J. Exp. Biol. (1967), **46**, 195–203
With 1 plate and 6 text-figures
Printed in Great Britain

HEPATIC VEIN SPHINCTERS IN ELASMOBRANCHS AND THEIR SIGNIFICANCE IN CONTROLLING HEPATIC BLOOD FLOW*

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(*Received* 25 *July* 1966)

The liver exerts its influence in general cellular metabolism and homeostatic control within limits set by the quality and quantity of blood perfusing it. Hepatic outflow reflects hepatic portal flow which is influenced by the diverse blood flow requirements of other visceral organs engaged in metabolic and absorptive functions. In addition, the hepatic blood flow constitutes a sizeable fraction of the total venous return to the heart and is important in the maintenance of an effective overall circulation.

The large size of the liver and the hepatic vasculature is as conspicuous in lower aquatic vertebrates as in higher vertebrates. This suggests an important hepatic role in vertebrate cardiovascular dynamics in general. The present paper concerns the discovery of discrete muscular sphincters of the hepatic veins‡ of elasmobranch fishes and their possible role in controlling hepatic blood flow.

In a large shark, *Selache maximus* (weight 1500 kg.), conspicuous muscular sphincters were discovered on the hepatic veins where they empty into the sinus venosus. Pl. 1, fig. 1 demonstrates these from a posterior view after ablation of the liver and the hepatic veins. In Pl. 1, fig. 2 the sphincters are viewed from the opened sinus venosus. The ducts of Cuvier which connect the common cardinal sinus system to the heart showed regular valves which prevent regurgitation during contraction of the sinus venosus.

The functional significance of the sphincters was investigated during studies of the general mechanics of venous return in elasmobranch fishes.

MATERIAL AND METHODS

Hepatic vein sphincters were dissected from pithed Pacific dogfish, *Squalus suckleyi*, and fixed in neutral buffered formalin or in Bouin's fixative diluted 1/3 with sea water. The embedded tissues were sectioned (10 μ) and stained by Patay's modification of Masson's technique.

In the Pacific skate, *Raja binoculata*, the heptatic veins empty into the common cardinal sinuses rather than directly into the sinus venosus. However, similar

* Supported by grant GB-4038 from the National Science Foundation and grant GM-1194-02 from the U.S. Public Health Service.

† This work was done during the tenure of an Established Investigatorship of the American Heart Association and supported by the Northeastern Chapter of the Washington State Heart Association.

‡ Hepatic veins often referred to as hepatic sinuses in lower vertebrates (Hyman, 1942).

muscular sphincters are located at their junctions with the cardinal sinuses. These were excised, fixed, sectioned and stained like the dogfish tissues.

Physiological studies were made on fourteen dogfish and two skates at the Friday Harbor Laboratories. Intravascular pressures were measured via chronically implanted polyethylene catheters. Pressures were compared in the sinus venosus, the common and posterior cardinal sinuses and in the hepatic portal and hepatic veins. In some cases the systemic arterial blood pressure was also recorded. The intravascular pressures were measured by means of sensitive electromanometers (Statham Strain Gauge Transducers, Model P23BB) and recorded with a Beckman Offner-type RS Dynograph.

During surgery the fishes were artificially ventilated with aerated sea water at 9° C. Cutaneous infiltration with a local anesthetic (Xylocaine, 2%) provided adequate anesthesia.

A mid-line incision 5–7 cm. long ventral to the anterior portion of the liver permitted access to the hepatic veins, hepatic portal vein and sinus venosus. In most dogfish a portion of the left hepatic vein was visible through the liver capsule about 3–6 cm. caudal to the transverse septum. In skates the liver was displaced mediad to expose an hepatic vein dorsal to the liver where it was cannulated. Polyethylene catheters 60–100 cm. long (0·86 mm. I.D.) were prepared with side holes cut in the terminal 1 cm. Catheters of equal length were used for each animal. Each catheter was inserted through a hole made in the wall of the vein and was secured by a purse-string suture which prevented blood leakage around the catheter with negligible constriction of the vessel. Portal veins were easily identified and were similarly cannulated.

Posterior cardinal and common cardinal sinuses were cannulated by inserting a 15 gauge needle into each sinus from the dorsal side, passing a catheter into the sinus through the needle and withdrawing the needle while leaving the catheter in place. Sinus venosus pressures were recorded by means of catheters passed from the hepatic vein through the sphincter to the sinus venosus and by indwelling catheters inserted through the wall of the sinus venosus during exposed heart surgery. The fish were free to swim in aquaria while the pressures were being recorded.

