Contributions of restricted length reporting original and significant findings of immediate interest

THE LOCATION OF SODIUM IN THE TRANSVERSE TUBULES OF SKELETAL MUSCLE

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Recent studies on the sarcoplasmic reticulum and transverse system of skeletal muscle have led to the conclusion that both systems are independent ones and that the transverse tubules (T system) are continuous with the external membrane of the muscle fiber (1). Ferritin penetrates into the lumen of the transverse tubule but is not found inside the sarcoplasmic reticulum (2). Thus, the fluid contained in the transverse tubules could easily be identical in composition with the extracellular fluid.

The distribution of sodium in skeletal muscle assessed with analytical methods indicates that the actual free sodium inside the limits of the fiber is very small, of the order of 1 mmole/kg of cell water, if a special region with high concentration of sodium at the level of the sarcolemma is taken into consideration (3).

More knowledge on the detailed location of different ions inside the membranous system of skeletal muscle is desired in view of their role in the process of excitation as well as in the coupling of excitation to contraction (4).

In an attempt to localize sodium by means of electron microscopy in skeletal muscle, the method utilized by Komnick and Komnick (5) and by Kaye et al. (6, 7) was applied with slight modifications. This method permits the observation of a fine precipitate of sodium pyroantimonate in sections of tissues fixed in OsO_4. The principle of this method, used in the past to determine sodium in biological fluids as a microtitration method (8), consists in the precipitation of sodium in solution as its pyroantimonate salt when in contact with potassium pyroantimonate. The latter salt is soluble in water whereas the sodium one is not.

The preservation of the muscles fixed in the presence of the pyroantimonate reagent is not very good when compared to the preservation of muscles treated with the fixatives alone and used as controls. Nevertheless, the images were clear enough to detect the precipitate and to recognize with certainty the spatial relationships of the precipitate to the fine structure of the muscles.

The precipitate of sodium pyroantimonate was observed inside the transverse tubules of the frog sartorius muscles which confirms the assumption that the composition of the fluid filling these tubules could resemble that of the extracellular fluid. No precipitate was found in the sarcoplasmic reticulum in any of its portions, and a considerable amount was seen on the outside of the sarcolemma of the fibers.

MATERIALS AND METHODS

Freshly dissected sartorius muscles of the frog (*Rana pipens*) were divided into halves. One part was fixed in the presence of reagent grade $KSb(OH)_6$ (potassium pyroautimonate) and the other used as control. The muscles were fixed for 1 hr in a medium containing 3% glutaraldehyde (9) and 2% $KSb(OH)_6$ in a 0.1 M potassium phosphate buffer. This mixture was brought to the boiling point in order to dissolve the pyroantimonate, then it was cooled, and its pH readjusted to 7.4. This fixation was followed by a rinse of 2 hr in phosphate buffer with the addition of 10% sucrose. The first fixation and the rinse were made at room temperature. The muscles were then cut into small pieces of about 1 mm and postfixed in 1% OsO_4 in phosphate buffer, pH of 7.4, at 2°C, for 1 hr. After dehydration in a graded series of diluted ethanol, embedding was done in Maraglas and, after staining with lead citrate (10), sections were studied in a Siemens Elmiskop I. The osmolarity of the first fixative was 513 milliosmols per liter, the one of the rinse 466 milliosmols per liter, and that of the second fixa-

