survey the presence of ischemic areas and their size. Particularly, the basal ganglia region appeared colourless in 2 animals, the cortical-subcortical region in 1 animal and the combined basal ganglia and cortical-subcortical regions in 5 animals. Lastly, in 1 rat there was no evidence of colourless areas (figure 2). No mortality occurred in 4 sham operated animals, and no colourless brain regions were observed.

The main limitations of this model lie in the need for general anesthesia and skull opening[16]. The advantage is the production of a focal cerebral ischemia without additional insults of hypoxia[1,11], hypotension[13,14] and handling of carotid and vertebral arteries[16]. Moreover, this model can be easily reproduced. Conversely, the model of transpalatine approach[7] involves a high rate of intra-operative accidents, mortality and postoperative complications consisting of wound infections and keratitis. The use of carbon microspheres for brain arterial embolization[10] involves multiple focal ischemic lesions. Furthermore, the permanent occlusion of many small vessels compromises the natural adjustments of the collateral cortico-pial circulation[18] and differentiates this model from the usual pathophysiological course of cerebral ischemia in man[19].

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The effect of cyclic AMP on dog renal function[1]

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Summary. The infusion of dibutyryl-cyclic AMP into the dog renal artery in vivo leads to diuresis, natriuresis and glucosuria. Addition of the nucleotide to the incubation medium bathing dog renal cortex slices in vitro causes inhibition of p-amino-hippurate accumulation and stimulation of glycine and β-methyl-glucoside transport. The results are interpreted in terms of the development of a blood-lumen flux of sodium and water in the renal proximal tubule, analogous to that seen in the intestine.

It is well established that a rise in the intracellular concentration of cyclic AMP in enterocytes is associated with an increase in the blood-lumen fluxes of sodium and chloride[2], though it is still unclear how the nucleotide exerts these effects. The direct influence of cyclic AMP on renal electrolyte transport mechanisms has not been examined in such detail; for that reason, we have studied the response of the dog kidney to an administration of the nucleotide via the renal artery using a recently described technique[3].

Methods. The experiments were performed on 4 mongrel dogs weighing around 30 kg. At the first intervention, the right kidney was removed and the left kidney was transplanted into the right iliac fossa, by means of end-to-end anastomoses to the iliac vessels[3]. 6–10 weeks later, the dog was re-anaesthetized, and the jugular vein was cannulated for the constant infusion of creatinine and p-amino-hippurate (PAH). After laparotomy, the ureter was cannulated, the sacral artery was ligated, and a catheter for the infusion of cyclic AMP was inserted[3]. For the 1st h, Krebs bicarbonate buffer alone was infused into the kidney at a rate of 0.5 ml/min; then dbcAMP (cyclic N^6,2′-O-dibutyryl-adenosine 3′:5′-monophosphate) was added to the infusion fluid at a dose level of 6 mg/kg · h and the infusion was continued for a 2nd h. Plasma and urine samples were taken every 15 min throughout the experiment for the determination of creatinine and PAH clearances, and sodium, potassium, chloride and glucose excretion rates.

At the end of the experiment, the kidney was excised for examination in vitro. Renal cortex slices of 0.4 mm thickness were incubated for 1 h in a ^{14}C-labelled solution of glycine, β-methyl-D-glucoside or p-amino-hippurate at a concentration of 0.1 mM and the uptake of these substrates into the tissues was determined as described previously[4].

The effect of an addition of dbcAMP to the incubation medium on the uptake of the above substrates was assessed in a separate series of experiments using cortex slices from normal dog kidneys. Incubations were also performed in a sodium-free, choline-substituted incubation medium in the presence and absence of dbcAMP.

Results. From the onset of the infusion of dbcAMP, there was a rise in the urine flow and a slight fall in the percentage reabsorption of sodium and chloride, but no consistent modification in the glomerular filtration rate. These changes were accompanied by a marked glucosuria (figure 1). When the perfused kidney was excised and studied in vitro, the uptakes of glycine, β-methyl-glucoside and PAH into cortical slices all fell within the normal range (results not shown).