RESULTS

Text-fig. 1*a* and *b* show a cross-section and a longitudinal section of hepatic vein sphincters in elasmobranchs. Each sphincter consists of a smooth-muscle ring at the junction of the hepatic vein and the venous sinus. Text-fig. 1*b* shows the prominent smooth muscle which constitutes the majority of the thickened vascular wall.

In vitro studies

Freshly excised hepatic veins including the sphincter were immersed in elasmobranch physiological saline solution and suspended in such a way that the sphincter and the lumen of the vein could be observed. Acetylcholine chloride (10 μg./ml.) caused the sphincter muscle to close the lumen firmly. Rinsing the tissues with fresh saline solution relaxed the sphincter. Adrenaline chloride solution increased the rate of relaxation.

In the presence of both acetycholine and adrenaline (both at 10 μg./ml.) the sphincter alternately constricted and relaxed, repeatedly for at least 5 min.

In vivo studies

Text-figure 2 shows simultaneous records of blood pressures from a dogfish posterior cardinal sinus (P.C.S.) and hepatic vein (H.V.). A negative (subatmospheric)

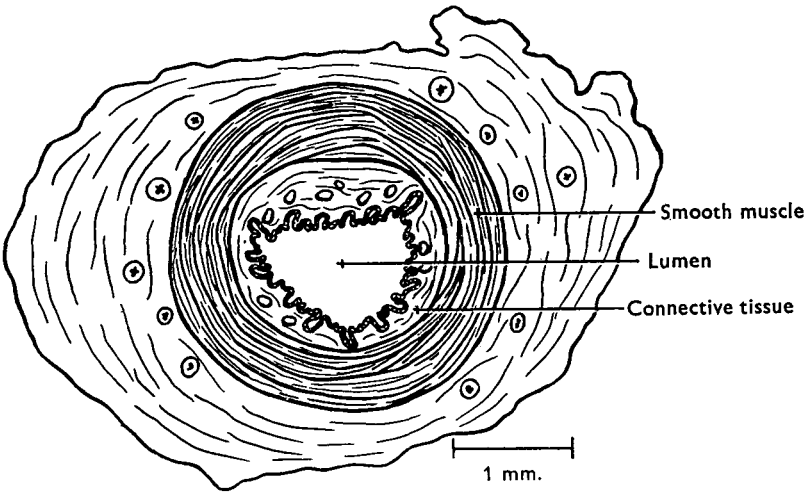


Fig. 1*a*

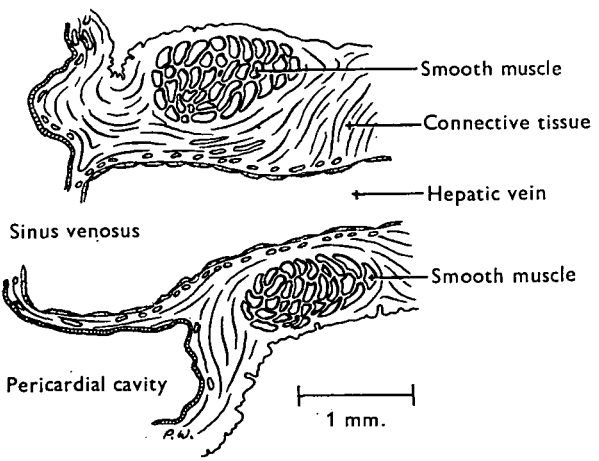
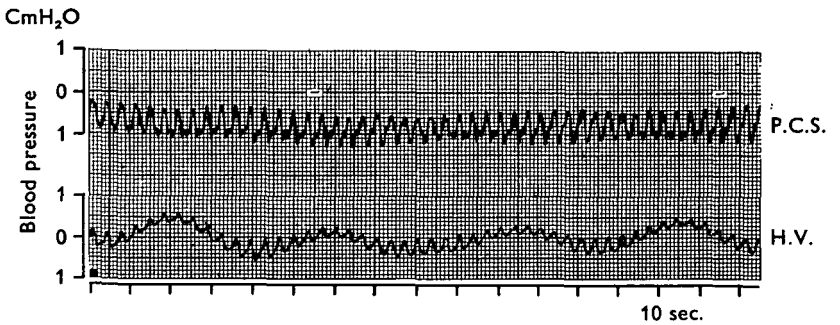


