

THE EFFECTS OF SODIUM-FREE  
SOLUTIONS ON THE FAST ACTION POTENTIALS OF  
*VIVIPARUS CONTECTUS* (MILLET)  
(GASTROPODA: PROSOBRANCHIA)

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INTRODUCTION

In conventional excitable cells, such as the squid axon, the inward current of the action potential is attributable to a rapid influx of sodium ions (cf. Hodgkin, 1951, 1964). The replacement of sodium in the saline bathing this preparation by electrolyte or non-electrolyte substitutes results predictably in a rapid decline of the action potential (Hodgkin & Katz, 1949). Effects of sodium-free solutions on conduction processes in a number of other molluscan nerve preparations have also been explored (cf. Kostyuk, 1968; Treherne & Moreton, 1970). The results obtained are complicated, however, by the discovery in the nerve cell bodies of several pulmonate and opisthobranch gastropod molluscs of action potentials which depend on a transient entry of one or more species of cation other than sodium. In the cell bodies of *Onchidium verruculatum* (Oomura, Ozaki & Maeno, 1961) and *Helix pomatia* (Gerasimov, Kostyuk & Maisky, 1964, 1965*a, b*; Meves, 1966, 1968; Krishtal & Magura, 1970), for example, calcium ions appear to account for the inward component of the action current. Action potentials dependent upon an influx of both sodium and calcium have been demonstrated in *Aplysia californica* (Junge, 1967; Geduldig & Junge, 1968) and certain neurones of *Helix aspersa* (Chamberlain & Kerkut, 1967, 1969; Moreton, 1972). Sodium, calcium and magnesium ions have been implicated in the active membrane response recorded from nerve cell bodies of *Limnaea stagnalis* (Jevlova, Krasts & Veprintsev, 1971; Sattelle, 1973*a*). Conventional sodium-dependent action potentials have been reported for certain neurones of *Helix aspersa* (Moreton, 1968, 1972) and *Tritonia diomedea* (Magura & Gerasimov, 1966; Veprintsev *et al.* 1966; Krasts & Veprintsev, 1972).

Further problems of interpreting the effects of sodium-free solutions on conduction processes in molluscan neurones arise with the demonstration of a functional sodium reservoir, available to certain axons within the central nervous system of the fresh-water lamellibranch *Anodonta cygnea* (Treherne, Carlson & Gupta, 1969). This sodium store has been identified using electronmicroscopical, radioisotopic and spectrophotometric techniques in addition to electrophysiological investigations which have explored the different effects of electrolyte and non-electrolyte sodium substitutes on the conducted action potentials (Carlson & Treherne, 1969; Mellon & Treherne, 1969; Treherne, Mellon & Carlson, 1969). Sodium from this source is

available only to the larger (2–4  $\mu\text{m}$  diameter) axons of the cerebro-visceral connectives and has tentatively been located as follows: a readily accessible component associated with non-diffusible anions in the immediate vicinity of the axon surfaces; also a component not readily accessible, requiring mobilization, and sequestered in the glial elements which exhibit much stronger association with the relatively few larger diameter axons than with the bulk of the smaller axons of the connectives (Gupta, Mellon & Treherne, 1969; Treherne, Carlson & Gupta, 1969).

An investigation into the ionic basis of axonal function in the central nervous system of the prosobranch gastropod *Viviparus contectus* has established a strong dependence of the conducted spike on the presence of sodium in the bathing medium (Sattelle, 1972*a*). In spite of this, dextran Ringer or isotonic dextran solutions are able to sustain compound action potentials for extended periods (Sattelle, 1972). Differences have emerged between the findings for *Anodonta* connectives and *Viviparus* connectives. Whilst in the latter preparation both fast and slow fibres can maintain conduction in dextran solutions by a sodium-dependent process (Sattelle, 1972), this ability is confined to the fast fibres of *Anodonta* (Treherne *et al.* 1969). Any such reservoir of sodium in the central nervous system of *Viviparus* would therefore need to be more extensive than that postulated to exist in *Anodonta* connectives. Also, the tentative location of the less accessible component of the sodium store in the glial elements associated with the larger axons of *Anodonta* could not apply to *Viviparus*, where a sparse distribution of glial elements has been observed throughout the connective (Sattelle & Lane, 1972). An intimate glial investment of all axons would be expected if a glial-mediated mechanism controlled the ionic composition of the extracellular fluid in the connective of this prosobranch mollusc. This paper describes an investigation into the effects of electrolyte and non-electrolyte sodium substitutes on the fast action potentials of the pleural-supraintestinal connective of *Viviparus contectus*. Experiments have been confined to the fast component of the compound action potential because it is much less variable than the slow component (Sattelle, 1972). The purpose of this study is to determine whether or not the maintenance of conduction processes under non-electrolyte, sodium-free conditions constitutes a physiological mechanism for regulation of the ionic micro-environment of the nervous elements.

