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EXPERIMENTS WITH THE ISOLATED HEART OF THE GASTROPOD *HELIX POMATIA* IN AN ARTIFICIAL PERICARDIUM

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INTRODUCTION

The gastropod heart consists of two chambers, which regularly alternately dilate (during diastole) and contract (during systole). It can be seen *in vivo* that the auricle and ventricle appear to act in an antagonistic way, so that the dilation of one is synchronous with the contraction of the other. The heart is suspended within the fluid-filled coelomic pericardial cavity which possesses a ciliated connexion with the kidney via the narrow reno-pericardial canal. Provided that the pericardial wall remains undamaged, the motion of the heart may be observed for some hours in an opened specimen. But it can be readily demonstrated that if the wall of the pericardium is ruptured, or if the heart is taken from the pericardium and placed in a culture medium in an open dish, the pattern of heart activity is disturbed. In particular, the diastolic dilation of the chambers virtually ceases, although systolic contractions may continue for some time. The integrity of the pericardial wall appears, therefore, to be essential for diastolic dilation of the chambers of the heart *in vivo*.

Various attempts have been made to explain this fact, and some older theories are reviewed by Krijgsman & Divaris (1955). They suggest that the most likely explanation is based upon hydrodynamics. Briefly, they state that the gastropod pericardium can be considered to be a closed chamber if it is accepted that (*a*) the wall is relatively rigid and (*b*) the reno-pericardial canal, with its restricted lumen, does not permit rapid fluid flow. Thus, by means of forces transmitted through the pericardial fluid, contractions of the ventricle automatically help to bring about dilation of the auricle, and vice versa.

Krijgsman and Divaris also consider that dilation of the ventricle is helped by a rise in the internal pressure within the ventricle consequent upon auricular systole. This, however, appears unlikely, because any rise in internal pressure would be distributed throughout much of the circulatory system in the known absence of valves at the entry of the great veins into the auricle. Krijgsman and Divaris' arguments based simply upon a consideration of the hydrodynamical conditions would be sufficient to explain the rhythmic diastolic dilation of the heart chambers. Hill & Welsh (1966) have considered that this 'seems to be a satisfactory explanation...although it needs quantitative testing'.

It is remarkable that, when preparing their review, Krijgsman and Divaris missed an earlier contribution to this discussion made by Ramsay (1952), and referred to it only in a footnote. Ramsay clearly stated that it is 'open to the snail to take advantage

of any negative pressure which may be set up in the pericardium by the expulsion of blood from the ventricle... Whether or not this is the true explanation of the filling of the snail's heart must remain undecided in the absence of experimental evidence.'

It was felt that it might be interesting to take up Ramsay's suggestion and try to furnish some experimental evidence.

GENERAL METHODS

The saline recommended by Jullien *et al.* (1955) was employed throughout. It was made up 1 l at a time and stored at room temperature. It contained the following salts, dissolved in 1 l of de-ionized water: NaCl 5·786 g, KCl 0·149 g, $CaCl_2$ 1·110 g.

SPECIAL METHODS AND RESULTS

Several methods were investigated for maintaining the heart of *Helix pomatia in vitro*, so that performance tests could be carried out.

Simple explantation

Whole hearts with or without intact pericardia were transferred to saline in conical flasks, Carrel flasks, and Petri dishes, at controlled temperatures in the range 6–25 °C. In all these tests the heart initially responded by vigorous contractions for about 1 min. Co-ordination was soon lost, the ventricle usually contracting less rapidly than the auricle, but more steadily. Spontaneous regular contractions stopped within 1 h in most cases, although one heart continued for 4 h at 25 °C. Unpredictable, unco-ordinated twitches of the heart might occur spontaneously over a longer period, up to 115 h at 19 °C in one favourable explant. Stretching the heart with needles or hooks maintained a regular heart-beat for a longer time, but the stretch had to be increased periodically for the effect to be maintained. This phenomenon has been noticed by many other investigators (reviewed by Krijgsman & Divaris, 1955).

In these simple experiments the heart contractions did not appear to be effective, and no fluid was pumped through the heart after co-ordination began to deteriorate.

