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Excretion and Tubular Reabsorption of Sodium, Glucose and Phosphate by Isolated Dog Kidneys. Influence of Blood Dilution

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Summary. Sodium, glucose and phosphate reabsorption and excretion have been investigated in totally isolated dog kidneys. The potentiation by furosemide of the natriuresis caused by saline loading of the preparation is quantitatively comparable to that observed in the whole animal; the decrease of proximal reabsorption together with its partial distal compensation correspond primarily to a direct renal effect of blood dilution. Glucose reabsorption is proportional to glomerular filtration rate; it does not differ from the values found in the whole dog. Phosphate reabsorption is proportional to the filtered phosphate load up to a value of the latter of 10 mg P/min/100 g; at higher values a true $TmPO_4$ is reached which is higher than in normal or thyroparathyroidectomized dogs. Blood dilution exhibits no influence on TmG. The influence of blood dilution on fractional P reabsorption depends on the P filtered load. At low values of this load, P reabsorption is not influenced; at high values, reabsorption is significantly reduced by dilution. No relationship is demonstrated between glucose and sodium reabsorption inasmuch as the latter is under direct dependence on blood dilution. Furosemide (10^{-4} M) induces no significant change in either TmG, P reabsorption or glomerular filtration rate.

Key words: Sodium Excretion — Glucose Excretion — Inorganic Phosphate Excretion — Blood Dilution — Isolated Dog Kidney.

Dilution of the blood impairs the tubular reabsorption of sodium [17] partly through the decrease of post glomerular plasma oncotic pressure [16]. Moreover, extracellular fluid expansion (ECFE) reduces tubular reabsorption and enhances excretion of glucose and inorganic phosphate [9, 12, 15, 19, 22, 23]. The purpose of the present work was to investigate the direct influence of blood dilution on proximal reabsorption of sodium, glucose and phosphate by totally isolated kidneys. The two kidneys of the same dog were perfused independently and simultaneously under identical conditions; blood was diluted on one

side, the other serving as a reference. Such a comparative procedure represents a prerequisite for reliable investigations on isolated kidneys [18].

Methods

The technique was described previously [5, 17, 18]. A blood volume of 450 ml was used in all experiments and the arterial pressure was held constant at 130 mm Hg. The average weight of kidneys was 35.7 g ($\sigma = 9$). Besides the usual scheme of urine collection and replacement [18], a different procedure was used in case of high urine flows. Urine was recirculated into the blood; small urine samples were collected at intervals during periods comprized between 1 and 5 min. These small samples were quantitatively replaced by half-diluted Ringer's solution. Continuous infusion of replacement solutions was unnecessary; changes in blood composition were small and were controlled by periodical blood sampling. No significant differences were observed between the results obtained by either procedure. Besides the usual chemical analysis [17, 18], glucose determinations were performed by the hexokinase technique and inorganic phosphorus by the technique of Fiske and Subbarow adapted to Technicon auto-analyzer. Dilution of blood was obtained by addition of 150 ml of a water solution containing 9 p. 1000 NaCl and 5 mEq/l KCl. This solution is referred to as "NaCl-KCl solution". Potassium was added in order to avoid excessive lowering of plasma potassium concentration which is not observed after saline infusion in vivo [24].

Results

1. Cumulative Influence of Blood Dilution and Furosemide on Reabsorption and Excretion of Sodium. At the beginning of each experiment 150 ml NaCl-KCl solution was added on one side; 2—4 urine samples were collected on both sides at corresponding time intervals. Furosemide was then added to both sides up to a blood concentration of 10^{-4} M and a second series of urine collections was performed. Average results are presented in Table 1.

The natriuretic effect of blood dilution is strongly enhanced by furosemide, in the absence of significant variations of basic parameters. The point will be discussed later.

