response can be related to the increase in cardiac cycle length and no valid conclusion about vasoconstrictor activity in response to L-epinephrine can be drawn. The decrease in pressure pulse transmission time would be evidence for significant vasoconstrictor activity (increased arterial tone) only if arterial pressure had remained constant since increased arterial pressure per se will decrease transmission time.

The responses to calcium chloride injection resembled those obtained with L-epinephrine and saline loading. However, the responses were less consistent, particularly in respect to effect on cardiac cycle time (3 increases, 2 decreases), with a corresponding inconsistency in pulse pressure change. $CaCl_2$ effects are probably predominently cardiac.

Isoproterenol (2×10^{-5} mgm/Kgm) consistently decreased both ventral and dorsal aortic systolic, diastolic and pulse pressure. Ventral aortic systolic pressure decreased more than dorsal aortic pressure (-12.4 vs -10.4 mmHg) but dorsal aortic diastolic pressure decreased more than ventral aortic pressure (-4.2 vs -5.5 mmHg). This again suggests that the ventral aortic system is less compliant than the dorsal aortic system, or that the dorsal aortic system possesses more vasoconstrictor tone. However, the most important factor in the reduction of arterial pressure was undoubtedly a decrease in cardiac stroke volume since pulse pressure decreased markedly.

These data are too few to warrant firm conclusions but the data do suggest that the arterial systems of Squalus acanthias function as simple pressure/volume systems which are little affected by vasomotor activity. This evidence indicates a need for further research on the vasomotor control of the circulation in this species.

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1969 #28

TOTAL BODY PERFUSION OF S. acanthias USING A MEMBRANE LUNG

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Efforts to develop a technique of perfusing the gills of S. acanthias were described in 1962 by Peirce and Dabbs (Bull. MDIBL 5:19, 1965). Attempts to limit the perfusion to a portion of the fish proved to be too difficult because of blood leakage from cut surfaces. An additional problem was the poor gas permeability of the 0.5 mil Teflon R then available. A total body perfusion technique was developed to permit more precise analysis of a gill vascular reflex elicited when 2 to 5% CO_2 in air was equilibrated with the seawater perfusing the gills (Bull. MDIBL 8:35, 1968). A membrane of high permeability, MEM-213, manufactured by General Electric Corporation (Trans. Amer. Soc. Artif. Int. Organs 14:220, 1968) was available and made it possible to use a small artificial lung. The preparation was extremely satisfactory, being easily controlled and stable. Its use should simplify obtaining a variety of cardiovascular and metabolic data.

The apparatus and fish were arranged as shown in Figure 1. Donor fish were heparinized (500 units of aqueous heparin/kg) and bled from a needle in the dorsal aorta while gill perfusion

