Hexamethonium, a potent ganglionic blocker, was used to determine the contribution of the spinal ganglia toward generation of vascular tone. Hexamethonium gave only a slight decrease in DAP. The reduction in pulse pressure was due largely to the increase in HR. The fact that hexamethonium mimicks, in part, the effect of atropine, a parasympathetic (vagal) blocker, indicates that in the dogfish hexamethonium is acting primarily on parasympathetic-type ganglia. Atropine gave no decrease in mean DAP (pulse pressure diminished, due to an increase in HR, as before). Since hexamethonium decreased systolic and diastolic pressures, but atropine did not, this suggests that some degree of sympathetic-type vasomotor control exists.

Phentolamine acts primarily on the vascular alpha adrenergic receptors and causes a marked decrease in DAP by inducing arteriolar vasodilation. The action of this drug suggests that major control of vasomotor tone lies either below the level of the spinal ganglia, or, that control is not neurogenic and is due to other factors. The conclusion made by others, as well as ourselves, that tonus is maintained by circulating catecholamines released from chromaffin tissue (which is abundant in this species) is likely to be correct.

A higher central nervous system link does not appear to be necessary for AII to exert a pressor response, since the pressor response is not blocked by brain-freeze. In addition, AII action is not blocked by hexamethonium, but it is effectively eliminated by phentolamine. This lends support to previous evidence presented by our group that AII acts through the direct release of catecholamines from chromaffin tissue. Higher neurogenic control seems to be absent in the dogfish, both in respect to the maintenance of vasomotor tone and as a factor in the pressor response to angiotensin II. Supported by USPHS Grant No. 18868.

CARDIAC FUNCTION IN A NEW DOGFISH ISOLATED HEART PREPARATION

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A new type of dogfish heart preparation has been developed in which cardiac output of the isolated heart can be observed under controlled conditions without physical disturbance to the heart itself. Cardiac output in this preparation can be determined either from the product of heart rate and stroke volume, or by direct measurement. It can be compared to values for cardiac output obtained from intact fish by indicator dilution methods (Murdaugh et al. Am. J. Physiol. 209:723-725, 1965). The maximum cardiac stroke volume and cardiac output theoretically possible in an intact dogfish was approximated by measuring both pericardial cavity volume and empty heart displacement volume. Comparisons of cardiac outputs calculated for the resting intact fish and cardiac outputs measured from the isolated heart of the same fish show a large discrepancy at any pre- or after-load.

Fish weighing between 4.2 and 7.0 kg were submerged in a trough of seawater. All subsequent dissections and cannulations were performed under water in order to avoid aspiration of air into the cardiac chambers. All experimental procedures were also made with the preparation completely submerged in a bath of seawater. The surface level of the bath was used as the zero hydrostatic pressure reference plane for pressure measurements. The sequential steps in making the preparation are now described. The head was severed with a single quick cut just anterior to the spiracles (approximate level of the spino-medullary junction). This exposed a length of the ventral aorta anterior to the pericardial wall. A second cut was made posterior to the last gill slit. This exposed the posterior cardinal sinus, the Ducts of Cuvier and the posterior wall of the pericardial cavity. Fluid-filled Foley balloon catheters (Fr. 18, 5 ml) were inserted into the Ducts of Cuvier and inflated. The hepatic veins and lateral abdominal veins were securely clamped. This permitted control of inflow into the sinus venosus by perfusion through the Foley catheters. Another cannula, connected to a

vertical tube of adjustable height, was inserted into the ventral aorta and any pairs of branchial arteries between the tip of the cannula and the anterior wall of the pericardium (usually only one pair) were ligated. Perfusion was started using oxygenated (95% O_2 - 5% CO_2) isotonic elasmobranch saline. Evan's blue dye added to the perfusion fluid did not leak into the bath, indicating no loss of perfusate. Pressure was measured on the inflow side (sinus venosus) and outflow side (ventral aorta) with Statham transducers and a CRO recorder. Inflow pressure (pre-load) was controlled by adjusting the level of the perfusate in a reservoir which also collected the outflow. Outflow was monitored by an electromagnetic flow probe and measured directly, and more accurately, by timed flow into a graduated cylinder. After-load (corresponding to ventral aortic diastolic pressure) was controlled by adjusting the height of the overflow level of the vertical outflow tube. Both pre-load and after-load were varied over a wide range on either side of normal pressures for intact fish (normal range: pre-load, 0-3 Torr; after-load, 10-30 Torr). Following the conclusion of the cardiac output studies, the ventral aortic cannula was removed and a small opening was made in the anterior pericardial wall just large enough to permit the heart to be excised and withdrawn from the cavity. The volume of the empty excised heart was obtained by measuring the volume displaced when it was immersed in a saline-filled graduated cylinder. The volume of the pericardial cavity was determined by noting the amount of saline necessary to completely fill it (exit from the cavity was blocked by the inflated Foley catheters). The difference between pericardial cavity volume and heart displacement volume was called "free pericardial cavity volume."