#### MATERIAL AND METHODS

Records of fast action potentials were obtained from isolated pleural-supraintestinal connectives of *Viviparus contectus*. Techniques employed for dissection and isolation of the connectives and extracellular recording and display of the fast component of the compound action potential were as described in a previous report (Sattelle, 1972). That account included a description of the perspex preparation chamber used in the present study and details of its perfusion. Changes in weight of the preparation in different bathing media were investigated using dissected lengths (8–10 mm) of pleural-supraintestinal connective. These were carefully blotted and rapidly weighed on an electrobalance (range 0–2.5 mg). Each weighing recorded was the average of three successive readings. The normal Ringer solution used was based on a saline devised for *Viviparus viviparus* (Little, 1965) with the following composition: 24.0 mM/l NaCl; 9.0 mM/l  $\text{NaHCO}_3$ ; 1.2 mM/l KCl; 5.5 mM/l  $\text{CaCl}_2$ ; pH 7.4. This saline main-

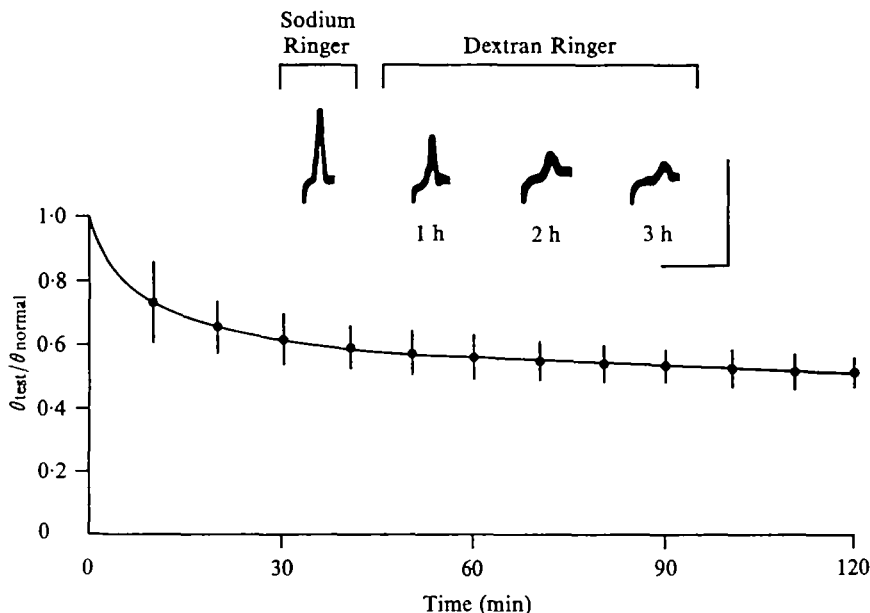


Fig. 1. Effects on the fast action potentials of prolonged exposure to dextran Ringer. A plot of relative conduction velocity ( $\theta_{\text{test}}/\theta_{\text{normal}}$ ) against time following replacement of the sodium salts of normal Ringer by dextran. The vertical lines represent twice the standard error. Inset shows records of fast action potentials; scale bars represent 100 msec (horizontally) and 100  $\mu\text{V}$  (vertically).

tained conduction in the fast fibres of *Viviparus connectus* for extended periods with little change in conduction velocity (Sattelle, 1972).

## RESULTS

### Effects of dextran and tris

When the sodium salts of normal Ringer were replaced by dextran (to maintain isosmotic conditions\*) fast action potentials were maintained for long periods. The conduction velocity ( $\theta$ ) fell to about 50% of its value in normal Ringer during a 2 h exposure to dextran Ringer. The decline in  $\theta$  was greatest over the first 30 min of the experiment and thereafter a much steadier decline was observed (Fig. 1).

Experiments were conducted in which the sodium salts in normal Ringer were replaced in stages by increasing concentrations of either tris chloride or dextran, thus maintaining isosmotic conditions, and the effects on  $\theta$  were observed. The results were plotted as relative conduction velocity ( $\theta_{\text{test}}/\theta_{\text{normal}}$ ) against the square root of the relative external sodium concentration ( $\text{Na}^+_{\text{test}}/\text{Na}^+_{\text{normal}}$ )<sup>†</sup> following the method used for *Anodonta* connectives (Carlson & Treherne, 1969; Treherne *et al.* 1969) and enabling a comparison with the data obtained for squid (*Loligo*) axons (Hodgkin & Katz, 1949) (Fig. 2). When tris replaced sodium the fast fibres of *Viviparus* exhibited

\* The terms *isosmotic* and *isotonic* as used in this paper follow the definitions given by Potts & Parry (1964). 'Isosmotic conditions' describe, for example, the replacement of sodium salts in normal Ringer by a concentration of electrolyte or non-electrolyte substitute calculated to maintain normal osmolarity. 'Isotonic conditions' refer to the use of an experimentally determined concentration of substitute which produces neither swelling nor shrinkage of the connective.