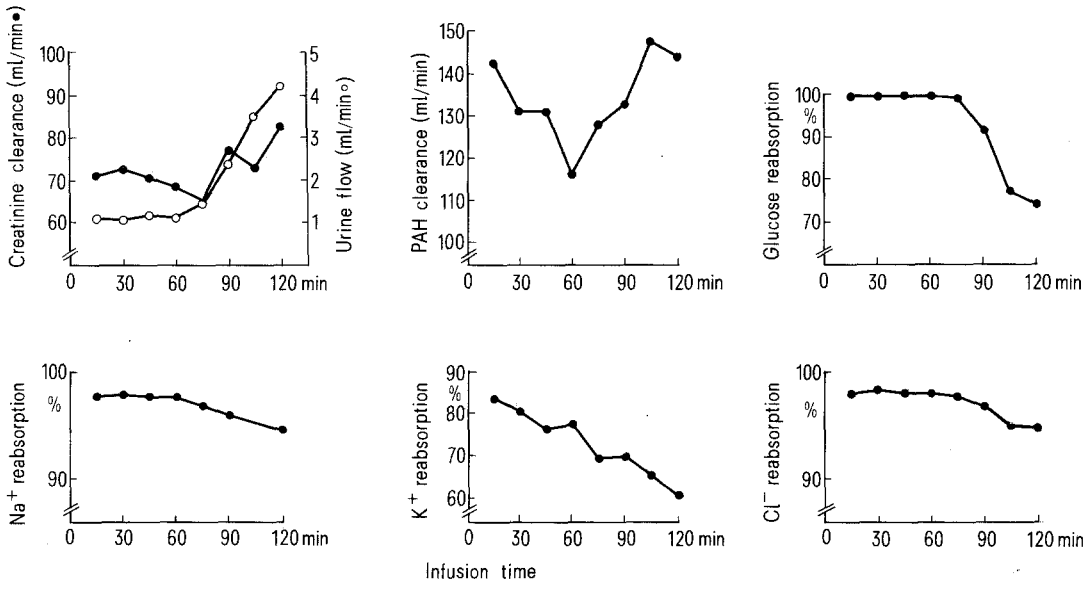

Fig. 1. Clearances after infusion of dbcAMP. During the 1st 60 min, the kidney received Krebs bicarbonate buffer, and for the 2nd h, the buffer contained dbcAMP at a dose of 6 mg/kg · h. The exogenous creatinine and PAH clearances were determined from blood and urine levels following i.v. infusion of the substances[3]. The results are the means of 4 experiments.

On the other hand, when dbcAMP was added to the incubation medium in vitro, a very marked inhibition of PAH accumulation in the presence of sodium ions was observed (figure 2). In contrast, the nucleotide enhanced the uptakes of glycine and β-methyl-glucoside, an effect that was maintained, albeit at a low level, in the absence of sodium ions.

Discussion. The changes in net water and electrolyte flows that occur when dbcAMP is delivered into the tubular lumen are reminiscent of those observed in the intestine. In agreement with the findings of other authors[5,6], diuresis and natriuresis develop, perhaps as a result of a stimulation of the blood-lumen fluxes of sodium, chloride and water, as has been demonstrated in the gut[2]. In our experiments, there was no great change in the glomerular filtration rate, though it certainly did not fall, as claimed by other workers[7]. The most important development provoked by the infusion of dbcAMP was glucosuria; this was apparently not studied in previous investigations on the effect of dbcAMP on the kidney, although its appearance has been described following renal infusion of cholera toxin or prostaglandins which are believed to act by virtue of an increase in intracellular cAMP[8,9].

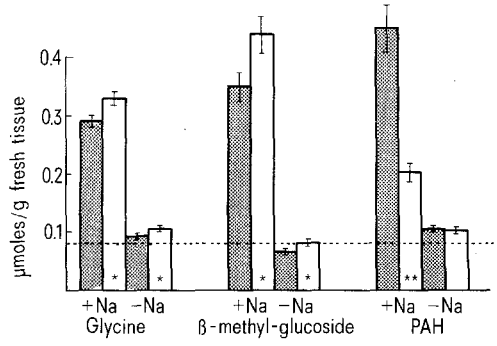

Fig. 2. Uptake of organic solutes by normal renal cortex slices in the presence (open columns) and absence (striped columns) of 1 mM dbcAMP. The effects of the nucleotide are significant at the 5% level (*) or 1% level (**), according to paired data analysis. The results are means ± SEM of 7 experiments. The dotted line represents a distribution ratio of unity.

The stimulation of sugar and amino-acid transport in renal cortex slices by cyclic AMP also finds an analogy in the small intestine[10,11]. The enhancement of organic solute accumulation in an enterocyte in the secretory state, such as occurs after treatment with cholera toxin, has been interpreted in terms of decreased efflux across the basolateral membrane because of the collapse of the intercellular spaces[11]. If this explanation also holds true in the renal proximal tubular cell, it could also help to elucidate the reasons for the glucosuria: if glucose efflux across the peritubular membrane is hindered, the net passage of sugar across the cell will be reduced and glucosuria will result. On the other hand, the maintenance of the enhancement of sugar and amino-acid accumulation in the absence of sodium ions, which contradicts reports of other workers[12], is difficult to reconcile with this interpretation, unless sufficient sodium was trapped in the interstitial spaces at the onset of the experiment to permit at least partial collapse of the paracellular pathways on contact with dbcAMP.

