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HISTOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL STUDIES ON THE HEART OF TWO CYCLOSTOMES, HAGFISH (*MYXINE*) AND LAMPREY (*LAMPETRA*)

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In the present paper an account is given of histological and experimental studies on the innervation of the heart in *Myxine* and *Lampetra* (*Petromyzon*). The reactions to drugs of the isolated hearts and the presence of acetylcholine (ACh), cholinesterases, histamine and catechol amines have been investigated.

Green (1902) and Carlson (1904) during experimental work with the cyclostome *Bdellostoma* found that this vertebrate is peculiar in having no cardioregulative nerves. Fänge & Östlund (1954) found that the isolated heart of another myxinoid, *Myxine*, gives no or only weak responses to acetylcholine, adrenaline and noradrenaline. Östlund (1954) reports that the heart of *Myxine* contains surprisingly great quantities of catechol amines.

In contrast to myxinoids the petromyzontids have been shown to possess nervous elements in the heart. The presence of ganglion cells in the heart of *Lampetra* (*Petromyzon*) has been observed by Owsiannikof (1883), Ransom & Thompson (1886) and Tretjakof (1927). According to Ransom & Thompson (1886) and Julin (1887) the heart receives fibres from the vagus nerve, and Tretjakof (1927) noticed a rich network of sympathetic fibres in the sinus venosus. Gaskell (1912) investigated chromaffin cells in *Lampetra* and noticed that the heart contains some substance with an adrenaline-like effect upon blood pressure of the cat. Otorii (1953) reports that the isolated heart of *Entosphenus* is accelerated by acetylcholine, pilocarpine, physostigmine and choline muscarine, whereas adrenaline retards the heart frequency.

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METHODS

Animal material: adult hagfish (*Myxine glutinosa*) and lamprey (adult specimens of *Lampetra fluviatilis*, adult and larval specimens of *L. planeri*).

Histological. The fixatives were Bouin's fluid and no. 2 of Bodian fixative for protargol staining: and in order to demonstrate chromaffin tissue, Müller's and Orth's fluids. Paraffin embedding and sectioning (5–15 μ) were followed by staining according to the Bodian method (Bodian, 1936, 1937) or the modifications of this method by Ziesmer (1951) or Johnels (1955).

Nerve stimulation. Stimulation was given from a thyratron stimulator by the aid of silver electrodes. Stimuli of 5 msec duration and 1·5 V were used in the experiments on *Lampetra* and of 2 msec duration and 4·5 V in the experiments on *Myxine*. The animals were anaesthetized with urethane.

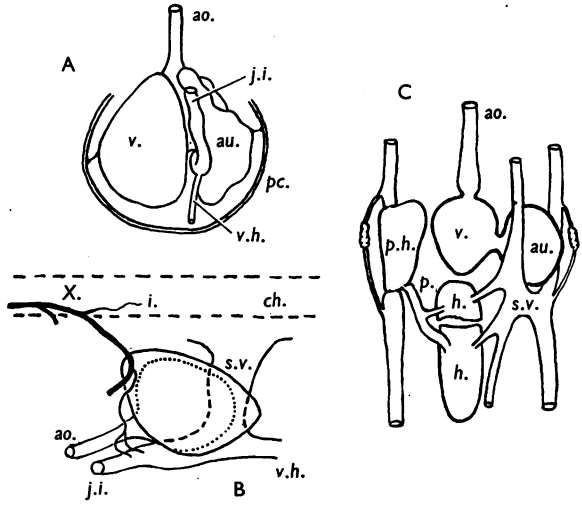

Fig. 1. A: *Lampetra fluviatilis*, adult. Diagram of the heart, ventral view. B: *L. fluviatilis*, larva. Reconstruction based on serial sections. Left side view. C: *Myxine glutinosa*. Diagram of the heart and adjacent structures. *ao*, ventral aorta; *au.*, auricle; *ch.*, notochord; *h.*, liver; *i.*, intestinal branch of the vagus; *j.i.*, ventral (median) jugular vein; *p.*, portal vein; *p.h.*, portal vein heart; *pc.*, pericardium; *s.v.*, sinus venosus; *v.*, ventricle; *v.h.*, hepatic vein; *X*, nervus vagus.

Heart perfusion. The apparatus described by Fänge & Östlund (1954) was used. The perfusion fluids were frog Ringer (*Lampetra*) and a 2/1 mixture of sea water and distilled water (*Myxine*). During work with the *Lampetra* heart the cannula was inserted into the dorsal part of the sinus venosus (Fig. 1 A, B). The *Myxine* heart was cannulated through one of the hepatic veins reaching the sinus venosus (Fig. 1 C). Temperature of the perfusion fluid was kept at about 18° C. Doses of drugs are expressed in the text below as g/ml. perfusion fluid. All values refer to the salts.

Substances used were: acetylcholine chloride, adrenaline hydrochloride, DL-noradrenaline hydrochloride, atropine sulphate, butyrylcholine chloride, cocaine hydrochloride, hexamethonium chloride, eserine salicylate, nicotine hydrochloride, tubocurarine chloride, propionylcholine chloride, 5-hydroxytryptamine creatinine sulphate.

Cholinesterase activity. The Warburg technique was employed (Augustinsson, 1948) using the chloride of acetylcholine (ACh), acetyl-β-methylcholine (mecholyl), and butyrylcholine as substrates. Enzymic activity is expressed in μmole of ester hydrolysed in 30 min by 1·0 g tissue (wet weight), corrections being made for spontaneous hydrolysis of the substrates.

Estimation of choline, choline esters and histamine. Paper chromatography was carried out according to the method described recently by Augustinsson & Grahn (1953). Extracts containing tissue bases were prepared in the following manner: the isolated organs (see Table 2) were homogenized in 10% trichloroacetic acid. After filtration the residue was washed with 7% trichloroacetic acid. The acid was removed by repeated extraction with ether (final pH 3·5). The solution was evaporated at a temperature not exceeding 40° C. The isolation of tissue bases followed the description by Kapfhammer & Bischoff (1931) with slight modifications. The reineckates were dissolved in aqueous acetone (pH 4) and converted to chlorides (Augustinsson & Grahn, 1954), the solutions of which were used for paper chromatography.

Provisional biological testing was carried out with various areas of the chromatograms using the isolated guinea-pig ileum as a test object. A paper strip containing a certain area of the chromatogram was put directly into the organ bath. By this method the presence of contracting substances in the chromatograms could easily be demonstrated.

