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# RESTING AND SPIKE POTENTIALS OF SKELETAL MUSCLE FIBRES OF SALT-WATER ELASMOBRANCH AND TELEOST FISH

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## SUMMARY

1. Membrane properties of the muscle fibre were studied in twitch motor system of sea-water elasmobranch (*Taeniura lymma*, *Himantura uarnak* and *Pastinachus sephen*) and teleost fish (*Periophthalmodon barbarus*, *Tetradon immaculata*, *Hemiramphus welsby*, *Parexocoetus brachypterus* and *Conger labiatus*).

2. The resting potential of the elasmobranch fibre is mainly determined by the $Cl^-$ concentration difference between inside and outside the membrane whereas the $K^+$ conductance is the determining factor in teleost fibres.

3. The resting membrane of the elasmobranch fibre is permeable not only to $Cl^-$ ions but also several other anions ($Br^-$, $I^-$, $NO_3^-$, $SCN^-$, $ClO_4^-$, $ClO_3^-$) of large limiting conductivities in the aqueous solution.

4. The spike potential of the elasmobranch fibre always shows a significant overshoot in normal saline while no significant overshoot is generally found in teleost fibres.

5. In both elasmobranchs and teleosts the spike is produced by the permeability increase of the membrane to $Na^+$ ions and is effectively suppressed by tetrodotoxin at a concentration of $0{\cdot}5$–$1{\cdot}0 \times 10^{-7}$ g/ml. of the external solution with one exception, i.e. the $Na^+$ spike of *Tetradon* fibre is not suppressed by the toxin even when the concentration is above $5 \times 10^{-4}$ g/ml.

## INTRODUCTION

Electrical properties of the muscle fibre membrane have been studied extensively in amphibian skeletal muscle (Ling & Gerard, 1949; Nastuk & Hodgkin, 1950; Adrian, 1956; Hodgkin & Horowicz, 1959). However, little is known about the properties of skeletal muscle fibres of fish. The present

paper is concerned with the analyses of resting and spike potentials of muscle fibres in salt water elasmobranch and teleost fish. The results have been discussed from the comparative viewpoint.

## METHODS

*Elasmobranchs.* For these experiments pelvic fin muscle preparations of three species of stingrays commonly found in the Great Barrier Reef of tropical Australia were used. They were blue spotted lagoon ray (*Taeniura lymma*), coach-whip ray (*Himantura uarnak*) and fantail ray (*Pastinachus sephen*). The fish were usually speared by native hunters. Pelvic fins were removed from the fish immediately after spearing and brought to the laboratory in cold elasmobranch saline.

A group of muscles located at the rostral and proximal part of the pelvic fin was always used. Since these muscles are loosely connected to the skin they can be prepared with little damage to the surface fibres. One muscle bundle was isolated from the fin with the attached cartilage, and the connective tissues covering the muscle surface were carefully removed. The diameter of the fibres measured in fresh preparation are shown in Table 1*A*. The fibre diameter is particularly large in Blue spotted lagoon rays. Therefore, the preparation offered very favourable material for the study of membrane physiology. Membrane potentials were recorded as the potential difference between two 3 M-KCl filled micropipettes, one inside and the other just outside the fibre. A third micropipette was often introduced into the fibre to apply currents across the membrane. The intensity of the current was monitored by recording the voltage drop across a resistor between the bathing solution and earth.

The composition of the normal and NaCl elasmobranch salines (*A* and *B*) is shown in Table 2*A*. The $Na^+$ concentration was altered by replacement with choline. The $Cl^-$ was replaced with the anions, methanesulphonate, $SCN^-$, $N_3^-$, $NO_3^-$, $Br^-$, $I^-$, $F^-$, acetate, or $ClO_4^-$, etc. When the external saline was changed the preparation was washed quickly four times with the new test solution within about 1 min.

*Teleosts.* For these experiments fin muscles of several teleosts commonly available in the Great Barrier Reef of tropical Australia were used. They were giant mudhopper (*Periophthalmodon barbarus*), a certain species of toados fish (*Tetradon immaculata* var. *virgatus*), flat-sided gar-fish (*Hemiramphus welsby*), short-finned flying fish (*Parexocoetus brachypterus*) and Australian conger eel (*Conger labiatus*). They were either caught from the deck of the laboratory vessel or netted on the coral reef.

The pectoral fin was removed together with *cleithrum* and *coracoidea* and the *Mm. adductor superficialis et profundus* were exposed. Some fibres in these muscles were white whereas others were more or less red. The electrical properties of these two groups of fibres, however, seemed to be similar. The diameters of the muscle fibre for these species are shown in Table 1*B*. The strong pectoral fins of giant mudhopper enable these fish in locomotion on land and those of the flying fish are used for gliding in air. The techniques for recording membrane potential and applying membrane current were similar to those used for elasmobranch muscle fibres. For the *Conger* preparation the same internal micropipette was used for the recording and polarization by the aid of the electronic circuit described by Takahashi (1965), since the diameter of the fibre was too small (15–30 $\mu$) to use two micropipettes.

The composition of the normal saline used for teleosts is shown in Table 2*B*. Choline or methanesulphonate was used to replace $Na^+$ or $Cl^-$. Tetrodotoxin used was supplied by Sankyo Co., Ltd., Tokyo. The experiments were carried out at room temperature (20–22° C).

TABLE 1. Diameter of the muscle fibre

| | Species | Diameter ($\mu$) |
|---|---|---|
| *A* | *Pastinachus sephen* | 50–80 |
| | *Himantura uarnak* | 50–100 |
| | *Taeniura lymma* | 140–200 |
| *B* | *Periophthalmodon barbarus* | 50–80 |
| | *Hemiramphus welsby* | 50–100 |
| | *Parexocoetus brachypterus* | 30–60 |
| | *Tetradon immaculata* | 50–100 |
| | *Conger labiatus* | 15–30 |

TABLE 2. Composition of salines

| *A* | NaCl (mM) | Urea (mM) | KCl (mM) | K-MS* (mM) | $CaCl_2$ (mM) | Ca-G** (mM) |
|---|---|---|---|---|---|---|
| Normal elasmobranch saline | 281 | 345 | 12 | — | 10 | — |
| NaCl elasmobranch saline (*A*) | 454 | — | 12 | — | 10 | — |
| NaCl elasmobranch saline (*B*) | 454 | — | — | 12 | — | 10 |
| *B* | | | | | | |
| Normal teleost saline | 231 | — | 2 | — | 6 | — |

The pH was adjusted to 7·7 for elasmobranch salines and 7·1 for teleost saline by adding 10 mM tris-maleate plus the required amount of NaOH.

