A SALINE MEDIUM FOR MAINTAINING ISOLATED HEART OF *PILA GLOBOSA*, SWAINSON

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ABSTRACT

Some known standard salines for molluscs were found unsuitable for maintaining the heart of *Pila in vitro*. A new saline for *Pila* has been devised keeping in view the ionic composition, pH and osmotic pressure of blood of *Pila*. A saline having the composition of blood was not found suitable for maintaining isolated heart of *Pila* but reduction of percentage of potassium made it so. The osmotic pressure was adjusted with addition of glucose which also provides energy. Changes of pH between 5 and 8·5 did not have an effect on the heart-beat of *Pila*. It was suggested that a saline containing only the principal ions of blood was not suitable because other trace and organic substances present in the blood were perhaps playing an important role in the regular beating of the heart. Adjustment in the proportion of different ions and addition of glucose did partly compensate for the absence of these substances.

A SUITABLE saline is always necessary for carrying out experiments on isolated organs. In experiments with marine animals sea-water serves as a very good perfusion medium and isolated organs of marine animals can be kept for very long periods in sea-water. Pilgrim (1953) and Fange (1955) have used diluted sea-water as a perfusion medium for a freshwater lamellibranch (*Anodonta*) but for work on isolated organs of terrestrial and freshwater animals it becomes necessary to find a suitable saline before performing various experiments. The present paper gives an account of processing a suitable saline for maintaining the isolated heart of *Pila in vitro* for experimental purpose.

The details of the technique followed are given here.

The pericardium was carefully slit open so that the heart remained undamaged. A piece of surgical ligature silk of '0' thickness was tied around the visceral aorta, a little away from the bifurcation of the aortic trunk. The cephalic aortal end was left open for the perfusion fluid to pass. Care was taken to include some of the surrounding mantle wall while tying the thread so that the silk thread may not cut through the thin-walled visceral aorta.

A second silk thread was now passed from below the auricle in its middle and a small transverse incision was made in the auricle a little behind the thread. A fine glass canula attached to the perfusion assembly was now passed into the auricle through the incision and the thread was securely tied around it.

The heart was now detached from the body of *Pila* by cutting through the cephalic and visceral aortae and the auricle a little behind the point of the insertion of the canula. The aortic end of the heart was tied to a writing lever in order to record the heart-beats. The heart along with the assembly was now put in a bath filled with saline and the heart-beat was studied during and after perfusion (Fig. 1).

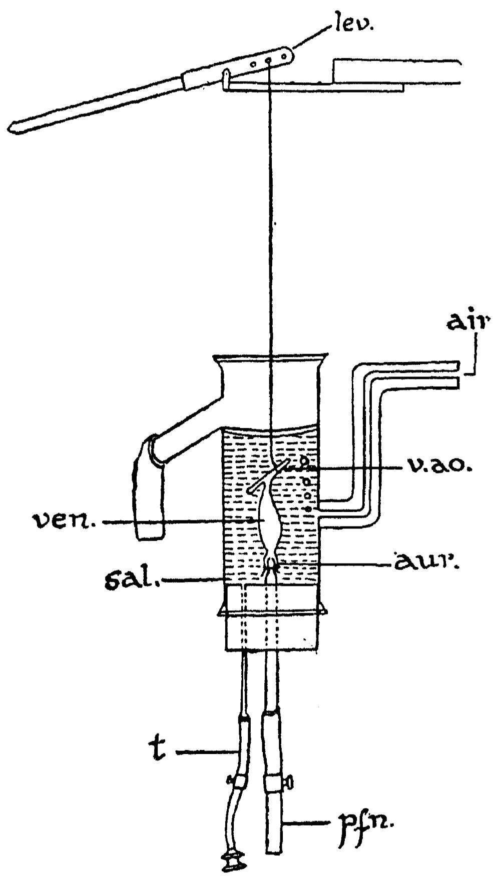

FIG. 1. Diagram of the bath used for perfusing the heart of *Pila globosa*.

air., inlet for aeration; *aur.*, auricle; *lev.* lever; *pfn.*, tube for perfusion; *sal.*, saline; *t.*, tube for experimental fluid and for washing; *v.ao.*, visceral aorta; *ven.*, ventricle.