Estimation of catechol amines. The material consisted of about ninety hearts of *Lampetra*. After suction filtration the extracts were concentrated and purified (Euler, 1948; Östlund, 1954) and examined by biological assay on cat's blood pressure and fowl's rectal caecum (Euler, 1949; Östlund, 1954). Paper chromatography of purified extracts was carried out by the ascending method with *n*-butanol saturated with N-hydrochloric acid (Östlund, 1954).

RESULTS

Lampetra

Distribution of nervous elements

The external shape of the heart is seen in Fig. 1A. In the adult it is completely enclosed by a cartilaginous pericardium. Between the ventricle and the auricle passes the sinus venosus as a curved tube in a mainly vertical direction (Favaro, 1908). Nerve fibres destined for the heart run in the epibranchial trunk of the vagus. The caudal extremity of this nerve curves ventrally in a loop caudal to the last branchial opening, forming the hypobranchial branch. The thin heart branch separates from the main trunk caudally to the hindmost branchial opening and runs in a mainly ventral direction to the dorsal wall of the median jugular vein (Fig. 1B). The nerve fibres are distributed in a loose network in the wall of the vein and are mainly oriented in a longitudinal direction (Fig. 2). As a rule one or two main bundles of fibres are discernible among the scattered single fibres. The diameters of the heart nerve fibres are distinctly greater in the adult than in the larva.

In the larva the jugular vein is embedded in the ventral body wall. The nerve fibres pass almost exclusively in the dorsal part of the vessel which is free from the body wall. In the adult the median jugular vein is connected ventrally with the body wall by a thin membrane (mesocardium), which also extends along the ventral and caudal surface of the sinus venosus. In the adult the fibres of the heart nerve are distributed in all parts of the wall of the vein. Further, nerve fibres have been observed which turn ventrally and run through the mesocardium to the body wall. These fibres return to the wall of the jugular vein, usually after only a short deviation from it: no contact with other neurons by these extravenous loops was observed. Finally the fibres spread and terminate in the wall of the sinus venosus.

No distinct nerve fibres (but numerous ganglion cells) have been observed in the walls of the Cuvierian ducts. A few of the fibres in the median jugular vein may continue along the hepatic vein. In the two ligaments (Fig. 1 A) which laterally attach the ventricle and the auricle to the pericardial wall no nervous elements have been observed.

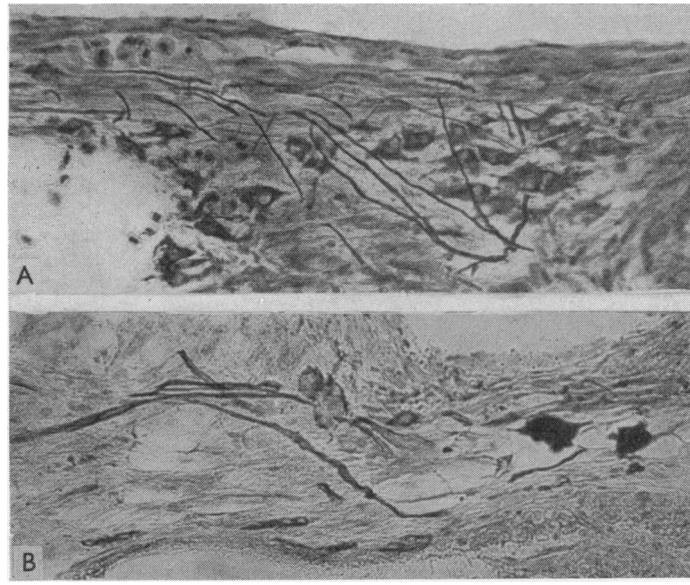

Fig. 2. A: *Lampetra planeri*, larva. Cardiac branch of the vagus nerve at the place where it enters the dorsal wall of the median jugular vein. Ganglion cells are scattered among the nerve fibres. Transverse section. B: *L. planeri*, adult. Same structures. The two dark bodies to the right are pigment cells. Microphotographs, × 260.

All parts of the *Lampetra* heart contain numerous cell bodies which resemble, and presumably are, ganglion cells and will be referred to as such. These cells are located mainly along the interior surface of the heart cavities. Tretjakoff (1927) observed in the sinus venosus multipolar and bipolar ganglion cells. In the present material the multipolar type is the rule. The shape of the cells is very varying (Fig. 3), which is partly explained by the fact that they are likely to change shape in conformity to the state of contraction of the muscle fibres on which they are situated.

In the *Lampetra* heart two types of ganglion cells occur: large and small (Fig. 4 A, B). The average size of the large cell type is usually 13–14 μ and never below 12, whereas the small type has an average size of about 7·5 μ and never exceeds 9 μ. The ventricle contains a few cells smaller than 5 μ. The measurements were made across the cells at right angles to the longitudinal

axis and through the nucleus. The variation in size of the small type of ganglion cells is demonstrated in Table 1.

The large type of ganglion cells is rare. Only about 20–30 have been counted in the heart of *L. planeri*. The total number of ganglion cells in a larva of about 10 cm in length (*L. planeri*) is about 22,000. Of these about 10,500 are in the auricle, about 8000 in the ventricle and about 3500 in the median

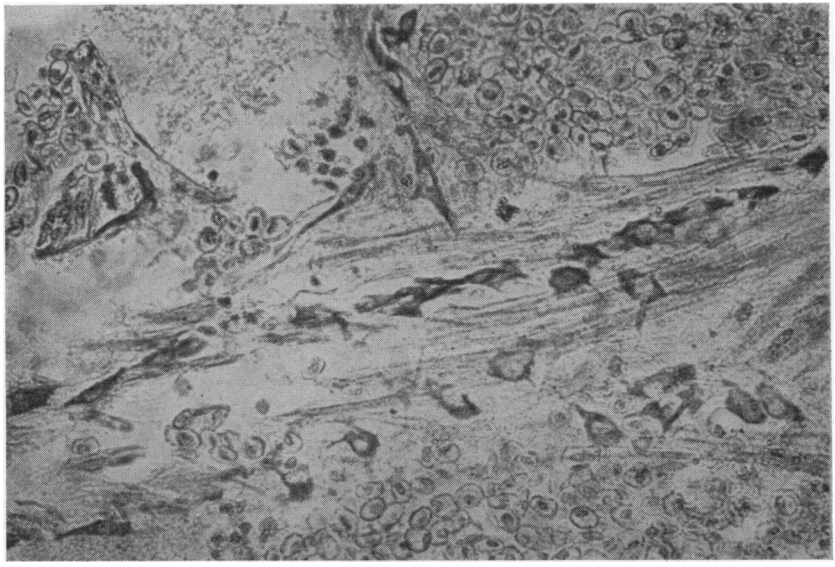

Fig. 3. *L. planeri*, adult. Ganglion cells on the surface of a muscular bundle in the auricle. Microphotograph, ×425.

