178—196° (dec.), $[\alpha]_D^{26}$ −54.0° (c=1.0, 10% AcOH). Rf^1 0.16, Rf^2 0.18, single ninhydrin- and Ehrlich-positive spot. Amino acid ratios in the 4 N p-toluenesulfonic acid hydrolysate: Phe 0.88, Val 0.91, Glu 0.87, Trp 0.81, Leu 1.01, Met 0.89, Asp 0.93, Thr 0.87. (average recovery 82%); amino acid ratios in the AP-M digest: phe 0.91, Val 0.89, Gln 0.82, Trp 0.84, Leu 0.95, Met 0.80, Asn 0.91, Thr 0.83.

Inhibitory Activity of Glucagon Fragments on Glucose-6-phosphate Dehydrogenase (EC 1.1.1.49)—— The procedure is described here for a Gilford 2400 recording spectrophotometer equipped with a temperature-controlled curvette compartment held at 25° by circulating constant-temperature water. Place triethanolamine buffer, NADP and glucose-6-phosphate reagents (3.0 ml) and the peptide in a test tube and equilibrate in a water bath at 25° for 4—5 min. During this period, keep the cuvettes in the instrument's cuvette compartment so that they reach temperature equilibrium. Add glucose-6-phosphate dehydrogenase (100 mU), mix, and transfer as quickly as possible to the prewarmed cuvette which is rapidly reinserted into the cuvette compartment. The cuvette compartment should be left open for as short a time as possible. Start automatic recording of the change in absorbance at 340 nm.

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Alterations in Renal Enzyme Activities following Sodium Restriction in the Rat

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Changes in reference enzyme activities of subcellular particles of the rat kidney cortex were followed during periods of sodium restriction. Animals were maintained on a sodium-deficient diet for 28 days. Dietary sodium deprivation resulted in a marked decrease in daily urinary excretion of sodium and potassium from the second day, although the urine volume did not change significantly. The kidney cortex homogenate of sodium-restricted rats was analyzed for the activities of reference enzymes. Acid phosphatase (lysosomal enzyme) and succinate dehydrogenase (mitochondrial enzyme) activities, and protein concentration remained unchanged throughout the experimental period. However, increases in the specific activities of glucose-6-phosphatase (microsomal enzyme), D-amino acid oxidase and catalase (peroxisomal enzymes) were observed after the 7th day of experiments.

Keywords——sodium restriction; urinary excretion of sodium and potassium; rat kidney cortex; catalase; D-amino acid oxidase; glucose-6-phosphatase; succinate dehydrogenase; acid phosphatase

Introduction

An important role of the kidney in dehydration following salt restriction is to regulate the volume and osmotic concentration of extracellular fluids. Sodium restriction has been shown to cause parallel activation of the renin angiotensin system and of aldosterone secretion,[2] accompanied by increased reabsorption of sodium in the renal tubules. However, under conditions of sodium restriction, little is known about the metabolism in renal cortical tissue. The present study is an attempt to approach this problem, starting with the determination

1) Location: *2-10-65 Kawai, Matsubara, Osaka, 580, Japan.*
2) J.O. Davis and R.H. Freeman, *Physiol. Rev.*, **56**, 1 (1976); W.S. Spielman and J.O. Davis, *Circul. Res.*, **35**, 615 (1974).

of reference enzyme activities of the principal subcellular particles. In addition, the plasma concentrations and urinary excretion of sodium and potassium were determined simultaneously during the sodium restriction.

Materials and Methods

Animal Experiments——Male Wistar rats, weighing from 150 to 280 g, were used. For at least 7 days prior to the experiments, the rats were fed a standard diet and received tap water *ad libitum*. The animals were separated into control and experimental groups. The control animals were given tap water and a standard diet (290.7 mg of sodium/100 g), while the experimental animals were maintained on distilled water and a sodium-deficient diet (23.2 mg of sodium/100 g) for 28 days. In the first experiments, all rats were housed individually in metabolic cages to collect daily urine samples. Arterial blood samples were taken through the aorta with a syringe wetted with heparin under pentobarbital anesthesia, cooled immediately and then centrifuged. Urine and plasma samples were analyzed for sodium and potassium.