Fig. 1*b*

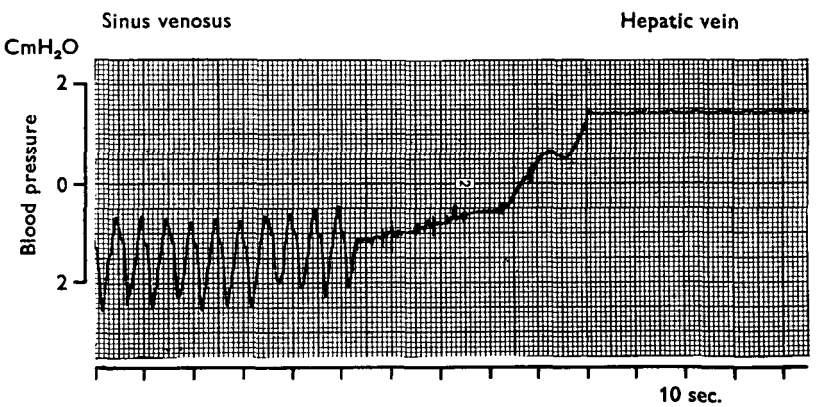
Text-fig. 1. Drawings from histological sections of hepatic veins. Tissues were fixed in Bouin's fixative and stained by Patay's modification of Masson's technique. *a*, Cross-section through a sphincter of *Raja binoculata*. *b*, Longitudinal section through a sphincter of *Squalus suckleyi*.

pressure (0 to −1 cm. H_2O) prevails in the posterior cardinal sinus, while the hepatic vein pressure fluctuates slowly between +0·5 cm. H_2O and −0·5 cm. H_2O. The pulse pressure increases significantly when the mean pressure drops. The mean pressure fluctuations represent spontaneous, intermittent closure and opening of the hepatic vein sphincter. In many cases the hepatic venous pressures did not fluctuate but

remained positive and well above the subatmospheric pressure in the other central systemic veins, indicating closed or nearly closed hepatic vein sphincters. Text-fig. 3 depicts a comparison of the pressure inside the sinus venosus and the hepatic vein. A catheter extended into the sinus venosus from the hepatic vein. A mean negative pressure and large pressure pulses with each heart-beat are apparent. Each drop in pressure is synchronous with ventricular systole. The smaller changes of the wave-



Text-fig. 2. Simultaneous records of intravascular pressures in a posterior cardinal sinus (P.C.S.) and an hepatic vein (H.V.) of *Squalus suckleyi*.



Text-fig. 3. Blood pressures recorded from the sinus venosus and an hepatic vein in *Squalus suckleyi*.

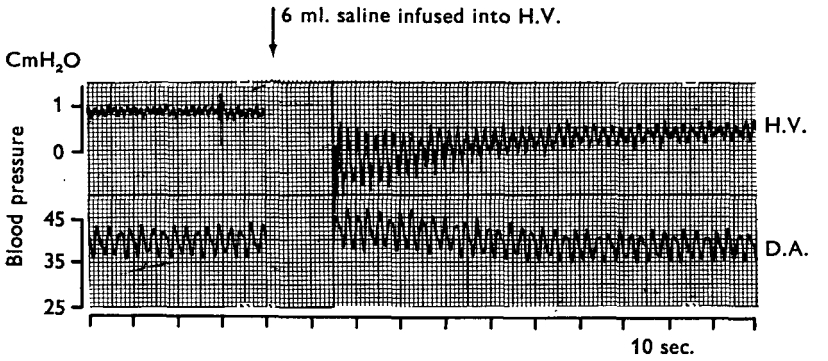
form are caused by respiratory movements. The catheter was slowly pulled back into the hepatic vein leaving the tip a few millimetres past the sphincter. The pressure in the hepatic vein is elevated and the fluctuations apparent in the sinus venosus pressure record are not transmitted through the closed sphincter.

Text-fig. 4 demonstrates the result of infusing saline solution into the hepatic vein. Prior to infusion the hepatic venous pressure is positive with a small pulse pressure, indicating that the hepatic vein sphincter is closed. Following infusion of 6 ml. the pressure declined and the pulse pressure increased accordingly. The rise back to the pre-injection pressure level is slow. The lower tracing in Text-fig. 4 represents dorsal aortic pressure which showed a small transient increase during and immediately following infusion.

