lowing intravenous inoculation early in pregnancy. There was no indication that this virus is able to cross the placenta. It was, however, able to proliferate there and also in fetuses, if these were inoculated directly, late in gestation. Directly inoculated fetuses surviving birth developed illness characteristic of RV-infections in the suckling period. Rats inoculated at birth might develop an acute disease characterized by a distended intestinal tract and die within 8 to 12 days or survive 40 or more days, dwarfs in size and with deformed teeth. One individual developed an extensive hydrocephalus. Additional findings were the recovery of RV in relatively high titers from lung, intestine, and urine and the fact that sucklings inoculated directly *in utero* might develop RV-infection or disease, even though the mother was RV-immune.

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Threshold for Pressor and Renal Ischemic Effects of Angiotensin.* (29724)

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The threshold dose for slowly infused angiotensin in man is in the neighborhood of 2-5 mμg/kg/minute(1,2,3). The renal ischemic effect has a threshold perhaps somewhat lower than this: in the study of Bock, Dengler, Krecke and Reichel(1), an infusion rate of 2 mμg/kg/min, the lowest administered, still depressed the PAH clearance to 70% of normal. In their study, an indirect method of measuring renal blood flow was employed, that of PAH clearance. Because with reduced renal blood flows, the indirect methods sometimes give erroneous results, it was thought desirable to ascertain the threshold dose for the renal ischemic effect when measuring renal blood flows directly. At the same time the threshold dose for the pressor effect was ascertained, under our conditions, for comparison with the renal ischemic effect.

Methods. Dogs anesthetized with sodium pentobarbital, 30 mg/kg, were suspended in the prone position and both kidneys dissected free except for the hilar blood vessels and the ureter. The left ureter was cannulated so that urine flow could be observed. Then the right kidney was removed, leaving a good exposure of the right side of the vena cava. A shunt from the left renal vein to the jugular vein was next established: a special trocar

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TABLE I. Effects of Angiotensin.

No.	Control blood flow, ml/g/min	Minimal effective dose			Maximal effective dose		
		mμg/kg/min	Blood pressure rise, %	Blood flow fall, %	mμg/kg/min	Blood pressure rise, %	Blood flow fall, %
1– 7	2.1	2.35	11	23	1.17	0	17
2– 9	3.5	2.50	11	15	1.00	0	0
3–11	2.6	5.26	5	13	2.10	0	0
4–17	2.0	5.00	28	15	2.00	0	0
5–18	2.5	5.80	8	3	2.30	0	0
6–19	3.6	4.34	11	13	1.70	5	0
7–20	2.6	4.50	9	13	1.80	0	0
Avg	2.7	4.25	12	14	1.72	1	2

was thrust through the wall of the cava across its lumen and into the left renal vein, this being accomplished after a serrafine had been put on the left renal artery. The trocar was then connected to plastic tubing which led to a cannula placed into the right jugular vein. Flow was quickly established through the shunt from the renal to the jugular vein; usually no more than 2 to 3 minutes elapsed between the arrest of renal blood flow and its reestablishment. In the dog a valve leaflet is present at the ostium of the left renal vein. To cut through the cava wall and through this valve a special trocar, with a cutting point, was utilized.

In order to measure renal blood flow, a side-arm was placed in the special shunting tube. When blood flow was to be measured, the shunt was clamped so that all renal blood was diverted into the side-arm and thence into a graduate. Blood flow was measured during a 6-sec period. Then the jugular shunt was reestablished, the volume of blood which had been collected was measured, and the blood sample returned to the animal's general circulation. During the course of the experiment 2 ml per minute of 6% mannitol were slowly infused. Blood pressure was observed with a mercury manometer. Synthetic angiotensin† was infused into the contralateral jugular vein in a volume of ½ to 2 ml saline/minute by means of a syringe pump. Infusion periods lasted from 1 to 5 minutes: blood pressure was recorded continuously and renal blood flows were measured for 6 seconds during each successive one-minute period.

† "Hypertensin CIBA" furnished through the courtesy of CIBA Pharmaceutical Co.

