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DECREASED Na^+-GRADIENT-DEPENDENT D-GLUCOSE TRANSPORT IN BRUSH-BORDER MEMBRANE VESICLES FROM RABBITS WITH EXPERIMENTAL FANCONI SYNDROME

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The effect of anhydro-4-epitetracycline on sodium gradient-dependent D-glucose transport of rabbit renal brush-border membrane vesicles was studied. The purity of isolated brush-border membrane vesicles as judged by enzyme activities was not different between normal control and anhydro-4-epitetracycline-administered rabbits. There was no difference in estimate of intravesicular volume, either. When NaCl was used for sodium gradient, the overshoot of D-glucose uptake into brush-border membrane vesicles isolated from anhydro-4-epitetracycline-treated rabbits was significantly smaller than that of normal control rabbits. In the cases of NaSCN or Na_2SO_4, the former was also smaller than the latter, but not significantly so. To avoid the possible effect of membrane potential on D-glucose uptake, the voltage-clamp method was applied. Even in the voltage-clamped condition, the overshoot of D-glucose uptake into vesicles from anhydro-4-epitetracycline-treated rabbits was decreased compared to that of normal rabbits. In vitro incubation of brush-border membrane vesicles with 20 mM anhydro-4-epitetracycline caused no alteration in sodium gradient-dependent D-glucose uptake. Our results demonstrate that there exists a disorder in sodium gradient-dependent D-glucose uptake of renal brush-border membrane in anhydro-4-epitetracycline-treated rabbits, and suggest that this disorder is one of the underlying mechanisms of experimental Fanconi syndrome.

Introduction

The renal Fanconi syndrome is characterized by a generalized disorder in proximal renal tubule transport affecting glucose, amino acids and phosphate, as well as uric acid, bicarbonate and other substances [1]. An understanding of the basic underlying mechanisms of the Fanconi syndrome is important not only to the clinician dealing with the consequences in the patient, but also to the renal physiologist and cell biologist attempting to fathom the cellular processes of membrane transport. Numerous substances have been described which cause the acquired Fanconi syndrome [2–10]. The use of outdated tetracycline has led to a reversible Fanconi syndrome [7–10] and the intoxicating substance has been shown to be anhydro-4-epitetracycline, formed from tetracycline under the influences of heat, moisture and low pH [10]. This substance has been used to experimentally induce a Fanconi syndrome [11,12]. However, the basic underlying mechanisms of this experimental Fanconi syndrome are still obscure.

Glucose is essentially completely reabsorbed from the urine in the proximal tubule via a sodium-coupled secondary active transport system located in the brush-border membrane [13,14]. Procedures have recently been developed for preparing highly purified membrane vesicles from cell

Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

surfaces, and a number of transport studies have been carried out taking advantage of the relative simplicity of these systems [15]. Therefore, we have been investigating possible impairment of the sodium-coupled secondary active D-glucose transport system of brush-border membrane vesicles isolated from the experimental Fanconi syndrome induced in rabbits by anhydro-4-epitetracycline.

Materials and Methods

Male albino rabbits, weighing about 2 kg were catheterized into the urinary bladder and their urinary glucose was checked. Animals which did not show glucosuria were used for the study. Fanconi syndrome was induced by intravenous injection of 100 mg/kg body weight of anhydro-4-epitetracycline, which had been dissolved in 10 ml of 0.9% NaCl. 1 h after injection, urine was collected through the catheter, and glucosuria was detected in all animals. In some rabbits, urinary amino acids were analyzed using an amino acids analyser (Hitachi 835), and they show marked amino aciduria (unpublished data).

In normal control rabbits, 10 ml of 0.9% NaCl was injected intravenously. 1 h after injection, urine was collected and glucosuria was checked, revealing no glycosuria.

