pear in conjunction with converted tubules, which would suggest an influence towards maleness emanating from this type of testis, it also happens that only male ducts appear on the rare occasions of the differntiation of ovarian tissues, the reverse of what one would expect if the gonads are really exercising any influence. There is, of course, no evidence as to whether the gonads in these grafts produce endocrine substances at all. If they do, there is no consistency of endocrine effect upon the ducts; if they do not, the independence of the ducts is self-evident. On the positive side, this randomness of differentiation of the ducts coupled with the fact that good gonad differentiation as often as not fails to elicit or support any duct differentiation whatsoever suggests the essential independence of the ducts.

Conclusion. Reviews(11,12) of the vast literature on gonadal control of embryonic differentiation of sexual ducts in mammals reveal 3 general viewpoints: (a) that the developing testes and ovaries produce sex hormones and that each has 2 opposing actions, stimulation of ducts of corresponding sex and inhibition of ducts of opposite six; (b) that the testes produce hormone which stimulates male parts while suppressing the female, but the ovaries do not influence sexual differentiation; (c) that sexual differentiation is not governed by gonad hormones. The results here submitted, namely, absence of correlation between duct development and hormonal environment provided by host and the similar lack of correlation between ducts and accompanying gonads, support the third view. This conclusion conforms in general to that reached by Bronski(10).

1. Jost, A., *Arch. Anat. micr. et Morph. exp.*, 1947, v36, 242.

2. ———, *Gynec. et Obstet.*, 1950, v49, 44.

3. Jost, A., Bosic, B., *Compt. Rend. Soc. Biol.*, 1951, v145, 647.

4. Moore, C. A., *Embryonic Sex Hormones and Sexual Differentiation,* Thomas, Springfield, 1947.

5. Moore, C. A., *J. Clin. Endocrinol.*, 1950, v10, 942.

6. Torrey, T. W., *Am. J. Anat.*, 1943, v72, 113.

7. ———, *ibid.*, 1945, v76, 375.

8. ———, *ibid.*, 1947, v81, 139.

9. ———, *J. Exp. Zool.*, 1950, v115, 37.

10. Bronski, M., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 426.

11. Colloques Internat. du Center National de la Recherche Scient. XXXI: La Differenciation sexuelle chez les Vertébrés, Paris, 1950.

12. Burns, R. K., *Analysis of Development,* Chap. 6. Saunders, 1950.

Received November 10, 1958, P.S.E.B.M., 1959, v100.

Release of Norepinephrine from the Isolated Heart.*† (24555)

J. A. RICHARDSON AND E. F. WOODS‡

Dept. of Pharmacology and Therapeutics, Medical College of S. Carolina, Charleston

Hoffmann *et al.*(1) reported the presence of an epinephrine-like substance in the perfusate of isolated mammalian heart treated with acetylcholine or nicotine. Similar observations were made by McDowell(2). More recently Paasonen and Krayer noted a marked decrease of norepinephrine content of mammalian heart treated with reserpine(3). These observations were based on bioassay technics. The present report concerns chemical identification and assay of biologically active agents liberated from the isolated heart during the interval of stimulation following administration of acetylcholine.

Methods and materials. Isolated rabbit hearts were perfused by means of a modified Langendorff apparatus with oxygenated

* This investigation was supported by research grants from Nat. Heart Inst. and Am. Heart Assn.

† Preliminary report in *Fed. Proc.*, 1957, v16, 330.

‡ The authors gratefully acknowledge technical assistance of J. L. Stewart, Jr., (Medical Student Fellow, Fn. for Infantile Paralysis) and F. C. Derrick.

Ringer-Locke solution containing 1 mg atropine/liter of perfusion fluid. Test doses of acetylcholine (25 to 200 μg) were introduced into the perfusion fluid at intervals. Perfusates from the heart were collected before administration of acetylcholine and during interval of stimulation which followed. They were analyzed fluorimetrically for their catechol amine content(4). Also, fluorescence emission spectra were recorded by means of Aminco-Bowman spectrophotofluorometer. Biologic activity of the perfusate was assessed by means of its effect on isolated segments of rabbit intestine. More decisive indications of biologic potency were obtained by injections of appropriately treated perfusates into a dog prepared for recording blood pressure and heart contractile force(5).

Results. Large doses of acetylcholine (25 to 200 μg) increased the amplitude of contraction of the isolated perfused heart and correspondingly produced substantial increments in concentration of norepinephrine in the perfusion fluid. In general, increasingly larger doses were required to elicit subsequent comparable degrees of stimulation. Positive inotropic effects were consistently obtained in 18 hearts in which 83 administrations of acetylcholine were made. In 6 of these preparations chemical analysis was done on 14 separate samples taken during stimulation. As contrasted with control determinations there were constant and substantial increases in the norepinephrine fraction with essentially no change in the epinephrine fraction. Mean levels of maximal norepinephrine values were

TABLE I. Catechol Amine Content of Perfusion Fluid from 6 Isolated Rabbit Hearts.

