J. Exp. Biol. (1966), **44**, 163–175
With 8 text-figures
Printed in Great Britain

A LEPIDOPTERAN SALINE: EFFECTS OF INORGANIC CATION CONCENTRATIONS ON SENSORY, REFLEX AND MOTOR RESPONSES IN A HERBIVOROUS INSECT

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(*Received* 15 *September* 1965)

INTRODUCTION

Owing to the great variability in ionic composition of insect haemolymph (Boné, 1944; Duchâteau, Florkin & Leclerq, 1953; Sutcliffe, 1963), it is probably desirable to use haemolymph itself as an experimental medium whenever this is possible. In the present studies of proprioception in *Antheræa pernyi* the long time spent in dissection and the necessity for aeration rendered the use of a simple synthetic medium essential. Certain phytophagous insects, among them the giant silkworms, possess haemolymph characterized by much lower Na concentrations and higher K and Mg concentrations than those found in the majority of other animals. Lockwood (1961) gave the composition of thirty-six different insect salines selected from published data, but of these only four were designed for such phytophagous insects with any reference to the cation concentrations present in their haemolymph.

Previous electrophysiological work on Lepidoptera is very scanty. Finlayson & Lowenstein (1958) worked on *Antheræa* pupal muscle receptor organs (MRO) as part of a study of stretch receptors in various insect groups, but in their experimental work they used Ephrussi and Beadle's *Drosophila* saline, a high-Na type medium, which has also been widely used in studies on diapause in *Hyalophora cecropia*. Belton (1958), in a report on studies of the muscle physiology of adult Saturniidae of several species, described a saline supposedly based on the concentrations of inorganic cations present in the species studied, though probably with appreciable deviations from any one of them (their haemolymph compositions were not given). Van der Kloot (1958), in a study of diapause in *Hyalophora* with particular reference to the activity of the C.N.S. during diapause, and again in a study of pupal spiracular function in the same species (van der Kloot, 1963), used Ephrussi and Beadle's saline. The only experimental medium closely similar in inorganic cation concentration to the haemolymph of a lepidopteran was that used by Wyatt (1956) for ovarian tissue culture in *Bombyx mori*. Wyatt also analysed some of the organic constituents of haemolymph, including protein and amino acids, which among Holometabola largely replace chloride, the more usual major anion component (Sutcliffe, 1963).

The work most relevant to the present problem has been done, not on Lepidoptera but on another phytophagous group, the Phasmidae. *Carausius morosus*, the representative of this group studied, has like the Saturniidae low Na, moderately high K

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and extremely high Mg concentrations. Wood (1957), using a saline based on the haemolymph of this species, examined the effects of changing the cation and osmotic concentrations individually. He found, perhaps not surprisingly, that the electrical parameters of muscular activity indicated in most cases optimal function at the concentrations present in haemolymph. The exceptions to this finding were Na and Ca, which in concentrations greater than normal exaggerated the size of the muscle action potential, and to a lesser extent the resting potential. As a result of Wood's findings it was necessary to examine the responses of the systems to be studied in *Antheræa* both in its own haemolymph and in the lepidopteran media which have been used previously.

MATERIALS AND METHODS

Dissection

Several procedures were used in the dissection of the last instar larvae of *Antheræa pernyi* which were the experimental animals for the major part of this study. (In the experiment of Table 3, larvae of *Antheræa mylitta* were used.) Initially carbon dioxide

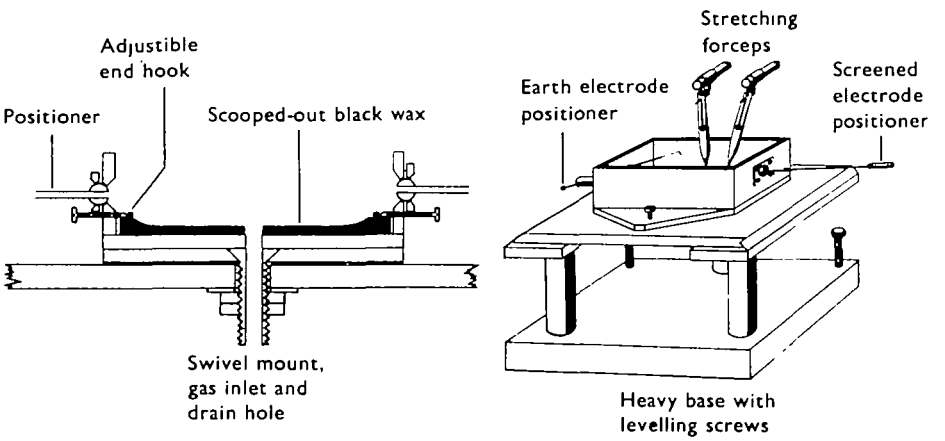

Fig. 1. Diagram of the Perspex experimental chamber; shown on the right with the forceps which were used to hold and stretch the MRO in position, and on the left in section.

anaesthesia was used for 2 or 3 min. to immobilize the caterpillar for the first mid-dorsal longitudinal incision. It was found that without some such precaution contraction of the animal was liable to rupture the gut. However, as shown by Ralph (1960) the use of this gas as an anaesthetic is undesirable owing to considerable effects on activity and excitability. Nitrogen is more satisfactory in this respect, but a longer exposure is necessary to produce immobility. For certain experiments the caterpillar was chilled for 1–2 hr. and the initial stages of dissection were performed at 0–3° C. All methods were greatly facilitated by the use of a 'rack', a device for holding the caterpillar at a constant length. Tungsten-wire loops with hooked and sharpened ends were inserted into the head capsule in regions where there are no major nerves, and into the flaps of integument lying dorsal to the anus. These were used as attachment points for the end hooks, both of the rack and of the preparation holder. The distance between these hooks could be varied as appropriate to the size of the animal on both devices. The preparation holder is shown in Fig. 1.

