PERFUSION OF ISOLATED FROG,S HEART WITH RINGER-TRIS SOLUTION

By

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Bhargava *et al* (1) reported that the efficiency of the isolated frog's heart improved on reduction of the sodium content to 60*mM/L* in the Ringer perfusate, the isotonocity being maintained by the addition of requisite amounts of various mono-and disaccharides. But the series of experiments brought up a question whether improvement in the performance of the heart was due primarily to reduction of soduim in the perfusate (which spared the energy otherwise to be expended on active extrusion of sodium) or due to beneficial effect, if any, of the sugars added. To clear this doubt, it was surmised that a substance chemically inert should be used to maintain the isotonicity. Such a chemical substance was *Tris* buffer.

MATERIALS AND METHODS

Perfusion experiments were performed on isolated frog's (*Rana tigrina*) heart, following the technique as given by Harris (2).

A series of modified Ringer solutions, *E*2—*E*8, was prepared with gradual reduction of sodium content and corresponding increase in the amount of *Tris* buffer so as to compensate for fall in tonicity. *E*1 was ordinary Ringer solution, whereas *E*8 contained no sodium chloride, the latter being completely replaced by *Tris*. The other constituents in modified Ringer solutions were kept unaltered. The composition of Ringer solution (labelled as "R" in cardiograms) was as follows :

1% Potassium chloride	0.75 *ml*
1% Calcium chloride	1.00 *ml*
1% Sodium bicarbonate	1.00 *ml*
0.6% Sodium chloride	to 100 *ml*

Chemicals used were of analytical grade.

Tris buffer is chemically 2-amino-2-(hydroxymethyl)-1:3 propane diol or Tris-(hydroxymethyl)-amino methane with the formula NH_2-C $(CH_2$-$OH)_3$' having a molecular weight of 121.14 *gm*.

Tris, being an inert organic substance and containing no metallictions, is a suitable chemical for the perfusion purpose and can replace other constitutents within limits without changing the molar or osmotic concentration. Also, its solutions can be so adjusted as to have any desired *pH*.

Table I

Volumes of sodium chloride and Trin buffer added and their concentrations as m M/L and the total millimolar concentration in the perfusion fluid.

Solution	Sodium chloride		*Tris* buffer		Perfusion fluid
	Vol. of 1% sod. chlor.	Conc. *mM/L*	Vol. of 1.2114%	Conc. *mM/L*	Millimolar concentration
E_1	58.35 *ml*	99.8	..	..	99.8
E_2	52.61 *ml*	90.0	0.98 *ml*	9.8	99.8
E_3	46.76 *ml*	80.0	1.98 *ml*	19.8	99.8
E_4	40.92 *ml*	70.0	2.98 *ml*	29.8	99.8
E_5	35.07 *ml*	60.0	3.98 *ml*	39.8	99.8
E_6	29.23 *ml*	50.0	4.98 *ml*	49.8	99.8
E_7	23.38 *ml*	40.0	5.98 *ml*	59.8	99.8
E_8	..	..	9.98 *ml*	99.8	99.8

The room temperature during the period of investigations varied from 19° to 24°C. Perfusion was carried out with all the seven solutions of a different concentrations of *Tris* under identical experimental conditions, ten observations being made with each solution. Records were taken on slowly moving drum.