2. Glucose Reabsorption and Excretion. Relation to Glomerular Filtration Rate. Influence of Blood Dilution. Comparison with Na Reabsorption. Paired experiments were performed as described above. On one side, blood was diluted by addition of 150 ml NaCl-KCl solution containing 6,7 g p. 1000 glucose. Moreover, 1,5 g glucose dissolved in 10 ml NaCl-KCl solution was added on both sides. Some of the experiments involved bilateral addition of furosemide (10^{-4} M). In Fig. 1 is represented the correlation between TmG and glomerular filtration rate (GFR) with undiluted and diluted blood. A strong correlation is observed between TmG and GFR, together with a straight regression line within a range of GFR of 9—72 ml/min/100 g. Blood dilution induces but a trivial modification of the regression line.

Table 1. *Natriuresis caused by blood dilution. Influence of furosemide*

		Undiluted blood		Diluted blood		Differences	
Without furosemide	Renal plasma flow (ml/min/g)	3.3	(0.8)	3.8	(0.5)	+ 0.5	(0.4)
	Plasma Na (mEq/l)	147.6	(3.4)	149.4	(2.8)	+ 1.8	(3.9)
	Plasma K (mEq/l)	4.3	(0.3)	4.5	(0.2)	+ 0.2	(0.2)
	Haematocrit (p. 100)	47.3	(3.5)	41.2	(1.6)	− 6.1	(3.1)
	Post glomerular plasma proteins (g/l)	79.5	(4)	50.2	(2)	− 29.3	(3.5)
	Glomerular filtration rate (ml/min/100 g)	48.7	(5.8)	70.8	(10.3)	+ 22.1	(8)
	Na excreted (p. 100 of filtered load)	7	(1.6)	17.8	(3.5)	+ 10.8	(3)
With furosemide (10^{-4} M)	Renal plasma flow (ml/min/g)	3.6	(0.7)	4.6	(0.5)	+ 1	(0.4)
	Plasma Na (mEq/l)	148.3	(2.8)	151.1	(1.7)	+ 2.8	(2.9)
	Plasma K (mEq/l)	3.8	(0.2)	4.3	(0.1)	+ 0.5	(0.2)
	Haematocrit (p. 100)	48	(3)	40.9	(2)	− 7.1	(2.2)
	Post glomerular plasma proteins (g/l)	79.3	(3.8)	48.9	(1)	− 30.4	(3)
	Glomerular filtration rate (ml/min/100 g)	44.7	(3.4)	67.7	(8.7)	+ 23	(7.1)
	Na reabsorbed (μEq/min/100 g)	5243	(533)	5556	(656)	+ 323	(860)
	Na reabsorbed (μEq/min/ml GFR)	117	(5.5)	84	(8.1)	− 33	(6.9)
	Na excreted (p. 100 of filtered load)	21.2	(4.1)	44	(5.8)	+ 22.8	(4.7)

Average values of 6 experiments and 20 collection periods. Intervals of confidence for $2\,P = 0.05$ are given between brackets; they are calculated for differences on the basis of paired experiments.

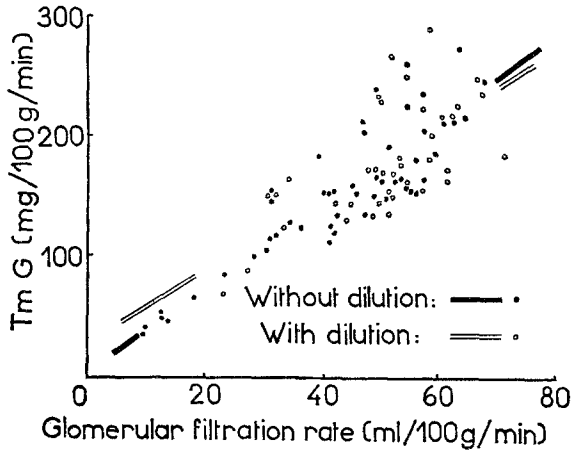

Fig. 1. Correlation between glomerular filtration rate (GFR) and tubular reabsorption of glucose (TmG) without and with blood dilution. *Without blood dilution:* n of clearance periods = 53; average GFR = 42.6 ml/min/100 g; average TmG = 154 mg/min/100 g; TmG = 3.47 GFR + 6.07; $r = 0.89$; $2\,P < 0.001$. *With blood dilution:* n of clearance periods = 39; average GFR = 51.6 ml/min/100 g; average TmG: 185 mg/min/100 g; TmG: 3.03 GFR + 28.5; $r = 0.63$; $2\,P < 0.001$