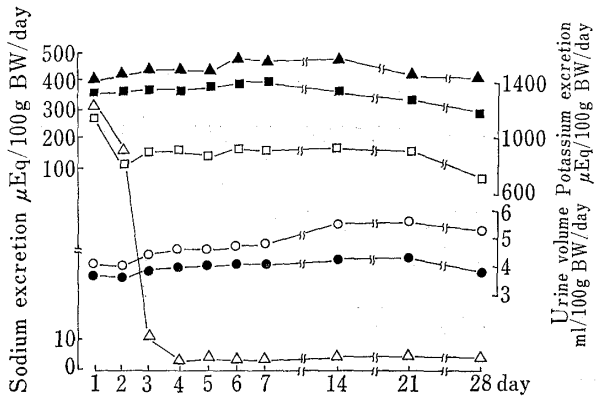

Fig. 1. Effects of Restricted Sodium Intake on the Urine Volume, and on the Urinary Excretion of Sodium and Potassium

The control and experimental rats were maintained on a standard diet and a sodium-deficient diet, respectively, for 28 days. Parameters of renal function were determined on daily urine. The filled symbols represent the control values and the open symbols represent experimental values. ●—●, ○—○, urine volume; ▲—▲, △—△, sodium excretion; ■—■, □—□, potassium excretion. The values are the averages for seven rats.

Preparation of Kidney Cortex Homogenate and Assays of Enzyme Activities——Next, the effects of sodium restriction on the reference enzyme activities of subcellular particles were investigated. Under pentobarbital anesthesia, both kidneys were removed and immediately cooled. After weighing each kidney, the cortex was sectioned into thin slices, rinsed throughly with ice-cold physiological saline, and then homogenized with cold 0.45 M sucrose solution. The homogenate (12.5% w/v) was analyzed for the following enzymes: glucose-6-phosphatase (G-6-Pase, EC 3.1.3.9) for microsomes, acid phosphatase (acid Pase, EC 3.1.3.2) for lysosomes, succinate dehydrogenase (succinate DH, EC 1.3.99.1) for mitochondria, catalase (EC 1.11.1.6) and D-amino acid oxidase (D-AAO, EC 1.33.99.1) for peroxisomes. The activities of these enzymes were assayed as described in our previous paper.[3] Except for catalase, 1 unit of enzyme activity was defined as the amount of enzyme causing the disappearance of 1 μmol of substrate per min. The unit of catalase, which obeys first-order kinetics, was defined as the amount of enzyme causing the logarithm of the hydrogen peroxide concentration to decrease by one unit per min in a volume of 1 ml. The concentration in the homogenate was determined by the method of Lowry *et al.*[4] Statistical significance was determined by means of Student's *t*-test.

Results and Discussion

Throughout the experimental period, no significant difference could be detected in average body weight or kidney weight per 100 g of body weight between the control and experimental rats. The first experiments were carried out to evaluate quantitatively the effects of sodium restriction on the plasma concentrations of electrolytes and on renal functions. On the first day of experiments, the plasma sodium level decreased from a mean control value of 134.4±4.7 mEq/l to 127.2±2.8 mEq/l ($p<0.01$), while plasma potassium level increased from a mean control level of 4.85±0.20 mEq/l to 5.60±0.38 mEq/l ($p<0.01$). However, these values returned to the control levels on the third day, and the levels subsequently remained unchanged. No significant changes were observed in daily urine volume, although there seemed to be a slight increase (Fig. 1). Daily urinary excretion of sodium

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4) O.H. Lowry, N.J. Rosebrough, A.L. Farr, and R.J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

decreased markedly from the second day, and the minimum level (1.11±0.14 μEq/100 g of body weight/day) was subsequently maintained. Daily urinary excretion of potassium (866.1 ±77.6 μEq/100 g of body weight/day) was also significantly lower in sodium-restricted rats, but was not reduced to the same degree as that of sodium. Accordingly, urinary sodium/potassium ratios were significantly lower in sodium-restricted groups compared to the control group. It is suggested that there is a greater reabsorption of distal tubular sodium in the sodium-restricted rat, since urine sodium/potassium ratios can provide a qualitative estimate distal function.