Perfusion apparatus

The apparatus may best be described by reference to Fig. 1 A. The upper and lower Perspex chambers both measured 12 × 5 × 5 cm. The height of one chamber relative to the other could be varied. Saline was pumped from the lower chamber to the upper by a simple compressed-air pump. An overflow tube leading back to the lower chamber kept the saline levels constant. Each chamber had a fitted Perspex lid and the whole apparatus when in use was encased in a polyethylene bag.

Before use the whole apparatus was sterilized with Milton's solution, then washed with distilled water. After filling with saline and removing air bubbles from the perfusion tube, the height of the upper tank was adjusted to give the required simulated venous return pressure, which is the difference in the height of the saline levels, in cm of saline. The apparatus could be fitted into a cooled incubator at 15 or 20 °C.

When pericardium-free hearts were placed in the perfusion apparatus, with a simulated venous pressure of 8 cm saline (lower pressures proved inadequate to stimulate

sustained regular heart-beat), the number of beats at first (for 2–3 h) was high, about 10/min, but then it slowed, reaching 2–3/min after 3 h. In very favourable individual preparations, regular contractions of 1/min or slightly less were detectable after 2 days. At any time following 1 h after explantation the cessation of perfusion led immediately

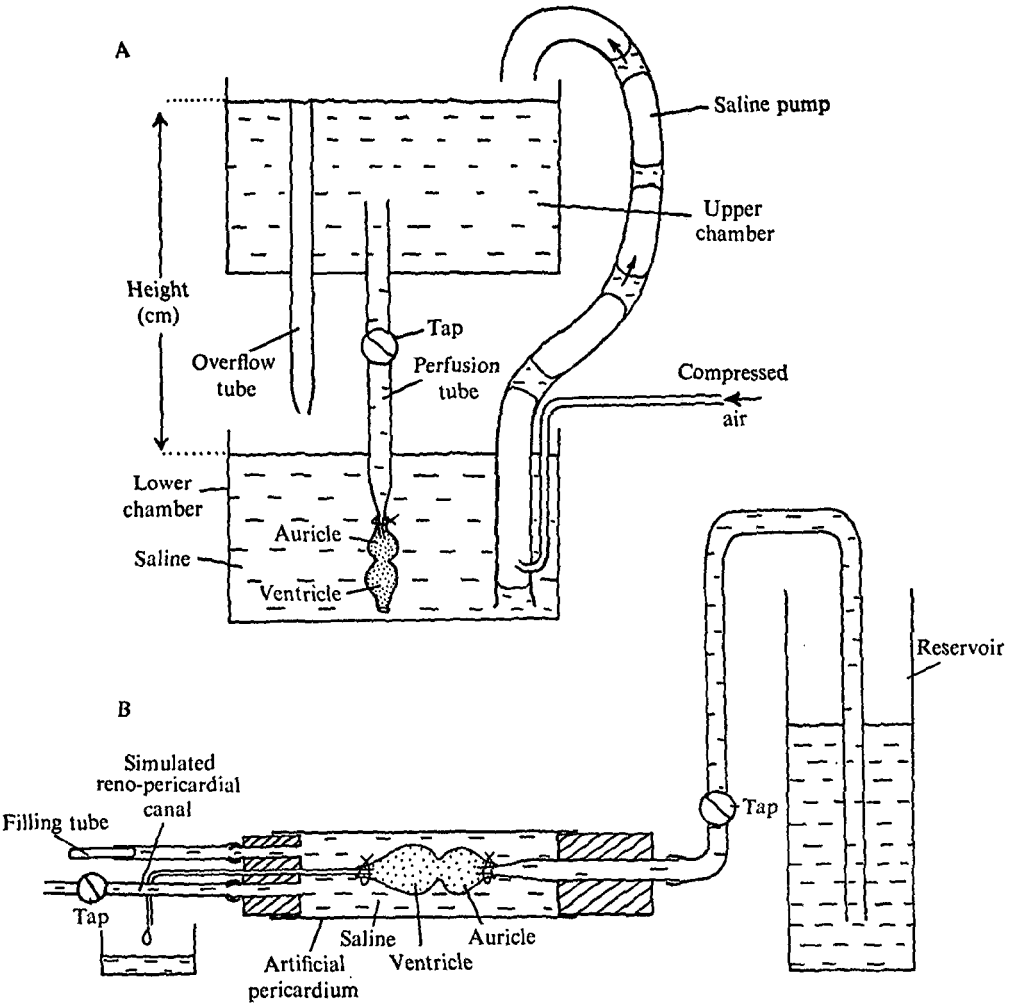

Fig. 1. A, Diagram of the perfusion apparatus; B, diagram of the artificial pericardium apparatus.

to the stoppage of the heart. If, however, perfusion was re-commenced, regular contractions began at once. It was noted that the contractions decreased in vigour as they decreased in rate. The results obtained with three different hearts are shown in Fig. 2.