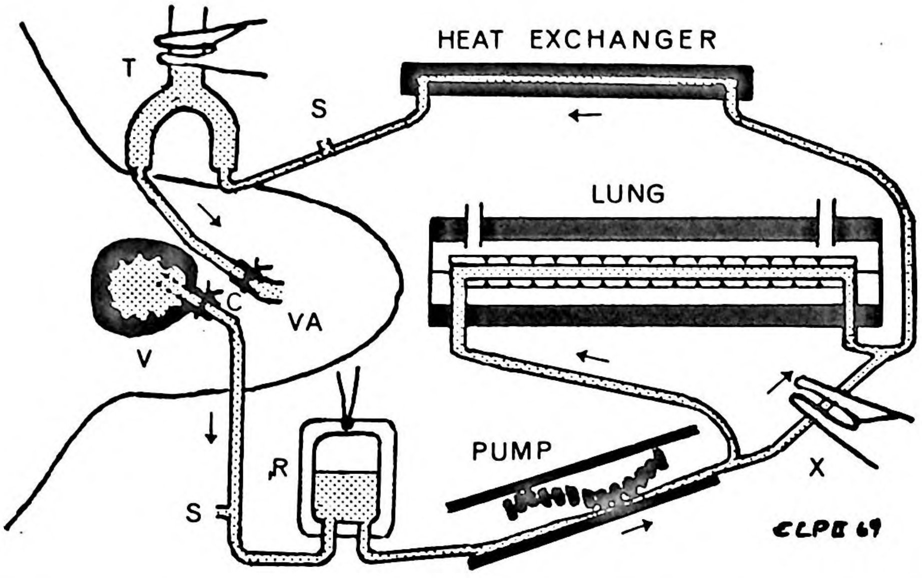

Figure 1. The ventricle (V) is cannulated through the divided aortic conus (C) for gravity delivery to the venous reservoir (R). Blood is returned to the ventral aorta (VA) via the other end of the cut conus. Blood may pass through the lung or be diverted through the shunt (X). Air trap (T) and sample ports (S). The finger pump is a small Harvard Apparatus unit. The heat exchanger was described previously (Arch. Surg. 84:329, 1962).

with fresh seawater was maintained. In one instance, the fish was bled without gill perfusion. The circuit was rinsed before and after use with dogfish Ringer's, emptied as completely as possible, and primed with fresh donor blood (all but one fish). The circuit, containing a single layer membrane lung of 0.125 meters^2, required about 100 ml of blood. The pump was run slowly, after priming, to keep the blood cold and well oxygenated since the nucleated red blood cells of the dogfish exhibit a strong Pasteur effect. The fish was heparinized and, after obtaining a control cardiac output ($\dot{Q}_B$) with an electromagnetic flow probe (Bull. MDIBL 7:40, 1967), the aortic conus was divided and cannulated proximally and distally with rigid plastic cannulas, 3/16 in. OD. The proximal cannula had multiple side holes and was inserted into the ventricle. Circulation was interrupted for less than 5 minutes. The perfusion flow ($\dot{Q}_E$) was set to conform to the control $\dot{Q}_B$.

In some experiments, periodic additions of 10 to 25 ml of Ringer's solution were necessary. The circuit was arranged to permit blood to go through the membrane lung or to be shunted around it. The lung was supplied with either air, oxygen, 3% CO_2 in air, or 3% CO_2 in oxygen. The gills were supplied, at a constant flow, with either fresh seawater, or seawater equilibrated with one of the above gases. In addition, the gill perfusion, via the spiracles, could be turned off leaving only a slight to and fro perfusion of the gills by seawater.

The dorsal aortic pressure (DAP) and the ventral aortic pressure (VAP) were measured alternately with a single low volume Statham pressure transducer (P23Gb). The aero was placed at the level of the heart. Fish temperature, blood acid-base data, hematocrit, and, in one experiment, pO_2 and O_2 and CO_2 content by Van Slyke (courtesy Dr. Eugene Robin) were obtained. In addition to the basic studies, pressure flow curves, and some drug curves were obtained.

Three perfusions, in fish averaging 2.9 kg, were carried out for a total duration of 12 hours. An additional perfusion of 1 hour and 40 min was carried out without any blood prime. This was a pilot study to demonstrate the feasibility of the method, but the data from it are not included. No circuit problems were encountered. Donor blood volume varied from 75 to about 225 ml for fish between 3 and 4 kg. Initial hematocrit (hct) varied from 14 to 17% and final values were below 10 in two experiments. Additional blood would have been helpful in preventing a decline in the hct. Control $\dot{Q}_B$ averaged 1.44 L/kg/hr and sustained $\dot{Q}_E$ 1.34 L/kg/hr (Table 1).

Table 1

#	Blood flow ml/kg/hr	
	Control $\dot{Q}_B$	Sustained $\dot{Q}_E$
26-69	1.54	1.69
38-69	1.19	1.12
40-69	1.57	1.20
	1.44	1.34

The lung, supplied with oxygen, had an estimated O_2 and CO_2 exchange ability of at least 5 volumes percent at the flows employed in these experiments. This compares with a total O_2 need of about 2 to 3 volumes percent. Using air instead of O_2, O_2 exchange should be 1 volume percent or a little more, resulting in considerable hypoxia when seawater was not supplied to the gills. Fish remained quite uniformly responsive to the various interventions which could be carried out repeatedly:

1. Pressure flow curves: A typical set of curves, obtained during a 12 min interval is shown in Figure 2. The lung was on 100% O_2 and the gills were supplied with fresh seawater. The response of both the gill and systemic vessels was basically passive but there was considerable hysteresis which varied from fish to fish. Resistance values (arbitrary units) fell as flow rose. Systemic resistance (R_S) comprised the larger share of total resistance (R_T) and

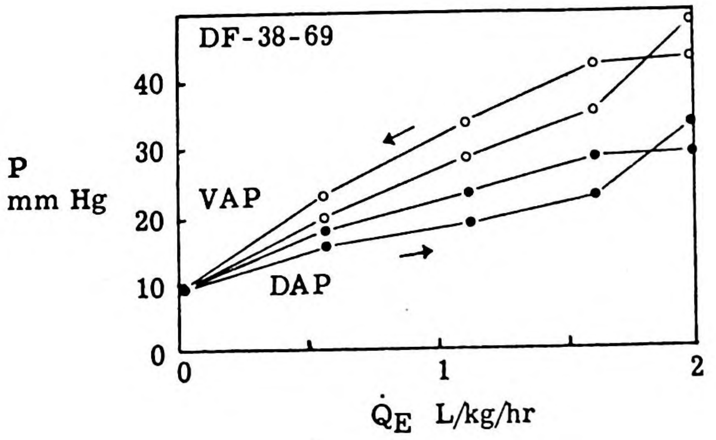

Figure 2.

generally changed more with flow than gill resistance (R_G) (Figure 3). A response to hypoxia at low flow could have been a contributing factor to the hysteresis.

2. Responses to high pCO_2 and to hypoxia: This subject is more fully discussed in report #13, this volume. Animals supplied with seawater equilibrated with 3% CO_2 in air have shown a marked rise in R_G with little change in R_S. This is accompanied by cardiac slowing, a fall in $\dot{Q}_B$, and a moderate rise in VAP. Because of the Bohr effect, some degree of blood desaturation results with 3% CO_2 in air so that the fish are exposed to a mixed hypoxia and hypercapnia. The response is blocked by atropine or vagotomy. Although this is clearly a vagal reflex, the afferent limb could not be deduced from the earlier experiments.

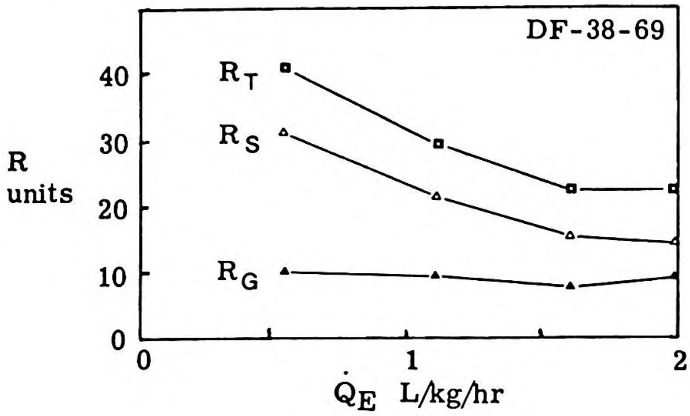

Figure 3.

In the present perfusion studies, changes in gill resistance could be studied at constant blood flow and several possible afferent pathways could be tested by varying the place in which the test gas was equilibrated with the blood. Sixteen interventions using 3% CO_2 in air or in O_2 supplied either to the membrane lung or to the gill were carried out. The basic response to 3% CO_2 in air in seawater in a perfused fish at constant $\dot{Q}_E$ is shown in Figure 4. Note the nearly 100% rise in both VAP and R_G. Figure 5 shows that the response was not elicited when the lung was supplied with 3% CO_2 in air as long as the gills were well perfused (A). When gill perfusion was stopped (B), a typical response pattern was seen. This indicates that pre-gill chemoreceptor afferents were probably not involved. When the lung was supplied with 3% CO_2 in oxygen,