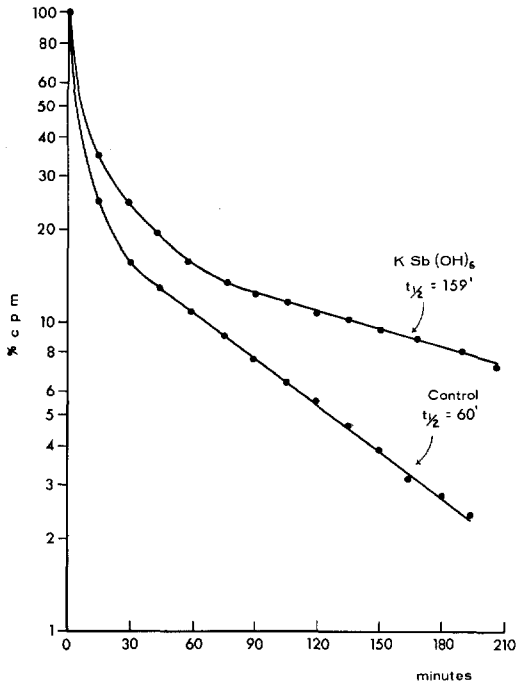

FIGURE 1 The loss of Na^{22} from 2 sartorius muscles of the frog while being fixed in glutaraldehyde is shown here. The muscle exposed to potassium pyroantimonate during fixation shows a slower efflux of radioactive sodium than the control. The muscles were previously equilibrated overnight with Na^{22} in a Ringer's solution in the cold.

tive 263 milliosmols per liter. To keep the osmolarity at this level in the fixative containing no $KSb(OH)_6$, sucrose was used in equiosmolar amounts.

The replacement of potassium for sodium in the buffer utilized gave comparatively similar results in the controls; in all instances, the muscles fixed in the presence of $KSb(OH)_6$ did not show such a good preservation as the controls. With another method of fixation consisting of a cold 1% solution of OsO_4 buffered with 0.1 M potassium acetate buffer, and containing 2% potassium pyroantimonate, the preservation of the structure was found to be poor, though the precipitate of sodium pyroantimonate was observed.

The efflux of Na^{22} was determined in companion muscles of the same frog immersed in the first fixative. First, the muscles were immersed in Conway-Ringer's fluid (11) at 2°C containing $Na^{22}Cl$ and left overnight for the radioisotope to reach the same specific activity in the muscle as in the medium. Conway-Ringer's fluid was utilized because it very closely imitates the composition of the frog plasma. Next, one muscle was placed in 5 ml of the glutaraldehyde fixative and used as control, and the other was simultaneously immersed in the same fixative but containing the potassium pyroantimonate. The loss of Na^{22} from the muscles during fixation was measured with a liquid scintillation counter.

RESULTS

Fig. 1 shows the rate of loss of Na^{22} from two muscles. In one, the reaction between the pyroantimonate and sodium is occurring, and the halftime