Previous to the first incision the dorsal integument of the caterpillar was thinly smeared with silicone grease. This prevented saline, contained as a pool inside the preparation, from leaking into the air space below and blocking the spiracles. The animal was placed in the preparation holder, restrained at either end by the hooks mentioned above, and the edges of the cut dorsal integument were pinned out on either side on to black wax blocks, also coated with silicone grease. The gut was removed, from the proctodaeum to the stomodaeum, taking care to avoid any escape of its contents, and then likewise the paired silk glands. From this stage onwards the preparation was irrigated with the saline medium to be described. This was circulated and aerated with the usual type of air-lift circulation pump. The fat body and muscles lying over the regions on which experiments were to be performed were then carefully removed as necessary.

The dissection of the pupa was much simpler because the gut and silk glands degenerate. The dorsal integument was again smeared with silicone grease and opened by a median longitudinal incision. No end hooks were required, but otherwise the same procedure was followed as with the caterpillar.

Recording equipment and methods

The electrodes used to stimulate and to record from the nerves were of the hook type made from platinum-iridium wire, insulated except for the angles of the hooks with K–Na–Pb glass. These were well suited for recording from small fibres and for stimulation of nerves below the surface of the saline. Muscle action potentials were recorded intracellularly with KCl-filled glass micropipettes having impedances from 10–40 MΩ, led via a cathode follower to a Tektronix type 122 capacity-coupled preamplifier, and displayed on the screen of a Telequipment type D31 oscilloscope. Permanent records were made with a Cossor model 1428 mark II oscillograph camera. Since an a.c. preamplifier was used for simplicity, muscle resting potentials were determined from the sudden positive deflexion recorded when an electrode was removed from its intracellular location. This method is probably less accurate than the usual one but gave reasonably consistent results.

Action potentials were recorded from many different muscles but all were essentially similar, so measurements were made only on the ventral longitudinal muscles in the abdomen since these are the most accessible. They were stimulated by 0·1–0·5 msec. rectangular pulses at 4–12 V. delivered via an RF stimulus-isolating unit to the middle the three segmental nerves (here called nerve 2) which had been pinched proximally to sever connexions with the C.N.S.

The muscle receptors (MRO) were carefully exposed and then lifted up in forceps (Fig. 1). The apparatus used to move the forceps in a controlled manner will be described in another paper.

Analysis of haemolymph

Since Duchâteau *et al.* (1953) quote incomplete data only for the pupa of *A. pernyi* it was necessary to analyse the ionic composition of the haemolymph of all developmental stages used in the present investigation.

The concentrations of inorganic cations in the haemolymph of *A. pernyi* were analysed with a Unicam SP 900 flame spectrophotometer. Owing to possible inter-

ference by other elements present in haemolymph the procedure shown in Fig. 2 was followed in all analyses to check the quantitative recovery of ions added to haemolymph. Osmotic concentrations were determined using the apparatus of Ramsay & Brown (1955).

In a later experiment *A. pernyi* were not available so *A. mylitta* were used instead. Ca was analysed with a Coleman model 21 flame photometer and Mg by a colorimetric method (Taras, 1948) with a Zeiss PMQ II spectrophotometer.

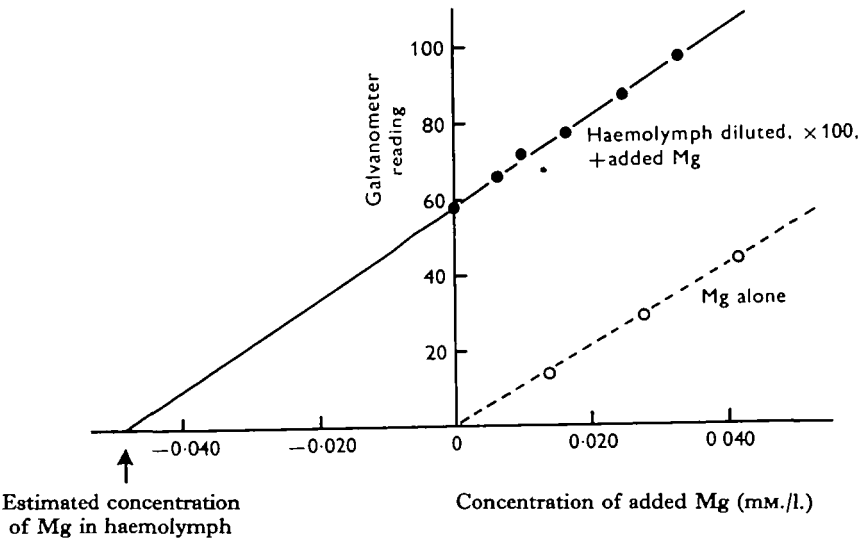

Fig. 2. The measured recovery of magnesium added to aliquots of caterpillar haemolymph. The negative intercept of the full line on the abscissa gives the corrected magnesium concentration in haemolymph.

