RADIOLOGICAL OBSERVATIONS ON THE CARDIO-VASCULAR SYSTEM IN ANGUILLA ANGUILLA

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(With Plates 8–10 and Two Text-figures)

INTRODUCTION

Comparatively little work appears to have been done on the circulation in the intact living fish, particularly in the bony fish. This paper describes the circulation in the common eel using angiographic methods. This species is convenient as it can be fairly easily obtained in the autumn and winter, is tolerant of laboratory conditions and can survive for some time out of water. As no general description of the circulation in the eel appeared to exist, it proved necessary, in order to interpret the angiographs obtained in the living animal, to make a number of dissections, of which a general account has been given elsewhere (Mott, 1950).

MATERIAL AND METHODS

The silver eels used were obtained through the good offices of the Freshwater Biological Association, and were kept in a large tiled tank in running tap water.

The method involved the radiographic recording at frequent intervals of a radiopaque substance introduced into the blood stream. The techniques of indirect and direct cineradiography have been discussed by Barclay et al. (Barclay, Franklin & Prichard, 1944). For various reasons their techniques were not suitable for a small animal in which good definition of the vessels was of the first importance. The technique used consisted of the intermittent passage by hand of a strip of cardboard carrying wrapped Ilfex non-screen X-ray films through a shallow wooden tunnel, the top of which was made of sheet lead with a transparent plastic window on which the animal was laid, as in Text-fig. 1. The X-ray exposures were initiated by depression of a foot key which also operated a marker on a smoked drum revolving at a known speed, from which the intervals between exposures could be calculated. Exposures were made at any desired rate up to a maximum rate of about three in every 2 sec. in the hands of a practised operator. This system has now been superseded by a machine designed by Dr E. H. J. Schuster for moving the film at a rate of two exposures per second. Exposures were made at 35 kV. and 300 mA. Under these conditions, and at an anode distance of about 16 in., the exposure required was of the order of 0.1 sec.

The eel was anaesthetized by immersion in 2½ % urethane until it was limp and had lost its righting reflex. In a few eels, when it was desired to increase the number
Radiological observations on cardiovascular system in Anguilla anguilla

of angiographs obtainable during each heart beat, the animal was immersed in colder water to slow the heart. Otherwise, no attempt was made to control the temperature at which the experiments were performed, but the heart rate and temperature were noted at intervals during the experiment. The eel, in common with many fish, has no superficial veins convenient for cannulation. Two vessels were used for injection; the caudal vein for the circulation from the heart to the anterior gut arteries, and the ventral aorta, for the circulation of the gut and associated organs.

In order to cannulate the caudal vein the greater part of the eel’s tail was removed and the caudal artery and vein clamped; this avoids a stagnant section of circulation in the tail. The vein was approached through a mid-ventral incision and a metal cannula, filled with eel ringer (Keys, 1931) with a stopper in the proximal end, was tied in. When the injection was to be made, the stopper was replaced by a syringe containing Thorotrast (a colloidal suspension of thorium dioxide). Most of the eels weighed between 500 and 750 g., and 1 c.c. of Thorotrast proved adequate for reasonable contrast. The use of the caudal vein had two advantages:

(a) The injection was made well away from the heart and on the distal side of the hepatic portal and/or renal portal systems, so that the injection was unlikely to produce an abnormally high pressure in veins immediately adjacent to the heart.

Text-fig. 1. Diagrams showing: (A) sectional view of apparatus for making serial radiographs; (B) plan of wooden tunnel through which wrapped X-ray film is pushed.
In fact, no appreciable reflux was ever seen in any of the veins entering the sinus venosus.

(b) The operator’s hands are well away from the X-rays.

Injections were made into the exposed ventral aorta using a fine curved hypodermic needle which was inserted into the vessel and tied to the neighbouring muscle. Thorotrast (1 c.c.) was again used for the injection and was introduced fairly slowly in an attempt to allow the normal flow in the ventral aorta to carry the injected material with it.

