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A COMPARISON OF RADIOACTIVE THALLIUM AND POTASSIUM FLUXES IN THE GIANT AXON OF THE SQUID

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SUMMARY

1. The influx and the efflux of ^{204}Tl and ^{42}K were measured in intact squid giant axons.

2. The resting efflux of ^{204}Tl was found to be about one half of ^{42}K and to have a temperature coefficient (Q_{10}) of 1·3 as compared to 1·1 for K.

3. The extra efflux of ^{204}Tl associated with nerve impulses was 30% greater than ^{42}K.

4. From either Cl or NO_3 sea water, the resting influx of ^{204}Tl was about three times that of ^{42}K. Ouabain reduced the influx of either isotope by about two thirds without changing the Tl/K ratio of the fluxes. This indicates that the Na pump can transport Tl.

5. From NO_3 sea water the extra influx of ^{204}Tl associated with nerve impulses was about the same as ^{42}K. From Cl sea water there was no detectable extra influx of ^{204}Tl.

6. The flux ratio, ouabain-insensitive influx/efflux, was different for the two ions. The resting flux ratio for Tl was consistent with a passive non-interacting flux, whereas K movements were consistent with 'single file' passage through the membrane.

7. The extra flux associated with nerve impulses is different from the resting flux both in Tl/K selectivity and in the effect of anion in the sea water. There is also a much higher flux per unit time during the nerve impulse. These differences suggest differences in the mechanisms underlying ion permeability at rest and during nervous activity.

INTRODUCTION

The movement of K across the axon membrane is very important to the functioning of a nerve cell. At rest, the membrane permeability to K ions primarily determines the resting potential and during the action potential the increase in movement of K across the membrane is responsible for the

repolarization of the membrane. The membrane is also able to transport K actively against a concentration gradient.

In order to study the interactions which occur between K and the membrane it is interesting to ask how does the membrane respond to other monovalent cations. The Tl^+ ion is of similar radius to K^+ and is quite stable in solution. This has led to its use as a substitute for K ions in studying membrane function (Mullins & Moore, 1960). Recently Hagiwara, Eaton, Stuart & Rosenthal (1972) and Hille (1973) have reported that the permeability of Tl is about twice that of K based on electrical measurements. In red blood cells Gehring & Hammond (1964) and Lishko, Kolchynska & Parkhomenko (1973), have shown that Tl is actively transported. This paper reports measurements of Tl movements with radioactive tracers in the resting and stimulated nerves in the presence and absence of ouabain. A preliminary communication has appeared (Landowne, 1973).

METHODS

These experiments were designed to measure unidirectional fluxes of radioactive tracers across the membrane of giant nerve fibres of *Loligo pealii*. Data from eighty-eight axons are included in this paper. Influx experiments were performed on paired axons, one of which was stimulated for 5–10 min at 20–100 shocks per second. Lower stimulation rates were used at low temperatures. The axoplasm was extruded and the influx into each axon was calculated in p-mole/cm^2.sec. The difference between these two values divided by the average rate of stimulation (number of impulses/total time of influx) was taken as the extra influx associated with nerve impulses.

Efflux experiments were performed in a chamber similar to that described by Caldwell, Hodgkin, Keynes & Shaw (1960) with the addition of a water jacket to allow control of the axon temperature. The isotopes were microinjected into the axoplasm. Sea water was pumped past the axon at 0·5 ml./min. The action potential was monitored in the cannula.

^{204}Tl was obtained from New England Nuclear as Tl $(NO_3)_3$ in 3N-HNO_3. It was diluted sixfold with distilled water and then bubbled for half an hour with SO_2 to reduce the thallic ion to thallous. The solution was then neutralized with NaOH for influx experiments and KOH for efflux experiments. If a precipitate formed, indicating the presence of the insoluble thallic hydroxide, the solution was discarded. ^{42}K was obtained as a KCl solution at pH 7. The stock solutions of the radioactive isotopes were dried and then made up into either an artificial sea water (ASW) with a final composition of (mM) Na^+, 460; K^+, 10; Ca^{2+}, 10; Mg^{2+}, 55; HEPES (N-2-hydroxyethyl piperazine N′-2-ethanesulphonate) buffer pH 7·4, 2; Cl^- or NO_3^-, 600, or an injection media made of neutralized isotope solution and 5 mM-HEPES. Sea waters with various Tl or K concentrations are described in the text.

The ability of Tl to cross the membrane, or its equivalent flux measured in 10 K, minimal Tl ASW was in the presence of 1–30 μM-Tl. In the minimal K, 4 Tl ASW the bulk K concentration was 50–100 μM. ^{42}K prepared from ^{41}K enriched K was used for this series. The flux of Tl and K respectively in these solutions was negligible but the equivalent flux was easily measured. The calculation of the equivalent flux does not depend on the specific activity of the added isotope as it is calculated directly from the ratio of radioactivity on the two sides of the membrane, as described in the Results section.

^{42}K was assayed by counting the Cerenkoff radiation, or in a well-type gamma counter, and ^{204}Tl was measured with a low background gas flow beta counter. In double label experiments the ^{42}K was allowed to decay for 10–15 days before counting the Tl.