RESULTS

The distortion of proprioceptive responses by a high-Na saline

Fig. 3 shows the importance of performing neurophysiological experiments on the MRO in the correct saline medium. The *Drosophila* saline of Ephrussi & Beadle (1936) (NaCl 128 mM./l., KCl 4·7 mM./l., $CaCl_2$ 1·9 mM./l.) greatly exaggerated both sensory and reflex responses. The caterpillar saline to be described closely matched haemolymph.

The composition of haemolymph

Table 1 shows the inorganic cation content and osmotic concentration of haemolymph from larvae and pupae. The compositions of the salines eventually used are included for comparison. The values found are similar to those reported by Duchâteau *et al.* (1953) for this species and also to the concentrations used by Wyatt (1956) in a saline for *Bombyx mori*. The differences between larvae and pupae are considerable.

These analyses do not in themselves constitute sufficient information for the preparation of a saline, since there remains for consideration the extent of ion activity or ion 'binding'. Two procedures were used in an attempt to resolve this question in relation to *Antheræa*. First a solution was made up which contained the major inorganic cations as analysed in larval haemolymph. Amino acids and protein were

then added in the concentrations found by Wyatt (1956) in *Bombyx* haemolymph. The second procedure was more empirical. Salines were made up based on the cation concentrations found in haemolymph but containing less of the divalent cations, since the ionic activities of these might be reduced by 'binding' or chelation.

All media were compared with haemolymph in their effects on the intracellularly recorded action potential. This criterion of adequacy was chosen because in *Carausius*

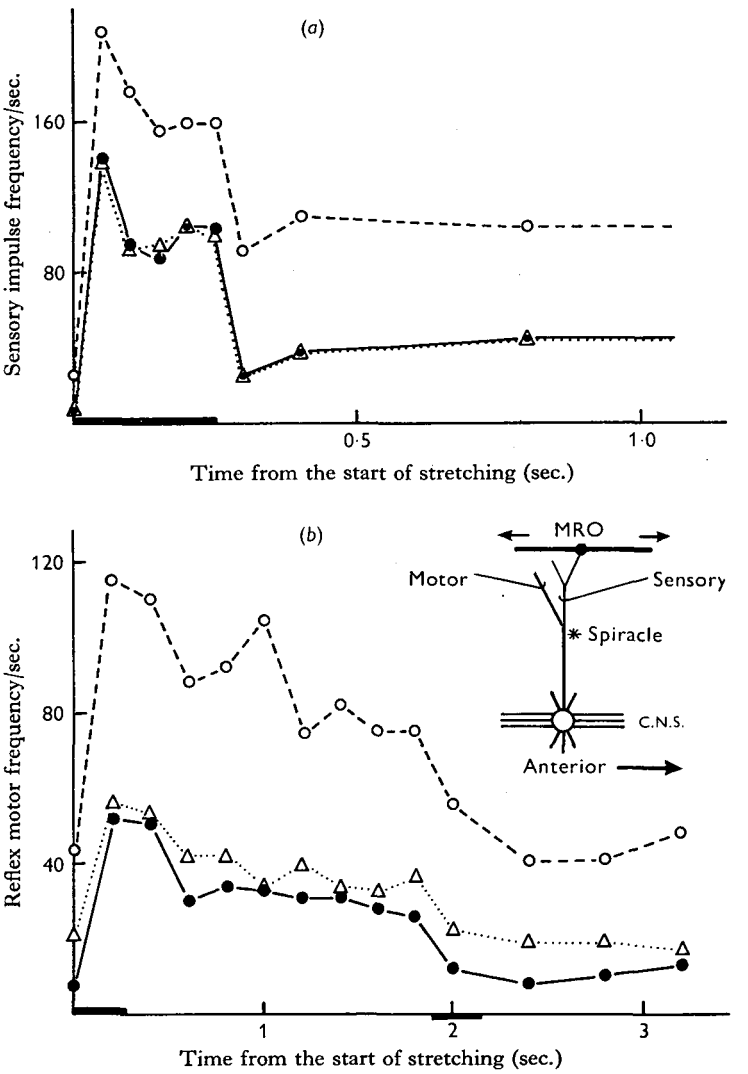

Fig. 3. (*a*) The discharge frequency of the same larval MRO plotted against time in three different media. ○, Ephrussi & Beadle's *Drosophila* saline; ●, caterpillar saline; △, haemolymph. (*b*) The reflex discharge evoked by the above stimuli, recorded simultaneously with the sensory discharge in the same three media (symbols as above). A heavy line above the abscissa indicates stretching of the MRO at 0·6 cm./sec. from 0·33 to 0·52 cm., and a heavy line below the abscissa indicates releasing at the same rate. Each curve is the mean of two tests. The time-scale of (*a*) is expanded relative to (*b*) in order to show the details of the phasic sensory response to stretching. The inset diagram shows the main branches of nerve 2 in the vicinity of the receptor and the location of the recording electrodes.

each of the major inorganic cations has a distinct and qualitatively different effect on the electrical parameters of muscular activity (Wood, 1957).

