injected via the left vertebral artery, filled the vascular bed of the left neck muscles (Figure 1). In the basilar artery, particularly at its caudal end, a mixture of blue and red material was observed. Mixed material was also found in the territories which were retrogradely filled via the right vertebral artery.

However, the smaller vessels of the brain, even in the ponto-medullary area were filled with red-coloured methylmethacrylate coming from the carotid artery (Figure 2).

In conscious, renal hypertensive rats, administration of α-methyldopa by the i.p. route (10–800 mg/kg) caused a dose-dependent decrease in blood pressure (7–53 mm Hg). After infusion of α-methyldopa (25 mg/kg) into the jugular vein also a fall in blood pressure was observed. Blood pressure decreased gradually with a maximum 5 h after starting the infusion (-27 ± 5 mm Hg). The decrease in blood pressure after infusion of the same dose of α-methyldopa into the vertebral artery was less pronounced (-16 ± 3 mm Hg after 5 h). The difference, however, was not significant at 2–6 h. Intravertebral fusion of α-methyldopa in a dose of 10 mg/kg caused no change in blood pressure.

Discussion. The blood pressure lowering effect of α-methyldopa in the rat is mediated by a central action of the drug[16,18,19] and is still present after midcollicular transection of the brainstem[20]. In cats, and to a lesser degree in dogs, the major part of vertebral artery blood goes to the ponto-medullary structures of the brain[10–12]. In cats, the intravertebral injection of α-methyldopa lowers the blood pressure at doses which do not lower blood pressure after i.v. injection[21]. We found no differences between intravertebral and i.v. administration of a low dose of α-methyldopa in renal hypertensive rats. An explanation for this finding was provided since the radioactive microspheres, injected into the vertebral artery of the rat were mainly found in the neck muscles on the injected side and not in the brain. This means that drugs, when injected in a similar way, will arrive mainly in the extracranial tissues. Consequently, a centrally induced hypotensive effect of α-methyldopa cannot be demonstrated with this injection technique in rats. The quantitative findings of microsphere distribution were further confirmed by cerebro-vascular methylmetacrylate casts of the rat. In these casts, the sidebranches of the basilar artery providing the pontomedullary structures were mainly filled by material injected via the common carotid artery. It is concluded that injection of compounds into the vertebral artery of the rat is not an appropriate procedure for the evaluation of their pharmacological activity at the level of the rhombencephalic structures.

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Effect of (+)-Catechin on Renal and Intestinal Transport

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Summary. The ability of dog renal cortex slices to accumulate β-methyl-glucoside or glycine is enhanced by the flavonoid (+)-catechin at a concentration of 3.5 m*M*. This stimulatory effect is apparently due to a decreased rate of efflux of either substrate. On the other hand, the uptake of p-amino-hippuric acid and N^1-methyl-nicotinamide is inhibited by (+)-catechin. The drug at the same concentration is without action on amino-acid transport by guinea-pig intestine in vitro.

The therapeutic and pharmacological effects of flavonoids have been related to their action at the level of the vascular endothelium[2], and some of these effects may be a consequence of the interaction of these drugs with membranous structures.

Certain effects of flavonoids on membrane permeability have been studied. Tesi and Forssmann[3] have shown that the passage of inulin across isolated rat mesentery was inhibited by a soluble derivative of (+)-catechin, namely Na(+)-epicatechin-2-sulphonate. Ring et al.[4] demonstrated that the passive penetration of thiourea and amino-acids into bacterial and animal cells was reduced by (+)-catechin, whereas the polar derivative used in above-mentioned study had no effect. In the present paper, we report the effect of (+)-catechin on the active transport and accumulation of substrates in epithelial cells of renal cortex and small intestine in vitro.

Methods and materials. Studies on renal cortex slices: The experiments were performed on kidneys from healthy mongrel dogs. Much of the methodology closely follows that described by Robinson[5]. After removal of the renal capsule, the cortex was dissected and cut with a spring-loaded guillotine[6] into slices of 0.4 mm thickness. The uptake of β-methyl-glucoside, glycine, *p*-amino-hippuric acid (PAH) or N^1-methyl-nicotinamide (NMN) was determined after incubation of the tissue slices in a solution of the labelled substrates in Krebs bicarbonate buffer at 37 °C for 60 min. (+)-Catechin {(+)-cyanidanol-3} was added at a concentration of 3.5 m*M* to the appropriate solutions. After the incubation, the slices were briefly

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rinsed, blotted, weighed and dissolved in 0.1 ml 30% KOH, prior to radioactivity determination[7]. Efflux of a previously accumulated substrate was determined as described by SEGAL et al.[8]. The slices were incubated as for the uptake experiments for 60 min, after which the tissues were removed, blotted and transferred to a thermostatically controlled double-walled chamber containing 5 ml Krebs buffer. Gas (95%/5% O_2/CO_2) was bubbled from below to ensure mixing as well as oxygenation. 50 µl samples of the incubation fluid were taken at 10 min intervals. After 40 min, the slices were removed and their radioactivity was assessed. By adding together the quantities in the medium and in the tissue at 40 min, a value of R_o, the original activity in the tissue at the beginning of the second incubation, was calculated. The results are presented graphically as $1 - Rt/Ro$ against t on a normal or a log scale, where Rt is the activity in the tissue at time t.