Results. The preinfusion levels of blood pressure and of blood flow were given a value of 100%; the effects of angiotensin were then calculated in terms of deviations from this level. Table I shows results obtained in 7 dogs. Blood flows averaged 2.7 ml per gram per minute, with the lowest flow being 2.1 ml. The Table shows the minimal effective dose and the maximal ineffective dose of angiotensin for each dog. Infusion of 4.25 mμg/kg/min caused an average rise in blood pressure of about 12% and an average decrease in blood flow of about 14%. An infusion rate of 0.4 times this rate was found to be the maximal ineffective dose: as the Table shows, there was no rise in blood flow pressure and no renal ischemia with an infusing dose of 1.72 mμg/kg/min. The threshold then, for the pressor effect and the renal ischemic effect is somewhat less than 4.3 mμg and somewhat more than 1.7 mμg. We shall adopt the figure of 3 mμg/kg/min as the average threshold dose.

Discussion. The data are in good agreement with the levels found for man by Bock *et al*(1): infusion rates of 4 mμg/kg/min depress renal blood flow in both man, deduced from PAH clearances, and the dog, as measured directly. The thresholds for the renal ischemic effect and the pressor effect are at approximately the same level, 3 mμg/kg/min.

This finding has an interesting implication: the teleological reasoning that so often is used for angiotensin production is questioned by this experiment. It is often argued that angiotensin, following on renin secretion, raises the blood pressure, in response to renal ischemia, and the higher perfusion pressure then corrects the ischemia. However, it is

shown here that even at the threshold level of angiotensin infusion, angiotensin causes, apparently in and of itself, a renal ischemia. Hence, it may be tentatively argued, angiotensin, if it does get into the systemic circulation, does not correct renal ischemia but, if anything, depresses renal blood flow. (Part of its pressor effect is due to the fact that it constricts, among others, the renal vascular bed(4,5). In renal ischemia, then, its secretion would be, if anything, damaging. This general suggestion receives support from the persistent and continuing failure to obtain a consistent and wholly satisfactory proof for the presence of angiotensin in systemic blood, or even in renal venous blood, during the experimental renal hypertensions which follow partial constriction of the renal artery, etc. (6). It is, however, perhaps present in lymph (7,8).

Summary and conclusions. The threshold dose for both the pressor effect and the renal ischemic effect of angiotensin was found to be, in the dog, 3 mμg/kg/min. It is deduced that angiotensin, if it is produced in sufficient quantity to raise the blood pressure, would aggravate, not benefit, a pre-existing renal ischemia.

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Inhibition of Bone Collagen Synthesis by Salicylates.* (29725)

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Salicylates have frequently been employed in the treatment of connective tissue diseases (1,2). Several different salicylates have been used as analgesic and antipyretic agents in treatment of rheumatoid arthritis, and p-aminosalicylic acid has been used extensively in treatment of pulmonary tuberculosis. Many anti-inflammatory drugs, including certain salicylates, have been found to inhibit the metabolism of cartilage and other connective tissues both *in vivo* and *in vitro*(3).

Recent studies from our laboratory have dealt with the interaction between parathyroid hormone and bone metabolism. Therefore, we have been interested in possible inhibitors of bone formation as a means by which bone resorption could be studied apart from the simultaneous influences of formation.

The *in vivo* and *in vitro* use of radioactive amino acid precursors of bone collagen as indicators of bone and bone collagen formation is well documented(4-6). In the studies reported here, radioproline uptake into bone matrix was used to assess the formation of bone collagen, and evidence is presented which demonstrates that this formation is inhibited by salicylates.

Materials and methods. A. *Incubation of rat femora. Procedure.* Adult male Holtzman rats, 150-200 g, were used in these studies. Femoral metaphyseal bone chips were prepared by the method of Borle, Nichols, and Nichols(7). The preparations from both femora were pooled for each animal and incubated for 4 hours at 37°C with constant shaking in 3.0 ml pooled rat serum (pH 7.4).

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