Preparation of brush-border membrane vesicles

Rabbits were killed by decapitation immediately after checking glucosuria. The kidneys were perfused in situ with Dulbecco's phosphate-buffered saline (Gibco Laboratories, Grand Island, NY) until blood-free, and then removed and placed in ice-cold phosphate-buffered saline. Cortices were dissected and renal brush-border membrane vesicles were prepared by the Ca-precipitation method of Turner et al. [16]. Typically, the vesicles were preloaded by suspending them in 100 mM mannitol/10 mM Hepes-Tris, (pH 7.4) (10 mM Hepes buffered with Tris to pH 7.4) to give a protein concentration of 2–4 mg/ml. Protein was measured using the Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA) with bovine γ-globulin as the standard.

Determination of enzyme activities

The activities of enzymes known to be characteristics of brush-border microvilli (alkaline phosphatase, trehalase, γ-glutamyl transpeptidase and 5′-nucleotidase), basal-lateral membrane ($(Na^+ + K^+)$-ATPase), mitochondria (succinate dehydrogenase) and endoplasmic reticulum (glucose-6-phosphatase) were measured. Alkaline phosphatase was measured by the method of Kind and King [17]; trehalase was measured according to the method of Carnie et al. [18], and liberated glucose was assayed using the Glucose glucose oxidase-Perid test (Boehringer-Mannheim Co., Mannheim); γ-glutamyl transpeptidase was assayed by the method of Orlowski and Meister [19]; $(Na^+ + K^+)$-ATPase was assayed according to the method of Morel and co-workers [20] and liberated phosphate was assayed by the method of Fiske and SubbaRow [21]; 5′-nucleotidase and glucose-6-phosphatase were assayed by the method of Aronson and Touster [22]; and succinate dehydrogenase was assayed by the method of Baginsky and Hatefi [23].

Measurement of D-glucose uptake

Uptake of D-glucose was measured by the Millipore filtration technique [24]. A 50 μl aliquot of vesicles was placed in a glass test-tube and at time zero a 100 μl aliquot of incubation medium was added. Typically the incubation medium was 100 mM mannitol/10 mM Hepes-Tris (pH 7.4) containing NaCl and ^{14}C-labelled D-glucose to give a final concentration of 100 mM NaCl and 0.1 mM D-glucose. Brush-border membrane vesicles and incubation medium were preincubated at 37°C, and incubation was carried out at 37°C. After an appropriate time the reaction was terminated by the addition of 1.5 ml ice-cold stop solution (150 mM NaCl/0.3 mM phlorizin/10 mM Hepes-Tris (pH 7.4)). The vesicles were immediately filtered through a Millipore filter (HAWP 0.45 μm) under light suction, and then washed with an additional 4.5 ml of stop solution. All incubation was carried out in triplicate at least. The filter, which retained brush-border membrane vesicles, was dissolved in a liquid scintillation fluid, and then the radioactivity was measured along with samples of the incubation medium and appropriate standards.

Chemicals

Anhydro-4-epitetracycline was prepared by the

method of McCormick et al. [25] and purified by a high-performance liquid chromatography. D-[^{14}C]Glucose was obtained from New England Nuclear Corp. Phlorizin was from Sigma Chemical Co. and valinomycin was from P-L Biochemicals Inc. Other chemicals were highest purity available from commercial sources. All solutions were filtered before use. Results were presented as mean ± S.E. and were evaluated statistically by independent *t*-test.

Results

Effect of anhydro-4-epitetracycline adminsitration on enzyme activity of renal homogenate and brush-border membrane

Table I shows the enzyme activity of renal homogenate and of isolated brush-border membrane vesicles from control and anhydro-4-epitetracycline-treated rabbits. The enrichment of brush-border marker enzymes was approx. 10-fold relative to renal cortical homogenate. Contamination by basal-lateral membrane, endoplasmic reticulum and mitochondria, as assayed by ($Na^+ + K^+$)-ATPase, glucose-6-phosphatase and succinate dehydrogenase, was quite low. Moreover, no enzyme activities of renal homogenate and brush-border membrane vesicles from anhydro-4-epitetracycline-administered rabbits were inhibited or activated significantly compared to those from control rabbits.