These experiments with the perfusion apparatus showed that (*a*) isolated pericardium-free hearts could be kept alive and actively (if slowly) contracting in such an apparatus for up to 2 days, (*b*) a steady simulated venous return pressure was necessary for the maintenance of heart activity.

The question then arose: are these heart-beats in the perfused explants effective

in translocating fluid through the heart? After a few minutes in the perfusion apparatus to ascertain that heart-beat was apparently normal, a heart preparation was rapidly taken and suspended by the perfusion tube in a weighing bottle. This bottle contained sufficient depth of saline to immerse the heart totally. In order to maintain a constant auricular pressure, the meniscus of the saline in the bottle was supported at the same level as that of the saline in the lower chamber of the perfusion apparatus. After exactly 1 min the heart was re-suspended in the lower chamber, and the weight of saline which had passed through the heart into the bottle was ascertained. The experiment was repeated a number of times. For purposes of comparison a control without a heart was run in the same way.

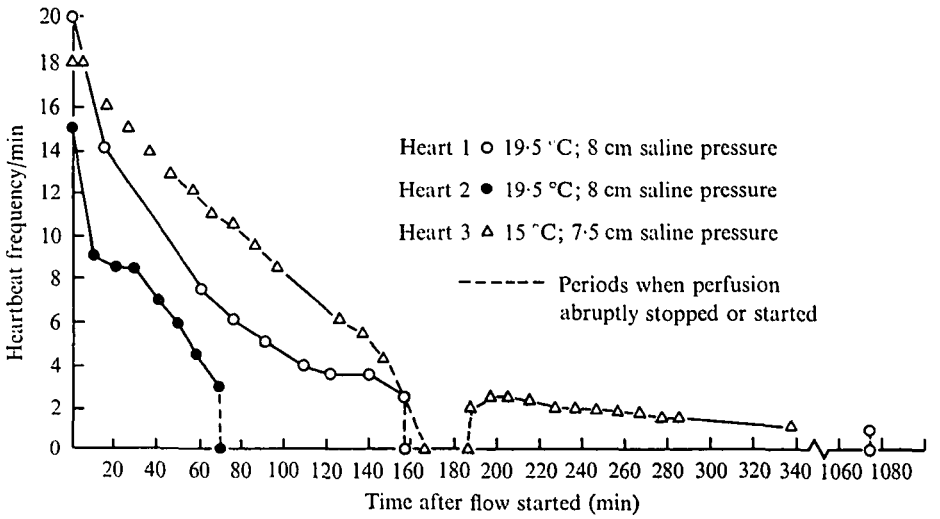

Fig. 2. Heart-beat frequency measured in three hearts mounted in the perfusion apparatus. The fall in frequency with time was similar in these and in other, similar, preparations, although the initial frequency after explantation showed considerable variation (and this was always higher than the rate recorded before operation). The illustration shows that continued perfusion is necessary for the maintenance of a steady heart-beat *in vitro*. This method allowed observations to be made on the beating heart for more than 18 h.

The results showed (Fig. 3) that the flow through the perfusion tube without a heart attached averaged 0·6 ml/min. With a heart in position on the perfusion tube the rate of passage of saline was very similar to this for over 1 h, although the heart-beat frequency during this period showed a steep decline. The rate of passage of saline, in fact, varied little during a frequency change from 10/min to 4/min. The rate of flow through the heart in this situation is not a function of the frequency of contraction; the heart neither assists the flow nor offers any resistance to it. In one individual experiment, however, the heart stopped after 18 h with the ventricle contracted and the auricle dilated. In this state the heart did offer some resistance to the flow, lowering it to 0·26 ml/min.