The cardiac outputs of isolated hearts were observed in 22 experiments over a wide range of pre- and after-loads. The pre-load (ventricular end diastolic filling pressure) averaged 6.1 ± 2.6 Torr; the after-load (ventral aortic diastolic pressure) averaged 11.9 ± 2.3 Torr. The cardiac output averaged 27.5 ± 18.3 ml/min. The cardiac output *expected* from a fish weighing 5.7 kg (the average weight of the fish in these experiments) can be calculated by using the cardiac index obtained by Murdaugh et al. The calculation is as follows: 1.60 liters/kg/hr x 5.7 kg = 9.12 liters/hr, or, 152 ml/min. Obviously, the average output of the isolated heart is much less than the cardiac output of an intact fish of the same weight (only 18% of expected output). Experiments with adrenergic drugs, calcium ion concentration and experimental variations of pre- and after-loads demonstrated that the output of the isolated heart can be significantly increased over the average outputs shown above, but the maximum outputs we were able to achieve by any means (40.3 ± 23.0 ml/min for all experiments) fell far short of that expected in intact fish.

TABLE 1

CORRELATION OF PERICARDIAL CAVITY VOLUME, HEART DISPLACEMENT VOLUME AND FREE PERICARDIAL CAVITY VOLUME WITH DOGFISH BODY WEIGHT

Pericardial Cavity Volume (A)	Heart Displacement Volume (B)	Free Pericardial Cavity Volume*
19.4 ± 8.4 ml †	6.5 ± 1.0 ml	12.9 ± 6.1 ml
r = 0.66 $p < 0.01$**	r = 0.73 $p < 0.01$	r = 0.53 $p < 0.01$

* Free pericardial volume = A - B; † Mean ± 1 S.E.

** Correlation coefficients relating volumes to body weight; df = 21

Table 1 presents the data on pericardial cavity volume, heart displacement volume, free pericardial cavity volume and the correlation coefficients relating these volumes to fish body weight. As expected, there is a highly significant correlation between these volume parameters and body weight.

An interesting calculation can be made from these data. The free pericardial cavity volume is the physical factor limiting maximum ventricular stroke volume. The product of free pericardial cavity volume and the maximum heart rate characteristic of intact fish, or the maximum heart rate observed in the isolated heart preparations (about 34 beats/min in either case) gives a theoretical maximal achievable cardiac output in a dogfish, assuming that the heart fills to the limit imposed by the free pericardial cavity volume and empties completely at each stroke. Using our data, this value would be about 440 ml/min, or about 2.9 times the calculated resting cardiac output derived by use of Murdaugh's cardiac index. This can be compared to a factor of about 6x in man or dog.

The discrepancy between actual cardiac output from isolated heart preparations and the output of hearts in intact fish raises a serious question about the viability and performance of *all* isolated heart preparations. The preparation described here should be superior to traditional isolated heart preparations of the Langendorff type because the hearts are not subjected to physical trauma, since they operate in the unopened pericardial cavity. However, it is evident that essential components of cardioregulatory function must have been removed. These could include neural, hormonal and chemical stimulators of cardiac activity, critical nutrients and an imbalance of respiratory gases, with attendant acid-base disturbances. The preparation offers an excellent opportunity to study the factors responsible for maintaining normal cardiac function. The future challenge is to make this isolated preparation perform on a par with the heart of an intact dogfish. Supported by USPHS Grant No. 18868.

EXPERIMENTS WITH ANGIOTENSIN ANTAGONISTS IN DOGFISH

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The curious circumstance that the dogfish, *Squalus acanthias*, exhibits a pressor response to a variety of natural and synthetic angiotensins I and II, even though this species is not known to make either renin or angiotensin, caused us to examine the action of some potent angiotensin antagonists. The situation is even more interesting because the pressor response to AII in dogfish is apparently mediated only by release of adrenergic catecholamines. There is no evidence for the presence of vascular angiotensin receptors in dogfish which directly cause vasoconstriction. Therefore, any antagonist activity exhibited in dogfish would involve the mechanism for catecholamine release. Three synthetic angiotensins that inhibit the pressor response to [Asn^1-Val^5] AII (teleost AII) in mammals were tested in dogfish. These were [Sar^1-Thr^8] AII, [Sar^1-Ile^8] AII and [Ile^8] AI. All were synthesized by the Research Division, Cleveland Clinic Foundation.

The dorsal aortas of dogfish were catheterized via the caudal artery. The fish were then lightly anesthetized with 10 mg/kg sodium pentobarbital and the gills bathed in running seawater through tubes placed in each spiracle. Another catheter was placed in the lateral abdominal vein under local anesthesia (procaine) and advanced to the posterior cardinal sinus. Dorsal aortic pressure and heart rate were monitored via the dorsal aortic catheter. Infusions of the antagonists were given through the lateral abdominal vein, but challenge doses of AII were injected through the dorsal aortic catheter. The test procedure was as follows: Challenge doses of AII were given at 3 dose levels, 1.5, 3.0 and 6.0 µg/kg at 10 min intervals and in random order before beginning the infusion of an antagonist. These dose levels cover the range of doses producing submaximal to maximal pressor responses. The pressor response is brief (1-2 min). The dogfish does not exhibit tachphylaxis to AII. Infusion of one of the antagonists was commenced at a rate of 2 µg/kg/min ten min after the last challenge dose of AII. Five min after beginning the antagonist infusion the sequence of challenge doses of AII was repeated at 10 min intervals. The responses were recorded continuously during each trial.