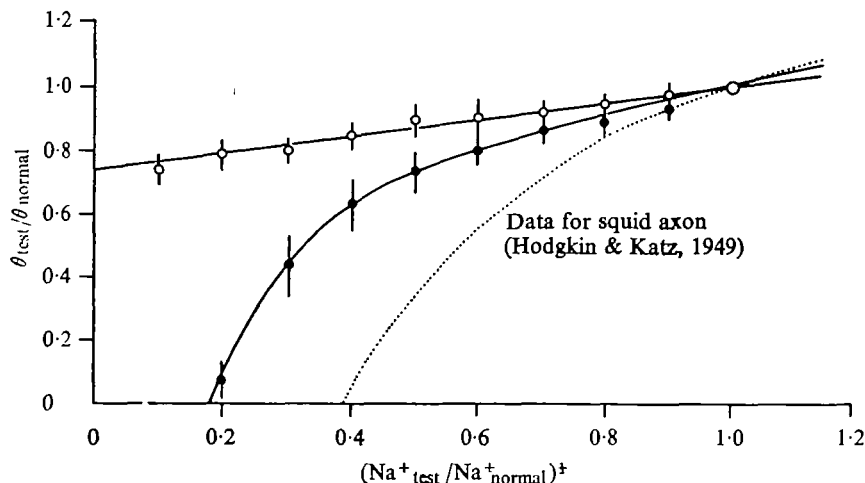


Fig. 2. Effects on axonal conduction of varying the external sodium concentration. Tris chloride (closed circles) and dextran (open circles) replace the sodium salts of normal Ringer. The curve for the squid (*Loligo*) axon is derived from measurements of the maximum rate of rise of the action potentials based on the assumption that, in a simplified theoretical system, the conduction velocity ( $\theta$ ) is related to the square-root of the rate of rise of the action potential (Hodgkin & Katz, 1949). The vertical lines represent twice the standard error.

a similar response to the squid axon. In fact, the curve obtained was somewhat displaced and approximated more closely to the data obtained for *Carcinus* axons (Katz, 1947). With dextran as a substitute for sodium salts, however, a straight-line relationship was obtained and when all the sodium had been replaced action potentials were still conducted (Fig. 2). These effects of tris and dextran on the fast action potentials of *Viviparus* closely correspond to those observed for the fast action potentials of the cerebro-visceral connective of *Anodonta* under similar conditions (Carlson & Treherne, 1969).

#### Effects of a range of non-electrolyte sodium substitutes

To investigate further the discrepancy between the effects on conduction velocity of tris and dextran, a range of non-electrolyte sodium substitutes was employed. The results have been summarized in Fig. 3 as plots of  $(\theta_{\text{test}}/\theta_{\text{normal}})$  against  $(\text{Na}^+_{\text{test}}/\text{Na}^+_{\text{normal}})^{\dagger}$ . Replacing the sodium salts in normal Ringer by mannitol, sucrose and raffinose produced much the same effect. In the presence of these non-electrolytes the fast action potentials were maintained at lower concentrations of external sodium ions than when tris was used to replace sodium. With inulin as the substitute, this trend continued and conduction block was only achieved in the complete absence of sodium in the bathing medium. Only dextran of the range of non-electrolyte substances investigated supported fast action potentials in the complete absence of sodium in the bathing medium.

#### Changes in weight of connectives in sodium-free Ringer

To test whether or not truly isotonic\* conditions prevailed during exposure to sodium-free solutions, weight changes of connectives were followed. Plots of  $W_t/W_i^{\dagger}$

$\dagger$  Test weight ( $W_t$ ) is the weight of the connective (average of three successive readings on the electrobalance) in the test (sodium-free) solution and initial weight ( $W_i$ ) is the weight of the connective in normal Ringer at the start of the experiment.

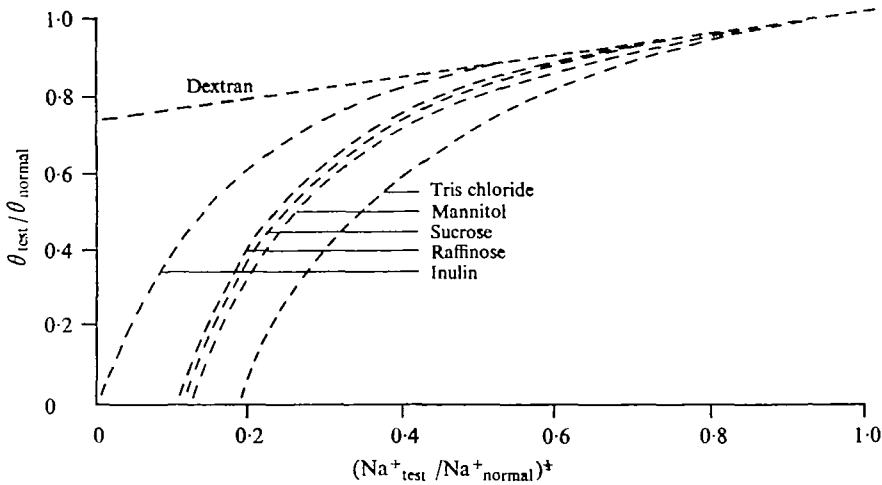


Fig. 3. Effects on axonal conduction of a range of sodium substitutes, each of which replaces in stages the sodium salts of normal Ringer. Results are summarized as plots of the relative conduction velocity ( $\theta_{\text{test}}/\theta_{\text{normal}}$ ) against the square root of the relative sodium concentration of the Ringer ( $\text{Na}^+_{\text{test}}/\text{Na}^+_{\text{normal}}$ )<sup>1/2</sup>. To facilitate a comparison of the effects of the different non-electrolytes, individual points (standard errors less than 0.1 units on vertical axis) are omitted and the final curves only are represented. The graphs summarize experiments on 33 isolated, pleural-suprarentestinal connectives.