TmG is independent of glucose filtered load (GL). 21 collection periods have been selected with the same GFR (42.7 $\pm$ 3.28 ml/min/100 g) but with a wide range of GL (140–360 mg/min/100 g); the scattered points demonstrate no correlation between TmG and GL ($r = -0.02$; $2\,P > 0.9$). Moreover, TmG is strictly proportional to GFR; if TmG/GFR is plotted against GFR the regression line is horizontal (38 collection periods; TmG/GFR = 4.05–0.007 GFR; $r = -0.19$; $2\,P = 0.3$).

Average results of paired experiments are presented in Table 2. Blood dilution induces no change in the TmG/GFR; glucose reabsorption increases proportionally to GFR. In the same experimental conditions with furosemide, but without glucose load (Table 1), Na reabsorption per ml GFR is drastically reduced by blood dilution, absolute Na reabsorption being not significantly modified. Simultaneous measurements of glucose and sodium reabsorption (Table 2) show essentially the same dissociation between the behaviour of Na and glucose. It appears from Table 2 that furosemide has no influence on the value of TmG/GFR. This is confirmed by the average results of 31 paired collections with or without furosemide; TmG/GFR (mg/ml) is 3.57 $\pm$ 0.22 without furosemide; 3.4 $\pm$ 0.20 with furosemide (10^{-4} M); GFR is unchanged: 48.6 ml in both cases.

Table 2. *Influence of blood dilution on sodium and glucose tubular reabsorption and excretion*

		No dilution		Dilution		Difference	
Without furosemide. 8 experiments, 38 collection periods	Plasma Na (mEq/l)	151.60	(2)	152.30	(1.60)	+ 0.70	(1.60)
	Plasma K (mEq/l)	3.28	(0.16)	3.77	(0.12)	+ 0.49	(0.12)
	Plasma glucose (g/l)	4.66	(0.20)	5.06	(0.20)	+ 0.40	(0.12)
	Post glomerular plasma proteins (g/l)	79.05	(2.29)	53.26	(3)	− 25.79	(2.80)
	Haematocrit (p. 100)	41.03	(2.27)	34.53	(1.06)	− 6.50	(2.33)
	Renal plasma flow (ml/min/g)	3.57	(0.47)	4.28	(0.32)	+ 0.71	(0.45)
	Glomerular filtration (ml/min/100 g)	41.95	(5.48)	52.36	(3.35)	+ 10.41	(6.09)
	Filtered glucose (mg/min/100 g)	205	(31)	270	(21)	+ 65	(28)
	Excreted glucose (mg/min/100 g)	47	(14)	82	(17)	+ 35	(12)
	Reabsorbed glucose (mg/min/100 g)	158	(21)	188	(17)	+ 30	(22)
	(mg/min/ml GFR)	3.75	(0.21)	3.63	(0.26)	− 0.12	(0.24)
With furosemide (10^{-4} M). 5 exp., 17 collection periods	Glomerular filtration (ml/min/100 g)	35.80	(8.70)	48.70	(5)	+ 12.90	(10.50)
	Filtered Na (μEq/min/100 g)	5492	(1305)	7564	(789)	+ 2072	(1570)
	Reabsorbed Na (μEq/min/100 g)	4400	(865)	5223	(451)	+ 823	(848)
	(μEq/min/ml GFR)	126	(9)	110	(11)	− 16	(9.80)
	Filtered glucose (mg/min/100 g)	171	(50)	242	(26)	+ 71	(50)
	Reabsorbed glucose (mg/min/100 g)	132	(28)	170	(13)	+ 38	(34)
	(mg/min/ml GFR)	3.70	(0.39)	3.57	(0.33)	−0.13	(0.29)

Average values. Limits of confidence for 2 P = 0,05 are between brackets. For differences these limits are calculated from paired values.

