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# AN ISOLATED DOGFISH GUT PREPARATION FOR THE STUDY OF VASCULAR PARAMETERS

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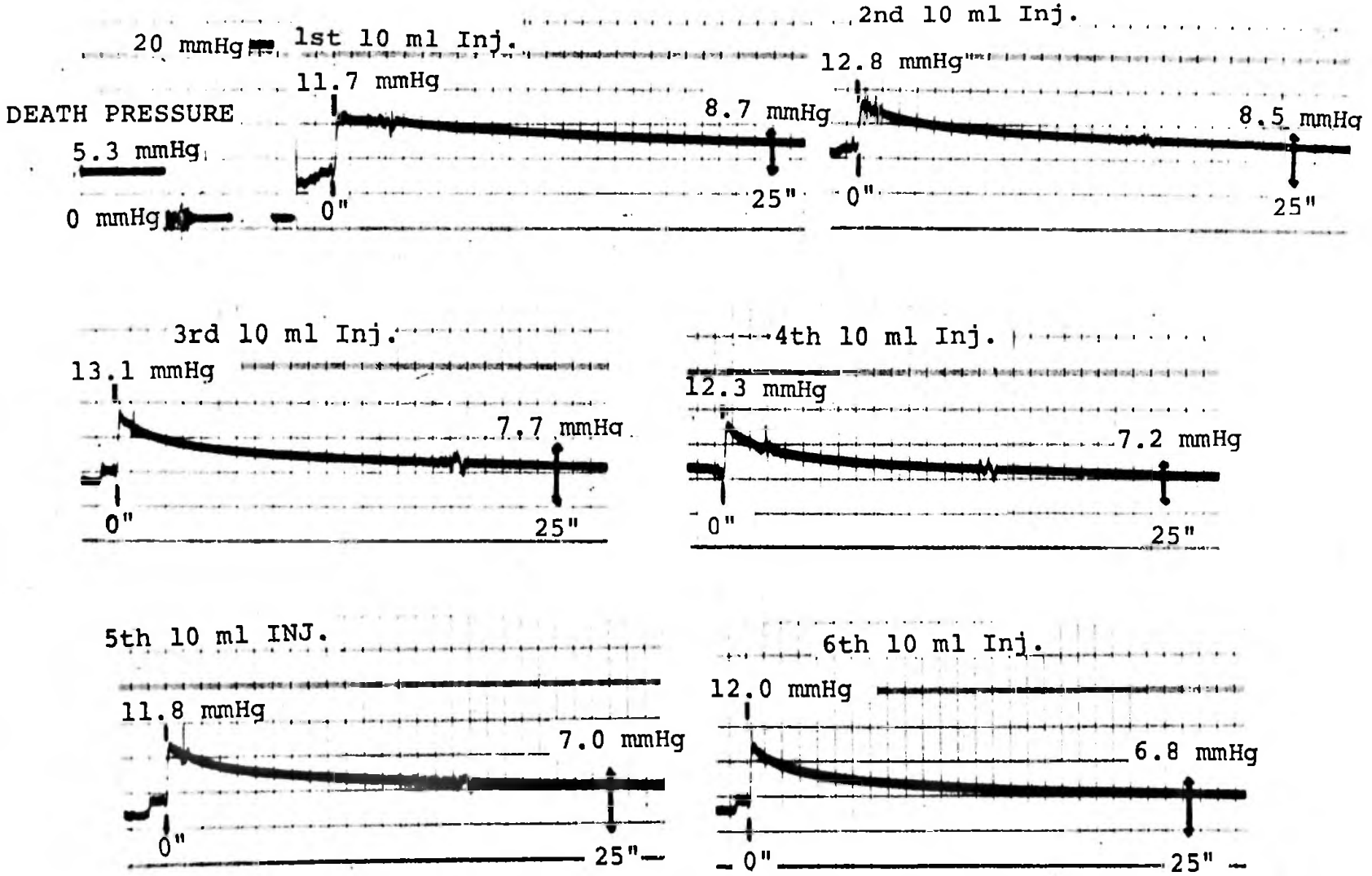
The circulatory system of the spiny dogfish, *Squalus acanthias*, exhibits what appears to be a marked smooth muscle stress relaxation phenomenon in response to multiple intra-arterial injections of elasmobrance saline solution administered at one minute intervals. A typical record from such an experiment is shown in Figure 1.

An alternate possibility is that the response is not due entirely to stress relaxation of vascular smooth muscle but to a rapid exit of plasma filtrate from the circulatory system into the interstitial space. An isolated gut preparation was developed to investigate this possibility. The purpose of this communication is to describe the isolated gut preparation and to present evidence that plasma filtrate does leave the vascular bed of the dogfish gut promptly under laboratory conditions where stress relaxation is usually observed.