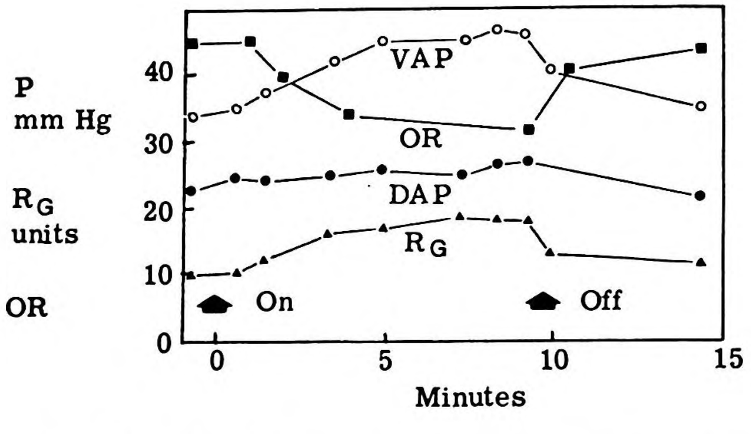

Figure 4. DF-38-69: 3% CO_2 in air supplied to gills by bubble oxygenator between arrows.

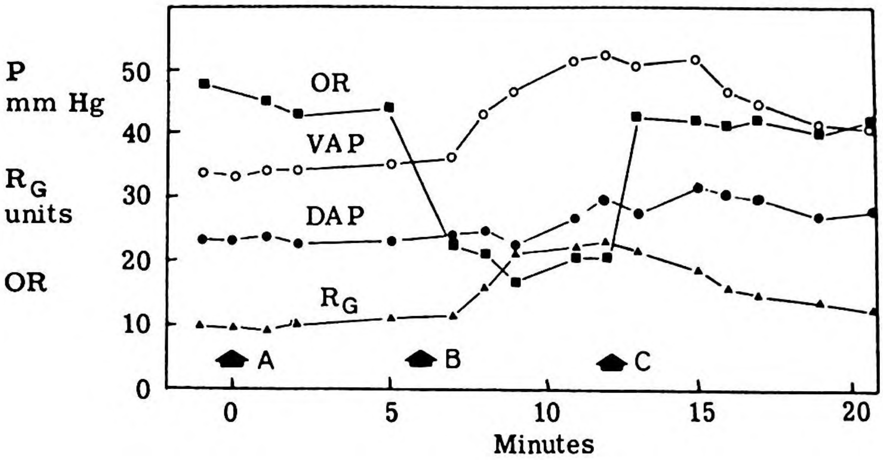

Figure 5. DF-38-69: 3% CO_2 in air to lung at A. Flow of seawater to gills stopped at B and resumed at C.

fully oxygenated hypercapnic blood entered the gills (Figure 6) (A) and again no response occurred. When gill perfusion was stopped (B), there was only a small response. A change to 3% CO_2 in air supplying the lung (C) produced a typical response which was reversed by switching to 3% CO_2 in O_2 (D).