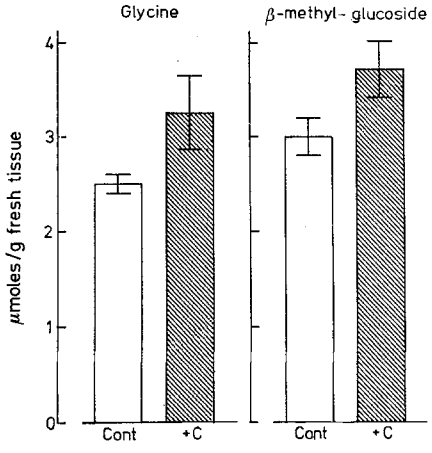

Fig. 1. Effect of (+)-catechin on glycine and β-methyl-glucoside uptake by dog renal cortex slices. Tissues were incubated for 1 h in either 0.1 mM substrate with or without 3.5 mM (+)-catechin. Results are the means of 6 experiments ± SEM. The stimulatory effect of (+)-catechin is significant at the 1% level.

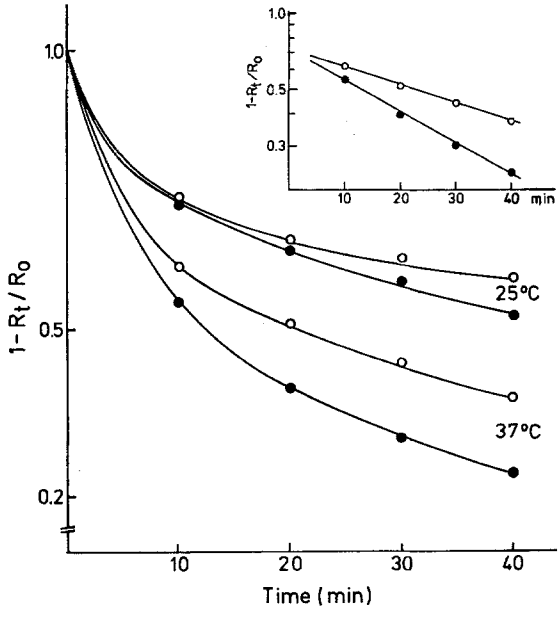

Fig. 2. Efflux of accumulated β-methyl-glucoside from dog kidney cortex slices. The tissues were preincubated at 37 °C in 0.1 mM ^{14}C-labelled β-methyl-glucoside in Krebs buffer for 1 h, then the slices were transferred to solutions with (○) or without (●) 3.5 mM (+)-catechin regulated either at 25 or 37 °C. Results are expressed as the fraction of initial radioactivity that remains on the tissue at a given time ($1 - Rt/Ro$). In the inset, results at 37 °C are presented on a semilogarithmic plot from which the time constants of efflux can be calculated. Results are the means of 6 experiments.

For studies on the small intestine, sections were removed from anaesthetised guinea-pigs and cut into rings. Initial velocity of uptake or accumulation at the steady-state was determined for L-phenylalanine as described previously[9].

Results. The uptake of glycine and β-methyl-glucoside by dog kidney cortex slices after 60 min incubations is shown in Figure 1. Their uptakes were significantly stimulated when (+)-catechin was added to the incubation medium ($p < 0.01$).

Efflux of β-methyl-glucoside from preloaded tissues was examined and the results are shown in Figure 2. The rate of β-methyl-glucoside efflux from slices was diminished when the experiment was performed in a medium containing (+)-catechin. The effect was smaller when the efflux was measured at 25 °C; at this temperature the efflux was diminished in accordance with previous findings[10], but the effects of the drug and the temperature are seen to be additive. As shown in the inset, the efflux data can be fitted to a closed two-compartment system. The rate constant of efflux of β-methyl-glucoside at 37 °C, calculated from the slopes of these two lines, decreased from 0.0289 min^{-1} in control efflux experiments to 0.0161 min^{-1} in experiments performed in the presence of 3.5 mM (+)-catechin. A similar action of the drug on glycine efflux was also observed (result not shown).

The effect of (+)-catechin on the uptake of PAH and NMN was also studied: These uptakes were very significantly inhibited by the drug ($p < 0.001$), as shown in Figure 3.

The possible influence of (+)-catechin on intestinal transport was also tested. The drug had no significant effect on the equilibrium uptake of L-phenylalanine by guinea-pig intestinal rings, the accumulations being respectively 8.1 ± 0.71 and 8.8 ± 0.58 µmoles/g fresh tissue ($n = 6$) in the absence and presence of 3.5 mM (+)-catechin. In addition no effect of the drug was detected on the initial rate of influx of the amino-acid across the brush-border membrane during 2 min incubations.

