bound to protein." The importance of Fraction V (albumin) in the binding and transport of estrogens in human plasma has been demonstrated(1,3,5,6). The results of these studies point to albumin as the major protein concerned with transport of estrogens and their metabolites in human plasma.

Summary. 16-C^{14}-estrone and 16-C^{14}-estradiol-17β were injected intravenously into normal human subjects. C^{14}-estrogens were injected either alone or following infusion of 50 mg unlabeled progesterone or estradiol-17β. Blood samples were drawn at 30 minutes and at 120 or 180 minutes after injection. The blood plasma was fractionated by the cold ethanol fractionation techniques and plasma fractions were examined for radioactivity. The delay of time of phlebotomy resulted in an increase in percentage of conjugated metabolites. Injection of a large amount of estradiol-17β resulted in a more rapid disappearance of conjugated radioactive metabolites of C^{14}-estradiol-17β in both protein-bound and unbound forms. No pronounced changes in distribution of radioactivity among the various plasma fractions occurred as a result of prior administration of large amounts of unlabeled steroid.

We wish to acknowledge the capable assistance of Mr. Lawrence Beecher and Mrs. Maria Karsay.

1. Sandberg, A. A., Slaunwhite, W. R., Jr., Antoniades, H. N., *Rec. Prog. Hormone Res.*, 1957, v13, 209.

2. Antoniades, H. N., Pennell, R. B., Slaunwhite, W. R., Jr., Sandberg, A. A., *J. Biol. Chem.*, 1957, v229, 1071.

3. Slaunwhite, W. R., Jr., Sandberg, A. A., *Endocrinology*, 1958, v62, 283.

4. ———, in preparation.

5. Bischoff, F., Stauffer, R. D., *Am. J. Physiol.*, 1957, v191, 313.

6. Slaunwhite, W. R., Jr., Sandberg, A. A., *J. Clin. Invest.*, 1959, v38, 384.

Received February 20, 1961. P.S.E.B.M., 1961, v106.

Evidence for a Central Mechanism in Angiotensin Induced Hypertension.* (26492)

ROBERT K. BICKERTON AND JOSEPH P. BUCKLEY (Introduced by L. V. Beck)
Cardiovascular Research Laboratories, Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pa.

Ever since Goldblatt(1) successfully produced sustained hypertension in dogs by partially constricting both renal arteries, investigators have been trying to isolate pressor substances from the blood of hypertensive animals and patients. Angiotensin II(2), a pressor octapeptide has been isolated in renal hypertensives(3) and has been synthesized by Bumpus *et al.*(4,5). Most investigators (4-6) have felt that the hypertensive properties of Angiotensin II were due entirely to the peripheral actions of the compound and have inferred that this agent did not act through the central nervous system. The purpose of this study was to determine if Angiotensin II possessed central hypertensive properties, and if so determine the possible mechanism of action.

Method. Six dog cross circulation preparations, modified after that described by Taylor and Page(7), were utilized in this study. Mongrel dogs were anesthetized by an intravenous injection of 35 mg/kg of pentobarbital sodium. The external jugular veins and common carotid arteries were isolated and covered with gauze moistened in normal saline solution. The internal jugular veins were doubly ligated and cut between the ligatures. The neck musculature was separated into sheets and removed, utilizing electrocautery, to expose the vertebral column from C-2 to C-5. A dorsal laminectomy, which included removal of the spinous processes of the more

* Supported in part by grants from Nat. Inst. Health, and from Ciba Pharmaceutical Products, Inc., Summit, N. J.,

TABLE 1. Effects of Angiotensin II on Donor and Recipient Blood Pressures in the Dog Cross Circulation Preparations.

Exp. No.	Dose (μg/kg)	Route	Donor Wt (kg)	Donor Sex	Donor Original B.P.‡ (mm Hg)	Donor Rise in B.P. (%)	Donor Duration (min.)	Recipient Wt (kg)	Recipient Sex	Recipient Original B.P.‡ (mm Hg)	Recipient Rise in B.P. (%)	Recipient Duration (min.)
1	1.0	IA	11.3	♂	125	40.0	4.0	8.6	♂	135	11.1	.5
	1.0	"			120	37.5	3.0			130	19.2	.5
	1.0	"			130	23.0	3.0			115	13.0	.5
	1.0	IV-R			130	.0	.0			115	52.1	6.0
2	.4	IA	11.0	♂	130	38.4	4.0	11.2	♀	140	14.2	1.0
	.4	"			125	24.0	4.0			140	14.2	.5
	.4	"			130	15.3	1.0			140	10.7	1.0
	.8	"			125	20.0	1.0			140	10.7	1.0
	1.2	"			150	33.3	2.0			125	20.0	1.0
	1.2	IV-R			150	.0	.0			125	60.0	2.5
	1.2	IA*			150	20.0	1.5			90	44.4	1.0
3	1.2	IA	16.6	♂	150	66.6	5.0	12.7	♀	200	25.6	5.0
	1.2	" *			100	50.0	3.0			200	12.5	2.0
4	2.0	"	10.5	♀	125	12.0	2.0	6.7	♀	80	25.0	.5
	2.0	"			150	§	§			135	14.0	1.0
	2.0	"			150	§	§			130	19.2	1.0
	2.0	"			140	§	§			70	42.8	1.0
5	1.0	"	23.7	♂	100	100.0	5.0	12.0	♂	100	50.0	.5
	1.0	"			100	100.0	5.0			100	50.0	.5
	1.0†	"			100	100.0	5.0			50	0.0	.0
6	1.0	"	§	§	90	78.0	5.0	§	§	90	50.0	1.0
	1.0	"			100	90.0	4.0			110	45.4	.7
	1.0†	"			75	86.6	4.0			65	7.7	.5
	.5	IV-R			60	.0	0.0			60	109.0	6.0

* Vagi were sectioned in cervical region of recipient.