Table 1. *Molecular dimensions and ratios of restricted ( $D_r$ ) to free ( $D$ ) diffusion coefficients for various sodium substitutes*

Molecular species	Radius of equivalent sphere (Å)	$D_r/D$ for 200 Å channel	$D_r/D$ for 100 Å channel
NaCl	1.4*	0.95	0.91
Sucrose	4.4*	0.86	0.75
Raffinose	5.7*	0.83	0.70
Inulin	15.3*	0.62	0.40
Dextran	22.5†	0.50	0.26

\* Data from Pappenheimer (1953).

† Data from Ingleman & Halling (1949).

against time were obtained (Fig. 4). During 30 min exposures to bathing media in which sodium salts were replaced by calculated isosmotic concentrations of tris chloride, mannitol, sucrose, raffinose, inulin and dextran, a loss in weight of the connectives occurred which could be abolished by re-exposure to normal Ringer. The most pronounced and rapid changes in weight of the connectives were observed when dextran replaced the sodium salts. This result is clearly of interest in the light of the electrophysiological findings for *Viviparus* axons in dextran Ringer.

#### *Changes in weight of connectives in mannitol and dextran*

To determine the truly isotonic concentrations of two of the non-electrolyte sodium substitutes, changes in weight of *Viviparus* connectives were studied during exposure to a range of concentrations of mannitol and dextran in distilled water (Figs. 5, 6). Connectives bathed in concentrations of mannitol in the region of 30 mM/l exhibited

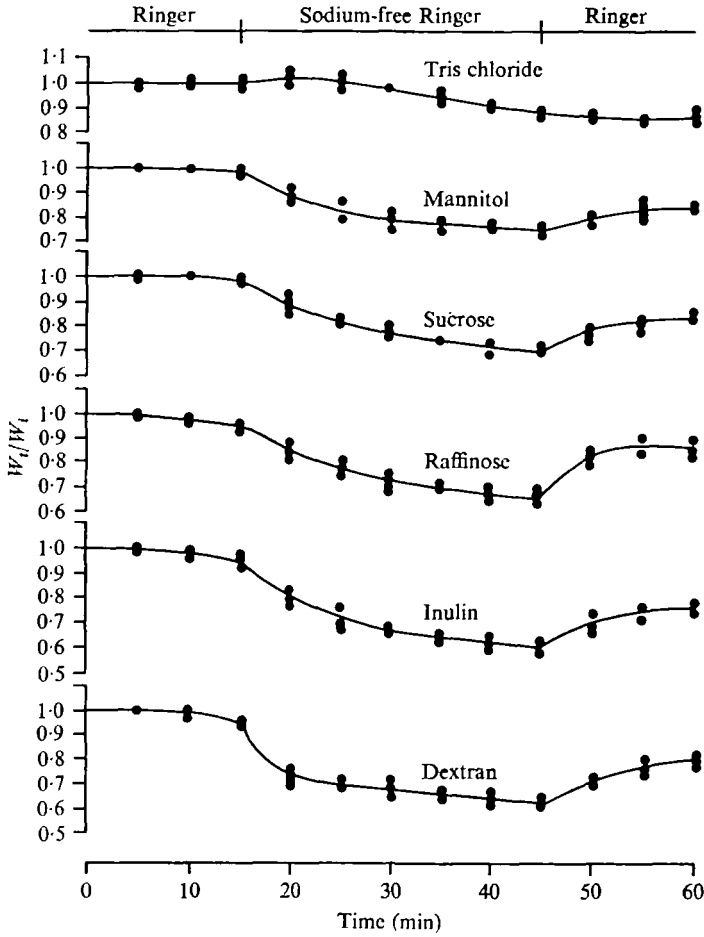


Fig. 4. Changes in weight of connectives during 30 min exposures to sodium-free Ringer. The various sodium substitutes are indicated on the graphs. Results have been expressed as plots of  $W_t/W_i$  (test weight/initial weight).

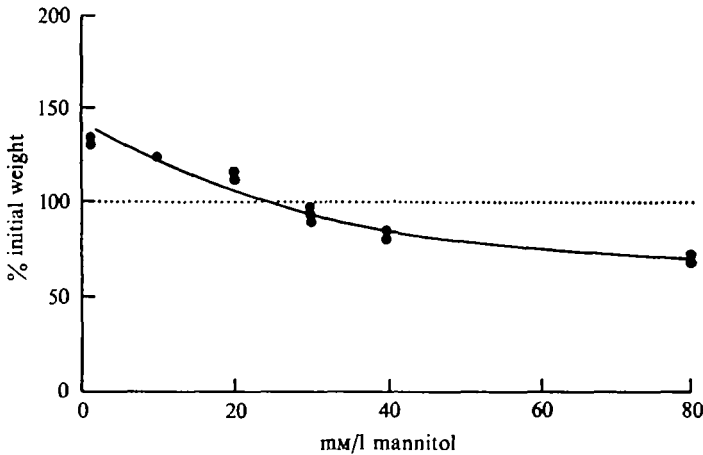


Fig. 5. Changes in weight of connectives in mannitol solutions. Weights are recorded as a percentage of the initial weight of the connective in normal Ringer. An equilibration time of 15 min is allowed before weighing.