Effect of anhydro-4-epitetracycline administration on D-glucose uptake into brush-border membrane vesicles

The time-course of D-glucose uptake into brush-border membrane vesicles is illustrated in Fig. 1. Three different sodium salts, NaSCN, NaCl and Na_2SO_4, were used to attain on initial 100 mM Na gradient between extravasicular and intravesicular space. In all cases, a transient overshoot of intravesicular D-glucose content was observed. However, the overshoot was biggest in NaSCN, and smallest in Na_2SO_4. This difference in the magnitude of the overshoot is ascribed to the permeability of the anion of sodium salts [16]. When NaCl was used, the uptake of D-glucose at 10 s and 20 s into renal brush-border membrane vesicles isolated from the anhydro-4-epitetracycline-treated rabbits was significantly lower compared to that into control vesicles. The uptake at 5 s was also lower, although not significantly so. When NaSCN or Na_2SO_4 was used, no significant change in the uptake of D-glucose between the vesicles isolated from anhydro-4-epitetracycline-treated rabbits and control rabbits was detected. However, the mean uptake of D-glucose into anhydro-4-epitetracycline-treated brush-border membrane vesicles in the first half-minute was less than that into control vesicles. When choline chloride was substituted for the sodium salts, the uptake of D-glucose into anhydro-4-epitetracycline-treated vesicles was not different from that into control vesicles. Intravesicular volume, estimated

TABLE I

EFFECT OF ANHYDRO-4-EPITETRACYCLINE ADMINISTRATION ON ENZYME ACTIVITIES OF RENAL CORTICAL HOMOGENATE AND BRUSH-BORDER MEMBRANE

Data are expressed as μmol/mg protein per min. All results are from ten determinations.

Enzyme	Homogenate		Brush-border membrane	
	control	anhydro-4-epitetracycline	control	anhydro-4-epitetracycline
Alkaline phosphatase	0.198 ± 0.011	0.184 ± 0.010	1.692 ± 0.119	1.500 ± 0.134
Trehalase	0.126 ± 0.010	0.143 ± 0.015	1.105 ± 0.123	1.255 ± 0.169
γ-Glutamyl transpeptidase	0.798 ± 0.037	0.711 ± 0.047	7.908 ± 0.647	7.012 ± 0.694
5′-Nucleotidase	0.033 ± 0.003	0.031 ± 0.004	0.209 ± 0.029	0.169 ± 0.015
($Na^+ + K^+$)-ATPase	0.056 ± 0.005	0.062 ± 0.006	0.009 ± 0.003	0.011 ± 0.005
Glucose-6-phosphatase	0.098 ± 0.005	0.084 ± 0.006	0.060 ± 0.004	0.052 ± 0.008
Succinate dehydrogenase	0.020 ± 0.002	0.022 ± 0.001	0.005 ± 0.001	0.006 ± 0.001