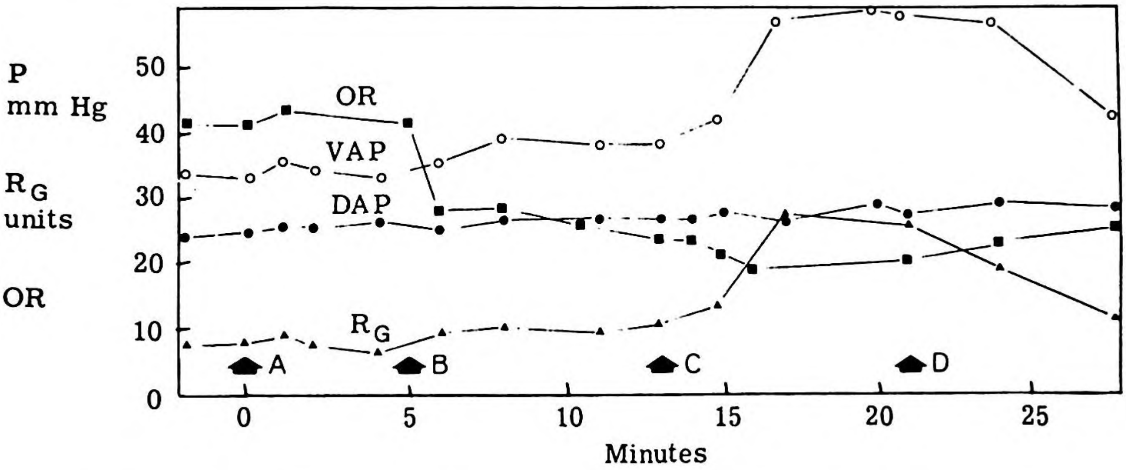

Figure 6. DF-38-69: 3% CO_2 in O_2 to lung at A. Gill perfusion with seawater stopped at B. 3% CO_2 in air to lung at C and 3% CO_2 in O_2 resumed to lung at D.

This study suggests that hypoxia is a much more potent stimulus than hypercapnia per se and indicates that the reflex does not involve, or at least require, direct pharyngeal or external gill stimulation. The implication is that there are chemoreceptors, responsive to hypoxia and probably to hypercapnia, in the gill afferents or more distally.

3. Opercular rate: Murdaugh and Robin have shown this to be correlated with rate of flow of seawater through the pharynx (Nature 211:1187, 1966). A pharyngeal baroreceptor mechanism has been proposed. In the present experiments, since the pump was relatively non-pulsatile, opercular rates were easy to record accurately. Opercular rate not only correlated with gill water flow but also varied with blood flow through the gills (Figure 7). Slowing occurred as $\dot{Q}_E$,

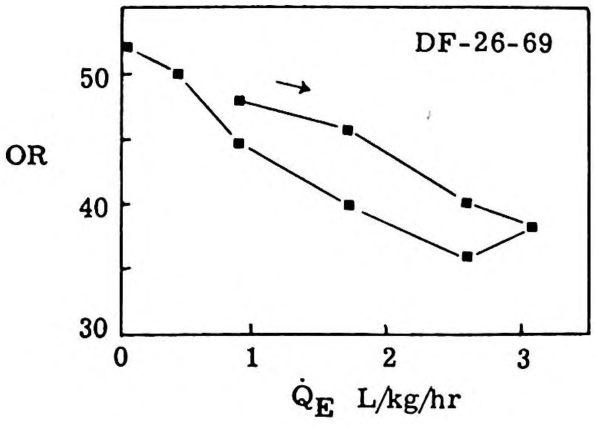

Figure 7.

VAP, and DAP increased, indicating the presence of a vascular baroreceptor response. Since opercular rate slowed with hypercapnia and hypoxia also, when VAP only was increased (Figure 4), it is likely that baroreceptors are located in the afferent gill arterioles as previously reported by Lutz and Wyman (Biol. Bull. Woods Hole 62:10, 1932). This would suggest that the primary response to hypoxia is an increase in gill resistance and that this occasions a secondary baroreceptor response slowing cardiac and opercular rate.

4. Drug responses: These are illustrated by curves for epinephrine and angiotensin from a single fish (Figures 8, 9). Both drugs gave diphasic responses primarily affecting systemic resistance. Epinephrine produced a modest sustained response (greater than 30 min) whereas the angiotensin response was of short duration (less than 10 min). The fish illustrated was the least reactive of the 3 studied but, nevertheless, shows the potential of the perfused preparation for drug response evaluations.

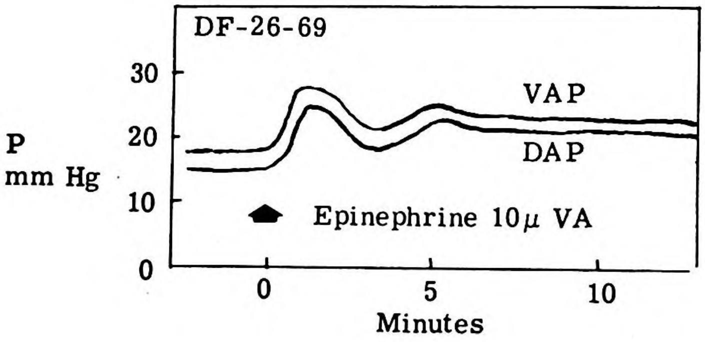

Figure 8.