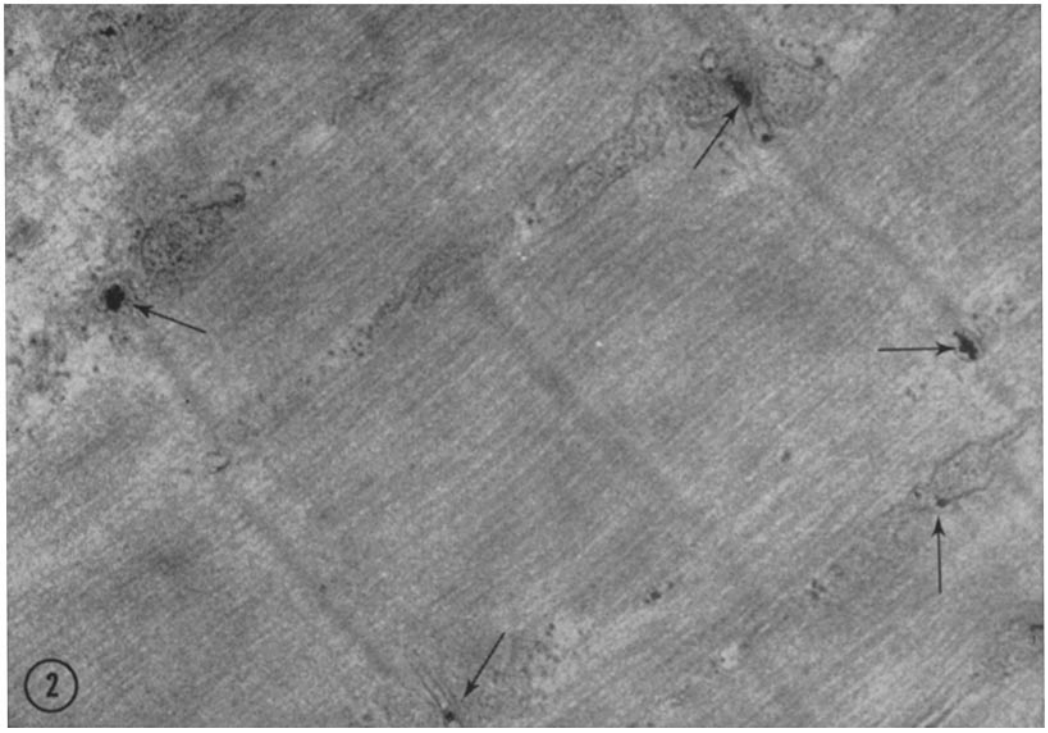

FIGURE 2 In this longitudinal section of frog sartorius muscle fixed in glutaraldehyde the precipitate of sodium pyroantimonate can be observed inside the transverse tubules indicated by the arrows. × 45,000.

for the loss of sodium from it is much greater than in the control. After 180 min, 10% of the sodium is still retained in the experimental muscle. Considering that the first portion of the curve corresponds to the exit of sodium from the extracellular fluid, this amount remaining has to be cellular sodium or sodium adsorbed to the muscle fibers, precipitated as sodium pyroantimonate. These experiments also indicate that the precipitate, once it is formed, does not leave the muscle fiber in an appreciable amount.

In Fig. 2, a longitudinal section of a muscle fiber is shown. It can be seen that a dark precipitate is contained inside the central element of the triads, the transverse tubule. No appreciable amounts of the precipitate are observed in the longitudinal tubules, inside the cisternae, or in the myofibrils. In Fig. 3, a more magnified view is given of the transverse tubules containing the precipitate. Extremely fine spots sometimes are seen inside the terminal cisternae (Figs. 3 and 4) and can be distinguished as different from glycogen granules or the sodium pyroantimonate precipitate. They do not have the intense, grumous quality of the latter.

Fig. 4 gives another example of the localization of the precipitate inside the transverse tubule, and in Fig. 6 these tubules appear somewhat dilated and fully occupied by the precipitate. In the transverse section of Fig. 5, it is possible to see a transverse tubule cut along its length, and containing a row of dots of the sodium precipitate. In none of the material examined was the precipitate localized in a region other than the transverse tubule and the sarcolemma. These findings were very reproducible, though some of the sections of the transverse tubules did not show precipitate in them, whereas in some located very close to these the precipitate was seen.

Fig. 7 shows a view of the region of the sarcolemma of a fiber. A very dark and highly concentrated precipitate is seen attached to the mucoid region lining the plasma membrane. In Fig. 8 the precipitate is again seen close to the sarcolemma, as well as inside of the transverse tubules located near the fiber surface.

DISCUSSION

The presence of the precipitate inside the transverse tubules, with a density in some cases almost equal to the one observed between the fibers, that is, in the extracellular space, can be taken as an indication that the fluid inside (the T system) has a high sodium content. The fact that neither the longitudinal system nor any other region of the sarcoplasmic reticulum shows the precipitate could be the consequence of a very low concentration of sodium in the fluid occupying this system, or the lack of penetration of the reagent into it during fixation. Though it is possible that the membranes of the sarcoplasmic reticulum are impermeable to the pyroantimonate ion, this would constitute