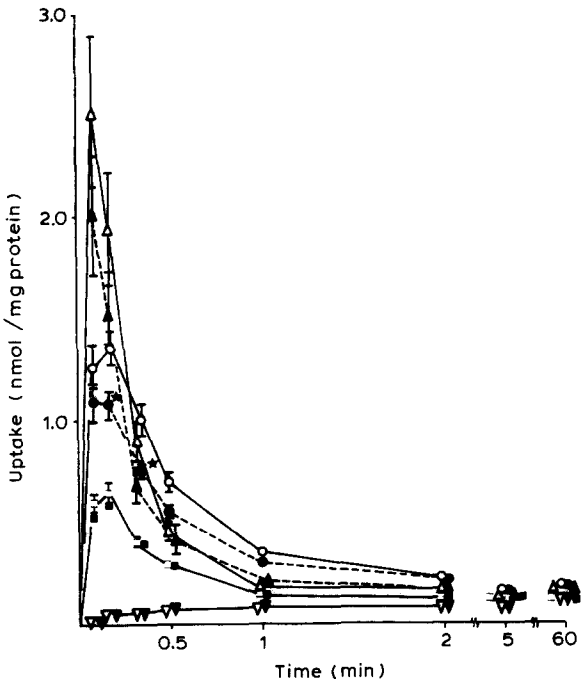

Fig. 1. Time-course of D-glucose uptake into brush-border membrane vesicles. Brush-border membrane vesicles were preloaded with 100 mM mannitol/10 mM Hepes-Tris (pH 7.4); and incubation medium was 100 mM mannitol/10 mM Hepes-Tris (pH 7.4) containing 0.15 mM ^{14}C-labelled D-glucose and 150 mM NaCl (○, ●), 150 mM NaSCN (△, ▲), 75 mM Na_2SO_4 (□, ■) or 150 mM choline chloride (▽, ▼). Open symbols represent uptake into vesicles from normal control rabbits ($n = 5$) and closed symbols represent uptake into vesicles from anhydro-4-epitetracycline-treated rabbits ($n = 5$). Asterisks indicate significant difference between uptake into vesicles from control rabbits and that of anhydro-4-epitetracycline-treated rabbits ($P < 0.05$).

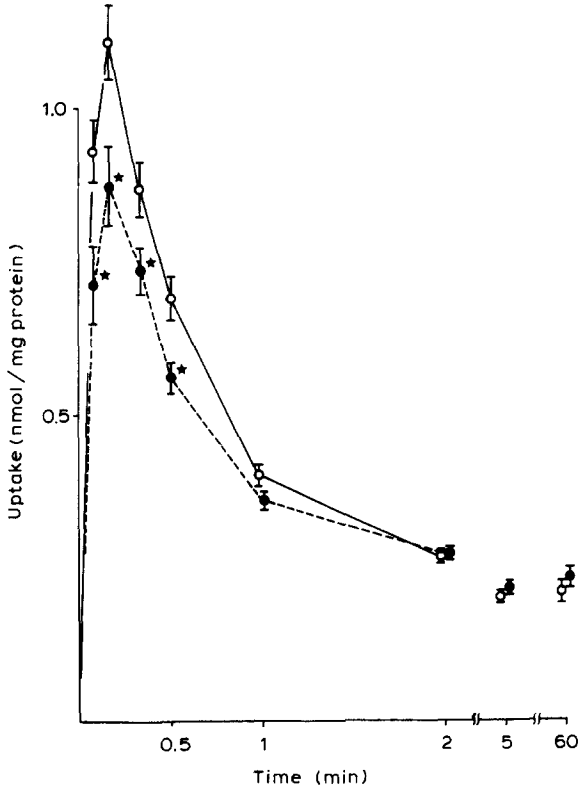

Fig. 2. Time-course of D-glucose uptake into voltage-clamped brush-border membrane vesicles. Brush-border membrane vesicles were preloaded with 300 mM mannitol, 10 mM Hepes-Tris (pH 7.4), 100 mM KSCN and 12.5 μg valinomycin/mg protein. Incubation medium was 300 mM mannitol, 10 mM Hepes-Tris (pH 7.4), 100 mM KSCN containing 150 mM NaCl and 0.15 mM ^{14}C-labelled D-glucose. Open circles and closed circles represent uptake into vesicles from control rabbits ($n = 8$) and that of anhydro-4-epitetracycline-treated rabbits ($n = 8$), respectively. Asterisks indicate significant difference between uptake into vesicles of normal rabbits and that of anhydro-4-epitetracycline-treated rabbits ($P < 0.05$).

from equilibrated D-glucose uptake at 60 min, was affected neither by anhydro-4-epitetracycline administration nor by salt substitution.

Effect of anhydro-4-epitetracycline administration on D-glucose uptake into voltage-clamped brush-border membrane vesicles

Fig. 2 shows the time-course of D-glucose uptake into voltage-clamped brush-border membrane vesicles. The vesicles used in this experiment were preloaded with 100 mM KSCN and 12.5 μg valinomycin/mg protein to clamp the transmembrane potential at zero. The uptake of D-glucose into the brush-broder membrane vesicles isolated from anhydro-4-epitetracycline-treated rabbits was significantly less than that from control rabbits at 5 s, 10 s, 20 s and 30 s. The estimate of intravesicular volume was not affected by anhydro-4-epitetracycline administration.

