J. Physiol. (1953) 120, 171–204

THE ELECTRICAL PROPERTIES OF CRUSTACEAN MUSCLE FIBRES

By P. FATT and B. KATZ

From the Department of Biophysics, University College, London

(*Received* 21 *October* 1952)

Crustacean muscle has been of special interest to physiologists because its myoneural junctions show the properties of central nervous synapses. The muscles are supplied by excitatory and inhibitory nerve fibres which can be stimulated separately, and whose interaction can be studied at the level of the nerve-muscle junction. As was pointed out in recent reviews (Wiersma, 1941; Katz, 1949), much valuable information has been obtained about the organization of the crustacean nerve-muscle system, but little progress has been made in elucidating the mechanisms by which antagonistic nerve impulses exert their effects on the muscle fibres.

As a first step, it seemed necessary to study the properties of crustacean muscle fibres, quite apart from their nerve junctions. Hitherto, most of the elementary properties of the crustacean muscle membrane have remained unexplored, largely because of technical difficulties in obtaining suitable fibre preparations. With the help of intracellular electrodes, this difficulty can be overcome, and in the present work extensive use has been made of this method, for stimulating and recording across the surface membrane of individual muscle fibres.

The object of this paper is to present electrical measurements on the crustacean muscle membrane, in particular its resistance and capacity, its resting and action potential, and its electrical reactions in various ionic environments. As was indicated in previous notes (Fatt & Katz, 1951*b*, 1952), the electric response of crustacean muscle fibres, and especially its persistence in sodium-free media, differs from that of many other excitable tissues, and the evidence for this will be discussed in detail.

METHODS

Application of intracellular electrodes. The usual procedure was to introduce two micro-electrodes into the same fibre, one to pass current through the membrane, the other to record the membrane potential. The circuits were completed by separate external electrodes whose connexions are shown in Fig. 1. The micro-electrodes were operated by separate manipulators and

observed with a Greenough microscope (magnification ×35, field of view 5·5 mm). The distance between electrode tips was measured with an eyepiece scale. To check the insertion of both electrodes the switch in Fig. 1 was placed in position 2, in which each electrode was connected to one grid of the balanced voltage amplifier. The entry of an electrode into the fibre was indicated by the sudden appearance of the resting potential and, after returning the switch to position 1, by the presence of electrotonic potentials at one electrode when rectangular current pulses were passed through the other. The latter test was particularly useful, in that it gave a positive result only when both electrodes were inside the same fibre; it could also be employed, by *transverse* displacement of one intracellular electrode, to check the fibre diameter.

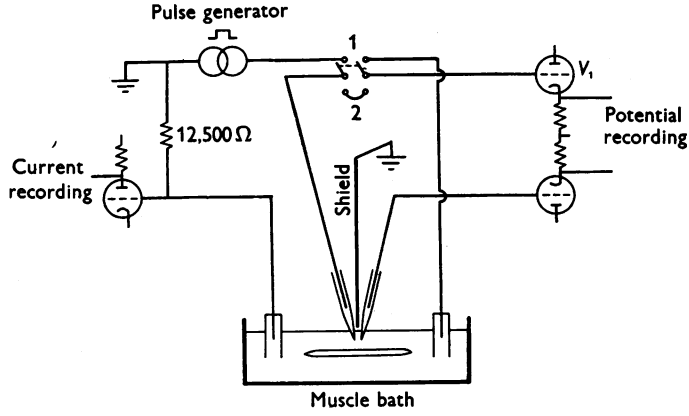


Fig. 1. Arrangement of electrodes for intracellular stimulation and recording. Note: double-pole double-throw switch; position 2 for testing electrode insertion, position 1 for normal use.

Current and potential across the fibre membrane were recorded by separate channels and displayed on a double-beam oscilloscope. The current intensity was measured as the p.d. across a 12,500 Ω series resistor. For calibration of the voltage amplifier, known voltages were introduced between this resistor and earth, while the grid of V_1 was earthed. Any errors which might arise from imperfect balance of the potential-recording system (failing to suppress the 'in-phase' signal across the 12,500 Ω resistor) were frequently checked and in most cases found to be negligible.

Preparation. The crabs (*Portunus depurator* and, occasionally, *Carcinus maenas*) were supplied by the Marine Biological Laboratory, Plymouth, and kept in the laboratory in jars of aerated sea water which was replaced at intervals of a few days. In keeping *Portunus* it was important to protect the animals against a rapid rise of temperature (cf. pp. 179 and 185). In most experiments the extensor muscle of the carpopodite was used. This muscle consists of a fairly thin layer of fibres attached to the shell of the meropodite. The fibres are several millimetres long and run obliquely from the shell to a chitinous tendon (apodeme) which is joined to the carpopodite. The fibre diameters vary between about 70 and 500 μ (*Portunus*), and 100 and 600 μ (*Carcinus*). To expose the muscle, the opposite part of the shell was removed, together with the flexor muscle and main limb nerve. The preparation consisting of meropodite and carpopodite, with the extensor muscle attached to shell and joint, was mounted under rubber bands on a glass slide, and the muscle gently stretched by flexing the joint. The preparation was immersed in saline in a Petri dish which was set up on a glass plate and attached to a mechanical stage, the whole being viewed in transmitted light.

Most experiments were carried out at room temperature (the preparation being 1–2° C cooler than recorded, cf. Fatt & Katz, 1951*a*); in special experiments, however, the temperature of the bath was varied by means of a cooling element and recorded thermo-electrically.

Solutions. The 'normal' saline had the composition shown in Table 1. It has been used successfully by many previous workers (cf. Pantin, 1934), but it was found to be a rather imperfect substitute for the normal environment of the muscle: the fibres often passed through a phase of weak contracture when the saline was first applied, and the frequent absence of electric responses may well have been accentuated by the use of this saline.

In many experiments some constituents of the solution were partly or wholly replaced. For example, Na was replaced by various quaternary ammonium ions, their osmotically equivalent concentrations being shown in Table 1. In other experiments NaCl was replaced by sucrose, but unfortunately prolonged soaking of the muscle in such electrolyte-deficient solutions produced irreversible damage. This could be prevented by substituting mixtures of sucrose and $MgCl_2$ for NaCl (Table 1), care being taken to check the effect of the raised Mg concentration. When changing solutions, the bath was stirred and sometimes the muscle 'syringed' under slight pressure, in order to accelerate washing the spaces between the loosely packed fibres and between fibres and shell. In the course of these experiments, the effects of several quaternary ammonium ions were examined, especially of choline, tetraethylammonium (TEA) and tetrabutylammonium (TBA).

TABLE 1. All concentrations in mM, i.e. millimoles per kg/H_2O. Intermediate concentrations were obtained by mixing known volumes of solutions *A* and *B*, or *C* and *D*, or *D* and *E*.

	A. Normal crab Ringer	*B*. Quaternary ammonium Ringer	*C*. (Na + Mg) Ringer	*D*. (Sucrose + Mg) Ringer	*E*. (Quaternary ammonium + Mg) Ringer
Na^+	513	—	423	—	—
R_4N^+	—	527	—	—	435
Sucrose	—	—	—	695	—
K^+	12·9	12·9	12·9	12·9	12·9
Ca^{2+}	11·8	11·8	11·8	11·8	11·8
Mg^{2+}	23·6	23·6	82·6	82·6	82·6
Cl^-	594	608	622	199	634
HCO_3^-	2·6	2·6	2·6	2·6	2·6

Choline chloride and acetylcholine chloride were used as supplied commercially (Roche, Light). Diethanoldimethylammonium chloride was provided by Dr R. Lorente de Nó of the Rockefeller Institute, New York. Tetramethylammonium, tetraethylammonium and phenyltrimethylammonium as iodides were obtained locally (Hopkin and Williams, Light). Tetrabutylammonium as the iodide and benzyltrimethylammonium as an aqueous solution of the hydroxide were supplied by Eastman (Rochester, New York). The iodides were converted to chloride by shaking them up in water (sufficient to make about an 0·6 M solution) with an excess of freshly precipitated silver chloride. The solid material was filtered off. The solution was then saturated with H_2S and filtered again in order to reduce further the silver ion remaining in solution. The benzyltrimethylammonium was similarly treated after having neutralized the hydroxide with HCl. The H_2S was removed by placing the solution in a desiccator over NaOH and carefully evacuating.

All the substances treated in this manner, except tetrabutylammonium, were then purified by crystallizing from concentrated solutions in either water or ethyl alcohol. Tetrabutylammonium which could not be made to crystallize from concentrated solution could be purified for the present purpose by crystallizing as a hydrate in the cold (below 14° C).

Because of the difficulty in obtaining the salts free of water, all the quaternary ammonium chlorides as well as $CaCl_2$ and $MgCl_2$ were usually made up to solutions of the desired concentration by titrating the chloride by the Volhard method, in an aliquot of solution of measured weight.

Other substances which were used in the experiments were glycine betaine, trimethylamine oxide and procaine, all obtained as the hydrochlorides (B.D.H.) and neutralized with NaOH.

