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Attempts to Produce Acute Cardiac Failure by Posterior Pituitary Extracts.

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The counterpart of clinical cardiac failure which develops after prolonged left ventricular strain as a result of working against an elevated arterial pressure has probably not been produced acutely in animal experiments. Until this is accomplished the fundamental cardiodynamic mechanisms involved will not have been fully elucidated. It remains problematical, for example, whether the typical congestive heart failure which often develops clinically can occur as a direct consequence of prolonged strain of the left ventricle and reduction in its blood supply, or whether it is due to secondary causes. In the search for a method for producing hypertensive cardiac failure rather acutely, the use of posterior pituitary principles suggested itself. Extracts of the posterior pituitary gland containing chiefly or solely the pressor principles, when injected into anesthetized animals, cause an elevation of arterial pressure by peripheral constriction, constriction of coronary vessels of an intense degree, and probably as a result of such action depression of the myocardium. Pulsus alternans which becomes significant in view of the cardiac slowing induced has also been reported. According to Starling's conception, myocardial failure means dynamically that the ventricles require a greater diastolic distention and higher initial tension in order to expel the same stroke volumes. Indeed, it is by virtue of such depressive action on the ventricles that caution has been urged in the clinical use of posterior pituitary preparations.

Cardiac Effects from Continued or Repeated Administration of Posterior Pituitary Principles. Fourteen dogs weighing from 10 to 18.5 kilos were anesthetized with morphine sulfate and sodium barbital. Aortic pressure pulses were recorded by a calibrated Gregg optical manometer of adequate sensitivity and frequency. As a first approach, central venous pressures were measured by inserting a glass cannula into the superior vena cava via an external jugular vein and connecting it with the saline manometer, the pressure readings being made, after flushing, during expiration. In some experiments peripheral venous pressures were recorded as well. It was realized that this is a crude procedure and quite inadequate for dynamic studies, but it was sufficient to determine whether a pronounced rise of venous pressure such as is evident during clinical types of decompensation occurred. A number of different pituitary preparations were used and we were not able to detect any special difference between them.* In different experiments pituitary extracts were injected in two different ways, namely, by administration of fractional doses of 2 to 5 P.U. with an occasional administration of a larger dose and by slow continuous intravenous injection of a dilute solution over a period of hours. In the latter a solution containing 1 pressor unit per 10 cc was injected into a femoral vein by use of a Mariotte burette.

Results. Trials with different preparations revealed that a prolonged and marked hypertension cannot be realized from the use of pituitary extracts alone. This was due in a certain measure to the development of an apparent refractoriness to repeated injections of the extracts. Elevation of pressure to high levels was also prevented by the cardiac slowing which supervened and the myocardial depression which accompanied this slowing. However, at the moderate elevations of pres-

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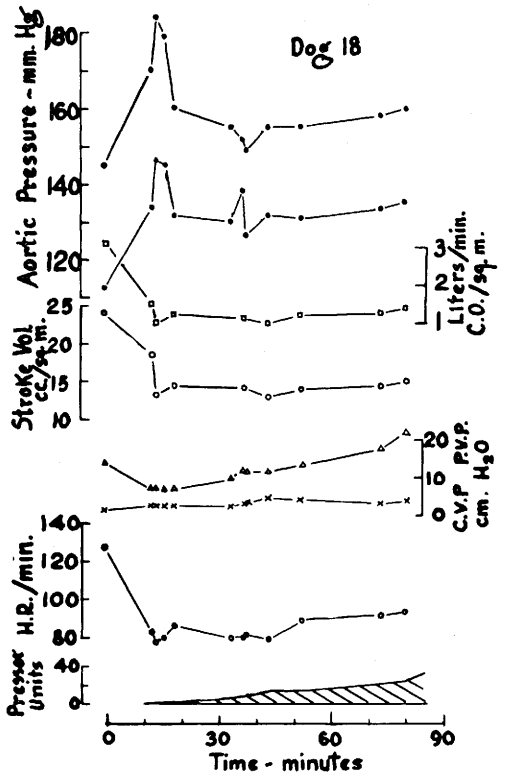

FIG. 1.

sures no consistent elevation of superior vena cava pressure was found to occur which in any way could be interpreted as congestive heart failure according to clinical standards.