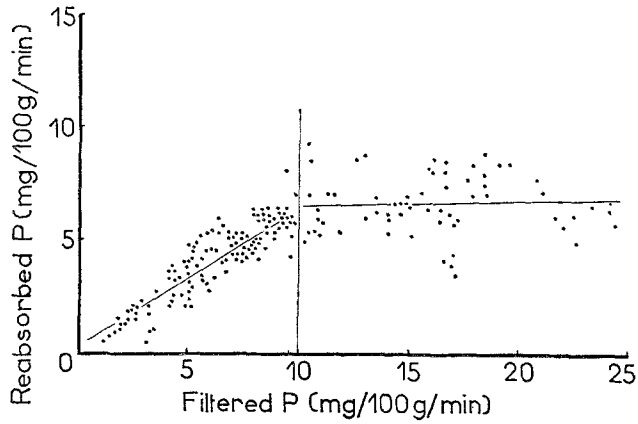

Fig. 2. Correlation between P filtered load (PL) and P tubular reabsorption (PR). *With* PL $\leq$ *9.9 mg/min/100 g: n* of clearance periods = 170. Average plasma inorganic phosphorus = 14.65 mg p. 100. Average GFR = 31.2 ml/min/100 g. Average PL: 5.74 mg/min/100 g. Average PR: 3.90 mg/min/100 g. PR = 0.575 PL + 0.60; $r = 0.90$; $2\,P < 0.001$. *With PL* $\geq$ *10 mg/min/100 g: n* of clearance periods = 62. Average plasma inorganic phosphorus = 45.5 mg/p. 100. Average GFR = 40 ml/min/100 g. Average PL: 15.94 mg/min/100 g. Average PR = 6.67 mg/min/100 g. PR = 0,016 PL + 6.424; $r = 0.04$; $2\,P = 0.9$

3. Inorganic Phosphate Reabsorption and Excretion. Relation to Filtered Phosphate Load. Influence of Blood Dilution. Phosphate excretion and reabsorption were investigated either at normal plasma level or after addition of isosmotic phosphate buffer ($NaHPO_4$-KH_2PO_4, pH 7.4, M/15) in volumes comprized between 14 and 125 ml. All values presented here are expressed as inorganic phosphorus (P in the text). Fig. 2 presents the overall pattern of correlation between P filtered load (PL) and P reabsorption (PR) and gives the regression equations. The amount reabsorbed depends on PL up to a value of 10 ml/100g/min. At higher values a true Tm is reached which is independent of the filtered load.

Contrarily to glucose, PR depends on PL and not on GFR: 15 collection periods have been selected with equal PL (5.67 $\pm$ 0.18 mg/min/100g) but with a range of GFR comprized between 32 and 48 ml/min/100 g. The scattering of the points reveals no correlation between PR and GFR ($r = -0.05$; $2\,P = 0.5$). Moreover, on the basis of 53 measurements, fractional P reabsorption (PR/PL) was also found independent of GFR (PR/PL = 0.81—0.0003 GFR; $r = -0.01$; $2\,P > 0.9$).

Fig. 3 indicates the correlation between filtered and reabsorbed P, with undiluted and with diluted blood and after phosphate buffer