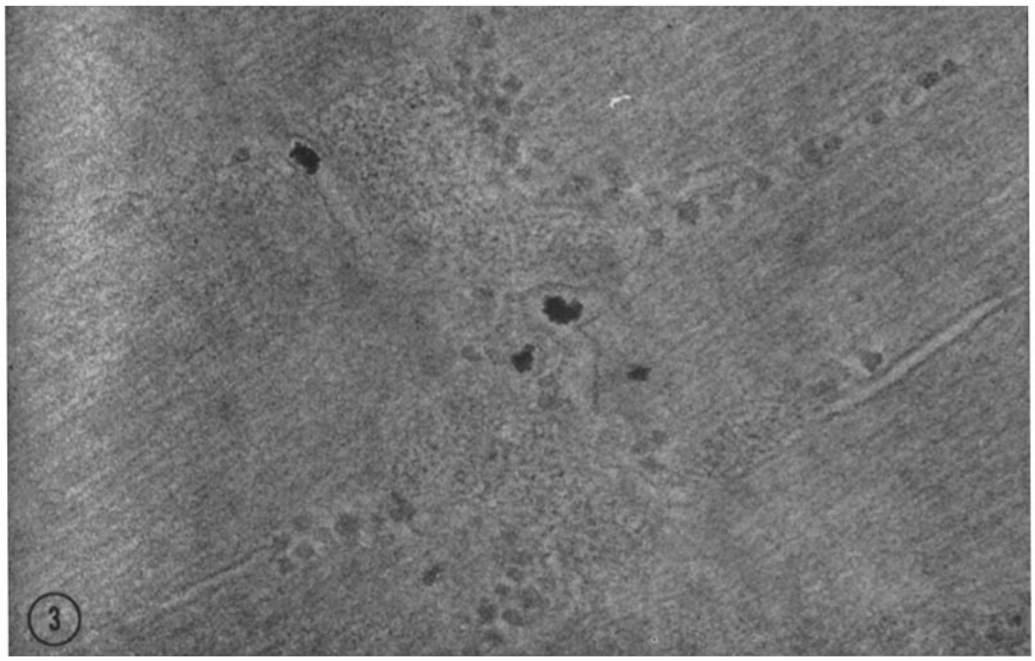

FIGURE 3 At the level of the Z band the terminal cisternae, the beginning of the longitudinal tubules, and the transverse tubules can be clearly observed in this section. The precipitate appears only inside the transverse tubules. × 100,000.

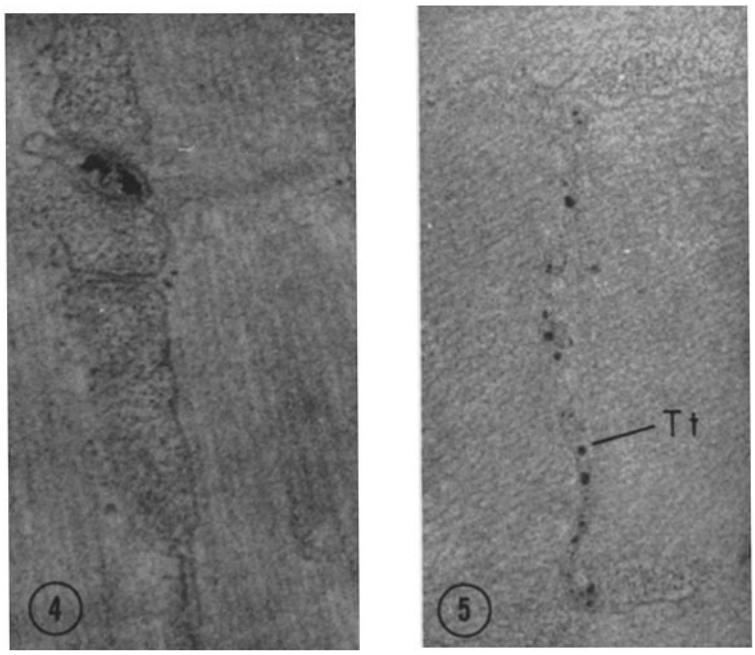

FIGURE 4 Another view of a triad shows the precipitate inside the transverse tubules. × 105,000.

FIGURE 5 This section, at the level of the Z band, shows a transverse tubule running vertically in the print. It is occupied by a row of dots of precipitated sodium. *Tt*, transverse tubule. × 69,000.

