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MICROELECTRODE STUDY OF THE RESTING AND ACTION POTENTIALS OF THE COCKROACH GIANT AXON WITH SPECIAL REFERENCE TO THE RÔLE PLAYED BY THE NERVE SHEATH

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INTRODUCTION

Until recently the main contribution to the study of the insect nerve membrane has been made by the use of intracellular microelectrodes. Boistel & Coraboeuf (1954) were the first to adopt this technique to record resting and action potentials of the cockroach giant axon and were followed 3 years later by Yamasaki & Narahashi (1957). Fine-tipped micro-electrodes inserted into giant axons of *Periplaneta americana* have subsequently been extensively used for the study of electrical and ionic properties of insect nerve (Boistel & Coraboeuf, 1957; Boistel, 1959; Yamasaki & Narahashi, 1959*a*, *b*; Boistel, 1960; and others). More recently, the same technique has also been employed for the study of the electrical activity of cockroach giant axons induced either by electrical stimulation of cercal nerves (Pichon & Boistel, 1965*b*), by electrical polarization of the last abdominal ganglion (Pichon & Boistel, 1965*a*, 1966*a*), or by the application, at the level of this ganglion, of high potassium salines (Pichon & Boistel, 1965*a*, *c*). A similar technique has also been adopted by Callec & Boistel (1965*a*, *b*, 1966*a*, *b*, 1967) for the electrophysiological study of the sixth abdominal ganglion of the cockroach.

In all these experiments easy insertion of the microelectrode was achieved by the prior removal of the tough fibrous and cellular nerve sheath. Thus, although numerous results concerning electrical properties of insect axons *in vitro* have been reported, there was until recently very little information concerning *in vivo* membrane potentials. We have recently shown (Pichon & Boistel, 1966*b*) that the use of very fine-tipped and mechanically resistant microelectrodes made possible reliable recordings of intracellular potentials in sheathed nerve cords of cockroaches. A similar technique has been used by Treherne & Maddrell (1967*a*) with the nerve fibres of *Carausius*. The results of the latter investigation showed that, whereas action potentials obtained in intact conditions were very similar to those previously recorded in desheathed nerve cords, the potential difference between the inside of a resting axon and physiological saline (62·0 ± 1·4 mV.) was 15 mV. lower than the resting membrane potential recorded in the same saline for desheathed nerve cords (77 ± 0·7 mV., Narahashi & Yamasaki, 1960). This difference was first attributed to the existence of a potential across the nerve sheath, the inside being positive with respect to outside, for such a potential sometimes has been recorded when the microelectrode was removed from the preparation. Similar potentials have also been recorded by Maddrell & Treherne (1966)

across the fat-body sheath of *Carausius*. The above explanation was however not the only possible one; Treherne (1962*a*, *b*) has shown, for example, that the ionic composition of the extracellular fluid surrounding the nerve cells of *Periplaneta* differs from that of the haemolymph, a Donnan equilibrium maintaining an excess of inorganic cations in the extracellular fluid. In sheathed nerve cords this high concentration of cations might result in some modifications of the electrical behaviour of the giant axons. So, according to the curves given by Yamasaki & Narahashi (1959*a*), the resting potential would be reduced to approximately 54 mV. (Treherne, 1962*b*). Moreover, some modifications of the shape and the height of the action potential were also to be expected. It is clear, therefore, that further investigations are needed in this direction.

The aim of the present experiments was to determine more accurately these modifications of the membrane potentials in cockroach nerve cords studied *in vitro*. A subsequent paper will describe experiments on intact nerve cords bathed in the insect's own haemolymph.

METHODS

In this study membrane potentials were recorded under various experimental conditions. Male adult cockroaches (*Periplaneta americana*) reared at room temperature were used.

Recording apparatus. Microelectrodes were drawn from Pyrex tubing (1·5 mm. external diameter) and filled with 3 M-KCl. They had a resistance ranging from 15 to 25 MΩ. Since good mechanical strength of the tip is required for proper impalement of the giant fibres through the nerve sheath, only fresh-filled microelectrodes were used. Experiments have shown, indeed, that, whereas tip diameter does not change when the microelectrode is kept for many days in KCl solution (Boisseau & Boistel, 1965), mechanical strength greatly decreases with time.

The microelectrode was connected to the input of a high inpedance and low grid-current amplifier (Bioelectric Instruments) by means of an agar–Ringer bridge and an Ag–AgCl wire. The reference electrode consisted of an Ag–AgCl wire embedded in agar–Ringer and was immersed in the bathing fluid. The output potential of the amplifier was recorded on one beam of an oscilloscope (Tektronix type 502 A) and by an ink-writer (Beckman Instruments). This potential was differentiated in a passive RC network with a time constant of about 1·4 μsec. and recorded on the other beam of the oscilloscope.

Solutions. The ionic composition of the normal and modified salines are given in Table 1. The same Ringer solution as that one praised by Yamasaki & Narahashi (1959*a*) has been used as normal saline throughout our experiments. The concentrations of K^+ and Ca^{2+} ions in the 'haemolymph-like' saline (H) and the 'extracellular-like' saline (E) were those used by Treherne (1962*c*) and corresponded respectively to his 'basic saline 1 A' and 'extracellular saline 1 B'. Na^+ concentrations were based upon a Na^+ concentration of 214 mM/l. in the normal saline. Increase of K^+ and Ca^{2+} concentrations in 'H' saline were compensated for by a corresponding decrease of Na^+ concentration. For the 'E' saline, the sodium concentration Na_E was calculated from the sodium concentration Na_H in 'H' saline and by taking the ratio $Na_E/Na_H = 1{\cdot}8$, this value corresponding to the mean value of the ratio of the extracellular sodium concentration to the external sodium concentration (Treherne, 1962*b*).

