

# Sodium, Potassium, and Chloride Concentrations in the Schwann Cell and Axon of the Squid Nerve Fiber

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**ABSTRACT** Sodium, potassium, and chloride concentrations were determined in the sheath cells and axoplasm of the nerve fiber of the squid *Sepioteuthis sepioidea*. The sheaths were obtained by slitting the nerve fiber, the extracellular electrolytes were washed out in isotonic sucrose solution, and the concentrations in the cells were determined after different soaking times in the sucrose solution. Values for the Schwann cell were calculated by extrapolation to zero time from the plots of the logarithms of the concentrations in the cells as a function of soaking time in sucrose solution. The Schwann cells made up 84 per cent of the sheath's total cellular volume. The Schwann cell concentrations in millimols per liter, are: 312 (404–241) for sodium, 220 (308–157) for potassium, and 167 (208–138) for chloride. The concentrations in the axoplasm (mean  $\pm$  SE), in millimols per liter are:  $52 \pm 10$  for sodium,  $335 \pm 25$  for potassium, and  $135 \pm 14$  for chloride. The possibility that some fraction of the Schwann cell electrolytes, especially of sodium, is bound, cannot be discarded.

The present work deals with the determination of sodium, potassium, and chloride in the Schwann cell and axoplasm of the nerve fiber of the squid *Sepioteuthis sepioidea*.

For the Schwann cell (Del Rio Hortega's peripheral glial cell) of the squid fiber, Schmitt and Geschwind (1) suggested that the sodium and potassium concentrations should be essentially similar to those of the axon, that is, low sodium and high potassium. On the contrary, Sjöstrand (2) proposed that the Schwann cell should have, as the blood and interstitial fluid of the squid, high sodium and low potassium concentrations. Subsequent work of Coelho, Goodman, and Bowers (3) presented direct evidence of the high potassium concentration in the Schwann cell of the squid fiber. No determinations of sodium and chloride have been made in this cell. The concentrations of these electrolytes are needed to obtain further knowledge on the nature of the

Schwann cell electrical potential (4), and on the role of this cell in the functioning of the axon.

The results of the present work indicate that the Schwann cell of *S. sepioidea* has (a) a high sodium concentration, (b) a high potassium concentration, as previously found by Coelho *et al.* in *Loligo pealii* (3), and (c) a concentration of chloride similar to that of the axoplasm.

Preliminary results of this work were reported (5) as part of a presentation to the International Biophysics Meeting in Paris (June, 1964).

#### EXPERIMENTAL METHOD

##### 1. Sheath Cells

The giant nerve fiber from the first stellar nerve of the tropical squid *S. sepioidea* was used. Immediately after decapitation of the squid, the giant nerve fiber was dissected from the mantle and placed in a lucite holder containing artificial sea water. The artificial sea water contained, in millimols per liter (*cf.* reference 6): NaCl, 442; KCl, 10; CaCl<sub>2</sub>, 11; MgCl<sub>2</sub>, 53; NaHCO<sub>3</sub>, 2.5. Small nerve fibers and the outermost portion of the giant fiber endoneurium were carefully removed under the microscope, leaving a periaxonal sheath about 6  $\mu$  thick (see below: Estimation of cellular volume). Only fibers which were fully excitable after isolation and cleaning were used. The inner diameter of the sheath cylinder (equal to the axon diameter) was measured with 3  $\mu$  precision with a microscope equipped with an ocular micrometer. Slitting of sheaths was accomplished by the insertion of sharp Cawthorne scissors into the axon and then cutting the cleaned sheath longitudinally. The length of slit sheath was measured with 1 mm precision with a vernier micrometer. Isolation, cleaning, and slitting of the fiber took approximately 15 minutes. Immediately after slitting, the axon sheath, carefully handled with fine stainless steel forceps, was soaked while gently shaken in a series of three baths, each containing 5 ml of sucrose solution, isosmolar with the artificial sea water. Total soaking times of 3, 5, 10, 15, or 20 minutes were used. After soaking the sheath was blotted on filter paper, placed on a piece of vycor glass, and analyzed as described below. The temperature varied between 22–23°C.

##### SODIUM AND POTASSIUM ANALYSES

The sodium and potassium were extracted in 1 ml of 10 per cent aqueous nitric acid solution for 12 hours at room temperature. The sodium and potassium concentrations in the extract were determined by flame photometry with a spectrophotometer (Zeiss PMQ II). Photometer wavelength settings were 589 m $\mu$  for sodium and 768 m $\mu$  for potassium. Slit width settings were 0.02 mm for sodium and 0.65 mm for potassium. An acetylene-oxygen flame was used.

##### CHLORIDE ANALYSES

The chloride was extracted from the slit sheath in 1 ml of 50 per cent aqueous acetic acid solution (v/v) for 12 hours at room temperature. The chloride in the extraction fluid was titrated potentiometrically in an acetic acid-ethanol (v/v) solution (7).