* K-methanesulphonate. ** Calcium gluconate.

## RESULTS

### *Elasmobranchs*

*$Cl^-$ and $K^+$ permeabilities of the resting membrane.* The resting potential of the stingray muscle fibre ranged between $-70$ and $-75$ mV when the preparation was surrounded with the normal elasmobranch saline which contained 345 mM urea. The resting potential became about 10 mV more negative when the urea in the saline was replaced with an osmotically equivalent amount of NaCl. This shift was reversed on returning the muscle to the normal elasmobranch saline. However, no change of resting potential occurred when the urea was replaced with Na-methanesulphonate. This indicates that the change of the resting potential found for the replacement with NaCl is due to the change in the external $Cl^-$ concentration. When the NaCl in the saline was replaced with an osmotically equivalent amount of either Na-methanesulphonate or urea the resting potential shifted in the positive direction. These experiments show that the resting potential of the stingray muscle fibre is sensitive to the external $Cl^-$ concentration. In order to find out the relation between the resting potential and the $Cl^-$ concentration the resting potentials were observed when varying amounts of the NaCl in NaCl elasmobranch saline (*B*) was replaced with Na-methanesulphonate and they were plotted as a function of the $Cl^-$ concentration (logarithmic scale) in Fig. 1*A* (filled circles). Each circle

represents a mean value of resting potential obtained from 6 to 7 different fibres as measured within a few minutes of changing the solution. Occasionally the preparation was brought back to the normal saline to check whether any long term change had occurred. The same figure shows the

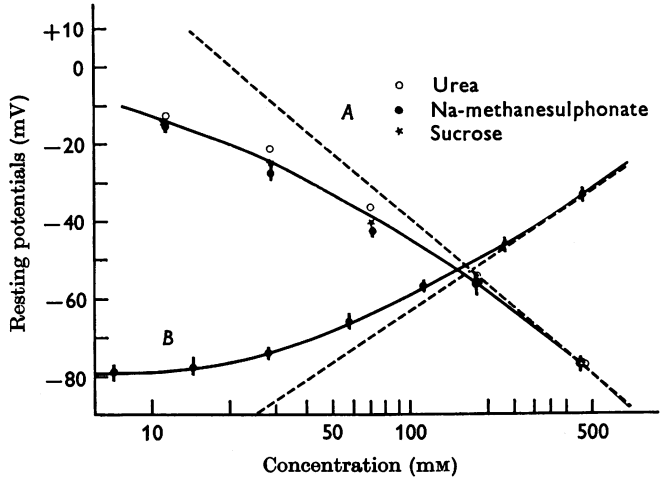


Fig. 1. Relations between the resting potential of the elasmobranch muscle fibre and the external $Cl^-$ (*A*) and $K^+$ concentrations (*B*). Open and filled circles, and stars in *A* represent the data obtained by substituting the NaCl in the NaCl elasmobranch saline (*B*) with urea, Na-methanesulphonate and sucrose respectively. For *B* the $K^+$ concentration was altered by replacing NaCl in NaCl saline (*A*) with KCl. *Taeniura lymma*. The vertical bar for each circle shows the standard deviation. Interrupted lines indicate 58 mV slope for a 10 fold change in the concentration.

results obtained from the same preparation as that used for the methanesulphonate substitution when the external $Cl^-$ concentration was altered by replacing NaCl in the NaCl elasmobranch saline (*B*) with urea (open circles) and sucrose (crosses). The result shows that the resting potential was a function of the external $Cl^-$ concentration and the relation was independent of the substitute used for $Cl^-$. The change of the resting potential per 10-fold change in $Cl^-$ concentration increased with increasing concentration and in the neighbourhood of the $Cl^-$ concentration of the normal elasmobranch saline (313 mM) it was about 50 mV. In these experiments the $K^+$ concentration was kept constant at 12 mM throughout.

The relation between the resting potential and the external $K^+$ concentration when a part of the NaCl in the NaCl elasmobranch saline (*A*) was replaced with KCl is shown in Fig. 1*B*. In this experiment the $Cl^-$ concentration was kept constant (486 mM). The slope of the relation was about 45 mV/10-fold concentration change at high $K^+$ concentrations and became smaller as the concentration was decreased. In the neighbourhood

of the normal $K^+$ concentration (12 mM) it was found to be about 7 mV. When the $K^+$ and $Cl^-$ concentrations were altered in such a way as to keep the product $[Cl^-]_o.[K^+]_o$ constant, the resting potential showed a linear relation to the logarithm of the concentration with a slope of 58 mV/10-fold

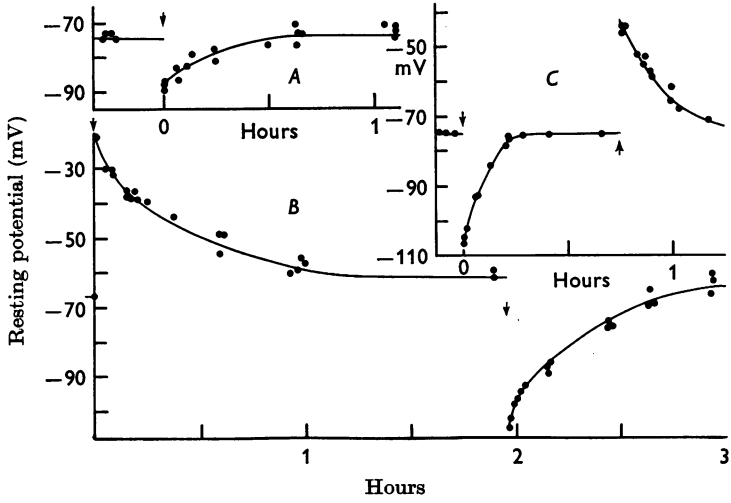


Fig. 2. Time course of the resting potential of the elasmobranch muscle fibre following the change of the external solution. *Taeniura lymma*. *A*. Change from the normal elasmobranch saline to the NaCl elasmobranch saline (*A*). *B*. 454 mM-$Cl^-$ in the NaCl elasmobranch saline (*B*) was reduced to 12 mM at first and then back to 454 mM again by substituting NaCl with Na-methanesulphonate. *C*. The $Cl^-$ in the NaCl elasmobranch saline (*B*) was replaced with $SCN^-$ and then back to $Cl^-$ again. Arrows indicate the moments of exchange of the external solution.

change in the concentration. In this experiment the resting potential became zero when $[K^+]_o$ was about 360 mM. Therefore, the internal $K^+$ concentration of the fibre in the normal saline can be considered equal to 360 mM which is close to the external $Cl^-$ concentration (313 mM) of the normal saline. It has been mentioned above that the resting potential changes with $[Cl^-]_o$ with a slope of about 50 mV in the neighbourhood of the normal external $Cl^-$ concentration. The corresponding slope for $K^+$ ions is about 7 mV. Since $[K^+]_i$ is very close to $[Cl^-]_o$ the ratio between these slopes (about 7) should be roughly equal to the permeability ratio $P_{Cl}/P_K$ (Hodgkin & Horowicz, 1959). This indicates that the resting membrane is far more permeable to $Cl^-$ ions than to $K^+$ ions.