RESULTS

I. Electric properties of resting muscle fibres

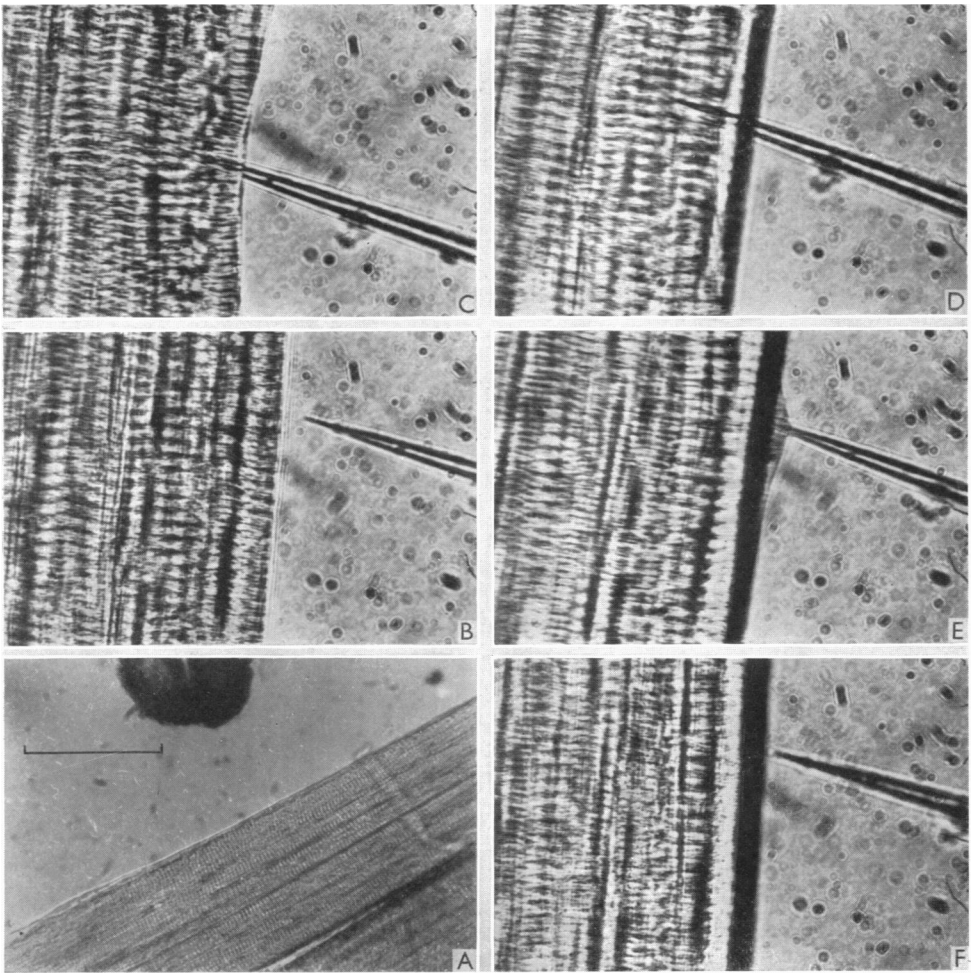
Resting potential

When the microelectrode entered a muscle fibre, a potential drop of about 70 mV was recorded, the inside of the cell being negative. In 144 fibres (normal saline, 20° C) variations in resting potential between 56 and 90 mV were observed (mean 70 mV, S.E. ±0·5 mV). Often, the p.d. appeared (and disappeared) instantly, during penetration (and withdrawal) of the electrode and remained constant for many minutes while the electrode stayed inside the fibre. At times, however, there was an irregular or step-wise increase of the p.d. during 'difficult' penetrations, suggesting the presence of obstructions (e.g. nerve branches, connective tissue, sarcolemma; cf. Pl. 1). Occasionally, when an electrode was withdrawn the whole of the p.d. did not disappear at once, but there was a slowly subsiding remainder of about 10–20 mV. It is possible that, in such cases, the tip of the electrode may have retained a 'plug' of myoplasm, and that the residual p.d. outside the fibre arose at the junction between this plug and the saline bath (a junction potential of this order has been estimated previously, see Hodgkin & Katz, 1949). In all these respects, crustacean muscle fibres do not differ from other cells to which the microelectrode technique has been applied.

There was, however, one interesting phenomenon which has apparently not been seen elsewhere: often, during a very small forward excursion of the electrode (small compared with the fibre size), the resting potential appeared and abruptly vanished again, *as though* the tip of the electrode had entered and passed through the cell. When the movement of the electrode was reversed, there was again a transient appearance of the resting potential. This happened in some locations, not in others, but at those points where it was seen, it could usually be repeated many times. One possible explanation may be the presence of superficial folds in the muscle fibre so that a minute forward movement might cause the electrode to go into and through the cell. Nerve fibres are said to spread along and penetrate more or less deeply into crustacean muscle fibres (d'Ancona, 1925) and it may be that convolutions are formed around the nerve terminations. The possibility of such complicated surface structures must be borne in mind: it might affect estimates of surface area, as well as be a source of retention or slow diffusion of solutes.

Effect of potassium. Crustacean muscle is known to have a high potassium content (Scholles, 1933; Katz, 1936), and it is of interest to inquire whether its resting potential is related to the potassium gradient across the fibre membrane, similarly to that in other nerve and muscle tissues.

When the crab-Ringer containing 12·9 mM-K was replaced by 'isotonic KCl' (580 mM), immediate depolarization occurred, e.g. the resting potential of twelve fibres fell from 70 mV (S.E. ±1·5) to −1·5 mV (S.E. ±0·57). The depolarization was not completely reversible, even though the muscle had remained in the K-rich solution for only a few minutes. After return to crab-Ringer the resting potential rose to 40 mV and more slowly increased to 51 mV.



EXPLANATION OF PLATE

Photomicrographs of crustacean muscle fibres. *Portunus*, M. extensor of carpopodite. The shell of the meropodite was removed far enough to see details of a fibre at the edge of the muscle, in transmitted light. Scale: 200 μ for *A*, 54 μ for *B–F*. In *A*, the whole width of a fibre (approximately 200 μ) is shown with low magnification. Above the fibre, the tip of a coarse external capillary electrode is visible. In *B–F*, with higher magnification, the insertion (between *B* and *C*) and, at a different level of the fibre, the withdrawal of a micro-electrode (between *E*–and *F*) are shown. In *C*, *D* and *E* the resting potential was between 60 and 70 mV. During insertion, the pressure of the electrode indents the fibre, a barely visible 'dimple' being formed round the tip (in *C* and *D*); during withdrawal the electrode pulls a 'bulge' (in *E*) which vanishes as soon as the tip is completely withdrawn (in *F*). The dark band at the edge of the fibre, in *D–F*, resulted from a change in the focusing level; this was readjusted to keep the electrode tip, rather than the fibre edge, in focus.

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With smaller changes of the external K concentration, intermediate effects were observed, illustrated in Fig. 2. Unfortunately, no great reliance can be placed on absolute quantities or the exact slope of the relation, because even after small alterations there was always some 'hysteresis', the membrane potential failing to return completely to the original Ringer level. This was particularly noticeable at the beginning of the experiments when the effect of

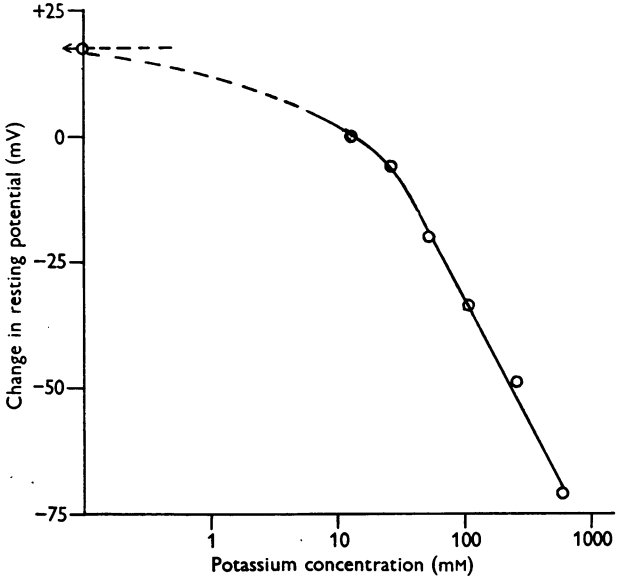

Fig. 2. Effect of potassium on resting potential. Abscissa: external potassium concentration $[K_x]$ in mM, on logarithmic scale. Ordinate: change of resting potential $E_{[Kx]} - E_{[Kn]}$, the normal concentration $[K_n]$ being 12·9 mM. The increase of potential was obtained in K-free solution. Each value is a mean from twelve fibres of one muscle, the same preparation being used for all concentrations except the highest (580 mM). In determining the ordinates for 0, 26, 53 and 105 mM, the mean values of two series of measurements in normal Ringer, before and after changing to $[K_x]$, were used.

K removal was studied. As shown in Table 2, the resting potential rose by some 15 mV in the K-free solution, but failed to decline to the original level, and in one experiment continued to rise after the muscle was returned to Ringer. The highest value obtained in this way in crab-Ringer (12·9 mM-K) was 95 mV. During repeated exposure to a K-free medium, the membrane potential could be driven up to 100 mV, and in some fibres as high as 110 mV. The reason for this curious phenomenon is not clear; it might have been due to slow diffusion of potassium and chloride across some unidentified hypolemmal spaces, but it seems to us more probable that slow changes in the membrane permeability were responsible for these 'hysteresis' effects.

Effect of temperature. The resting potential showed a small positive temperature coefficient. Twenty measurements at 2·6° C (varying between −0·4 and +6·5° C) gave a mean potential of 64 mV (S.E. ±1 mV, range 54–72 mV), compared with 70 mV at 20° C (±0·5 mV, 144 measurements). In sixteen experiments, the same fibres were used for comparative measurements at different temperatures: in these an increase from 2 to 14° C was accompanied by a 9 mV (±2 mV) increase of the resting potential (from 64 to 73 mV).

TABLE 2. Effect of K concentration on resting potential

Mean values and S.E. of means, from twelve fibres of the same muscle. Temperature 19° C. K was increased by replacing Na. Measurements started after 5 (or more) min soaking in the solution; each set of readings was completed within about 5 min.

Time of first reading (min)	K concentration (mM)	Resting potential (mV)	
		Mean	S.E.
0	12·9	68	±1·4
13	0	85	±1·2
23	12·9	79·5	±1·2
36	0	94·5	±0·9
50	0	98	±0·8
64	12·9	86·5	±0·9
83	12·9	83	±0·8
99	26·3	73·5	±0·7
113	12·9	75	±0·7
126	12·9	77	±0·9
139	53	54	±0·65
152	12·9	70	±0·85
165	105	32	±0·7
180	12·9	61	±0·95
194	263	12	±0·4

Electric constants of the resting fibre

In several experiments the subthreshold cable properties of the fibres were examined using the rectangular pulse technique (Hodgkin & Rushton, 1946; Lorente de Nó, 1947; Katz, 1948). Two intracellular electrodes were used for passing current and recording potential differences across the membrane. The technique was the same as described by Fatt & Katz (1951*a*; see also Methods), except in the following details:

(i) Measurements were made at several distances, keeping the 'current' electrode fixed and moving the 'voltage' electrode along the fibre.

(ii) Fibres of clear outlines were chosen whose size could be measured with the microscope. In several cases, this was checked by transverse displacement of the two internal electrodes: as long as both remained in the same fibre, subthreshold potential changes were recorded with only slight attenuation, as soon as one electrode entered a neighbouring fibre, the potential changes vanished.

This result incidentally showed that the different muscle fibres behave as separate cable units and not as a 'syncytium'. If there are protoplasmic connexions between them, their resistance must be so high as to render local current spread undetectable.

While it was not difficult to measure the thickness of the fibres, as exhibited *in situ*, estimates of circumference and cross-sectional area were less certain. They were based on the assumption that the fibres are cylindrical, and no account could be taken of surface folding or departure from circular cross-section. Whenever practicable, the 'polarizing' electrode was inserted at the middle of the fibre, a few millimetres from its 'shell' and 'tendon' ends.

When examining isolated muscle fibres, elliptical cross-sections were often found, especially with large fibres whose axes might differ by a factor of two. This may have caused a serious error in the estimate of fibre volume and specific internal resistance. It is possibly reflected in the large scatter of R_i (Table 3), and we suspect that the values of R_i in the fibres of largest *observed* diameter may have been overestimated in this way.

(iii) The analysis of the results depends on the theory of a linear cable and requires that the diameter of the fibre should be small compared with its length constant. This was not always fulfilled, especially not with large fibres where two kinds of discrepancy from the cable theory occur: (*a*) the spatial decrement of the electrotonic potential is exponential only at some distance from the 'current electrode' (Fig. 4). In the area immediately surrounding this electrode, the decrement is much steeper because electric charge spreads along the fibre surface circumferentially as well as longitudinally. (*b*) In the same region, an 'instantaneous' p.d. is developed across the transverse resistance of the cell interior, which interferes with the recording of the electrotonic potential. In practice it was necessary to restrict the analysis to fibres of less than 250 μ diameter, in which a fairly good agreement between the results and the simple cable theory was usually found.