RESULTS

Electrical recordings

Tl has long been known to be toxic and to produce neurological symptoms (Lamy, 1863). Recently, it has been suggested that Tl was directly toxic to the mechanism underlying Na movements during action potentials in frog nerves (Hille, 1972). It therefore seemed appropriate to look for alterations in the action potential in sea waters containing Tl. Two difficulties arose in these experiments. First, thallous chloride is only sparingly soluble. This problem was circumvented by the use of artificial sea water

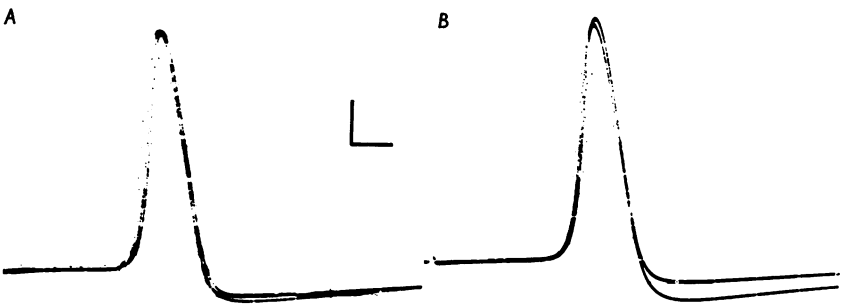

Fig. 1. Effect of replacing K in sea water by Tl. *A*, action potentials in 10 K ASW and 1 Tl, 7·5 K ASW. The second action potential has been shifted slightly to the left and has a larger undershoot. *B*, action potentials in 10 K ASW and 4 Tl ASW. The second action potential has a lower amplitude and larger undershoot. Calibration 20 mV and 0·5 msec. Temperature 18° C. All records in NO_3 ASW.

in which all of the Cl^- was replaced by NO_3^-. This replacement by itself increased the amplitude of the action potential 3–5 mV and also reduced the undershoot by a similar amount. Both of these effects would be expected if there were a slight change in the resting potential in the hyperpolarizing direction.

This hyperpolarization is what would be expected from the constant field equation if NO_3^- were more permeable then Cl^-. In skeletal muscle, however, Cl^- is more permeable than NO_3^- (Woodbury & Miles, 1973). The hyperpolarization is consistent with the reduced K influx from NO_3 ASW as compared to Cl ASW (Table 6).

The second difficulty arises because Tl^+ depolarizes the axon more effectively than K^+ and the depolarization would be expected to alter the

form of the action potential. Accordingly the three solutions tested contained altered K concentrations based on the finding that 4 mM-Tl^+ is as effective as 10 mM-K^+ in maintaining the resting potential (Hagiwara *et al.* 1972).

Fig. 1*A* shows the response in 10 K (NO_3) ASW and in 1 Tl, 7·5 K (NO_3) ASW. The trace with Tl has been shifted slightly to the left. The only obvious difference between the two records is a 3 mV increase in the undershoot in Tl. This effect is more dramatically shown in Fig. 1*B* which compares 10 K (NO_3) ASW with 4 Tl (NO_3) ASW. This shows a 8 mV larger undershoot in 4 Tl (NO_3) ASW and also a diminution of the amplitude of the action potential. These changes in the undershoot are more readily discussed after considering the results of the flux experiments.

The changes seen in the action potential were fully reversible for exposures up to 30 min. Longer exposures were not attempted. Furthermore, the solution did not display any obvious effects on heavily stimulated nerves. The amplitude of the action potential decreased 2–3 mV after 30000 impulses in either 10 K (NO_3) ASW or 4 Tl (NO_3) ASW. It seems unlikely, therefore, that any toxic effects on the action potential mechanism are exhibited in the course of the flux experiments reported here.

Efflux

Resting efflux. At room temperature the resting axon lost approximately one one thousandth of the injected ^{204}Tl each minute (Table 1). As each individual experiment progressed, the fractional loss increased, primarily following stimulation. This is also the case for the ^{42}K efflux as is shown in Fig. 2. This increase in K efflux following stimulation can also be seen in the data of Caldwell & Keynes (1960, Fig. 4) perhaps resulting from a small residual depolarization of the axon following stimulation which raises the permeability of K.

The experiment shown in Fig. 2 is from an axon which was injected with both ^{42}K and ^{204}Tl. It is apparent that the ^{204}Tl leaves the axon less easily than the ^{42}K. At room temperature the ratio ^{204}Tl efflux/^{42}K efflux is equal to $0{\cdot}62 \pm 0{\cdot}02$ (S.E.). A similar effect can be seen in muscle by comparing the data of Mullins & Moore (1960) for Tl efflux with the data of Hodgkin & Horowicz (1959) for K. If the Tl data is normalized to the internal K value found by Hodgkin & Horowicz (1959) one obtains a Tl efflux/K efflux ratio of 0·7 for the muscle membrane.

The rate of loss of thallium increases as the temperature is increased with a Q_{10} of about 1·3. While this temperature coefficient is smaller than values seen for active transport, it is larger than the value of about 1·1 reported by Hodgkin & Keynes (1955*a*) for K efflux. If K efflux and Tl efflux have different temperature coefficients their ratio should also change with

temperature. In the double labelled experiment the ratio ^{204}Tl efflux/^{42}K efflux at 7° C was 0·52 ± 0·04 (S.E.). This change in the ratio with cooling is quantitatively consistent with the different temperature coefficients for the two fluxes.

Stimulated efflux. As can be seen in Fig. 2 when the axon was stimulated at 20 or 60 shocks/sec the rate of loss of Tl and K was dramatically increased. The ratio of the two effluxes approaches one. The extra efflux of Tl per impulse, that is, the difference between the stimulated efflux and

TABLE 1. The efflux of ^{204}Tl from squid giant axons

Expt.	Time (min)	$k_r \times 10^3$ (min^{-1})	$k_s \times 10^6$ (impulse^{-1})	Rate (sec)	Temp. (° C)	Diam. (μ)	Q_{10} k_r	Q_{10} k_s
20J3	60	0·95	1·09	20	26	533		
	115	0·60	1·80	20	11		1·36	1/1·56
	175	0·94	0·84	20	26			
	230	1·30	0·77	60	26			
	260	1·43	0·50	120	26			
	290	1·62	0·21	180	26			
26J3	40	0·77	1·08	20	25	574		
	85	0·78	1·05	20	25			
	120	1·08	0·81	60	25			
	185	0·66	1·23	20	7			
11Y3	12	1·81	2·25	60	29	470		
	44	1·96	2·00	30	29			
	66	0·99	3·47	30	10		1·27	1/1·39
	94	1·19	4·03	30	10			
	118	1·40	2·14	60	27			
	140*	1·49	2·06	60	27			
Mean†		1·29	1·40		26	526	1·32	1/1·47
S.E.		± 0·11	± 0·20					

* Isethionate sea water was used.

