

## COMPARISON BETWEEN ACTIVE TRANSPORT OF $Tl^+$ , $K^+$ AND $Rb^+$ ACROSS THE ISOLATED SHORT-CIRCUITED FROG SKIN

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### SUMMARY

Active transport of  $Tl^+$  by frog skin was compared with transport of  $K^+$  and  $Rb^+$ .  $Tl^+$  was transported actively (using a Na,K-ATPase) by the epithelium and the adrenalin-stimulated glands.

In the epithelium,  $K^+$ ,  $Tl^+$  and  $Rb^+$  competed for transport in the ratio 1:1.7:0.9; in the glands the ratio was 1:1:1.

### INTRODUCTION

Thallos ions are very similar to potassium ions with respect to charge, hydrated radius and mobility in water. The two ions are also transported in similar fashion by muscle cells (Mullins & Moore, 1960).

In the midgut of the American silkworm, there is active transport of potassium but not of thallium, and the passive flux of thallium is ten times that of potassium (Zerahn & Koefoed, 1979). Rubidium ions also have similar properties to potassium ions, and are transported to a similar extent in many systems (Zerahn, 1980). Transport of  $Tl^+$ ,  $Rb^+$  and  $K^+$  by the frog skin are compared in this paper.

### METHODS

Potential difference, short-circuit current and fluxes were measured in frog skin, using techniques essentially as previously described (Ussing & Zerahn, 1951). Skins were dissected from brown frogs, *Rana temporaria*, obtained from Robert Stein, Lauingen, W. Germany, and kept in shallow water at 4 °C. Frogs were obtained in October and experiments were carried out between the months of October and March. Ringer's solution consisted of 115 mM-NaCl, 2 mM-KHCO<sub>3</sub> and 1 mM-CaCl<sub>2</sub>. Thallium was added, as the nitrate, at a concentration of 0.1 mM. This concentration produces only a small inhibition of potential difference and short-circuit current. Rubidium and barium were added as chlorides; Rb at 0.1 mM or 1 M, and barium at 2 or 5 mM. The isotopes <sup>42</sup>K, <sup>86</sup>Rb and <sup>204</sup>Tl were obtained from Risø (Denmark) and did not contain significant amounts of other radioisotopes. Antidiuretic hormone (ADH) in the form of arginine vasotocin was obtained from Calbiochem; ADH as

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vasopressin from Alfred Benzon, Denmark; adrenalin from Nordisk Droge & Kemikalie; amiloride from Merck, Sharp & Dohme, West Point, PA, and ouabain from Meco Benzon, Denmark.

Experiments were performed with paired half skins, either to compare fluxes in opposite directions, or compare experimental treatment with a control. The isotopes were added to one side of the skin and the samples taken from the other side at the desired time. Plastic tubing was used instead of rubber, because rubber may react with thallium.

When the flux experiments were finished the skin was usually removed, blotted on filter paper and weighed before it was placed in a vial with 1 ml 0.3 M perchloric acid for measurement of  $^{42}\text{K}$ ,  $^{204}\text{Tl}$  or  $^{86}\text{Rb}$ . The  $^{42}\text{K}$  was measured with a NaI crystal, 'Selectronic' amplifiers and scalers and a pulse height analyser, adjusted so that very little  $^{86}\text{Rb}$  and no  $^{204}\text{Tl}$  were counted.  $\text{K}^+$  was determined by flame photometry (Unicam SP 90 B). The extracellular space in the skins was assumed to be 50%. Inaccuracy in this estimate is insignificant in comparing the ratio between different isotopes. Later an aliquot was taken for measurement of  $^{204}\text{Tl}$  and  $^{86}\text{Rb}$ . When all three isotopes were used simultaneously the  $^{42}\text{K}$  samples of 30 min and 1 h periods were often measured with an error greater than  $\pm 2\%$  (due to low count-rates), but accuracy of the later samples was always better than  $\pm 2\%$ . The calculated net values for the later samples varied accordingly, from 5 to 10% for  $^{42}\text{K}$  samples. The  $^{204}\text{Tl}$  and  $^{86}\text{Rb}$  samples were measured with a Packard Tricarb liquid scintillator with an accuracy greater than 2%.