Examples of the results are shown in Figs. 3 and 4, and a summary of eight experiments is given in Table 3. The most interesting result is the very large size of the membrane capacity, about 40 μF/cm^2, and the low resistance, about 100 Ωcm^2. Twenty-one other, less complete, experiments were made which were not included in Table 3. In all these experiments, the membrane capacity was of the same large order (average 50 μF/cm^2, range 24–83 μF/cm^2), but the membrane resistance and, therefore, the length and time constants varied greatly, depending upon the type of muscle, temperature and other conditions (see p. 198). The lowest values were: λ 0·4 mm, τ_m 1–2 msec, R_m about 20 Ωcm^2; the highest (observed in the opener of the claw of a Hermit crab *Eupagurus bernhardus*): λ 2·65 mm, τ_m 55 msec, R_m about 2000 Ω cm^2. It should be noted that the resting potential was *higher* in the former case (85 and 65 mV respectively).

Effect of temperature. In several experiments the effect of temperature on the fibre constants was investigated, using a simplified procedure with measurements at only two or three positions along the fibre. An interesting result was obtained, namely that the membrane resistance *falls* greatly as the

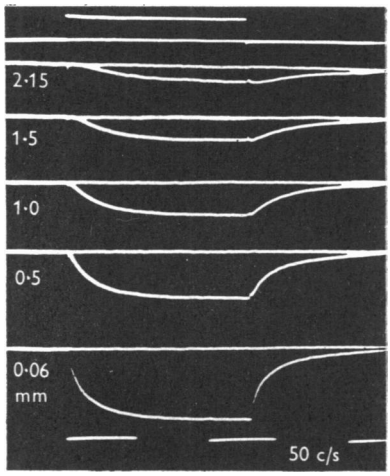

Fig. 3.

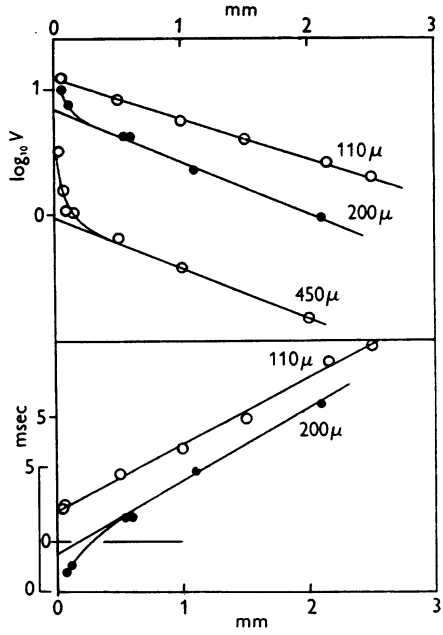

Fig. 4.

Fig. 3. Electrotonic potentials. *Carcinus*. 110 μ fibre. Top record: anodic current pulse. The other records show membrane potential changes at the indicated distances from the point of current application.

Fig. 4. Electrotonic potentials. *Carcinus* muscle fibres. Abscissa: distance between intracellular 'current' and 'voltage' electrodes. Upper part: spatial decrement of steady anelectrotonic potential. Ordinate: relative size of electrotonic potential in logarithmic units. Three fibres of different sizes: the smallest shows simple exponential decrement, the larger ones show a steep 'origin distortion' (see text). Lower part: variation of 'half-time' of electrotonic potential with distance (used for determining membrane time-constant). Ordinates: 'half-time' in msec. The small fibre shows a linear relation, the larger one shows 'origin distortion'.

TABLE 3. Electric constants of crustacean muscle fibres

λ, length constant; τ_m, time constant; R_i, specific resistance of myoplasm; R_m and C_m, specific membrane resistance and capacity.

Species	Temp. (° C)	Fibre diameter (μ)	Resting potential (mV)	λ (mm)	τ_m (msec)	R_i (Ω cm)	R_m (Ω cm^2)	C_m (μF/cm^2)
Carcinus	17	110	72	1·3	6·7	37	230	29
Carcinus	18	185	76	1·16	6·5	58	170	38
Carcinus	18	190	67	0·95	4·2	50	95	44
Portunus	20	150	76	0·78	4	52	86	47
Portunus	24	220	64	0·6	3·3	125	83	40
*Portunus**	21	255	72	0·74	3·5	112	97	36
*Portunus**	21	165	72	0·78	2·6	32	48	55
*Portunus**	21	210	70	0·83	5·8	89	117	50
Mean	20	180	71	0·9	4·6	69	116	42

* In these experiments solution *C*, in all others solution *A* was used.

temperature is raised, while the resting potential *increases*. There was no significant effect of temperature on the membrane capacity. In seven experiments, in which the same fibres were examined at 2° C (0·5–3·5° C) and 17° C (15–18° C), the following mean values were found: (i) the resting potential increased with temperature from 64 to 80 mV; (ii) the membrane resistance fell from 194 to 52 Ωcm^2; (iii) the specific resistance of the myoplasm fell from 152 to 111 Ωcm, (iv) the membrane capacity was 58 and 59 $\mu F/cm^2$ respectively. The values of resistances and capacity were less accurate than those in Table 3, but give a useful indication of temperature effects. Thus Q_{10} values are about 1·2 (S.E. $\pm 0{\cdot}11$) for myoplasm resistance, and 2·5 ($\pm 0{\cdot}27$) for membrane resistance. The former value does not differ significantly from that of ordinary electrolytes, while the latter is well above it.

If the temperature of the muscle was raised several degrees above 20° C it was often found, especially when the crabs had been kept previously in cool sea water, that the membrane resistance dropped irreversibly. This effect will be discussed further in § II.

Voltage/current relation. When measuring fibre constants, the applied current through the membrane was inward ('anodic') throughout; the intensity was about 1 μA which raised the membrane potential locally by about 10 mV. An increase of current strength to several microamperes produced a proportional increase of the potential change. With outward ('cathodic') current, however, the relation between voltage and current became non-linear when the depolarization exceeded about 20 mV; this was observed in all fibres which had retained some vestige of electric response (§ II); it showed itself in a gradual increase of the voltage/current slope and in the appearance of local and eventually propagating action potentials (Figs. 5–7).

II. The electric response of crustacean muscle fibre to direct stimulation

Qualitative observations

The electrical properties of resting crustacean muscle differ in important respects from those of other excitable tissues. The fibres are of about the same size as the largest nerve axons of cephalopods, but their membrane capacity is some 40 times greater and the resistance of the unstimulated membrane much smaller. This in itself must have an important effect on the electric excitability of crustacean muscle, and it is worth considering what kind of action potential one might expect from such fibres. Suppose the mechanism of their resting and action potentials is the same as that found in the squid giant axon and various other types of nerve and muscle fibres (Hodgkin, 1951), with a relatively high K (and Cl) permeability of the resting muscle fibre (accounting for the resting membrane conductance) and a sudden pulse of inward current during activity (due to a specific increase of Na permeability)

giving a maximum of about 1 mA/cm^2. As a consequence of the large membrane capacity, other factors being equal, the action potential of crustacean muscle could propagate at only 1/40 of the speed of a *Sepia* or *Loligo* axon (cf. Offner, Weinberg & Young, 1940; Katz, 1948). The situation is even less favourable, for the pulse of inward current generated by the active membrane must not only discharge a very large capacity (40 μF/cm^2), but is opposed by the relatively high leakage current which has been shown to pass through the resting membrane (100 Ωcm^2). On these grounds, small and slow action potentials are all that can be expected.

Propagated responses have previously been observed in crustacean muscle (Katz & Kuffler, 1946), but they did not invariably occur; quite often the muscle responded to an electric stimulus only locally. When propagation took place the conduction velocity of the spike was very low, about 20 cm/sec at 20° C.

During the present work we encountered great variability in the size of the action potential in different muscles, and even in different fibres of the same muscle. In some preparations, only electrotonic depolarization could be produced; these muscles usually came from animals of poor condition, and their fibres had a very low membrane resistance. With sufficiently strong depolarization a local contraction was still seen, similar to that in a narcotized or sodium-deprived frog muscle, without being associated with an action potential. A condition of this kind could be brought about by raising the temperature of the animal, or of the isolated muscle, and by certain chemical changes, e.g. by calcium withdrawal. But sometimes this state of electric inexcitability appeared for no obvious reason nor could it be overcome by the use of chemical 'activators' described on pp. 186 *et seq.*, below.

In the majority of the muscles, used with normal Na-saline, local action potentials were obtained when a sufficient outward ('cathodic') current was sent through the fibre membrane, and in many cases the action potential was large enough to propagate itself along the whole length of the fibre (Figs. 5–7). At the point of stimulation the local action potentials were found to 'take off' above a level of depolarization amounting to 20–45 mV (Fig. 5). With prolonged current pulses, the activity became repetitive; this is illustrated in Fig. 5. (records 7–11), where an 'oscillatory' as well as an 'intermittent-spike' type of electric response is shown.

Using the Na-saline, it was difficult to predict whether a particular fibre would give a small non-conducted spike of, say, 5 mV amplitude, or a large propagating potential of 60–80 mV. There was, however, some relation to the general state of the animal, and a fair consistency was often obtained between fibres of any one muscle. It may be noted that propagated responses were seen more frequently in *Portunus* than in *Carcinus* muscle.

There was an interesting relation between action potential and mechanical response. A local miniature spike was accompanied by a distinct local twitch,

even though the spike amounted to only a small increment in the local depolarization (sometimes as little as 4 or 5 mV, added to some 40 or 50 mV of electrotonic potential). It seemed surprising that a small and brief abortive spike was accompanied by an apparently much more powerful local contraction than a similar increment in the electrotonic potential, but it should be noted that a local electric response spreads further than a 'passive' electrotonic potential (Hodgkin, 1938) and thus a greater length of fibre would be involved in the twitch.

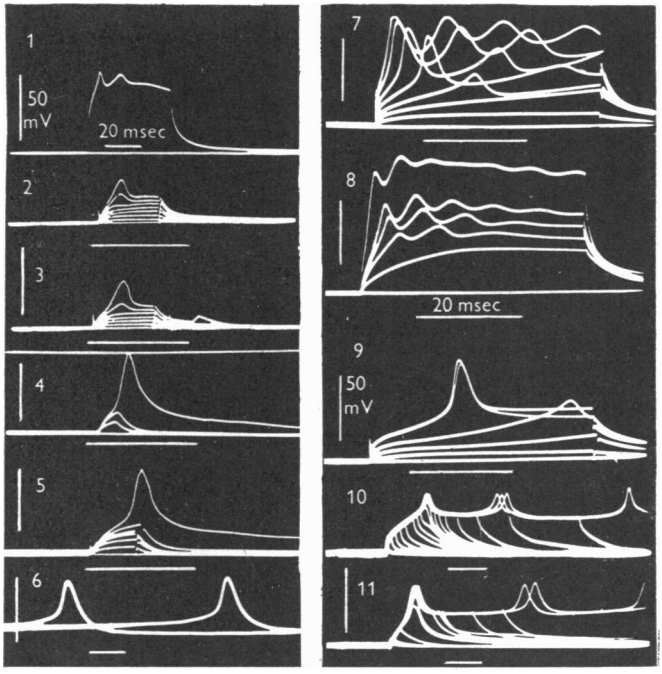

Fig. 5. Action potentials in crab-Ringer. Records 2–5 from *Portunus*, all others from *Carcinus*. Record 6 shows 'injury' discharges in a *Carcinus* muscle fibre, the other records show different types of response to cathodic currents (see text). In records 2–5 and 7–11 several current pulses were applied during successive time base sweeps (pulses of constant duration and varying intensity in 2–5 and 7–9, varying duration and constant intensity in 10–11). Distances between intracellular stimulating and recording electrode varied between 100 μ (records 1, 7 and 9) and 700 μ (records 2 and 3). Record 4 shows a 'zero potential' line: the spike happens to be equal to the resting potential. Scales: 20 msec and 50 mV.