Table 1. *Ionic composition of solutions* (mM./l.)

	NaCl	KCl	$CaCl_2$	NaH_2PO_4	Na_2HPO_4	$NaHCO_3$
Normal saline	210·2	3·1	1·8	0·2	1·8	—
'H' saline	198 3	12·3	4·5	0·2	1·8	—
'E' saline	358 2	17·1	17·6	0·1	—	2·1

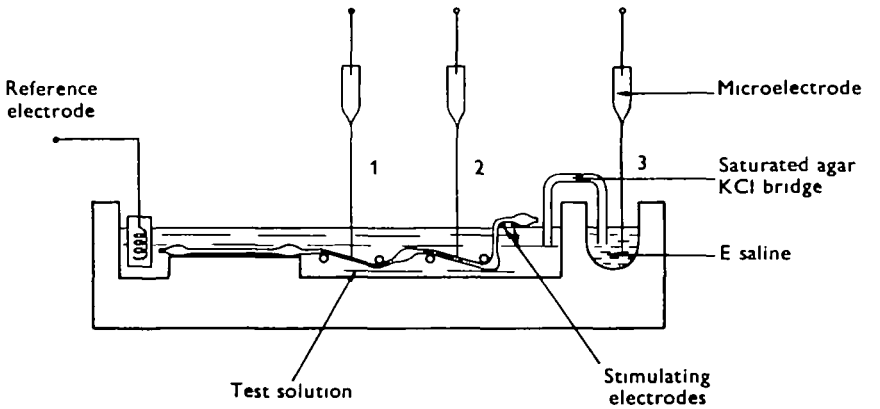

Fig. 1. Schematic diagram of the experimental arrangement. The microelectrode is shown in three different positions: position, 1, impalement of the desheathed part of the nerve cord (between 4th and 5th ganglia); position 2, impalement of a sheathed part of the nerve cord (between 3rd and 4th ganglia); position 3, measurement of the microelectrode tip potential in 'E' saline. The nerve cord was stimulated electrically between 2nd and 3rd ganglia by means of a pair of Ag–AgCl electrodes.

Experimental procedure. The abdominal nerve cord, including the five last abdominal ganglia, was removed from the animal. In some experiments it was desheathed between the 4th and the 5th ganglia by means of fine needles; in others, the nerve sheath was kept intact. The preparation was then mounted in the nerve chamber shown in Fig. 1. Antidromic electrical stimulation was applied by means of a pair of Ag–AgCl electrodes located in the right part of the chamber and at the surface of the saline. Microelectrodes were inserted either into the desheathed region (position 1 of the electrode), or into a sheathed region (position 2 of the electrode). Electrode tip potentials were measured, when needed, as the difference between the potentials recorded with the microelectrode tip dipping on one hand in the test solution and on the other hand in the 'E' saline (position 3 of the microelectrode). If the saturated KCl bridge is assumed to abolish junction potentials, this should give correct measurements of the tip potential between the test saline and 'E' salines. Measurements of the membrane potentials of the giant axons were made after an equilibration time of more than 30 min. in the test solution. DC potentials were recorded on the ink-writer. Measurements of resting potential were made 2–3 min. after the impalement. Measurements of 'sheath potential' were made when the microelectrode was slowly withdrawn from the nerve cord.

Experiments were carried out at room temperature ranging from 19 to 27° C. Temperature was never changed by more than 1° C. during the course of one experiment.

RESULTS

(1) *Introduction of a microelectrode into a sheathed nerve cord*

The nerve cord was mounted in the nerve chamber and slightly stretched. The microelectrode was then advanced until its tip caused a small depression in the connective. A small vibration of the holder led to a rapid penetration of the microelectrode through the sheath into an underlying giant axon. Under these conditions a negative potential was recorded and adequate electrical stimulation gave rise to a large action potential (Fig. 2). If the microelectrode was kept in this position no modification of the resting or action potential was noticed.

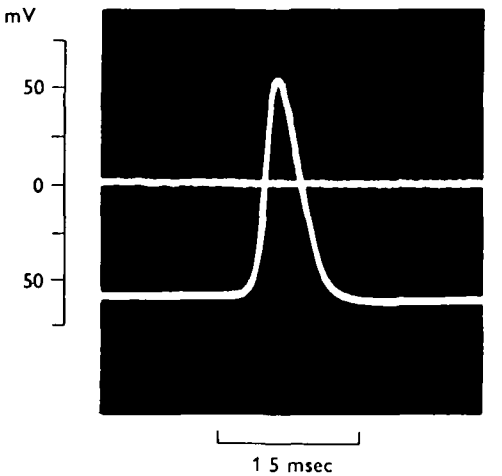

Fig. 2. Superimposed tracings of the potential at the tip of the microelectrode before and after impalement of a giant axon in a sheathed nerve cord. An action potential was elicited by electrical stimulation of the nerve cord. Notice that a low resting potential (55 mV.) was associated with a large action potential (110 mV.). 19° C. Normal saline.

When the microelectrode was removed from the preparation the potential returned to its original zero level. However, when the movement was carried out slowly enough, it was possible, in most cases, to record two successive changes of the microelectrode tip potential: a positive deflexion (70–75 mV.) followed by a rapid negative deflexion (10–15 mV.) to the original zero potential (Fig. 3). It was agreed to call 'sheath potential' this last negative deflexion and resting potential the potential difference between inside of a giant axon and external solution. The meaning of this 'sheath potential' will be discussed in further detail in the last section of this paper.