TABLE 1. Sizes of small type of ganglion cells.

Microns	4·0	4·5	5·0	5·5	6·0	6·5	7·0	7·5	8·0	8·5	9·0
Auricle (100 cells)	—	—	6	8	10	14	15	32	8	4	3
Ventricle (100 cells)	1	1	17	10	10	12	14	26	4	2	2

jugular vein and the ventral portion of the sinus venosus. In the valve region of the aortic bulb there are about 100 cells, but none has been found in the aorta itself. In the walls of the dorsal part of the sinus venosus and the cardinal veins there are numerous ganglion cells.

In the median jugular vein groups of ganglion cells, as well as single cells, may be embedded in the smooth muscular and fibrous connective tissue of the wall. In the sinus venosus the ganglion cells are distributed on the interior surface of the muscles.

In the auricle and ventricle the ganglion cells often group themselves together, forming chains along the muscular bundles (Figs. 3, 4 B). The chains of ganglion cells are strongly developed along the surfaces of the main, central

cavities of the ventricle and auricle, whereas in the peripheral meshes of the loose network of myocardial bundles the ganglion cells appear singly, more remote from each other and connected by long processes.

There is a direct connexion between the cells in the sinus wall, the cell chains within the auricle and, via cells in the atrioventricular junction, the cells of the same kind in the ventricle. In the atrioventricular junction the ganglion cells are present within the connective tissue at the base of the valves and also on the surfaces of the valves. Thus the system of ganglion cells forms a continuum throughout the whole of the heart. The system of ganglion cells is also in contact with ganglion cells in the Cuvierian ducts probably belonging to the sympathetic nervous system.

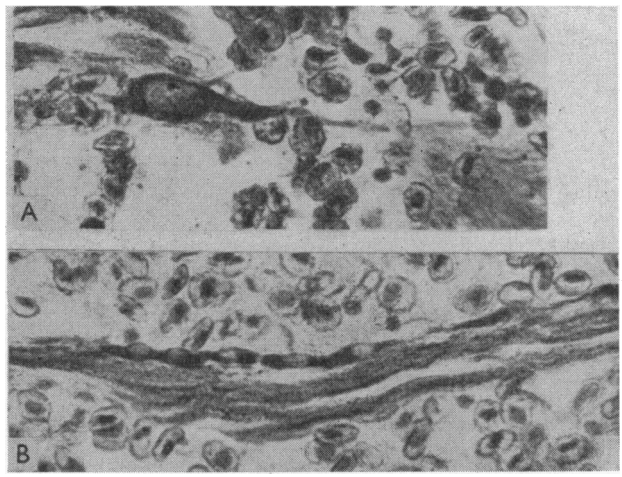

Fig. 4. *L. planeri*, larva. A: large ganglion cell in the ventricle. B: small ganglion cells forming a chain alongside a muscle bundle in the auricle. Microphotographs, ×515.

The cytoplasm of both the large and the small types of ganglion cells contains granules which are heavily stained with protargol. The cytoplasm itself appears light bluish pink. The granules become dark blue when stained by chromium haematoxylin-phloxin (Gomori, 1941).

Giacomini (1902) and Gaskell (1912) observed chromaffin cells along the cardinal veins in *Lampetra* (*Petromyzon*), and Gaskell has described chromaffin cells in the wall of the sinus venosus. In the present investigation it was observed that there are cells in all parts of the heart which give a distinct chromaffin reaction. These chromaffin cells are easily seen in the auricle, the sinus venosus and the wall of the median jugular vein: in the ventricle they are less obvious. The position and shapes of these chromaffin cells are similar to those of the ganglion cells described above, and there is good reason to

suppose that they are identical with what has been termed the small type of ganglion cell. All ganglion cells of the small type appear to be chromaffin. The ability of the small ganglion cells to be stained by the Bodian method is not incompatible with their supposed chromaffin nature because it is well known that chromaffin cells are stainable by silver methods (cf. Pearse, 1953).

Not only the chromaffin cells but also the heart muscle tissue itself shows a weak chromaffin reaction. In preparations of the *Lampetra* heart which have been treated with Müller's fluid the muscle fibres take on a distinct yellow tint. It was occasionally observed that in one heart which had been exhausted by perfusion experiments the myocardium was of a paler yellow tint than in other cases. This may indicate that the chromaffin material within the heart muscle fibres is utilized or released under certain conditions.

Myxine

In this species there exist two hearts, the ordinary branchial heart and the portal vein heart (Fig. 1 C). The pericardial cavity has an open communication with the abdominal cavity (Favaro, 1908; Pietschmann, 1934). Fibres from the intestinal branch of the right and left vagus nerve pass close to the heart and the portal vein heart. Like Carlson (1904) we have been unable to follow, either by dissection or histologically, any nerve fibres passing from the vagus nerve into the heart or portal vein heart although the intestinal fibres of the vagus nerve and the ganglion cells in the intestinal wall stained very distinctly. Nor has it been possible to detect any ganglion cells or chromaffin cells in the *Myxine* heart. However, according to Giacomini (1904) chromaffin cells probably occur along the cardinal veins in *Bdellostoma*. In *Myxine*, as in *Lampetra*, the heart muscles show a weak chromaffin reaction after treatment with Müller's fluid.

Effect of nerve stimulation

Lampetra

The epibranchial trunk is thin, non-myelinized and difficult to find macroscopically in the highly haemorrhagic preparation. We did not succeed in stimulating this nerve, but the presence of cardioregulative nerves was established by stimulation of the medulla oblongata (floor of the fourth ventricle; see Fig. 5 A) or the median jugular vein. The result was the same with both methods of stimulation: great acceleration during, and a period of slowing after cessation of stimulation. With low-frequency stimulation (5 impulses/sec) there was only acceleration, but with high-frequency stimulation (up to 60 impulses/sec) acceleration was always followed by pronounced bradycardia lasting 1–2 min. We have never seen slowing during the period of stimulation, as occasionally observed by Carlson (1906) and Zwaardemaker (1924) in corresponding experiments in which they also found a cardio-accelerating effect of stimulation.