Effect of incubation of brush-border membrane vesicles with 20 mM anhydro-4-epitetracycline on D-glucose uptake

Addition of 20 mM anhydro-4-epitetracycline (the final concentration) to an incubation medium had no effect on D-glucose uptake into brush-

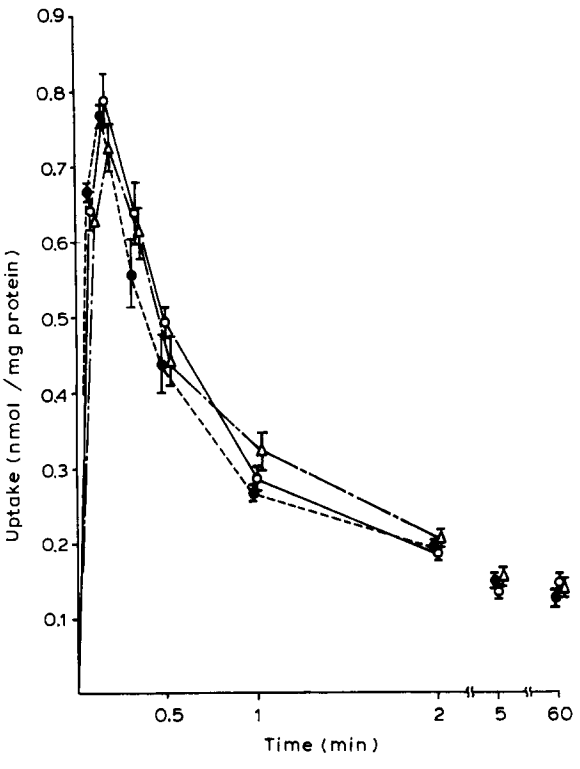

Fig. 3. Effect of incubation of brush-border membrane vesicles with 20 mM anhydro-4-epitetracycline on D-glucose uptake. Preloading medium and incubation medium were identical to those used in Fig. 2, except that 30 mM anhydro-4-epitetracycline was added to incubation medium in closed circles. Open triangles represent uptake into vesicles preincubated with 20 mM anhydro-4-epitetracycline for 60 min. Open circles represent the control. Vesicles were prepared from the same normal rabbit. Each point represents the mean of five measurements.

border membrane vesicles isolated from a control rabbit. Preincubation of brush-border membrane vesicles of a normal rabbit with 20 mM anhydro-4-epitetracycline for 60 min also had no effect (Fig. 3).

Discussion

The term Fanconi syndrome describes a disturbance of the renal tubular function characterized by generalized hyperaminoaciduria, renal glycosuria and hyperphosphaturia, as well as renal loss of potassium, bicarbonate and water [1]. The basic underlying mechanisms of Fanconi syndrome have been attractive subjects, but the underlying mechanisms still remain obscure. Numerous substances are known to cause an acquired Fanconi syndrome [2–10]. The experimental Fanconi syndrome induced by these substances has been used investigating the mechanisms of Fanconi syndrome. Solutes such as glucose [13,14], phosphate [26], bicarbonate [27] and amino acids [28] are known to be reabsorbed from the urine in the proximal renal tubule via the brush-border membrane. One possible mechanism is the disordering of solute reabsorption through brush-border membrane. Recently, methods for purification of renal brush-border membrane vesicles have been established and they have made it possible to study the transport of many solutes across the brush-border membrane without the interference of the cellular metabolism or of the basal lateral membrane [14,15]. However, Silverman reported that D-glucose uptake into brush-border membrane vesicles isolated from dogs with experimental Fanconi syndrome induced by maleic acid was not decreased compared with that from normal control dogs [29]. Moreover, Bergerson et al. [30] reported that in rats with Fanconi syndrome induced by maleic acid, the net influx of amino acids appeared normal at proximal nephron (using microinjection technique) and the efflux of molecules from the cell appeared enhanced through the proximal and the distal tubule (using stop-flow technique).

However, the underlying mechanisms of other types of experimental Fanconi syndrome still remain unknown. In the present study, we investigated the effect on D-glucose uptake into brush-border membrane vesicles in the experimental Fanconi syndrome rabbits induced by anhydro-4-epitetracycline. The use of brush-border membrane vesicles has the advantage of permitting the study of solute transport independent of the possible effect of anhydro-4-epitetracycline on the cellular metabolism and, even more important, on solute transport across the basal lateral membrane.