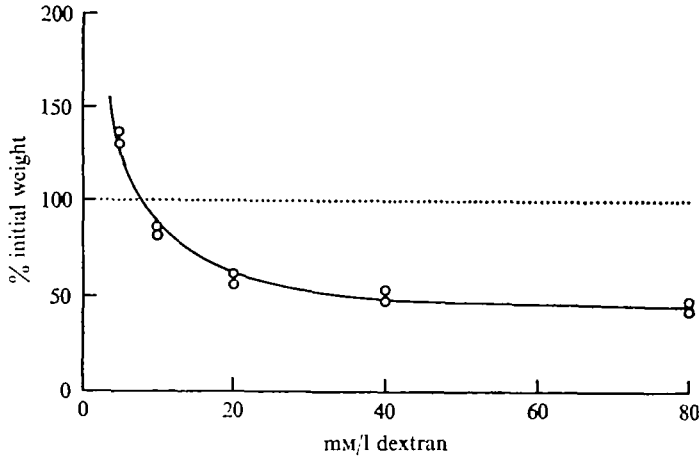


Fig. 6. Changes in weight of connectives in dextran solutions. Weights are recorded as a percentage of the initial weight of the connective in normal Ringer. An equilibration time of 15 min is allowed before weighing.

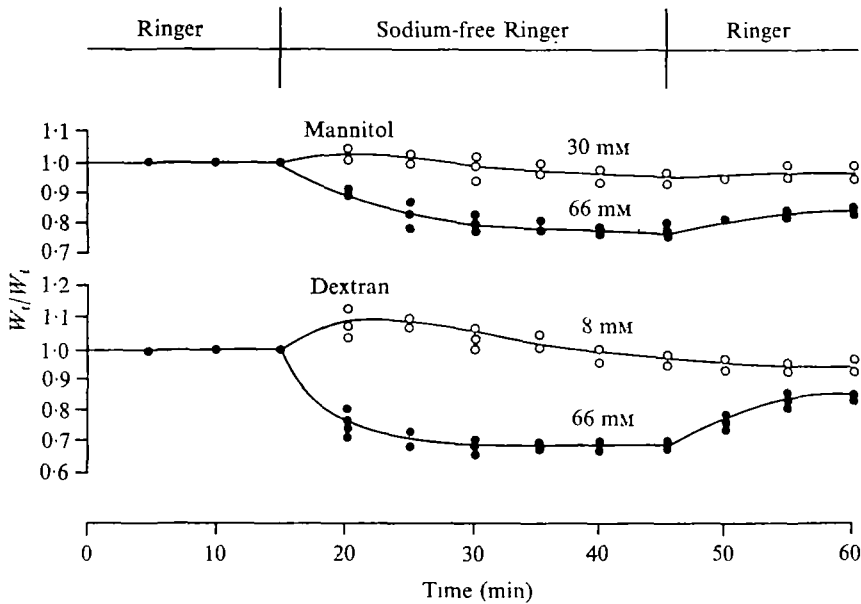


Fig. 7. Changes in weight of connectives during exposure to Ringer solutions in which isotonic (open circles) and isosmotic (closed circles) concentrations of mannitol and dextran replace the sodium salts.

minimal weight changes. When exposed to a range of dextran solutions, connectives maintained constant weight at concentrations of about 8 mm/l. Ringer solutions in which the truly isotonic concentrations of mannitol and dextran have been used to replace sodium salts induced, over a period of 30 min, very small changes in weight of the connectives (Fig. 7).

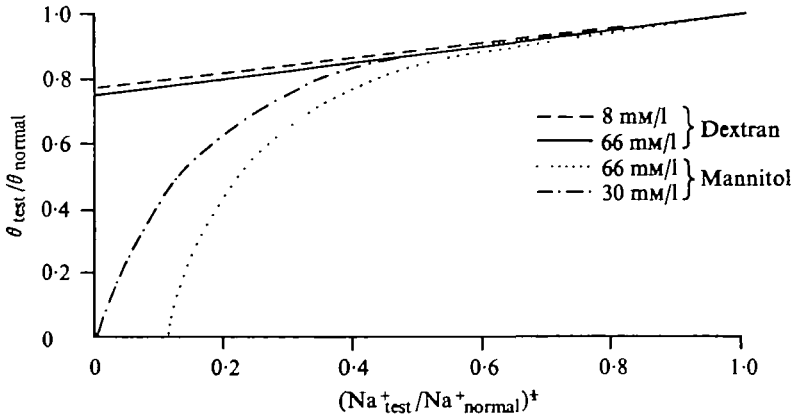


Fig. 8. Effects on axonal conduction of using isosmotic and isotonic concentrations of mannitol and dextran to replace in stages the sodium salts of normal Ringer. Individual points (standard errors less than 0.1 units on vertical axis) are omitted and final curves only are represented.