If the effective area of the peritubular membrane is reduced by a collapse of the intercellular spaces, the passage of substances across this membrane will be greatly diminished. This explanation might be sufficient to account for the inhibition of PAH accumulation by cAMP. However, it has also been claimed that cAMP is a competitive inhibitor of PAH uptake into rabbit kidney cortex slices[13]; such an effect would clearly contribute to the large inhibition caused by the nucleotide, but in this case, it is surprising that no inhibition occurs in the absence of sodium ions.

In conclusion, the results of the present experiments can most likely be explained in terms of an action of cAMP in provoking a flux of sodium, chloride and water across the proximal tubular cell from blood to lumen, analogous to that known to occur in the small intestine when intracellular cAMP levels are increased. Since the epithelium of the proximal tubule is relatively homogeneous, this hypothesis would indicate that both lumen-blood and blood-lumen

fluxes of sodium are mediated by a single cell type. In the intestine, it has been proposed that secretion is a function of the crypt cells and absorption a property of the villus epithelium[2]; it appears to us to be more likely[14] that each cell possesses both transport processes but their relative capacities change during maturation. The present observations with the kidney appear to justify the assumption that a single cell can harbour both absorptive and secretory mechanisms.

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Significance of the variation in haemolymph copper-protein ratio in the crab *Scylla serrata* (Forskal) during different hours of the day

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Summary. In *Scylla serrata,* haemolymph copper is bound to protein and it can be more reliably determined by the 2,2′ biquinoline method than by other spectrophotometric methods. Ionic or free copper is absent from the haemolymph. The lack of significant time-of-day variation in copper concentration and the occurrence of variation in total protein concentration and copper-protein ratio, indicate fluctuations in copper-free proteins, which may either be periodically sequestered or released by the tissues during different hours of the day.

A number of authors have shown that factors that influence the haemolymph protein concentration of decapod also affect the haemolymph copper concentration[1-3]. Since the amount of ionic copper in the haemolymph of decapods is negligible[4,5] a strong association of haemolymph copper with protein is indicated. The copper-bound proteins have been shown to range from 17% to 93% of the total haemolymph protein concentration[1-3,6,7]. The copper-bound proteins are also shown to vary under the influences of various physiological conditions like moulting and starvation[1,3], suggesting that the haemolymph copper/protein ratio is not a constant feature under these conditions. The variations in copper/protein ratio would thus indicate the relative fluctuations in copper-bound proteins and copper-free proteins under the respective conditions. Therefore, the haemolymph copper/protein ratio was studied at different times of day in *Scylla serrata,* in which the haemolymph protein has been shown to vary in relation to the time of day[8,9], after selecting a reliable and consistent method for determination of total haemolymph copper concentration.

For this purpose normal, intermoult, male specimens of the crab *Scylla serrata* (Forskal) of 175–200 g were used. Haemolymph was collected directly into a micropipette after cutting the tip of the dactylus. The sodium diethyl dithiocarbamate (SDDC) method[10], the oxalyl dihydrazide (ODH) method[11]and the 2,2′ biquinoline (BQ) method[12] were compared for determination of total copper in the haemolymph. In the first 2 methods copper was liberated from haemolymph protein by incubating in 6 N HCl[13]. Results presented in table 1 reveal that the ODH method gave poor results. With the SDDC method the absorbance after adding amyl alcohol-ether was highly variable. The BQ method was precise, consistent and reliable. This method has also previously been employed by a number of crustacean hematologists[2-5]. The haemolymph copper concentration determined in 12 crabs ranged from 35.0 to 153.3 µg/ml. This is in accordance with the range reported for other decapods[2,3,16]. No ionic copper is found in the haemolymph, confirming earlier reports[4,5]. The haemolymph protein concentration, determined by Biuret method[17] ranged from 27 to 150 mg/ml.

To study the effect of time of day on haemolymph copper and protein concentrations, the animals were kept tied and placed in plastic buckets containing 3 cm of water. Soon after haemolymph collection, the wound was cauterized. After 2 h, another appendage was cut; the same amount of haemolymph sample was collected 5 times at intervals of 2 h from one and the same animal. 5 animals were used to collect the data. The experimental animals were alive and active after the experiments. Table 2 shows the results of measurements of time-of-day variation in haemolymph copper, protein concentration and copper/protein ratio.

The copper concentrations remained somewhat constant throughout, whereas the protein concentration increased from an initial value of 53.2 ± 8.7 mg/ml at 10.30 h to 86.2 ± 7.4 mg/ml at 12.30 h and declined steadily thereafter. The pattern of variation in haemolymph protein concentration confirms an earlier report on *Scylla serrata*[9].

Analysis of variance of the results pertaining to copper and protein concentration showed that the influence of the time of day on haemolymph copper is insignificant. The influence on haemolymph protein concentration is, however, significant at $p = 0.05$ level, confirming earlier reports on *Scylla serrata*[8,9]. The significant variation in the haemolymph protein concentration could be due to injury and/or the loss of haemolymph. To what extent the protein concentration as well as the copper concentration were influenced by injury and/or the loss of haemolymph was