QUANTITATIVE RELATIONSHIPS BETWEEN GLUCOSE REABSORPTION AND MUTAROTATION BY DOG KIDNEY IN VIVO[1]

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SUMMARY

When α or β glucose are infused into the renal artery of dogs the anomeric composition of the glucose found in urine due to exceeding the renal threshold is not substantially different from that of the glucose which entered the kidney. Glucose found in the renal vein blood plasma, however, has been subjected to catalyzed mutarotation. The data are consistent quantitatively with the proposal that mutarotase (aldose 1-epimerase) action is involved in the reabsorption of glucose.

INTRODUCTION

There have been many theories advanced for the mechanism by which glucose is reabsorbed by kidney. While the kidney possesses many enzymes, none of these have definitely been shown to be involved in reabsorption of glucose.

Mutarotase catalyzes the interconversion of the anomers of various sugars (1). The presence of mutarotase in various animal tissues was reported in 1954 and a unitary theory of glucose transport involving this enzyme was postulated (2). Evidence has been presented consistent with the control of the limiting rate of carbohydrate metabolism and transport by mutarotase (3,4,5).

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The quantity of mutarotase in kidneys and intestines and other tissues has been shown to be proportional to their rate of glucose transport (kidney and intestine) or glycolysis (other tissues; brain, retina, etc.). The Km's and inhibitor constants (K_i's) for various mutarotases from kidneys and intestines of various species of animals have been shown to be concordant with a substantial body of previously reported data on the transport of sugar and the inhibition of this process. Phlorizin was found to inhibit mutarotase of kidney and intestine in a competitive manner (5). The K_i's for phlorizin for various sugars have been shown to be in accordance with the apparent K_i's for the corresponding *in vivo* transport processes.

This report describes experiments which indicate that concurrently with the reabsorption of glucose, catalyzed mutarotation of glucose does occur. Furthermore, it indicates that glucose which appears in the urine due to exceeding the renal threshold was not appreciably mutarotated.

MATERIALS AND METHODS

Creatinine-HCl was from Eastman, p-amino hippuric acid (PAH), 2g/10ml ampoules from Merck, Sharpe and Dohme, α and β glucose were gifts from Corn Products Co., Argo, Illinois.

Infusion solution: One liter normal saline (Abbott Laboratories) containing 5g creatinine-HCl, 3ml PAH solution and 0.5g of ammonium chloride.

Anomeric glucose solutions: 20g of α or β glucose were dissolved in ice cold water (100ml) with vigorous stirring. The fresh solutions were kept cold during the infusion into the renal artery. The anomeric composition of the α and β glucose were determined by a glucose oxidase method (6) and by gas chromatography of trimethyl silane derivatives (7). The original α glucose was found to be 95% α and 5% β. The β glucose was found to be 99% β and 1% α. Determinations were carried out immediately after the solutions were prepared.

Female dogs were anesthetized with pentobarbital and the left renal

vein and renal artery were exposed through a small lumbar incision. The ureters were ligated through a small midline incision and separately cannulated for collection of urine. The animal was prepared for kidney studies by infusion with PAH and creatinine and ammonium chloride via femoral vein. Fresh cold solutions of α or β glucose were injected into the renal artery through a 25 G needle employing a calibrated Sage pump. The renal plasma flow, plasma glucose concentration, and filtration rate were determined at various times before, during and after the sugar infusion. Samples of renal arterial blood, renal vein blood and urine were taken at various times during the experiments. Two milliliters of blood were collected in a tube containing 0.2 ml of an acetate buffer (acetate/acetic acid - 1, pH 4.8, 0.2M). The tubes were kept in an ice bath and centrifuged at 0^{o}, and the plasma samples obtained were immediately analyzed for α glucose (6). Another aliquot of the blood was collected in a siliconized heparinized tube, centrifuged and the plasma analyzed for total glucose (8) according to the glucose oxidase, peroxidase, o-dianisidine method (see for example the similar method described in Bergmeyer, H. U., Ed., "Methods of Enzymatic Analysis", Academic Press, New York, N.Y., 1963, p. 123). Standard methods were used to determine creatinine (9) and PAH (10).

The renal plasma flow was calculated from the amount of PAH found in the urine, and the difference between arterial and venous PAH concentrations. The rate and concentration of the anomer being injected into the renal artery was calculated from the calibrated pump flow rate and the concentration and composition of the anomer solution. The anomeric composition of the plasma entering the kidney was calculated from the rate of infusion into the renal artery, the renal blood flow, the determined amount of glucose in each and their anomeric composition. Complete mixing of the infused glucose solution and the renal artery blood flow was assumed.