These results showed that in the perfusion apparatus at the simulated venous pressure used (8 cm saline) saline flowed at a steady rate through the isolated heart (without pericardium) at all times except possibly when the ventricle was maximally contracted. It was plainly necessary to design an apparatus that would make possible continuous

Measurement of the rate of blood flow through actively beating hearts at various simulated venous return pressures, and with the role of the pericardium more clearly defined.

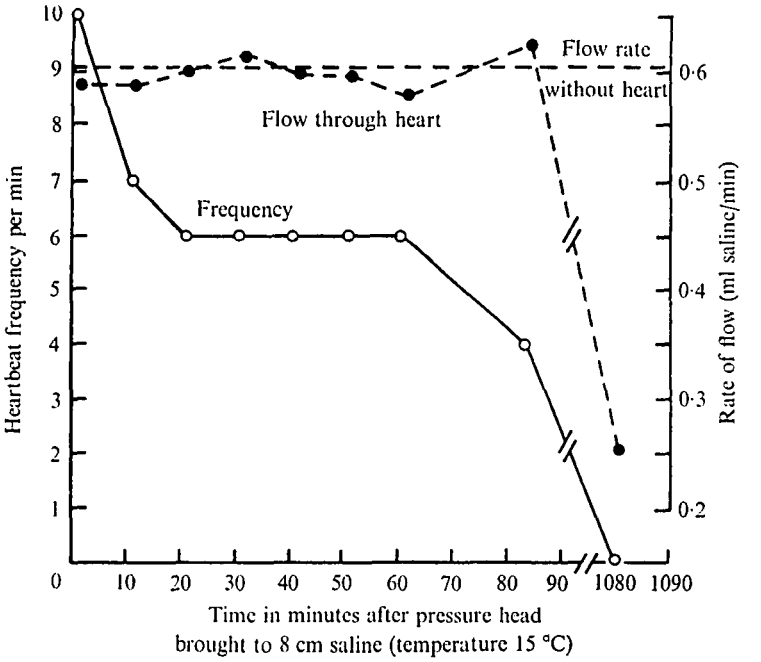

Fig. 3. Simultaneous observations in the perfusion apparatus on flow through the heart and the heart-beat frequency, with a comparison of flow through the same apparatus in the absence of a heart (approximately 0·6 ml saline/min). The results indicate that the flow through the heart in this apparatus is not closely related to the frequency of contraction.

The artificial pericardium apparatus (APA)

An apparatus was developed which allowed the heart to be ligatured into an artificial pericardium (Fig. 1 B). A reservoir of saline, from which runs a polyethylene tube with a tap, leads into a glass tube, finely drawn at one end and ligatured into the auricle. This glass tube passes through a rubber bung which itself fits into one end of a wider glass tube. The wide tube completely encloses the heart and has a bung fitting into the other end. Through this passes a narrow tube ligatured into the ventricle, and two other glass tubes of a slightly wider bore. Two short lengths of rubber tubing are attached to these. One is closed off by a piece of glass rod, the other (the simulated reno-pericardial canal) by a tap.

When a heart is ligatured properly in position and all the tubes are full of saline and without air bubbles, the apparatus simulates a pericardium such as is found in many gastropods.

Before use the apparatus was thoroughly cleaned with detergent and rinsed with distilled water. It was also found necessary to boil the rubber bungs in distilled water in order to remove surface toxins. Without this treatment the chances of the heart behaving normally were greatly reduced.

Setting up a heart in the apparatus usually took about 40 min. The drawn-glass

tube with bung and polyethylene tube fitted was force-filled with saline and the tap closed. This drawn tube was ligatured into the auricle of a recently explanted heart using boiled nylon thread. The other fine glass tube, also filled with saline, was then similarly ligatured into the ventricle. These operations were performed in a Petri dish of saline under a binocular microscope.

The wide-bore tube which formed the pericardial wall was held upright in a clamp. The heart was lifted from the Petri dish and introduced into the apparatus, the rubber bung being firmly pushed into the end of the tube ensuring a leak-proof seal. Great care was necessary at this stage not to tear or puncture the heart.

The other rubber bung, already fitted with the two wider tubes and their rubber extensions, was then introduced into the lower end of the artificial pericardium. The narrow tube leading from the ventricle was carefully worked into its hole in the bung. When the bung was fully in position this tube was adjusted so that the heart was supported at its natural length.