Large female dogfish, 2-5 kg, were anesthetized with 20 mg/kg sodium pentobarbital injected into the caudal artery and placed belly up in a trough. The gills were perfused via the spiracles with cold, fresh sea water until after the gut was removed. A long ventral mid-line incision gave access to the abdominal cavity. The following maneuvers were performed in quick succession (three to five minutes total elapsed time): 1. The spiral intestine was doubly ligated and divided as near the cloaca as possible. 2. The common bundle containing the lienogastric and anterior mesenteric arteries and associated structures was doubly ligated near its dorsal origin


20 mmHg
1st 10 ml Inj.
11.7 mmHg
DEATH PRESSURE
8.7 mmHg
5.3 mmHg
0 mmHg
0"
25"
2nd 10 ml Inj.
12.8 mmHg
8.5 mmHg
0"
25"
3rd 10 ml Inj.
13.1 mmHg
7.7 mmHg
0"
25"
4th 10 ml Inj.
12.3 mmHg
7.2 mmHg
0"
25"
5th 10 ml INJ.
11.8 mmHg
7.0 mmHg
0"
25"
6th 10 ml Inj.
12.0 mmHg
6.8 mmHg
0"
25"

and sectioned. 3. The mesentery (practically bloodless) was cut to free the dorsal aspect of the stomach, intestine, pancreas, and spleen. The liver was excluded from the preparation. 4. Double ligatures were placed around the anterior portion of the stomach. 5. The long, free coeliac artery was cannulated with a polyethylene catheter (Intramedic PE 190) filled with heparinized saline. 6. The bundle containing the portal vein, hepatic artery, and common bile duct was ligated near the gall bladder and the portal vein cannulated (Intramedic PE 260) distal to its junction with its main tributaries and the bundle divided between the site of cannulation and the liver. 7. The cardiac portion of the stomach was sectioned between the previously placed ligatures, the gut lifted out and weighed on a tarred triple beam balance. 8. The preparation was transferred to a perforated porcelain plate connected to the blood perfusion system and perfusion commenced.

Blood perfusion was accomplished by connecting the heparin-saline filled arterial cannula to a volume calibrated, blood filled Mariott bottle which provided a constant hydrostatic pressure head. Blood (150-400 ml) was collected from heparinized donor dogfish via the caudal artery and filtered through several layers of surgical gauze before use. In some experiments the preparations were perfused with homologous blood. No instances of blood-blood or blood-tissue incompatibility were observed. The perfusion inflow pressure head was set arbitrarily by adjusting the height of the Mariott bottle air inflow tube to a carefully measured distance (27.2 cm = 20 mm Hg) above the zero hydrostatic plane of the preparation (surface of the porcelain plate). The bubbling of air from the air inflow tube served adequately to keep the RBC's suspended in the bottle. Whenever blood was recycled through the system it was first re-oxygenated by gently bubbling 100 percent $O_2$ through the blood contained in a tall cylinder. All preparations visibly reduced oxygen satura-

tion of the perfused blood during a single passage.

Venous outflow was collected in a flask or cylinder from the lateral arm of a T tube, the open end of which was positioned at the same horizontal level as the porcelain plate on which the preparation rested. Venous outflow rate was quantified by measuring outflow volume for accurately timed periods. As a practical point the hydrostatic level of the venous outflow cannula could not be placed so as to provide a negative outflow pressure because the portal vein would flutter at the junction of the vein and catheter and thus act as a valve. The upstream outflow pressure, which did not always correspond to zero hydrostatic because of the vis a tergo of venous flow was recorded from the straight-through arm of the T tube by a P23AA Statham strain gauge. Direct measurement of upstream and downstream portal vein pressures via a 25-gauge needle attached to a single limb saline manometer indicated that in the anesthetized, open abdomen dogfish placed belly up in a trough, the portal venous pressure averaged 1.9±0.7 (SEM) mm Hg upstream and 1.3±0.6 mm Hg downstream when the zero reference level of the manometer was placed at the level of the portal vein. These values are in agreement with hepatic portal vein pressures reported in a related species (Satchell, Circulation in Fishes, page 32. Cambridge Press, 1971). When the venous outflow cannula was adjusted to zero hydrostatic pressure (catheter tip at the same horizontal plane as the portal vein) the dynamic upstream outflow pressure recorded by the transducer averaged 2.0±0.2 mm Hg (N=25).

Meticulous attention was given to measurements of blood volume. The initial volume of blood available for perfusion in each experiment was carefully recorded. A running account of all partitions of the original blood volume was kept throughout each experiment including allowance for blood in the tubing of the inflow and outflow systems and blood discarded or unavoidably

lost. There was no measurable loss of blood or fluid externally nor any accummulation of blood or fluid in the lumen of the gut. The fluid content of the post-absorptive guts was observed to be scanty (5-10 ml of thick, mucoid secretion) both before and after experimentation. (Peristaltic action of the stomach and intestine often appeared during the course of the experiments. Peristaltic action in the spiral intestine of the dogfish is worth seeing.) It appeared that extravasated fluid from any source did not enter the lumen of the gut in these experiments. Petechial hemorrhages were not observed. The preparations were carefully weighed at the beginning and at the end of each experiment. Thus changes in gut weight could be detected accurately.

