uterine smooth muscle. Calculations similar to those performed by Marshall and Kroeger[13] based on the findings of -8.9 mV hyperpolarization in these experiments would give a Ca efflux of 0.7×10^{-9} m Ca min^{-1} cm^{-2}. It is therefore possible that electrogenic Ca efflux can account for the hyperpolarization observed[14].

4. During the 1st few min following readmission of Na, rapid replenishment of Na_i occurs enhanced by the abundant amount of Ca_i available for exchange[2,13]. Thus, within min there may be marked enhancement of electrogenic Na-K exchange mediated by the Na-K ATPase[15]. Increased Ca_i may also stimulate the Na pump.

5. Ca_i is thought to modulate K permeability in many excitable cells and increasing Ca_i might increase K permeability in CPF[16]. However, a change in K permeability is probably not the sole cause of the hyperpolarization observed because a) this effect should be observed during Na-free perfusion whereas hyperpolarization invariably occurred 4–10 min after Na-readmission; and b) K probably accumulates in the intracellular clefts during Na-free perfusion resulting in a transient decrease of the K-equilibrium potential[10].

The hyperpolarization following Na-readmission to ionophore-treated fibres is probably both a result of enhanced electrogenic Na efflux that is related to the ionophore-mediated increase in Ca_i and electrogenic Ca efflux. Preliminary experiments with readmission of Li-substituted solution and in fibres exposed to acetyl-strophanthidin during ionophore-exposure support this suggestion.

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2 H. Reuter and N. Seitz, J. Physiol. *195*, 451 (1968).
3 I.R. Wendt and G.A. Langer, J. molec. cell. Cardiol. *9*, 551 (1977).
4 H. Jundt, H. Porzig, H. Reuter and J.W. Stucki, J. Physiol. *246*, 229 (1975).
5 J.M. Gelles, Experientia *32*, 906 (1976).
6 J.M. Gelles, Circulation Res. *41*, 94 (1977).
7 P.W. Reed and H.A. Lardy, J. biol. Chem. *247*, 6970 (1972).
8 C.M. Armstrong and B. Hille, J. gen. physiol. *59*, 388 (1972).
9 J.J. Van Der Walt and E. Carmeliet, Archs int. Physiol. Biochem. *79*, 149 (1971).
10 D.S. Miura, B.F. Hoffman and M.R. Rosen, J. gen. Physiol. *69*, 463 (1977).
11 B.C. Pressman and N.T. deGuzman, Ann. N.Y. Acad. Sci. *264*, 373 (1975).
12 H. Reuter, Prog. Biophys. molec. Biol. *26*, 3 (1973).
13 J.M. Marshall and E.A. Kroeger, Phil. Trans. R. Soc. Lond. B *265*, 135 (1973).
14 J.R. Wiggins and P.F. Cranefield, J. gen. Physiol. *64*, 473 (1974).
15 H.G. Haas, in: Electrical Phenomena in the Heart, p. 163. Academic Press, London 1972.
16 J.B. Bassingthwaighte, C.H. Fry and J.A.S. McGuigan, J. Physiol. *262*, 15 (1976).

Localization of renal kallikrein in the dog

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Summary. Renal kallikrein was estimated in glomerular, tubular and medullary fractions of dog kidneys. It was found primarily in the cortex, the highest level of activity being detected in a glomeruli-rich fraction. These results support previous observation that kallikrein may be associated with the juxta-glomerular complex.

Renal kallikrein is probably the source of kallikrein found in the urine because the 2 enzymes are indistinguishable, while both differ from the kallikreins of plasma[1–3]. Renal kallikrein has been localized primarily to the cortex of the rat[4] and dog kidney[5–7], the highest concentration being found in the outer cortex[7]. Early studies by indirect methods indicated localization within the proximal tubule[4,8], however, more recently, utilizing stop-flow techniques in the dog[9] and fluorescent antibody histochemistry in the rat[10], kallikrein has been localized to the distal tubule and possibly the macula densa of the juxtaglomerular apparatus.