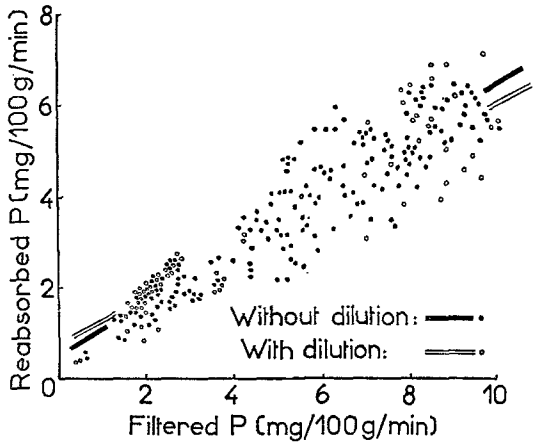

Fig. 3. Correlation between P filtered load (PL) and P tubular reabsorption (PR) without and with blood dilution. *Without blood dilution: n* of clearance periods = 170. Average plasma inorganic phosphorus: 14.65 mg p. 100. Average GFR = 31.2 ml/100 g/min. Average PL = 5.74 mg/min/100 g. Average PR = 3.90 mg/min/100 g. PR = 0.575 PL + 0.60; $r = 0.90$; $2\,P < 0.001$. *With blood dilution: n* of clearance periods = 53. Average plasma inorganic phosphorus = 13.3 mg p. 100. Average GFR = 55.5 ml/100 g/min. Average PL = 5.96 mg/min/100 g. Average PR = 3.86 mg/min/100 g. PR = 0.535 PL + 0.68; $r = 0.89$; $2\,P < 0.001$

supplementation (16 ml on control side, 26 ml in blood diluted by 150 ml NaCl-KCl solution).

More accurate results are provided by paired experiments. Average data are presented in Table 3. A first series of experiments were performed without phosphate supplementation; another series involved addition of phosphate buffer (16 ml on control side, 26 ml in diluted blood). At normal plasma levels and low filtered load of phosphate, fractional reabsorption of this ion is not altered by blood dilution. At the abnormally high plasma concentrations and high PL obtained after phosphate buffer supplementation, blood dilution induces a significant decrease of fractional reabsorption.

31 comparative measurements demonstrated that furosemide (10^{-4}M) has no influence on fractional phosphate reabsorption (0.72 ± 0.04 with or without furosemide).

Discussion

1. Influence of Furosemide on the Natriuresis Caused by Blood Dilution. The effect of ECFE on fractional sodium excretion is considerably enhanced by diuretics with predominant distal action, thus suggesting

Table 3. *Influence of blood dilution on tubular reabsorption and on excretion of inorganic P*

	Normal plasma P levels 4 exper.; 15 collection periods						Phosphate buffer loading 9 exper.; 39 collection periods					
	No dilution		Dilution		Differences		No dilution		Dilution		Differences	
Plasma Na (mEq/l)	146.70	(1.90)	150.80	(1.60)	+ 4.10	(2)	144	(2.09)	148.20	(1.18)	+ 4.20	(2.07)
Plasma K (mEq/l)	3.50	(0.20)	3.90	(0.20)	+ 0.40	(0.20)	4.07	(0.16)	4.24	(0.12)	+ 0.17	(0.16)
Plasma P (mg p. 100)	5.69	(0.77)	4.28	(0.42)	− 1.41	(0.50)	16.48	(0.79)	15.83	(0.49)	− 0.65	(0.79)
Post glomerular plasma proteins (g/l)	80.60	(4.80)	50.20	(2.10)	− 30.40	(3.50)	77.58	(2.68)	53.38	(3.15)	− 24.20	(4.06)
Haematocrit (p. 100)	50.20	(3.30)	40.30	(2)	− 9.90	(1.70)	44.34	(1.68)	36.24	(1.73)	− 8.10	(1.93)
Renal plasma flow (ml/min/g)	3	(0.80)	3.90	(0.50)	+ 0.90	(0.60)	3.34	(0.49)	4	(0.41)	+ 0.66	(0.37)
Glomerular filtration rate (ml/min/100 g)	35.80	(6.50)	48.10	(10.20)	+ 12.30	(8.80)	36.84	(4.12)	48.69	(4.63)	+ 11.85	(3.80)
P filtered load (mg/min/100 g) (PL)	1.91	(0.29)	1.92	(0.32)	+ 0.01	(0.46)	6.07	(0.69)	7.86	(0.85)	+ 1.79	(0.57)
P excreted (mg/min/100 g)	0.07	(0.02)	0.09	(0.03)	+ 0.02	(0.03)	1.63	(0.30)	2.78	(0.49)	+ 1.15	(0.30)
P reabsorbed (mg/min/100 g) (PR)	1.84	(0.29)	1.83	(0.30)	− 0.01	(0.45)	4.48	(0.55)	5.02	(0.55)	+ 0.54	(1.10)
PR/PL	0.96	(0.01)	0.95	(0.01)	− 0.01	(0.02)	0.74	(0.04)	0.65	(0.04)	− 0.09	(0.20)