At this stage the whole apparatus was held vertically and filled with saline. This was introduced via the longer of the rubber extension tubes, using a syringe. Any air bubbles which formed were carefully removed. When the shorter rubber tube overflowed, it was sealed with the glass rod. More saline was added until the longer tube overflowed. This was then closed with the tap. Any leaks were quickly sealed with molten paraffin wax.

The apparatus was rotated to a horizontal position and the level of the reservoir was adjusted to give the required venous pressure. The tap between the reservoir and the auricle was then opened. Often at this stage both chambers of the heart were collapsed. They were dilated slightly by opening the tap, allowing one or two drops of saline to escape from the artificial pericardium, and closing the tap again.

The venous pressure and degree of dilation of the heart were experimentally altered while the frequency and flow rate were measured at intervals. The flow rate was estimated at the same time as the frequency by collecting and weighing the saline pumped out of the tube ligatured into the ventricle.

In calculating the flow rate in terms of volume, the saline was assumed to be of unit density. Dividing the flow rate by the frequency gives the volume produced by each contraction (the stroke volume), a measure of the effective amplitude of the contractions.

Good preparations maintained regular strong alternating contractions for some hours at 15 °C. The apparently normal pattern of contractions was unaffected by experimental changes in the simulated venous pressure, even when this was lowered to a negative value. A measurable flow of saline was produced by the heart under these conditions. This continuance of heart-beat after drastic lowering of venous pressure is in marked contrast to what is observed in the perfusion apparatus, and this difference must be due to the simulated closed pericardium. To confirm this, the tap on the rubber filling tube was opened and the tube was held horizontally, so that no fluid escaped, thus transforming the artificial pericardial cavity into an open system. At venous pressures around zero or lower this greatly decreased the effectiveness of the heart by preventing the adequate filling of the auricle. Under these conditions the heart was soon observed to stop beating.

The amount of saline passed by a single contraction of the ventricle depends on the amplitude of the movements. An important factor affecting the potential amplitude

of the two heart chambers is the extent of dilation permitted by the volume of fluid in the pericardial space. Fig. 4 illustrates how, for different overall dilations of the heart, the stroke volume could be altered by changing the simulated venous pressure. Increasing the dilation generally produced a greater stroke volume for a given simulated venous pressure. By these means the effectiveness of a heart undergoing testing may be varied at will, within wide limits. The flow rate is of necessity a direct function of the frequency and the stroke volume. It also follows experimental variations in venous return pressure fairly closely.

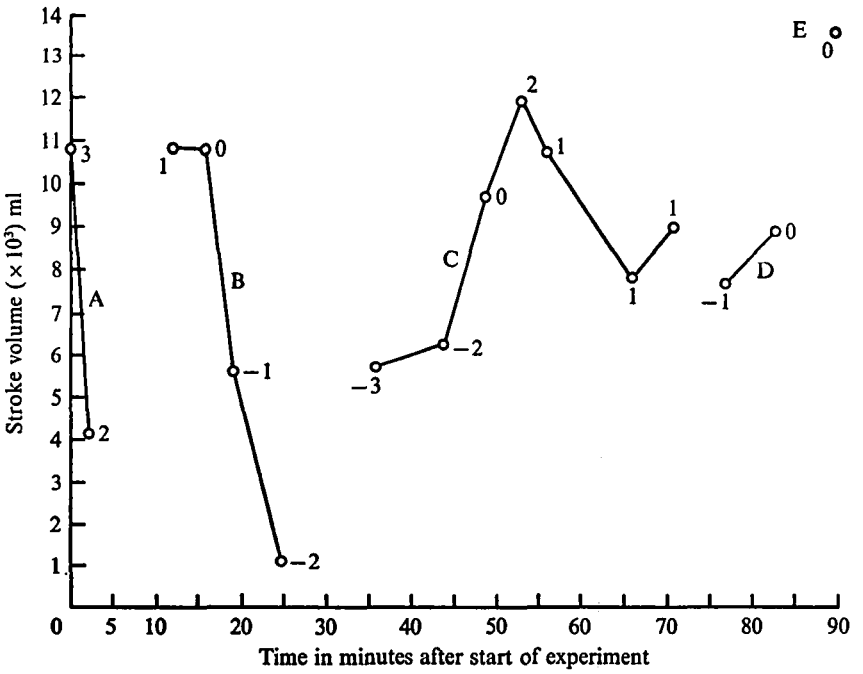

Fig. 4. Relationship between stroke volume and pericardial pressure in the artificial pericardium apparatus. Pericardial pressure was lowered by allowing saline to escape from the artificial pericardium, and this immediately led to greater dilation of the heart. The letters A–E indicate series of observations made during stepwise increases of heart dilation brought about in this way. Numbers alongside the points indicate the venous return pressures (cm saline).