It was possible to make at least two injections in any one animal, but in the absence of any mechanism which eliminates Thorotrast from the circulation, such as is present in many mammals (Barclay et al. 1944), the Thorotrast previously introduced causes some haziness.

A few experiments were done under water in a plastic box with a thin bottom virtually transparent to X-rays, but there did not seem to be any difference from the results obtained in air. As the anaesthetic usually inhibits the respiratory movements of the opercula this is perhaps not surprising.

The eel was kept in water when anaesthetized, except when being radiographed and cutaneous respiration seemed to be quite adequate for maintaining strong and regular contractions of the heart for the duration of the experiments.

The interpretation of the angiographs required some practice, and considerable help was obtained from the anatomical studies already mentioned. The calibre of vessels was measured when required by a modified pair of vernier callipers devised by the late Dr Barclay (Barclay et al. 1944).

It proved difficult in some instances to distinguish bone and injected vessels, both of which cast shadows. One way of overcoming this is to make a ‘positive’ from the X-ray film of an earlier uninjected frame of the series and then print through both ‘positive’ and a later frame of the series. In this way, as in Pl. 8, fig. 1, a print is obtained showing vessels only; but the procedure is time-consuming and is not worthwhile except in special instances.

RESULTS

The circulation through the heart and gills as shown by the injection of Thorotrast into the caudal vein

The time taken for the Thorotrast injected into the caudal vein to reach the ventricle was $4.2 \pm 1.8$ sec. in fifteen animals. This time is of no physiological significance, but it is of course of practical importance to know it.

On anatomical grounds, the easiest route from the caudal vein to the heart might well appear to be via the caudal vein—hepatic portal vein anastomosis and the hepatic portal system (Mott, 1950). In fact, however, in all but two of fifteen experiments, the bulk of the injection travelled via the renal portal system to the right posterior cardinal vein (which is much larger than the left) and thence to the heart.
Radiological observations on cardiovascular system in Anguilla anguilla

The heart

The precise picture obtained of the heart naturally depends on the phase of contraction when the Thorotrast reaches it. If the sinu-auricular valves are closed, the Thorotrast is held up. If they are open, a varying amount will get through to the auricle and perhaps to the ventricle. Pl. 8, fig. 3, shows the ventricle in diastole containing a small amount of contrast medium, and the sinus well filled with the sinu-auricular valves obviously closed.

The auriculo-ventricular valves can be seen in the ventrodorsal projection, Pl. 8, fig. 7, and the jet of blood from the auricle entering the ventricle in Pl. 8, fig. 2. Emptying of the ventricle is shown in Pl. 8, fig. 4.

Text-fig. 2. Diagram showing changes with time in size of various parts of the heart shadow.

Measurements made as indicated in Pl. 8, fig. 6, of: (i) diameter of extreme anterior end of bulbus arteriosus; (ii) diameter of bulbus arteriosus at mid-point; (iii) length of auricle and ventricle shadow, most of which is contributed by the ventricle; (iv) width of auricle and ventricle shadow, most of which is contributed by the auricle. 0 = auriculo-ventricular valves open; x = auriculo-ventricular valves shut.

The most remarkable feature noticed in connexion with the heart beat were the 'contractions' of the bulbus arteriosus, which were often very considerable, particularly at its anterior end. Plate 8, figs. 6, 7, shows two of a series of radiographs taken as fast as possible from which the dimensions of various parts of the heart have been measured and plotted in Text-fig. 2. The curve representing the greatest width of the bulbus arteriosus approximates to a mirror image of that representing the greatest length of the shadow of the auricle and ventricle (the greater part of which is contributed by the ventricle). This is a striking indication of the elasticity of the
bulbus arteriosus. In extreme cases the anterior end of the bulbus arteriosus appears to be able to shut down almost completely; it is possible that in this way it supplements the action of the ventriculo-bulbar valves (Pl. 8, fig. 6).