Average values. Limits of confidence for 2 P = 0.05 are between brackets. For differences these limits are calculated from paired values.

that the influence of ECFE on proximal reabsorption is greater than on overall reabsorption, this interpretation being confirmed by micropuncture experiments [2,6,8,14,21]. The potentiation of saline natriuresis by furosemide in the isolated kidney is quantitatively comparable to that found in the whole animal submitted to preferential distal blockade [6,7]. It appears therefore that the proximal effect of ECFE as well as its partial distal compensation correspond both primarily to an autonomous response of the kidney to blood dilution and that they are not primarily monitored by changes in body fluid volumes.

2. Glucose Reabsorption and Excretion. The present experiments demonstrate the adjustment of glucose reabsorption to GFR, thus confirming the previous findings of Van Liew *et al.* [25] and Bowman *et al.* [1] in the rat, of Keyes *et al.* [13] in the dog and of Gekle *et al.* [10] in the man. The values found for the TmG per ml GFR (3,75 $\pm$ 0.21 mg) are identical to those obtained in the whole dog by Shannon *et al.*: 2.2—4.63 mg [20], by Handley *et al.*: 3.76 mg [11] and by Keyes *et al.* 3.55 $\pm$ 0.31 mg [13]. It is therefore unlikely that TmG is modulated by extrarenal factors—at least in normal conditions—. The present experiments did not demonstrate any influence of blood dilution on TmG per ml GFR, the observed increase in glucose excretion being related only to the increase of GFR. A degree of blood dilution inducing a major impairment of Na reabsorption per ml GFR remains without influence on TmG per ml GFR, thus indicating that glucose reabsorption is not linked to that part of Na reabsorption which is controlled by blood dilution. The observation by Robson *et al.* [19] of a decrease of TmG in the rat after saline loading seems to require a different mechanism.

3. Inorganic Phosphate Reabsorption and Excretion. Our experiments demonstrate that phosphate reabsorption is proportional to the filtered load and not directly dependent on the filtered volume. A true TmP is reached for a filtered load equal or higher than 10 ml/min/100 g, with a value (6.67 $\pm$ 0.37 mg/min/100 g) definitely higher than that measured in the intact dog (2.82 mg) or in the thyroparathyroidectomized dog (4.48 mg) [4]. This suggests the interference of other major limiting humoral factors in the whole organism. The effect of blood dilution on P reabsorption depends on the filtered P load. At moderate values, no influence at all is observed. At higher values, fractional P reabsorption is significantly reduced. It is conceivable that the inhibitory influence of blood dilution appears only when P reabsorption is near to its saturation level.

References

1. Bowman, R. H., Maack, T.: Functional studies on the isolated perfused rat kidney with particular attention to glucose transport. Abstracts, p. 398. IVth International Congress of Nephrology, Stockholm, 1969.