The effects of some organic constituents of haemolymph on the muscle action potential

The experiment shown in Table 2 was performed in an attempt to decide how far and to what components of haemolymph the divalent cations might be bound. It was found that the first partially synthetic procedure described above failed to produce a

Table 1. *The major inorganic cations and osmolarity of haemolymph from larvae and pupae compared with larval and pupal salines*

Developmental stage	Dissolved constituent	Mean concentration ± S.D. Haemolymph mM./l.	Saline mM./l.	Number of animals
Last instar	Sodium	11·8 ± 2·5	12	5
	Potassium	29·8 ± 2·7	30	
	Magnesium	48·6 ± 4·1	18	
	Calcium	6·7 ± 1·7	3	
	Osmolarity	280 ± 70	280	
Pupa	Sodium	3·6 ± 1·0	4	6
	Potassium	45·5 ± 3·5	40	
	Magnesium	27·1 ± 2·9	18	
	Calcium	7·3 ± 0·6	3	
	Osmolarity	394 ± 4·5	394	

Experimental media were made isotonic with glucose and buffered to pH 6·6 with 1·5 mM each of $NaHCO_3$ and NaH_2PO_4 in 4 ml. of solution, made up to 100 ml. of saline for each experiment. Inorganic cations were added as their chlorides in the required amounts from 300 mM. stock solutions kept at 0° C. Calcium was added last after dilution to 95 ml. to avoid precipitation of the phosphate.

Table 2. *The effect of some organic constituents of haemolymph on the muscle action potential in a last instar larva*

Medium	Junction potential (mV.) Mean ± S.D.		Number of fibres	Active membrane response (mV.) Mean ± S.D.	
(1) Haemolymph	40·5	1·3	4	17·5	0·8
(2) 'Heat-treated' haemolymph*	40·8	2·0	4	16·5	1·1
(3) Cations as in haemolymph + amino acids and bovine serum albumen	28·0	—	2	26·0	—
(4) Cations as in haemolymph without added amino acids or protein	30·0	—	2	26·0	—
(5) Basic saline	42·2	2·3	6	18·6	2·1

* Haemolymph was heated at 100° C. for 10 min. The protein precipitate was then spun down hard, the supernatant solution being 'heat-treated' haemolymph.

medium with properties similar to haemolymph. Both the medium with cations as in haemolymph and the medium with added amino acids and protein exaggerated the active membrane response and reduced the junction potential compared with the

normal. By contrast caterpillar saline containing reduced divalent cation concentrations (see Table 1) matched haemolymph quite well. Thus the present bioassay techniques give indirect evidence for a reduction in the ionic activities of the divalent cations in haemolymph. This does not appear to be due to the presence of amino acids; bovine serum albumen likewise cannot be used to render the saline more similar to haemolymph.

If haemolymph is heated to 100° C. and the coagulum removed by centrifugation it is found that the supernatant fluid has an effect on the muscle action potential very similar to that of haemolymph (Table 2). Two alternative conclusions may be drawn from this observation: either protein and the heat-labile constituents of haemolymph are not the components responsbile for reducing the ionic activities of the divalent cations; or else a substantial proportion of the total Ca and Mg must be removed after heat-treatment, 'bound' to the protein coagulum. Analysis of the divalent cations in haemolymph before and after heat-treatment should distinguish between these alternative possibilities. Unfortunately a different species of *Antheræa* had to be used for this experiment so the data are only useful qualitatively. Table 3 shows the results of this experiment.

Table 3. *Divalent cations in heat-treated haemolymph from larvae of* Antheræa mylitta *as compared with untreated haemolymph*

	Magnesium mM./l. ± S.D.		Calcium mM./l. ± S.D.	
Untreated haemolymph	63·2	7·0	2·4	0·7
Heat-treated haemolymph	45·3	10·3	2·1	0·8

Samples of haemolymph from three caterpillars were divided into two parts one of which was analysed directly and the other after heat treatment as described previously. Two analyses of magnesium and two of calcium were performed on each of these solutions.

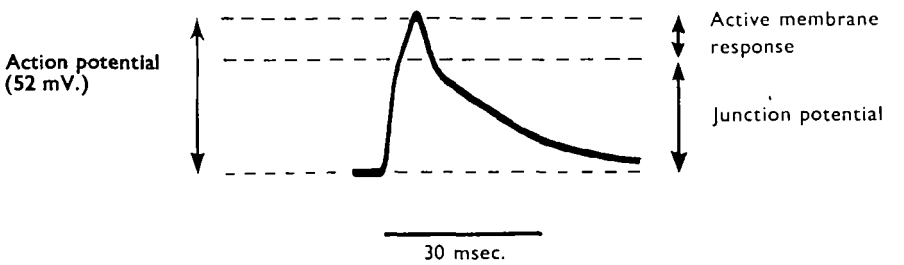

Fig. 4. Tracing of a caterpillar muscle action potential recorded in haemolymph with an intracellular glass micropipette; labelled to show the subdivisions used in Table 2 and in Figs. 5 and 6.

