.8) containing mercaptoethanol (0.005 M) in fractions of 2 ml. 2 peaks of activity were observed with the initial peak being MM and the 2nd peak mitochondrial creatine kinase.

Results and discussion. The final fraction from CM-sephadex was shown by electrophoresis on cellulose acetate to exhibit a single band which was positively charged and free of MM creatine kinase (figure 1). On SDS-polyacrylamide gels, a single protein band was observed with a mol.wt of about 44,000 (figure 2). On a G-200 sephadex column, the mol.wt was estimated to be 84,000 (figure 3), thus mitochondrial creatine kinase is a dimer composed of 2 monomers of identical molecular weight. Repeated freezing and thawing (× 6) of solutions of MM and BB creatine kinase consistently exhibited the hybrid form MB (figure 4), however silimar experiments performed between mitochondrial creatine kinase and that of BB or MM consistently exhibited no hybrid form (figure 5).

Antiserum developed to mitochondrial creatine kinase exhibited binding to ^{125}I-mitochondrial creatine kinase which was dependent on the antibody concentration but exhibited no binding to MM or BB creatine kinase (figure 6). Antiserum to MM and BB creatine kinase exhibited no binding to ^{125}I-mitochondrial creatine kinase (figure 7).

Thus, in conclusion mitochondrial creatine kinase does not hybridize with B or M subunits and exhibits biochemical and immunological properties not shared by that of the B or M subunits. Whether it is synthesized in the mitochondria by its own DNA or transported following synthesis in the nucleus is unknown.

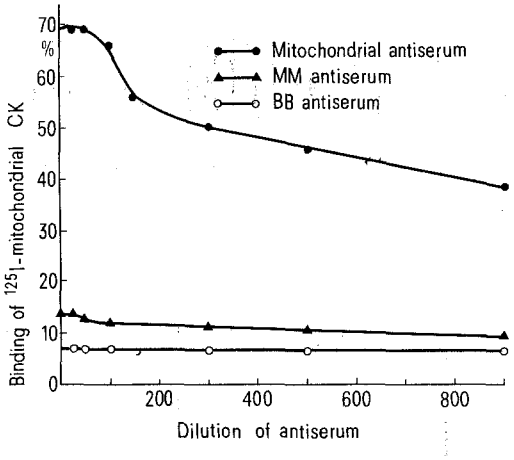

Fig. 7. Shown here is further evidence for the specificity for mitochondrial antiserum. The upper curve shows the binding curve between ^{125}I-mitochondrial creatine kinase and that of mitochondrial antiserum. However, antiserum to MM or BB exhibited no binding to mitochondrial creatine kinase indicating complete lack of cross-reactivity.

1 Supported in part by the Special Center of Research in Ischemic Heart Disease (1 P17 HL 17646).
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Effect of fluoride on in vivo nitrate reduction in rice leaves (*Oryza sativa* L.)

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Summary. Fluoride had no effect on in vitro nitrate reductase activity in rice leaves, but in vivo activity was strongly inhibited. It is suggested that fluoride brings about this inhibition by adversely affecting the physiological NADH generating system required for in vivo nitrate reduction.

Fluoride has long been known to be injurious to plants. Large scale damage to vegetation in some areas by industrial waste has been shown to be due to accumulation of fluoride[2]. Effects of fluoride on plants are diverse. Visible symptoms of fluoride injury like retarded growth, necrosis and chlorosis of tissues are preceeded by pronounced biochemical changes. The effects of fluoride on protein and nucleic acid metabolism[3,4], respiration[5–7] and photosynthesis[8,9] have been reported. At the enzyme level, a number of enzymes including ATPase[10], hexokinase[11], enolase[12], phosphoglucomutase[13], succinic and malic dehydrogenases[14,15] are inhibited by fluoride, while other enzymes such as glucose-6-phosphate dehydrogenase, catalase, peroxidase and cytochrome oxidase are stimulated[16]. Very little information is, however, available on the effect of fluoride on nitrate assimilation in plants. Nitrate reductase, an enzyme which catalyses the first step in nitrate assimilation is considered to be the rate limiting step in the

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

1 Acknowledgments. We thank Dr M.S. Naik for useful suggestions and discussion. C.S.R. is grateful to Indian Council of Agricultural Research for a Senior Research Fellowship.

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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