A schematic drawing of the DC potential changes associated with vertical movements of the microelectrode tip together with photographic recordings of the electrical events following electrical stimulation of the preparation are shown in Fig. 4.

(2) *Membrane potentials in sheathed and desheathed nerve cords bathed in normal saline*

The magnitude of the action potential, resting potential, 'sheath potential', overshoot, and the rates of rise and fall of the action potential of cockroach giant axons have been measured in intact and in desheathed nerve cords bathed in normal saline

(Table 2). Typical tracings of action potentials and their derivatives are given in Fig. 5 (A and B). It is clearly seen that, whereas action potentials were higher in sheathed nerve cords (103·0 ± 5·9 mV.) than in desheathed preparations (85·9 ± 4·6 mV.), resting potentials were lower in sheathed preparations (58·1 ± 5·4 mV.) than in desheathed ones (67·4 ± 6·2 mV.); thus, the mean value of the overshoot was of 44·3 ± 5·8 mV. in the first case instead of 18·5 ± 7·1 mV. in the last case. This very important difference

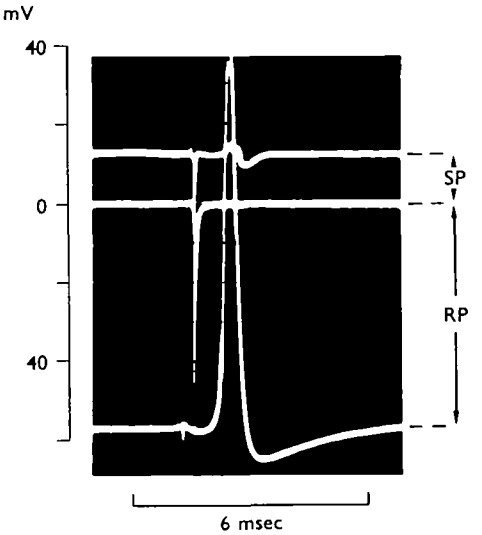

Fig. 3. Superimposed tracings showing changes in the tip potential of the microelectrode as it was withdrawn from a giant axon in a sheathed nerve cord. Before being withdrawn, the microelectrode was inside a giant axon and a full-sized action potential (110 mV.) was elicited by electrical stimulation (lower tracing). After the microelectrode has been partly withdrawn, a positive deflexion was recorded (upper tracing). After complete withdrawal of the microelectrode its tip potential again reached the initial zero potential (middle tracing). The intracellular action potential is truncated. 19° C. Normal saline.

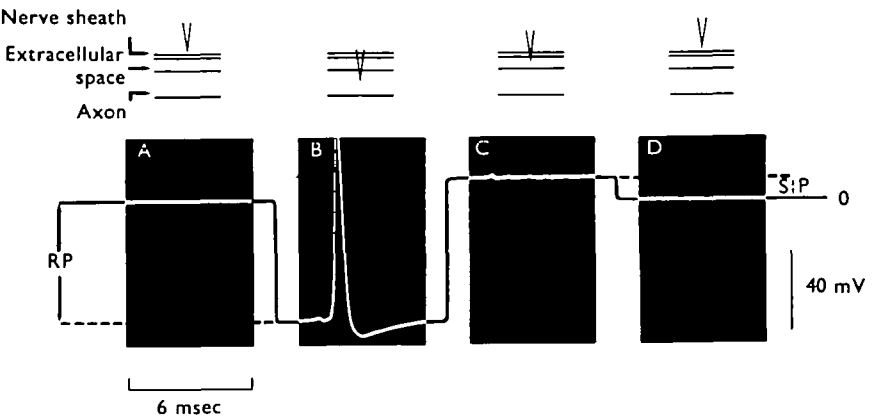

Fig. 4. Potential changes associated with vertical movements of the microelectrode. The solid line represents the DC potential variations of the microelectrode tip. Photographic recordings show electrical events following electrical stimulation of the preparation for postulated positions of the microelectrode tip (upper drawings). The intracellular action potential is truncated. 19° C. Normal saline.

in the overshoots (25·8 mV.) may be attributed to the 'sheath potential' only with difficulty, the mean recorded value of this potential being only of 6·7 mV. It is not quite impossible, however, that the true value of this 'sheath potential' has been higher than the recorded one. Maximum rates of rise and rates of fall of the action potential were slightly higher in sheathed nerve cords (928·1 ± 122·0 and 321·9 ± 38·0 V./sec.) than in desheathed ones (804·7 ± 128·9 and 320·3 ± 49·4 V./sec.) but no significant change in the shape of the action potential has been noted.

Table 2. *Membrane potentials of cockroach giant axons in normal saline*

Temp. (° C.)	Serial no.	Action potential (mV.)	Resting potential (mV.)	'Sheath' potential (mV.)	Over-shoot (mV.)	Maximum rate of Rise (V./sec.)	Maximum rate of Fall (V./sec.)
			(A) Nerve sheath removed				
26	1	84	60	—	24	775	350
26	2	90	62	—	28	1000	400
26	3	90	67	—	23	950	400
26	4	80	69	—	11	800	350
26	5	85	68	—	17	800	350
26	6	80	70	—	10	700	275
26	7	83	67	—	16	850	350
26	8	90	74	—	16	960	350
26	9	96	76	—	20	1025	300
26	10	88	67	—	21	850	275
26	11	82	74	—	8	800	300
27	12	87	77		10	750	350
25	13	90	62	10	28	750	300
25	14	80	68	7	12	600	275
25	15	84	55	—	29	625	275
25	16	85	62	—	23	650	225
Mean:		85·9	67·4	—	18 5	804·7	320·3
			(B) Intact nerve sheath				
26	1	110	—	—	—	1050	400
26	2	105	60	—	45	1000	350
22	3	105	62	—	43	1000	350
22	4	95	57	10	38	—	—
22	5	95	50	9	45	700	300
22	6	95	57	8	38	—	—
22	7	107	72	2	35	1025	350
19	8	110	55	0	55	850	300
19	9	105	62	5	43	1000	300
19	10	105	57	5	48	900	300
19	11	100	56	2	44	—	—
19	12	95	53	9	42	—	—
19	13	110	57	11	53	—	—
19	14	105	57	13	48	—	—
Mean:		103 0	58·1	6·7	44·3	928·1	321·9

(3) *Effects of 'H' saline on membrane potentials*

Previous results having been obtained with a low potassium saline, it was therefore of interest to test the effects of a modified saline containing higher concentrations of K^+. This has been done using 'H' saline, the ionic composition of which is close to that of haemolymph for K^+ and Ca^{2+}.