Fig. 1 illustrates the main effects of a continuous accelerating infusion.‡ After a preliminary large rise of systolic and diastolic pressures to a maximum of 187/146 mm Hg (2 upper plots) these pressures decrease to somewhat lower ranges (average 155/132 mm Hg) owing partly to the marked retardation of the heart from 128 to about 80 beats per minute. Estimation of cardiac output by analysis of aortic pressure pulses after the method of Hamilton and Remington[2] indicates that the high arterial pressures are maintained despite the large reduction in stroke volume and cardiac output. Since the stroke volume is decreased despite the marked cardiac slowing it is apparent that the myocardium of the left ventricle is definitely depressed. This accords with observations previously reported by Wiggers[3] with regard to similar action on the right ventricle. Despite these changes, central venous pressure was not increased materially, although peripheral venous pressures measured by a catheter in the iliac veins rose moderately.

The nature of the myocardial depression induced by continuous action of posterior pituitary principles is revealed by a study of the aortic pressure pulses. These underwent changes in contour which were fairly constant and are exemplified by 8 segments in Fig. 2. Segment 1 is a normal control. Segment 2 shows an initial pressor effect, and segment 3 a subsequent depressor effect following an injection of 2 units of pitressin. Segment 4 represents the recovery but with the slowing maintained after this injection. Obviously, the effects of a single dose of posterior pituitary principles on rate persist longer than those causing depression of the myocardium. It is observed in segments 2 and 3 that regardless of whether the diastolic pressure rises or falls the pulse pressure is materially decreased. The isometric contraction phase indicated by the interval between the small preliminary vibration and the rise of the pressure pulse is definitely prolonged. Some recovery is noted in segment 4. The curve rises much more gradually to a summit in segments 2 and 3, indicating that the velocity of systolic discharge is reduced and its volume decreased. The magnitude of the change in stroke volume estimated according to the method of Hamilton and Remington is indicated directly on the record (S.V.). It will be noted that although the systolic discharge or stroke volume is restored in segment 4 the minute volume remains decidedly reduced owing to the continued reduction in heart rate (HR). Segments 5 to 8 indicate reactions during a later phase of the experiment after administration of pitressin had been discontinued for some time. Segment 5 shows some characteristics of pituitary action after another injection made after the curves of segment 4

‡ Indicated by cross-hatched lower graph.

[2] Hamilton, W. F., and Remington, J., *Am. J. Physiol.*, 1947, **148**, 14.

[3] Wiggers, C. J., *Am. J. Physiol.*, 1914, **33**, 382.

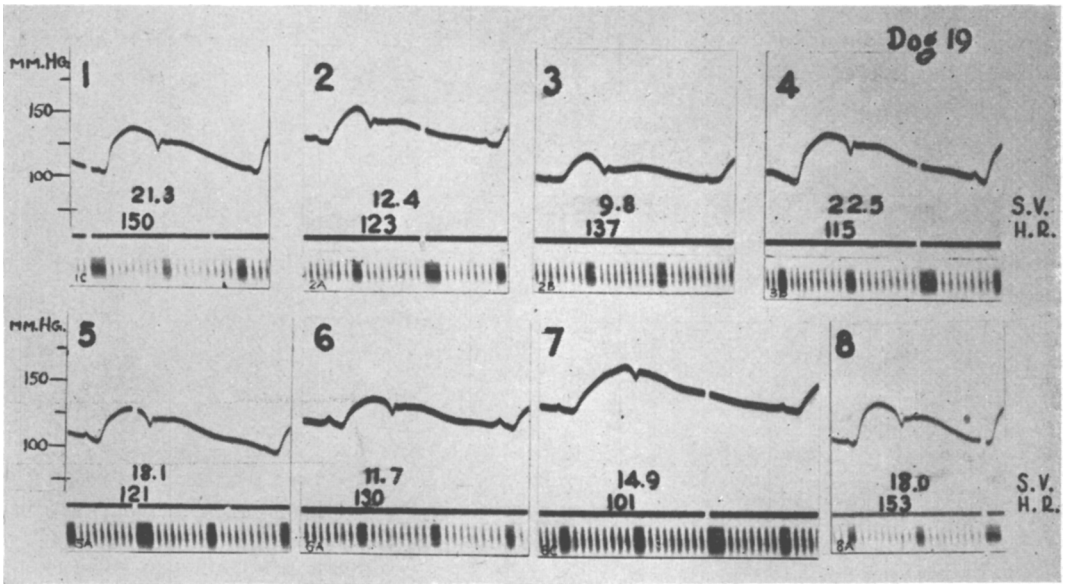

FIG. 2.

had been recorded. Segments 6 and 7 indicate additional and progressive effects of a continuous infusion. It will be noted that the stroke volumes and velocity of ejection by the left ventricle reduce significantly and that in segment 7 the stroke volume was diminished, although the pulse pressure was increased. Segment 8 shows considerable recovery, 52 minutes after the infusion was stopped.