**Passage of blood through the gills**

Part of a typical series of radiographs from which the circulation times were estimated is shown in Pl. 9. The afferent branchial arteries fill in from 1 to 2 sec. after the ventricular contraction and before the next heart beat. Sometimes an appearance of streamlining (Pl. 9, fig. 2) can be seen in the ventral aorta. This presumably indicates incomplete mixing of blood from the right posterior cardinal and other veins entering the heart. The filling of the afferent branchials is not always quite simultaneous, and similarly with the efferent branchials. To get an estimate of the time of passage of the blood through the gills, the times have been recorded of the arrival of the injection at the two posterior afferent and two posterior efferent arteries respectively. In fourteen experiments the circulation time through the gills was $5.6 \pm 1.9$ sec.

**The circulus cephalicus and cranial arteries**

These arteries are not nearly so conspicuous in the living animal as post-mortem radiographs of specimens injected with 10% bismuth carbonate suspension might lead one to expect. An examination of ‘double prints’ suggests that the blood coming up the second efferent branchial artery flows only caudally—the first two efferent branchial arteries contribute to the circulus cephalicus in the eel (Ridewood, 1899)—and it is probable that much of that coming up the first efferent branchial also flows backwards. Certainly all the emphasis is on the caudal flow, and the

<table>
<thead>
<tr>
<th>Artery</th>
<th>Time (in seconds)</th>
<th>Number of Experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronary artery (cephalic, proximal)</td>
<td>4.2 ± 1.1</td>
<td>(3)</td>
</tr>
<tr>
<td>Coronary artery (cephalic, distal)</td>
<td>10.0 ± 4.4</td>
<td>(2)</td>
</tr>
<tr>
<td>Coronary artery (caudal)</td>
<td>3.9 ± 1.4</td>
<td>(6)</td>
</tr>
<tr>
<td>Brachial artery</td>
<td>3.7 ± 0.7</td>
<td>(8)</td>
</tr>
<tr>
<td>Cranial arteries</td>
<td>8.1 ± 4.4</td>
<td>(5)</td>
</tr>
<tr>
<td>Orbital artery</td>
<td>4.6 ± 1.3</td>
<td>(2)</td>
</tr>
<tr>
<td>Ophthalmic artery</td>
<td>5.3 ± 0.9</td>
<td>(3)</td>
</tr>
<tr>
<td>Retinal artery</td>
<td>5.9</td>
<td>(1)</td>
</tr>
<tr>
<td>Anterior gut arteries</td>
<td>3.8 ± 0.7</td>
<td>(8)</td>
</tr>
</tbody>
</table>

(Times (in seconds) taken by Thorotrast, introduced into the caudal vein, to pass from the efferent branchial arteries to other anterior arteries. The time at the efferent branchial arteries is arbitrarily taken as zero.) The number of experiments is given in brackets.

The quantity of blood made visible by the radiopaque injection in the head is very small indeed. Table 1 gives an indication of the times elapsing between the appearance of the blood in the posterior efferent branchial arteries and its appearance in various arteries in the anterior part of the body. It must be remembered that the smaller the artery, the greater is the possibility of missing its earliest filling on the radiograph, since there may not be a sufficient quantity of radiopaque material in it to cast a shadow.
Radiological observations on cardiovascular system in Anguilla anguilla