The differences between sheathed and desheathed nerve cords were more pronounced than in preceding experiments (Table 3, Fig. 5C, D). Whereas resting potentials were only slightly higher in desheathed preparations (57·3 ± 5·3 mV. instead of 55·6 ± 4·2 mV.), action potentials were three times smaller in desheathed preparations (36·5 ± 7·6 mV.) than in sheathed ones (107·9 ± 6·0 mV.); in the first case there was an undershoot of 20·7 ± 8·4 mV., whereas in the second there was an overshoot of 52·2 ± 7·1 mV. It should be noted that the action potentials recorded in desheathed preparations were often in two phases (Fig. 5C). The mean values for the maximum rate of rise were 244·2 ± 106·4 V./sec. in desheathed nerve cords for 1071·6 ± 159·5 V./sec. in sheathed ones; the maximum rates of fall were 100·0 ± 3·6 V./sec. in the first case and 398·7 ± 44·0 V./sec. in the second. The mean value of the 'sheath potential' was 11·5 ± 8·2 mV.

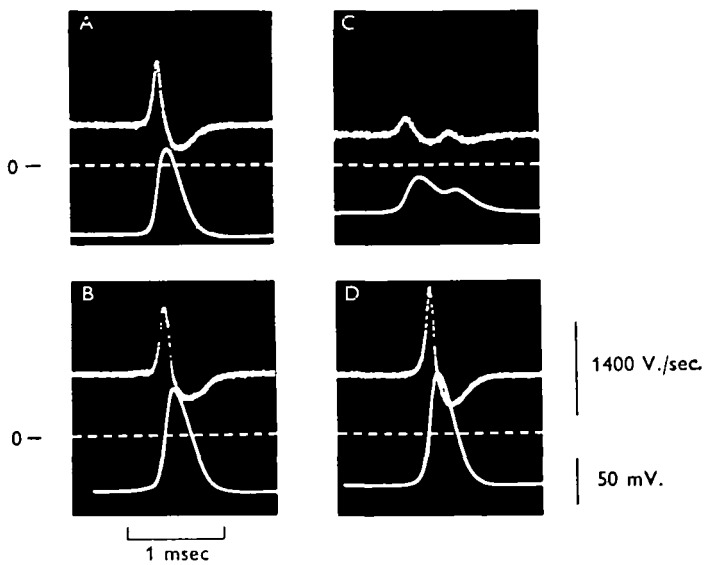

Fig. 5. Effects of changing ionic composition of the external solution on the action potential (lower tracings) and its derivative (upper tracings) of sheathed (B and D) and desheathed (A and C) nerve cords. A and B, normal saline; C and D, 'H' saline. Interrupted line indicates zero potential. A, B and D: 26° C.; C: 27° C.

(4) *Effects of desheathing procedure upon electrical properties of giant axons*

The preceding results raised the question of the extent to which local desheathing of the nerve cord altered the electrical properties of the giant axons along their whole length. Experiments were performed in which measurements of membrane potential were made in sheathed and desheathed regions of the same nerve cords. Similar differences to those mentioned above were observed, despite the fact that recordings in the sheathed part were made more than 2 hr. after the desheathing had been carried out (Fig. 6, Table 4): in the sheathed portion the action potentials were 62 mV. higher and the resting potentials 18·5 mV. lower than in desheathed portions of the nerve cord. It will therefore be seen that, whereas a solution having the same ionic composition as the haemolymph in respect of K^+ and Ca^{2+} produced a drastic decrease in the

magnitude of the action potential in desheathed preparations, removal of the nerve sheath led only to local changes, thus demonstrating that diffusion processes along the extracellular spaces were very slow.

Table 3. *Membrane potentials of cockroach giant axons in 'H' saline*

Temp. (° C.)	Serial no.	Action potential (mV.)	Resting potential (mV.)	'Sheath potential' (mV.)	Overshoot (mV.)	Maximum rate of Rise (V./sec.)	Maximum rate of Fall (V./sec.)
			(A) Nerve sheath removed				
27	1	35	50	—	−15	530	125
27	2	30	58	15	−28	—	—
27	3	30	62	7	−32	200	50
27	4	34	59	—	−25	225	125
27	5	30	56	—	−26	200	50
27	6	35	55	—	−20	350	100
27	7	35	55	—	−20	150	50
27	8	58	67	—	−9	230	150
27	9	30	61	—	−31	150	100
27	10	40	64	—	−24	200	100
27	11	30	62	—	−32	—	—
27	12	42	51		−9	300	100
27	13	40	56	—	−16	275	100
27	14	44	53	—	−9	300	150
27	15	35	50	—	−15	—	—
Mean:		36·5	57 3	—	−20·7	244·2	100
			(B) Intact nerve sheath				
25	1	115	50	26	65	1250	375
25	2	104	57	16	47	900	350
26	3	105	58	9	47	925	350
24	4	104	49	14	55	1025	375
26	5	102	58	—	44	1050	425
26	6	98	58	7	40	1050	425
26	7	110	55	5	55	1300	475
26	8	104	51	9	53	1200	400
26	9	115	58	20	57	1200	425
26	10	117	52	20	65	1350	475
26	11	118	60	20	58	1125	400
26	12	110	55	8	55	1050	450
26	13	110	62	0	48	875	375
26	14	107	62	0	45	775	400
26	15	105	53	17	52	1050	340
26	16	102	52	2	50	1020	340
Mean:		107 9	55·6	11·5	52·2	1071·6	398·7