Effect of anhydro-4-epitetracycline administration on enzyme activity

Our results indicate that the activities of marker enzymes of brush-border membrane and other cellular fractions in renal cortical homogenate and in isolated brush border membrane vesicles were not

affected by anhydro-4-epitetracycline administration. Therefore, these results suggest that renal brush-border membrane vesicles from anhydro-4-epitetracycline-treated rabbits can be isolated by the same Ca-precipitation methods that have been used with normal rabbits.

Effect of anhydro-4-epitetracycline administration on D-glucose uptake into brush-border membrane vesicles

The overshoot of intravesicular D-glucose content in the anhydro-4-epitetracycline-treated rabbits was significantly smaller than that in control rabbits when chloride salt was used to obtain the sodium gradient. However, no difference in sodium-independent (choline chloride-dependent) D-glucose uptake or in the estimate of intravesicular volume between the brush-border membrane vesicles of anhydro-4-epitetracycline-treated rabbits and that of normal rabbits was detected. These results suggest that sodium gradient-dependent D-glucose uptake into brush-border membrane vesicles was inhibited in anhydro-4-epitetracycline-treated rabbits.

When NaSCN was used for sodium gradient, the overshoot of intravesicular D-glucose in anhydro-4-epitetracycline-treated rabbits was smaller than that in control rabbits, but not significantly so. In this condition, SCN ion is considered to be more permeable than Na^+ ion, and SCN diffusion potential (inside negative) would facilitate D-glucose uptake [16]. Therefore, D-glucose uptake would be too rapid to detect any significant difference between the uptake into brush-border membrane vesicles isolated from anhydro-4-epitetracycline-treated rabbits and that from normal rabbits. On the other hand, sulphate ion is considered to be less permeable than sodium ion, and Na^+ diffusion potential (inside positive) would suppress D-glucose uptake [16]. Therefore, D-glucose uptake would be too slow and the overshoot would be too small to detect any significant difference when Na_2SO_4 was used.

Effect of anhydro-4-epitetracycline administration on D-glucose uptake into voltage-clamped brush-border membrane vesicles

As discussed above, the sodium-coupled D-glucose transport is affected greatly by membrane potential, and this phenomenon complicates investigation. We solved this problem by using the voltage-clamp method [16]. Brush-border membrane vesicles were suspended in 100 mM KSCN and 12.5 μg valinomycin/mg protein, and the incubation medium also contained 100 mM KSCN. Therefore, the membrane potential could be held at zero.

As shown in Fig. 2, the overshoot of D-glucose content in anhydro-4-epitetracycline-treated rabbits was significantly smaller than that in control rabbits under the voltage-clamped condition and the estimate of intravesicular volume was not different between the two groups. We confirmed that sodium gradient-dependent D-glucose uptake into brush-border membrane vesicles was inhibited by anhydro-4-epitetracycline administration.

Effect of incubation of brush-border membrane vesicles with 20 mM anhydro-4-epitetracycline on D-glucose uptake

Another question is whether anhydro-4-epitetracycline inhibits the sodium gradient-dependent D-glucose uptake directly or indirectly. Neither the addition of 20 mM anhydro-4-epitetracycline to incubation medium nor the preincubation of brush-border membrane vesicles with 20 mM anhydro-4-epitetracycline affected the sodium gradient-dependent D-glucose uptake of brush-border membrane vesicles isolated from a normal rabbit. A much lower concentration (1 mM anhydro-4-epitetracycline) had no effect on D-glucose uptake, either (unpublished data). Therefore, either anhydro-4-epitetracycline would inhibit sodium gradient-dependent D-glucose uptake through affecting cellular metabolism or some metabolite of anhydro-4-epitetracycline would be a direct inhibitor of D-glucose transport.