*Long-term change of the resting potential.* The foregoing results on the resting potential were concerned only with the immediate change following the alteration of the external $Cl^-$ or $K^+$ concentration from the normal value. As described before the resting potential became about 10 mV more

negative just after the replacement of the urea in the normal saline with NaCl. If the preparation was kept in this solution the resting potential slowly returned to the original value found in the normal saline (Fig. 2*A*). A plot of the resting potential as a function of elapsed time from the moment at which the test solution was applied is shown in Fig. 2*A*. Each circle represents a single measurement of a different fibre in the same preparation. The half decay time of the potential change was about 11 min. A similar plot after the external $Cl^-$ concentration was reduced from 454 mM (NaCl saline, *B*) to 12 mM by substituting NaCl with Na-methanesulphonate is shown in Fig. 2*B*. The initial positive shift of the resting potential was followed by a slow return to the original value. After the potential returned to almost the original level the preparation was brought back to the original high $Cl^-$ medium (454 mM) and this resulted in a negative shift of the resting potential which also delayed with a slow time course. The half decay time of the potential change was about 15 min after the decrease of the $Cl^-$ concentration and about 14 min after the increase. The times were not much different from those found for the change associated with the replacement of urea with NaCl.

Similar results have been found for frog muscle fibres (Hodgkin & Horowicz, 1959) and also for crayfish muscle fibres (Giradier, Reuben & Grundfest, 1961; Grundfest, 1962). Although the membrane is less permeable to $K^+$ ions than it is to $Cl^-$ ions, KCl and water move across the fibre membrane when $[Cl^-]_o.[K^+]_o$ is altered. The movement ceases when $[Cl^-]_i.[K^+]_i$ becomes equal to $[Cl^-]_o.[K^+]_o$. Since $K^+$ ion is the major cation inside the fibre the movement of KCl results in little change in $[K^+]_i$, whereas the change in $[Cl^-]_i$ is significant. When the preparation is equilibrated to the original solution $[K^+]_i.[Cl^-]_i = [K^+]_o.[Cl^-]_o$, therefore the $Cl^-$ equilibrium potential is equal to the K-equilibrium potential. After changing the solution, this condition once again prevails, after a suitable delay and since $[K^+]_o$ and $[K^+]_i$ are not altered the membrane potential is the same as found originally. Thus the resting potential should return to the original level during the prolonged immersion in the test solution if the $K^+$ concentration is common between the control and test solutions.

The magnitude of the resting change in potential following an increase of the $Cl^-$concentration was usually the same as that following a decrease in concentration (Fig. 2*B*). In this experiment the change in $Cl^-$ concentration from 454 to 12 mM was followed by a positive shift of the potential of about 45 mV and the change of 12 to 454 mM caused a negative shift of about 44 mV. This result contrasts with those found in frog muscle fibres (Hodgkin & Horowicz, 1959) and crayfish muscle fibres (Giradier *et al.* 1961) in which the potential change shows a marked asymmetry, i.e. the amplitude

of the change for the increase in the $Cl^-$ concentration is always much smaller than that associated with the decrease. According to Hodgkin & Horowicz (1959) this is due to the rectifying property of the $K^+$ channel in the resting membrane. The $K^+$ conductance increases as the resting potential becomes more negative, and under these conditions the change of the $Cl^-$ equilibrium potential has less effect on the potential. The symmetrical relation found in the stingray muscle fibre may be due to the fact that the $Cl^-$ conductance is far greater than the $K^+$ conductance or to the absence of the rectifying property of the $K^+$ conductance.

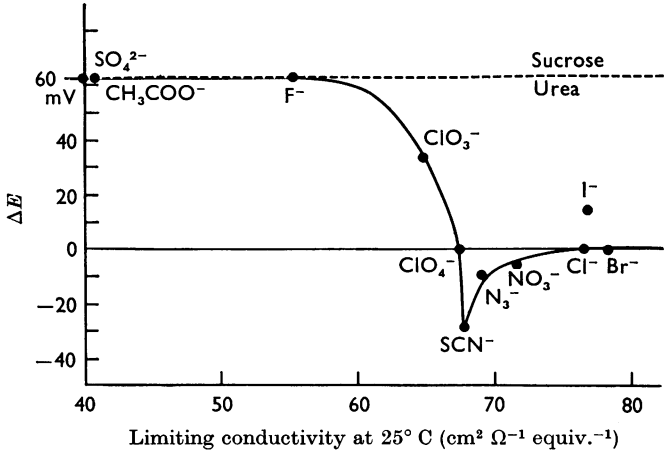


Fig. 3. Relations between $\Delta E$ and the limiting equivalent conductivity of anion in the aquatic solution at 25° C. Data of conductivities are from Robinson & Stokes (1959) in most cases and from *International Critical Tables of Numerical Data*, VI (1929) and Miller (1956) for $SCN^-$ and $ClO_4^-$. *Taeniura lymma*.

*Effect of various anions on the resting potential.* When the $Cl^-$ ions in the external solution were replaced with various other anions the resting potential showed a positive or negative shift according to the anion species. The preparation was equilibrated in the NaCl saline (*B*) before the experiment and test solutions were made up by replacing the NaCl with NaBr, NaSCN, etc. Resting potentials of six to seven different fibres were observed before and after the application of the test solution and then the preparation was brought back to the NaCl saline. After the resting potential had returned to the original value another test solution was applied. The preparation was kept in each test solution for no more than 4 min. The change of the resting potential is plotted against the limiting conductivity of respective anion species in the aquatic solution in Fig. 3. In the presence of anions having limiting conductivity smaller than 60 mho $cm^2$ $equiv^{-1}$ such as $F^-$, acetate, methanesulphonate and $SO_4^{2-}$, there was a positive shift of the resting potential of about 60 mV and practically the same

change was found after the substitution of the NaCl in the NaCl saline ($B$) with sucrose or urea. The result, therefore, indicates that the membrane is impermeable to those anions. Some of these anions, such as $F^-$ and $SO_4^{2-}$ remove $Ca^{2+}$ ions from the external medium. However, this is not the basis of the reduction of the resting potential due to $F^-$ or $SO_4^{2-}$, since removal of $Ca^{2+}$ by ethyleneglycol bis(aminoethylether)tetra-acetate (EGTA) was without effect on the resting potential.