Table I. Specific Activities of Glucose-6-phosphatase and Peroxisomal Enzymes in the Rat Kidney Cortex Homogenate

Days	Glucose-6-phosphatase units/mg protein		Catalase units/mg protein		D-Amino acid oxidase units/mg protein	
	Control	Experimental	Control	Experimental	Control	Experimental
0	0.702±0.035		2.52±0.10		2.07±0.06	
4	0.676±0.015	0.687±0.020	2.47±0.07	2.61±0.31	2.36±0.27	2.46±0.09
7	0.666±0.031	0.823±0.021[a)]	2.39±0.09	3.10±0.25[a)]	2.11±0.15	2.76±0.09[a)]
14	0.809±0.052	0.951±0.013[a)]	2.57±0.11	3.14±0.03[a)]	2.21±0.12	2.74±0.09[a)]
21	0.812±0.021	0.971±0.019[a)]	2.51±0.12	3.51±0.28[a)]	2.03±0.10	3.08±0.12[a)]
28	0.818±0.029	1.112±0.044[a)]	2.38±0.26	4.66±0.23[a)]	2.49±0.11	4.27±0.10[a)]

The control and experimental rats were maintained on a standard diet and on a sodium-deficient diet, respectively. The values are means ± S.E. for seven animals.
a) Values significantly different from the control ($p<0.05$).

The amount of protein (15.1±0.49 mg/ml) and the specific activities of succinate DH (2.13±0.04 units/mg protein) and acid Pase (1.46±0.04 units/mg protein) in the kidney cortex homogenate remained unchanged throughout the experimental period. In contrast, restricted sodium intake caused a significant increase in the specific activity of G-6-Pase after the 7th day (Table I). It is well known that this enzyme is one of the key enzymes for gluconeogenesis and that renal cortical tissue has a high capacity for producing glucose. Therefore, there is a possibility that the rate of renal gluconeogenesis may be enhanced during sodium restriction.

Since morphological observations[5)] have demonstrated the presence of peroxisomes in renal tubules, their reference enzymes were also investigated in the present study. No significant changes were observed in the specific activity of catalase of the control rats during the experiments. However, after the 7th day of sodium restriction, a significant increase was found in the specific activity of catalase, reaching approximately twice the control value on the 28th day. In these rats, the specific activity of D–AAO also increased significantly in parallel with the changes of catalase activity, suggesting the development of kidney peroxisomes following sodium restriction. Morphological observations in numerous studies have established that peroxisomes are formed *via* the endoplasmic reticulum. Svoboda *et al.*[6)] reported that clofibrate caused an increase in the number of peroxisomes in proximal tubules. On the other hand, Long and Jones[7)] found a marked increase in the quantity of smooth-surfaced endoplasmic reticulum in the zone glomerulosa cells of sodium-depleted opossums. These findings might be related to our observation that sodium restriction led to significant increases in catalase, D-AAO and G-6-Pase activities. Although peroxisomes are abundant

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in the kidney and liver, their physiological role remains to be elucidated. It has recently been reported[8] that the liver peroxisomes are capable of oxidizing palmitoyl-CoA and that treatment with clofibrate enhances the peroxisomal system of fatty acid oxidation. If this is also the case in kidney peroxisomes, increased activities of peroxisomal enzymes on sodium restriction may be associated with fatty acid metabolism, which is a major source of energy for renal processes. Further studies are required in this area.

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Sustained Release of Theophylline from Konjac Gels[1]

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The possible use of konjac gels to achieve sustained release of theophylline on oral administration was examined *in vitro*. Theophylline was trapped in the gels by soaking the gels in a hot drug solution and subsequently drying them. Sustained release of the drug from the dried konjac gels was obtained. No marked difference was observed in the release patterns in the range of pH values expected in the gastrointestinal tract, and the drug contained in the gels was completely released.

Keywords——hydrogel; konjac gel; theophylline; bronchodilator; sustained release; solubility; swelling rate

Theophylline, a bronchodilator, has been used for the management of chronic asthma for prophylactic purposes. Its plasma half-life ($t_{1/2\,\beta}$) has been reported to be 4.4—6.2 hr.[3-5] In order to obtain effective plasma theophylline concentrations, a dose of 200—300 mg of theophylline is administered three or four times daily. The rapid absorption and elimination characteristics of theophylline result in large variations in plasma theophylline concentration during dosing in patients receiving the chronic therapy. Therefore it has been suggested that sustained release preparations might decrease the variations in plasma concentration by slowing the rate of absorption.[6]

In an attempt to examine the applicability of hydrogels for sustained release preparations, the release of dibucaine, a local anesthetic, from konjac gels gelatinized with borax has been examined.[7] The results indicated that release of the drug dispersed in konjac gels was sustained. The release experiments *in vivo* following rectal administration of the gels

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