## RESULTS

In the isolated short-circuited frog skin,  $\text{Tl}^+$  fluxes were as shown in Table 1. The net  $\text{Tl}^+$  flux was very small compared to the short-circuit current of 700 nequiv  $\text{Na}^+$   $\text{h}^{-1} \text{cm}^{-2}$ .

### *Effect of antidiuretic hormone*

When the skin was treated with 0.04  $\mu\text{g ml}^{-1}$  vasotocin or 0.04 i.u.  $\text{ml}^{-1}$  vasopressin the  $\text{Tl}^+$  flux from inside to outside was increased (Table 2) indicating that the epithelium is involved in the active  $\text{Tl}^+$  flux across the skin. The skins contained  $4.5 \pm 0.9 \mu\text{equiv Tl}^+ \text{g}^{-1}$  of cells when  $\text{Tl}^+$  was taken up from the inside solution, and only  $0.045 \pm 0.008 \mu\text{equiv g}^{-1}$  when taken from the outside ( $N = 5$ ). When measuring

Table 1.  $^{204}\text{Tl}$  flux across the isolated short-circuited frog skin

| Time (h) | Inside $\rightarrow$ outside | Outside $\rightarrow$ inside | Net flux (nequiv $\text{h}^{-1} \text{cm}^{-2}$ ) |
|----------|------------------------------|------------------------------|---|
| 1        | 0.149 $\pm$ 0.037            | 0.057 $\pm$ 0.016            | 0.092 $\pm$ 0.048                                 |
| 2        | 0.253 $\pm$ 0.057            | 0.116 $\pm$ 0.027            | 0.137 $\pm$ 0.041                                 |
| 3        | 0.381 $\pm$ 0.096            | 0.151 $\pm$ 0.031            | 0.230 $\pm$ 0.085                                 |
| 4        | 0.521 $\pm$ 0.080            | 0.209 $\pm$ 0.027            | 0.312 $\pm$ 0.103                                 |
| 5        | 0.443 $\pm$ 0.051            | 0.183 $\pm$ 0.024            | 0.260 $\pm$ 0.053                                 |

$\text{Tl}^+$  concentration, 0.1  $\text{mmol l}^{-1}$ . Mean values for five experiments, mean value of  $I_{sc}$  700 nequiv  $\text{h}^{-1} \text{cm}^{-2}$ .

Table 2. *Effect of ADH*

| Time (h) | $\frac{\text{Tl}^+ \text{ flux (ADH)}}{\text{Tl}^+ \text{ flux (control)}}$ | $\frac{I_{sc} \text{ ADH}}{I_{sc} \text{ control}}$ |
|----------|---|---|
| 1        | 1.79 ± 0.15   | 1.33 ± 0.11   |
| 2        | 1.95 ± 0.20   | 1.36 ± 0.11   |
| 3        | 2.08 ± 0.30   | 1.56 ± 0.26   |
| 4        | 1.75 ± 0.15   | 1.73 ± 0.40   |
| 5        | 1.65 ± 0.03   | 1.52 ± 0.38   |

Mean values of four experiments, three with 1  $\mu\text{g}$  arginine vasotocin; one with 1 i.u. vasopressin. Skins short-circuited, 0.1  $\text{mmol l}^{-1}$   $\text{Tl}^+$ .  $\text{Tl}^+$  flux from inside to outside.

Table 3. *Tl<sup>+</sup> from inside to outside of isolated short-circuited frog skin*

|   | Time (h)    |             |             |             |             |             |
|---|-------------|-------------|-------------|-------------|-------------|-------------|
|   | 0.5         | 1           | 2           | 3           | 4           | 5           |
| Flux<br>( $\text{nequiv h}^{-1} \text{cm}^{-2}$ ) | 0.32 ± 0.10 | 0.33 ± 0.15 | 0.85 ± 0.22 | 0.71 ± 0.08 | 0.69 ± 0.15 | 0.82 ± 0.30 |
| Ratio against control<br>flux                     | 3.5 ± 0.6   | 3.1 ± 0.4   | 3.1 ± 0.9   | 2.1 ± 0.5   | 2.0 ± 0.2   | 2.2 ± 0.4   |