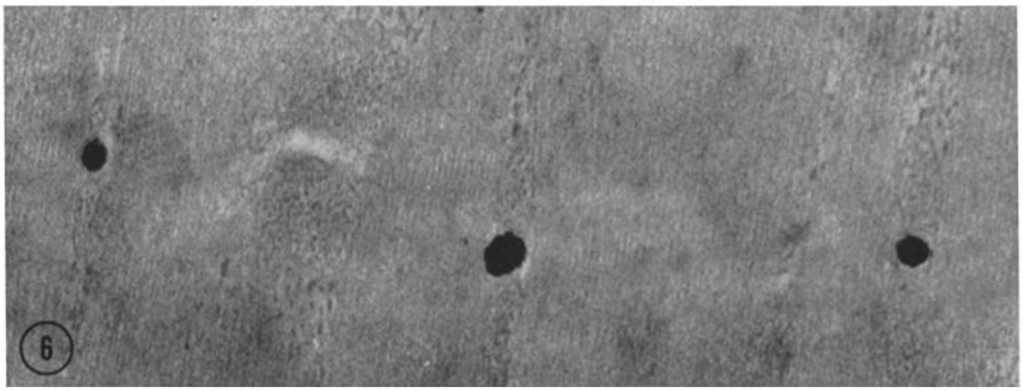

FIGURE 6 In another longitudinal section the precipitate is again seen inside the transverse tubules which, in this case, appear dilated and completely occupied by the dense precipitate. × 40,000.

more an exception than the rule, since the precipitate is easily observed in other tissues throughout the cytoplasm (6, 7). We are inclined to think that the pyroantimonate penetrates through the muscle fiber and that the concentration of sodium in regions other than the transverse tubule and the basement membrane of the sarcolemma is too low to reveal any precipitate. However, the evidence presented here does not exclude completely the possibility that the reagent utilized did not penetrate through the plasma membrane of the muscle fibers.

The efflux of sodium from the muscles immersed in the glutaraldehyde fixative demonstrates that

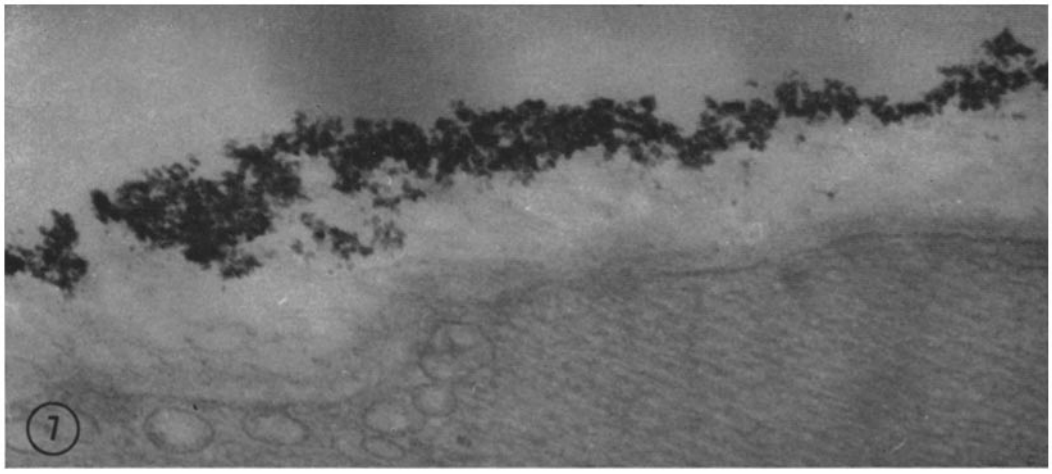

FIGURE 7 A view of the sarcolemma. The dense and concentrated precipitate is observed on the outer region of the sarcolemma. Large transverse tubules at the surface of the fiber are empty. × 100,000.