† Mean includes only data at room temperature and at stimulation rates of 60/sec and lower.

the resting efflux, is approximately 25 % larger than that of K. The Tl/K selectivity of the membrane is altered when the nerve is stimulated. In frog muscle Mullins & Moore (1960) found that about the same amount of Tl was lost per impulse as K. The extra efflux of Tl increased as the temperature was reduced with a Q_{10} of about 1/1·5, as does the extra efflux of potassium (D. Landowne, unpublished).

Influx

The influx of ^{204}Tl and ^{42}K was measured from a series of sea waters with different Tl and K concentrations chosen to maintain the same resting

potential. The data are presented as the fluxes of the ions, expressed in p-mole/cm^2.sec in Table 3 and as the ability of an ion to cross the membrane in Table 2. This ability to cross the membrane has been called the *equivalent flux* after Kohlrausch's equivalent conductance. It is the flux across the membrane divided by the concentration from which the flux is

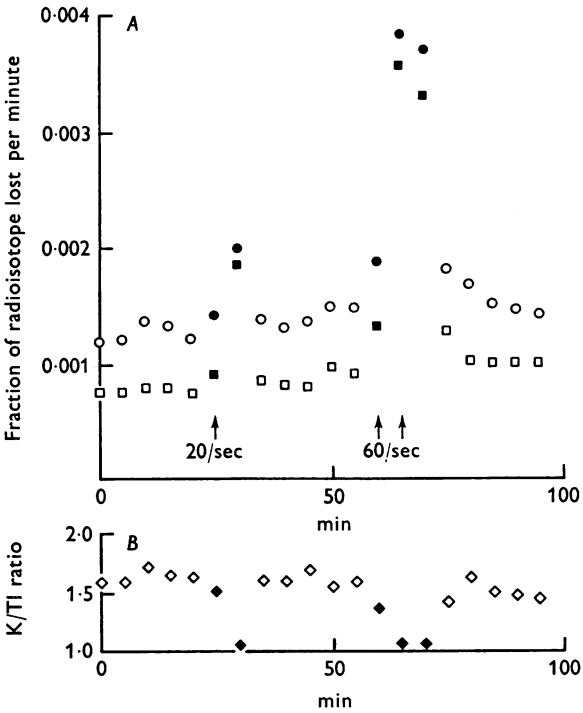

Fig. 2. The efflux of radioactive K and Tl from a squid giant axon which was microinjected with both of these isotopes. *A*, the fraction of the K (circles) and the Tl (squares) which leaves the axon per minute. During the 5 min collection periods indicated by the arrows the axon was stimulated at 20/sec and 60/sec respectively. The filled symbols represent the collection periods which were considered as extra efflux associated with the stimulation, being the stimulated periods and the first following period. Temp. 25° C, axon diameter 574 μm. *B*, the ratio of the fractional losses plotted in *A*.

measured. The units are cm/sec, the same as membrane permeability. It seems preferable not to label these measurements as permeability for at least two reasons. First, no account is taken of the charge on the ion or the membrane potential which can be expected to interact to change the equivalent fluxes but not alter the permeabilities as described by Goldman (1943) and Hodgkin & Katz (1949). Secondly, it is known that some of the ions cross the membrane by active transport mechanisms which contri-

butes to the measured equivalent flux but, again, is not the kind of permeability used in the constant field equations.

Influx measurements were made in artificial sea waters with either Cl^- or NO_3^- as the major anion. Thallous chloride is relatively insoluble which limits the range of Tl concentrations which can be used in Cl sea water. Furthermore, there will be a considerable amount of TlCl associated in solution. Therefore, the results from nitrate sea waters will be described first and then compared with those from chloride sea water.

TABLE 2. Equivalent influxes of ^{42}K and ^{204}Tl into resting squid axons. The equivalent influx of each isotope is the measured influx divided by the concentration in the bathing medium. The result, expressed as cm/sec, has been multiplied by 10^7. The numbers indicate the mean ± S.E. (numbers of experiments). The Tl/K ratio is only from double-label experiments. All experiments were in NO_3 sea water at 20–22° C

	10 K ASW	+ Ouabain
K	19 ± 2 (5)	5·9 ± 0·4 (3)
Tl	40 ± 6 (8)	12 ± 2 (6)
Tl/K	3·5 ± 0·9 (3)	2·8 ± 0·3 (3)
	1 Tl 7·5 K ASW	+ Ouabain
K	18 ± 2 (5)	4·3 ± 0·5 (5)
Tl	48 ± 3 (5)	14 ± 2 (5)
Tl/K	2·8 ± 0·3 (5)	3·8 ± 0·9 (5)
	4 Tl ASW	+ Ouabain
K	15 ± 3 (3)	3·4 ± 0·4 (3)
Tl	49 ± 6 (3)	10 ± 1 (3)
Tl/K	3·2 ± 0·1 (3)	3·1 ± 0·3 (3)
	Combined data	
K	18 ± 1 (13)	
Tl	45 ± 3 (16)	12·6 ± 1·2 (14)
Tl/K	3·1 ± 0·3 (11)	3·3 ± 0·4 (11)

Resting equivalent influx. Table 2 shows the resting equivalent influx of ^{42}K and ^{204}Tl in standard NO_3 artificial sea water and in sea water where some or all of the K is replaced by Tl with 1 Tl replacing 2·5 K ions. The ratio of the equivalent fluxes has been calculated for all double label experiments and is also tabulated. The measurements were made on separate axons in the presence and absence of ouabain.

