

GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND PYRUVATE KINASE ACTIVITIES IN RABBIT ERYTHROCYTE FOLLOWING FLUORIDE INGESTION

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It is well recognized that fluoride ions "activate" as well as "inhibit" enzyme function. Fluoride ions activate adenyl cyclase both *in vitro* and *in vivo* conditions (1–3). Fluoride has also been recognized as an inhibitor for carbohydrate metabolism in *in vitro* and in intact cells (4). Altered G-6-P dehydrogenase activity in liver and uterus after fluoride ingestion has been reported (5, 6). Changes in serum enzyme levels due to fluoride ingestion have also been reported (7, 8). Excessive ingestion of fluoride is known to raise circulating levels of fluoride in rabbits as well in human subjects afflicted with fluorosis a disease caused by excessive ingestion of fluoride (9, 10). The present report reveals the effect of fluoride ingestion on carbohydrates metabolism in rabbit erythrocytes. The erythrocyte membrane is known to lodge important and significant carbohydrate moieties and associated enzymes. The objective of the present investigation was to explore the influence of fluoride on G-6-P dehydrogenase and pyruvate kinase activities of rabbit erythrocytes.

Materials and Methods: Adult, healthy female rabbits maintained on standard diet and water *ad libitum*, were given daily 10 mg sodium fluoride/kg body weight through the intragastric route for a period of 6 months. Control rabbits maintained under similar laboratory conditions were not given sodium fluoride. Blood was drawn in heparinized tubes from the ear vein of the rabbit at intervals of 3 and 6 months. Blood was stabilized and plasma was separated. The packed cell volume was washed with 0.9% saline and the buffy coat was removed and samples rewashed. By repeated washing and centrifugation at 0–4 °C the leucocytes and platelets were estimated and the erythrocytes thus obtained were used for enzyme assays.

Assay of G-6-P dehydrogenase activity: The erythrocytes were suspended in 30 volumes of hemolyzing solution. The hemolyzing solution consisted of 10 μ NADP; 7 mM β -mercaptoethanol and 2.7 mM EDTA (sodium, pH 7.0). The erythrocyte suspension and hemolyzing solution were mixed well over a period of 10 min and centrifuged at 2000 g for 30 minutes at 0–4 °C. The enzyme G-6-P dehydrogenase activity of the hemolysate was assayed according to the method reported in the WHO technical report (11) adopting a 3 cuvette system in which the first cuvette contains the blank, the second cuvette phosphogluconic acid and the third one contains both phosphogluconic acid and G-6-P as substrates. The final concentration of the reaction mixture was as follows: NADP 0.2 mM; 0.1 M Tris HCl pH 8.0; MgCl₂ 0.01M; G-6-P 0.6 mM; 6 phosphogluconic acid 0.6 mM. The reaction was started by adding a known volume of the hemolysate to the reaction mixture and the rate of the reaction was recorded at 340 μ . The results are expressed as IU/g hemoglobin.

Assay of pyruvate kinase activity: The erythrocytes were suspended in approximately 10 volumes of hemolysing solution prepared by mixing 0.05 ml of β -mercaptoethanol and 10 ml of neutralized 0.27 M EDTA to a total volume of one litre. The assay was carried out by the method of Bucher and Pfeideter (12). The assay mixture was contained in a final volume of 1 ml comprising of 0.2 M/litre of Tris HCl pH 8.2 at 25 °C, 65 mmol/litre KCl; 20 mmol/litre MgSO₄; 0.1 mg lactate dehydrogenase; 0.1 mmol/litre NADH and 2 mmol/litre of ADP. Phosphoenolpyruvate was in the range of 0.1–10 mmol/litre. The activity of the enzyme pyruvate kinase in hemolysate was assessed by the addition of 0.1 ml ADP solution (final concentration 3mM) 5 min after the addition of lactic dehydrogenase. Optical density was recorded at 366 m μ . The readings were taken after 1, 2, 3 and 4 min. The mean of the extinction changes/min was calculated. Results were expressed as units/g hemoglobin.

Hemoglobin content of the hemolysate was measured using the Drabkin's method using the Sigma Kit no. 525 (Colorimetric). Plasma fluoride content was estimated by the method of Hall *et al.* (13). The statistical significance of the data was evaluated by Student's *t* test.

Effect of sodium fluoride ingestion on G-6-P dehydrogenase and pyruvate kinase activities of rabbit erythrocytes (means \pm SD, n = 5)

	G-6-P dehydrogenase IU \times 1000/g Hb	Pyruvate kinase units \times 100/g Hb	Fluoride (ppm/g% Hb of hemolysate)
Normal	6.0 \pm 0.21	6.52 \pm 0.57	0.0062 \pm 0.004
3 months treated animals	5.12 \pm 1.27*	5.8 \pm 0.48*	0.081 \pm 0.013
6 months treated animals	4.3 \pm 0.62*	5.1 \pm 0.08*	0.082 \pm 0.013

*Significant difference (P < 0.05).

Results and discussion: The table reports the data on G-6-P dehydrogenase and pyruvate kinase activities of rabbit erythrocytes before and after sodium fluoride ingestion. The circulating levels of fluoride are also reported. It is evident from the data that due to fluoride ingestion, there is a reduction in the enzyme activity. The enzyme activity is much more reduced in 6 months-treated animals than three months-treated animals. This report provides evidence on the inhibitory effect of sodium fluoride. The results obtained in the present study on G-6-P dehydrogenase activity are consistent with the observations reported by Carlson and Suttie (5) revealing a decrease in enzyme level in the liver of fluoride fed rats. But contrary to their report suggesting that the changes in enzyme levels are directly related to diet and reduced dietary

intake, our data (unpublished results) has shown an increase in body weight of rabbits after fluoride ingestion and it is unlikely that diet and dietary intake have an influence on the enzyme activity.

The present report also describes the influence of fluoride upon erythrocyte pyruvate kinase activity. Pyruvate kinase activity has also been reduced by fluoride ions and is directly proportional to the concentration of fluoride in circulation. From an earlier report it is evident that fluoride ions inhibit metalloenzymes (14). Pyruvate kinase being a metalloenzyme, the reduction in enzymic activity is likely due to the inhibition by fluoride ions.

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