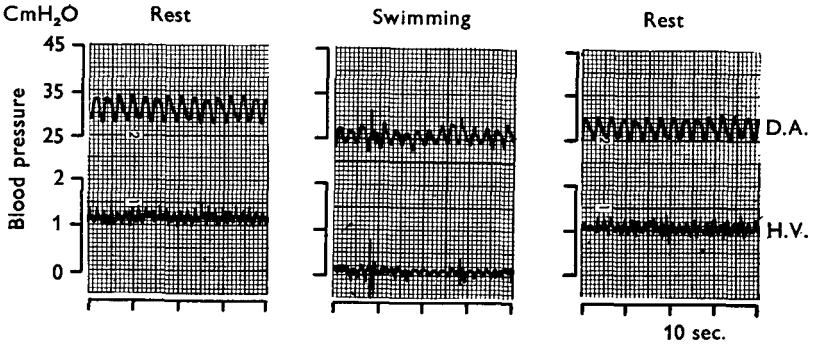
Text-fig. 5 shows dorsal aortic and hepatic venous blood pressures during rest,

swimming and subsequent rest. The swimming causes a decreased dorsal aortic pressure indicating a reduced resistance in the systemic arterial bed which lasts long into the recovery. The hepatic venous pressure similarly dropped, but increased promptly when the fish stopped swimming.

Text-fig. 6 illustrates the result of an attempt to influence the contractile state of the hepatic sphincter by injection of acetylcholine into the hepatic vein. The sphincter started to relax after 30 sec., resulting in a progressive drop in the hepatic vein



Text-fig. 4. Intravascular pressures recorded simultaneously from the hepatic vein (H.V.) and the dorsal aorta (D.A.) in *Squalus suckleyi* prior to and following infusion of saline solution into the hepatic vein.



Text-fig. 5. Comparison of dorsal aortic and hepatic vein pressures during rest, swimming and subsequent rest in *Squalus suckleyi*.

pressure. The response was slow and was probably secondary to an effect of the acetylcholine on the systemic circulation. Injection of drugs *in vivo* has the disadvantage that general systemic effects may obscure the specific effect of the drugs on the sphincter.

DISCUSSION

It is generally recognized that the capacitative function of the vascular system resides dominantly in its venous portion. In lower vertebrates an extensive system of large venous sinuses is interposed between the terminal arteries and capillaries and the heart, especially in cyclostome and chondrichthian fishes. In teleosts and higher vertebrates the venous sinuses are much reduced, but they persist as liver sinusoids and portions of the cerebral venous drainage even in mammals.

An adequate venous blood return to the heart is essential to maintain effective circulation since adjustments of cardiac output are largely effected by changes in cardiac inflow. Evaluation of the role of liver blood flow in the maintenance of effective circulation requires an appreciation of the major factors responsible for the return of venous blood. The haemodynamics of venous return are influenced by local vasomotor activity in the smooth muscles of the vascular walls, resulting in changes of calibre and capacitance (venomotor activity), and by the pressure that remains after the gradual dissipation of the energy imparted to the arterial blood by cardiac contraction (*vis a tergo*). Suctional attraction for the venous blood is also operational in the mechanics of venous return in elasmobranch fishes (*vis a fronte*). A negative pressure at least partly caused by ventricular contractions within the rigid pericardium is largely responsible for this effect. The subatmospheric pressure is transmitted to the atrium, sinus venosus and cardinal sinus systems (Johansen, 1965; Sudak, 1965*a*, *b*). Branchial respiratory movements also contribute to the *vis a fronte* effect.

Specialized mechanisms that aid the return of venous blood to the heart are apparent in all vertebrate classes. The large venous sinuses of the lowest vertebrates offer little structural basis for capacitance changes by venomotor activity since the sinus walls apparently lack smooth muscles. However, a number of auxiliary venous pumps aid the venous return in these fishes. The portal, cardinal and caudal hearts of myxinoid fishes are striking examples in this respect (Johansen, 1963).

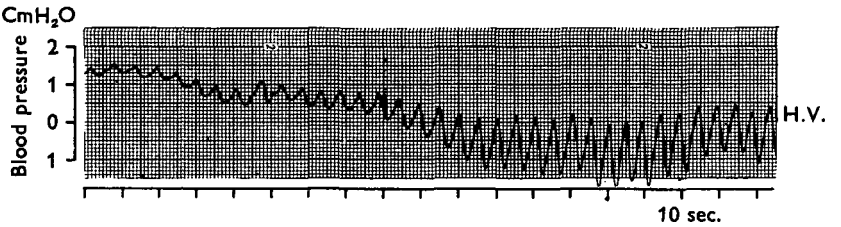
Venous valves primarily located in dependent extremities of terrestrial vertebrates translate extramurally applied forces into propulsive energy (venous muscle pump). The belief has been held that venous valves have largely developed in response to the gravitational forces acting on columns of blood in dependent extremities and should hence not occur in aquatic animals where gravitational effects are virtually nil. Recently, intravascular valves have been demonstrated in elasmobranch fishes in veins as well as in the segmental arteries (Satchell, 1965). Extramural forces resulting from muscle contractions during swimming have long been thought to aid venous return in fishes (Schoenlein, 1895). The recent finding of intravascular valves re-emphasizes the functional significance of such a mechanism for venous return.