The concentration of Ca in the haemolymph of *A. mylitta* is lower than in the other species. The observation that the concentration of Ca in heat-treated haemolymph was little different from that in the haemolymph itself (Table 3) is therefore of dubious significance in relation to *A. pernyi*. The results of the Mg analyses are of more interest. Although a different method of analysis was used the concentration of this ion was still found to be abnormally high in comparison with other insects, comparable in fact with the value found in *A. pernyi*. Thus it is probably significant that although the

concentration of Mg is less in heat-treated haemolymph it is only reduced by one-quarter. Table 2 suggests that in the haemolymph of *A. pernyi* the ionic activity is as low as two-fifths of the total concentration of Mg. Thus although some of this ion may be bound to proteins, some other heat-stable component of haemolymph is probably as important or more important in lowering the ionic activity of Mg.

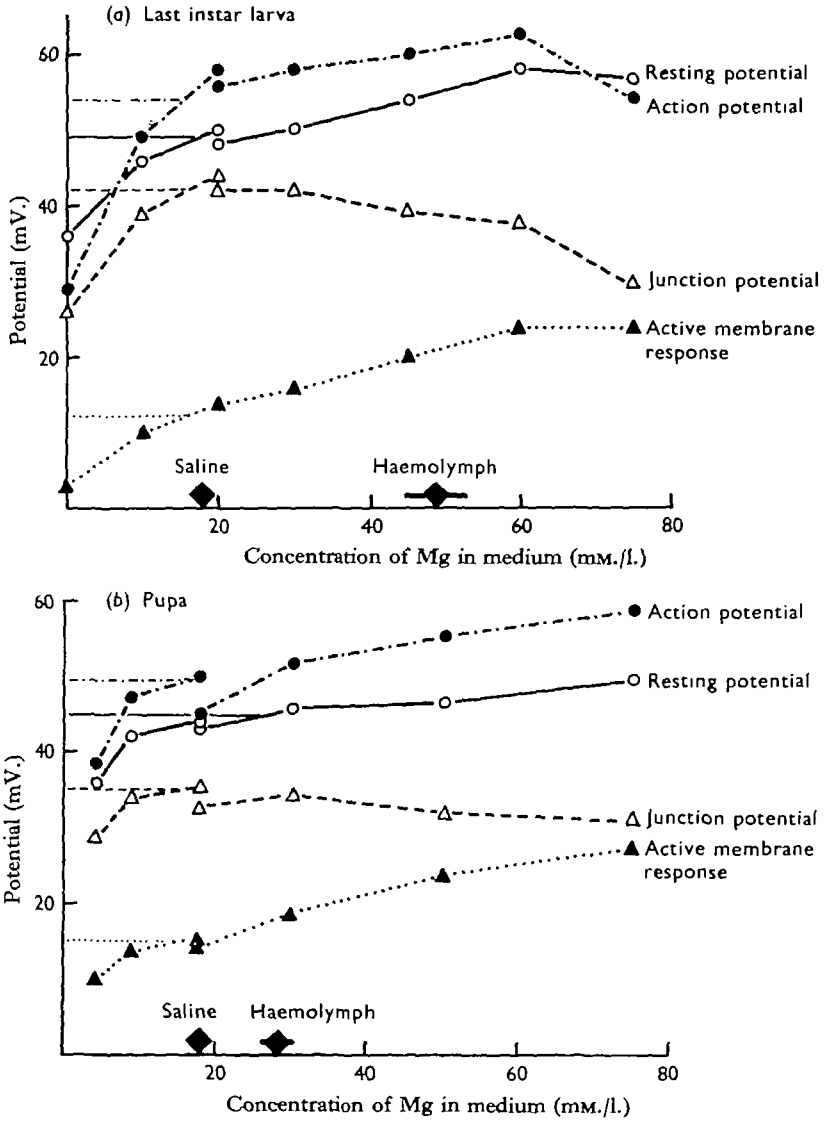

Fig. 5. The resting potential and three parameters of the action potential in the ventral longitudinal muscles of *Antheræa pernyi*, plotted against the concentration of Mg in the experimental medium. The concentration of Mg in haemolymph and in the prepared salines are shown above the abscissa in both (*a*) and (*b*). In (*a*) a single record was taken at each Mg concentration; (*b*) shows the averaged results from three different muscle fibres. The first records were made in haemolymph followed by saline. Then the low-Mg media were tested followed by a check in basic saline before proceeding to the high-Mg media. The muscles were bathed in each medium for 30 min. before taking a reading. All media were made isotonic with haemolymph by adding the appropriate amount of glucose.

Bioassay of divalent cations

Selecting the best salines was a process of trial and error which would be difficult and rather tedious to report satisfactorily. Figs. 5–8 show the effects of deviation from the optimum saline on the parameters of muscular and nervous activity used as criteria of the adequacy of the media tested.

(1) *The muscle action potential*

Fig. 4 is a tracing of a caterpillar muscle action potential. It shows the subdivisions of the action potential recognized by Hoyle (1955), which are plotted in Figs. 5 and 6.