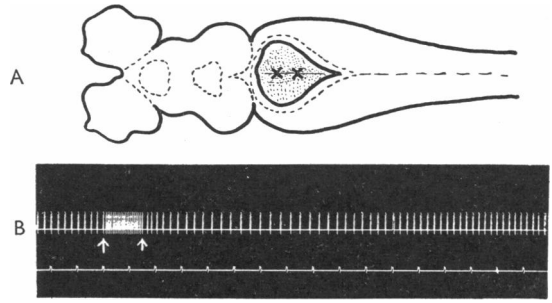

Fig. 5. Stimulation of the floor of the fourth ventricle of the brain of *Lampetra fluviatilis*. A: diagram of the brain showing place of application of electrodes (crosses). B: effect of stimulation. Auricular systoles recorded by pulling down a morse key connected with a magnet signal. Between the arrows stimulation. Time in 5 sec. Duration of stimuli 5 msec. Intensity 1·5 V. Frequency 40 impulses/sec.

Myxine

In this species the vagus nerve can easily be exposed in the gill region by macroscopical preparation. No cardiac effects were obtained during stimulation of one or both vagi, of the medulla oblongata, or of the large veins in the vicinity of the heart. In a few cases, however, stimulation of the right vagus for 1 min was followed by a reversible auriculo-ventricular block reaching its maximum 8–10 min after cessation of stimulation. The rate of the ventricular beat decreased but without any change in the auricular rhythm. It is probable that this block did not result from a direct effect of the nerve on the heart but was caused by some mechanical or humoral mechanism. It may be remembered in this connexion that adrenaline and noradrenaline in relatively weak concentrations sometimes cause a block in the isolated *Myxine* heart (Fänge & Östlund, 1954). Whether vagal stimulation in *Myxine* causes a release of catecholamines, however, is not known. A mechanical effect upon the *Myxine* heart certainly occurs during vagal stimulation, owing to the strong contraction of the gill apparatus and the striated muscles at the cardia (cardiac sphincter).

Drug actions on the isolated hearts

Lampetra

The isolated heart of the lamprey beats spontaneously with a frequency of 40–60 beats/min. The frequency of the heart *in vivo* (anaesthetized or decapitated specimens) is generally lower, 25–40 beats/min.

Adrenaline. Adrenaline added to the perfusion fluid generally caused a slight initial depression followed by a slowly developing positive inotropic and sometimes also chronotropic action (Fig. 6A). The same result has been obtained in the heart of *Entosphenus* by Otorii (1953). Relatively high concentrations (about 10^{-5}) were necessary in order to obtain definite responses.

The stimulating effect of adrenaline on the heart of *Lampetra* is decidedly weaker than that on the hearts of selachians, teleosts (Fänge & Östlund, 1954), or higher vertebrates.

Noradrenaline. On the isolated *Lampetra* heart DL-noradrenaline had the same effect as adrenaline. Owing to the weak reactions the activity ratio adrenaline:noradrenaline could not be established with certainty.

Heart homogenate. Freshly prepared homogenate of a *Lampetra* heart extracted with a small volume of Ringer's solution produced the same effects as adrenaline or noradrenaline solutions. For the occurrence of catechol amines in the *Lampetra* heart, see p. 271.

5-*Hydroxytryptamine.* A slight, positive chronotropic and inotropic effect was generally obtained (concentration 10^{-5}).

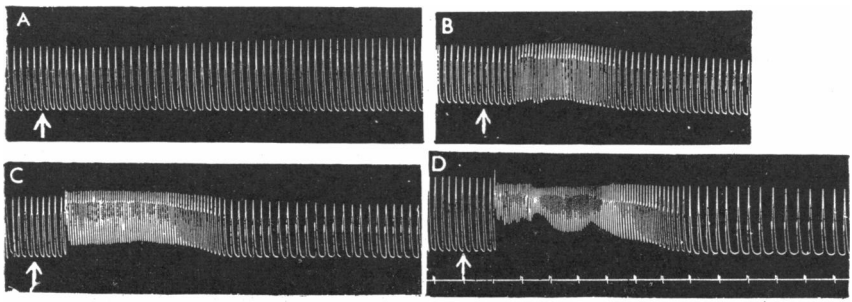

Fig. 6. Perfusion of isolated heart of *Lampetra fluviatilis*. Effect noradrenaline 10^{-5}, A; of acetylcholine $2{\cdot}5 \times 10^{-8}$, B; 5×10^{-8}, C; and $7{\cdot}5 \times 10^{-8}$, D. Time in 5 sec.

Acetylcholine. Otorii (1953) found acceleration of the heart of *Entosphenus* by parasympathomimetic drugs. We found, similarly, that the heart of *Lampetra* was greatly accelerated by acetylcholine and was very sensitive to it. Effects were obtained with a concentration of 10^{-9}. In stronger concentrations acetylcholine produced, in addition, pronounced increase of tone, irregular rhythm, and a tendency for the heart to stop in a kind of systolic flutter or fibrillation. The period of acceleration produced by a small dose of acetylcholine was followed by a period of slowing with long diastolic pauses (Fig. 6). This subsequent period of bradycardia became more pronounced with higher doses of acetylcholine.

A few experiments were performed on a larval specimen (*L. planeri*, body length about 10 cm) with the heart *in situ*. In this specimen the heart showed a spontaneous frequency of 24–30 beats/min. When a drop of Ringer's solution containing acetylcholine in a concentration of 10^{-6} was placed upon the outside of the exposed heart the frequency immediately increased to about 100/min. When the acetylcholine was washed away with Ringer's solution slowing of the heart to about 15/min occurred for about 2 min.

Propionylcholine and butyrylcholine. The effects were the same as those caused by acetylcholine, propionylcholine being slightly more active than butyrylcholine (10^{-7}). Only a few experiments were made with these substances.

Nicotine. Nicotine had the same effect as acetylcholine (Fig. 7) but concentrations about twenty times stronger were needed to produce an effect. Tobacco smoke blown gently upon the isolated heart produced the same acceleration as addition of nicotine to the perfusion fluid.