The primitive cardinal veins, which persist in adult elasmobranch fishes, appear in the embryos of all vertebrates. Vertebrate phylogeny is often illustrated by the gradual transformation of the cardinal system to the venae cavae system of higher vertebrates. An integral part of this transformation is related to the hepatic portal circulation. In the lower vertebrates possessing cardinal veins the circulation through the liver is connected directly to the sinus venosus (sharks) or to a central portion of the common cardinal vein (skates). In the higher vertebrates (tetrapods) the hepatic veins take part in the formation of the posterior venae cavae.

The prominent suctional attraction aiding the return of central venous blood to the heart in elasmobranch fishes and the minimal structural basis for venomotor control of regional venous return create a special case for the hepatic circulation. It is to be expected that the vital homeostatic role of the liver would depend upon an effective means of controlling blood flow through the liver. The prevailing negative pressure in the sinus venosus of these fishes would exert a pull on the blood in the hepatic system leaving little opportunity for regulation of blood flow. We contend that the hepatic

sphincters offer a remarkable structural adaptation for controlled release of hepatic blood. A sphincteric control of hepatic flow is eminently designed to permit delicate adjustments of the volume and transit time of blood in the liver while it provides a structural basis for sudden mobilization of blood from the massive liver in situations demanding an increase of cardiac output. Text-fig. 5 implies such a sphincteric control of hepatic blood mobilization.

Text-fig. 2 shows a phasic release of the contracted sphincter which suggests that the normal control of the sphincter is mediated by the volume of blood flowing through the liver. Such a volume-dependent sphincter control is also indicated by the relaxation of the sphincter when the hepatic vein is infused with saline solution. Recent work has also disclosed that acetylcholine administered intravascularly to elasmobranchs may cause an increased flow due to a decreased resistance in the systemic vascular bed (Hanson, 1967). Text-fig. 6 shows a gradual relaxation of the sphincter that may be caused by such an increased systemic perfusion.



Text-fig. 6. Changes of hepatic vein pressure following administration of 5 μg. of acetylcholine into the hepatic vein of free-swimming *Squalus suckleyi*.

The hepatic sphincters may also prevent regurgitation of blood during contraction of the sinus venosus. To the authors' knowledge, they are the only sphincter-type cardiac valves ever reported.

The idea of a sphincteric release of hepatic blood flow in higher vertebrates has long been promulgated and several review articles on the subject have been written (Arey, 1941; Snyder, 1942; Knisely, Harding & Debacker, 1957). Inlet and/or outlet sphincters on the hepatic vessels have been described for several species of carnivores, rodents and cetaceans among the mammals, in one species of turtles (Tyler, 1941), amphibians (frogs) and one species of teleosts (Elias, 1955). However, there has been no earlier report of such discrete and prominent sphincters as those described for elasmobranch fishes in the present investigation. Hepatic sphincters have been variously described as sluice valves, sluice channels or throttle veins (*Drosselvenen*). Knisely *et al.* (1957) have pointed out that the majority of papers dealing with hepatic outflow mechanisms make use of the above terms from results obtained by physiological experimentation rather than from anatomical evidence of discrete sphincters. The different types of hepatic outflow mechanisms have been categorized into five different groups (Knisely *et al.* 1957). One of these types is an arrangement of the smooth muscle fibres as annular bands, especially where the hepatic venules empty into the hepatic veins or on the hepatic vein itself. In a number of aquatic mammals (e.g. seals, whales, and hippopotamuses) the annular bands are located around the inferior vena cava and the outlet orifices of the large hepatic veins.

