min A within sufficient time to allow storage showed many green fluorescing inclusions in epithelial and Kupffer cells, and chemically more than 1000 international units of vitamin A per gram of liver tissue. Two other animals which received 3300 and 6600 units within 3 hours before killing showed only very little green fluorescence.

The adrenals of positive controls and deficient animals after repletion revealed many small droplets with green fading fluorescence in the epithelial cells of the cortex, especially in the middle layer. In the deficient animals no such green fluorescence was present.

In the liver of 6 newborn rats there was no green fluorescence in the cytoplasm and only a few fluorescing droplets in epithelial and Kupffer cells. This is in agreement with chemical determinations of Ellison and Moore.[4] In the adrenals there was no green fluorescence.

In rats deficient in vitamin B_1 (2 rats), B_2 (2 rats), and D‡ (4 rats) normal amounts of fluorescing inclusions were found in liver and adrenal.

The liver of rabbits, monkeys, dogs, guinea pigs, mice, and frogs revealed essentially the same findings as the liver of normal rats.

11121

Production of Renin by Constricting Renal Artery of an Isolated Kidney Perfused with Blood.

K. G. KOHLSTAEDT AND IRVINE H. PAGE.

From the Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana.

Experimental hypertension resulting from constricting the renal artery with a clamp (Goldblatt, Lynch, Hanzel and Summerville[1]) or as a result of perinephritis produced by cellophane or silk (Page[2]) is believed by many investigators to be of humoral origin and specifically to be caused by the liberation of renin from the kidneys. This renin may in turn interact with renin-activator (Kohlstaedt, Helmer

4 Ellison, J. B., and Moore, T., *Biochem. J.*, 1937, **31**, 165.

‡ For these animals I wish to thank Dr. H. J. Cannon, Director of the Laboratory of Vitamin Technology, Chicago.

1 Goldblatt, H., Lynch, J., Hanzal, R. F., and Summerville, W. W., *J. Exp. Med.*, 1934, **59**, 347.

2 Page, I. H., *Science*, 1939, **89**, 273.

and Page[3]) to produce a highly active pressor substance, angiotonin (Page and Helmer[4]). It is, therefore, important to know whether renin is in fact liberated from the kidneys and if so under what circumstances.

To answer this problem it seemed desirable to employ experimental conditions as simple as possible. For this purpose isolated dogs' kidneys were perfused with blood by means of a Dale-Schuster pump.

A dog's lungs and kidneys were perfused with defibrinated blood by means of a double Dale-Schuster pump.[5] Blood flow from the renal vein was measured by a Gaddum recorder and urine flow by collection in a balanced spoon.

Samples of the renal vein blood (100 cc) were taken at intervals during perfusion of the kidney. This blood was perfused through a rabbit ear with pulsatile pressure. Injections of renin, prepared by the method of Helmer and Page[6] and renin-activator prepared according to Kohlstaedt, Helmer and Page[7] were made through a side arm in the apparatus directly into the cannula which connected the artery of the ear to the perfusion apparatus.

Forty-four experiments have been performed, typical examples of which are given.

In Experiment I, a control experiment, a kidney was perfused 240 minutes. Blood flow remained unchanged and there were only slight changes in blood pressure and urine flow (Table I). Renal vein blood collected at the beginning and at the end of the experiment was perfused through a rabbit's ear and when renin was injected into the perfusing blood, intense constriction occurred (Table II). When renin-activator was injected there was no reduction in flow.

In Experiment II after 105 minutes of perfusion a clamp was applied to the renal artery sufficiently tight to reduce pulse pressure 60%. Mean blood pressure distal to the clamp was kept constant by increasing the output of the pump. Immediately after application of the clamp blood flow was reduced 23% and there was some decrease in urine flow (Table I). In some experiments blood flow was not reduced.

---

3 Kohlstaedt, K. G., Helmer, O. M., and Page, I. H., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 214.

4 Page, I. H., and Helmer, O. M., *Proc. Central Soc. Clin. Invest.*, November, 1939.

5 Dale, H. H., and Schuster, E. H., *J. Physiol.*, 1928, **64**, 356.

6 Helmer, O. M., and Page, I. H., *J. Biol. Chem.*, 1939, **127**, 757.

7 Kohlstaedt, K. G., Page, I. H., and Helmer, O. M., *Am. Heart J.*, 1940, in press.

TABLE I.
Conditions Employed During Perfusion of Kidney.

Exp. I—Without Constriction of the Renal Artery
Kidney wt 38 g.
Blood Pressure 132/92 mm Hg. at beginning of perfusion
136/86 mm Hg. after 240 min. perfusion
Mean Pressure increased from 102 mm to 111 mm Hg.
Pulse Pressure 40 mm Hg. throughout the experiment
Total Blood Flow 180 cc/min. 30 min. after perfusion started
180 cc/min. after 240 min. perfusion
Urine Flow .3 cc/min. 30 min. after perfusion started
.2 cc/min. after 240 min. perfusion
Urea Clearance 5.5 cc blood cleared per minute.
Blood Sample Taken (1) after 30 min. perfusion
(2) after 240 min. perfusion

Exp. II—With Constriction of the Renal Artery
Kidney wt 43 g
Perfused 105 min. before constricting renal artery
Blood Pressure 138/88 mm Hg.
Mean Pressure 113 mm Hg.
Pulse Pressure 50 mm Hg.
Total Blood Flow 168 cc/min.
Urine Flow 1.1 cc/min.
Urea Clearance 5.16 cc/min. blood cleared
Blood Sample Taken (3) after 30 min. perfusion; (4) 105 min. perfusion

Conditions Initiated by Application of Clamp to Renal Artery and Adjustment of Pump to Maintain Mean Pressure
Blood Pressure 120/100 mm Hg.
Mean Pressure 110 mm Hg.
Pulse Pressure 20 mm Hg. (60% reduction)
Total Blood Flow 129 cc. (23% reduction in blood flow)
Urine Flow 0.8 cc/min.