Discussion. The results presented in this study show that (+)-catechin is able to stimulate sugar and amino-acid accumulation in vitro by renal cortex slices. This action appears to be due to an inhibition of the efflux processes of the substrates. A similar explanation has been suggested to account for the differences in steady-state uptake of α-methyl-glucoside at 25 and 37 °C in rat kidney cortex slices[10], a fact that is confirmed in Figure 2.

Although (+)-catechin appears to influence the efflux of glycine and β-methyl-glucoside, the actual localisation of these effects at a given anatomical site remains uncertain. The exit of sugars and amino-acids from the proximal cell appears to occur primarily across the lateral/basal membrane[11,12]. That fact suggests that

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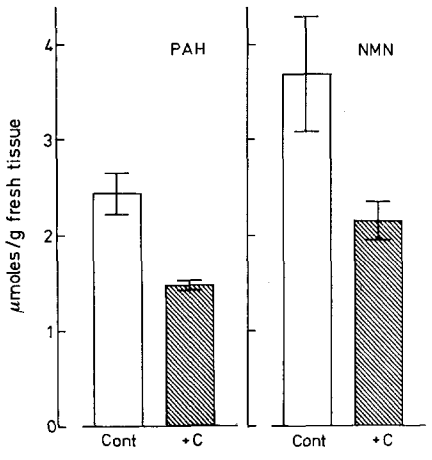

Fig. 3. Effect of (+)-catechin on PAH and NMN uptake by dog renal cortex slices. Tissues were incubated for 1 h in 0.1 m*M* PAH or 0.1 m*M* NMN with or without 3.5 m*M* (+)-catechin. The inhibitory effect of (+)-catechin is significant at the 0.1% level in both cases. Results are the means ± SEM of 6 experiments.

(+)-catechin may act preferentially on this membrane, where the transport mechanisms for NMN and PAH are thought to be located[13–15]. The inhibition of the uptake of both these substrates by (+)-catechin provides further support for the hypothesis of an interaction with the basal membrane.

Ring et al.[4] suggested that (+)-catechin reduces the passive permeability of membranes by interaction with some of the lipophilic membrane components. Such an explanation would agree with the decreased passage of sugars and amino-acids across the peritubular membrane. Equally, a direct effect of (+)-catechin at that locus would affect the accumulation of PAH and NMN across that membrane. It remains to be elucidated why (+)-catechin appears specifically to affect the peritubular membrane, rather than that of the brush-border. It is probable that the different compositions of the two membranes may be responsible for this specificity.

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Histamine Receptors in the Cat Mesenteric Circulation

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Summary. The distribution of histamine receptors was studied in the isolated perfused vascular bed of the cat terminal ileum. The results indicated that the depressor effect of histamine is mediated through the stimulation of metiamide-sensitive H_2-receptors, while the pressor effect of the amine is mediated by the stimulation of mepyramine-sensitive H_1-receptors.

The distribution of histamine receptors in the tissues could easily be studied using classically known H_1 and recently discovered H_2-receptor blockers[2,3]. Both receptors have been shown to be present in different regional vessels. Using H_2-receptor blockers, we have recently shown the presence and the role of H_2-receptors in the vascular bed of the guinea-pig lung[4], rabbit kidney[5], guinea-pig heart[6] and rabbit ear[7].

In continuing these studies, we recently indicated the presence of histamine H_2-receptors in the mesenteric circulation of the cat terminal ileum. The present report contains the data of this investigation.

Material and method. The terminal ileums from adult mongrel cats, anesthetized with sodium pentobarbital (30 mg/kg i.v.), were isolated according to the method described previously[8,9]. The ileal segment was perfused with oxygenated (5% CO_2 in O_2) and warmed (37 °C)

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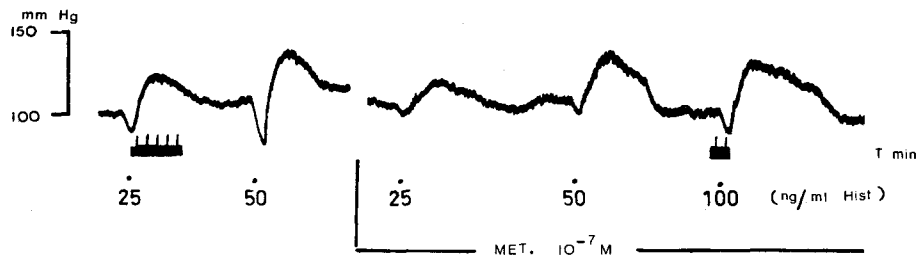

Fig. 1. A recorder tracing fromisolated perfused cat terminal ileum showing the effect of histamine on perfusion pressure before and after addition of metiamide (Met) to the perfusion medium.