(5) *Effects of 'E' saline on membrane potentials*

The differences observed between intact and desheathed nerve cords may be partly attributed to different ionic concentrations in the extracellular fluid as compared with the bathing solutions. In order to determine whether this was the case membrane potentials of giant axons were recorded in desheathed preparations bathed in 'E' saline (Table 5, Fig. 7). This saline was hypertonic to the nerve fibres, and although desheathing procedure gave rise to a shrinking of the connective such a preparation continued to function satisfactorily for many hours. The resting potential was $64{\cdot}6 \pm 3{\cdot}3$ mV. and the action potential $90{\cdot}9 \pm 7{\cdot}2$ mV., giving an overshoot of

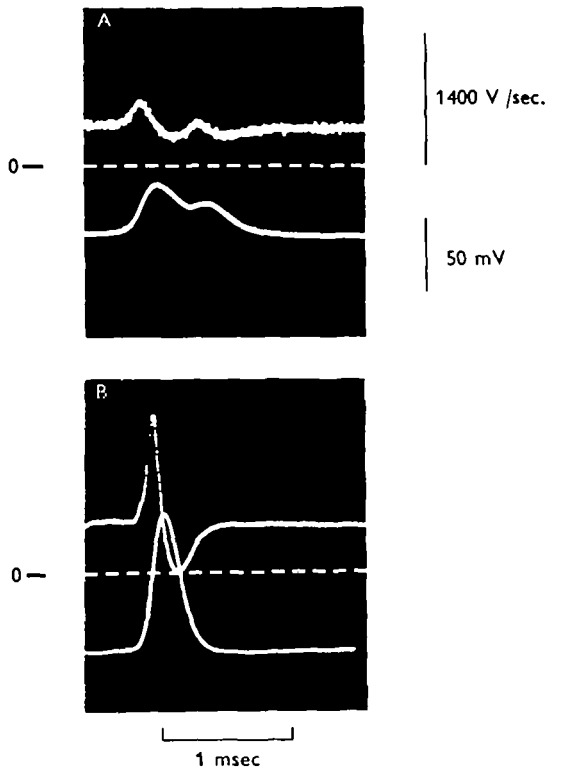

Fig. 6. Effects of haemolymph ions on the action potential and its derivative in a desheathed part (A) and in a sheathed part (B) of the same nerve cord. Same preparation as in fig 5C. 27° C.

Table 4. *Membrane potentials of giant axons in a partly desheathed nerve cord bathed in 'H' saline*

Temp. (° C)	Serial no.	Action potential (mV.)	Resting potential (mV.)	'Sheath potential' (mV.)	Over-shoot (mV.)	Maximum rate of Rise (V./sec.)	Maximum rate of Fall (V./sec.)
			(A) Desheathed part				
27	1	58	67	—	−9	230	150
27	2	30	61	—	−31	150	100
27	3	40	64	—	−24	200	100
27	4	30	62	—	−32	—	—
27	5	42	51	—	−9	300	100
27	6	40	56	—	−16	275	100
27	7	44	53	—	−9	300	150
Mean:		40·6	59·1	—	−18·6	242 5	116·6
			(B) Sheathed part				
27	1	95	32	35	63	1130	420
27	2	95	40	33	55	1250	340
27	3	113	45	10	68	1600	410
27	4	110	44	28	66	1560	480
27	5	100	42	27	58	1420	410
Mean:		102·6	40·6	26·6	62 0	1392·0	412·0

26·3 ± 7·2 mV. The maximum rates of rise (830·9 ± 116·8 V./sec.) and rates of fall (343·6 ± 35·3 V./sec.) were of the same order of magnitude as those recorded for desheathed preparations in normal saline.

Table 5. *Membrane potentials of cockroach giant axons in 'E' saline*

Temp. (° C.)	Serial no.	Action potential (mV.)	Resting potential (mV.)	'Sheath potential' (mV.)	Over-shoot (mV.)	Maximum rate of Rise (V./sec.)	Maximum rate of Fall (V./sec.)
			Nerve sheath removed				
27	1	85	67	—	18	775	360
27	2	90	60	—	30	875	370
27	3	95	69	—	26	1020	410
27	4	80	67	—	13	730	340
27	5	87	62	—	25	730	340
27	6	90	64	—	26	770	340
27	7	90	65	—	25	770	340
27	8	98	67	—	31	840	340
27	9	83	60	—	23	700	260
27	10	102	62	—	40	1050	340
27	11	100	68	—	32	880	340
Mean:		90·9	64·6	—	26 3	830·9	343·6

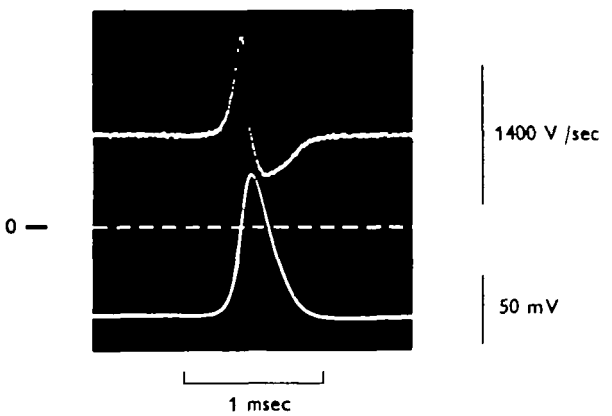

Fig. 7. Effects of extracellular ions on the action potential and its derivative of a giant axon in a desheathed nerve cord. 27° C.