For the other anions the relation between the change of the resting potential, $\Delta E$, and the limiting conductivity was not a monotonic function. $Br^-$ usually showed no change of the resting potential, $I^-$ always showed a positive shift of 12–14 mV. For $NO_3^-$, $N_3^-$ and $SCN^-$, $\Delta E$ was $-5$, $-10$ and $-30$ mV respectively. These values varied slightly between preparations but the range was only a few mV and the order was always $SCN^- < N_3^- < NO_3^-$ ($SCN^-$ giving the greatest negative shift). $ClO_4^-$ did not show any appreciable change of the resting potential from the $Cl^-$ potential. For $ClO_3^-$ $\Delta E$ was positive but the value was substantially less than that found for the other impermeable anions.

The resting potential, $E_{Cl}$ in the $Cl^-$ medium, is given by

$$E_{Cl} = \frac{RT}{F} \ln \frac{P_K [K^+]_o + P_{Cl} [Cl^-]_i}{P_K [K^+]_i + P_{Cl} [Cl^-]_o} \quad (1)$$

Here $R$, $T$ and $F$ have the usual meanings and $P_K$ and $P_{Cl}$ are permeability constants (Goldman, 1943; Hodgkin & Katz, 1949). The resting potential, $E_A$, obtained after the replacement of the external $Cl^-$ with the test anion $A^-$ is given by,

$$E_A = \frac{RT}{F} \ln \frac{P_K [K^+]_o + P'_{Cl} [Cl^-]_i}{P_K [K^+]_i + P_A [A^-]_o} \quad (2)$$

since $[Cl^-]_i$ has not been altered and $[A^-]_i$ is zero at this stage. $P'_{Cl}$ is the permeability constant for $Cl^-$ in the presence of $A^-$. For the permeable anions such as $SCN^-$, $NO_3^-$, etc., the $K^+$ conductance can be neglected and hence,

$$\Delta E_A = E_A - E_{Cl} = \frac{RT}{F} \ln \frac{P'_{Cl}}{P_A} \quad (3)$$

If $P'_{Cl} = P_{Cl}$, the result indicates that $P_{SCN} > P_{N3} > P_{NO3} > P_{Cl}, P_{Br}$; i.e. the permeability increases as the conductivity decreases. If the size of anion is assumed to increase as the conductivity decreases the above result implies that within a certain range of ion size the permeability increases as the ion size increases. However, measurement of the membrane resistance of the muscle fibre indicates that this does not seem to be the case.

The membrane resistance was determined in a muscle fibre before and after the substitution of $Cl^-$ ions. The negative shift of the resting potential

following the substitution of external $Cl^-$ with anions such as $SCN^-$ or $NO_3^-$ was associated with two- to three-fold increase in the effective membrane resistance. Since the internal resistance likely did not change just after the substitution, the membrane resistance increased by a factor of 4–9. The increase of the membrane resistance has also been shown in frog

TABLE 3. Cable constants of the muscle fibre

| | Time constant (msec) | Space constant (mm) | Effective input resistance (kΩ) | Fibre diameter ($\mu$) |
|---|---|---|---|---|
| *A* | | | | |
| *Himantura uarnak* | 6–7 | 3·0–3·5 | 360–460 | 50–100 |
| *Taeniura lymma* | 1·5–2·5 | 2·5–3·0 | 60–80 | 140–200 |
| *B* | | | | |
| *Periophthalmodon barbarus* | 5–9 | 2·5–3·5 | 250–350 | 50–80 |

muscle fibre (Hutter & Padsha, 1959) when the external $Cl^-$ is replaced with $Br^-$, $NO_3^-$, etc. If equation (1) is to hold, the foregoing result should indicate that $P'_{Cl}$ becomes smaller than $P_{Cl}$ when the fibre is bathed with $SCN^-$ or $NO_3^-$ saline. As described before $Br^-$ did not cause any observable change in the resting potential and similarly no appreciable change in the membrane resistance was found. Thus the negative $\Delta E$ does not indicate that the permeability of the anion in question is higher than $P_{Cl}$ found in the $Cl^-$ saline. The membrane resistance measurements suggest that $P_{SCN}$ and $P_{NO3}$ are smaller than $P_{Cl}$ found in the $Cl^-$ saline. However, they were not complete enough to obtain a quantitative conclusion.

The replacement of $Cl^-$ by $SCN^-$ after equilibration in $Cl^-$ was followed by an immediate negative shift of the resting potential, as described before, and then the potential returned slowly to the initial $Cl^-$ resting potential level (Fig. 2*C*). After the potential had returned to the original level the preparation was again placed in $Cl^-$ saline and this was followed by a positive shift of the resting potential which again decayed slowly to the original level. The time courses of the resting potential change were substantially faster than those found for similar treatments with impermeable anions. As described before the slow return of the resting potential for impermeable anions or urea is associated with the movement of KCl across the membrane. In the present case, however, the membrane is permeable to both $Cl^-$ and $SCN^-$ ions so that the return of the resting potential is mainly due to the exchange between the internal $Cl^-$ and the external $SCN^-$ and therefore the time courses can be faster than those found for impermeable anions.

*Cable constants and current–voltage relations of the membrane.* The cable properties of a few muscle fibres of *Himantura uarnak* and *Taeniura lymma* were examined. The results are mentioned in Table 3*A*. The membrane

time constant found for *Taeniura* was always much smaller than that found for *Himantura*. The calculation showed that this seemed to be due to a smaller specific membrane resistance. Although the specific membrane

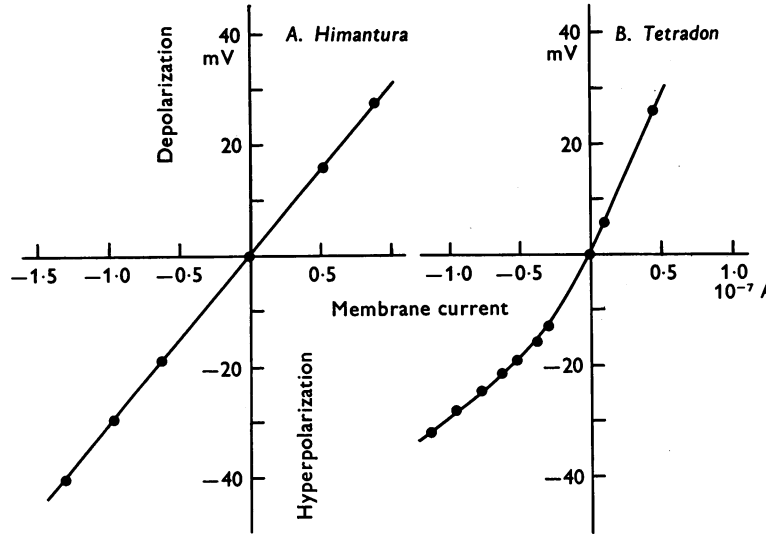


Fig. 4. Current–voltage relations of the resting muscle fibre membrane at the steady state obtained in the normal salines. *A*, Elasmobranch (*Himantura uarnak*). *B*, Teleost (*Tetradon immaculata*).