† 1.0 mg/kg of piperoxan inj. into recipient's peripheral circulation.

‡ Mean arterial blood pressure $\left(\frac{\text{systolic} + \text{diastolic}}{2}\right)$.

§ Not recorded.

IA = Administration *via* arterial inflow to recipient's head. IV-R = Administration *via* recipient's femoral vein.

caudad vertebrae and the transverse processes of each of the 2 respective vertebrae, was then performed either between C-2 and C-3 or between C-3 and C-4. A suitable length of 21-gauge stainless steel wire was inserted lengthwise through a soft rubber sponge (0.75 by 5 by 0.25 cm), until the wire protruded through the sponge. The wire and sponge were pulled under the spinal cord, then the wire was placed in such manner that when brought to the ventral surface between the carotid arteries, it fell into correct position in the intervetebral spaces and when tightened occluded the vertebral venous sinuses and vertebral arteries. Circulation was established between the left common carotid artery of the anesthetized donor dog and the 2 common carotid arteries of the recipient, and the 2 jugular veins of the recipient and left jugular vein of the donor. Two to 4 ml of heparin (1,000 μ/ml) were administered I. V. to the donor. Blood pressure was recorded from a femoral artery of each dog via a Statham transducer (P23AC) onto a Grass polygraph. Ten microcuries of I^{131} were administered into the arterial circulation to the recipient's head after thyroid glands of both animals had been saturated with iodine utilizing 0.25 ml of Lugol's solution, I. A., to the recipient's head. Circulatory leakage between head and body of the recipient was determined periodically by obtaining 3 ml blood samples from a femoral vein of the donor and recipient and determining amount of radioactivity per minute in each sample. There was absolutely no leakage observed in the 6

preparations utilized. Synthetic Angiotensin II† was administered either into the arterial inflow to the recipient's head or via the femoral vein to the recipient's peripheral circulation.

Results. The effects of Angiotensin II on donor and recipient blood pressures in the dog cross circulation preparation are summarized in Table I. Angiotensin II administered into the arterial inflow to the recipient's head in doses varying from 0.2 to 4 μg/kg produced consistent pressor responses in both recipient and donor animals with pressure increments ranging between 12% and 50% above normal levels in recipient and 30% to 100% above pre-drug level in donor. Central hypertensive response was shorter in duration (0.5 to 5.0 min.) than pressor response in the donor animal (2.0 to 5.0 min.). Intravenous administration of Angiotensin II into the peripheral circulation of the recipient produced marked hypertensive responses of relatively long duration (2.5 to 6.0 min.), in the recipient only. In 2 preparations, 2 doses of Angiotensin II, 1 μg/kg, were injected into the head of the recipient and the hypertensive effect in both animals recorded. Subsequently, 1.0 mg/kg of piperoxan was slowly infused into the recipient's femoral vein. Angiotensin, 1 μg/kg, was then administered into the arterial inflow to the recipient's head and produced pressor responses in the donor only. Intravenous administration of Angiotensin into the peripheral circulation of the recipient produced the usual pressor response. Vasopressin (0.1 μ/kg) administered *via* the arterial inflow to the untreated recipient's head produced hypertensive effects in the donor animal only.

Summary. Angiotensin II appears to produce an increase in blood pressure by 2 mechanisms: 1. A direct peripheral action on the vascular smooth musculature producing a marked increase in peripheral resistance, which is not blocked by piperoxan. 2. A central hypertensive effect, probably due to stimulation of central sympathetic structures and evoking peripheral sympathetic discharges, which are blocked by administration of a sympatholytic agent into the peripheral circulation.

† Kindly supplied as Hypertensin by Ciba Pharmaceutical Products, Inc., Summit, N. J.

1. Goldblatt, H., Lynch, J., Hanzal, R. F., Summerville, W. W., *J. Exp. Med.,* 1934, v59, 347.

2. Braun-Menendez, E., Page, I. H., *Science,* 1959, v127, 242.

3. Braun-Menendez, E., Paladini, A. C., *Circulation,* 1958, v17, 668.

4. Bumpus, F. M., Schwarz, H., Page, I. H., *ibid.,* 1958, v17, 664.

5. ———, *Science,* 1957, v125, 886.

6. Bianchi, A., DeSchaepdryver, A. F., Vleeschouwer, G. R., Preziosi, P., *Arch. Internat. Pharmacodyn. Therap.,* 1960, v124, 21.

7. Taylor, R. E., Page, I. H., *Circulation,* 1951, v4, 563.

Received November 21, 1960. P.S.E.B.M., 1961, v106.

Production of Streptolysin S by Streptococci Before and After Mouse Passage.* (26493)

IRVIN S. SNYDER† AND TOM R. HAMILTON

Department of Medical Microbiology, University of Kansas Medical Center, Kansas City, Kansas

No difference was found by Stollerman and Bernheimer (1) in streptolysin S production by strains of group A beta hemolytic streptococci isolated from patients with streptococcal pharyngitis or with rheumatic fever. That streptolysin S was produced in presence of yeast nucleic acid by strains of Lancefield's group A as well as by certain strains of groups D, E, G, H, and L was reported by

* This investigation was carried out during the tenure of a Predoctoral Fellowship from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S.

† Present address: Dept. of Bacteriology, State University of Iowa, Iowa City.