Our investigation demonstrated the disorder of sodium gradient-dependent D-glucose uptake of brush-boder membrane vesicles in Fanconi syndrome rabbits induced by anhydro-4-epitetracycline. This disorder must be one of the underlying mechanisms of experimental Fanconi syndrome by anhydro-4-epitetracycline. Anhydro-4-epitetracycline has unique properties as a transport inhibitor and may be a useful tool not only for elucidating the pathophysiology of Fanconi syndrome but also for investigating the cellular processes of membrane transport.

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References

1 Stein, J.H. and Kunau, R.T.J. (1979) in Strauss and Welt's Disease of the Kidney, 3rd Edn. (Earley, L.E. and Gottschalk, C.W., eds.), pp. 1071–1085, Little Brown, Boston
2 Clarkson, T.W. and Kench, J.E. (1956) Biochem. J. 62, 361–372
3 Goyer, R.A., Tsuchiya, K., Leonard, D.L. and Kalryo, H. (1972) Am. J. Clin. Pathol. 57, 635–642
4 Rothstein, A. and Berke, H.J. (1949) J. Pharmacol. Exp. Ther. 96, 179–187
5 Otten, J. and Vis, H.L. (1968) J. Pediatr. 73, 422–425
6 Harrison, H.E. and Harrison, H.C. (1954) Science 120, 606–608
7 Gross, J.M. (1963) Ann. Int. Med. 58, 523–528
8 Ehrlich, L.I. and Stein, W.S. (1963) Pediatrics 31, 339
9 Frimpter, G.W.,Timpanelli, A.E., Eisenmenger, W.J., Stein, W.S. and Ehrlich, L.I. (1963) J. Am. Med. Assoc. 184, 111–113
10 Lowe, M.B. and Tapp, E. (1966) Arch. Pathol. Lab. Med. 81, 362–363
11 Benitz, K.F. and Diermer, H.F. (1964) Proc. Soc. Exp. Biol. Med. 115, 930–935
12 Lindquist, R.R. and Fellers, F.X. (1966) Lab. Invest. 15, 864–876
13 Mudge, G.H., Berndt, W.O. and Valtin, H. (1973) in Handbook of Physiology. Renal Physiology (Orloff, J. and Berliner, R.W., eds.), pp. 587–653, Am. Physiol. Soc., Washington, DC
14 Silverman, M. (1976) Biochim. Biophys. Acta 457, 303–351
15 Kinne, R. and Schwartz, I.L. (1978) Kidney Int. 14, 547–556
16 Turner, R.J. and Moran, A. (1982) Am. J. Physiol. 242, F406–414
17 Kind, P.R.N. and King, E.J. (1954) J. Clin. Pathol. 7, 322–326
18 Carnie, J.A., Porteous, J.A. and Porteous, J.M. (1962) Biochem. J. 85, 620–629
19 Orlowsky, M. and Mesiter, A. (1963) Biochim. Biophys. Acta 73, 679–681
20 Doucet, A., Katz, A.I. and Morel, F. (1979) Am. J. Physiol. 237, F105–F113
21 Fiske, C.H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
22 Aronson, N.N.J. and Touster, O. (1974) Methods Enzymol. 31, 90–102
23 Baginsky, M.L. and Hatefi, Y. (1969) J. Biol. Chem. 250, 5313–5319
24 Aronson, P.S. and Sacktor, B. (1975) J. Biol. Chem. 250, 6032–6039
25 McCormick, J.R.D., Fox, S.M., Smith, L.L., Bitler, B.A., Reichenthal, J., Origoni, V.E., Muller, W.H., Winterbottom, R. and Doerschuk, A.P. (1957) J. Am. Chem. Soc. 79, 2849–2858
26 Sacktor, B. and Cheng, L. (1981) J. Biol. Chem. 256, 8080–8084
27 Kinsella, J.L. and Aronson, P.S. (1980) Am. J. Physiol. 238, F461–F469
28 Evers, J., Murer, H. and Kinne, R. (1976) Biochim. Biophys. Acta 426, 598–615
29 Silverman, M. (1981) Membrane Biochem. 4, 63–69
30 Bergerson, M., Dubord, L. and Hausser, C. (1976) J. Clin. Invest. 57, 1181–1189