2  $\text{mmol l}^{-1}$   $\text{Ba}^{2+}$  on the inside.  
Mean values for five experiments.

outward flux a 7  $\text{cm}^2$  skin of about 200 mg with 50% extracellular space will contain 0.45  $\mu\text{equiv Tl}^+$ . Thus it is clear that the flux of about 0.001–0.002  $\mu\text{equiv h}^{-1}$  (Table 1), taking 1–2 h before equilibrium is obtained, must involve an appreciable exchange of  $\text{Tl}^+$  between cells and inside solution.

#### *Effect of Ba<sup>2+</sup>*

Addition of 2  $\text{mM-Ba}^{2+}$  to the inside solution induced a transient inhibition of the active transport of  $\text{Na}^+$ , reaching a minimum in 5–15 min, then increasing over several hours, as previously shown (Natochin & Skulskii, 1971; Nielsen, 1979) and increased the active outward transport of  $\text{Tl}^+$  by 2 to 3.5 times (Table 3). Uptake of  $\text{Tl}^+$  by skins was unaffected by barium, being 0.71 ± 0.11  $\mu\text{equiv g}^{-1}$  in 30 min for 2  $\text{mM-Ba}^{2+}$  experiments and 0.85 ± 0.11  $\mu\text{equiv g}^{-1}$  for controls ( $N = 3$ ), (Fig. 1).

To test whether the decreased  $I_{sc}$  was due to a decreased  $\text{K}^+$  permeability (Sperelakis, Schneider & Harris, 1967; Nagel, 1979)  $\text{K}^+$  flux and  $\text{K}^+$  content of skins were measured with  $^{42}\text{K}$  in the inside solution. Determinations were made after 30 min to ensure that  $^{42}\text{K}$  was far from being in equilibrium with the skin  $\text{K}^+$ . With 2  $\text{mM-Ba}^{2+}$ , there was a decrease of 10–20% in  $\text{K}^+$  outflux (Table 4). This flux represents an exchange rather than an uptake, since the  $\text{K}^+$  contents of the skins were not changed significantly.  $\text{K}^+$  concentration in the cell (with an arbitrary value of 50% for extracellular space) was 22.7 ± 1.4  $\text{mmol l}^{-1}$  in the presence of  $\text{Ba}^{2+}$  and 22.1 ± 1.0  $\text{mmol l}^{-1}$  in the control.

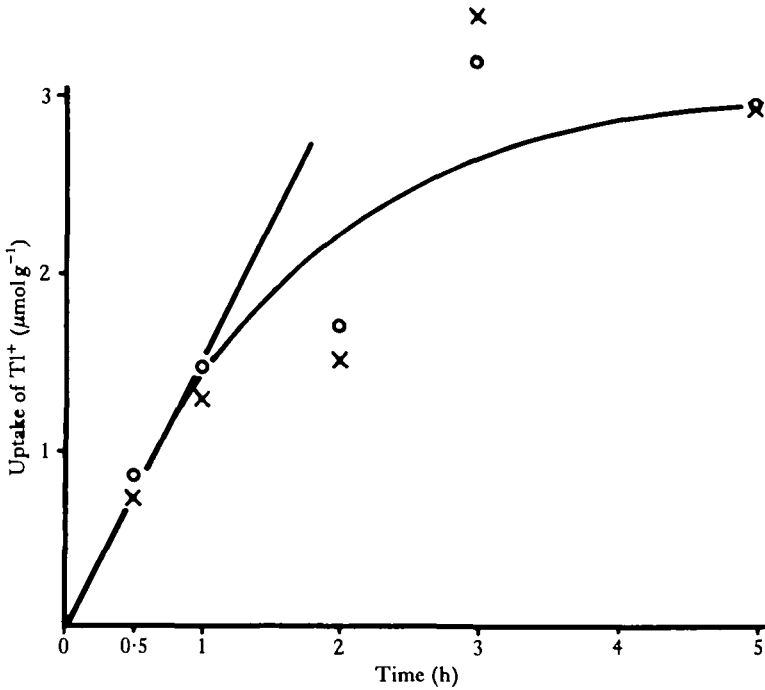


Fig. 1. Uptake of  $^{204}\text{Tl}$  from the inside solution.  $\text{Tl}^+$  concentration  $0.1 \text{ mmol l}^{-1}$  on both sides of short-circuited frog skin. For calculations extracellular space is assumed to be 50% and is deducted from whole skin. Control,  $-\text{O}-$ ;  $2 \text{ mmol l}^{-1} \text{ Ba}^{2+}$  on inside,  $-\text{X}-$ .