The resting influx of potassium from 10 K (NO_3) ASW is slightly lower than from 10 K(Cl)ASW (Table 6). 10^{-5} M ouabain reduces the influx dramatically in both Cl and NO_3 solutions. The magnitudes of K influxes are similar to previously reported experiments (Caldwell *et al.* 1960; Caldwell & Keynes, 1960; Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969; Mullins & Brinley, 1969).

The ^{204}Tl equivalent fluxes from 10 K(NO_3)ASW are about three times as large as ^{42}K both in the presence and absence of ouabain. There is clearly a ouabain-sensitive Tl influx which suggests that Tl is actively transported into the axon in the same way as K. This has been shown to be the case in red blood cells (Gehring & Hammond, 1964; Lishko *et al.* 1973).

As the concentration of Tl and K are altered the equivalent influxes do not change with the possible exception of K in the presence of ouabain. This decrease is statistically significant but the linear regression coefficient (r^2) is only 0·4. The other data have been pooled at the bottom of Table 2 which emphasized the finding that ^{204}Tl crosses the resting membrane three times as easily as ^{42}K.

TABLE 3. Resting influx of K and Tl in squid axons in p-mole/cm².sec. Other conditions as in Table 2

	10K ASW	+ Ouabain
K	19	5·9
Tl	—	—
Tl + K	19 ± 2 (5)	5·9 ± 0·4 (3)
	1 Tl, 7·5 K ASW	+ Ouabain
K	13·5	3·2
Tl	4·8	1·4
Tl + K	18 ± 2 (5)	4·6 ± 0·3 (5)
	4 Tl ASW	+ Ouabain
K	—	—
Tl	20	4·2
Tl + K	20 ± 3 (3)	4·2 ± 0·3 (3)

In contrast to the equivalent fluxes, the actual fluxes of Tl and K do change as the concentrations are changed (Table 3). However, in the absence of ouabain the total influx, Tl plus K, remains constant in the three different solutions. This reflects the reason for choosing these particular solutions for their ability to maintain the same resting potential. In the presence of ouabain the total flux declines slightly with increasing Tl replacement but again r^2 is only 0·4.

Extra influx associated with nerve impulses. When one axon of a pair was repetitively stimulated there was always a greater uptake of ^{42}K by the stimulated axon than by the resting axon in either Cl or NO_3 sea water (Tables 4, 5, 6). The extra influx of K is larger from NO_3 sea water than from Cl sea water. The extra K influx in squid axons in Cl sea water was 0·2 p-mole/cm².impulse, about one half of the value found for *Sepia* (Keynes, 1951). In NO_3 sea waters it can be seen that the extra equivalent influx of ^{204}Tl was slightly greater than that of K and that the Tl/K ratio was not dependent on the composition of the sea water (Table 4). The

addition of 10^{-5} M ouabain reduced the Tl/K ratio for the extra equivalent influx from 1·4 to 1·0, both far less than the value of 3·2 found for the ratio of the resting equivalent influxes.

TABLE 4. Extra equivalent influxes of ^{42}K and ^{204}Tl associated with nerve impulses in squid axons expressed in cm/impulse and multiplied by 10^7. Other conditions as in Table 2

	10 K ASW	+ Ouabain
K	0·43 ± 0·07 (4)	0·43 ± 0·06 (3)
Tl	0·55 ± 0·10 (8)	0·28 ± 0·07 (6)
Tl/K	1·6 ± 0·9 (3)	0·8 ± 0·2 (3)
	1 Tl, 7·5 K ASW	+ Ouabain
K	0·48 ± 0·11 (5)	0·25 ± 0·04 (5)
Tl	0·62 ± 0·04 (5)	0·26 ± 0·03 (5)
Tl/K	1·4 ± 0·2 (5)	1·1 ± 0·2 (5)
	4 Tl ASW	+ Ouabain
K	0·21 ± 0·02 (3)	0·17 ± 0·05 (3)
Tl	0·25 ± 0·16 (3)	0·18 ± 0·05 (3)
Tl/K	1·2 ± 0·7 (3)	1·1 ± 0·2 (3)
	Combined data	
K	0·40 ± 0·05 (12)	0·28 ± 0·04 (11)
Tl	0·51 ± 0·07 (16)	0·25 ± 0·03 (14)
Tl/K	1·44 ± 0·29 (11)	1·02 ± 0·10 (11)

TABLE 5. Extra influx of K and Tl associated with stimulation expressed in p-mole/cm^2.impulse. Other conditions as in Table 2

	10 K ASW	+ Ouabain
K	0·43	0·43
Tl	—	—
Tl + K	0·43 ± 0·07 (4)	0·43 ± 0·06 (3)
	1 Tl, 7·5 K ASW	+ Ouabain
K	0·36	0·19
Tl	0·06	0·03
Tl + K	0·42 ± 0·07 (5)	0·21 ± 0·03 (5)
	4 Tl ASW	+ Ouabain
K	—	—
Tl	0·10	0·07
Tl + K	0·10 ± 0·04 (3)	0·07 ± 0·01 (3)

Table 5 shows that the total extra influx of Tl and K decreases as Tl replaces K in the bathing medium. This is consistent with the enhancement of the undershoot seen in Tl sea waters (Fig. 1). Reducing the extra influx is equivalent to increasing the net efflux or outward movement of charge which tends to hyperpolarize the axon. The reduction in influx comes

primarily from the reduced external concentrations but also it appears as if the extra equivalent influxes are smaller in 4 Tl ASW than in 10 K ASW. This trend can be seen for both K and Tl in the absence and presence of ouabain but the differences are small and there is too much variability in the data to attempt to quantitate this trend. Ouabain also seems to reduce the extra equivalent influx of both ions with a larger effect on Tl. Thus, if all of the data are pooled, ouabain reduces the extra K equivalent influx by about one quarter ($P < 0{\cdot}1$) and the extra Tl equivalent influx by about one half ($P < 0{\cdot}001$). This effect of ouabain on stimulated fluxes was also seen for sodium efflux by Cohen & Landowne (1974).