The objective of this study was to examine the distribution of kallikrein in glomerular and tubular fractions derived from dog renal cortex. Studies with rat kidney indicate that, when compared with tubular tissue, glomerular kallikrein concentration may be relatively high[11]; however, in another study glomeruli devoid of juxtaglomerular apparatus accounted for only 15% of the activity of the total cortex[7].

Methods. Dogs (8–12 kg mongrel) were anaesthetised with sodium pentobarbital (20 mg kg^{-1} i.v.) and the kidneys perfused in situ with sucrose (0.25 moles l^{-1}). The method described by Cook and Pickering[12] was then used to separate a glomerular fraction from the tubular tissues of the cortex. 3 fractions were obtained; cortical tissue rich in glomeruli, cortical tissue poor in glomeruli (mainly tubules) and medulla. An aliquot of each sample was taken for histological observation and the remaining tissue lyophilised. The lyophilised tissue was homogenised in distilled water (25–50 mg ml^{-1}) followed by centrifuging (10,000 × g, 4 °C, 20 min). The supernatant was acidified to pH 2.0–2.5 with HCl (0.1 moles l^{-1}) for 20 min at room temperature (to inhibit kininase). It was then adjusted to pH 9.0 with NaOH (0.1 moles l^{-1}) and centrifuged. Aliquots of the supernatant (0.05–0.4 ml) were incubated at 37 °C for 20 min with 15 mg dog kininogen (modified from Rocha E. Silva et al.[13]) 50 mg ml^{-1} in bicarbonate buffer[14] at pH 9.0. Incubates were applied to an isolated guinea-pig ileum preparation suspended in magnesium-free Tyrode solution containing atropine and mepyramine (both 2×10^{-8} g ml^{-1}) aerated with 95% O_2, 5% CO_2. Kinin present in the incubates was determined by comparison with doses of synthetic bradykinin (BRS 640, Sandoz) by 6 point bio-assay. The kallikrein content of an extract was expressed as the amount of synthetic bradykinin equivalent in potency to the kinin liberated by the extract per mg of protein during 20 min incubation. Protein was measured by the method of Lowry et al.[15].

Qualitative tests. To exclude the possibility of contamination by smooth muscle stimulants, the extracts and kininogen were incubated separately at pH 9.0 for 20 min with an equal volume of 0.16 moles l^{-1} NaCl, and the incubates

applied to an isolated guinea-pig ileum preparation. Kininase inhibition was confirmed by incubating synthetic bradykinin with an aliquot of the acidified extract at pH 7.0 for 20 min at 37 °C and recoverable bradykinin determined by bio-assay. The ability of extracts to liberate kinin in preference to angiotensin was confirmed by 2 methods; firstly, incubates of extract with kininogen were applied to an isolated rat duodenum preparation suspended in DeJalons solution. Secondly, 1×10^{-4} g carboxypeptidase A or 1×10^{-5} g carboxypeptidase B (Worthington) dissolved in 0.16 moles l^{-1} NaCl were incubated for 20 min, pH 7.0 at 37 °C with incubates containing the suspected kinin activity and remaining activity determined by bio-assay.

Results. The pH optima of dog renal kallikrein was found to be pH 9.0. Extracts and substrate possessed no extraneous activity. The acidification procedure produced effective inhibition of renal kininase, 95–100% of added bradykinin was recoverable following incubation with renal extracts. The activity liberated by the extracts had properties characteristic of a kinin[16]. It produced relaxation of an isolated rat duodenum preparation, was destroyed by carboxypeptidase B, but unaffected by carboxypeptidase A. Synthetic bradykinin exhibited the same properties; whereas, synthetic angiotension II (Hypertensin Ciba) was inactivated by carboxypeptidase A and produced an increased tonus of rat duodenum.

Histological examination revealed that the glomerular fraction contained a small amount of arteriolar and tubular tissue, whereas, the tubular fraction was relatively pure. The activity detected in the glomerular, tubular and medullary fractions of 6 dog kidneys is shown in the table. The medulla contained consistently low levels of activity. Both glomerular and tubular fractions were found to contain kallikrein, a significantly greater level of activity being detectable in the glomerular-rich fraction ($p < 0.001$, paired t-test).