Table 4. *Effect of  $\text{Ba}^{2+}$  on exchange of cell  $\text{K}^+$  with inside solution*

| Experimental time (h)                  | $\text{Ba}^{2+}$ ( $\text{mmol l}^{-1}$ ) | $\mu\text{equiv K}^+$ |
|--|---|-----------------------|
| 0.5                                    | 0   | 1.44                  |
|  | 2   | 1.12                  |
| 0.5                                    | 0   | 1.52                  |
|  | 2   | 1.46                  |
| 0.5                                    | 0   | 1.96                  |
|  | 2   | 1.26                  |
| 0.5                                    | 0   | 1.28                  |
|  | 5   | 1.16                  |
| 0.5                                    | 0   | 1.85                  |
|  | 5   | 1.90                  |
| Mean value for first three experiments |   | $1.63 \pm 0.20$       |
|  |   | $1.28 \pm 0.12$       |
| Mean value for last two experiments    |   | 1.57                  |
|  |   | 1.53                  |

Skin short-circuited.

*Dependence of  $Tl^+$  transport on  $Na^+$  transport*

Table 5 shows that, in skins treated with  $2\text{ mM-Ba}^{2+}$  in the inside solution to stimulate  $Tl^+$  flux the addition of  $10^{-6}\text{ M}$  amiloride to the outside solution produced a fall in the  $I_{sc}$  from  $100\ \mu\text{A}$  to less than  $10\ \mu\text{A}$  in 1–2 min.  $Tl^+$  flux was affected more slowly and was significantly decreased after 1–2 h (Table 5). Only the active transport of  $Tl^+$  was affected, while the flux from outside to inside seemed constant.

Table 5. *Effect of  $10^{-6}\text{ M}$  amiloride on  $Tl^+$ -fluxes across the isolated, short-circuited frog skin*

| Time (h) | Inside→outside (nequiv $Tl^+$ $\text{h}^{-1}\text{cm}^{-2}$ ) | Outside→inside (nequiv $Tl^+$ $\text{h}^{-1}\text{cm}^{-2}$ ) | Net $Tl^+$ flux (nequiv $\text{h}^{-1}\text{cm}^{-2}$ ) |
|----------|---|---|---|
| 1        | $1.671 \pm 0.39$  | $0.099 \pm 0.006$   | $1.586 \pm 0.4$   |
| 2        | $2.386 \pm 0.46$  | $0.224 \pm 0.017$   | $2.157 \pm 0.5$   |
| 3        | $1.929 \pm 0.39$  | $0.371 \pm 0.036$   | $1.557 \pm 0.4$   |
| 4        | $1.471 \pm 0.30$  | $0.354 \pm 0.033$   | $1.129 \pm 0.3$   |
| 5        | $0.943 \pm 0.15$  | $0.357 \pm 0.027$   | $0.586 \pm 0.2$   |

Amiloride added after 2 h.  $0.1\text{ mmol l}^{-1}\text{ Tl}^+$  and  $2\text{ mmol l}^{-1}\text{ Ba}^{2+}$  in inside solution. Mean values of six experiments.