Influx from Cl sea water. Tl Cl is sparingly soluble and 1–2 m-mole Tl^+ can be added to sea water without forming a precipitate. Table 6 shows the equivalent influxes of ^{204}Tl and ^{42}K measured in Cl sea water, and comparable experiments in NO_3 sea water.

TABLE 6. Comparison of equivalent influx of ^{42}K and ^{204}Tl from Cl or NO_3 sea waters. All sea water contained 10 mM-K and trace amounts of Tl. Other conventions as in Tables 2 and 4, except that the measurements in 10 K (Cl) ASW were at 24–27° C

	Resting		Stimulated	
	ASW	ASW + ouabain	ASW	ASW + ouabain
		Cl		
K	25 ± 2 (4)	11 ± 1 (8)	0·18 ± 0·06 (4)	0·23 ± 0·03 (8)
Tl	65 ± 5 (4)	30 ± 3 (8)	−0·09 ± 0·08 (4)	0·00 ± 0·05 (8)
Tl/K	2·6 ± 0·1 (4)	2·8 ± 0·2 (8)		
		NO_3		
K	19 ± 2 (5)	5·9 ± 0·4 (3)	0·43 ± 0·07 (4)	0·43 ± 0·06 (3)
Tl	40 ± 6 (8)	12 ± 2 (6)	0·53 ± 0·10 (8)	0·28 ± 0·07 (6)
Tl/K	3·5 ± 0·9 (3)	2·8 ± 0·3 (3)	1·6 ± 0·9 (3)	0·8 ± 0·2 (3)

The resting flux of ^{42}K is somewhat greater in Cl than in NO_3 sea water. The Cl experiments in the absence of ouabain were done at higher temperature (25° C) than the rest which may account for some of the difference in equivalent influxes. However, the ouabain-insensitive influx of both ^{204}Tl and ^{42}K is significantly greater from Cl sea water than from NO_3. This is consistent with the idea that the axon hyperpolarizes in NO_3 ASW but does not allow one to decide if the hyperpolarization reduces the flux by reducing the driving force on K or if the reduced influx hyperpolarizes the cell. The ouabain-sensitive influx of both isotopes is not significantly changed by this change of anions. There is too much variability in the data to decide if the Tl/K ratios are altered.

The pattern of stimulated influx is quite different from the resting influx. The extra influx of ^{42}K was smaller from Cl sea water compared to

NO_3 whereas the resting influx was larger from Cl. Even more strikingly there was no detectable extra influx of ^{204}Tl. In many cases, the stimulated fibre had less influx than the control. This was not the case for K where every axon was found to gain ^{42}K when stimulated. The resting influx of ^{204}Tl is considerably larger than ^{42}K and it might have been that the apparent lack of extra influx was due to the larger variability of the resting influx. That this is not the case can be seen in the experiments done in the presence of 10^{-5} M ouabain. Here the resting influx of ^{204}Tl is approximately the same as ^{42}K influx in sea water but still there is no detectable extra influx of ^{204}Tl with stimulation.

Correcting the data for the efflux which occurs during the influx experiments (Keynes, 1951) does not significantly alter the findings although the correction is larger in the case of ^{204}Tl influx.

In almost every experiment there was less extra ^{204}Tl influx than ^{42}K. The difference between extra influxes was highly significant ($P < 0{\cdot}001$). With 90% confidence intervals the largest ratio of ^{204}Tl extra influx/^{42}K extra influx is 0·65 at room temperature.

Experiments at low temperature. In Cl sea water cooling the axons to 7° C reduced the resting ^{204}Tl equivalent influx to $20 \pm 2 \times 10^{-7}$ cm/sec (eight experiments). The Tl/K ratio was $2{\cdot}4 \pm 0{\cdot}2$. The effect of cooling on the Tl/K influx ratio is not statistically significant ($0{\cdot}2 < P < 0{\cdot}3$) but there is so much variability in the measurements that one cannot say that it is different than the effect on the efflux ratio ($0{\cdot}4 < P < 0{\cdot}3$) where cooling reduced the ratio by about 20%. The extra equivalent influx was $0{\cdot}3 \pm 0{\cdot}2$ cm/impulse. When the experiment was repeated in NO_3 sea water it was found that the axons fired repetitively in response to a single shock or fired spontaneously. This was probably due to effectively lowering the free Ca^{2+} by formation of $CaNO_3^-$ ion pairs. The association constant for $CaNO_3^-$ is 0·5 M at 18° C (Sillen & Martel, 1964) which means about one half of the Ca is complexed and presumably lowering the temperature favours pair formation. Adding sufficient $Ca(NO_3)_2$ to raise the total Ca to 100 mM while reduced Mg and Na stabilized the axon. No flux measurements were made in this altered sea water.

Flux ratios. Ussing (1949) has shown that in a passive system with freely moving ions the ratio of the influx to the efflux of a univalent ion is given by

$$\frac{M_i}{M_o} = \frac{f_o}{f_i}\frac{C_o}{C_i}\exp\,(eV/kT),$$

where M_i is the influx; M_o, the efflux, f, the activity coefficient; C, the concentration, V, the voltage across the membrane, e, the charge on the electron, k, Boltzman's constant, and T, the absolute temperature. If the activity coefficients are the same on both sides of the membrane, as is likely to be the case in the squid giant axon, then the ratio of the equivalent fluxes should be related to the membrane potential by

$$V = \frac{kT}{e}\ln\frac{M_i/C_o}{M_o/C_i},$$

where M_1/C_0 is the equivalent influx and M_0/C_1 is the equivalent efflux. Equivalent effluxes were obtained by multiplying the rate constant for loss of isotope (Table 1) by the volume/surface area ratio (i.e. the diameter/4) and dividing by 60 sec/min. If the activity coefficients are different then the potential calculated from the fluxes is given by $V = \tilde{V} - kT/e \ln (f_1/f_0)$, where $\tilde{V}$ is the potential calculated on the assumption that $f_1 = f_0$. Table 7 shows $\tilde{V}$ calculated from the ratio equivalent influx from Cl ASW to equivalent efflux into Cl sea water. It can easily be seen that the flux ratios are quite different for the two cations.