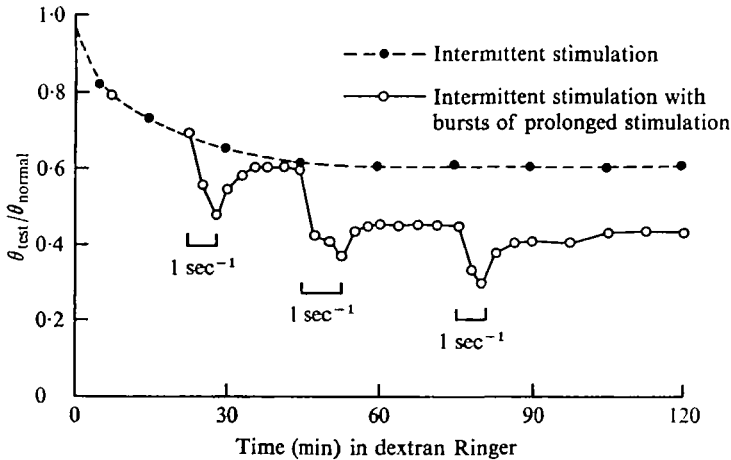


Fig. 9. Effects of stimulation on axonal conduction in dextran Ringer. Bursts of stimulation at  $1 \text{ sec}^{-1}$  and of 5 min and 7.5 min duration are applied to a connective exposed to dextran Ringer. The resultant changes in relative conduction velocity are recorded (open circles). For comparison, the changes in relative conduction velocity incurred during exposure to dextran Ringer with only intermittent stimulation are illustrated (closed circles).

### *Effects of isotonic (experimentally determined) concentrations of mannitol and dextran on fast action potentials*

When 30 mM/l concentrations of mannitol were employed to replace the sodium salts in normal *Viviparus* Ringer, the relationship between  $(\theta_{\text{test}}/\theta_{\text{normal}})$  and  $(\text{Na}^+_{\text{test}}/\text{Na}^+_{\text{normal}})^{\dagger}$  shown in Fig. 8 was obtained. With this concentration of mannitol as a substitute, fast action potentials were maintained at somewhat lower sodium concentrations than was the case when 66 mM/l mannitol was used. There was very little difference between the effects of 8 and 66 mM/l concentrations of dextran when these replaced the sodium salts of normal Ringer. The findings using 30 mM/l concentrations of mannitol emphasized the difference, partly obscured in earlier work by the use of higher concentrations, between the effects of mannitol and of tris chloride (cf. Fig. 3) on the fast action potentials.



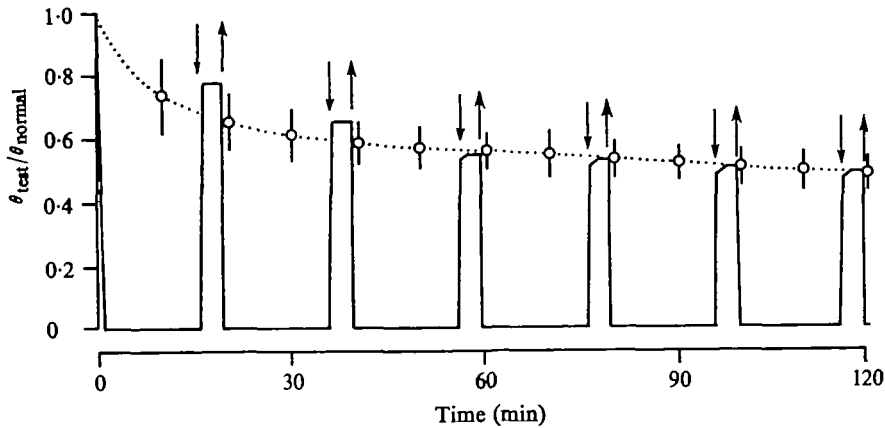


Fig. 10. Effects on axonal conduction of intermittent exposure to dextran Ringer. A connective is exposed to tris Ringer for 2 h apart from 5 min periods (at 15 min intervals) when it is subjected to dextran Ringer. Changes in relative conduction velocity are followed throughout the experiment (solid line) and the results have been compared to those obtained when connectives are exposed continuously to dextran Ringer (open circles, dotted line). Arrows indicate the beginning ( $\downarrow$ ) and the end ( $\uparrow$ ) of a period of substitution of tris Ringer by dextran Ringer. Vertical lines represent twice the standard error.

#### *Effects of stimulation on the fast action potentials*

The effect of stimulation on the ability of the fast fibres to maintain their function in dextran Ringer was investigated (Fig. 9). A 5 min burst of stimulation at  $1 \text{ sec}^{-1}$  following a 20 min exposure of connectives to dextran Ringer produced some reduction in the conduction velocity of the fast axons which recovered when stimulation ceased. After 45 min, stimulation at the same rate for  $7\frac{1}{2}$  min produced an irreversible decline in conduction velocity and a similar reduction was induced by a 5 min burst of stimulation following 75 min of exposure to dextran Ringer. Whatever the source of sodium maintaining fast action potentials in dextran Ringer, this cation is clearly abundant in the extra-neuronal fluid, although stimulation may lead to some depletion of the available sodium.