RESULTS AND DISCUSSION

Figure 1 shows graphically the percent α glucose for: the arterial blood being infused into the kidney, the venous blood leaving the kidney

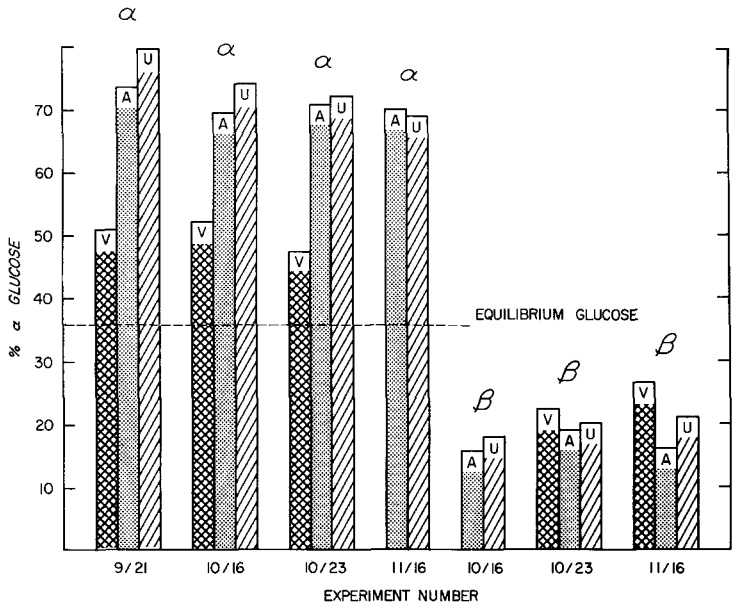

Figure 1: Percent α glucose in urine, renal artery and renal vein, during infusion of α or β glucose (as indicated) into renal artery.

and that appearing in the urine from exceeding the renal threshold during the infusion of either α or β glucose. Glucose appeared in the urine in about 90 seconds after the start of glucose infusion. The glucose anomeric composition found in the urine was similar to that infused, indicating that glucose which was excreted was not mutarotated, while the glucose appearing in the renal vein had been mutarotated. The data in Table I shows that when β glucose was infused a change in anomeric composition had occurred in the glucose appearing in the urine, compared to the original arterial glucose composition. However, the venous glucose shows a greater mutarotation. The percent errors are expected to be larger for β infusion since a change could only occur from 16.7% arterial α glucose to the maximum of 36% α glucose at equilibrium. When α glucose was infused, the urine anomeric glucose composition differed only by 3.4% from the arterial composition, while the venous differed by 46.3%. These findings are consistent with the enzyme mutarotase being involved in the reabsorption of glucose, since as predicted, the glucose appearing in the urine is largely unmutarotated while that found in the renal vein was mutarotated.

TABLE I

% α Glucose in Urine, and Renal Arterial and Venous Plasma During Infusion of Glucose Anomers into Renal Artery of Dogs

Anomer of Glucose Infused	% α Glucose[1]		
	Artery	Urine	Vein
α	71.4 ± 1.4 (4)	73.8 ± 3.4 (4)	50.5 ± 1.6 (3)
β	16.7 ± 1.6 (3)	19.8 ± 0.9 (3)	24.4 ± 2.1 (2)

[1] ± average deviations. Figures in ( ) refer to number of dogs used.

Glucose at equilibrium is comprised of 36% α glucose and 64% β glucose.

The fact that venous plasma is not at mutarotational equilibrium (see figure 1) was expected since the glucose in the renal vein is derived from both the perfused and reabsorbed glucose. Our results clearly show that mutarotation of glucose occurs during passage through the kidney.

A mechanism which involved phosphorylation and dephosphorylation conceivably could lead to a change in the anomeric composition of glucose. The work of Crane has shown that 6-deoxyglucose and glucose share the same intestinal transport mechanism (14). His findings are also concordant with the kinetic data obtained in inhibition studies with mutarotase and these sugars (5) Because the number 6 position is blocked in 6-deoxyglucose, Crane and Krane (15) have ruled out phosphorylation as being involved in the transport of this sugar.

The postulate that the flow of glomerular filtrate carries glucose to the mutarotase locus in the tubule, seems to be the simplest explanation of our findings with respect to the anomeric composition of urinary glucose and that of renal vein plasma. These findings are consistent with those of Hill et al. (11) that the anomer infused in excess is excreted in excess. This is expected on the basis that mutarotase is involved in glucose reabsorption and not in excretion. This laboratory has previously

reported that mutarotase distribution in kidney is consistent with the distribution of the proximal tubules (12). Bailey et al. (13) state that their results for kidney, "confirm those of Keston and are not inconsistent with a concentration of mutarotase in kidney tubules. The cellular distribution remains to be determined."

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