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## Vascular Volume, Blood Flow, and Resistance in Dog Kidneys During Constriction of the Renal Artery* (33877)

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It is generally accepted that renal blood flow remains relatively constant despite considerable changes in arterial pressure. Previous studies by Selkurt *et al.* (1) and by Shipley and Study (2) indicated that the constant blood flow is primarily due to changes in tone of the afferent arterioles while efferent arteriolar and postarteriolar resistance remain essentially unchanged. The observations by Thurau and Wober (3) were in accordance with these conclusions: they found a constant pressure in proximal convolutions and peritubular capillaries during autoregulation. The studies by Schmid *et al.* (4) indicated, however, that a change in postglomerular pressure contributed a little, although the results did not give support to the tissue pressure theory especially argued for by Hinshaw (5).

Recent investigations in man by Pedersen and Ladefoged (6) indicate that a greater part of the renal vascular bed other than the afferent arterioles changes its volume during regulation of blood flow in hypertension and renal artery stenosis. The aim of the present investigation was to measure to what extent renal vascular volume changes with resistance during constriction of the renal artery in acute experiments.

*Material and Method.* Three dogs, each weighing approximately 20 kg, were anesthetized by injecting 30 mg/kg of pentobarbital intravenously. Following endotracheal intubation, the left kidney was exposed by an oblique incision below the left curvature and the renal artery was isolated. A curved needle cannula was introduced into the artery peripheral to a tourniquet and pointed against the direction of the blood. The pressure in the renal artery was determined by connecting the cannula to a strain gauge manometer. The pressure in the aorta was measured by passing a catheter into the aorta via the femoral artery. After an injection through the cannula of 1–2 mCi of $^{133}Xe$ into the renal artery, renal blood flow was determined with the $^{133}Xe$ desaturation method described by Ladefoged (7). Mean circulation time for blood through the kidney was measured by use of $^{131}I$-albumin as described by Pedersen

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TABLE I. Cortical Vascular Volume in Dog Kidneys with Constriction of the Renal Artery.[a]

| Dog no. | Period no. | MABP (mmHg) | RABP (mmHg) | $cRBF_{Xe}$ (ml/g·min) | $\bar{t}_{pl}$ (sec) | $V$ (ml/100 g) |
|---|---|---|---|---|---|---|
| | | | Control | | | |
| $S_{II}$ | 1 | 125 | 115 | 5.4 | 3.0 | 27 |
| | | | Constriction of renal artery | | | |
| | 2 | 121 | 103 | 6.0 | 3.3 | 33 |
| | 3 | 122 | 65 | 6.0 | 3.4 | 34 |
| | | | Control | | | |
| $S_{III}$ | 1 | 125 | 113 | 6.3 | 2.2 | 23 |
| | | | Constriction | | | |
| | 2 | 138 | 62 | 3.5 | 5.4 | 32 |
| | 3 | 140 | 52 | 5.4 | 3.6 | 32 |
| | | | Control | | | |
| | 4 | 153 | 135 | 6.0 | 2.4 | 24 |
| | | | Constriction | | | |
| | 5 | 130 | 52 | 5.2 | 4.9 | 42 |
| | 6 | 155 | 58 | 4.1 | 4.5 | 31 |
| | | | Control | | | |
| $L_I$ | 1 | 128 | 116 | 4.8 | 4.3 | 34 |
| | | | Constriction | | | |
| | 2 | 129 | 56 | 4.0 | 4.0 | 27 |
| | 3 | 152 | — | 4.5 | 4.0 | 30 |
| | 4 | 144 | — | 2.5 | 9.9 | 41 |
| | | | Control | | | |
| | 5 | 108 | 108 | 3.3 | 3.1 | 17 |

[a] Blood flow, mean circulation time for plasma, and vascular volume in the cortex of dog kidneys during control periods and during constriction of the renal artery. Abbrev.: MABP = mean arterial blood pressure; RABP = mean renal arterial blood pressure behind stenosis; $cRBF_{Xe}$ = renal cortical blood flow; $\bar{t}_{pl}$ = mean circulation time for plasma; and $V$ = renal cortical vascular volume.

and Baerenholdt (8). For both determinations a scintillation detector was placed 5–7 cm over the kidney in a collimator with such a bore that the collimator field comprised the whole kidney. The renal vascular volume, $V$, was calculated from the formula $V = cRBF_{Xe} \cdot t_{pl}$, where $cRBF_{Xe}$ denotes the renal cortical blood flow in ml/g·min calculated from the most rapid component of the $^{133}Xe$ wash-out curve, and $t_{pl}$ is the mean circulation time for $^{131}I$-albumin through cortex. The vascular volume is given in ml/100 g. The time of passage through the kidney for albumin being somewhat slower than for whole blood results in a small systematic overestimate of the size of the vascular volume [Zierler, (9)].

The measurements were performed first during a control period, then during 2–4 periods where the renal artery was constricted to varying degrees. Two of the experiments were followed by another control period.

In dog $L_1$, the pressure behind the stenosis could not be obtained during the last two constriction periods possibly because the cannula tip was located against the vessel wall.