**The circulation through the viscera as shown by the injection of Thorotrast into the ventral aorta**

After injection into the ventral aorta, the time taken for the radiopaque material to reach the level of the anterior gut arteries was exceedingly variable (2.9–16.6 sec.), and is probably of no significance. Only in one eel did the injection take a time (7.7 sec.) of the order expected from the previous experiments (injection into the caudal vein); otherwise it either tended to be rather short (2.9–3.6 sec.) which may mean the injection was made too rapidly, or it took 11–16 sec. This latter result was probably due to temporary inhibition of the heart beat following the injection, through the operation of a branchial depressor reflex which was discovered in later experiments (Mott, unpublished). The distribution of blood to the various parts of the gut and other viscera is on the whole slow (Pl. 10). The various arteries do not always fill in the same order, or at all, and it is not unusual for an artery to fill with radiopaque material, which then moves only slowly. Such ‘rootless’ branches of the dorsal aorta present rather a curious aspect when abandoned by the main bulk of the injection which has proceeded down the dorsal aorta. One would not, of course, expect the gut circulation in a starving animal to be particularly active, but it is evident that it is by no means non-existent, particularly in the region of the pylorus. Some circulation times, calculated from the arrival of the blood at the level of the anterior gut arteries in the dorsal aorta, are given in Table 2. These times are necessarily very rough, as the frames are separated by long intervals of up to 10 sec. and it is more than usually difficult to be consistent in interpreting radiographs of this region because the circulation is so very slow. In general, a vessel has been considered reached by the contrast medium when about two-thirds of its length is easily visible; thus though the splenic artery is a branch of the lienointestinal, the latter may not fill until after the former, according to these criteria. It is not unusual for the proximal parts of the anterior mesenteric and anterior renal arteries to fill and the radiopaque material to remain static, in which case the time taken is that at which no further progress seems to be made by the injection.

**Table 2**

(Times (in seconds) elapsing between the appearance of Thorotrast, introduced into the ventral aorta, in the dorsal aorta at the level of the anterior gut arteries (taken as zero time) and some visceral arteries.)

<table>
<thead>
<tr>
<th>Artery</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coeliac artery</td>
<td>8.4± 6.2 (9)</td>
</tr>
<tr>
<td>Lieno intestinal artery</td>
<td>10.5± 9.8 (10)</td>
</tr>
<tr>
<td>Pneumogastric artery (intestinal branch)</td>
<td>11.0±10.4 (10)</td>
</tr>
<tr>
<td>Gas bladder artery</td>
<td>8.2± 9.6 (8)</td>
</tr>
<tr>
<td>Splenic artery</td>
<td>10.9± 8.5 (9)</td>
</tr>
<tr>
<td>Anterior mesenteric artery</td>
<td>17.3±10.3 (10)</td>
</tr>
<tr>
<td>Anterior renal artery</td>
<td>19.7±10.1 (10)</td>
</tr>
<tr>
<td>Meeting of mesenteric and lienointestinal arteries</td>
<td>45.9±36.6 (6)</td>
</tr>
</tbody>
</table>

The pneumatic duct and gas gland circulation

There are two morphologically distinct routes by which blood entering the gas gland artery can return to the heart (Mott, 1950). These are the pneumatic duct
vein, which receives blood from the capillary network in the wall of the pneumatic duct and runs straight back to the heart; and the gas gland vein receiving blood from the red bodies which empties into the hepatic portal vein. It appears from the radiographic records that, as a rule, one or other of these circulations is in action, or, sometimes, both together. The relevant circulation times are given in Tables 2 and 3, calculated from the arrival of the injection at the level of the anterior gut arteries in the dorsal aorta.

**The posterior venous return**

The time which elapses before the radiopaque material appears in the veins is often very great, especially when the heart rate is slow. Some of these times are given in Table 3 calculated from the arrival of the blood at anterior gut artery level in the dorsal aorta.

**Table 3**

(Times (in seconds) elapsing between the appearance of Thorotrast, injected into the ventral aorta, in the dorsal aorta at the level of the anterior gut arteries and in the visceral veins.

<table>
<thead>
<tr>
<th>Hepatic portal vein at the level of:</th>
<th>(Times in seconds)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>16.8 ± 10.6</td>
<td>(9)</td>
</tr>
<tr>
<td>Spleen</td>
<td>11.8 ± 3.4</td>
<td>(5)</td>
</tr>
<tr>
<td>Mid-intestine</td>
<td>44.4 ± 35.0</td>
<td>(7)</td>
</tr>
<tr>
<td>Branches of the hepatic portal vein in liver</td>
<td>20.5 ± 15.9</td>
<td>(4)</td>
</tr>
<tr>
<td>Pneumatic duct vein</td>
<td>19.4 ± 14.9</td>
<td>(7)</td>
</tr>
<tr>
<td>Gas bladder vein</td>
<td>19.0 ± 7.8</td>
<td>(4)</td>
</tr>
<tr>
<td>Posterior end of posterior cardinal vein</td>
<td>61.1 ± 36.5</td>
<td>(3)</td>
</tr>
</tbody>
</table>