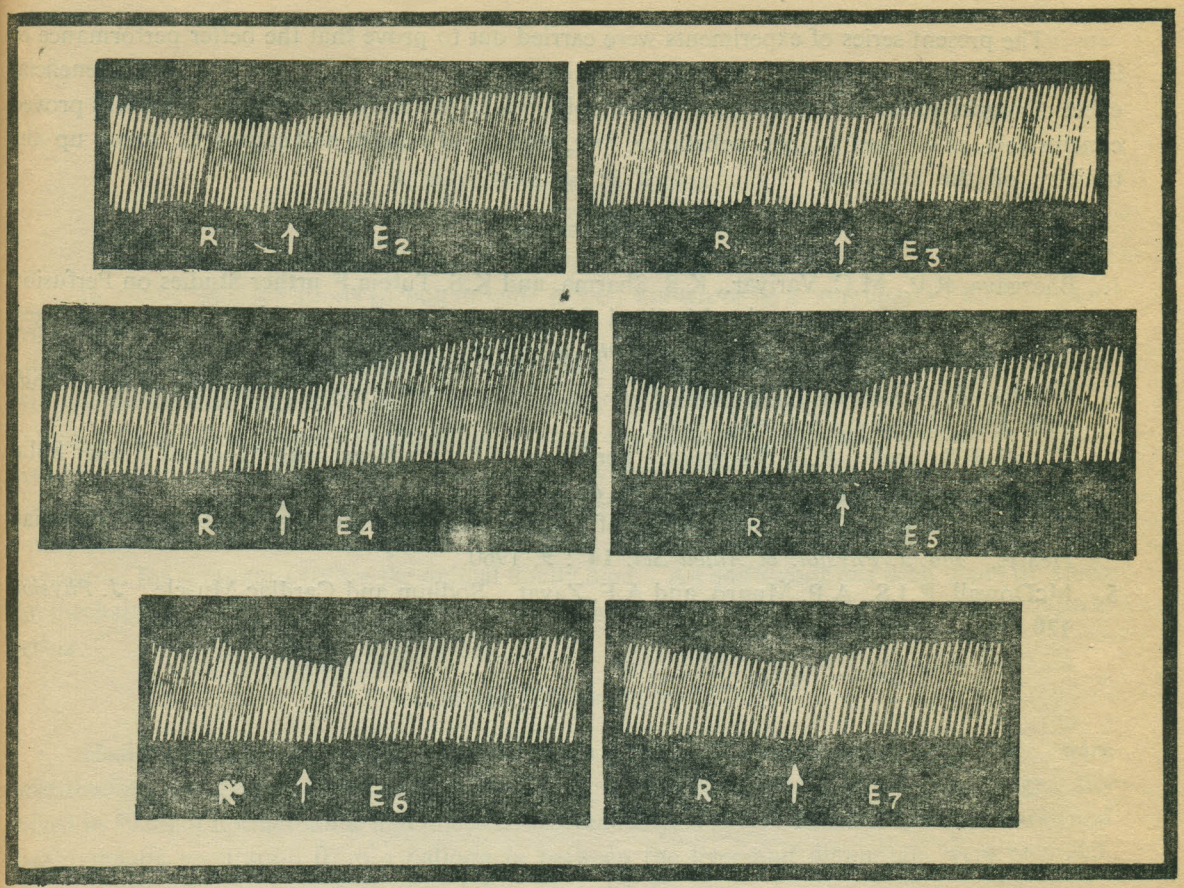
OBSERVATIONS AND RESULTS

It was observed that the amplitude of heart beat increased considerably when modified Ringer solutions, E_2 to E_7, were used for perfusion. But the most well-marked effects were

Table II

The observed amplitude values in mm for Ringer and test (Ringer-Tris) solutions.

Solution No.	Observed values in millimeters		Calculated for 10 *mm* of Ringer solution curves
	Ringer	*Tris*	
2	14	19	13.6 *mm*
3	14.5	19	13.1 *mm*
4	13	20	15.4 *mm*
5	12.5	19	15.2 *mm*
6	14.5	17	11.7 *mm*
7	13.5	15	11.1 *mm*



elicited by E_4 and E_5 . The heart stopped in systole when perfused with solution E_8, which contained *Tris* in place of sodium chloride.

DISCUSSION

It was Ringer who first showed that it is not only the osmolarity but the composition as well of the perfusate which is important for the isolated frog's heart.

McDowall *et al* (5) suggested that energy expenditure can be cut by reducing the sodium content, within limits, of the perfusion fluid which spared the heart from dissipating energy on active extrusion of sodium during the recovery phase.

Kahali and Bhargava (3) observed that the performance of isolated frog's heart improved by perfusion with Ringer-sucrose solution.

The experiments performed by Kahali and Kothari (4) and Bhargava *et al* (1) corroborate the previous workers' findings.

The present series of experiments were carried out to prove that the better performance of isolated frog's heart was due to reduction of sodium content in perfusate and not due to beneficial effect of sugars added in the modified Ringer solutions to maintain isotonicity. This was proved conclusively, as we used an inert substance, *Tris*, in place of sodium and sugars to make up the tonicity of the solution.

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