The effects of different cations on the action potential

Sodium

Examples of electric responses obtained in normal crab-Ringer are illustrated in Figs. 5–7, which show the diversity between small (local) and large (propagated) spikes in different fibres. The shape of the action potential

varied as well as its amplitude: at 20° C brief spikes, of 2–3 msec duration (measured between points of maximum slope), and broad, sometimes humped or double-peaked potentials of up to 17 msec duration were observed. It will be shown below that the duration of the action potential is susceptible to a thousand-fold change, depending upon experimental conditions, and it is therefore not very surprising that a several-fold variation occurs under apparently unchanged conditions.

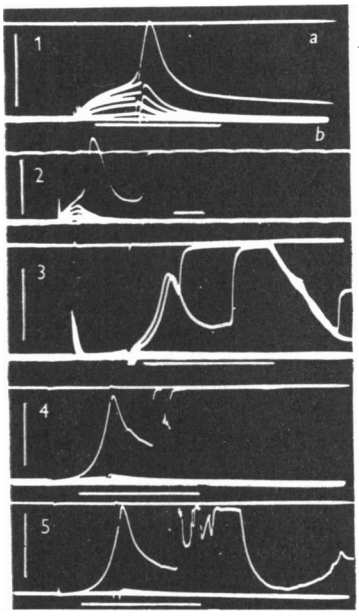

Fig. 6.

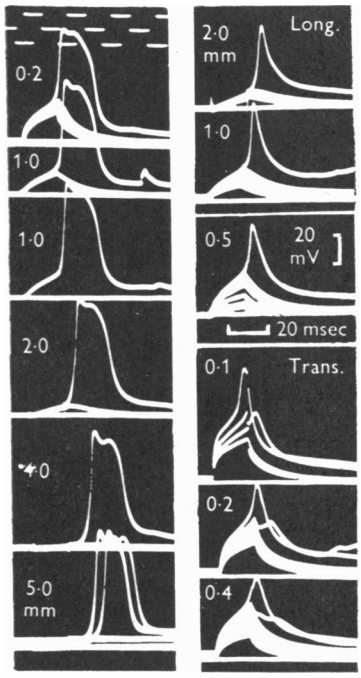

Fig. 7.

Fig. 6. Action potentials and movement artifacts. *Portunus*. Five different muscle fibres. Each record shows resting and action potentials (*a* with recording micro-electrode outside, *b* resting potential). Different appearances of movement artifacts in 2–5. Temperature: 20–26·5° C in 1 and 3–5; 0·5° C in 2. Distance between internal stimulating and recording electrodes: 0·5 mm in 1 and 2; 2·5 mm in 3, and 2 mm in 4 and 5. Scales: 20 msec and 50 mV.

Fig. 7. Propagation of muscle impulse. *Carcinus*. Distances between stimulating and recording electrodes shown in figure. Left part: note decline of electrotonic potential and delay of action potential with increasing distance. Calibration: 50 c/s with 10 mV spacing between sweeps. Right part: another fibre. Upper portion showing longitudinal propagation of impulse; lower portion: transverse separation of stimulating and recording electrodes along fibre circumference.

The most commonly observed 'sodium spike' had a sharp, almost cusped peak with a high initial rate of decline. At times it appeared that the ascending phase of the action potential was 'cut short' abruptly and collapsed more rapidly than it rose.

The propagated action potential is followed by a quick twitch of all-or-none type (disregarding localized mechanical responses which occur below the propagation threshold). The question arises whether the intracellular record of the spike may be vitiated by movement artifacts. Such artifacts occurred frequently, but could always be recognized (i) by their delayed appearance, beginning at the earliest some milliseconds after the spike peak, provided the recording electrode is close to the point of stimulation, (ii) by their irregular occurrence, and (iii) by the fact that the electrical change was of the kind which accompanies a partial or complete withdrawal of the internal electrode, or a series of transient withdrawals and re-entries. The point is best brought out by Fig. 6 which shows the unmistakable differences between late, irregular, movement artifacts and initial response.

TABLE 4. Resting and action potentials of crustacean muscle fibres

Action potentials: only propagated potentials have been included. Solutions: for Na, solution *A* (Table 1), for choline and TEA solutions *B* were used. With TBA the effect was irreversible and measurements have, therefore, been included which were made in solution *B* or other solutions after the TBA effect had been established. Duration of action potential was measured between points of maximum slope. With TBA, some longer potentials were seen, but exceeded the length of the photographic record. Mean values and S.E. of mean (or range, for duration) are shown with number of fibres in brackets

Principal cation	Temp. (° C)	Resting potential (mV)	Action potential (mV)	Active-membrane potential (mV, inside minus outside)	Maximum rate of rise (V/sec)	Duration (msec)
Na	22	*72*±0·65 (110)	*61*±1·2 (59)	*−11*	*20·5*±1 (37)	*3·5* [2–17] (106)
Choline	22	*69*±1·1 (31)	*79*±1·4 (30)	*+10*	(*28*±3) (3)	*24* [7–45] (26)
TEA	23	*63·5*±1·4 (27)	*96*±2·9 (27)	*+33*	*36*±5 (6)	*102* [23–260] (14)
TBA	20	*58*±1·45 (27)	*70*±1·85 (27)	*+12*	*4*±0·65 (8)	*1100* [430–2160] (20)

Measurements made on a large number of fibres (temperature 16·5–26° C) are summarized in Table 4. As was previously stated, the amplitude of the action potential varied between nil or a few mV and 84 mV. In Table 4, the sizes of action potentials were taken from propagated spikes; non-conducted responses were excluded. Furthermore, the mean value of 61 mV was derived from records made at some distance from the point of stimulation, or some time after the end of the pulse, so that any residual electrotonic component must have been very small compared with the measured spike height. In a less exclusive series of measurements, a mean amplitude of 63 mV (S.E. ±1 mV, 80 observations) was found. These values are about 10 mV less than the resting potential, and the mean difference would become much larger if the size of the non-conducted spikes had been taken into account.

Duration. The duration was measured between the points of maximum slope during rise and fall of the spike potential. The mean value (3·5 msec) was taken from eighty propagated and twenty-six non-propagated action potentials. This value is not very accurate, because with a 'cusped' spike of the kind shown in Fig. 6 the inflexion points are close to the peak of the wave, and a relatively large change is produced by small errors in selecting points of maximum slope. When the width of the spike was measured at a lower level (about 50% of the peak), a mean value of 5·2 msec was obtained. The main object of these measurements was to provide a comparison with the very much larger and relatively more accurate values obtained with certain quaternary ammonium ions (see Table 4), and for this purpose it makes little difference whether 3·5 or 5·2 msec is taken as a representative figure for 'sodium-spikes'.

Maximum rate of rise. This was taken from thirty-seven fibres, only those records being used in which any local electrotonic component was negligible. The mean value of 20·5 V/sec may seem small compared with the 500–1000 V/sec in a squid axon (Hodgkin & Katz, 1949), but it is probably more pertinent to compare the ionic membrane current associated with the rising phase of the action potential. If we make use of the argument of Hodgkin & Katz (1949, p. 55), we find a net ionic inward current of

$$20{\cdot}5 \text{ V sec}^{-1} \times 42 \ \mu\text{F cm}^{-2} = 0{\cdot}86 \text{ mA/cm}^2,$$

which is close to the current density estimated in the squid giant axon.

The 'active' inward current must have been even greater, because a large 'passive' outward current was presumably flowing through the depolarized membrane at that instant. The maximum slope was measured at about 50 mV of spike height: with a leakage resistance of the *resting* membrane of 116 Ωcm^2 (Table 3) the ionic outward current would have been at least (i.e. disregarding the onset of 'delayed rectification', cf. Hodgkin, Huxley & Katz, 1949) 50 mV/116 $\Omega\text{cm}^2 = 0{\cdot}43$ mA/cm^2. Hence, the observed rate of rise required an 'active' inward current of at least $0{\cdot}86 + 0{\cdot}43 = 1{\cdot}29$ mA/cm^2.

Conduction velocity. In twenty-one fibres (at 21° C) estimates of the propagation velocity were made by recording the action potential at different distances along the fibre (e.g. Fig. 7). The method was not very accurate because only currents of moderate intensity, and several milliseconds duration, could be used with the intracellular electrode, and random changes in latency are therefore likely to occur in successive records. The results, however, were fairly consistent, the mean value being 29 cm/sec, with variations in different fibres between 19 and 42 cm/sec. This confirms the low speed of propagation (25 cm/sec) observed by Katz & Kuffler (1946) in a limb muscle of the Australian swimmer crab (*Portunus pelagicus*). In Table 5 two individual experiments are listed on a 510 μ and a 300 μ fibre. The velocities in

these fibres are little more than 1/100 of those obtained in the only slightly larger squid giant axon (500–700 μ, 25 m/sec at 20° C).

It is of interest to compare the propagation velocities in crab muscle fibres with that in a 30 μ crab axon (Hodgkin, 1939; Katz, 1947) which, in a large volume of saline, conducts impulses at 450 cm/sec. On a simple local circuit theory (Offner *et al.* 1940), velocities of similarly constructed non-medullated fibres which produce the same inward current density during excitation, should be related as $(d/R_i)^{\frac{1}{2}}C^{-1}$, d being fibre diameter, R_i specific internal resistance and C membrane capacity per cm^2. That this relation is approximately obeyed, is shown in Table 5. Thus the extremely low velocity in a large crustacean muscle fibre is consistent with its very large membrane capacity.

TABLE 5. Relation between conduction velocity and resting fibre constants (at about 20° C)

Fibre	Radius (μ)	C_m (μF/cm^2)	R_i (Ωcm)$_i$	(Radius/R_i)$^{\frac{1}{2}} \times C_m^{-1}$ (rel. units)	Conduction velocity (m/sec)	References
Squid giant axon	250	1·1	30	26	25	Curtis & Cole (1938) Hodgkin (1939), Cole & Hodgkin (1939)
Crab axon	15	1·1	60	4·5	4·5	Hodgkin (1939, 1947), Katz (1948)
Crab muscle fibre*	*A*255	42	69	0·46	0·4	—
	*B*150			0·35	0·31	—
	*C*125 (7)			0·32	0·29 (21)	—

* *A* and *B* are individual fibres, *C* the mean of several experiments (number in brackets).

Effect of temperature. In a few experiments the effects of temperature changes were studied. These preparations happened to be taken from animals kept at about 12° C and, therefore, the results must be considered separately from those summarized in Table 4. These crabs did not survive a sudden rise of temperature to 22° C or above. Similarly, the isolated muscles became completely and irreversibly inexcitable when their temperature was raised to this level. Under these conditions the resting potential was high, the membrane resistance very low (§ I), and no action potential could be obtained. By contrast, the muscles of animals which had acclimatized to warm surroundings often gave large propagated spikes at temperatures as high as 26–27° C.