There is often an appearance of retrograde flow in the hepatic portal vein. Whether this is genuine or not is extremely difficult to ascertain, as the more anterior part of the vein naturally becomes opaque first, since the arteries whose contents contribute to its opacity are themselves opaque before those whose contents eventually appear in the posterior parts of the hepatic portal vein. In several eels, however, there was a definite indication of a forward flow in the posterior half of the hepatic portal vein whose shadow joined up with the shadow in the anterior part of the vessel. It seems likely, therefore, that any retrograde movements are only apparent.

**The anterior venous return**

No contrast medium has been detected in the anterior venous return so that all that can be stated is that it does not occur within about 40 sec. of the blood leaving the ventricle. So little blood seems to enter the cranial arteries, however, that it is quite possible that some time would have to elapse before any contrast medium became visible in the anterior veins. That the contrast medium does reach them is shown by the fact that they eventually become just as opaque as other parts of the venous system.

**DISCUSSION**

The general picture obtained of the behaviour of the eel's circulation from the serial radiographs described above does not differ markedly from what would be assumed from a knowledge of its morphology. There are, however, some subsidiary points of interest.
Radiological observations on cardiovascular system in Anguilla anguilla

The bulbus arteriosus is well known to be composed of elastic tissue, but it does not appear to have been generally realized that its behaviour can be literally elastic, though von Skramlik (1935) described it as a 'windkessel'. It will help to spread out the rise of pressure produced by ventricular systole over a longer period of time than would otherwise be the case, and thus avoid subjection of the proximal ends of the gills to too extensive fluctuations in pressure as von Brucke (1852) suggested. Pressure curves in the bulbus arteriosus and ventral aorta were later recorded with a condenser manometer, amplifier and cathode-ray oscilloscope. There was considerable flattening of the peak pressure recorded in the ventral aorta compared with that in the bulbus arteriosus. The functional analogy with the aortic arch of mammals is obvious.

It is clear that the gills offer a considerable resistance to the passage of the blood though not perhaps as much as might have been expected. The various parts of the heart-gill complex, should not, however, be considered separately. The pressure developed in the ventral aorta and afferent branchials must depend on the resistance of the gills as well as on the force of the heart beat and the resultant of both these factors may be reflected in the behaviour of the bulbus arteriosus. It is possible that complete mixing of the venous blood from various parts of the body does not occur in the ventricle, since streamlines were observed on occasion in the ventral aorta.

The amount of blood reaching the head seems small in view of the great importance always attached to this part of the circulation. It must be remembered, however, that the brain of a fish only forms a very small part of its body weight. Even so, this part of the circulation is less in evidence than one might expect from its morphology. All the emphasis is on the caudal flow of blood from the gills, certainly from the second, third, and fourth gill arches.

Interest in the visceral circulation chiefly attaches to that part of it supplying the pneumatic duct and gas glands, and to the fact that there appear to be two alternative circulations here. From what is known of the function of the gas glands and the homologues of the pneumatic duct in fish, it is tempting to associate activity in the pneumatic duct circulation with gas absorption and that in the red body circulation with gas secretion. This hypothesis is perhaps supported by the fact that the latter tends to be more in evidence when the duration of the experiment has been less. The blood in the pneumatic duct vein of the anaesthetized fish often appears very red, as if well oxygenated.