The renal conductance to flow, $L$, was calculated as $L = cRBF_{Xe}/P_a$, where $P_a$ is the pressure in the renal artery measured via the cannula. Renal vein pressure was ignored. The kidney was removed and weighed after the experiment.

*Results.* The results appear in Tables I and II. During the control periods, the mean blood pressure averaged 128 mm Hg in the aorta and 117 mm Hg in the renal artery.

TABLE II. Mean Values of Data in Table I.

| Periods (no.)[a] | | MABP (mmHg) | RABP (mmHg) | $cRBF_{Xe}$ (ml/g·min) | $\bar{t}_{pl}$ (sec) | $V$ (ml/100 g) |
|---|---|---|---|---|---|---|
| Control | Mean | 128 | 117 | 5.2 | 3.0 | 25 |
| (5) | SD | 16.1 | 10.3 | 1.2 | 0.8 | 6.2 |
| Constriction | Mean | 137 | 64 | 4.6 | 4.8 | 34 |
| (9) | SD | 12.3 | 17.8 | 1.2 | 2.0 | 5.0 |
| $t$[b] | | 1.18 | 5.92 | 0.91 | 1.86 | 3.00 |
| $p$ | | >0.20 | <0.01 | >0.30 | 0.05–0.10 | 0.01 |

[a] Number of periods is given in parentheses.

[b] $t$ calculated for samples with different numbers in each group: $t=(\bar{x}_1-\bar{x}_2)\{[n_1 n_2(n_1+n_2-2)]/[(n_1+n_2)\Sigma x^2]\}^{1/2}$.

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The cortical blood flow as 5.2 ml/g·min, and the mean circulation time for plasma was 3.0 sec. The vascular volume was 25 ml/100 g of renal tissue. After constriction of the renal artery, the pressure there fell on an average to 64 mm Hg, whereas the pressure in aorta was unchanged. The cortical blood flow did not change significantly, but the mean circulation time for plasma was increased to 4.8 sec. The vascular volume rose from 25 to 34 ml/100 g, which is significant ($p < 0.01$-$.02$). At the end of the experiments the respective kidney weights were 61, 65, and 47 g.

The relation between the renal conductance of flow, $L$, and the cortical vascular volume, $V$, from control and test periods appears in Fig. 1. There was a significant positive correlation, $r = 0.67$, $p < 0.05$.

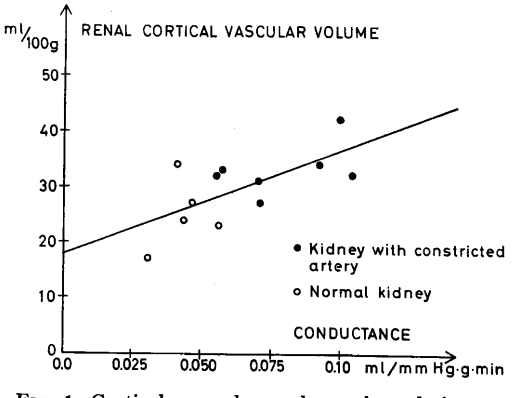


FIG. 1. Cortical vascular volume in relation to cortical vascular conductance in control periods and during constriction of the renal artery. The correlation line $V = 0.018$ liter $+ 17.95$ is shown.

*Discussion.* Previous works rendered it probable that by use of the external counting technique, the mean circulation time and the blood flow apply to blood passing through the renal cortex [Pederson *et al.* (10); Ladefoged *et al.* (11)]. The vascular volume consequently comprises the blood in cortex and in those parts of the renal arteries and veins that are situated within the collimator field. During the control periods, the vascular volume averaged 25 ml/100 g, which agrees with previous measurements of the vascular volume in kidneys of the dog [Selkurt (12) and Ofstadt (13)]. When the artery was constricted, which caused the perfusion pressure to fall, the vascular volume increased on the average from 25 to 34 ml/100 g. As cortex makes up about 70% of the total kidney weight [Weaver *et al.* (14)], and as the kidney weights in the present experiments averaged 57 g, this change in vascular volume per 100 g must mean either an increase in the total volume by approximately 4–5 ml, or the unlikely possibility of an acute fall in the weight of cortex by 26%. Though the kidney weight changes with the perfusion pressure [Swann (15)], it does not likely change to the stated degree. A decrease in total volume of 2–3% was observed in the pressure range of the "full" organ to the value at 64 mm Hg. The present findings therefore indicate that the renal vascular volume increases when a renal artery is acutely stenosed. It is obvious that this big increase in volume is not due only to a dilatation of the afferent arterioles. The increase must be accomodated

by the glomerular bed and possibly postefferent arteriolar vessels.