TABLE 7. Calculation of flux ratios for ^{204}Tl and ^{42}K in squid axons. The equivalent fluxes are in units of cm/sec and have been multiplied by 10^7. All measurements at room temperature

	Tl	K
Ouabain-insensitive equivalent influx	30 ± 3	11 ± 1
Equivalent efflux	2·8 ± 0·4	4·6
Ratio	10·6	2·4
(kT/e) ln (ratio)	59 mV	22 mV

The influx data are from Table 6. The efflux of Tl is the average value at room temperature from Table 1. The efflux of K has been computed from this average using the Tl/K ratio. At the end of two separate efflux experiments ouabain raised the efflux of Tl and K by 18 and 15% respectively. The effect was not investigated thoroughly and is not as large as the coefficient of variation of the data presented here.

The potentials calculated from these ratios without correcting for any difference in activity coefficients on the two sides of the membrane are also different for the two cations. The potentials for Tl are consistent with freely moving ions distributing across the membrane whose potential is about 60 mV as in the case of the squid giant axon. Correspondingly, Mullins & Moore (1960) found at equilibrium, when the influx must be equal to the efflux, the ratio of internal to external Tl was about 40. This means the ratio of the equivalent fluxes is also 40, the value expected for a resting potential of 90 mV. This is not the case with K, as has been previously shown in *Sepia* axons by Hodgkin & Keynes (1955*b*). Thus there would appear to be a qualitative difference between the resting movements of Tl and the movements of K. The influx of Tl is higher and the efflux is lower than K.

DISCUSSION

There is considerable reason to expect that Tl will behave like K in the squid giant axon, as has been shown for many biological and other systems (Mullins & Moore, 1960; Gehring & Hammond, 1964; Lishko *et al.* 1973; Spencer, Peterson, Madrid & Raine, 1973). This is due to the similarity of their crystal radii and their limiting conductance in solution. It seems valuable therefore to discuss the results of this paper in comparison with the movements of K ions considering that Tl is a probe for the particular sites of interaction of K with the membrane (Williams, 1970). The Tl flux data reported here resemble the expected K fluxes much more than Na fluxes both in temperature sensitivity and in the effect of ouabain. The equivalent influx of Na is less than 1 % of the corresponding value of K or Tl. The resting Na influx has a Q_{10} of 1·4 (Hodgkin & Keynes, 1955*a*), whereas Tl and K effluxes have low Q_{10}. During the action potential, the increased conductances associated with Na and K are approximately equal (Hodgkin & Huxley, 1952) and correspondingly the extra equivalent fluxes are approximately equal (Tasaki, Teorell & Spyropolous, 1961). There is need, therefore, for more caution in associating the stimulated Tl fluxes with K fluxes.

The ouabain-sensitive influx. It is convenient to distinguish between the ouabain-sensitive influx and ouabain-insensitive influx of K and attribute the former to active transport (Baker *et al.* 1969; Mullins & Brinley, 1969). It can be seen from Tables 2 and 6 that the ouabain-sensitive proportion of the equivalent influx of Tl is about 3 times that of K.

Baker & Connelly (1966) found that Tl was 1·4 times as effective as K in stimulating the pump in crab nerve. In the rabbit vagus nerve (Rang & Ritchie, 1968), the Tl seems to be about 3 times as effective as K and in human erythrocytes (Lishko *et al.* 1973) 5–10 times as effective as K in stimulating the Na pump.

Comparable experiments have not been reported for squid axons. However, when Tl replaced K at a ratio of 1:2·5 the total (Tl + K) ouabain-sensitive influx remain approximately constant (Table 3) suggesting that the pump is operating at the same rate. One might suggest that the site for K stimulation of pump activity is the same as for K influx and Tl binds about 3 times better than K to this site.

The suggestion that a cation's ability to cross the membrane is well correlated with its ability to stimulate the pump (Baker & Connelly, 1966) can perhaps be extended to its ability to be pumped into the cell. At least for these two ions the ratio of equivalent influxes is the same in the presence and absence of ouabain (Tables 2 and 6). One model which is consistent with this idea is that the process which gives the selectivity to the

resting (ouabain-insensitive) influx is external to and in series with the pump mechanism.

Comparison with electrical measurements

Resting fluxes. Hagiwara *et al.* (1972) reported that the permeability ratio P_{Tl}/P_K as calculated from the constant field equation is 1·8. They found that 4 mM-Tl^+ was equivalent to 10 mM-K^+ in depolarizing the axon. The ability to depolarize the axon should be related to the ability to enter the axon and accordingly the ratio of the ouabain-insensitive influxes is about 3 (Table 2) and this total (Tl + K) influx remains approximately constant when 10 K is replaced by 4 Tl ASW. The similarity between these numbers indicates that Tl crosses the membrane in a form which is able to carry current and depolarize the axon, but does not offer any further clue as to the mechanisms by which it enters.

Stimulated fluxes. In frog myelinated nerve Hille (1973) has found a P_{Tl}/P_K ratio of 2·3 in voltage-clamp experiments using the reversal potentials for current and the constant field equation. This does not seem to be the case in squid axons. If the constant field equation is used to analyse the undershoot of the action potential then the change, ΔV, in transferring the axon from 10 K ASW to 4 Tl ASW (Fig. 1*B*) is given by

$$\Delta V = \frac{kT}{e} \ln \frac{\frac{P_{Na}}{P_K}[Na]_o + \frac{P_{Cl}}{P_K}[Cl]_i + \frac{P_{Tl}}{P_K} \times 4}{\frac{P_{Na}}{P_K}[Na]_o + \frac{P_{Cl}}{P_K}[Cl]_i + 10}$$

(Hodgkin & Katz, 1949). Clearly if P_{Tl}/P_K is 2·5 there will be no change in the undershoot whereas an 8 mV change was observed. If one assumes $P_{Na}/P_K = 0$ (Hodgkin & Katz, 1949), $P_{Cl}/P_K = 1/5{\cdot}6$ (Caldwell & Keynes 1960) and $[Cl]_i = 60$ mM (Steinbach, 1941), then an 8 mV change corresponds to a value of 1·1 for P_{Tl}/P_K. Higher estimates for these three parameters lead to a smaller calculated value of P_{Tl}/P_K.