From these results, it was therefore concluded that the ionic composition of the extracellular fluid might be at least partly responsible for maintaining normal resting and action potentials despite the relatively high potassium concentrations. It is not impossible, however, that osmotic pressure itself might play some rôle in avoiding the enlargement of extracellular spaces and in concentrating ions (by water loss) in the extracellular fluid.

(6) *Errors in measurements of resting potential and 'sheath potential'*

It has been seen that in sheathed nerve cords the apparent resting potential (i.e. the potential difference between the inside of a giant axon and the external solution) was always smaller than in desheathed preparations although bathed in the same saline.

Part of this difference might be attributed to the 'sheath potential', the origin of which needed to be studied in more detail.

We have seen that the inside surface of the nerve sheath is positive with respect to outside. This sign is not the one which should be expected if the sheath acted as a semi-permeable barrier with a Donnan equilibrium operating between the extracellular fluid and the bathing fluid. The recorded values of this 'sheath potential' were ex-

Table 6. *Tip-potential differences between 'E' and 'H' salines*

Temp. (° C.)	Serial no.	Tip potential (mV.)
20	1	5
20	2	6·5
20	3	8 5
20	4	4
Mean		6·0

Table 7. *Membrane potentials and 'sheath potentials' in a sheathed nerve cord in 'H' saline and microelectrode tip potentials between 'E' and 'H' salines*

Temp. (° C.)	Serial no.	Action potential (mV.)	Resting potential (mV.)	'Sheath potential (mV.)	Tip potential (mV.)
21	1	107	50	19	10
21	2	105	54	10	3
21	3	95	50	0	0
21	4	95	49	11	11
21	5	100	55	3	1
21	6	80	55	0	3
21	7	92	50	0	3
21	8	100	57	10	4
21	9	100	53	9	9
21	10	96	50	9	6
21	11	99	52	14	3
Mean		97·2	52·3	7·7	4·8

tremely variable (0–35 mV.), whereas membrane potentials remained nearly constant. 'Sheath potentials' have sometimes been recorded after removal of the nerve sheath (see Tables 2 and 3). These facts led us to think that the 'sheath potential' might not exist *per se* but that the recorded potential differences were to be attributed to variations of the microelectrode tip potential in contact with different ionic solutions. Adrian (1956) has shown, indeed, that tip potentials of microelectrodes filled with 3 M-KCl are logarithmically related to the external concentrations of NaCl or KCl. Table 6 shows that, whenever unused microelectrodes were tested, the recorded tip potentials in 'E' saline were 6 mV. more positive than in 'H' saline. It was important to determine whether these values agreed or not with recorded 'sheath potentials'. It is seen (Table 7) that, whereas 'sheath potentials' and tip potentials were of the same sign, there was no complete agreement between the values of these two potentials, 'sheath potentials' being generally greater than tip potentials. It is nevertheless possible that other ions than those contained in E saline (Na^+, K^+, Ca^{2+} and Cl^-) are responsible for this difference.

The possible error in measurements of resting potential in sheathed nerve cords brought about by this 'sheath potential' will be discussed later.

DISCUSSION

The above results have shown that whereas action potentials recorded in sheathed nerve cords are of greater magnitude than those recorded in desheathed preparations, resting potentials are always lower in the first case (Table 8). It has also been demonstrated that diffusion processes along the extracellular spaces are very slow and that the potential difference existing across the nerve sheath seems to be extremely variable from one impalement to another. Moreover, it has been pointed out that the use of

Table 8. *Effects of ions on sheathed and desheathed nerve cords*

	Action potential (mV.)	Resting potential (mV.)	'Sheath potential' (mV.)	Over-shoot (mV.)	Maximum rate of Rise (V./sec.)	Maximum rate of Fall (V./sec.)
			(A) Normal saline			
(*a*) Desheathed nerve cords	85·9 ± 4·6	67·4 ± 6·2	—	18·5 ± 7·1	804·7 ± 128·9	320·3 ± 49·4
(*b*) Sheathed nerve cords	103·0 ± 5·9	58·1 ± 5·4	6·7 ± 4·2	44·3 ± 5·8	928·1 ± 122·0	321·9 ± 38·0
			(B) 'H' Saline			
(*a*) Desheathed nerve cords	36·5 ± 7·6	57·3 ± 5·3	—	−20 7 ± 8·4	244·2 ± 106·4	100· ± 3·6
(*b*) Sheathed nerve cord	107·9 ± 6·0	55·6 ± 4·2	11·5 ± 8·2	52·2 ± 7·1	1071·6 ± 159·5	398·7 ± 44 0
			(C) 'E' saline			
Desheathed nerve	90·9 ± 7·2	64 6 ± 3·3	—	26·3 ± 7·2	830·9 ± 116·8	343·6 ± 35 3

a saline containing cations at the extracellular level results in quite normal resting and action potentials in desheathed preparations despite of a relatively high potassium concentration. The maximum rates of rise and rate of fall recorded in different conditions show that there is no significant difference in the shape of the action potentials except for desheathed nerve cords bathed in 'H' saline.