Applying the same principle as used in this investigation, Lochner and Ochwadt in 1954 (16) measured the renal vascular volume in dogs. They found that the vascular volume diminished with increasing perfusion pressure and that the resistance rose simultaneously. Their conclusion consequently was that an active vascular reaction was contributing to the regulation of the renal blood flow. Lochner and Ochwadt's investigations as well as ours thus show that, when the perfusion pressure varies, changes in the resistance are accompanied by changes in the size of the vascular volume, as shown in Fig. 1. However, none of the investigations substantiates the idea that the reduced resistance is caused by the demonstrated vasodilatation, although Poiseuille's law for laminar flow might be an indication thereof. Under certain circumstances, the resistance and the vascular volumes in the kidney seem able to vary independently. Thus, Mehrizi and Hamilton (17) found that during infusion of levarterenol the vascular volume increased simultaneously with a rise in the resistance. This was probably due to a contraction of the veins and a consequent dilatation of the prevenous vessels. The contributions to the total resistance by the individual vascular segments must therefore be independently variable, and, according to the above, the distribution of the resistance in the individual segments must differ during autoregulation and during influence of levarterenol.

Our finding that a normal blood flow is maintained in kidneys with pronounced arteriostenosis indicates that vasodilatation takes place in the afferent arterioles, but the dimension of the dilatation indicated by the volume change suggests that other vessels—probably capillaries and veins—are dilated, too. Similar changes have been observed in the hind limb of the cat by Mellander (18).

The possible duration of such vasodilation is not known. It is possible that securing an adequate blood flow in the long run necessitates development of a collateral circulation.

It has been shown in hypertensive patients with renal artery stenosis that the vascular volume was higher in severely stenosed kidneys than in the contralateral ones and in kidneys with moderate stenosis; but compared with normal kidneys, no actual dilatation was demonstrable [Pedersen and Ladefoged (6)]. Long-termed experiments with dogs may possibly show the same.

*Summary.* The vascular volume in dog kidneys was determined by combining injections into the renal artery of $^{133}Xe$ and $^{131}I$-albumin with an external counting technique. During control periods, where the renal blood flow averaged 5.2 ml/g • min, the vascular volume was 25 ml/100 g of cortex. In the present investigation the vascular volume was systematically overestimated, by a few percent, for technical reasons.

Constriction of the renal artery resulting in a fall in the perfusion pressure from 117 to 65 mm Hg caused no change in the blood flow. The decrease in resistance during constriction of the artery was followed by a dilation of the renal vessels, and the vascular volume in the kidney rose from 25 to 34 ml/100 g, when the artery was stenosed. A dilation reflected by this volume change indicates that other vessels than the afferent arterioles expand their volumes, and suggests the possibility that the postglomerular resistance most likely is also reduced.

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# Biosynthesis of Lysophosphatidic Acid from ATP and 1-Monoolein by Subcellular Particles of Intestinal Mucosa* (33878)

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In a previous report (1), we presented evidence for the biosynthesis of lysophosphatidic acid by rat intestinal mucosa during absorption of a mixture of doubly labeled 1-monopalmitin ($^{14}C$ glycerol and $^{3}H$ palmitic acid) and free fatty acids. Isotopic ratios of both phosphatidic and lysophosphatidic acids indicated that direct phosphorylation of the 1-monopalmitin occurred, followed by acylation to produce phosphatidic acid. This result was obtained under *in vivo* conditions and requires additional *in vitro* evidence. For this purpose, we have adopted an experimental procedure described by Pieringer and Hokin (2) for brain and liver homogenates, modified as follows: 98% pure ATP$\gamma^{32}P$ (C.E.A., Saclay, France) is substituted for the ATP generating system and incubations are carried out with intact subcellular particles (i.e., microsomes and mitochondria) instead of their deoxycholate extracts.

*Materials and Methods.* Subcellular fractions were obtained by fractional sedimentation of homogenates of the intestinal mucosa of 6 rats by the method of Hübscher *et al.* (3). Homogenates were prepared from mucosal scrapings and 0.3 *M* sucrose, pH 7.4 (1/9, w/v).

Conditions of incubation are described in Table I. The reaction was stopped by addition of 16 ml of methanol followed by 64 ml of methylal. After 2 hr the mixture was filtered, dried over $Na_2SO_4$ and concentrated under vacuum at a temperature not exceeding 50°. Total $^{32}P$ radioactivity of the extract was determined by liquid scintillation counting of an aliquot.

Fractionation of the total extract into different phospholipid classes was carried out by thin-layer chromatography on Kieselgel G using chloroform–methanol–water (65/25/4, v/v/v) as the developing solvent. The different phospholipids were identified by comparison with known compounds [phosphatidic acid, cephalins and lecithins extracted from rat liver and lysophosphatidic acid synthesized according to the method of Kabashima (4)].

*Results and Discussion.* The data in Table I clearly show that incorporation of labeled phosphate is greater with 1-monoolein than with 1, 3-diolein as acceptor. From this observation, one can conclude directly that monoglyceride phosphokinase is present in the mucosa of the small intestine of the rat and that its activity is higher than that of diglyceride phosphokinase.

The distribution of the $^{32}P$ radioactivity on the different phospholipids separated by thin-layer chromatography (Expt. 1) clearly indicate that lysophosphatidic acid is the main product (83 and 71% with mitochondria and 76 and 68% with microsomes). This observation serves as additional proof for direct