The circulation times in the anaesthetized eel are obviously subject to considerable variation and all are slow when compared with those known for higher Vertebrates. On the other hand, it must be remembered that these observations have been recorded in anaesthetized eels, which are naturally quiescent. The venous return is slow under these conditions, and it may well be that the active eel maintains a more rapid venous return through the effect of its continuous co-ordinated bodily undulations on the veins.
SUMMARY

1. A description is given of an angiographic method for studying the circulation of blood in the anaesthetized common eel.
2. The valves of the heart are concluded to be competent.
3. The extreme elasticity of the bulbus arteriosus is noted, especially of its anterior end.
4. The circulation time through the gills was $5.6 \pm 1.9$ sec.
5. The circulation in the head is relatively inconspicuous; all the emphasis is on the backward flow of blood from the gills.
6. The circulation times to the viscera are very variable.
7. It appears that the two alternative routes of venous return from the pneumatic duct artery, which are the pneumatic duct vein and the gas gland vein, may both be operating together or either may operate alone.

My thanks are due to Dr G. S. Dawes for helpful criticism, to the late Dr A. E. Barclay who introduced me to the techniques of cineradiography, and to Mr Maurice Tuckey for technical assistance. This work was carried out during the tenure of a D.S.I.R. maintenance grant.

REFERENCES


MOTT, J. C. (Unpublished).


EXPLANATION OF PLATES

All the plates are made from contact prints of the original radiographs.

PLATE 8

Fig. 1. Eel 55, run 1, dorsoventral projection: a positive of frame taken 6.1 sec. after injection of 1 ml. Thorotrast into caudal vein printed together with frame taken 14.4 sec. after injection. Note virtual elimination of skull and afferent branchial system; l.o.a., left orbital artery; d.a., dorsal aorta; c.a., root of left cephalic coronary artery.

Fig. 2. Eel 25, run 2 (1st run failed), lateral projection 6.3 sec. after 4 ml. Thorotrast injected per caudal vein. Shows jet of blood entering ventricle from auricle, a.; bulbus arteriosus, b.a., filled at previous heart beat; p.c., right posterior cardinal vein; v., vertebral column.

Figs. 3–5. Eel 38, run 1, lateral projection, three consecutive frames 3.9, 5.6 and 7.3 sec. respectively after injection of 1 ml. Thorotrast into caudal vein. Heart rate 50–5 per min. Fig. 3 shows ventricle in diastole and accumulation of ThO$_2$ in sinus venosus; Fig. 4 shows ventricle in systole and auricle well filled; Fig. 5 shows ventricle in diastole and ventriculo-bulbar valves shut, s., sinus venosus; v., ventricle; b., bulbus arteriosus; v.b., ventriculo-bulbar valves; c., cleithrum; a', afferent branchial artery.

Figs. 6, 7. Eel 52, run 2 B. Two consecutive frames from series from which Text-fig. 2 was constructed, ventrodorsal projection. Lines indicate distances measured. Heart rate 15 per min. a.v., auriculo-ventricular valves.
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Radiological observations on cardiovascular system in Anguilla anguilla

PLATE 9
Eel 55, run 1, dorsoventral projection. Six frames taken respectively 1·6, 2·4, 3·1, 8·3, 14·4 and 17·5 sec. after an injection of 1 ml. Thorotrast into the caudal vein. Heart rate 32 per min. a., auricle; b., bulbus arteriosus; v.a., ventral aorta; v., ventricle; o., otolith; 1, 2, 3, afferent branchial arteries; g., gill filaments; e., efferent branchial artery; d., dorsal aorta; c., origin of cephalic coronary artery; b.a., brachial artery; h., hepatic artery; c.a. coeliac artery; r., retina outlined by vessels; o., orbital artery; op. ophthalmic artery. Note in frame 2, jet of blood from ventricle entering bulbus arteriosus.

PLATE 10
Eel. 72, run 1. Five frames taken respectively 3, 6, 12, 24 and 39 sec. after the injection of 1 ml. Thorotrast into the ventral aorta. Heart rate 62 per min. h., hepatic artery; c., coeliac artery; p., pneumogastric artery; d., pneumatic duct; g., gas bladder; r. (frame 1), red body; t., lienointestinal artery; s., splenic artery; b., branch of pneumogastric artery; p.v. pneumatic duct vein; m., mesenteric artery; r. (frame 3), anterior renal artery; h.p.v., hepatic portal vein; g.v., gas bladder vein.