In view of the results shown in Tables 2 and 3 the biggest problem in devising a

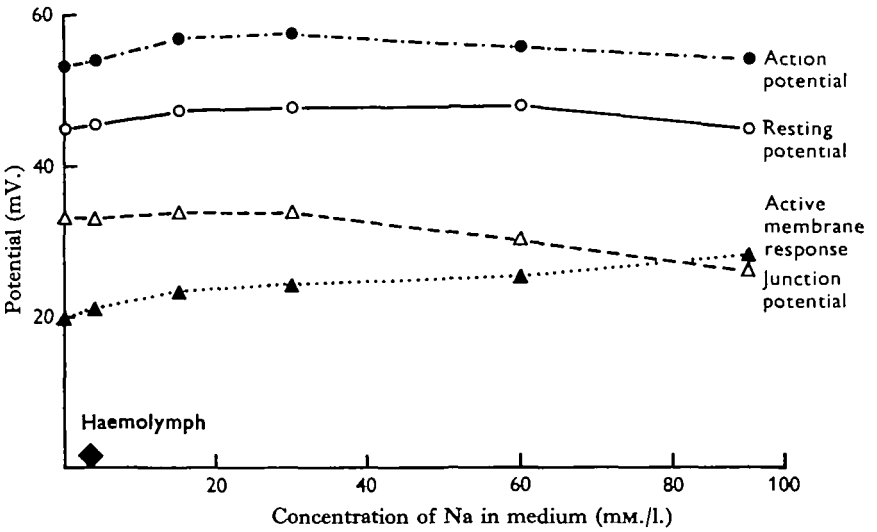

Fig. 6. The resting and action potentials in the ventral longitudinal muscles of the pupa, plotted against the concentration of Na in the experimental medium. Results were obtained in the same way as those of Fig. 5, except that the muscles were first immersed for 50 min. in a medium lacking Na, then in basic saline followed by increasing concentrations of Na. The concentration of Na in haemolymph is shown above the abscissa.

lepidopteran saline seems to be how far to reduce the Mg concentration. Thus Fig. 5 shows the effects of Mg on larval and pupal muscles; the size of the active membrane response in particular is closely related to Mg concentration, and the size of the action potential is much reduced in the absence of Mg.

It is of interest that when the concentration of Na in the medium is varied the effects on the muscle action potential are qualitatively rather similar to those of Mg. Thus high concentrations of Na exaggerated the active membrane response. Provided that 20 mM./l. of Mg is present the action potential is not greatly reduced by prolonged immersion in Na-free media. If Mg is low or absent, Na must be high (greater than 90 mM./l.) for the preparation to survive. This probably explains why high-Na media have mostly been used previously.

(2) *The response of the MRO to stretch*

In Fig. 3 it was shown that the response of the MRO to a standard stretch is exaggerated in *Drosophila* saline. This effect is largely due to the high concentration of Na but also in part to the absence of Mg. Fig. 7 shows the effects of changing the

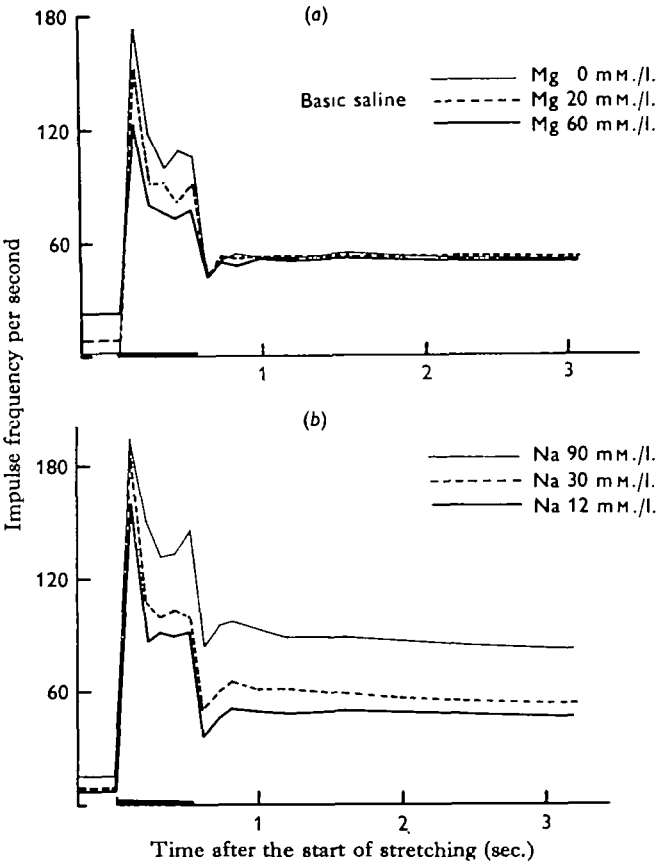

Fig. 7. The discharge frequency of the larval MRO plotted against time in media containing varying amounts of (*a*) Mg and (*b*) Na. The stimulus was the same in each case: stretching from 0·30 to 0·49 cm. at 0·35 cm./sec. The period of stretching is shown by a heavy line above the abscissa.

concentrations of these two cations individually in a saline based on haemolymph. Low Mg diminishes the phasic response, elevates the unstretched discharge frequency and leaves the tonic discharge unchanged. High Na elevates the discharge frequency during all phases of the stimulus about equally.