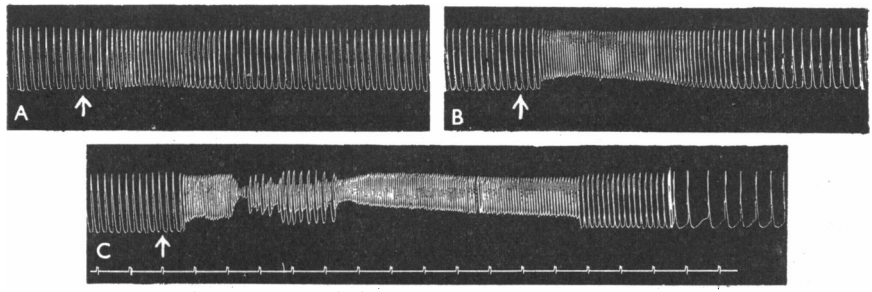

Fig. 7. Perfusion of the isolated heart of *Lampetra fluviatilis*. Effect of nicotine $0{\cdot}5 \times 10^{-6}$, A; $1{\cdot}0 \times 10^{-6}$, B; and $1{\cdot}5 \times 10^{-6}$, C. Time in 5 sec.

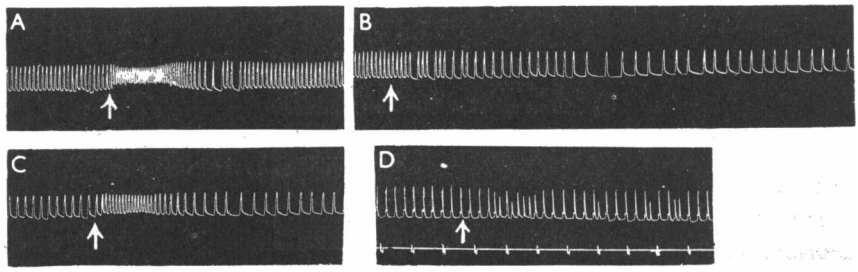

Fig. 8. Perfusion of isolated heart of *Lampetra fluviatilis*. Effect of atropine 5×10^{-5}, B; and of acetylcholine 10^{-8}, A, C and D; at C immediately after several minutes' perfusion with atropine 5×10^{-5}; at D during perfusion with cocaine 2×10^{-5}. Time in 5 sec.

Hexamethonium chloride. Perfusion with a hexamethonium solution in a concentration of 10^{-4} did not influence rate or amplitude of the heart beats, but after a few seconds the responses to acetylcholine were significantly reduced. Perfusion for 5–15 min with hexamethonium greatly reduced the sensitivity of the heart to acetylcholine so that concentrations about 1000 times stronger were required to produce an effect.

Tubocurarine. A few experiments showed that perfusion with tubocurarine (10^{-4}) rendered the heart insensitive to acetylcholine.

Atropine. Perfusion with a concentration of 5×10^{-5} had a negative chronotropic effect (Fig. 8B). Sometimes atrioventricular block or irregularities of

rhythm occurred. After perfusion with atropine (5×10^{-5}) for 10 min the heart still reacted to acetylcholine (10^{-8}), although the response seemed to be slightly reduced (Fig. 8C). According to Otorii (1953), atropine does not antagonize the accelerating action of acetylcholine, pilocarpine, and similar drugs on the *Entosphenus* heart.

Cocaine. In a concentration of 2×10^{-5}, cocaine hydrochloride produced essentially the same effects as atropine.

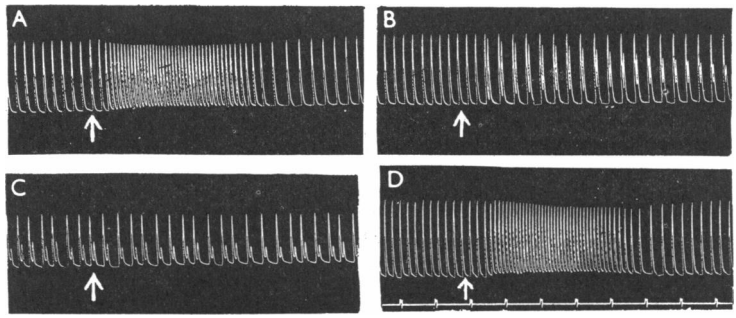

Fig. 9. Perfusion of isolated heart of *Lampetra fluviatilis*. Effect of eserine 10^{-4}, B; and of acetylcholine 10^{-8}, A, C and D; at C during and at D 1 min after perfusion with eserine 10^{-4}. Time in 5 sec.

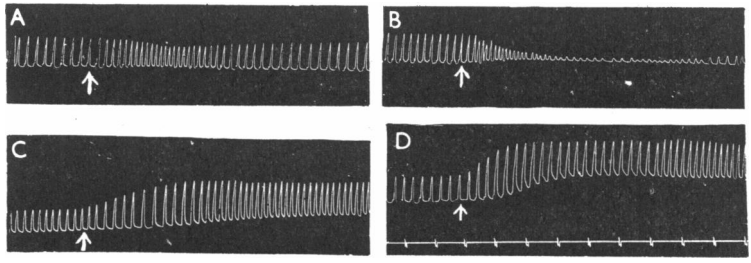

Fig. 10. Perfusion of isolated heart of *Lampetra fluviatilis*. Effect of KCl 10^{-4}, A, and 5×10^{-4}, B; and of $CaCl_2 + 6H_20$ 2×10^{-4}, C, and 4×10^{-4}, D. Time in 5 sec.

Eserine. Perfusion with eserine salicylate in a concentration of 10^{-6}–10^{-5} had a slight, positive, inotropic effect. In higher concentrations (10^{-5}–10^{-4}) there occurred a retardation of the rhythm (Fig. 9B, C). No enhancement of the acetylcholine effect could be observed after treatment of the isolated heart with eserine (Fig. 9D). Eserine in high concentration rendered the heart insensitive to acetylcholine (Fig. 9C).

Potassium. Potassium chloride caused initially a brief period of acceleration and decrease of amplitude. Large concentrations caused the heart to stop in diastole (Fig. 10A, B).

Calcium. Addition of calcium chloride caused an increase in the amplitude of the contractions and incomplete diastolic relaxation, so that the heart contracted in a more systolic position (Fig. 10C, D). Large doses caused irregular rhythm and a tendency of the heart to systolic arrest.

Myxine

The reactions of the isolated heart of *Myxine* to adrenaline, noradrenaline, di-hydro-ergotamine, acetylcholine, calcium, and potassium ions have been studied by Östlund (1954) and Fänge & Östlund (1954).

