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Kidney Enzymes of Gluconeogenesis, Glycogenesis, Glycolysis and Direct Oxidation.* (27018)

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Studies of the past two decades demonstrated that the kidney carries out manifold metabolic functions of primary physiologic importance in addition to urine production. The various metabolic contributions of the kidney have been reviewed(1). One of the striking properties of kidney, shared only by liver, is its capacity to produce sugar. Earlier studies involving glucose measurement in arterial and renal vein and recent work utilizing kidney slice incubation techniques to demonstrate net synthesis of carbohydrate were discussed(1,2). More recent investigations on the alternate pathways of glucose metabolism of kidney employed labeled glucose or pyruvate and conclusions were reached on the behavior of metabolic pathways by determination of metabolites and end products of the various pathways(1,2). Thus, it has become of interest to obtain detailed information regarding the activities and behavior of the enzyme systems involved in glucose metabolism in this tissue.

The present work describes the carbohydrate enzymes involved in gluconeogenesis, glucose production, glycogenesis, glycolysis and direct oxidative pathway in the kidney. The enzymes studied are responsible for channeling glucose-6-phosphate into four alternate pathways: glucose-6-phosphatase (glucose release); phosphoglucomutase (glycogen synthesis); phosphohexose isomerase (glycolysis); and glucose-6-phosphate and 6-phosphogluconate dehydrogenases (direct oxidative pathway). The activities of other enzymes of gluconeogenesis and glycolysis (fructose-1,6-diphosphatase and lactic dehydrogenase) are also given. These carbohydrate metabolizing enzymes were studied in the kidney cortex of rats and values were compared with activities found in the liver. The investigation revealed a distinct enzyme pattern in kidney cortex which can be correlated with the behavior of metabolic pathways in this organ as elucidated by isotope approaches.

Materials and methods. Male Wistar rats of 250 g of weight were maintained on Purina Laboratory Chow and water *ad libitum*. The animals were stunned, decapitated and exsanguinated. Kidneys were quickly removed,

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placed in beakers standing on cracked ice, chilled for 5 minutes and sliced in two. The kidney cortex areas were removed, placed in ice cold beakers, minced with scissors and 10% homogenates were prepared in isotonic KCl solution. Homogenization was carried out with a teflon plastic pestle turning at about 600 r.p.m. for exactly 90 seconds. The supernatant fluid was obtained by centrifuging the tissue homogenates at 100,000 g for 30 minutes at 0°C. A refrigerated Spinco model L centrifuge was used.

Cell counts and biochemical assays. The cellularity of the kidney was determined by counting the cell nuclei in cortex homogenates following previously described procedures employed in counting liver nuclei(3). Nitrogen content was determined by the micro-Kjeldahl procedure. Glucose-6-phosphatase activity was measured in the homogenate(4,5), and all other enzyme activities were assayed in the supernatant fluid. Phosphoglucomutase activity was assayed according to Najjar(6); phosphohexose isomerase, glucose-6-phosphate and 6-phosphogluconate dehydrogenase activities were followed by the methods of Glock *et al.* (7) and Glock and McLean(8). Fructose-1, 6-diphosphatase and lactic dehydrogenase were measured according to Weber and Cantero(9). All enzyme assays were carried out at 37°C under linear kinetic conditions. Activities are expressed per average cell.

Results and discussion. A comparison of the cellularity, nitrogen content and carbohydrate enzyme activities in the average cell of rat liver and kidney is presented in Table I. The cellularity of the kidney was 58% higher than that of the liver. However, nitrogen content of the average kidney cell was about one-half of that of the average hepatic cell.