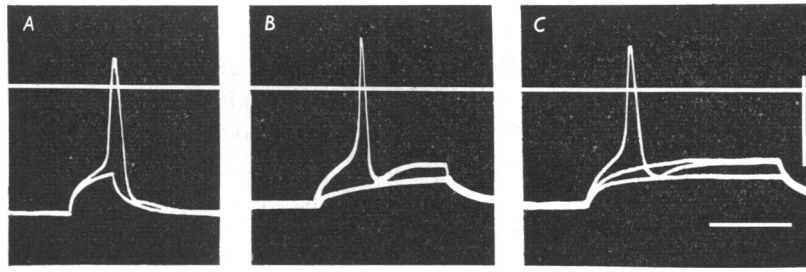


Fig. 5. Spike potentials of elasmobranch muscle fibres obtained in the normal elasmobranch saline. *A*, *Taeniura lymma*; *B*, *Himantura uarnak*; *C*, *Pastinachus sephen*. Voltage, 50 mV; time, 10 msec. The upper trace in each record indicates the reference membrane potential level.

resistance was smaller in *Taeniura*, the space constant obtained for *Taeniura* was not much smaller than that obtained for *Himantura* because of the larger diameter of the muscle fibres in *Taeniura*. The steady state current–voltage relation of the membrane in the normal saline obtained with a muscle fibre of *Himantura* is shown in Fig. 4*A*. The relation is linear for hyperpolarizations and subthreshold depolarizations. In other words

the slope conductance is the same for hyperpolarizing and subthreshold depolarizing currents.

*Action potential.* Three records in Fig. 5 show spike potentials of the muscle fibre in the normal elasmobranch saline for three different species of stingrays (*A*: *Taeniura lymma*; *B*: *Himantura uarnak*; *C*: *Pastinachus sephen.*) An appreciable overshoot was invariably found in the normal

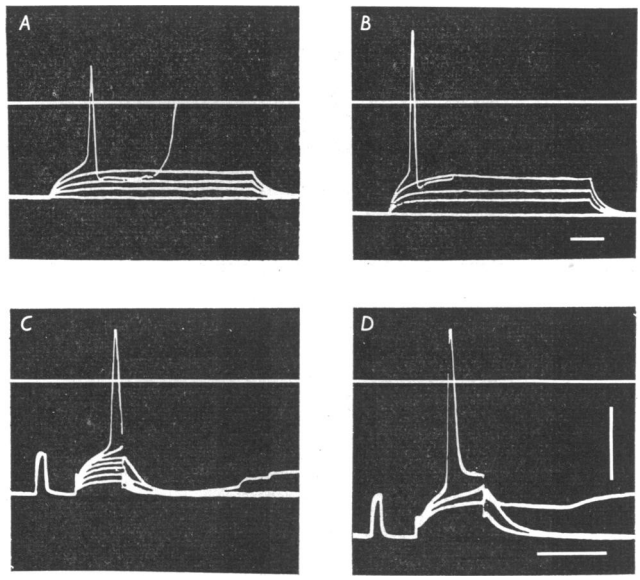


Fig. 6. Spike potentials of the elasmobranch muscle fibre. *A* and *B*, spike potentials before (*A*) and after (*B*) the substitution of the external urea with an osmotically equivalent amount of NaCl. Note increased overshoot and increased resting potential. *Himantura uarnak*. *C* and *D*, spike potentials obtained before (*C*) and after (*D*) the substitution of the external $Cl^-$ in the NaCl elasmobranch saline (*B*) with $SCN^-$. Note increased resting potential with an unaltered overshoot. *Taeniura lymma*. *A* and *B*, and *C* and *D* were obtained from different fibres of the same preparations respectively. Voltage, 50 mV; time, 10 msec.

saline. It usually measured 25–30 mV for *Pastinachus* and *Himantura* but somewhat smaller values were found for *Taeniura*. With a prolonged suprathreshold depolarization repetitive spikes were elicited only rarely. Even when two spikes were obtained the amplitude of the second one was much smaller than the first (Fig. 7*A*). Record *A* of Fig. 6 shows a spike potential obtained in the normal saline and record *B* was obtained from a different fibre of the same preparation after the urea in the saline had been replaced with NaCl. The replacement was associated with a negative shift of the resting potential and at the same time the overshoot of the spike increased. The increase of the overshoot is due to the increase of the

external Na concentration as will be shown below and is not a secondary effect of the higher resting potential. Records *C* and *D* of the same figure were obtained from two different fibres of the same preparation before and after the NaCl in the NaCl saline (*B*) had been replaced with NaSCN. The replacement resulted in a large negative shift of the resting potential, but the overshoot of the spike was not changed, probably because the $Na^+$ concentration had not been altered.

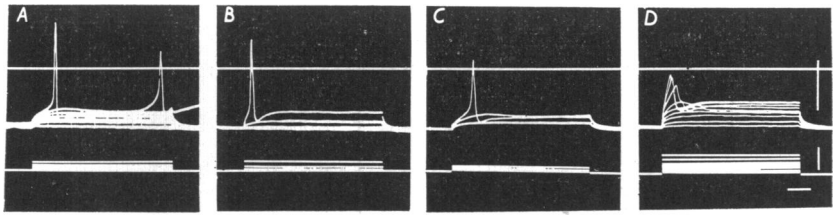


Fig. 7. Effect of the external NaCl concentration upon the spike potential of the elasmobranch muscle fibre. The $Na^+$ concentration was altered by substitution of the NaCl with choline chloride. The $Na^+$ concentration was 464 mM in *A*, 237 mM in *B*, 124 mM in *C* and 67 mM in *D*. The upper, middle and lower traces in each record shows reference potential level, membrane potential and applied membrane current respectively. *Himantura uarnak*. Voltage, 50 mV; current $2 \times 10^{-A}$; time, 10 msec.