Mean kallikrein activity of 3 dog kidney fractions

Dog	Kidney	Glomerular fraction	Tubular fraction	Medulla
1	Left	-	3.73 (2)	0.61 (2)
	Right	8.07 (5)	3.13 (4)	0.91 (4)
2	Left	6.22 (2)	1.35 (3)	0.56 (2)
	Right	9.74 (3)	2.27 (3)	0.28 (2)
3	Left	6.26 (1)	3.04 (1)	0.56 (1)
	Right	5.77 (1)	1.87 (1)	-
Mean ± SEM		7.2 ± 0.74	2.57 ± 0.36	0.98 ± 0.1

Units of activity. µg (10^{-6}) synthetic bradykinin equivalent in potency to the kinin liberated by the extract per mg protein during 20 min incubation. Protein concentration (10^{-6} g ml^{-1}). Glomerular fraction 201 ± 40 (18); tubular fraction 616 ± 85 (19); medulla 772 ± 125 (17); mean ± SEM. Figures in parenthesis indicate the number of determinations performed.

Discussion. Kallikrein was found primarily in the cortex of the dog kidney. The highest level of activity was found in the glomeruli-rich fraction, which contained some juxtaglomerular tissue. Activity was also detected in the tubular fraction. In the rat kidney, kallikrein has been localized to the distal tubule near the juxtaglomerular apparatus[9,10] and glomeruli devoid of juxtaglomerular tissue contain little kallikrein[7]. These observations on dog kidney find significant quantities of kallikrein in glomerular fractions contaminated with juxtaglomerular tissue, supporting the notion that kallikrein may be associated with the juxtaglomerular complex.

1 K. Nustad, Br. J. Pharmac. *39*, 72 (1970).
2 I.F. Carvalho, in: Kidney Hormones, p. 585. Ed. J.W. Fisher. Academic Press, London 1971.
3 K. Nustad, K. Vaaje and J.V. Pierce, Br. J. Pharmac. *53*, 229 (1975).
4 K. Nustad, Br. J. Pharmac. *39*, 87 (1970).
5 A. Scicli, O. Carretero, A. Hampton, P. Cortes and N. Oza, Physiologist *17*, 327 (1974).
6 C. Moriwaki, K. Miyazaki, Y. Matsuda, H. Moriya, Y. Fujimoto and H. Ueki, J. Biochem. *80*, 1277 (1976).
7 A. Scicli, O. Carretero, N. Oza and M. Schork, Proc. Soc. exp. Biol. Med. *151*, 57 (1976).
8 E. Werle and R. Vogel, Archs int. Pharmacodyn. Thér. *126*, 171 (1960).
9 A. Scicli, O. Carretero, A. Hampton, P. Cortes and N. Oza, Am. J. Physiol. *230*, 533 (1976).
10 T. Örstavik, K. Nustad, P. Brandtzaeg and J. Pierce, J. Histochem. Cytochem. *24*, 1037 (1976).
11 K. Mann, R. Geiger and E. Werle, Adv. exp. Med. Biol. *70*, 65 (1976).
12 W. Cook and G. Pickering, Nature, Lond. *182*, 1103 (1958).
13 M. Rocha, E. Silva, W. Beraldo and G. Rosenfield, Am. J. Physiol. *156*, 261 (1951).
14 G. Delory and E. King, Biochem. J. *39*, 245 (1945).
15 O. Lowry, N. Rosebrough, A. Farr and R. Randell, J. biol. Chem. *193*, 265 (1951).
16 M. Schachter, Physiol. Revs. *49*, 509 (1969).

Influence of age on the renal renin response to a high salt intake in the rat

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Summary. Saline drinking combined with DOCA-treatment was found to decrease renal renin in weanlings at a higher rate than in adult rats, with a comparable saline consumption level. The decrease was not potentiated by uninephrectomy.

Renin-angiotensin system (RAS) activity decreases during postnatal ontogeny in the rat, presumably because of maturation of water, salt and circulatory homeostasis[1]. There is evidence that, in suckling rats, increasing body sodium does not influence RAS activity[2]. However, no data are available about RAS reactivity in the weaning period. Kidney function is more developed at this period than in suckling rats, but is still not fully mature[3]. Rats of this age are more sensitive than adults to the hypertensogenic effects of a chronically increased salt intake[4], particularly