Lower resting potentials in sheathed cords may be due to the excess potassium ions in the extracellular fluid demonstrated by Treherne (1962*b*). The mean recorded values of 58·1 and 55·6 mV. in sheathed cords bathed respectively in normal saline and in 'H' saline are indeed close to the theoretically predicted value of 54 mV. Now, if the slowness of diffusion processes in the very restricted extracellular channels of the nerve cord is taken into account it seems very likely that, at the time of the measurements, the ionic composition of the extracellular fluid was not in equilibrium with the ionic composition of the test solution, but was also determined by the ionic composition of the haemolymph before the dissection. Hence, measurements of resting potential made on sheathed nerve cords were not directly related to the ionic composition of test solution; this is especially the case for the measurements in the normal saline, the potassium concentration of which is very different from that of the haemolymph.

A possible error in measurements of the resting potentials in sheathed nerve cords has been pointed out earlier: the true resting potential of axons bathed in extracellular

fluid might correspond to the measured resting potential plus the measured 'sheath potential'. In that case the true resting potential of the axons would be higher than the measured one. This possibility has been provisionally discarded for two reasons. First, it has been shown that measured resting potentials were rather constant for the same preparation whereas 'sheath potentials' varied widely from one impalement to another. Secondly, it was observed that potentials of the same sign as 'sheath potentials' have been recorded in desheathed cords whose axons had a quite normal resting potential. One could suspect therefore the so-called 'sheath potential' to be an artifact determined by variations of the microelectrode tip potential between two different ionic solutions, similar to those recorded between 'H' and 'E' salines. Moreover, one could imagine the 'sheath potential' as originating not from the nerve sheath itself but from the underlying perineurial and glial cells. It has, in fact been suggested that these cells might be involved in the extra-axonal sodium regulation in *Carausius* nerve cords (Treherne & Maddrell, 1967*b*). The suggested movement of sodium ions across these cells would result in a potential difference relative to the outside medium. The polarity of this potential difference would depend upon the mobility of sodium ions relative to that of the anion which would be secreted with sodium in the channels of the mesaxon folds. Experiments on the isolated nerve sheath, being carried out at the present time, should permit us to decide whether the 'sheath potential' exists or not. It must be noted here that possible errors in measurements of resting potential may arise from microelectrode tip potentials, the importance of which cannot be well appreciated. Proper elimination of tip potentials using thorium chloride (Agin & Holtzman, 1966) would perhaps permit more reliable results.

If the measured values are considered as corresponding to the true resting potentials, this raises the question of how axons with so low resting potentials may give rise to action potentials as large as those that we have recorded. We have shown in fact, that, in the 'H' saline the resting potential was 57·3 mV. in desheathed nerve cords (this value being higher than that recorded in sheathed nerve cords) and that, in these conditions, the mean recorded action potential was only of 36·5 mV. This last result is in good agreement with the curves given by Yamasaki & Narahashi (1959*a*) and Narahashi (1966). A possible explanation is that extracellular calcium acts in such a way as to stabilize the membrane. The greater magnitude of action potentials in sheathed nerve cords may be interpreted in different ways. According to Yamasaki & Narahashi (1959*a*), increasing sodium concentration results in an increased active membrane potential; it was supposed that an excess of sodium ions in the extracellular fluid (Treherne 1962*a*, *b*) would result in higher action potentials in sheathed preparations. Narahashi (1966) has shown that an excess of calcium ions leads to an increased overshoot; now extracellular fluid also contains an excess of calcium ions (Treherne, 1962*b*). On the other hand, it seems very likely that the desheathing procedure slightly damages the nerve fibres which for this reason give rise to smaller action potentials. Moreover, it must be pointed out that impalement of a giant axon in a desheathed nerve cord may involve a greater injury than in a sheathed nerve cord. Indeed it has been noticed that, in desheathed preparations, if the microelectrode was maintained inside a nerve fibre for some minutes after the time of the impalement, the resting potential often gradually increased by some mM. whereas the action potential increased by 10–15 mV. This increase did not occur in sheathed preparations.

Experiments with a saline containing Na^+, K^+ and Ca^{2+} at the extracellular level have clearly shown that quite normal action potentials can be obtained, despite a relatively high potassium concentration. This finding is in good agreement with results of Treherne (1962*c*) who has shown that elevation of the cation concentration to the extracellular levels resulted in delayed conduction block in desheathed cockroach nerve cords irrigated with high potassium saline.* Great care must be exercised, however, in interpreting our results, for the relatively high resting potential in extracellular saline may be responsible for the production of these action potentials, whereas in sheathed nerves, large action potentials were associated with much lower resting potentials. The mean resting potential recorded in extracellular saline (64·6 mV.) cannot be interpreted in terms of external potassium concentration. Moreover, it has been demonstrated (Yamasaki & Narahashi, 1959*a*) that in the case of cockroach axons the magnitude of the resting potential undergoes little change when the external sodium concentration is varied over a wide range. It must be noted here, however, that an increase in the resting potential in high-sodium salines has been reported by Seyama & Irisawa (1967). These authors have shown that, with constant external potassium concentrations, the resting potential of the skate heart was 14 mV. higher in 200% sodium solutions than in 50% ones. The major difference might be due, in that case, to the variations of the microelectrode tip potential and to an increase of potassium equilibrium potential, E_K, caused by an increase of intracellular potassium in hypertonic solutions. Such an explanation might be valuable in the case of the giant axon of the cockroach bathed in extracellular saline. On the other hand, Narahashi (1966) has shown that there was little or no change in resting potential when external calcium concentration was varied from 0·18 to 54 mM./l. It is possible, however, that high calcium concentrations may play some rôle in preventing membrane depolarization at high potassium concentrations (cf. Stämpfli & Nishie, 1956). Osmotic pressure may also play a similar rôle (Stämpfli & Nishie, 1955). On the other hand, it does not seem unlikely that chloride ions may be partly responsible for the increased resting potential; in that case, an excess of chloride ions in the extracellular saline would result in a higher chloride equilibrium potential, E_{Cl}, and consequently to an increased resting potential. Further investigations concerning these problems will be carried out using the voltage-clamp technique.