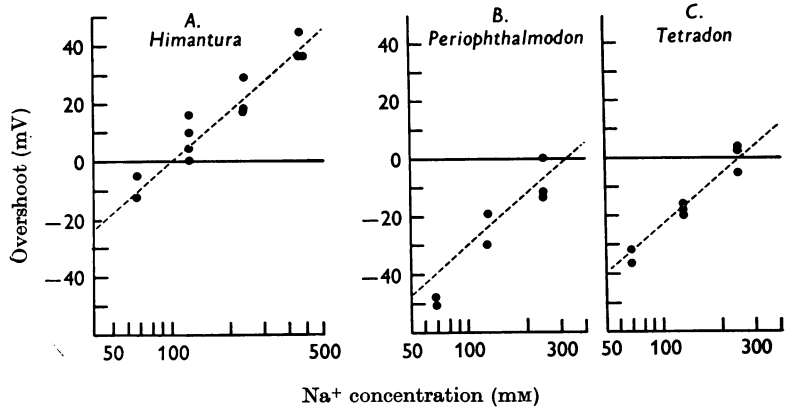


Fig. 8. Relations between the overshoot of the spike and the external $Na^+$ concentration in elasmobranch fibre (*Himantura uarnak*, *A*) and teleost fibre (*Periophthalmodon barbarus*, *B*; *Tetradon immaculata*, *C*).

To investigate the effect of $Na^+$ concentration on the spike overshoot, the NaCl concentration of the NaCl saline (*A*) was altered by replacement with choline chloride. The substitution did not alter the resting potential because the $Cl^-$ concentration was constant. Records *A–D* of Fig. 7 were obtained from different fibres of the same preparation during the decrease of the $Na^+$ concentration. The overshoot decreased with decreasing $Na^+$

concentration and when the $Na^+$ concentration was less than 50 mM the membrane was no longer capable of initiating an all-or-none spike. The relation between the overshoot and the external $Na^+$ concentration is shown in Fig. 8*A*. Although some variations were seen, the relation indicates that the overshoot has a slope of 58 mV/10-fold change in the concentration. Therefore the spike in the stingray muscle fibre is considered to be a $Na^+$ spike in which the permeability change of the membrane to $Na^+$ ions plays the major role for the spike potential initiation.

It has been shown by a number of workers that the $Na^+$ spike is abolished in frog muscle fibre (Furukawa, Sasaoka & Hosoya, 1959; Narahashi, Deguchi, Urakawa & Ohkubo, 1960; Nakajima, Iwasaki & Obata, 1962), squid giant axon (Nakamura, Nakajima & Grundfest, 1965) and lobster giant axon (Narahashi, Moore & Scott, 1964) by applying tetrodotoxin at a concentration of $1–5 \times 10^{-8}$ g/ml. of the external solution. A similar effect was also seen in the stingray muscle. The spike was abolished at a concentration of $5 \times 10^{-8}$ g/ml.

### *Teleosts*

*Resting membrane.* The resting potential of the muscle fibre immersed in the normal saline (2 mM-K) was 80–85 mV in all species examined except *Conger* in which smaller resting potentials were often found. The small potentials in *Conger* may be related to the small size of the muscle fibres in this species (15–30 $\mu$). Effects of $K^+$ and $Cl^-$ concentrations on the resting potential were examined mainly with *Periopthalmodon* preparation. A few experiments performed with other species indicated that the results were essentially similar among different species. Figure 9 shows the relation between the resting potential and the logarithm of the external $K^+$ concentration. Solutions of different $K^+$ concentrations were obtained by replacing NaCl in the normal saline with KCl and therefore the $Cl^-$ concentration was kept normal during the alteration of $K^+$ concentration. The slope of the relation is not much smaller than 58 mV/10-fold change in the concentration. In contrast changes in the external $Cl^-$ concentration showed little effect on the resting potential. The complete removal of $Cl^-$ from the normal saline by replacement with methanesulphonate shifted the potential by less than 8 mV. The above result indicates that in teleost muscle fibre membrane the $K^+$ conductance is more significant than the $Cl^-$ conductance.

The cable constants of the muscle fibre were observed with *Periopthalmodon* preparation and the result is shown in Table 3*B*. The steady state current–voltage relation of the fibre membrane obtained with *Tetradon* preparation is shown in Fig. 4*B*. The slope conductance for the inward current was larger than that found for the subthreshold outward current,

i.e. a kind of rectification was found. This rectification was more marked in *Hemiramphus* preparation and less marked in *Periopthalmodon* preparations. Nevertheless, the rectification was invariably seen in teleost muscle fibres.

*Spike potential.* Figure 10 shows spike potentials obtained in the normal saline with preparations of five different species. In most cases the overshoot of the spike potential was close to zero. A small positive overshoot

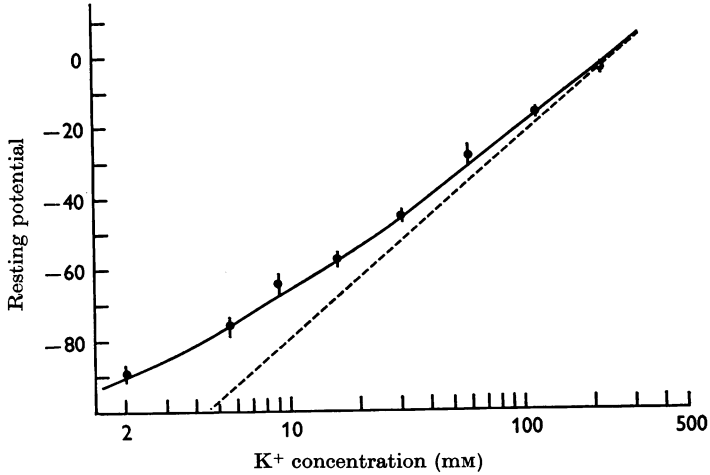


Fig. 9. Relation between the external $K^+$ concentration and the resting potential of the teleost muscle fibre (*Periophthalmodon barbarus*). The $Cl^-$ concentration was kept at 245 mM throughout. The vertical bar for each circle shows the standard deviation. The interrupted line indicates 58 mV slope for a 10-fold change in the concentration.