2. Brenner, B. M., Falchuk, K. H., Keimowitz, R. I., Berliner, R. W.: The relationship between peritubular capillary protein concentration and fluid reabsorption by the renal proximal tubule. J. clin. Invest. **48**, 1519—1531 (1969).
3. Corvilain, J.: Effet de l'hormone de croissance sur l'excrétion rénale des phosphates. Acta clin. belg. Suppl. 3 (1966).
4. — Abramow, M.: Effect of growth hormone on tubular transport of phosphate in normal and parathyroidectomized dogs. J. clin. Invest. **43**, 1608—1612 (1964).
5. Cuypers, Y., Nizet, A., Baerten, A.: Technique pour la perfusion de reins de chien avec du sang hépariné. Arch. int. Physiol. **72**, 245—255 (1964).
6. Dirks, I. H., Cirksena, W. J., Berliner, R. W.: The effect of saline infusions on sodium reabsorption by the proximal tubule in the dog. J. clin. Invest. **44**, 1160—1170 (1965).
7. — — — Micropuncture study of the effect of various diuretics on sodium reabsorption by the proximal tubules of the dog. J. clin. Invest. **45**, 1875—1885 (1966).
8. Earley, L. E., Martino, J. A., Friedler, R. M.: Factors affecting sodium reabsorption by the proximal tubule as determined during blockade of distal sodium reabsorption. J. clin. Invest. **45**, 1668—1684 (1966).
9. Frick, A.: Parathormone as a mediator of inorganic phosphate diuresis during saline infusion in the rat. Pflügers Arch. **325**, 1—13 (1971).
10. Gekle, D., Janovsky, M., Slechtova, R., Martinek, J.: Einfluß der glomerulären Filtrationsrate auf die tubuläre Glucosereabsorption bei Kindern. Klin. Wschr. **45**, 416—419 (1967).
11. Handley, C. A., Sigafoos, R. B., La Forge, M.: Proportional changes in renal tubular reabsorption of dextran and excretion of *p*-aminohippurate with changes in glomerular filtration. Amer. J. Physiol. **159**, 175—180 (1949).
12. Hellman, D., Baird, H. R., Bartter, F. C.: Relationship of maximal tubular phosphate reabsorption to filtration rate in the dog. Amer. J. Physiol. **207**, 89—96 (1964).
13. Keyes, J. L., Swanson, R. E.: Dependence of glucose Tm on GFR and tubular volume in the dog kidney. Amer. J. Physiol. **221**, 1—7 (1971).
14. Knox, F. G.: Effect of increased proximal delivery on furosemide natriuresis. Amer. J. Physiol. **218**, 819—823 (1970).
15. Massry, S. G., Coburn, J. W., Kleeman, C. R.: The influence of extracellular volume expansion on renal phosphate reabsorption in the dog. J. clin. Invest. **48**, 1237—1245 (1969).
16. Nizet, A., Baerten, A., Dujardin, A., Thoumsin, H., Thoumsin-Moons, J.: Quantitative influence of non hormonal blood factors in the control of sodium excretion by isolated dog's kidney. Kidney International **1**, 27—37 (1972).
17. — Cuypers, Y., Deetjen, P., Kramer, K.: Functional capacity of isolated dog kidney. Pflügers Arch. ges. Physiol. **296**, 179—195 (1967).
18. — Godon, J. P., Mahieu, P.: Quantitative excretion of sodium and water load by isolated dog kidney: autonomous renal response to blood dilution factors. Pflügers Arch. **304**, 30—45 (1968).
19. Robson, A. M., Srivastava, P. L., Bricker, N. S.: The influence of saline loading on renal glucose reabsorption in the rat. J. clin. Invest. **47**, 329—335 (1968).
20. Shannon, J. A., Farber, S., Troast, L.: Measurement of glucose Tm in normal dog. Amer. J. Physiol. **133**, 752—761 (1941).
21. Spitzer, A. Windhager, E. E.: Effect of peritubular oncotic pressure changes on proximal tubular fluid reabsorption. Amer. J. Physiol. **218**, 1188—1193 (1970).

22. Steele, T. H.: Increased urinary phosphate excretion following volume expansion in normal man. Metabolism **19**, 129—139 (1970).
23. Suki, W. N., Martinez-Maldonado, M., Rouse, D., Terry, A.: Effect of expansion of extracellular fluid volume on renal phosphate handling. J. clin. Invest. **48**, 1888—1894 (1969).
24. Toussaint, Ch.: Contribution à l'étude de l'excrétion des électrolytes chez le Chien. Rev. belge. Path. **28**, 5—139 (1961).
25. Van Liew, J. B., Deetjen, P., Boylan, J. W.: Glucose reabsorption in the rat kidney. Dependence on glomerular filtration. Pflügers Arch. ges. Physiol. **295**, 232—244 (1967).

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