Pilocarpine, in concentrations 10^{-8}–10^{-4}, is without effect. The same had previously been found for acetylcholine.

5-*Hydroxytryptamine*, in concentrations of 10^{-6}–10^{-5} generally produced a slight positive chronotropic and inotropic effect.

TABLE 2. Cholinesterase activity expressed in μmole of ester hydrolysed in 30 min by 1·0 g tissue (corrections made for spontaneous hydrolysis). Substrates: acetylcholine (ACh), acetyl-β-methylcholine (MeCh), and butyrylcholine (BuCh)

Animal	Tissue	ACh	MeCh	BuCh
Hagfish	Heart	9·1	2·0	1·3
(*Myxine*)	Auricle	10·5	2·5	0·5
	Ventricle	7·6	2·9	1·3
	Portal heart	10·2	1·8	1·1
Lamprey	Heart	24·6	9·6	1·8
(*Lampetra*)	Auricle	10·5	2·5	0·5
	Ventricle	11·2	—	—

TABLE 3. Cholinesterase activity at various acetylcholine concentrations. Activity values as in Table 2.

ACh concentration (M)	*Myxine*		*Lampetra*	
	Heart	Portal heart	Heart auricle	Heart ventricle
$1{\cdot}10 \times 10^{-1}$	8·0	—	6·9	3·1
$3{\cdot}30 \times 10^{-2}$	10·8	7·1	14·7	6·5
$1{\cdot}10 \times 10^{-2}$	11·5	9·5	18·2	10·1
$3{\cdot}30 \times 10^{-3}$	11·7	9·7	21·4	10·8

Cholinesterase content

The results are given in Table 2. The cholinesterase values for the tissues of *Myxine* are low, those for the tissues of *Lampetra* somewhat higher. Both types of cholinesterase are present in the extracts but the 'true' or acetylcholinesterase is probably responsible for most of the activity because of the higher rate of hydrolysis of acetyl-β-methylcholine as compared to butyrylcholine. This conclusion is supported by the finding that the enzyme activity of the crude extracts was not increased by high concentrations of acetylcholine (see Table 3), suggesting depression of enzyme activity by high substrate concentration which is characteristic for acetylcholinesterase.

Acetylcholine, choline, and histamine content of heart and brain

Lampetra. The amounts of reineckate obtained from the heart and brain were very small (Table 4). On chromatograms of brain extracts an acetylcholine area could be traced with difficulty and on those of heart extracts this area was hardly visible at all (Fig. 11).

TABLE 4. Amounts of reineckate precipitate obtained on isolation

Animal	Tissue	g	Approx. number of organs	mg of reineckate precipitate	mg reineckates per g tissue
Hagfish (*Myxine*)	Heart	13·6	450	240	15·5
	Portal heart	1·5	—	33·7	22·5
Lamprey (*Lampetra*)	Heart	17·1	110	14	1

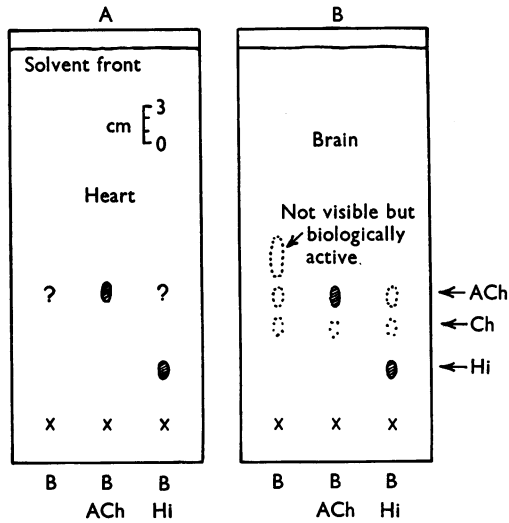

Fig. 11. Ascending chromatogram of bases (B) isolated from tissue of *Lampetra fluviatilis*. A, heart; B, brain. ACh = acetylcholine; MeCh = acetyl-β-methylcholine; PrCh = propionylcholine; Ch = choline; Hi = histamine; B = test solution.

The acetylcholine area of paper chromatograms of brain extracts was biologically active in contracting the guinea-pig ileum. The calculated acetylcholine content of the brain was not more than 0·05 μg/g fresh tissue. However, the brain may contain, in addition to acetylcholine, another biologically active principle precipitable with Reinecke salt. It was not visible on the chromatograms, but was detected biologically in an area of the chromatograms with the R_F interval 0·40–0·48. The nature of this unknown compound was not studied owing to lack of material.

The acetylcholine area for the heart was not active when tested on the isolated guinea-pig ileum, nor could a compound differing from acetylcholine

in R_F value, similar to that found in the brain, be detected in chromatograms of heart extracts (Fig. 11 A).

Histamine could neither be detected chromatographically nor by bio-assay in the heart or brain.

Myxine. On the chromatograms for the heart (Fig. 12 A) three areas were visible which could be analysed by comparison with pure compounds. The most easily detectable substance was that with the lowest R_F (0·16); it could be identified as histamine. The compounds with R_F 0·37 and 0·28 were traced with dipicrylamine as definite but not distinct areas and were identified as

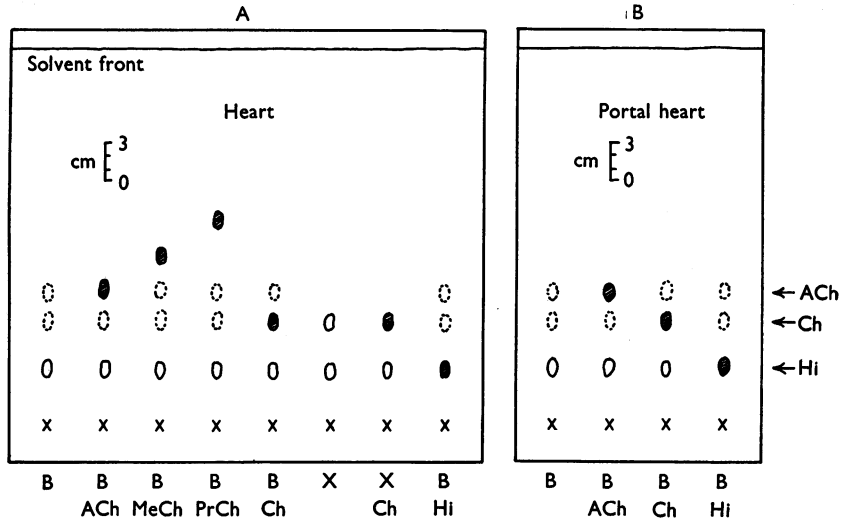

Fig. 12. Ascending chromatogram of bases isolated from heart of *Myxine glutinosa*. A, heart; B, portal vein heart. Symbols as in Fig. 11. X = test solution (B) treated with alkali before chromatography.

acetylcholine and choline. When the test solution was pretreated with alkali (X in Fig. 12 A), the acetylcholine area disappeared and the choline area became deeply coloured and pinker. Choline in alkaline solution always behaves in this manner.