There has been considerable conjecture about the purpose of the sphincteric mechanism of the hepatic veins in higher vertebrates. Its role in anaphylactic shock has been emphasized, but the minor biological significance of that condition has suggested the alternative that the relaxation and contraction of the smooth muscle on the hepatic veins enhances the overall circulation in the normal animal (Thomas & Essex, 1949). The numerous attempts to assess the functional significance of these sphincters do not have enough in common to warrant space in this discussion. It has been difficult to reconcile the presence of sphincters with the ecology and behaviour patterns of the species that possess them. However, Knisely *et al.* (1957) point out that hepatic outlet control mechanisms represent an important throttle valve between the main venous return route to the right heart and the very large and important blood reservoir of the liver and splanchnic vascular bed. Their principal function is hence thought to consist of allowing blood stored in the liver to be mobilized for increased demands of cardiac output. The thought presently advanced that the sphincters in elasmobranchs primarily control the blood flow through the liver to permit optimal functioning of that organ seems entirely original. Our view strongly opposes a statement by Elias (1952, 1955) that these muscles (sphincters) present one of those 'accidental curiosities of evolution which are of no use to their possessors, but which exist because they are not harmful enough to wipe out the species'. This far-reaching, and we feel presumptuous, statement evidently arose from the observation that anaphylactic shock and other experimental forms of shock were characterized by tightly closed outflow sphincters and pooling of large quantities of blood in the liver.

SUMMARY

1. Discrete muscular sphincters have been discovered on the hepatic veins of elasmobranch fishes. Their anatomy has been studied by gross dissection and by histological techniques. Their functional significance has been assessed by application of drugs to excised sphincters and by measurements of intravascular pressures in free-swimming fishes by means of chronically indwelling catheters.

2. Topical application of acetylcholine caused contraction of the sphincters and adrenaline facilitated relaxation.

3. Pressure measurements disclosed that the hepatic venous pressures in general exceed those in the central systemic veins, thus indicating partial or complete closure of the hepatic vein sphincters. The hepatic vein pressures showed spontaneous phasic changes reflecting closure and opening of the sphincters.

4. It is suggested that hepatic vein sphincters are of particular significance in elasmobranchs since negative pressures prevail in the sinus venosus and central venous sinuses, exerting suctional attraction for venous blood. A sphincteric release of hepatic blood provides a structural basis for delicate adjustments of the volume and transit time of blood in the liver and for mobilization of blood stored in the liver at times of increased demand on cardiac output.

5. The findings are discussed in relation to general mechanics of venous return in fishes and are compared with studies of hepatic vascular sphincters in other vertebrates.

The authors thank Mrs Deborah Christensen for her assistance in the histological portion of this investigation.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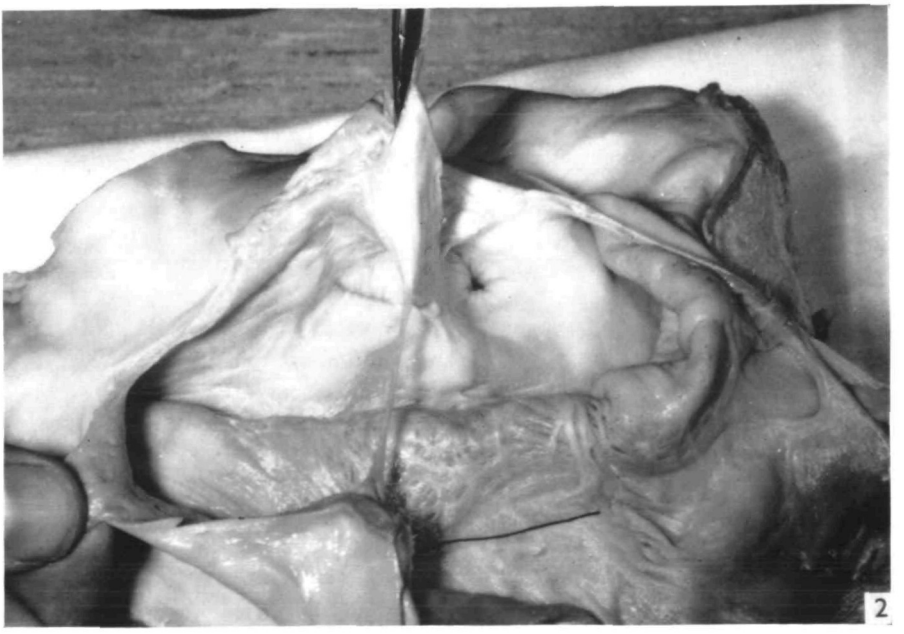
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EXPLANATION OF PLATE

Hepatic vein sphincters in *Selache maximus*. Fig. 1. Posterior view after ablation of the liver and the hepatic veins. Fig. 2. Anterior view from the opened sinus venosus.