In Table 6, an experiment on five fibres is summarized in which the temperature was lowered from 12 to −0·4° C and then raised in three steps to 12·8° C. In this range, a 10° C fall of temperature caused the resting potential to drop by 6 mV, from 69·5 to 63·5 mV, and the action potential to increase, in amplitude from 59 to 71 mV, and in duration from 5·1 to 19·6 msec. It is interesting that at a low enough temperature the action potential exceeded the resting potential (mean reversal point, in eleven fibres, at about 6° C): e.g. at −0·4° C it was 13 mV *greater*, while at 12–15° C, it was about 20 mV *less*.

TABLE 6. Effect of temperature on action potential

Mean values from several fibres of the same muscle. Experiment started at 12° C and proceeded in the sequence shown below.

Temp. (° C)	Resting potential (mV)	Action potential (mV)	Active-membrane potential (mV, inside minus outside)	Duration (msec)	No. of fibres
12	70·5	63	−7·5	5	4
−0·4	63	74	+11	24	5
3	64	68·5	+4·5	16	5
8·1	67	59	−8	6·2	5
12·8	69·5	50·5	−19	4	5

Choline

In spite of quantitative differences the electric response of crustacean muscle resembles that of other excitable tissues in many important respects: it is elicited by outward current (i.e. at the 'cathode') when a sufficient depolarization is attained, the fibre gives a local graded and a propagated, all-or-none, response which is associated with a wave of transient depolarization or reversal of the membrane potential.

The excitatory mechanism is less stable than that in crustacean or frog nerve fibres, but its variability and apparently low safety margin may, in part at least, be explained by the properties of the resting fibre membrane, its large capacity and low resistance. To obtain further evidence, the role of the external cations was studied, in particular the effect of sodium ions which are indispensable for the electric response of other excitable tissues (Overton, 1902; Hodgkin, 1951).

An attempt to replace NaCl by osmotically equivalent dextrose or sucrose was not wholly successful: the fibres became quickly inexcitable, but their resting potential was also considerably reduced, and the inexcitability may have been merely a consequence of depolarization. To prevent the latter, it was necessary to maintain a fairly high electrolyte content in the external medium, and various other substitutes were tried. Following the procedure of Lorente de Nó (1949) and others (Hodgkin & Katz, 1949; Nastuk & Hodgkin, 1950), sodium was replaced by choline which has been described as an 'inert' cation, being unable to maintain excitability of nerve or muscle fibres without altering their resting properties.

The effect of the substitution of choline for sodium was unexpected and striking; in no case were muscle fibres rendered inexcitable; on the contrary, the action potential became significantly larger, and many fibres which had previously given small local responses now produced large propagated potentials. The most noticeable change was the increased duration of the action potential which broadened, from a cusped spike of 3–4 msec between its points of maximum slope, to a 'rectangular' wave of about 25 msec duration. These changes were associated with an increase in the twitch response, of which a particularly striking example is illustrated in Fig. 10.

Typical 'choline-potentials' are shown in Fig. 8 and their measurements are summarized in Table 4. The resting potential is nearly the same as in sodium, the action potential is about 20 mV larger and, in contrast with the sodium response, significantly exceeds the resting potential. The difference in size between sodium and choline responses was on the whole even greater than indicated in Table 4 because the average value for sodium given there does not include small, non-conducted, action potentials. An example of successive measurements (Na-choline-Na) made on an individual fibre is shown in Fig. 8 (records 3–5), the mean values being: resting potential 73 mV in Na, and 71 mV in choline; action potential 65 and 85 mV; duration 3·1 and 32 msec respectively.

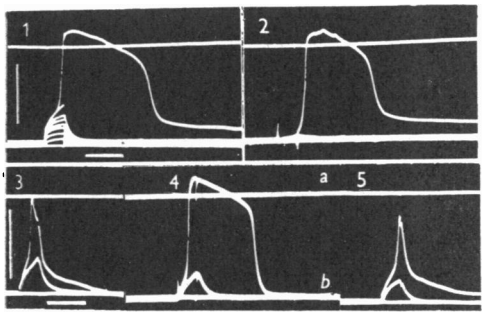

Fig. 8. Choline action potentials. *Portunus*. Upper part: 'choline-Ringer'. Distance between stimulating and recording electrodes: 0·13 mm in record 1; 2 mm in record 2. Note: action potential exceeds resting potential. Lower part: another fibre. Electrode separation 0·5 mm. Records 3 and 5 in 'Na', before and after washing in 'choline' (record 4). Scales: 20 msec and 50 mV. Resting potential shown by distance between lines *a* and *b*.

These results show that in crustacean muscle choline is not 'inert', but may be more properly described as an 'activating' ion which reinforces the excitatory process. The observation that the action potential is retained and, indeed, intensified when the external sodium had been totally replaced by choline is so surprising that we could not help suspecting some error. An obvious question is whether there may not have been a retention of sodium in spaces immediately surrounding the fibre membrane. As the fibres are loosely packed and freely exposed to the bath, such retention could only occur in the sarcolemma and minute spaces between sarcolemma and myoplasm, a layer perhaps some microns thick. We have no direct evidence about the ion permeability of this layer, but there are several reasons which make it appear very unlikely that any such retention of salt, in hypolemmal or other spaces, can account for the action of choline.

(i) The effect of choline substitution on the response was established quickly: it was observed as soon as the bath had been rinsed and replaced

and the electrodes reinserted, and there was no appreciable further change during the following two hours. When the muscle was returned to crab Ringer, the effect reversed equally promptly. When mixtures of sodium and choline were used (e.g. one-half of each) intermediate effects were obtained which developed with the same rapidity.

(ii) The qualitative effect was not of the kind which, by analogy with other tissues, one might expect to result from an incomplete sodium withdrawal. What we, in fact, observed was not the persistence of a reduced response, but a substantially increased response.

(iii) That there is an important difference in this respect between crustacean muscle and nerve, was confirmed by the following experiment. The same preparation was used, but instead of passing current with an intracellular electrode through a muscle fibre, a capillary stimulating electrode was placed externally against the nerve which runs along the exposed surface of the extensor muscle. The electrode was brought close to a point of the nerve near the proximal end of the meropodite, well away from the muscle fibre whose membrane potentials were recorded. It will be shown in a subsequent paper that, with this arrangement, 'end-plate potentials' are produced by nerve stimulation and can be recorded along the whole length of the muscle fibres. Now, when sodium was replaced by choline the 'end-plate potentials' disappeared at once, but when the current was made sufficiently strong, twitches were set up in muscle fibres near the capillary electrode, by direct stimulation. On returning to sodium-Ringer, the 'end-plate potentials' promptly reappeared. This observation is relevant, because it confirms, under the present experimental conditions, the findings of Hodgkin & Katz (1949), viz. that crab nerve fibres do not conduct in 'choline-Ringer'.

(iv) In some experiments the isolated limb was 'perfused' with the technique described by Pantin (1934) and the mechanical response of the flexor of the dactylopodite to brief direct shocks recorded. The effect of choline substitution was a large increase in the size and duration of the twitch (see Fig. 10), qualitatively the same as observed with the microscope on impaled fibres exposed to the choline bath. It evidently made no difference how the choline-Ringer was applied.

In these perfusion experiments, an interesting phenomenon (though not obviously related to the primary effect of choline) was observed. After a series of shocks, each producing a choline-twitch, a slow contracture developed which resembled that of an iodoacetate poisoned frog muscle. This was not, and could not very well have been, detected with intracellular electrodes because the fibres injured themselves after a few strong twitches, and the number of stimuli applied to individual fibres had to be kept to a minimum.

Because of the long-lasting action potentials, movement artifacts were a more serious technical problem, and many more records were spoilt in choline than in the ordinary saline. The criteria for differentiating between response and artifact were the same as described on p. 183, and there was no difficulty in distinguishing them.

The above arguments do not eliminate the possibility that traces of sodium may have been left on the outer surface of the fibre membrane; nevertheless it seems safe to conclude that the excitability of crustacean muscle is not only maintained when choline is substituted for sodium, but that choline is a more powerful 'activator' of the excitatory mechanism. To obtain further evidence the actions of several other quaternary ammonium ions were compared with those of sodium and choline. The substances which proved of greatest interest were tetraethylammonium (TEA) and tetrabutylammonium (TBA).

Tetraethylammonium (TEA)

This ion is much more powerful even than choline, as will be apparent from Tables 4 and 7. When substituted for sodium, it enables the fibres to give very large action potentials (up to 118 mV amplitude) rising at a greater rate (36 V/sec compared with 20·5 V/sec in Na), and much longer duration (up to 260 msec at 20° C, cf. Fig. 9). The action potential is accompanied by a very strong and long-lasting contraction (cf. Fig. 10) which might have been mistaken for a tetanus had the electric response not been recorded. The contraction was so powerful that at times fibres tore off at their point of attachment to the shell. The potency of TEA was further shown in an experiment in which the excitability of the muscle had been reduced to a very low level by soaking in a sodium-free medium in which an isotonic mixture of sucrose and $MgCl_2$ had been substituted for NaCl. In this solution, some fibres still gave minute local action potentials while others appeared to be inexcitable. The addition of as little as 2 mM-TEA was sufficient to restore large propagated responses in some fibres (Fig. 11).

The effect of TEA was as prompt as that of choline, and equally quickly reversed by washing in crab Ringer, provided a moderate concentration (e.g. 50 mM) had been used. When TEA was applied in a concentration of 527 mM (to replace 513 mM-Na), return to crab-Ringer did not immediately reverse the whole effect, presumably because traces of TEA were still present. This was made probable because it was found that the addition to crab Ringer of only a few millimoles of TEA produced a large increase in the size and duration of the action potential.

Table 4 indicates that the resting potential was somewhat reduced in the solution containing 527 mM-TEA. This depolarization was not apparent immediately, but developed slowly over a period of many minutes, in contrast with the characteristic change in the response which was observed as soon as records could be taken. The depolarization was progressive and irreversible and eventually lead to complete inexcitability.

It was difficult to ascertain whether this slow and progressive action was due to TEA itself or to impurities such as traces of silver or other heavy metals. We used at first a commercial preparation of TEA chloride which certainly contained traces of Ag and other impurities, while

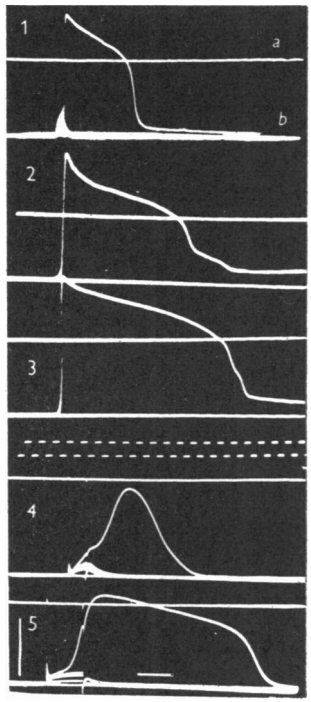

Fig. 9. Tetraethylammonium action potentials. Upper part: three different fibres in TEA solution (527 mM). Electrode separation: 0·2 mm in 1, 2·5 mm in 2, 1·6 mm in 3. Calibration sweeps: 50 c/s separated by 10 mV. Note: large excess of action potential over resting potential (shown as in Fig. 8). Lower part: effect of low concentrations of TEA. Record 4: muscle in a medium containing 4·4 mM-TEA (one part of solution *E*, Table 1, and 99 parts of *D* (sucrose + Mg)). Record 5: 22 mM-TEA (5 parts *E* + 95 parts *D*). Scales: 20 msec and 50 mV.