When the nerve sheath is removed from the cockroach nerve cord, the resting potential of the giant axons is increased whereas the amplitude of the action potential is decreased. This phenomenon seems different from that reported by Treherne & Maddrell (1967*b*). These authors have shown, indeed, that in *Carausius* nerve cord removal of the fat-body sheath does not affect the amplitude of the action potential whereas a positive potential of about 15 mV., probably associated with this fat-body sheath, is responsible for an apparent reduction of the resting potential in intact nerve cords (Maddrell & Treherne, 1966; Treherne & Maddrell, 1967*a*). A lower resting potential in the intact nerve cord of the cockroach might be associated with a positive

* Treherne has shown that the irrigation of the 4th abdominal ganglion of the cockroach nerve cord with K^+-rich solutions resulted in the development of a reversible conduction block. Whereas irrigation of the desheathed ganglion with a saline containing 70 mM/l. K^+, 99·3 mM/l. Na^+ and 4·4 mM/l. Ca^{2+} led to a conduction block at between 3 and 4 min., irrigation with a saline containing elevated cation concentrations (85·9 mM/l. K^+, 153·0 mM/l. Na^+ and 17·6 mM/l. Ca^{2+}) resulted in a slower loss of excitability even though the potassium concentration in the last saline exceeded that in the first one.

potential across the nerve sheath, the 'sheath potential', reported in a previous paper (Pichon & Biostel, 1966*a*). In that case, however, the magnitude of the action potential must be unaffected when the nerve sheath is removed. The possibility remains that some damage to the giant axons in removing the nerve sheath or a greater injury to these axons when they are impaled with a microelectrode in a desheathed nerve cord (as pointed out earlier) may be responsible for such a reduction of the action potential. This would agree with the fact that removal of the nerve sheath made the impalement of the axons of *Carausius* a virtual impossibility (Treherne & Maddrell, 1967*b*). It is logical to conclude, however, from the present experiments, that the elevated concentrations of cations in the extracellular fluid is sufficient to explain the differences between sheathed and desheathed preparations, the so-called 'sheath potential' being related to variations of the microelectrode tip potential. The mechanism responsible for the maintenance of this specialized ionic composition inside the extracellular spaces is not clear. This differential ionic distribution between external solution and extracellular fluid has been shown probably to result from a Donnan-like equilibrium (Treherne, 1962*a*, *b*). The nature of the anion causing this effect is not known, but if we assume this anion to be a large molecule in solution in extracellular spaces then the nerve sheath acting as a semi-permeable barrier would be polarized, the inside being negative with respect to the outside. Such a potential has never been recorded. On the other hand, if an extracellular fixed-charge system is involved, the activity coefficient of extracellular cations might be lower than that in a free solution and would be equivalent to that in the bathing medium as pointed out by Treherne & Maddrell (1967*b*). A possible local regulation by the glial cells at the region of the axon surfaces, also suggested by these authors for sodium ions, is not to be excluded.

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SUMMARY

1. The use of very fine-tipped and mechanically strong microelectrodes has allowed reliable recordings of resting and action potentials to be made in cockroach giant axons in sheathed and desheathed nerve cords.

2. When the microelectrode was withdrawn from a giant axon in an intact connective the first positive change in the potential from the resting level, was in most cases followed by a negative deflexion to the original zero level, the 'sheath potential'. The values of this 'sheath potential' together with the resting potential, the action potential, the maximum rate of rise and maximum rate of fall of the action potential have been measured in three different salines.

3. In normal saline, resting potentials were lower in sheathed preparations ($58{\cdot}1 \pm 5{\cdot}4$ mV.) than in desheathed ones ($67{\cdot}4 \pm 6{\cdot}2$ mV.), whereas action potentials were higher in the former ($103 \pm 5{\cdot}9$ mV.) than in the latter ($85{\cdot}9 \pm 4{\cdot}6$ mV.).

4. Elevation of K^+ and Ca^{2+} concentrations in the saline to the haemolymph level resulted in a decrease of resting and action potentials in desheathed cords, to $57{\cdot}3 \pm 5{\cdot}3$ mV. and $36{\cdot}5 \pm 7{\cdot}6$ mV. respectively. No alterations in the membrane potentials were recorded in intact connectives bathed in this saline, the mean resting potential

being $55{\cdot}6 \pm 4{\cdot}2$ mV. and the mean action potential $107{\cdot}9 \pm 6{\cdot}0$ mV. Local desheathing of the nerve cord led only to local disturbance of the resting and action potentials, thus indicating that diffusion processes along the extracellular spaces were very slow.

5. The use of a saline in which cation concentrations have been elevated to the extracellular level resulted in normal resting potentials ($64{\cdot}6 \pm 3{\cdot}3$ mV.) and action potentials ($90{\cdot}9 \pm 7{\cdot}2$ mV.) in desheathed cords, despite the relatively high potassium concentration (17·1 mM./l.).

6. Recordings of the maximum rates of rise and rates of fall showed that there was no significant modification in the shape of the action potential in these different experimental conditions.

7. The values of the 'sheath potential' were very variable from one impalement to another and it is suggested that this potential might be related to variations of the microelectrode tip potential bathed in different ionic solutions.

8. The low resting potentials and high action potentials of giant axons in intact nerve cords may result from an excess of inorganic cations in the extracellular fluid.

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