In squid axons the Tl/K ratio of extra equivalent/fluxes is 1–1·4 (Tables 1 and 4). Perhaps some of the Tl flux should be associated with extra sodium movements as Hille (1972) has reported a P_{Tl}/P_K ratio of 0·33. If this is the case, the Tl/K ratio of equivalent fluxes through the K channel is even less. Thus it seems as if the Tl/K selectivity of the active squid axon membrane is different than the frog node. Hille (1972) has also noted differences between his Rb/Na and Cs/Na selectivity ratios and those found in squid axons by Chandler & Meves (1965).

The flux ratios. The data in Table 7 indicate that the flux ratios, influx/efflux for K and Tl are different. K is as would be predicted from a single-file movement across the membrane (Hodgkin & Keynes, 1955*b*) whereas thallium behaves as if the ions do not affect each other's motion.

It would clearly be interesting to know if this pattern prevails at potentials other than the resting potentials. One might suggest that this difference in flux ratios means that there are different pathways for the two ions. A second indication of this kind of difference of the two ion fluxes is seen in Table 2 where in the presence of ouabain the ability for K ions to cross the membrane decreases as the K concentration decreases. Qualitatively, this is what one would expect from the single file model. On the other hand Tl seems to obey the 'independence principle'; its ability to cross the membrane is independent of its concentration, at least in the range studied. A third way the movement of these two ions can be distinguished is by the higher temperature coefficient for Tl efflux.

The simplest hypothesis to account for these differences in passive Tl and K movement is that there are entirely separate systems for the two ions. This is somewhat surprising since it suggests that the nerve membrane has efficient channels for an ion with which it normally never comes in contact and that these Tl channels are highly selective against K and the K channels are highly selective against Tl. Another possibility is that Tl and K use the same pathway but Tl does not interact with the channel in the same way that K does. It is not clear what type of channel could accommodate these two modes of passage. In any case, as it seems that the passive movements of Tl are different from K, one cannot assume that the Tl is acting as a probe for the K channel.

Chloride vs. NO_3 sea water. The dissociation constant for TlCl ion pairs is about 0·25 M (Sillén & Martell, 1964). Thus only about 29 % of the Tl in Cl sea water is in the form Tl^+. The dissociation constant for KNO_3 is 1·3–1·6 M and for Tl-NO_3, 0·40 M (Sillen & Martell, 1964). There is no evidence for KCl complex formation. Thus in NO_3 sea water one could expect about 68 % of the K and 45 % of the Tl to be free, the remainder being complexed with NO_3. The Tl/K ratio of activity coefficients should be 0·29 for Cl sea water and 0·40/0·68 = 0·59 in NO_3 sea water. In the case of the resting equivalent influxes the Tl/K ratio seems to stay about the same in both Cl and NO_3 sea waters in spite of the differing relative fractions of free ions. Apparently the resting membrane is unable to distinguish 'free' Tl^+ from Tl associated with Cl ions. It seems unlikely that TlCl is the species that crosses the membrane, at least in the ouabain sensitive pathway. A likely explanation is that the difference in free energy between an ion in solution and an ion in the membrane is large compared to the binding energy of the cation to the anion so that if an ion or an ion pair collides with the membrane with sufficient thermal energy it can cross the interface (without an anion) whether it was associated with an anion or with the H_2O molecules.

The stimulated fluxes on the other hand are dramatically affected by

the anion present. The Tl/K ratio of equivalent fluxes seems to vary as the Tl/K ratio of activity coefficients. Thus, from Cl sea water, where the ratio of activity coefficients is 0·29, the Tl/K equivalent flux ratio is near zero; from NO_3 sea water, the ratio of activity coefficients is 0·59 and the equivalent flux ratio is 0·8; and from axoplasm, assuming Cl_i is 60 mM, the ratio of activity coefficients is 0·80 and the ratio of equivalent effluxes is about 1·4. Qualitatively, it appears that the Tl/K ratio of the equivalent fluxes is related to the ratio of the activity coefficients. This indicates a difference between the resting ion movements and those activated by the passage of a nerve impulse, in that the ion membrane interaction is weaker in the latter case so that the presence of an anion which binds the cations reduces their flux in proportion to the binding.

The free energy of TlCl association is only a few hundred calories per mole whereas the free energy change associated with moving an ion from water to a region of low dielectric constant is many kcal/mole. It is unclear how small changes in the binding of the ions by the solution from which they come has an effect on the stimulated fluxes and not on the resting fluxes. There are two other differences in these two fluxes which may be related: firstly, the greater Tl/K selectivity of the resting flux and secondly, the greater magnitude of the ion movement per unit time during the nerve impulse than at rest. Thus the pathway of ion movement associated with nerve impulses has a higher flux, a lower Tl/K selectivity and is more easily affected by binding in the solution exterior to the membrane when compared to the pathway for ion movement in the resting nerve. All of these are consistent with the idea that the selectivity mechanism is less tenacious during the nerve impulse than at rest.

Tl has several other properties to make it attractive as a probe for the interactions of potassium with the membrane. It is fluorescent in aqueous solution and this fluorescence is sensitive to ligand binding (Steffen & Sommermeyer, 1968). ^{205}Tl has a high relative sensitivity for NMR and this has been used to study its binding to pyruvate kinase where Reuben & Kayne (1971) found direct evidence for a conformational change of the enzyme. Because of its high atomic weight also it has been suggested that it would be possible to observe bound Tl in the electron microscope (Britten & Blank, 1968; Spencer *et al.* 1973).