Structurally the portal vein heart resembles the auricle of the branchial heart; it is therefore not surprising that paper chromatograms of its extracts reveal the presence of the same compounds, i.e. acetylcholine, choline and histamine, as those found in extract of the branchial heart. The chromatographic results are recorded in Fig. 12 B.

The acetylcholine and histamine areas from chromatograms of tissue preparations of branchial heart and portal vein heart were active in contracting the isolated guinea-pig ileum. Owing to lack of material it was not possible to make an accurate quantitative assay of the active compounds. However, from

the bio-assay results the amounts of acetylcholine which could be extracted from the heart tissue could be estimated as being approximately 0·01–0·1 μg/g fresh tissue, and those of histamine as being a little higher.

Catechol amine content of heart

Paper chromatography of 2 g tissue extracts from the auricle of the *Lampetra* heart showed the presence of adrenaline, noradrenaline, and a third substance giving a spot with a lower R_F value than adrenaline or noradrenaline. This third substance was in all probability identical with 'catechol-4' which has formerly been demonstrated in interrenal organs of selachians, etc. (Östlund, 1954). Besides adrenaline, noradrenaline, and the presumed catechol-4, two other substances seemed to occur giving spots which were stained by potassium ferricyanide at pH 7·7 (Fig. 13).

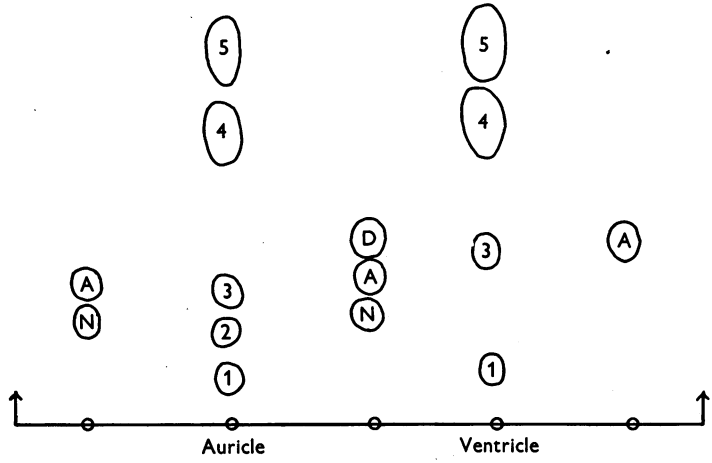

Fig. 13. Ascending chromatogram of catechol amines from the auricle and ventricle of the heart of *Lampetra fluviatilis*. A, adrenaline; D, dopamine; N, noradrenaline. 1, catechol-4; 2, noradrenaline; 3, adrenaline; 4 and 5, unidentified substances possibly decomposition products of catechol amines.

With 2 g extracts of the ventricle of the *Lampetra* heart, adrenaline, catechol-4 and the two above-mentioned, unidentified substances could be demonstrated (Fig. 13) but no noradrenaline. This is consistent with the results from the bio-assays (Table 5), according to which the content of noradrenaline is much lower in the ventricle than in the auricle.

In the *Myxine* heart noradrenaline, adrenaline, and catechol-4 could be identified by paper chromatography.

In Table 5 the content of noradrenaline and adrenaline for the heart of *Lampetra* is given. The figures were obtained by bio-assay of heart extracts on the cat's arterial blood pressure and on the fowl rectal caecum preparation.

The table shows that the heart of *Lampetra* contains high concentrations of catechol amine. In the auricle 41 μg adrenaline and 19 μg noradrenaline were found per gram tissue. The table also compares the content of these amines with that in the heart of other vertebrates.

TABLE 5. Content of adrenaline and noradrenaline in heart tissue of Lamprey compared with that of other vertebrates

Species	Tissue	Noradrenaline (μg/g)	Adrenaline (μg/g)	Adrenaline percentage	Author
Lamprey (*Lampetra*)	Total heart	5·0	19·1	79	Present paper
	Auricle	19·0	41·2	68	
	Ventricle	0·8	12·5	95	
Hagfish (*Myxine*)	Heart	0·83	5·0	86	Östlund (1954)
Ray (*Raja batis*)	Heart	0·087	0·025	22	
Dogfish (*Squalus ac.*)	Heart	0·049	0·031	39	
Codfish (*Gadus call.*)	Heart	0·06	0·043	42	
Frog (*Rana temporaria*)	Heart	0·19	0·89	82	
Rat	Heart	0·65	0·022	3·2	Hökfelt (1951
Rabbit	Heart	0·17–0·54	0·045–0·094	8–35	Holz, Kroneberg & Schümann (1951)
Dog	Heart	0·14	0·02	15	Raab & Gigee (1951)

DISCUSSION

With histological methods employed in the present study no trace of nervous elements could be detected in the heart of *Myxine*, whereas nerve cells and fibres appear distinctly in the vagal branches of the intestine. The fibres of the ramus intestinalis of the vagus are directed exclusively to the intestine and no cardiac nerve is detectable. The results of electrical stimulation of the vagus nerve confirm the idea that *Myxine* is peculiar among the vertebrates in possessing no cardiac innervation (cf. Greene, 1902; Carlson, 1904). No chromaffin cells could be detected in our preparations of the *Myxine* heart.

In *Lampetra* application of the same histological methods reveals the existence of a distinct cardiac branch from the vagus trunk on each side and a great number of ganglion cells, nerve fibres and chromaffin cells in the wall of the heart. Among the ganglion cells there can be distinguished a large type and a small type, the latter being more abundant. The large ganglion cells undoubtedly are real postganglionic neurones. It is questionable, however, whether the numerous small ganglion cells are true nerve cells because of the impossibility of distinguishing between these cells and chromaffin cells after treatment with dichromate. Perhaps the chromaffin cells of the *Lampetra* heart represent from a phylogenetical point of view a primitive type of sympathetic (adrenergic) neurone. It is worth mentioning in this connexion that in higher vertebrates the chromaffin cells of the adrenal medulla emerge ontogenetically from the same source as sympathetic neurones (Bachmann, 1954).