The responses of the MRO suggest that the maximum Ca concentration which should be used in the saline is about 4 mM./l. (The minimum concentration of about 2 mM./l. is better gauged from the effects on the muscle action potential.) Fig. 8 shows that when the receptor is released there is normally a post-excitatory pause of about 0·8 sec. But in media containing more than 4 mM./l. Ca this pause is shorter, and the discharge frequency takes some time to fall to the unstretched value.

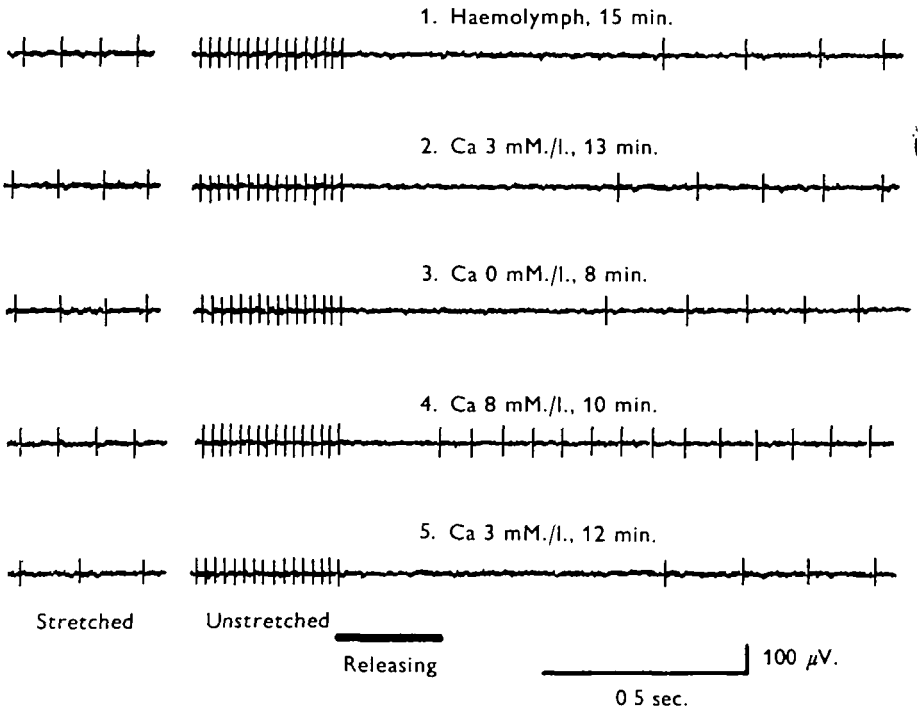

Fig. 8. Tracings from five consecutive oscillograph records. In this experiment the sensory discharge from a larval MRO was recorded in media containing different amounts of Ca. The duration of immersion in each medium before testing is shown above each record. The discharge is shown at a length of 0·30 cm., at the end of a 2 sec. period of maintained stretch to 0·49 cm. and during and following releasing. The heavy line on the figure shows the time of releasing.

DISCUSSION

Exaggeration of reflexes in high-Na media

Fig. 3 showed that the reflex resulting from stretch of the MRO is greatly influenced by the ionic content of the experimental medium. This reflex will be described in detail in a subsequent communication; it is clearly important for an understanding of its function that it should be studied under conditions which are as nearly normal as possible. For instance it might be that muscles which did not usually respond to MRO excitation would exhibit a stretch reflex when tested in a high Na saline. The exaggerated reflex in Ephrussi & Beadle's (1936) saline probably results largely from the supernormal sensory input, though careful testing suggested that over longer periods even the C.N.S. is not immune to changes in the ionic composition of the medium (cf. Treherne, 1965).

Abnormal Mg concentrations also affect the sensory input, but in a complex manner which is rather difficult to explain. Perhaps this is the result of independent actions by this ion on neural processes and on mechanical properties of the receptor (such as might result from effects on the state of hydration of proteins forming the receptor strand).

Ion 'binding'

Carrington & Tenney (1959) concluded from dialysis studies on the haemolymph of *Telea polyphemus* that no K, about 10% Ca and about 20% Mg were held to organic particles which did not pass through their dialysis membranes. The present observations suggest that nearer to 60% of the Mg and Ca are somehow excluded from acting on muscles and sense organs. It may be significant that in this investigation the experiment of Table 3 gave a value of 30% Mg bound to protein, quite close to the value reported by Carrington & Tenney. Of course it is improbable that the degree of cation binding by protein is the same before and after denaturation. The ionic activity of the divalent cations in haemolymph would repay investigation by the technique of Walser (1960) for measuring 'free' and total Mg and Ca.

In the present study it has been assumed that the activities of Na and K in haemolymph are not reduced by binding. However, it was found necessary to reduce the concentration of K in pupal saline to 40 mM./l., as higher concentrations rendered the afferent discharge from the MRO very irregular.