The *glucose-6-phosphatase* activity was 74% of that found in the liver which is in good agreement with previous reports(5,10). The *fructose-1,6-diphosphatase* activity was approximately one-third of the liver activity. The presence of these 2 specific phosphatases is necessary for the functioning of the gluconeogenic processes which were demonstrated by

TABLE I. Comparison of Carbohydrate Enzyme Activities.*

	Liver		Kidney	
Cellularity‡	178 ± 5	(100)†	281 ±20	(158)
Homogenate nitrogen§	1.7 ± .2	(100)	.94 ± .1	(55)
Supernatant " §‖	.9 ± .02	(100)	.47 ± .06	(52)
Phosphohexose isomerase	685 ±66	(100)	811 ±58	(118)
Lactic dehydrogenase	593 ±19	(100)	140 ±14	(24)
Phosphoglucomutase	127 ±11	(100)	2.8 ± .2	(2)
Glucose-6-phosphatase	36.3 ± 2.1	(100)	26.9 ± 4.9	(74)
Fructose-1,6-diphosphatase	26.5 ± 2.8	(100)	8.3 ± 1.4	(31)
Glucose-6-phosphate dehydrogenase	4.4 ± .4	(100)	.3 ± .03	(7)
6-Phosphogluconate dehydrogenase	12.3 ± .8	(100)	1.0 ± 0	(8)
	Ratios			
Phosphohexose isomerase/ Phosphoglucomutase	5.4	(100)	290	(5370)
Glucose-6-phosphatase/ Glucose-6-phosphate dehydrogenase	8.3	(100)	90	(1084)

* Mean values and standard errors represent 4 or more rats in each group.
† Data expressed in %. Liver values taken arbitrarily as 100%.
‡ In millions of nuclei counted/g wet weight of tissue.
§ Calculated as mg nitrogen/cellularity x 10^8.
‖ Enzyme activities given per cell x 10^7 μmoles of substrate metabolized/hr at 37°C.

pyruvate to glucose conversion utilizing isotope methods(1,2). *Phosphohexose isomerase* was in the same activity range in liver and kidney. The function of this powerful enzyme which channels glucose-6-phosphate into glycolysis is in line with the presence of glycolysis described in this tissue(1,2). *Lactic dehydrogenase* activity was less than one-third of that in the liver; however, it was shown that even an activity of less than 20% for this very active enzyme is compatible with production of large amounts of lactate(9,11). The presence of *glucose-6-phosphate* and *6-phosphogluconate dehydrogenases* indicates the capacity of this tissue to carry out direct oxidation of glucose-6-phosphate, channeling it into pentose production. However, since these dehydrogenases are less than 10% of activities found in the liver it appears that the direct oxidative pathway probably contributes comparatively little as an alternate metabolic pathway of glucose-6-phosphate metabolism. A similar low activity in the kidney cortex was noted for *phosphoglucomutase* activity which was less than 5% of the enzyme levels observed in liver. Experience obtained in the study of behavior of phosphoglucomutase in Novikoff hepatoma and Morris hepatoma 5123(12) as well as in adipose tissue(13) indicates that when this enzyme occurred in activities of less than 15% of those found in liver poor glycogenesis existed. This is in line with the low glycogen deposition in kidney(1,2,10) in comparison with the powerful glycogenic processes of the liver which has very active phosphoglucomutase activity. The difference in this glycogenic enzyme activity may explain the fact that cortisone administration results in glycogen deposition in liver(10) which contains high phosphoglucomutase activity, but not in kidney (2,14) where this enzyme activity is very low. On the other hand, cortisone administration resulted in increased gluconeogenesis in both liver and kidney(2,10,14), which may be explained by the similarity on the enzymatic level of the presence of glucose-6-phosphatase and fructose-1,6-diphosphatase in these 2 organs.