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REFERENCES

BAKER, P. F., BLAUSTEIN, M. P., KEYNES, R. D., MANIL, J., SHAW, T. I. & STEINHARDT, R. A. (1969). The ouabain-sensitive fluxes of sodium and potassium in squid giant axons. *J. Physiol.* **200**, 459–496.

BAKER, P. F. & CONNELLY, C. M. (1966). Some properties of the external activation site of the sodium pump in crab nerve. *J. Physiol.* **185**, 270–297.

BRITTEN, J. S. & BLANK, M. (1968). Thallium activation of the (Na^+-K^+)-activated ATPase of rabbit kidney. *Biochim. biophys. Acta* **159**, 160–166.

CALDWELL, P. C., HODGKIN, A. L., KEYNES, R. D. & SHAW, T. I. (1960). The effects of injecting 'energy-rich' phosphate compounds on the active transport of ions on the giant axons of *Loligo*. *J. Physiol.* **152**, 561–590.

CALDWELL, P. C. & KEYNES, R. D. (1960). The permeability of the squid giant axon to radioactive potassium and chloride ions. *J. Physiol.* **154**, 177–189.

CHANDLER, W. K. & MEVES, H. (1965). Voltage clamp experiments on internally perfused giant axons. *J. Physiol.* **180**, 788–820.

COHEN, L. B. & LANDOWNE, D. (1974). The temperature dependence of the movement of sodium ions associated with nerve impulse. *J. Physiol.* **236**, 95–111.

GEHRING, P. J. & HAMMOND, P. B. (1964). The uptake of thallium by rabbit erythrocytes. *J. Pharmac. exp. Ther.* **145**, 215–221.

GOLDMAN, D. E. (1943). Potential, impedance and rectification in membranes. *J. gen. Physiol.* **27**, 37–60.

HAGIWARA, S., EATON, D. C., STUART, A. E. & ROSENTHAL, N. P. (1972). Cation selectivity of the resting membrane of squid axon. *J. Membrane Biol.* **9**, 373–384.

HILLE, B. (1972). The permeability of the sodium channel to metal cations in myelinated nerve. *J. gen. Physiol.* **59**, 637–658.

HILLE, B. (1973). Potassium channels in myelinated nerve. Selective permeability to small cations. *J. gen. Physiol.* **61**, 669–686.

HODGKIN, A. L. & HOROWICZ, P. (1959). Movements of Na and K in single muscle fibres. *J. Physiol.* **145**, 405–432.

HODGKIN, A. L. & HUXLEY, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**, 37–77.

HODGKIN, A. L. & KEYNES, R. D. (1955*a*). Active transport of cations in giant axons from *Sepia* and *Loligo*. *J. Physiol.* **128**, 28–60.

HODGKIN, A. L. & KEYNES, R. D. (1955*b*). The potassium permeability of a giant nerve fibre. *J. Physiol.* **128**, 61–88.

KEYNES, R. D. (1951). The ionic movements during nervous activity. *J. Physiol.* **114**, 119–150.

LAMY, M. (1863). Sur les effets toxique du thallium. *C. r. hebd. Séanc. Acad. Sci., Paris*, **57**, 442–445.

LANDOWNE, D. (1973). Thallium fluxes in the squid giant axon. *Biol. Bull. mar. biol. Lab. Woods Hole* **145**, 445.

LISHKO, V. K., KOLCHYNSKA, L. I. & PARKHOMENKO, M. T. (1973). Thallium and sodium pump of erythrocytes. *Ukr. biokhim. Zh.* **45**, 42–46.

MULLINS, L. J. & BRINLEY, F. J. (1969). Potassium fluxes in dialyzed squid axons. *J. gen. Physiol.* **53**, 704–740.

MULLINS, L. J. & MOORE, R. D. (1960). The movement of thallium ions in muscle. *J. gen. Physiol.* **3**, 759–773.

RANG, H. P. & RITCHIE, J. M. (1968). On the electrogenic sodium pump in mammalian non-myelinated nerve fibres and its activation by various cations. *J. Physiol.* **196**, 183–221.

REUBEN, J. & KAYNE, F. J. (1971). Thallium-205 nuclear magnetic resonance study of pyruvate kinase and its substrates. *J. biol. Chem.* **246**, 6227–6234.

SPENCER, P. S., PETERSON, E. R., MADRID, A. R. & RAINE, C. S. (1973). Effects of thallium salts on neuronal mitochondria in organotypic cord-ganglia-muscle combination cultures. *J. cell Biol.* **58**, 79–95.

SILLÉN, L. G. & MARTELL, A. E. (1964). *Stability Constants of Metal–Ion Complexes*, Special Publ. no. 17, London: The Chemical Society.

STEFFEN, G. & SOMMERMEYER, K. (1968). Fluorescence of thallium (I) ion in aqueous solutions upon excitation with ultraviolet light. *Biophysik* **5**, 192–206.

STEINBACH, H. B. (1941). Chloride in the giant axons of the squid. *J. cell. comp. Physiol.* **17**, 57–64.

TASAKI, I., TEORELL, T. & SPYROPOLOUS, C. S. (1961). Movement of radioactive tracers across squid axon membrane. *Am. J. Physiol.* **200**, 11–22.

USSING, H. H. (1949). The distinction by means of tracers between active transport and diffusion. *Acta physiol. scand.* **19**, 43–56.

WILLIAMS, R. J. P. (1970). Biochemistry of sodium, potassium, magnesium and calcium. *Chem. Soc. Quart. Rev.* **24**, 331–365.

WOODBURY, J. W. & MILES, P. R. (1973). Anion conductance of frog muscle membranes: one channel, two kinds of pH dependence. *J. gen. Physiol.* **62**, 324–353.