The experiment was carried out in a temperature-controlled laboratory with temperature range of 16°–22° C. The aeration of the bath was, obtained by blowing fine bubbles of air from a side tube connected to an aspirator pump of the type commonly used in small fish aquarium. As the bubbles are small and are blown at a low rate they do not disturb the heart mechanically. At the bottom of the bath there is a tube in the perfusion assembly for drawing out the bath fluid and introducing experimental fluids. A slow constant drip of the perfusion fluid under constant pressure was maintained (Fig. 1).

In recording the heart-beat a muscle lever which was 13 cm. long and was counter-weighted to provide a tension of approximately 500 mgm. was used to write on a smoked Kymograph drum moving with a speed of about 1·2–1·5 cm./minute.

The following perfusion media were tried:

1. *Hedon fleig saline.*—This saline, which has a high pH and low potassium and calcium content, can retain the activity of the heart for only about 10 minutes, after which the heart stops (Fig. 2).

2. *Jullien's solution used for Helix.*—This solution (having a composition NaCl—6·5 gm.; KCl—0·14 gm.; $CaCl_2$—0·12 gm.; $NaHCO_3$—0·20 gm. dissolved in one litre of distilled water) does not maintain the isolated heart of *Pila* for more than 3–4 hours during which period also the beats are very irregular. The heart ultimately stops in diastole. A modification of the above solution by increasing its calcium content does not help much in maintaining the heart although the beats become more regular.

3. *Divaris and Krijgsman's solution used for Cochlitoma.*—This solution (having a composition NaCl—5·10 gm.; KCl—0·15 gm.; $CaCl_2$—0·75 gm.; $NaHCO_3$—0·20 gm.; $MgCl_2$—0·50 gm.; Glucose—1·00 gm. dissolved in one litre of distilled water) does not maintain the heart of *Pila* for more than an hour. Apparently these salines did not prove satisfactory because of the unsuitable Ionic composition, Osmotic pressure, and pH value of the fluid.

As the ionic composition of the saline is of foremost importance it is necessary that not only the correct quantity but the correct proportion of the different ions is used. Saxena (1957) gave the concentration of the different inorganic ions in mg./100 ml. of blood of *Pila*, but the preparation of a saline by suitable combination of these salts in which the total quantity of anions was lesser than the cations as given by Saxena (1957) was met with difficulties. Since for the proper functioning of the heart of molluscs the quantity and proportion of the cations like Na, K, Ca and Mg are more important than the anions, an attempt was made to keep the quantities of the basic ions constant and adjust the amount of anions accordingly. This was also necessitated from the fact that a combination of the salts, NaCl, KCl, $CaCl_2$, $MgCl_2$, Na_2HPO_4, $MgSO_4$ could not provide a saline in which the cations and anions were exactly in the same proportion as given by Saxena (1957). By a suitable adjustment, a modified saline was prepared (Composition: NaCl—3·168 gm.; KCl—0·373 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·254 gm.; $MgSO_4.7H_2O$—0.028 gm.; $Na_2HPO_4.12H_2O$—0·063 gm. dissolved in one litre of distilled water).

In the above solution the amount of Na^+, K^+, Ca^{++}, Mg^{++}, SO_4^{--} PO_4^{--} and Cl^- ions dissolved in 100 ml. of distilled water would correspond to Na—125·7 mg.; K—19·3 mg.; Ca—30·7 mg.; Mg—3·3 mg.; PO_4—1·7 mg.; SO_4—1·1 mg.; Cl—284·18 mg.

Moreover the quantities of the Na^+, K^+, Ca^{++}, Mg^{++}, SO_4^- and PO_4^{--} ions in this saline are the same as those found in the blood of *Pila*. Only the quantity of Cl^- is higher. This solution shows a pH range of 4·5–5·5. In this modified *Pila* saline the heart of *Pila* survives longer upto 5–6 hours but the heart-beats do not remain regular (Fig. 3).