	Control		After acetylcholine max values	
	Norepinephrine	Epinephrine	Norepinephrine	Epinephrine
	μg/l			
	1.6	6.2	12.7	4.9
	4.0	4.5	31.4	1.7
	2.6	1.5	36.2	4.2
	8.3	1.4	12.8	2.1
	.3	1.0	6.7	1.9
	.8	.3	32.4	2.0
Mean	2.9	2.5	22.0	2.8

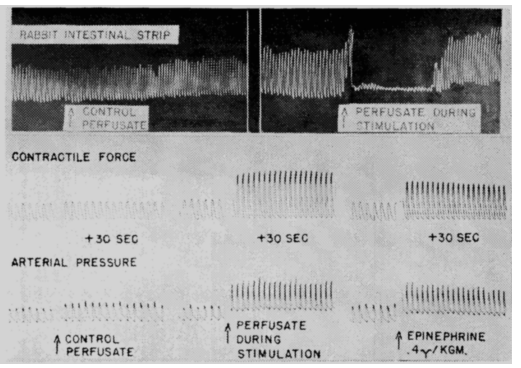

FIG. 1. Upper tracing: contractions of isolated rabbit intestinal segment suspended in Ringer solution. Perfusates collected from rabbit heart before (control) and during acetylcholine stimulation. Middle and lower tracings: effects of extracts of rabbit heart perfusates on heart contractile force and arterial blood pressure of a test animal (open-chest dog preparation).

about 10 times control figures (Table I). As recorded by Aminco-Bowman spectrophotofluorometer, the fluorescence emission spectrum of condensation products of rabbit heart perfusate with ethylenediamine was identical with emission spectrum of an authentic mixture of norepinephrine and epinephrine containing the same calculated amounts of catechol amines found in the perfusate. Biologic activity of perfusates collected during acetylcholine stimulation was clearly manifested on the small intestine and on the cardiovascular system. When added to the bath of isolated intestinal segments, marked inhibition of motor function was observed (Fig. 1); when injected intravenously into a dog prepared for recording blood pressure and heart contractile force, substantial increments were noted in these 2 parameters (Fig. 1). In the latter instance, pooled perfusates collected during successive periods of stimulation were adsorbed on a column of aluminum oxide and subsequently eluted with weak acetic acid. This eluate was then injected into the test animal. Increases in cardiac contractility and in blood pressure were similar in character and in the same range as those seen after intravenous administration of epinephrine 0.4 μg/kg.

Discussion. Increasing emphasis is being accorded the autonomic nervous system in controlling heart function. This control may

be modified by release of sympathomimetic substances from specialized tissue within the heart. The present report suggests the presence of ganglionic structures in the heart since the bulk of the sympathomimetic agent in the perfusate was norepinephrine. Schmitthenner *et al.*(6) concluded that increases in coronary blood flow, cardiac oxygen consumption, and left ventricular work elicited by nicotine infusions were due predominantly to stimulation of sympathetic ganglia of the heart with liberation of norepinephrine and epinephrine locally. West *et al.*(7) demonstrated that intracoronary arterial injections of nicotine produced increases in myocardial contractility and coronary blood flow qualitatively similar to effects produced by intracoronary injections of norepinephrine and epinephrine. Recently, Burn and Rand(8) demonstrated that isolated atria from rabbits pretreated with reserpine no longer exhibited positive inotropic and chronotropic effects when tested with nicotine. Recognizing the ability of reserpine to deplete norepinephrine stores of cardiac tissue, these authors concluded the stimulant action of nicotine was due to release of epinephrine and norepinephrine from the heart. The possibility of the existence of chromaffin tissue in the heart should not be excluded, however, since it would probably be stimulated by acetylcholine in the same manner as the adrenal medulla. If such were the case, however, it would be necessary to postulate a difference in composition of the chromaffin tissue at the 2 sites since the hormone of the adrenal medulla is predominantly epinephrine and the hormone liberated from the heart as demonstrated here is predominantly norepinephrine.

Activity of cardiac drugs may be dependent to some extent upon release of adrenergic amines within the heart. These amines may, likewise, play an important role in coronary artery disease. The significance of epinephrine and related compounds in heart muscle has been reviewed by Raab(9). Recent work in our laboratory has revealed elevated plasma levels of catechol amines in patients with angina pectoris after exercise and in myocardial infarction. Under such circumstances the damaged heart might conceivably contribute to these increments in circulating pressor amines.

Summary. Acetylcholine produces a prompt positive inotropic effect on the isolated atropinized heart. Norepinephrine is the agent largely responsible for this action as judged by biological and chemical assay of perfusates collected during the period of stimulation.

1. Hoffmann, F., Hoffmann, E. J., Middleton, S., Talesnik, J., *Am. J. Physiol.*, 1945, v144, 189.

2. McDowall, R. J. S., *J. Physiol.*, 1945, v104, 392.

3. Paasonen, M. K., Krayer, O., *J. Pharmacol. and Exp. Therap.*, 1958, v123, 153.

4. Richardson, J. A., Richardson, A. K., Brodie, O. J., *J. Lab. and Clin. Med.*, 1956, v47, 823.

5. Cotten, M. deV., Bay, E., *Am. J. Physiol.*, 1956, v187, 122.

6. Schmitthenner, J. E., Forte, I., Riegel, C., Hafkenschiel, J. H., *ibid.*, 1956, v187, 630.

7. West, J. W., Guzman, S. V., Bellet, S., *Circulation*, 1956, v14, 1017.

8. Burn, J. H., Rand, M. J., *British Med. J.*, 1958, v1, 137.

9. Raab, W., *Exp. Med. and Surg.*, 1943, v1, 188.

Received November 12, 1958. P.S.E.B.M., 1959, v100.