## CONTROL ANALYSES

In all analyses reagent blanks as well as known standards were run with the unknowns. Mutual sodium-potassium interferences were not found within the range of the concentrations in the samples. Various determinations between standards were used. The samples were carefully handled to avoid external contamination, evaporation, and contamination from the glass of the beakers. Vycor glass was always used.

## ESTIMATION OF CELLULAR VOLUME

The total cellular volume in a slit sheath was calculated from the diameter of the sheath cylinder, the length of the slit sheath, and the thickness of the cellular layers in the sheath. The total thickness of the cellular layers was measured in electron micrographs of 17 sections of 4 nerve fiber sheaths, similar to those utilized in the present work. We are indebted to Dr. Gloria M. Villegas for the electron microscopic work.

The tissue was fixed in 2 per cent osmium tetroxide as previously described (8). Methacrylate was used as embedding medium. Fine sections obtained in a LKB ultramicrotome (LKP Produkter AB, Stockholm, Sweden) were examined in a Siemens Elmiskop I (Siemens & Halske AG, Siemensstadt, Germany). The maximal reduction in the thickness of the cellular layers with these techniques is calculated to be 2.7 per cent (9). Therefore, no corrections for shrinkage were made to the electron micrograph measurements. The thickness measured at the nuclear regions was discarded to avoid the influence of relatively high values on the mean. This appears justified because the fraction of the total perimeter occupied by the nuclear regions is only 7.5 to 8 per cent.

The mean value obtained for the total thickness of the cellular layers was  $2.2 \pm 0.1 \mu$  ( $\pm$ SE), of which  $1.9 \mu$  corresponds to the Schwann cell layer and  $0.3 \mu$  to endoneurium cells. Thus, about 84 per cent of the total cell volume in the sheath is formed by Schwann cells. These values are rather constant, despite the variation in total thickness of the isolated sheaths due to extracellular material. The total thickness of the sheaths was 4 to  $7 \mu$  from the axolemma to the outer dissected boundary of the endoneurium. As described in a previous paper (4) the Schwann layer of *S. sepioidea* is formed by a single row of cells. The number of Schwann cell nuclei observed around the perimeter of the axon is usually 8. Thus, it was assumed that 25 per cent of the Schwann cells were damaged by the slitting procedure. In order to express the results in millimols per liter, a cellular water content equal to 80 per cent of the total volume of intact cells was used.

## 2. Axoplasm

Immediately after isolation from the squid mantle, the nerve fiber was soaked while gently shaken in 20 ml of sucrose solution during approximately 15 seconds. Only fibers which were fully excitable, prior to immersion in the sucrose solution, were used. At the end of the soaking period in the sucrose solution the fiber was taken out, blotted on filter paper, and its axoplasm extruded. Each axoplasm sample was

collected on a small piece of vycor glass (about 10 mg in weight) and weighed in a balance (Mettler S6), at 60, 90, 120, 150, and 180 seconds after extrusion, with an accuracy of  $\pm 2 \mu\text{g}$ . The initial weight of collected axoplasm was calculated by extrapolation and was used in the calculations. The electrolyte analyses were carried out by the same method described above for the sheath samples.

The values for the axoplasm are given in millimols per liter, using a predetermined water content of 773 mg per gm (10) and an axoplasm density of 1.05 gm per  $\text{cm}^3$  (11).

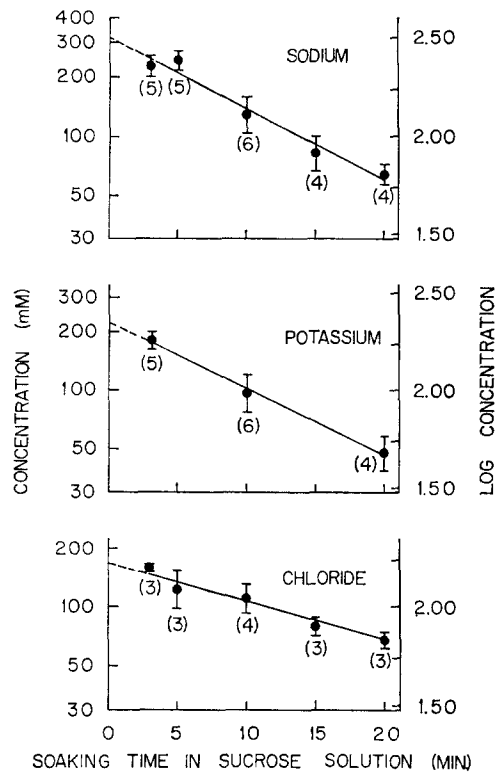


FIGURE 1. Sodium, potassium, and chloride concentrations in the cells of isolated nerve fiber sheaths plotted as a function of soaking time in sucrose solution, isosmolar with artificial sea water. The values are the mean  $\pm$  one standard error. The numbers in parentheses are the numbers of sheath samples analyzed. The total cellular volume in the nerve fiber sheaths is mainly formed by the Schwann cells (84 per cent). Thus the values shown in this figure may be taken as those of the Schwann cell concentrations.