At the beginning of each experiment the outflow level was set at zero hydrostatic pressure and the preparation perfused with blood at physiologically normal pressure gradient (20 mm Hg inflow pressure; 2 mm Hg outflow pressure) until inflow/outflow rate equilibrium had been established. The inflow was then shut off and the preparation allowed to drain until outflow ceased. The volume of the outflow collected after the end of perfusion was measured and designated as the drainage volume at zero hydrostatic pressure. The drainage volume in the perfusions just described was 7.3±0.5 ml (Table 1). Each preparation was then infused successively from the arterial side with 20, 40 and 60 ml blood after the outflow was occluded. Immediately upon completion of each infusion the inflow was occluded and the outflow cannula opened. The volume of blood that drained out spontaneously (drainage volume at zero hydrostatic pressure) was collected and measured after each infusion. The results are given in Table 1. Closed system pressures at the end of normal perfusion (outflow open), 20, 40, and 60 ml infusions (outflow closed) were 2.0±0.2, 6.8±0.3, 9.0±0.4, and 10.0±0.4 mm Hg. respectively. If the

TABLE 1

SUMMARY OF DATA DERIVED FROM BLOOD PERFUSION OR INFUSION IN 12 ISOLATED DOGFISH GUT PREPARATIONS

| Parameter Measured or Calculated | Mean ± 1 SEM |
|---|---|
| 1. Weight of gut preparation | 212.2±13.6 gm |
| 2. Change in weight of the preparation | 51.4±5.5 gm |
| 3. Initial blood perfusion volume | 329.3±24.4 ml |
| 4. Blood recovered or accounted for | 275.6±26.0 ml |
| 5. Blood unaccounted for | 59.1±6.4 gm* |
| 6. Weight gained minus weight of blood unaccounted for (item 2 - item 5) | -7.8±3.4 gm |
| 7. Residual blood volume (kerosene wash-out) | 5.1±0.5 ml |
| 8. Drainage volume at zero hydrostatic pressure | |
| a. after perfusion at normal pressure gradient, outflow unoccluded | 7.3±0.5 ml |
| b. after 20 ml infusion, outflow closed | 18.1±0.8 ml |
| c. after 40 ml infusion, outflow closed | 30.1±1.0 ml |
| d. after 60 ml infusion, outflow closed | 44.3±2.6 ml |

*Volume of blood accounted for times specific gravity of blood taken as 1.08 gm/cm$^3$.

opening of the outflow cannula was intentionally delayed the pressures at the arterial and venous sides equilibrated within five seconds.

After completion of the infusions described above the preparation was again perfused at the original normal physiological pressure gradient and the drainage volume collected as before. The blood remaining in the praparation

after drainage was then completely washed out by perfusing with a large volume of kerosene through the arterial cannula. The outflow was collected in a graduated cylinder in which the residual blood separated from the kerosene and could be easily measured. The residual volume in 12 preparations averaged 5.1±0.5 ml. Since this amounts to only approximately three percent of the total blood volume in a 3 kg dogfish, it is obvious that the gut of the dogfish is not the great blood reservoir it is reputed to be in mammals.

The results show that an increasing fraction of the infused blood amounting to as much as 25 percent of the infused volume could not be recovered from the vascular system by drainage at zero hydrostatic pressure. That the unrecovered blood was not sequestered within the vascular system was proven by the fact that it could not be recovered by kerosene washout. The gain in gut weight accounted for nearly all the weight of nonrecovered and unaccounted for blood (Table 1). The probability that the fluid lost was a plasma filtrate rather than whole blood is strengthened by the consistent results obtained in three experiments in which hematocrits were taken on the blood before and after the infusions. The means were as follows: Initial Hct = 15.7%; final Hct = 19.8%; initial blood perfusion volume = 235 ml; recovered and accounted for blood volume = 193 ml. The coefficient relating the ratio of cross products of the proportionality equation of 0.96.

It must be presumed that the lost fluid volume was sequestered in the interstitial space since there was no sign of fluid loss via unoccluded lymphatic vessels in the cut mesentery. Any lymph vessels accompanying major blood vessels entering or exiting from the preparation would have been tied off, but distended lymph vessels were not seen. However a rise in interstitial pressure could have prevented lymphatic filling.

It is concluded that fluid does leave the dogfish gut vascular compartment more promptly and freely when vascular volume is elevated. The loss of fluid appears to become significant when the resulting intra-vascular pressure approaches 6-7 mm Hg. This is a high venous and possibly capillary pressure for a dogfish, but well within the range observed in our saline-loading experiments. This finding may contribute to our failure to observe a sustained elevated vascular pressure after vascular volume loading.

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## RENAL CLEARANCE OF SUGARS BY THE WINTER FLOUNDER, *Pseudopleuronectes americanus*

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In recent years Kleinzeller et al. (Bull. MDIBL 13:67-69, 1973 and J. Gen. Physiol. 62:169-184, 1973) have shown that several distinct sugar transport systems of differing specificity are located at the peritubular (antiluminal) membrane of renal tubular cells in the winter flounder, *Pseudopleuronectes americanus*. Supporting data have been developed using the in vitro teased tubule preparation where the sugars are presented exclusively to the peritubular face of the cells. In the studies reported below, sugars are presented in vivo, and thus reach the luminal face of the tubular cell by filtration and the antiluminal face via the peritubular capillaries. Sugars characteristic of each of the three carrier systems described by