TABLE 7. Relative potencies of TEA and choline

Mean values, taken from three to four fibres of the same muscle. Measurements in Na made before and after application of R_4N (showing reversibility of effect). Action potential size: only propagated potentials included. Solutions were made up by mixing solutions *A* (≡100 Na) and *B* (≡100 R_4N).

Solution	Resting potential (mV)	Action potential (mV)	Active-membrane potential (mV)	Duration (msec)
(*a*) TEA				
100 Na	74	72	− 2	3·5
99 Na + 1 TEA	71	90	+19	22
90 Na + 10 TEA	70	103	+33	42
(*b*) Choline				
100 Na	77	69	− 8	3·3
90 Na + 10 choline	81	75	− 6	(4·5)
0 Na + 100 choline	73	82	+ 9	20

in later experiments the chloride was obtained from a commercial TEA iodide as described in 'Methods'. The immediate effect on the action potential was the same with either preparation, but the development of severe depolarization and inexcitability was much more rapid with the former (15–20 min) than with the latter (more than 1 hr).

Relation between TEA concentration and size of the electric response. The present experiments raise two separate problems: (*a*) why does crustacean muscle, unlike other nerve or muscle tissues (but see Lorente de Nó, 1949), remain excitable in the absence of sodium, and give action potentials of greater amplitude and rate of rise when certain quaternary ammonium ions are substituted for sodium? (*b*) What causes the enormous change in the

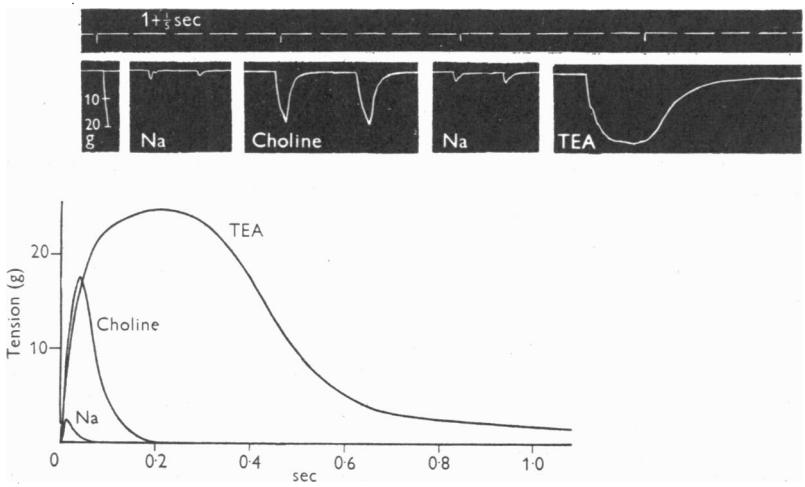

Fig. 10. Muscle contraction in Na, choline and TEA. Upper part: kymograph records of twitches of flexor of dactylopodite (*Portunus*), due to directly applied single induction shocks. Leg 'perfused' with Na, choline, Na and TEA. Lower part: superimposed drawings of contractions in the three media. Note: the Na twitch is very quick and recorded with some distortion.

duration of the electric response? The two phenomena do not necessarily depend upon a single factor: the first is concerned with the rise, the second with the fall of the action potential; and the recent work of Hodgkin & Huxley (1952) has shown that, at least in the giant axon of the squid and probably in many other systems, different types of permeability change are responsible for these two phases of activity. The squid axon becomes inexcitable in a sodium- (and lithium-) free medium, because sodium and lithium appear to be the only ions to which the axon membrane offers a negative resistance and which provide the inward current necessary for the rise of the spike. It is possible that, by a similar mechanism, sodium enters crustacean muscle fibres during the ascending limb of the action potential, but if this is the case the specific carrier mechanism of the membrane must operate even more effectively with choline and much more so with TEA.

To test this hypothesis, it is of interest to study the quantitative relation between external ion concentration and size of response. If the mechanism is similar to that of squid nerve, then a direct proportionality may be expected between maximum rate of rise and concentration, and a logarithmic relation between amplitude and concentration.

The difficulty with this experiment is that we have not yet found a completely 'inert' substitute for sodium, etc., in which crustacean muscle can be kept without either becoming partly depolarized or retaining some vestige of excitability. When NaCl was replaced by dextrose or sucrose the fibres became inexcitable, but the resting potential was substantially reduced (e.g. from 68 to 38 mV). During this treatment spontaneous slow contraction waves often appeared in individual muscle fibres of the type described by Rollett (1891) and Hürthle (1909).

With intermediate concentrations (e.g. $\frac{1}{4}$ NaCl + $\frac{3}{4}$ sucrose, or $\frac{1}{8}$ NaCl + $\frac{7}{8}$ sucrose) there was still appreciable depolarization (e.g. reduction of resting potential from 76 to 67 mV), but many fibres remained excitable. In some of these, the duration of the rising phase of the spike increased, and the action potential reached then the same or a somewhat greater amplitude than in normal sodium. The maximum rate of rise was always less in the Na-deficient medium, the reduction in five fibres, at a mean Na level of 0·3 of Ringer, being to 0·55 (varying between 0·37 and 0·7).

To remove the sodium completely and yet avoid drastic depolarization, a mixture of sucrose and excess $MgCl_2$ (plus usual amounts of KCl and $CaCl_2$) was finally adopted (see Methods, solution *D*). With this solution the resting potential often remained unaltered, but a small local response of a few millivolts amplitude was still present in some fibres and could not be reduced by repeated washing and stirring of the bath. Although this residual response was small, it was important to ascertain which, if any, of the external cations were essential for its occurrence. When removing, one by one, the different constituents of solution *D*, the most important ion appeared to be calcium, for its withdrawal caused a marked decline, or abolition, of the residual response, while a trebling of calcium concentration substantially increased its size. It may be added that presence of calcium was essential for the Na or TEA action potentials, which were greatly reduced or abolished, in a reversible manner, as soon as calcium was removed.

The effect of the high Mg concentration in solution *D* had to be checked separately: the replacement of 90 mM-Na by 59 mM-Mg (solutions *A* and *C* respectively) always diminished electric excitability, the rate of rise of the action potential was lower, and conduction of impulses ceased in many fibres.

In the experiment of Fig. 11, TEA was added to the bath (initially containing solution *D*) by replacing osmotically equivalent amounts of sucrose, retaining the concentrations of all other constituents. With 0·85 mM-TEA a greatly increased local action potential was obtained, while with 1·7 mM propagated responses (R.P. 73 mV, A.P. 55·5 mV) were recorded whose amplitude, rate of rise and duration increased as the concentration of TEA was raised further (e.g. with 435 mM-TEA, R.P. 75 mV, A.P. 95 mV). The relation, however, was not of the simple type predicted by the above hypothesis; for example, an increase of TEA concentration from 1·7 to 435 mM increased the rate of rise from 4·4 to only 24 V/sec (5·5 instead of 256 times) and the active-membrane potential by only 37·5 mV (instead of by 58 $\log_{10} 256 = 140$ mV).

Hence, the relation between TEA concentration and size of response certainly does not give unqualified support to the idea that the transfer of charge across the active fibre membrane is brought about by influx of TEA ions (or its less powerful substitutes). The finding of a residual trace of response in the presence of only Ca, Mg, K and Cl ions raised a further difficulty for this

hypothesis, and even more serious objections arose during a series of experiments in which the effect of tetrabutylammonium ions (TBA) was studied.

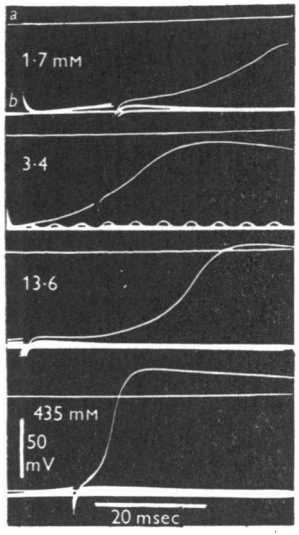

Fig. 11.

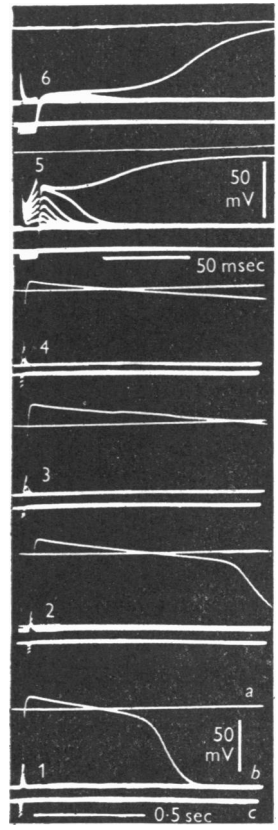

Fig. 12.

Fig. 11. Effect of TEA concentration on amplitude and rate of rise of action potential. Solutions are made up by mixing solutions *D* and *E* (Table 1). TEA concentrations (mM) shown in figure. *a*: 'zero potential' line; *b*: resting potential.

Fig. 12. Tetrabutylammonium action potentials. Upper beam shows resting (*a* and *b*) and action potentials, lower beam (*c*) shows applied brief current pulses. Records 1–4 (note slow time base) were obtained, successively, and from different fibres, in TBA (527 mM), record 1 after 7 min soaking, record 4 after another 7 min. There was barely visible contraction associated with the electric responses in 1 and 2, and no visible contraction during 3–6. Records 5 and 6 (faster time base) were obtained from another fibre, after 2½ hr soaking in a solution containing 87 mM-TBA (mixture of solutions *D* and *E*, Table 1). Separation between internal stimulating and recording electrodes: 0·45 mm (5) and 2·2 mm (6).

Tetrabutylammonium (TBA)

The effect of this substance differs from those of choline and TEA in several respects (see Fatt & Katz, 1951*b*, 1952), the most interesting being the irreversibility of its action. As a substitute during the excitation process, it

is less effective than the other ions, the maximum rate of rise of the TBA potential being much less (Table 4). But in prolonging the action potential, TBA is the most powerful agent which we have found: single electric responses lasting up to 18 sec (13° C) were observed.