DISCUSSION

Preliminary experiments showed the feasibility of maintaining explanted hearts of *Helix pomatia* for up to 3–4 days *in vitro* at 19 °C. Spontaneous regular contractions stopped within 1 h in most cases, but unpredictable twitches could be observed for periods up to 115 h. When stretched the heart beat regularly for more than 4 h. In these experiments contractions of the heart probably did not result in fluid translocation for more than a few minutes, because co-ordination was soon lost. This is consistent with Nisbet & Plummer's (1969) demonstration that the pulmonate auricle and ventricle are both subject to firm extrinsic neural control, both excitatory and inhibitory.

Experiments with pericardium-free hearts mounted in the perfusion apparatus, having a simulated venous return pressure of 8 cm saline, showed that in this apparatus

the heart-beat was maintained for up to 2 days at 15 °C. After the heart had settled down to a steady beat the cessation of perfusion led immediately to a reversible stoppage of the heart. The pericardium was unnecessary for the survival of the *Helix* heart in this apparatus, but experiments showed that under these conditions the heart did not apparently effect any translocation of fluid.

The artificial pericardium apparatus (APA) allowed experimental testing of the rate of flow through the heart at various simulated venous return pressures, and made possible a more accurate understanding of the role of the pericardium *in vivo*. In the APA good preparations maintained regular strong contractions for some hours at 15 °C, unaffected by changes in the simulated venous pressure (even when this was lowered to a negative value). Measurable translocation of fluid was observed in the APA (even in the absence of the normal neural control mentioned earlier). If the APA was experimentally transformed into an open system by opening up a simulated reno-pericardial canal, the effectiveness of the heart was greatly impaired, the diastolic filling of the auricle was seen to be much reduced, and the heart soon stopped beating. These results confirmed the often-stated view that *in vivo* the gastropod reno-pericardial canal (with its muscular reno-pericardial sphincter and restricted lumen) effectively controls fluid transfer from the pericardium to the kidney (and vice versa).

Experiments in the APA, in which the volume of fluid in the artificial pericardium itself (and hence the dilation of the heart chambers) was altered, showed that increasing dilation of the heart produced a greater stroke volume for a given simulated venous pressure. Such methods of altering the effectiveness of the heart *in vitro* suggest that similar controlling mechanisms (involving changes in the volume of fluid in the pericardium) may play a part in the normal physiological adaptation of the blood-pressure to the hour-to-hour activities of the individual.

Finally, it is clear that these results, obtained with the APA, give fullest support to Ramsay's (1952) theory of hydrodynamical coupling between the filling of the auricle and the contraction of the ventricle in the molluscan heart, with its functionally closed, rather rigid, pericardium.

SUMMARY

1. Isolated hearts of *Helix pomatia* could be maintained for 3–4 days at 19 °C in physiological saline. Co-ordination was soon lost, but irregular twitches could be observed for up to 115 h.

2. A perfusion apparatus was designed which supplied a simulated venous return pressure of 8 cm saline and enabled *in vitro* survival of pericardium-free hearts for up to 2 days at 15 °C. Cessation of perfusion led immediately to a reversible stoppage of heart-beat.

3. An artificial pericardium apparatus (APA) allowed the role of the pericardium to be studied. In the APA measurable translocation of fluid was effected by the heart, even when the simulated venous return pressure was negative.

4. If the APA was transformed into an open system by opening up a simulated reno-pericardial canal, the effectiveness of the heart was greatly reduced.

5. In the APA the greater the dilation of the heart (in consequence of decreased volume of pericardial fluid) the greater was the stroke volume for a given simulated venous return pressure.

6. Results obtained with the APA give support to the theory that in the gastropod heart the filling of the auricle is hydrodynamically coupled (through the pericardial fluid) to the emptying of the ventricle.

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