#### *Effects on the fast action potentials of intermittent exposure to dextran Ringer*

Connectives were exposed to sodium-free (tris) Ringer for a 2 h period. Conduction block in the fast fibres rapidly ensued, but exposure to dextran Ringer for brief periods at 15 min intervals restored activity (Fig. 10). The decline of conduction velocity in these restored fast fibres closely followed the normal pattern of decline when fibres were maintained in dextran Ringer throughout the same period.

### DISCUSSION

The effects of a range of sodium substitutes on the fast action potentials of the pleural-supraintestinal connective of *Viviparus contectus* have been investigated. The behaviour of these action potentials on exposure to a range of sodium-free (tris) solutions in which the proportion of tris is steadily increased up to the point of complete replacement of sodium resembles that of the active membrane responses recorded under comparable conditions from conventional excitable cells such as the squid (*Loligo*) axon (Hodgkin & Katz, 1949) and the crab (*Carcinus*) axon (Katz,

1947). With dextran as the sodium substitute, however, the behaviour of the fast fibres departs considerably from the conventional situation and complete replacement of the sodium salts of normal Ringer by this non-electrolyte does not produce conduction block. Fast action potentials are maintained by a sodium-dependent process for extended periods under these conditions (cf. Sattelle, 1972); a 2 h exposure to dextran Ringer producing only a 50% decline in the conduction velocity ( $\theta$ ) of the fast fibres. It has been observed that the effects of dextran on the fast axons of *Viviparus* are atypical for the range of non-electrolyte sodium substitutes employed. When mannitol, sucrose, raffinose and inulin replace sodium, fast action potentials are rapidly abolished, in contrast to the findings for connectives of *Anodonta cygnea* where both sucrose and dextran are able to maintain the conduction of fast fibres (Treherne *et al.* 1969; Carlson & Treherne, 1969). The different effects of non-electrolyte sodium substitutes on action potentials of *Viviparus contectus* are not, therefore, readily explained in terms of the functional sodium store proposed to account for the results obtained in the fast axons of *Anodonta cygnea* (Carlson & Treherne, 1969; Treherne, Carlson & Gupta, 1969).

The anomalous behaviour of *Viviparus* axons when exposed to various non-electrolytes may result from shrinkage of the nervous tissue. This possibility is raised with the demonstration that weight losses of connectives occur when isosmotic concentrations of mannitol, sucrose, raffinose, inulin and dextran replace the sodium salts of normal Ringer and the largest and most rapid changes accompany substitution by dextran. Adjusting to isotonic concentrations of mannitol and dextran does not, however, greatly affect the response of the fast fibres to these non-electrolytes. For example, axonal function is still maintained when isotonic dextran completely replaces sodium, suggesting that the changes in weight of the connectives associated with the use of isosmotic concentrations of dextran are not the primary cause of this phenomenon.

Clearly a major problem in interpreting the effects of dextran is that none of the other non-electrolyte sodium substitutes employed produces the same response in the large axons of *Viviparus*. In a first attempt to account for this it is assumed that dextran (M.W. 10000) alone of these non-electrolytes fails to penetrate the extracellular system. An explanation of the results may then be sought in terms of the withdrawal of ions and water from the nervous elements in response to concentration and osmotic gradients arising from the exclusion of dextran. The Stokes radius of this dextran, which refers to the hydrated form, has been estimated at 22.5 Å (Ingleman & Halling, 1949). The molecular size (45 Å in diameter) of this non-electrolyte is not, therefore, insignificant in comparison with the dimensions (100–200 Å in width) of the outwardly facing clefts of the extracellular system (Sattelle & Lane, 1972). Its complete exclusion from the extracellular system does nevertheless seem a remote possibility in the apparent absence of any ultrastructural evidence for restriction of the intercellular clefts (cf. Sattelle & Lane, 1972). Such a view is given support by the demonstration that the exogenous tracer molecule macroperoxidase (M.W. 40000) penetrates the extracellular system of the cerebro-visceral connective of *Anodonta* (Lane & Treherne, 1972) in which the dimensions of the extracellular channels (150 Å in width) (Gupta *et al.* 1969) closely resemble those referred to above for *Viviparus*.

If, as seems likely, dextran penetrates the extracellular system, the sodium involved in action potential production in dextran Ringer may be derived from sodium bound

to fixed negative charges located either on the axonal membrane or in close proximity to its surface. It is well known, for example, that membrane-bound fragments from vertebrate central nervous tissues contain tightly bound cations, and that bound sodium and potassium are available to the  $\text{Na}^+$ ,  $\text{K}^+$ -activated ATPase (Rodnight, Carrera & Goldfarb, 1969). More recently a cell-surface coating of an ionic nature has been detected as a 'surface potential' of lymphocytes and trophoblastic cells using conventional microelectrode recording techniques (Hause *et al.* 1970). Also the potential role of indiffusible anions as cation reservoirs in the central nervous system of molluscs has been suggested for *Helix aspersa* (Chamberlain & Kerkut, 1967, 1969) and *Anodonta cygnea* (Carlson & Treherne, 1969; Treherne *et al.* 1969). Sodium attached to such anion groups would be expected to exchange with tris ions but not with dextran, which might account for the observed effects of these sodium substitutes on the fast action potentials of *Viviparus*. Conduction failure in all the other non-electrolyte sodium substitutes nevertheless remains difficult to reconcile with this explanation.