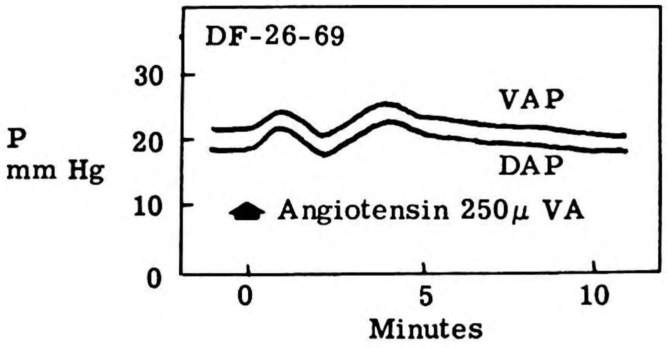

Figure 9.

5. A humoral vaso-active material: The possibility that a vaso-active material, affecting primarily gill resistance, may be elaborated during hypoxia is shown in Figure 10. This is an isolated curve obtained after donor blood was added from a fish bled without gill perfusion. There may, of course, be some other explanation such as a "transfusion reaction," but if there was a vaso-active material, it gave a response similar to the reflex response characteristic of hypoxia. This would suggest that both humoral and neural factors may take part in increasing

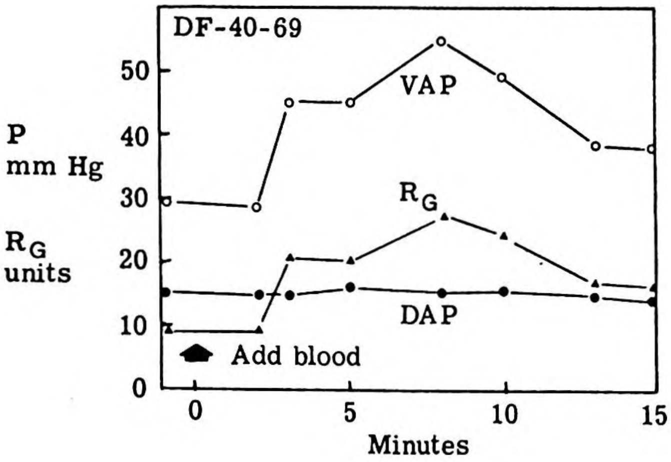

Figure 10.

gill vascular resistance if hypoxia is prolonged or profound. The substance was not epinephrine since epinephrine affects systemic resistance primarily.

6. <u>Gill exchange and metabolism</u>: The gills and systemic organs are in series so that constant flow perfusion of the gills can simplify studies of gill exchange or of whole body metabolism. Paired blood samples taken proximal to the perfusion circuit (mixed venous blood) and distal to the gills (dorsal aortic blood) provide data for blood entering and leaving both the gills and the systemic organs (Table 2). The lung may be bypassed, if its exchange function is not desired, or utilized to modify the level of O_2 and CO_2. No significant modification of non-volatile materials occurs in the lung.

Table 2

SOME EXCHANGE VALUES IN A PERFUSED DOGFISH

$\dot{Q}_E = 1.2$ L/kg/hr

Substance	Venous	Arterial	Exchange/kg/hr
O_2	0.24 vol%	2.41 vol%	23.8 ml
CO_2	6.60 vol%	5.27 vol%	14.6 ml
NCA (Control)	2.1 mEq/L	1.7 mEq/L	0.5 mEq
NCA (3% CO_2)	2.6 mEq/L	0.9 mEq/L	1.7 mEq

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1969 #29

PRELIMINARY STUDIES ON THE TOXICOLOGY OF ANTICANCER DRUGS IN *S. acanthias*

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A quantitative and qualitative comparison of the toxicity of typical antimetabolites and alkylating agents in poikilothermic vertebrates and mammals would be of interest. We report pre-