was often found for *Tetradon* (*A*) and *Hemiramphus* (*B*) and the overshoot was slightly negative or zero for *Periopthalmodon* ($C_1$). A positive overshoot of a relatively large amplitude was usually found with *Conger* (*E*) muscle fibres. Figure $10C_2$ was obtained with *Periopthalmodon* muscle fibre when the membrane was depolarized with outward current pulses of a relatively large duration. When the intensity was high the threshold for the spike was reached shortly after the start of the depolarization and this resulted in a full-sized spike potential. When the intensity was relatively low, however, the threshold was reached after a prolonged depolarization and this decreased the spike amplitude considerably (Fig. $10C_2$). A similar phenomenon has been found in the squid giant axon and explained in terms of the $Na^+$ conductance inactivation (Hodgkin & Huxley, 1952). The phenomenon was not marked in the elasmobranch muscle fibre. No repetitive spikes were produced by prolonged suprathreshold current. This property is common between teleost and elasmobranch muscle fibres. With

stimulation by a current pulse of long duration the fibre often showed a local contraction around the tip of the polarizing electrode even when the depolarization was subthreshold for the spike potential. This construction usually resulted in a movement artifact of the potential recording as shown

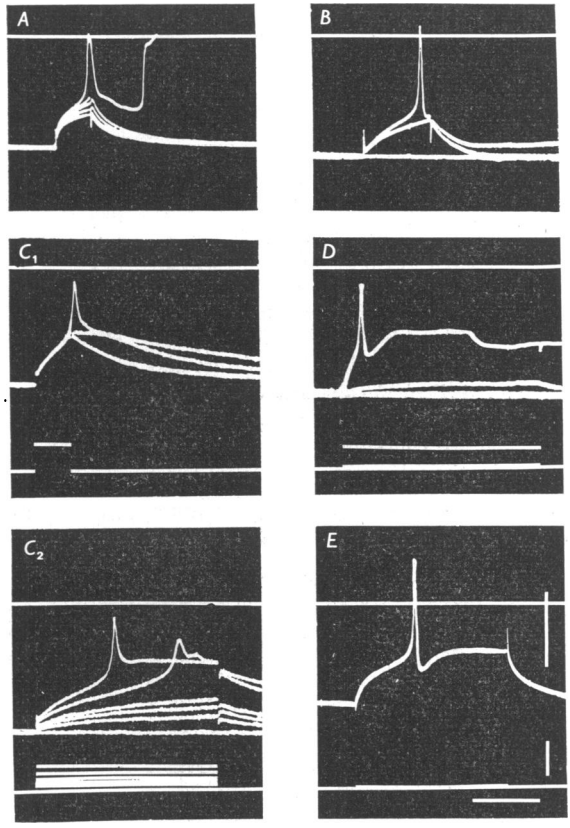


Fig. 10. Spike potentials of the teleost muscle fibre. *A. Tetradon immaculata*, *B. Hemiramphus welsby*; *C. Periophthalmodon barbarus*; *D. Parexocoetus brachypterus* and *E. Conger labiatus*. The upper trace in each record shows reference potential level; middle, membrane potential; and the lower, membrane current. Voltage, 50 mV; current, $2 \times 10^{-7}$ A and time, 10 msec.

in Fig. 11*Aa*. The fact indicates that for prolonged current pulses the threshold depolarization for the spike is higher than that for contraction. For current pulses of a small duration (less than 5 msec) the spike potential was always produced before the amplitude of depolarization reached the threshold for contraction.

*Effects of $Na^+$ concentration and tetrodotoxin on the spike.* The effect of the external $Na^+$ concentration upon the spike potential was examined by replacing various amounts of NaCl in the normal saline with choline

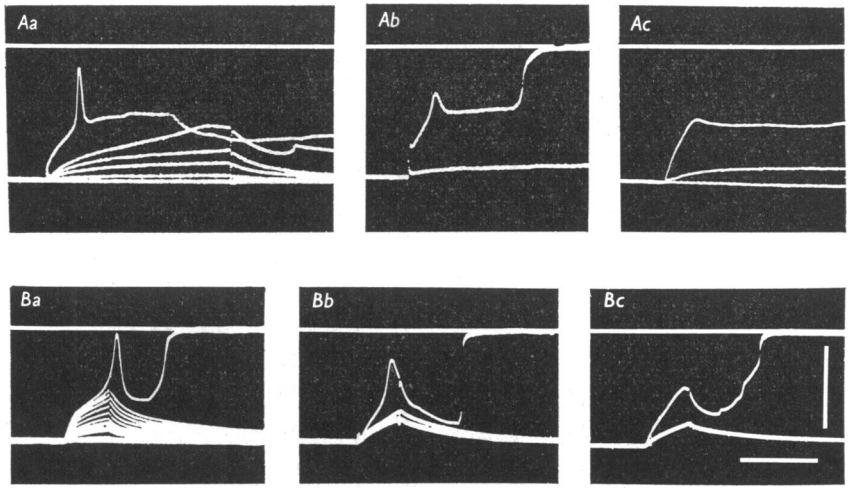


Fig. 11. Effect of the external $Na^+$ concentration upon the spike potential of the teleost muscle fibre. *A Periophthalmodon barbarus* and *B Tetradon immaculata*. The $Na^+$ concentration was altered by replacing NaCl in the normal saline with choline chloride. The $Na^+$ concentration was 241 mM in *a*, 126 mM in *b* and 68 Mm in *c*. Voltage, 50 mV; time, 10 msec.

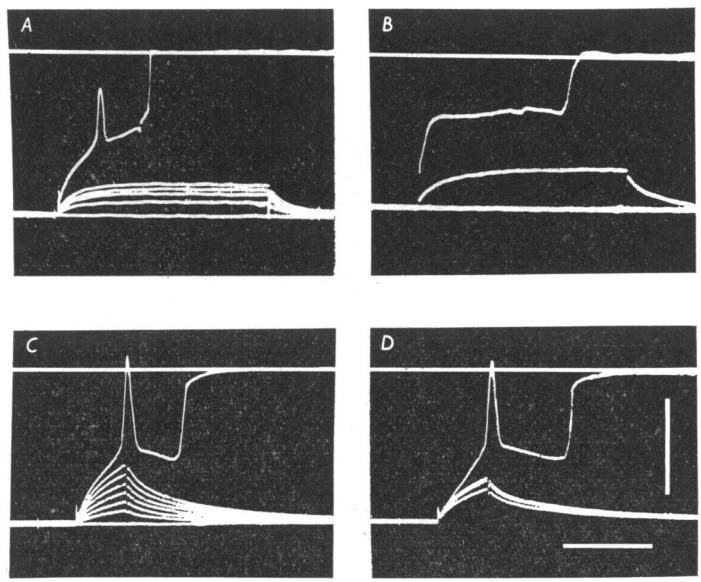


Fig. 12. Effect of tetrodotoxin upon the spike potential of the teleost muscle fibre. *A* and *B* obtained from *Periophthalmodon barbarus* preparation before (*A*) and after (*B*) the application of the toxin at $10^{-7}$ g/ml. of the external normal saline. *C* and *D*, obtained from *Tetradon immaculata* preparation before (*C*) and after (*D*) the application of the toxin at $5 \times 10^{-4}$ g/ml. of the external normal saline. *A* and *B*, and *C* and *D* were obtained from different fibres of the same preparations respectively. Voltage, 50 mV; time, 10 msec.