Attempts were made to vary the concentration of various ions (K, Ca, Mg and Na), and observe the effect on the regularity of the heart-beat. It was noticed that the absence of potassium from the saline causes an increase in amplitude and frequency of the heart-beats which ultimately become irregular and then the heart stops in diastole in about 50 minutes.

To such a solution (Composition: NaCl—3·197 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·276 gm.) completely lacking in potassium, varying quantities of potassium salt KCl were added. For this purpose a stock N/100 KCl solution was prepared and measured quantities of this were added to the above solution. It was found that only a little addition of KCl (2 c.c. N/100 ml. in 100 ml. of saline) greatly increases the survival period of the heart which could live for more than 24 hours. With the addition of different concentrations of potassium in this way, it was found that with 10 c.c. N/100 KCl in 100 ml. of the above saline the heart would beat regularly and remain alive for more than 36 hours. A solution was, therefore, prepared containing NaCl—3·197 gm.; KCl—0·074 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·276 gm. dissolved in one litre of distilled water.

Besides the ionic composition of the saline, its osmotic pressure and pH value are equally important.

It was, therefore, decided to balance the osmotic pressure and pH value of the above saline according to those present in the blood of *Pila*. The osmotic pressure of the above saline was found lower than that of the blood of *Pila*. Moreover, it did not contain any organic nutritive material to provide energy to the isolated heart under experiment. It was, therefore, thought desirable to make the above saline isotonic with blood of *Pila* by the addition of glucose, and adjustment of the osmotic pressure was done by comparing the depression of freezing point of the blood of *Pila*. The average lowering of the freezing point of blood was found to be 0·29° C.

The saline was accordingly adjusted by adding 1 gm. of glucose per 1,000 c.c. of saline. This saline now becomes nearly isotonic with the blood of *Pila*, as the freezing point in this case is also 0·29° C. The addition of glucose would provide energy to the heart during experimental *in vitro* condition for long periods. This saline after addition of glucose shows a pH value of 5–5·5. Since the normal blood of *Pila* is slightly alkaline small quantities of $NaHCO_3$ were added to this solution to bring the pH to near the normal condition although it was found that changes within the range 5–8·5 do not materially affect the activity of the heart of *Pila*. Addition of 0·25 gm. of $NaHCO_3$ to one litre of the saline solution makes its pH 7·5. It has been seen that the addition of extra Na^+ and CO_3^{--} ions does not affect the working of the heart. Thus the saline of the following composition was found to be the most suitable for the heart of *Pila* as was clear from the subsequent experiments: NaCl—3·197 gm.; $CaCl_2$—0·850 gm.; $MgCl_2.6H_2O$—0·276 gm.; KCl—0·074 gm.; Glucose—1·00 gm.; $NaHCO_3$—0·25 gm. disssolved in one litre of distilled water.

In the above saline it was possible to maintain the isolated heart of *Pila* living in almost normal condition for 48–72 hours (Fig. 4).

The preparation of physiological (balanced) salines should aim at maintaining *in vitro* isolated parts of experimental animals in as nearly a normal living state as possible. Inside the body of the animal the tissues are bathed in blood or haemolymph, yet as has been mentioned earlier, a saline resembling the inorganic composition of the blood of *Pila* was not found a satisfactory substitute for blood. In several cases fluids having the inorganic composition of blood have not been found suitable as physiological salines, although Jullien *et al.* (1955) have used the same ionic concentration of calcium, potassium and sodium as in the blood of Helicides for preparing saline, The role of different ions is still far from clear as in spite of the fact that blood is composed of a large number of inorganic substances, Zoond and Slome (1928) have used a simple solution of sodium chloride and calcium chloride as a physiological saline for *Palinurus* and *Octopus*. Hogben (1925) has used the same two salts for *Mya* and Otis (1942) for *Ostrea*.

The sea-water which closely resembles the composition of body fluids of marine animals also acts as an excellent saline for most of them. Harvey (1957) has pointed out that sea-water contains ten principal substances, which are Sr, Br, H_3BO_3, SO_4, and Na, K, Ca, Mg, Cl and CO_3 ions but out of these only six (*viz.*, Na, K, Ca, Mg, Cl and CO_3 ions) are generally used in physiological salines. Besides these a number of minor inorganic constituents are present in the sea; there are several organic substances in solution and a number of organic and inorganic particles in suspension.