There is also a difference in the response to acetylcholine and certain other substances between the heart of *Myxine* and of *Lampetra*. Thus the nerve-free heart of *Myxine* does not react to acetylcholine (Östlund, 1954), whereas the richly innervated *Lampetra* heart is accelerated by it. The richly innervated intestine of *Myxine* also reacts to acetylcholine which causes its muscles to contract (Fänge, 1948). The insensitivity to acetylcholine of the *Myxine* heart is reminiscent of conditions in nerve-free embryonic hearts. Armstrong (1935) reports that in early stages the heart of the *Fundulus* embryo does not respond to acetylcholine, and other authors have found that although embryonic hearts are influenced by acetylcholine they only react to very high doses (Cullis & Lucas, 1936; Prosser, 1942). According to Hiatt & Garrey (1943) the turtle ventricle, which apparently has no innervation, does not react to acetylcholine.

The isolated *Lampetra* heart is not inhibited by acetylcholine as are the hearts of higher vertebrates, but instead is accelerated in about the same way as the supposedly neurogenic heart of large arthropods such as lobsters (Welsh, 1942). Not only acetylcholine but also propionylcholine, butyrylcholine, and nicotine give this type of reponse. The problem arises whether acetylcholine and similarly acting substances stimulate the cardiac muscle fibres directly or whether the observed effects are due to stimulation of ganglion cells or chromaffin elements. Hexamethonium in rather high concentration (10^{-4}) does not disturb the rhythm of the isolated *Lampetra* heart but blocks effectively the effect of acetylcholine. Because hexamethonium is considered to have a specific ganglionic blocking property (Paton, 1951), the result indicates that acetylcholine acts by stimulation of ganglion cells in the heart. The acetylcholine effect is not blocked by atropine and may thus be characterized as a 'nicotinic action' rather than a 'muscarinic action'. Kottegoda (1953) and Ginzel & Kottegoda (1954) have found that the isolated auricle of the rabbit heart is accelerated by ganglion-stimulating drugs and it has been shown that acetylcholine is able to liberate adrenaline-like substances in the mammalian heart (Hoffmann, Hoffmann, Middleton & Talesnik, 1945; McNamara, Krop & McKay, 1948). It is unlikely that in the *Lampetra* heart the acceleration caused by acetylcholine and nicotine is due to stimulation of the abundant chromaffin cells and subsequent liberation of catecholamines, because adrenaline and noradrenaline have only a feeble effect on the *Lampetra* heart.

The acceleration of the *Lampetra* heart produced by acetylcholine or by vagal stimulation is usually followed by a period of slow rate. A similar phenomenon is noticed in teleosteans, where acetylcholine may produce alternate periods of acceleration and retardation of the heart (Fänge & Östlund, 1954, Fig. 4). If the acceleration of the *Lampetra* heart is assumed to be due to stimulation of ganglionic elements (chromaffin cells) the negative after-effect might possibly be a direct effect of acetylcholine upon the heart muscle fibres.

The hearts of *Myxine* and *Lampetra* contain strikingly high amounts of catechol amines in comparison with the hearts of other vertebrates. The high amounts of adrenaline and noradrenaline extractable from the hearts probably emanate partly from the muscle fibres, which in both species give a slight chromaffin reaction. The fact that the heart of *Lampetra* contains 4 to 5 times more catechol amines per gram than the *Myxine* heart corresponds well with the occurrence of numerous chromaffin cells in the former. It is probable that heart muscle fibres are able to absorb catechol amines from the surroundings (Raab & Gigee, 1953), but the possibility cannot be excluded that there is some production of catechol amines within the muscle cells. At any rate the presence of catechol amines in the heart muscle cells may indicate that these substances are of importance for the normal activity of the cells. The presence in the *Lampetra* heart, in addition to adrenaline and noradrenaline, of catechol-4 and two other substances probably related to catechol amines (3, 4-dihydroxyphenylacetic acid and 3, 4-dihydroxyphenyllactic acid?) indicates that there occurs a rapid metabolism of catechol amines in the heart.

In both *Lampetra* and *Myxine* the isolated heart shows remarkably weak and inconstant reactions to adrenaline and noradrenaline. Perhaps the weakness of response is dependent upon the fact that the isolated heart is already under influence of adrenaline-like substances liberated from its own tissue.

SUMMARY

1. The heart of *Lampetra* receives fibres from the vagus nerve reaching the heart along the median jugular vein. In the sinus venosus, auricle and ventricle there occur two types of cells of presumably ganglionic nature.

2. No nervous elements could be detected in the heart and the portal vein heart of *Myxine*.

3. The heart of *Lampetra*, especially the auricle, contains numerous chromaffin cells which appear to be identical with small ganglion cells. No corresponding cells were found in the *Myxine* heart. In both species the heart muscle fibres give a weak chromaffin reaction.

4. In *Lampetra* stimulation of the vagus produces intense acceleration followed by bradycardia.

5. Stimulation of the vagus, the medulla oblongata, or the large blood vessels near the heart of *Myxine* has no immediate effect upon the rhythm of the heart.

6. The isolated *Lampetra* heart is accelerated by acetylcholine, butyrylcholine, propionylcholine and nicotine. The acetylcholine effect is abolished by hexamethonium and tubocurarine but not by atropine. Eserine in high concentration produces a slow rhythm and blocks the acetylcholine acceleration.

7. The isolated *Myxine* heart is insensitive to pilocarpine even in large concentrations.

8. Adrenaline and noradrenaline produce only weak effects on the isolated heart of *Lampetra* and *Myxine*. 5-Hydroxytryptamine usually has a slight stimulatory effect.

9. The presence of low concentrations of acetylcholine, choline, and histamine was demonstrated by paper chromatography in extracts from the heart and portal vein heart of *Myxine*. None of these substances could be demonstrated in extracts from the *Lampetra* heart.

10. A low cholinesterase activity is found in the heart of *Lampetra* and the heart and portal vein heart of *Myxine*. The enzymic activity is mainly due to true or acetylcholinesterase.

11. The heart of *Lampetra* contains large amounts of adrenaline and noradrenaline. A third substance called catechol-4 and two other substances probably related to catecholamines could also be demonstrated.

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