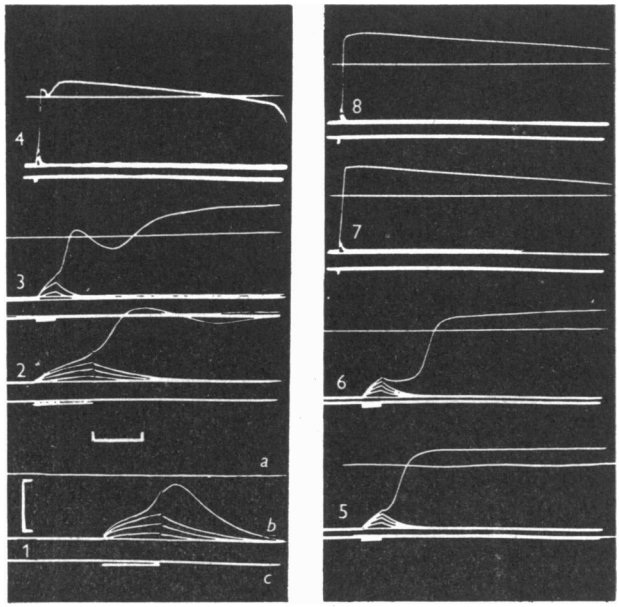

Fig. 13. Action potentials at low TBA concentrations. The muscle had first been made 'inexcitable' by soaking in solution *D* (Table 1). The bath was then replaced by mixture of 1/10 solution *E*, 9/10 solution *D* (containing 43 mM-TBA) and record 1 was obtained (time scale 10 msec; voltage scale 50 mV applying to all records). Records 2, 3 and 4 show action potentials, at different sweep speeds, obtained after replacing bath by 1/5 solution *E*, 4/5 solution *D* (time scale 10 msec for 2, 30 msec for 3, 232 msec for 4). Records 5–8 were obtained later in the same solution (time scale 30 msec for 5 and 6, 232 msec for 7 and 8). Note transient double-response in 2–4, later replaced by single slow action potential. *a* and *b* show resting potential; *c* applied current pulse.

TBA has several effects which develop in a slow and progressive manner, the time course depending upon the concentration. There are three main actions which appear in the following sequence: first, a large prolongation of the action potential (Figs. 12–14). This becomes noticeable immediately if TBA is used at a concentration of about 500 mM. At 100 mM, 30–45 min may elapse before the same degree of action is observed. In either case, returning the preparation to crab-Ringer, at any time, only tends to arrest the effects at the stage which they had reached, without reversing them. At an early stage, the action potential often shows a two-step, or double-peaked rise (Fig. 13): a fast spike component, followed by a slower rise to a plateau. Later,

during the experiment, the initial spike disappears, and a simple pulse-shaped action potential of relatively long latency, low rate of rise and long duration remains. It is difficult to account for the two-step response except by assuming that all parts of the membrane are not uniformly affected at all stages, e.g. the initial spike may be due to a membrane area upon which TBA has not yet exerted its full effect.

While the lengthening of the action potential was in progress, a second even

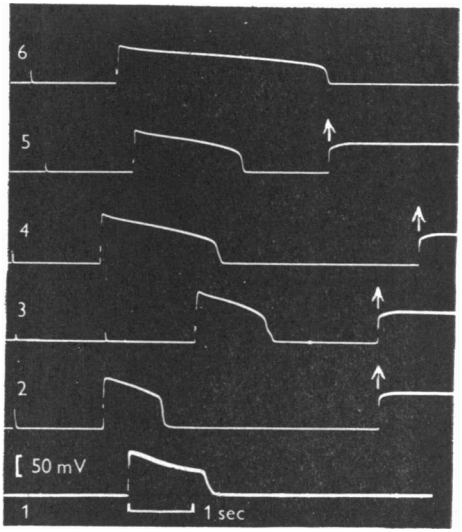

Fig. 14. Irreversible action of TBA. Fibres had been given the following treatment: (1) after preliminary immersion in 527 mM-TBA, muscle had been immersed and repeatedly washed in Na-Ringer; (2) the muscle was in a bath containing 109 mM-TBA, but no Na (mixture of one part of solution *E* and three parts of *D*, Table 1); (3) after previously soaking as in (2) muscle was washed and kept in solution *D*, containing neither TBA nor Na; (4) and (5), same treatment as in (3), but after adding 0·1% procaine hydrochloride; (6) same treatment as in (3) but after adding 0·5% procaine hydrochloride. At the arrows, the internal electrode was withdrawn from the fibre.

more surprising phenomenon made its appearance: the mechanical response (which had lengthened with the action potential) gradually vanished, and after a while the muscle gave prolonged action potentials of large amplitude without being able to contract. Return to crab-Ringer did not alter this situation which, from an experimental point of view, was very favourable as it eliminated the possibility of movement artifacts. Almost all the records in Figs. 12–15 were obtained from fibres whose contractile response had been abolished or reduced to a barely visible microscopic movement.

The third effect was an even more slowly developing depolarization, the resting potential falling gradually to less than 30 mV and the fibres becoming

electrically inexcitable. This whole sequence of events took some 20 min, with 527 mM-TBA at 20° C, but could be spread out over a much longer period by working at a lower temperature or lower TBA concentration. To avoid the last stage, viz. severe depolarization and inexcitability, it was best to start with 100–250 mM-TBA and return to Na as soon as the mechanical response had fallen to a conveniently small size.

Once the TBA solution had acted long enough to produce the characteristic changes in the action potential, the bath solution could be replaced by Na-Ringer, or by solution *D* (sucrose-Mg-Ringer) which contained neither TBA nor Na, without reversing the effect (Fig. 14). Resting and action potentials usually diminished somewhat when the sucrose-$MgCl_2$ mixture was applied, but there was no doubt that many fibres continued to propagate typical TBA potentials under these conditions without TBA or Na in the external medium. This is an important point because it makes it difficult to believe that influx of external Na or TBA can provide the transfer of charge across the active fibre membrane. It seems from the present experiments that TBA alters the response of the fibre by combining irreversibly with receptors in its surface. An obvious corollary is that TEA, choline and Na act on the same receptors, but in a reversible manner. Unfortunately, this leaves completely unanswered the question as to the identity of the ions which carry the inward current through the active membrane.

Changes of membrane resistance during the prolonged TBA response. The 'rectangular' shape of the action potential in TBA-treated crustacean muscle resembles rather closely that of vertebrate heart muscle (Weidmann, 1951; Woodbury, Hecht & Christopherson, 1951). It would be interesting to know what causes an action potential to broaden from a brief spike to a long-lasting pulse, and especially which factors are responsible for the maintenance of the plateau and its abrupt termination. It has been shown by Weidmann (1951) that during the plateau of the heart action potential, the membrane resistance has recovered from its initial 'breakdown'; in fact, the plateau can be described as a stable depolarized state, which is very similar to the resting state except that the excitability of the membrane has reversed its electric sign. Thus, a sufficiently strong inward (i.e. 'anodic') current pulse 'excites' the membrane and causes it rapidly to *repolarize* to its normal level of stability. In these respects, the TBA potentials resemble the responses of the mammalian heart studied by Weidmann (1951). We have used a similar method, viz. modulating the membrane potential by the application of sub-threshold current pulses (cf. Fig. 15), a third intracellular electrode being used for this purpose. A qualitative indication of the changes in membrane resistance is obtained from the amplitude and time course of the modulating potential. At the beginning of the plateau, this potential was much diminished, and rose and fell more rapidly than at rest, suggesting that the membrane

resistance at the height of the action potential was considerably less than at rest. This was followed by a gradual recovery, though often the membrane resistance remained below normal throughout the duration of the plateau. As its termination approached and the rate of fall of the action potential began

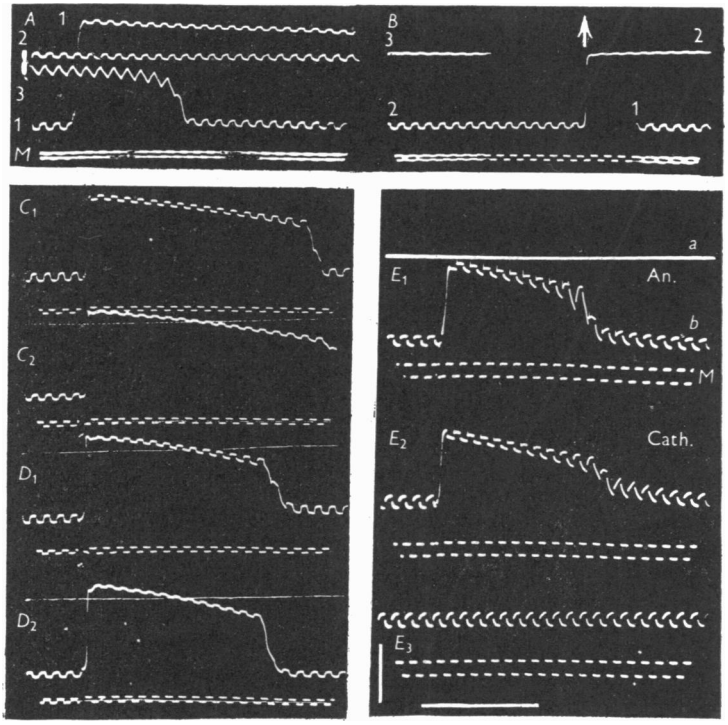

Fig. 15. Changes of membrane resistance during TBA action potential. Three intracellular electrodes used for stimulating, pulsing and recording respectively. *A* and *B*: records from one fibre giving a long action potential (about 7 sec) of which successive parts are shown in *A*, in three superimposed sweeps (1, 2 and 3). *M*: pulse monitor. In *B*, recording electrode withdrawn from resting fibre during second sweep (at arrow). Distance between pulsing and recording electrode 0·5 mm. C_1 and C_2: two records from one fibre, with 'pulse-to-recording' distance 0·2 mm (C_1) and 0·7 mm (C_2). D_1 and D_2: same from another fibre. *E*: anodic pulsing in E_1 (as in *A*, *C* and *D*). Note inverted 'partial response' during fall of action potential. Cathodic pulsing in E_2 (note prolongation of response, as compared with E_1). E_3: cathodic pulsing in resting fibre. *a*: 'zero-potential' line; *b*: modulated 'base-line'. Scales: 0·5 sec and 50 mV.

to increase, the membrane resistance appeared to become greater than normal (see Fig, 15, records *A*, *E*), but this may be attributed to a regenerative potential change, i.e. a local electric response which occurs near threshold in the usual manner, except that in the present case the polarity of the response is reversed. The occurrence of abortive repolarization, which takes off at a

certain level with a typical inflexion is evident in records A and E_1 (Fig. 15). The resemblance between these observations and Weidmann's findings is close, though the underlying ionic movement is probably not the same.

The depolarization caused by TBA. Although the ultimate depolarization, and inexcitability, of the TBA-treated muscle appeared to be a secondary phenomenon, its mechanism is of interest because it might throw some light on the other actions of this substance. It is conceivable, for instance, that a process which, in the end, manifests itself in irreversible depolarization, may be operative in a transient manner during the plateau of the action potential. In several fibres, the membrane constants were determined during the progressive action of TBA. The pertinent result was that the membrane resistance continued progressively to *increase* while the resting potential was falling. In Table 8, a summary of the mean results from three fibres of one muscle is given, which shows that during a progressive decline of the resting potential, from 66 to 38 mV, the membrane resistance rose from 133 to 521 $\Omega\,cm^2$. The membrane capacity and internal resistivity did not change significantly. In another experiment, a 'sodium-muscle' had been made inexcitable and its membrane resistance reduced to a low value by 'pre-heating' to 32° C (see § I); application of TBA temporarily restored excitability and progressively raised the membrane resistance from 37·5 to 141 $\Omega\,cm^2$ (20° C), while the resting potential fell from 78 to 27 mV.

TABLE 8. Progressive effect of TBA on membrane resistance

Mean values from three fibres of the same muscle. Solutions A (for Na) and E (for TBA, cf. Table 1) were used in this experiment. The effect of TBA is not reversed by the final return to Na.