Conditions After Kidney Perfused 165 *Min. with Renal Artery Constricted*
Blood Pressure 168/164 mm Hg.
Mean Pressure 166 mm Hg. (56 mm increase)
Pulse Pressure 4 mm Hg. (16 mm decrease)
Blood Flow 76 cc/min. (41% reduction in total blood flow)
Urine Flow 0
Blood Sample (5) After 165 min. perfusion with renal artery constricted by clamp (270 min. total time perfused)

As perfusion was continued with the renal artery constricted a gradual rise in mean pressure and a further decrease in pulse pressure was observed. After 165 minutes of perfusion under these circumstances mean pressure had increased 56 mm Hg and blood flow had been reduced 41% (Table I).

When renin was injected into a rabbit ear perfused with renal vein blood taken before application of the clamp, marked vasoconstriction occurred but when the ear was perfused with blood taken 165 minutes after constriction of the renal artery, renin did not cause reduction in blood flow. Renin-activator injected into the ear perfused with blood taken before application of the clamp did not reduce

blood flow but if injected into the ear perfused with blood taken after the clamp was applied, intense vasoconstriction occurred (Table II).

These results indicate that constricting the renal artery by a clamp caused the kidney to form or liberate a substance which caused further constriction of blood vessels in the renal parenchyma.

Additional evidence favoring the view that this substance may be renin is furnished by the observation that when excess renin is added to blood in which there is no available renin-activator, vasoconstriction does not occur and if renin-activator is added to blood containing an excess of renin then vasoconstriction occurs. Blood taken prior to clamping contained renin-activator and injection of renin into the ear during perfusion with this blood, caused vasoconstriction. Blood taken after clamping apparently did not contain available renin-activator because renin injected into the ear caused no reduction in flow but it did contain a substance which resembled renin because it reacted with the renin-activator to produce vasoconstriction.

Perfusion of a dog's leg for periods similar to those employed for

TABLE II.

Effect of Injection of Renin and Renin-activator into Artery of a Rabbit's Ear During Perfusion with Defibrinated Blood Taken from the Renal Vein During Perfusion of a Kidney.

	Constriction in min.	% reduction in flow
Control Experiment.		
Exp. I—Kidney Perfused 240 Min. without applying clamp		
Blood sample (1) taken after 30 min. perfusion through kidney		
1 cc renin-activator	0	0
1 cc renin	16	91
Blood sample (2) taken after 240 min. perfusion through kidney		
1 cc renin-activator	0	0
1 cc renin	11	98
Exp. II—Clamp applied to renal artery		
Blood sample (3) taken after 30 min. perfusion and before clamp applied to artery		
1 cc renin-activator	0	0
1 cc renin	12	76
Blood sample (4) taken after 105 minutes perfusion immediately before clamp applied (Dosage of renin reduced)		
.5 cc renin-activator	0	0
.5 cc renin	3	46
.5 cc renin-activator	0	0
Blood sample (5) taken 165 min. after clamp had been applied to renal artery		
1 cc renin	0	0
1 cc renin-activator	6	75
1 cc renin	0	0
1 cc renin-activator	8	69

perfusion of the kidneys did not cause liberation of renin when the femoral artery was constricted. Renin caused vasoconstriction when injected into the femoral artery during this perfusion.

Conclusion. Renin appears to be produced by normal isolated dog's kidney perfused with blood when pulse pressure and blood flow are reduced by constricting the renal artery. Reduction of mean pressure is not a necessary condition. It is not produced when the hind leg is perfused under similar circumstances nor by kidneys perfused by blood under normal pressure-flow conditions.

11122 P

Diminution of Acetylcholine Content of Retina After Prolonged Functional Disuse.

HSI-CHUN CHANG, WEI-MING HSIEH, LAO-YING LEE AND TSUNG-HAN LI.

From the Department of Physiology, Peiping Union Medical College, Peiping, China.

With aseptic precaution, the eyelids of one of the dog's eyes were sutured together, the other being left untouched as a control. After a period of blindfolding, the lids were opened up under ether anesthesia, the eyeball enucleated, and the retina carefully freed from the ciliary muscle and extracted with alcohol. All these procedures were done in the dark room under red light. The control eye was similarly prepared under ordinary illumination. The acetylcholine (AC) was identified by different tests, and the quantity determined by assay with the toad's rectus preparation.

While the AC content of the retina of the eye blindfolded for 7-49 days was found to be 11-25% less than that of the control in 5 experiments, that of the eye blindfolded for 160-170 days was over 58% less than the control in 4 experiments. The variation of the AC content of the retinae of the 2 normal eyes encountered in 12 experiments was $9.3 \pm 8.4\%$.

As there was no sign of infection, injury or irritation of any sort in the blindfolded eye, the diminution of the AC content after 160-170 days' blindfolding was apparently due to the prolonged functional disuse.