#### RESULTS AND DISCUSSION

Fig. 1 shows the sodium, potassium, and chloride concentrations in the cells of the slit sheaths at different soaking times in sucrose solution, isosmolar with artificial sea water. Each point corresponds to the mean  $\pm$  SE of the logarithms of the concentrations. They are well fitted by a single exponential function of time. The regression lines were calculated by the method of least squares. The intersections at zero time of the regression lines correspond to the cellular sodium, potassium, and chloride concentrations. Since the Schwann cells constitute 84 per cent of a sheath's total cellular volume, the intercepts are considered to represent mainly the Schwann cell concentrations. The uncertainties in the values of the intersections, represented by their standard

errors, would in all probability mask the contribution of the endoneurium cell electrolytes.

Table I shows the values of the concentrations in the Schwann cell. Because of the experimental difficulties involved in the determination of the cellular volume, no claim is made for extreme accuracy of these values. They represent the first direct quantitative estimation of the sodium and chloride concentrations in the Schwann cell. It should be pointed out that the ratio of sodium to potassium to chloride is 1.4 to 1 to 0.8 for this cell and should not be affected by the uncertainty in the estimation of the cellular volume.

The value for the Schwann cell potassium concentration is in agreement with that of the 210 millimols per liter obtained by us from measurements of

TABLE I  
SODIUM, POTASSIUM, AND CHLORIDE  
CONCENTRATIONS IN THE SCHWANN CELL AND  
AXON OF THE SQUID NERVE FIBER

	Concentration		
	Sea water	Schwann cell*	Axon†
	<i>mM/liter</i>	<i>mM/liter</i>	<i>mM/liter</i>
Sodium	444.5	312 (404-241)	52±10
Potassium	10	220 (308-157)	335±25
Chloride	580	167 (208-138)	135±14

\* Values for the Schwann cell were calculated by extrapolation to zero time from the plots of the logarithms of the concentrations in the cells as a function of soaking time in sucrose solution. Given values are antilogarithm of the intersection (and the antilogarithm of the intersection plus and minus one standard error of the intersection).

† Values for the axon are mean ± SE. They were obtained by analyses of six extruded axoplasms for each electrolyte.

the Schwann cell membrane potential as a function of the external potassium concentration (5, 12). Coelho *et al.* (3) have estimated a mean potassium concentration of 353 (range 100 to 800) millimols per kilogram of wet weight for the sheath cells of *L. pealii* fibers. Our values for *S. sepioidea* are not statistically different from theirs, though the *L. pealii* fibers have a Schwann cell layer about ten times thinner than that of the *S. sepioidea* fibers (4), and the sheaths utilized by Coelho *et al.* were shaken for 2 hours in a respirometer before the determination procedure was begun. Despite the difference in squid species, structure of the fibers, and handling of the preparations, the calculated means of the potassium concentrations are similar, and significantly higher in the Schwann cell than in the extracellular fluid.

The shortest soaking time of the sheaths in isotonic sucrose solution was 3 minutes. We have determined, in 5 well cleaned *S. sepioidea* fibers, that the action potential, which depends on the sodium concentration in the extra-

cellular fluid (6), is abolished after  $21 \pm 3$  seconds (mean  $\pm$  SE) when the sea water bath is replaced by isotonic sucrose solution. Therefore, a significant contribution of the extracellular electrolytes to the values obtained for the sodium and chloride concentrations of the Schwann cells, can be discarded.

Table I also shows the concentrations in the axoplasm. These axonal concentrations are similar to those found by Keynes and Lewis (11) and Koechlin (13) for sodium and potassium in *Loligo forbesi*, *Sepia officinalis*, and *L. pealii*, and by Koechlin (13), Deffner (14), and Keynes (15) for the chloride concentration in the axoplasm of *L. pealii*, *Dosidicus gigas*, and *L. forbesi*.

The outstanding feature of the results of the present work is the high sodium concentration in the Schwann cell, which is about six times that of the axoplasm. The potassium and chloride concentrations in the Schwann cell are about two-thirds and almost equal to the axoplasm concentrations, respectively. The possibility that some fraction of the Schwann cell electrolytes, especially of sodium, is bound, cannot be discarded at the present moment. The binding of alkali metal ions by several biologically important molecules, such as phosphate esters, polyphosphates, nucleic acid, and proteins is well known (16).

A pattern of electrolyte concentrations similar to that found for the Schwann cell in the present work, was found by Katzman (17), and Koch, Ranck, and Newman (18) in the glial cells of the central nervous system of mammals. However, Nicholls and Kuffler (19) have proposed for the leech central nervous system that the glial cells should have a low free sodium concentration. Further direct evidence is required to settle this point.

The high sodium content of the Schwann cell may have some physiological significance. It is tempting to suggest in view of the important role of sodium ions in the functioning of the axon, that the Schwann cell sodium may contribute to maintain a normal sodium concentration in the axolemma-Schwann cell space, under some conditions.

The present work should be considered as a base line for future experiments that may disclose the role of the Schwann cell in the functioning of the nerve fiber.

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