Temp. (° C)	Principal cation	Resting potential (mV)	R_m ($\Omega\,cm^2$)
12	Na	66	133
16·5	TBA (+Mg) after 18 min	49	314
18	TBA (+Mg) after 45 min	45	333
19·5	Na	38	521

Qualitatively, a similar effect, viz. partial depolarization accompanied by a large increase of membrane resistance, had previously been observed as a result of lowering the temperature (§ I), and it is possible that a common mechanism is involved in all these changes, for example a decrease of potassium permeability. It is conceivable that one of the principal actions of TBA is a large reduction of 'potassium conductance' (see Hodgkin & Huxley, 1952), which might be an important factor contributing to the enormous prolongation of the action potential.

To a lesser extent, the same kind of change (e.g. reduction of K permeability) may be produced by the other quaternary ammonium ions, for in an

experiment in which Na was replaced by choline the membrane resistance increased from 100 to 190 $\Omega\,cm^2$, while the resting potential fell slightly, from 72 to 69 mV.

Effect of temperature on TBA potentials. In a few experiments, the effect of temperature on the TBA response was studied, between 0 and 22° C. There were no obvious changes in amplitude, but the duration of the action potential varied in an interesting manner, reaching a maximum between 10 and 15° C, and falling sharply when the temperature was raised or lowered. Thus, in one experiment, the TBA response lasted for 0·5 sec at 0° C, 1·2 sec at 4·5° C, reached 4–6 sec at 11–15° C and fell to 1·5 sec at 20° C.

Other substances

The effects of several other ions which were used to replace sodium will be described briefly.

Acetylcholine. When this substance was added in a concentration of 1–5 mM to 'sucrose-$MgCl_2$-Ringer' (solution *D*), restoration of local action potentials was observed, but the effect was less powerful than that of equal concentrations of TEA. Thus, there was no evidence for any *specific* action of acetylcholine on crustacean muscle.

Diethanoldimethylammonium. This substance, which we obtained through the courtesy of Dr Lorente de Nó, was used to replace sodium entirely. Its action was found to be similar to that of choline though somewhat less potent. The results of one typical experiment (a raised Mg concentration was used throughout in this case) are shown in Table 9.

TABLE 9. Effect of diethanoldimethylammonium

Principal cation (solutions *C* and *E*, cf. Table 1)	Resting potential (mV)	Action potential (mV)	Duration (msec)
Na	65	Very small, local	
Diethanoldimethylamm.	69	62	7
Choline	64	76	11

Tetramethylammonium. The effect of this ion was comparable to that of choline, but in addition a slowly developing depolarization was observed. A similar result was obtained with phenyltrimethylammonium.

Benzyltrimethylammonium. The initial effect of this substance was similar to that of choline, but with prolonged soaking (1–2 hr) the action potential became very long (up to 800 msec), and like the TBA response could not be shortened by return to sodium-Ringer. There was also a slowly developing depolarization.

Procaine hydrochloride. This substance, though not a quaternary ammonium salt, is largely in the form of a substituted ammonium ion when dissolved in the crustacean Ringer. It was first used in concentrations of 0·1–0·5% (3·7–18·3 mM) in an attempt to 'paralyse' the fibres and abolish the prolonged

action potentials which were obtained, after preliminary treatment with TBA, in the 'sucrose-Mg' soaked muscles. The result, however, was a further lengthening of the response. In other experiments, 18·3 mM-procaine HCl was applied to sodium-muscles, the result being an increase in the amplitude and duration of the action potential (e.g. from 55 to 68 mV, and from 3 to 45 msec). Some fibres which gave only a small local response in 'sodium-Ringer' were found to give large conducted action potentials when as little as 1·8 mM-procaine HCl was applied. The substance appears, therefore, to be as effective as TEA, and more potent than choline, in its 'restoring' and intensifying action. This was further confirmed by the following observation: when 18·3 mM-procaine HCl was added to 'choline-Ringer', the duration of the action potential increased from 16 to 190 msec.

It seemed at first very surprising that a 'local anaesthetic' like procaine serves to activate the excitatory process of crustacean muscle fibres, but it falls into line with the previous evidence suggesting that we are dealing here with a different mechanism. It should, however, be recalled that a similar, though transient, restoring effect of procaine has previously been observed by Lorente de Nó (1950) in frog's nerve.

Other substances. Regarding other effects it is worth mentioning that lithium could be substituted for sodium without changing the response. A medium in which one-third of NaCl had been replaced by an isotonic solution of trimethylamine oxide produced no change; in another experiment in which betaine was used instead of one-third of NaCl, the fibres became gradually depolarized. The last two substances, though virtually unionized at a pH of 7·6, were of interest as they are known to be present in crustacean blood.

The action of calcium withdrawal in reversibly reducing or abolishing the electric response has already been mentioned (p. 192).

DISCUSSION

The purpose of these experiments was to gain some information on the electrical properties and excitability of crustacean muscle fibres, before proceeding with a study of their neuromuscular mechanism. In the course of this work some unexpected problems have been raised.

It is clear that crustacean muscle is made up of individual fibres which can respond to electric stimuli as units, with propagated action potentials of all-or-none type and of customary electric sign. There is also no doubt that the resting fibres have electric properties similar to those of other nerve and muscle cells: they are negatively charged, the resting potential is of the same order of magnitude, it depends on the external potassium concentration, and the subthreshold behaviour of the fibres is that of a highly-leaky and capacitative cable.

In two important respects, however, crustacean muscle fibres seem to be made up differently from other excitable tissues. The membrane capacity is so large that it is difficult to picture the underlying structure and its physical dimensions. A capacity of 40 μF/cm^2 is outside the order of magnitude which has previously been found in biological tissues. There are several factors which might contribute to this result: for example, a large 'dielectric constant' (concerning the definition of this term and its dependence on the phase angle of the capacity, see Cole, 1949) or excessive folding and convolutions of the surface membrane. There is evidence for a wide-spread innervation of these fibres (see van Harreveld, 1939), and it is conceivable that their surface may be deeply indented by numerous clusters of nerve endings causing the true surface area to be much larger than we have estimated. One may hope that this matter can be put to a test with the help of the electron microscope. It may be of importance also in other respects, for a 'glomerular' surface structure would introduce 'bottle-necks' and slow the diffusion of solutes between the bath and parts of the membrane surface.

The second unexpected finding was the maintenance of excitability in the absence of external sodium and the enlargement of the electric response by various quaternary ammonium ions. These observations are evidently related to the recent finding of Lorente de Nó (1949) who showed that a group of small nerve fibres of the frog remain excitable and conduct a modified action potential when sodium is replaced by TEA and certain other quaternary ammonium ions. The situations differ in important details: for example, choline and diethanoldimethylammonium ions, which do not sustain the excitability in frog axons (Lorente de Nó, 1949), were found to be more powerful than Na, though less potent than TEA, in supporting the electric activity of crustacean muscle fibres.

Two obvious question arise from these experiments: what is their bearing on the normal activity of the muscles in the living animal? Secondly, what is the mechanism of the action potential in crustacean muscle?

To the first question the answer seems fairly simple. The normal activity of crustacean muscle is built up largely by non-propagated responses (Wiersma, 1941; Katz & Kuffler, 1946) which cause effective development of tension, presumably because nerve endings are distributed along the whole length of the fibres (van Harreveld, 1939). The latter observation has been disputed (Holmes, 1943), and some doubt was expressed by Katz & Kuffler (1946) because sharp foci of 'end-plate potentials' were often found in crab muscle. We have recently re-examined the spatial distribution of the crustacean 'e.p.p.'s' with the help of intracellular recording and found that the variations in amplitude of these potentials along the length of a normally innervated fibre are only of the order of 40%. Thus it appears that many crustacean muscle fibres are innervated, at somewhat varying density, from

end to end. The generation of an all-or-none spike in such a fibre would only be called for when a sudden maximum effort is demanded; the effect of the spike would be to *eliminate inequalities* in the strength of response along the fibre, rather than to propagate excitation over long distances. Whether the crab depends for this purpose entirely on the sodium content of its haemolymph, or has available some more powerful substance which, like TEA, acts in low concentration is a question which remains to be decided.

The mechanism of the action potential, and the species of ions involved in the movement of charge across the membrane, remain a puzzling problem. It would not be very difficult to believe that in some tissues a number of univalent cations beside lithium can take the place which sodium normally occupies (see Hodgkin, 1951) and give rise to an action potential by entering the fibre as it becomes depolarized. But the difficulty arises with the observation that, after TBA, prolonged large action potentials occur in the presence of only calcium, magnesium and potassium chloride in the external medium.

There appear to be two possible ways of accounting for this observation: (i) it may be that TBA remains adsorbed to the fibre surface, but is mobilized during excitation and temporarily transferred into the cell interior; (ii) alternatively, influx of calcium or magnesium, or outflux of some internal anion may be responsible for the transport of charge. The second explanation would imply that in crustacean muscle, sodium and the quaternary ammonium ions play only an indirect role during the production of the action potential, 'conditioning' the excitatory reaction of the membrane without themselves being carriers of the action current. This may seem contrary to the convincing evidence for a direct sodium mechanism which has been obtained in cephalopod axons, and which very probably operates in the same manner in crustacean nerve, but there is no reason to believe that this mechanism is universal.

SUMMARY

1. The electrical properties of crustacean muscle fibres were examined with the help of intracellular stimulating and recording electrodes.

2. The fibres have a resting potential of about 70 mV at 20° C, the cell interior being negative to the surrounding bath. This p.d. varies with temperature (falling to about 60 mV at 0° C) and with the external K concentration, being about 90 mV in K-free saline and reversing to minus 1–2 mV in isotonic KCl solutions.

3. The membrane capacity of the resting muscle fibres is very large, about 40 μF/cm^2, and their membrane resistance very small, about 100 Ω cm^2. Lowering the temperature increases the membrane resistance, about 2·5 times for a change of 10° C, but has no significant effect on membrane capacity.

4. The electric response to a direct 'cathodic' current pulse varies greatly; in many muscle fibres only small non-conducted action potentials are observed.

In other fibres propagated spikes occur of about 60 mV amplitude, rising at a maximum rate of 20 V/sec and travelling at a speed of 30 cm/sec.

5. When the sodium content in the muscle bath is entirely replaced by certain quaternary ammonium ions, electric excitability of the muscle fibres is not only maintained, but a large increase is observed in the amplitude and duration of electrical and mechanical responses.

6. Using choline as a Na substitute, the action potential attains an amplitude of 80 mV, and its waveform changes from a brief spike to a long rectangular pulse of about 25 msec duration.

7. With tetraethylammonium as a Na substitute, the action potential increases to approximately 100 mV in height and 100 msec in duration.

8. With tetrabutylammonium, action potentials lasting for several seconds were recorded. The effect of tetrabutylammonium is irreversible, the enormously lengthened action potential being still obtainable when the bath has been replaced by a solution containing only calcium, magnesium and potassium chloride and sucrose.

9. The following problems have been discussed: (i) the interpretation of the large membrane capacity and its effect on impulse propagation, (ii) the mechanism of electric excitation of the crustacean muscle fibre, the ionic currents underlying it, and its role during normal muscle activity.

We are indebted to Mr J. L. Parkinson for his unfailing assistance. This work was carried out with the aid of a grant for scientific assistance made by the Medical Research Council.

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