Mg and the muscle action potential

It was shown in Fig. 5 that a lepidopteran saline must contain 20 mM./l. of Mg for normal muscle function. In higher concentrations the size of the action potential, particularly the active membrane response, is exaggerated. In *Carausius* the size of the muscle action potential is similarly related to the concentration of Mg, and this led Wood (1957) to propose that at least a proportion of the action current might be carried by this ion. The same could be true of lepidopteran muscle, for the qualitative similarity between the effects of Na and Mg on the action potential is quite striking. There is evidence that in crustacean muscles also, ions other than Na may contribute to the action current (Fatt & Katz, 1953; Fatt & Ginsborg, 1958). The results of Treherne (1965) suggest that the action current during nerve impulses in the C.N.S. of *Carausius* may also be partly carried by Mg.

SUMMARY

1. The inorganic cations in the haemolymph of *Antheræa pernyi* larvae and pupae were analysed by flame photometry.

2. Synthetic media based on these analyses were compared with haemolymph in their effects on the muscle action potential and on the response of the dorsal muscle receptors to a standard stretch.

3. The best match with haemolymph was obtained in all cases with concentrations of divalent cations considerably below the values found by flame photometric analysis. Binding to haemolymph proteins is probably not the major factor responsible for lowering the ionic activities of calcium and magnesium.

4. The role of magnesium in neuromuscular transmission is discussed. It is concluded that in phytophagous Lepidoptera this ion may carry a proportion of the muscle action current.

I am grateful to my supervisor, Prof. G. M. Hughes, for guidance and encouragement during this work, and to the D.S.I.R. for financial support in the form of a research studentship.

REFERENCES

BELTON, P. (1958). Membrane potentials recorded from moth muscle fibres. *J. Physiol.* **142**, 20–21 P.

BONÉ, G. J. (1944). La rapport sodium/potassium dans le liquid coelomique des insects. *Ann. Soc. Zool. Belg.* **75**, 123–32.

CARRINGTON, C. B. & TENNEY, S. M. (1959). Chemical constituents of haemolymph and tissue in *Telea polyphemus* Cram., with particular reference to the question of ion binding. *J. Insect Physiol.* **3**, 402–13.

DUCHÂTEAU, G., FLORKIN, M. & LECLERQ, J. (1953). Concentration des bases fixes, et types de composition de la base totale de l'hémolymphe des insects. *Arch. int. Physiol.* **61**, 518–49.

EPHRUSSI, B. & BEADLE, G. W. (1936). A technique of transplantation for *Drosophila*. *Amer. Nat.* **70**, 218–25.

FATT, P. & GINSBORG, B. L. (1958). The ionic requirements for the production of action potentials in crustacean muscle fibres. *J. Physiol.* **142**, 516–43.

FATT, P. & KATZ, B. (1953). The electrical properties of crustacean muscle fibres. *J. Physiol.* **120**, 171–204.

FINLAYSON, L. H. & LOWENSTEIN, O. (1958). The structure and function of abdominal stretch receptors in insects. *Proc. roy. Soc.* B, **148**, 433–49.

HOYLE, G. (1955). The effects of some common cations on neuromuscular transmission in insects. *J. Physiol.* **127**, 90–103.

VAN DER KLOOT, W. G. (1958). The control of neurosecretion and diapause by physiological changes in the brain of the cecropia silkworm. *Biol. Bull., Woods Hole*, **109**, 276–94.

VAN DER KLOOT, W. G. (1963). The electrophysiology and the nervous control of the spiracular muscle of pupae of the giant silkmoths. *Comp. Biochem. Physiol.* **9**, 317–34.

LOCKWOOD, A. P. M. (1961). 'Ringer' solutions and some notes on the physiological basis of their ionic composition. *Comp. Biochem. Physiol.* **2**, 241–89.

RALPH, C. L. (1960). Modification of activity rhythm of *Periplaneta americana* (L), induced by nitrogen and carbon dioxide. *Physiol. Zool.* **32**, 57–62.

RAMSAY, J. A. & BROWN, R. H. J. (1955). Simplified apparatus and procedure for freezing-point determinations upon small volumes of fluid. *J. sci. Instrum.* **32**, 372–5.

SUTCLIFFE, D. W. (1963). The chemical composition of haemolymph in insects and some other arthropods, in relation to their phylogeny. *Comp. Biochem. Physiol.* **9**, 121–35.

TARAS, M. (1948). Photometric determination of magnesium in water with brilliant yellow. *Anal. Chem.* **32**, 1156–8.

TREHERNE, J. E. (1965). Some preliminary observations on the effects of cations on conduction processes in the abdominal nerve cord of the stick insect. *Carausius morosus*. *J. exp. Biol.* **42**, 1–6.

WALSER, M. (1960). Determination of free magnesium ions in body fluids. Improved methods for free calcium ions, total calcium, and total magnesium. *Anal. Chem.* **32**, 711–17.

WOOD, D. W. (1957). The effect of ions upon neuromuscular transmission in a herbivorous insect. *J. Physiol.* **138**, 119–39.

WYATT, S. S. (1956). Culture *in vitro* of tissue from the silkworm, *Bombyx mori*, L. *J. gen. Physiol.* **39**, 841–52.