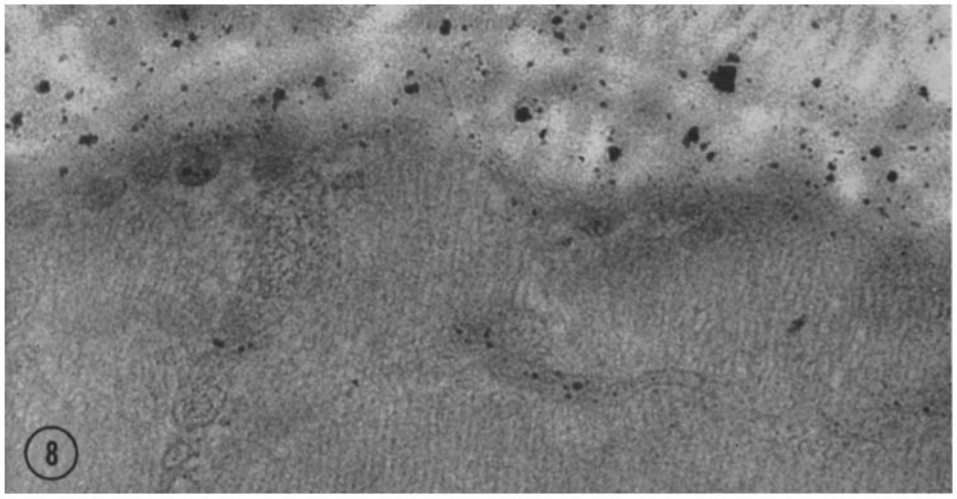

FIGURE 8 Another view of the sarcolemma. The precipitate appears as a fine granular material or with a grumous aspect throughout the outer portion of the sarcolemma. Transverse tubules near the surface contain the sodium precipitate. A triad is seen on the left of the picture very close to the surface of the fiber, with the precipitate only inside the transverse tubule. × 76,500.

the Na^{22} incorporated into them overnight in the Conway-Ringer's solution is retained to a greater extent in the muscle exposed to the potassium pyroantimonate reagent. This experiment simply shows that the reaction is taking place during fixation and that the precipitate probably does not diffuse out after it is formed.

The total volume of the transverse tubular system has been calculated to be between 0.2 and 0.5% of the total volume of the muscle fiber (2, 12). If the sodium inside these tubules is at the same concentration of 104 mM as in the extracellular fluid, then the total amount of internal fiber sodium calculated by Conway (3) to be about 1 mmole/kg is of the order of magnitude of the total sodium inside the transverse tubules. However, this 1 mmole/kg value for the internal fiber sodium was arrived at on the basis of the lack of exchange of all stable sodium with Na^{24}. If a free communication exists between the extracellular fluid and

the transtubular fluid, then complete exchange and release of radioactive sodium between these two fluids should occur and the transtubular sodium would be included in the fast first component of the sodium efflux from the muscle which has traditionally represented the extracellular fraction.

The free communication with the sarcolemma or the extracellular space of the transverse tubules, together with the lack of communication of this system with the sarcoplasmic reticulum and the overwhelming number of facts and correlations with electrical and ionic measurements in muscles or single fibers, during rest and excitation, has led to the view that besides the activation of the sarcolemma, by a sodium current, the membranes separating the transverse tubules from the terminal cisternae could also be activated. As a consequence, Ca ions would be released from the sarcoplasmic reticulum to initiate the contraction of the fibrils (4, 12–14). The present findings give further support to the above mentioned hypothesis and confirm the assumption of the high sodium concentration of the fluid contained in the transverse tubules.

SUMMARY

The location of sodium by electron microscopy was studied in the frog sartorius muscles fixed in a glutaraldehyde medium containing KSb $(OH)_6$ and postfixed in osmium tetroxide. A dense precipitate of sodium pyroantimonate only was observed inside the transverse tubules of the T system, whereas the sarcoplasmic reticulum and interfibrillar areas were completely free of the precipitate. The outer region of the sarcolemma showed profuse precipitate. It is concluded that the fluid inside the transverse tubules has a high or higher concentration of sodium than the rest of the muscle fiber and that, in all probability, this is a reflection of the communication of these tubules with the extracellular space. However, the evidence presented here does not exclude completely the possibility that the reagent utilized did not penetrate through the plasma membrane of the muscle fibers.

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