Retaining the assumption that dextran penetrates the extracellular system, it is necessary to consider whether or not its rate of entry into the connective is retarded either by its molecular dimensions in relation to the size of the outwardly facing intercellular clefts or by its diffusivity. A simplified analysis of the effects of steric hindrance and viscous drag for the case of spherical particles entering circular openings is discussed by Pappenheimer (1953), who accounts for these effects in terms of a restriction to free diffusion given by the equation

$$\frac{D_r}{D} = \frac{(1 - a/x)^2}{1 + 2.4a/x},$$

where  $D_r/D$  is the ratio of diffusion coefficients for restricted ( $D_r$ ) and free ( $D$ ) diffusion,  $a$  is the radius of the particle and  $x$  is the radius of the channel. The ratios  $D_r/D$  have been calculated for a number of the non-electrolyte sodium substitutes (Table 1) and allowance is made for the maximum and minimum observed widths of the intercellular clefts of *Viviparus contectus* (cf. Sattelle & Lane, 1972). Restriction to free diffusion appears to be considerably greater for dextran than for sodium chloride although it should be pointed out that the above analysis refers to circular openings rather than elongate clefts.

A value of  $1.483 \times 10^{-5} \text{ cm}^2/\text{sec}^*$  has been obtained for the diffusion coefficient of sodium chloride at a concentration of 0.1 M in aqueous solution at 25 °C. To a reasonable approximation, the diffusion coefficient ( $D$ ) varies inversely as the cube root of the molecular weight ( $M$ ) (cf. Clark, 1952). From a plot of  $D$  against  $M$  for various sugars and proteins, a diffusion coefficient of approximately  $0.135 \times 10^{-5} \text{ cm}^2/\text{sec}$  is obtained for dextran (M.W. 10000). Thus a combination of steric hindrance, viscous drag and low diffusivity could markedly slow down the entry of dextran into the central nervous tissues of *Viviparus*, which may contribute to its observed effects on axonal conduction. A direct effect of dextran in, for example, inducing a leakage of ions into the extracellular spaces even under isotonic conditions should not be discounted. It has been shown that dextran Ringer rapidly restores axonal function when applied in short bursts at fifteen minute intervals during a 2 h exposure to tris Ringer.

\* From *Handbook of Chemistry and Physics* (44th ed.), the Chemical Rubber Publishing Co. (1962).

The relative conduction velocity ( $\theta_{\text{test}}/\theta_{\text{normal}}$ ) quickly attains a level very similar to that produced by continuous perfusion of *Viviparus* connectives with dextran Ringer. Also, in this context it may be relevant to note the recent observations of Pooler & Oxford (1972) on the lobster axon under sucrose-gap conditions. Careful measurements of membrane current indicated the presence of a parallel leakage current attributable to a leaching of ions from the axon into the bathing sucrose solution. If a similar withdrawal of intracellular ions takes place in the connective of *Viviparus* it must be much greater in the presence of dextran than in the presence of the lower molecular weight non-electrolytes.

It nevertheless appears that under normal physiological conditions all the axons of the pleural-supraintestinal connective of *Viviparus contectus* exist in a fluid compartment that is readily accessible to ions and small molecules in the bathing medium (cf. also Sattelle, 1972). The maintenance of axonal conduction in sodium-free (dextran) Ringer is not therefore considered to be an example of a physiological mechanism of control of the ionic environment of the nervous elements of this prosobranch mollusc.

#### SUMMARY

1. A 2 h exposure of connectives of *Viviparus contectus* to sodium-free Ringer, in which dextran maintains osmolarity, results in a decline in the conduction velocity of the fast axons to 50% of the value obtained in normal Ringer.

2. The changes in conduction velocity of the fast axons in response to the replacement in stages of the sodium salts of normal Ringer correspond to those exhibited by conventional excitable cells when tris is the substitute, but depart considerably from this behaviour when dextran is the substitute.

3. Experiments employing mannitol, sucrose, raffinose, inulin and dextran to replace the sodium salts of normal Ringer reveal that dextran alone of these non-electrolytes maintains fast action potentials in the absence of sodium.

4. Weight losses of connectives under sodium-free (isosmotic) conditions are greatest and are most rapidly achieved when dextran (of the range of non-electrolytes studied) replaces the sodium salts of normal Ringer. Adjusting to isotonic conditions (8 mM dextran) does not diminish the ability of this non-electrolyte to maintain fast action potentials.

5. During prolonged perfusion of connectives by tris Ringer brief (5 min) exposures to dextran Ringer at 15 min intervals rapidly restore fast action potentials. The decline of conduction velocity in these restored fibres closely follows the normal pattern of decline when function is maintained continuously in dextran Ringer.

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