The predominance of the capacity of certain enzymes involved in channeling of glucose-6-phosphate into alternate metabolic pathways is shown by the ratios of these enzymes given in Table I. The ratio of phosphohexose isomerase/phosphoglucomutase compares the 2 enzymes which channel glucose-6-phosphate into the energy-yielding (glycolytic) and storing (glycogenic) pathways and the glucose-6-phosphatase/glucose-6-phosphate dehydrogenase compares the activities of the 2 enzymes which channel glucose-6-phosphate into hydrolysis (glucogenesis) or into pentose production (direct oxidative pathway). The phosphohexose isomerase/phosphoglucomutase ratio is 5.4 in the liver, but is 290 in the kidney. The glucose-6-phosphatase/glucose-6-phosphate dehydrogenase ratio is 8.3 in the liver; 90 in the kidney. These ratios indicate that in kidney cortex the enzyme capacity for channeling glucose-6-phosphate into glycolysis and glucose production markedly predominates over the enzymes involved in channeling this hexose ester into glycogenesis or pentose production.

The behavior of kidney carbohydrate enzymes under various physiologic and pathologic conditions is being studied.

Summary. The present work described the cellularity, nitrogen content and kidney enzymes involved in gluconeogenesis, glucose production, glycogenesis, glycolysis and direct oxidative pathway in kidney cortex. The results were compared with values found in the liver. The kidney contained 50% more cells per unit weight; however, the average kidney cell contained only about half the nitrogen content found in the liver cell. These studies demonstrated that the enzyme systems involved in glycolysis and gluconeogenesis, phosphohexose isomerase, lactic dehydrogenase, glucose-6-phosphatase and fructose-1,6-diphosphatase, are present in the kidney cortex at relatively high levels. On the other hand, the low activities for kidney phosphoglucomutase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase suggest that this tissue has poor glycogenic and direct oxidative pathways in comparison with the metabolic pathways of the liver. This investigation revealed a distinct carbohydrate enzyme pattern in kidney cortex and showed that the activities of these enzymes as determined in tissue homogenate and supernatant fluid can be well correlated with studies on the relative importance of glucose metabolic pathways as elucidated by

isotope techniques on kidney slices.

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Distribution of Cortical "Feedback" Fibers in the Nuclei Cuneatus and Gracilis.* (27019)

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In cat and monkey pyramidal fibers are distributed to the nuclei cuneatus and gracilis (1,2,3,4). The fiber distribution within these nuclei displays some degree of organization (3,4) which has been investigated further.

Experiments. A. In 2 cats the rostral pole of one hemisphere was removed, including the sensory-motor areas. Using the Nauta-Gygax impregnation technique(5), the degenerating cortical fibers were found to pass as recurrent bundles through the basal parts of the nucleus cuneatus, as previously reported(3). Similar bundles pass alongside the nucleus gracilis. At caudal levels, these degenerating fibers are distributed to the basal parts of the nucleus cuneatus (and gracilis) (Fig. 7,8). However, only few fibers are distributed to the characteristic dorsal cell clusters (Fig. 2, 3, 6, 7, 8). The fiber distribution in both the nuclei becomes more diffuse in passing from a few millimeters behind the obex rostrad. Some of the longitudinal fibers in the basal parts of the nucleus cuneatus move dorsally towards the surface (Fig. 6), thus cutting through the area of the cell clusters. This narrow fiber pathway widens rostrally at the expense of the clusters, resulting in a more widespread fiber distribution (Fig. 1, 4, 5). However, at rostral levels, some clusters persist, e.g., in the ventrolateral parts of the nucleus cuneatus and along its dorsal medial border (Fig. 5).

The cyto- and dendritic architecture of the nuclei, in Nissl and Golgi material (6-weeks-old kittens impregnated following Moliner(6)), likewise displays rostro-caudal differences, described by Cajal. The caudal cell clusters contain round cells, possessing short, repeatedly ramifying dendrites (Fig. 10, 11, 12). The diameter of the dendritic territories equals approximately 10 times that of the cell body. The dendrites in one cell cluster frequently interlace. The question arose whether this phenomenon could be related to the mutual inhibition characteristic for the cells at these levels (13). In the tail end of the nucleus gracilis these cells are more triangular, possessing longer dendrites (Fig. 13). The basal cells in the caudal part of the nucleus cuneatus are

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