All these substances make the sea-water, so that when sea-water is used for perfusion there are a large number of substances both organic and inorganic contributing towards the healthy existence of the heart. It is likely, therefore, that the substances present in traces in the sea-water exert some definite healthy role on cardiac metabolism, similar to the effect exerted by trace substances present in the blood. It is possible that by altering the proportion of the major ions of the blood the absence of the trace and organic substances is partly compensated.

The hydrogen ion concentraton also plays an important role in the metabolism. It has, however, been noticed by us and other workers like Meeter (1955), Zoond and Slome (1928), and Otis (1942), that small changes in pH do not affect the working of the heart very much. The studies of Kokubo (1929) have shown that in oysters the pH of blood decreases in summer and increases in winter; further, it also changes with the change of pH in the environmental sea-water. It is possible, therefore, that as the heart is subjected to such changes in pH during normal conditions, it is not sensitive to mild changes of pH.

The present studies reveal that for preparing a suitable saline in case of *Pila*, modifications in the ions as present in the inorganic composition of its blood and addition of glucose are essential. The modification probably compensates for the absence of many trace substances and the organic materials present in the blood.

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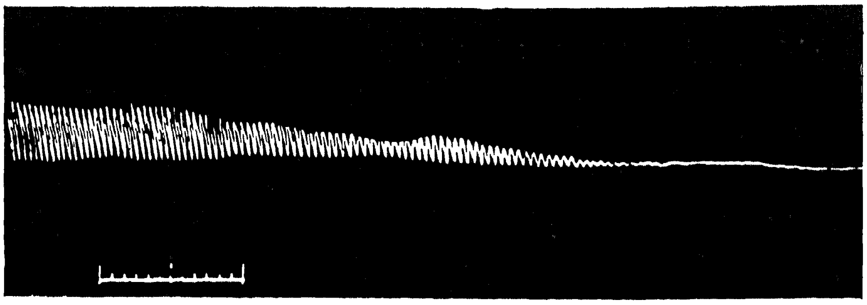

FIG. 2. Graph showing the heart beat of *Pila globosa* in Hedon Fleig's saline. Time scale—2 minutes.

FIG. 3. Graph showing the heart beat of *Pila globosa* in a saline resembling the inorganic ccmposition of its own blood. Time scale—2 minutes.

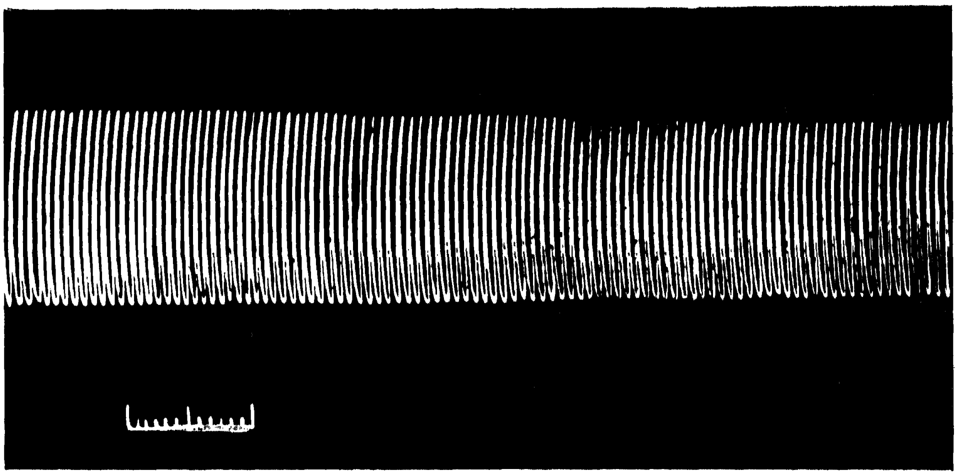

FIG. 4. Graph showing the regular beat of the isolated heart of *Pila globosa* in *Pila* salinc. Time scale—2 minutes.

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