Effects of Pituitary Extracts Plus Additional Hypertension. Since a sufficiently intense hypertension could not be produced by use of pituitary preparations alone, the technic was modified in two ways in an effort to make the left ventricle work against higher arterial pressure levels. In 4 dogs the carotid sinuses were excised and the vagi nerves cut. Thereupon, pitressin or pituitary extracts were again administered. Blood pressure was generally elevated at the start in such animals, although it tended to decline before pituitary extracts were administered. Progressive administration of pitressin either by continuous injection or given in stepwise doses failed to cause a pronounced elevation of arterial pressure required except very large doses which often proved treacherous. The observation was frequently made that after such deafferentation the heart rate, instead of decreasing, generally accelerated to an extreme degree. The mechanism remains enigmatic. The conclusion was reached that this also was not always a successful method for reproducing hypertensive cardiac failure acutely. A second modification consisted in opening the chest, maintaining a mild artificial respiration, and compressing the aorta to various degrees. This maneuver combined with injection of posterior pituitary extracts enabled us to produce higher systemic pressures although it involved certain complications as well. For example, too great compression of the aorta led to circulatory changes in the abdomen which tended to stagnate blood in the abdominal viscera. For this reason, such compression could not be maintained for too long a time. The typical responses following 3 periods of continued injection of pituitary extracts over a period of 3 hours are indicated in Fig. 3. Arterial pressures were maintained at rather high diastolic levels throughout, the heart rate became progressively slower, but central venous pressures remained remarkably constant. However, this may perhaps be accounted for in part by the reduction in venous return during aortic compression. Right ventricular failure in the clinical sense was

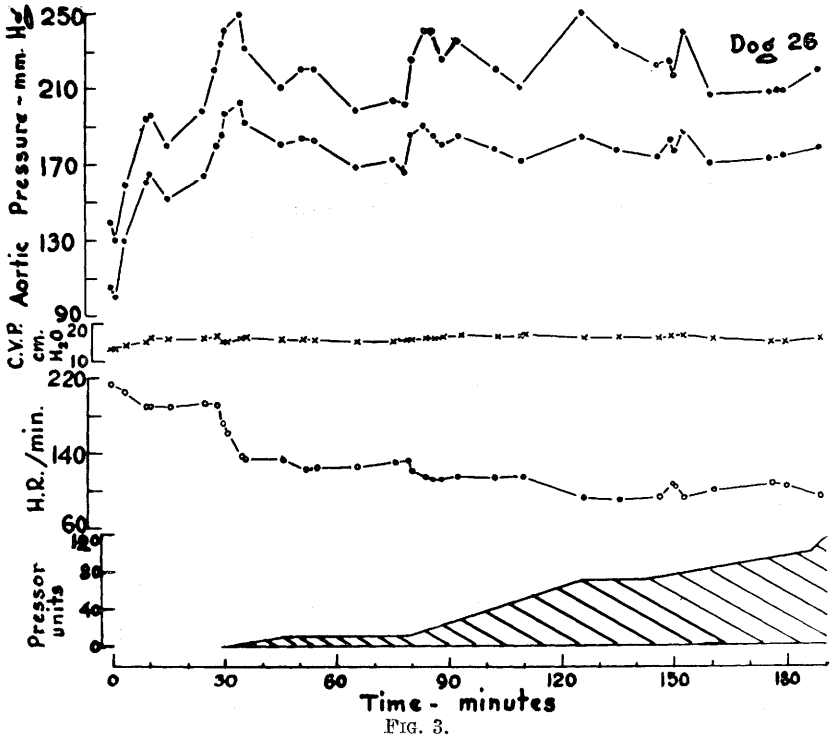

FIG. 3.

certainly not manifested in these experiments.

It was apparent after many trials that the congestive heart failure does not eventuate in dogs when a depressed left ventricle is required to work for 3 to 4 hours against high arterial resistance at the same time that the myocardium of the right heart is depressed and the coronary vessels are constricted. To assure ourselves that posterior pituitary preparations injected into a femoral vein is in sufficient concentration in the blood stream to reduce coronary blood flow while pressures are elevated, experiments were carried out in which flow in the left ramus descendens was measured either by the Opdyke flowmeter[4] or by a bubble flow meter. These experiments showed that significant reduction of blood flow occurred.