chloride. Records *a*, *b* and *c* in Fig. 11*A* and *B* show spike potentials obtained at 241, 126, and 68 mM-$Na^+$ respectively with *Periophthalmodon* (*A*) and *Tetradon* (*B*) preparations. In both cases the overshoot of the spike potential decreased with decreasing $Na^+$ concentration. The relations between the overshoot and the concentration are shown in Fig. 8*B* and *C* and they suggest that the spike potential of the teleost muscle fibre is also produced by the conductance increase of the membrane to $Na^+$ ions, i.e. the spike represents the so-called $Na^+$ spike.

Records *A* and *B* of Fig. 12 were obtained from two different fibres of the same *Periophthalmodon* preparation before and after the application of $10^{-7}$ g tetrodotoxin/ml. of the external normal saline. This concentration of the toxin abolished the spike potential completely. The threshold concentration for the abolition was $5 \times 10^{-8}$ g/ml. and similar values were also found for *Hemiramphus* and *Parexocoetus*. This value is similar to those found for $Na^+$ spikes in other tissues. In contrast to these results tetrodotoxin was ineffective on the spike potential of *Tetradon* muscle fibre. As shown in Fig. 12*C–D* the spike potential showed no change before (*C*) and after (*D*) the application of the toxin at $5 \times 10^{-4}$ g/ml. which was about $10^4$ times higher than ordinarily necessary to abolish $Na^+$ spike. Since the spike of the *Tetradon* muscle fibre represents a $Na^+$ spike this gives an example of a $Na^+$ spike which is insensitive to tetrodotoxin. This is probably related to the fact the *Tetradon* contains tetradotoxin. At the present stage, however, nothing can be said about the mechanism which protects against tetrodotoxin. A similar ineffectiveness of tetrodotoxin on the $Na^+$ spike of nerve fibre in a certain species of puffer has been reported by C. Y. Kao (personal communication).

## DISCUSSION

Barets (1955) described two distinct nerve-muscle systems in teleost fish. Takeuchi (1958) has shown that all-or-none spike potentials are seen only in muscle fibres of the one system which could be called the twitch fibre system. The histological examination showed that the fin adductor muscles of teleosts used in the present experiment belonged to the twitch type muscles and an all-or-none spike potential was always found in the fibre. The fibres of stingray fin muscle used are also spike producing or twitch fibres. However, the experimental results show that the membrane properties are different between elasmobranch and teleost twitch muscle fibres in several points. These differences will be discussed below.

The resting potential of elasmobranch muscle fibre is mainly dependent on the $Cl^-$ concentration difference between inside and outside the fibre and the $K^+$ conductance is far smaller than the $Cl^-$ conductance in the resting

membrane. In contrast in the teleost muscle fibre the $K^+$ conductance is more than the $Cl^-$ conductance. The steady state current–voltage relation of the fibre membrane is linear over the wide range of hyperpolarization and subthreshold depolarization in elasmobranch muscle. In the teleost twitch muscle fibre a higher slope conductance is always found for hyperpolarization. Adrian & Freygang (1962) have shown the occurrence of a similar rectification in frog muscle fibres, have called it anomalous rectification, and have attributed it to a $K^+$ conductance. This suggests that the lack of the rectification on elasmobranch muscle fibre may be due to the high $Cl^-$ conductance by which any change of the $K^+$ conductance is covered.

The spike potential of the elasmobranch twitch fibre always shows a relatively large overshoot in the normal saline, whereas little or no overshoot is found in teleost twitch fibre. Takeuchi (1958) has shown that the overshoot is also small or sometimes negative in the twitch fibre of fresh-water teleost. In cyclostome, however, a relatively large overshoot is found for the spike of the twitch fibre (Andersen, Jansen & Løyning, 1963). As described already prolonged depolarization produced by an outward current pulse often produces a local contraction in sea water teleost fibre before the amplitude of depolarization reaches the threshold for the spike. This suggests that depolarizations associated with a train of end-plate potentials may produce contraction even when the potential does not initiate a spike potential. Distributed neuromuscular junctions are found in twitch muscle fibre system in fresh-water teleosts (Barets, 1955; Barets, Fessard & Le Touzé, 1956; Takeuchi, 1958). Similar distributed junctions are also found in sea water teleosts (Hagiwara & Takahashi, unpublished). The fact indicates that the conduction of spike potential may not be of great functional significance for the spread of contraction over the muscle fibre in teleosts even though the fibre can produce an all-or-none spike. In this respect the teleost twitch fibre system resembles the crustacean muscle fibres.

A discrete end-plate is found for the twitch muscle fibre of elasmobranchs (Cavalié, 1902) and therefore the conduction of spike potential should have a great functional significance for the spread of contraction along those fibres. The neuromuscular junction is also a discrete end-plate in the twitch fibre of cyclostomes (Andersen *et al.* 1963). The overshoot of the spike is relatively large in elasmobranch as well as in cyclostome twitch fibres and in these respects they resemble amphibian twitch muscle fibres.

The resting membrane of the elasmobranch muscle fibre is permeable to $Br^-$, $I^-$, $Cl^-$, $NO_3^-$, $SCN^-$, $ClO_4^-$ and $ClO_3^-$. All these anions show large limiting equivalent conductivities in the aqueous solution. In contrast to this the membrane is impermeable to anions which have small equivalent conductivities such as $F^-$, methanesulphonate, acetate, $SO_4^{2-}$, etc. A similar

anion permselectivity has been found for the inhibitory subsynaptic membrane of the cat motoneurone (Coombs, Eccles & Fatt, 1955; Araki, Ito & Oscarsson, 1961). Therefore the present experimental result suggests that the mechanism of permselectivity in the resting membrane of the elasmobranch muscle fibre is similar to that in the inhibitory synaptic membrane. Coombs *et al.* (1965) and Araki *et al.* (1961) have proposed the idea that the permselectivity of ions at the subsynaptic membrane is determined by the size of ion in water and this is called the sieve hypothesis.

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