The conclusion was reached that if left ventricular strain accompanied by drastic reduction of coronary flow leads directly to cardiac decompensation the time factor must play an important role. Incidentally, our observations give some reassurance that while posterior pituitary preparations do induce coronary constriction and myocardial depression neither of these actions is apt to cause serious consequences when such preparations are administered in therapeutic doses to patients with normal coronary circulation and cardiac action. Careful continuous administration of 185 units during a period of 3 hours did not lead to permanent damage (Fig. 3).

Summary. An attempt was made to reduplicate clinical conditions in which a depressed left ventricle with a reduced coronary flow is required to work against high arterial pressures (a) by repeated or continuous infusions of posterior pituitary extracts, and (b) by creating additional arterial resistance through cutting of afferent moderator nerves or mechanical compression of the aorta. In all of these ways it proved impossible over periods of 3 to 4 hours to induce a state comparable to clinical congestive heart failure. If such failure is secondary to left ventricular strain plus reduction of coronary flow and

[4] Opdyke, D. F., and Foreman, *Am. J. Physiol.*, 1947, **148**, 726.

myocardial depression, the time element must play a dominant role.

Additional information regarding the nature of left ventricular depression induced by large doses of posterior pituitary extracts was obtained by an analysis of the aortic pressure pulses. Experimental evidence indicated that serious cardiac consequences are not apt to result from use of posterior pituitary preparations administered to patients with normal hearts in therapeutic doses.

We desire to express our appreciation to Professor C. J. Wiggers for his supervision of the experiments and help in interpreting the data.

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Effect of Estrogens on Early Development of Frog Embryos.*

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Boell[1] showed that cytochrome oxidase activity undergoes changes during the development of *Amblystoma punctatum* embryos, and McShan and Meyer[2] reported that certain estrogens inhibit the succinoxidase system in mammalian tissues. This inhibition by the estrogens was shown to be mediated through the cytochrome oxidase of the system. In the light of these results and the fact that cytochrome oxidase plays an important role in respiration, it became of interest to determine whether exposure of fertilized frog eggs to natural and synthetic estrogens would affect their normal development. Eggs of *Rana pipiens* and *Rana clamitans* were treated with diethylstilbestrol and with estrone in varying concentrations and for varying periods of time. The results of these preliminary experiments will constitute the data presented in this paper.

Frogs were procured from a dealer and stored in the laboratory at 4°C until used. Ovulation was produced and the eggs fertilized according to the standard technique of Rugh.[3]

A 10^{-3} M stock solution of diethylstilbestrol was prepared as reported by McShan and Meyer, and a solution of estrone of the same strength was made by dissolving 2.7 mg of estrone in 0.3 cc of 2 N NaOH plus 0.2 cc of 95% alcohol and diluting to a volume of 10 cc. The required strength of the estrogens was obtained by dilution with 20% Holtfreter's solution.[4] Proper control solutions of NaOH and alcohol were used.

The eggs were treated 1 hour after fertilization (after rotation and before the first cleavage) by dividing them into groups of 30 and placing them in 50 cc of the test solution. After exposure the eggs were rinsed with distilled water, transferred to Holtfreter's solution and maintained at 18°C for 5 days. Stages of development attained by normal and abnormal embryos were designated according to Shumway.[5] Abnormalities were recorded by number from Rugh's series of photographs.[3]

In the first series of experiments eggs of *Rana pipiens* and *Rana clamitans* were exposed to diethylstilbestrol in concentrations of 27, 13.5 and 6.8γ per cc for periods increasing by half hours from ½ hour to 4 hours. Control groups of eggs were placed in 6/100,000, 6/200,000 and 6/400,000 M NaOH and in 20% Holtfreter's solution. In

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1 Boell, Edgar J., *J. Exp. Zool.*, 1945, **100**, 331.

2 McShan, W. H., and Meyer, Roland K., *Arch. Biochem.*, 1946, **9**, 165.

3 Rugh, Roberts, *Experimental Embryology*, 1941, N. Y. Univ. Bookstore, 18 Washington Place, New York.

4 Holtfreter, J., *Arch. f. Ent. Mech.*, 1931, **124**, 404.

5 Shumway, Waldo, *Anat. Rec.*, 1940, **78**, 139.