Table 6. *Competition between  $K^+$ ,  $Rb^+$  and  $Tl^+$  for flux across the isolated short-circuited frog skin ( $7\text{ cm}^2$ )*

|               | $K^+$               | $Rb^+$              | $Tl^+$              | Flux ( $\mu\text{equiv h}^{-1}$ ) |
|---------------|---------------------|---------------------|---------------------|-----------------------------------|
| November 1979 | $0.0569 \pm 0.025$  | $0.0518 \pm 0.028$  | $0.0775 \pm 0.022$  | i→o                               |
|               | 100                 | 85                  | 163                 |                                   |
|               | $0.0133 \pm 0.0063$ | $0.0132 \pm 0.0064$ | $0.0258 \pm 0.0055$ | o→i                               |
|               | 100                 | 101                 | 209                 |                                   |
| December 1979 | $0.0422 \pm 0.019$  | $0.0407 \pm 0.021$  | $0.0498 \pm 0.014$  | $\Delta$                          |
|               | 100                 | 89                  | 150                 |                                   |
|               | $0.0268 \pm 0.0040$ | $0.0208 \pm 0.0023$ | $0.0364 \pm 0.0077$ | i→o                               |
|               | 100                 | 78                  | 137                 |                                   |
| January 1980  | $0.0052 \pm 0.0007$ | $0.0042 \pm 0.0003$ | $0.0095 \pm 0.0017$ | o→i                               |
|               | 100                 | 81                  | 178                 |                                   |
|               | $0.0216 \pm 0.0039$ | $0.0166 \pm 0.0023$ | $0.0261 \pm 0.0068$ | $\Delta$                          |
|               | 100                 | 78                  | 121                 |                                   |
| Mean values   | $0.0125 \pm 0.0038$ | $0.0103 \pm 0.0031$ | $0.0298 \pm 0.0057$ | i→o                               |
|               | 100                 | 83                  | 292                 |                                   |
|               | $0.0061 \pm 0.0015$ | $0.0038 \pm 0.0008$ | $0.0105 \pm 0.0016$ | o→i                               |
|               | 100                 | 64                  | 176                 |                                   |
| Mean values   | $0.0064 \pm 0.0041$ | $0.0065 \pm 0.0023$ | $0.0193 \pm 0.0039$ | $\Delta$                          |
|               | 100                 | 92                  | 225                 |                                   |
|               | 100                 | 82                  | 197                 | i→o                               |
|               | 100                 | 82                  | 188                 | o→i                               |
|               | 100                 | 86                  | 165                 | $\Delta$                          |

Fluxes for  $Rb^+$  and  $Tl^+$  are multiplied 20 times to get values comparable to the value for  $2\text{ mmol l}^{-1}\text{ K}^+$ ; fluxes measured by labelling with  $^{42}\text{K}$ ,  $^{86}\text{Rb}$  and  $^{204}\text{Tl}$  on one half skin from i→o, and the other half from o→i.

Mean values of three experiments in November 1979, three in December 1979 and three in January 1980. All skins treated with  $2\text{ mmol l}^{-1}\text{ BaCl}_2$  in the inside solution.

Percentages are mean values from percentages of single experiments.

*Competition between  $Tl^+$ ,  $K^+$  and  $Rb^+$* 

The competition for active transport between  $Tl^+$ ,  $K^+$  and  $Rb^+$  was studied by adding  $^{42}K$ ,  $^{86}Rb$  and  $^{204}Tl$  to the solution bathing the skins in which the  $Tl^+$  flux was stimulated by adding 2 mM-Ba to the inside solution. All fluxes decreased with the length of time that frogs had been kept in storage; on average  $Tl^+$ ,  $K^+$  and  $Rb^+$  competed in the ratio 1.7:1:0.9. (Table 6).

*Adrenalin*

Application of adrenalin to the inside of the short-circuited frog skin causes an active transport of  $Cl^-$  ion by the glands, amounting to several  $\mu\text{equiv h}^{-1}$  (Koefoed-Johnsen,

Table 7. *Active transport of  $Tl^+$  and  $Rb^+$  ( $\mu\text{equiv h}^{-1}$ ;  $7\text{ cm}^2$  skin), across the isolated, short-circuited frog skin induced by adding adrenalin to the inside solution ( $50\ \mu\text{g}$  in 25 ml)*

|                   | $Rb^+$ flux         | $Tl^+$ flux         | $Tl^+/Rb^+$ ratio |
|-------------------|---------------------|---------------------|-------------------|
| $i \rightarrow o$ | $0.109 \pm 0.011$   | $0.116 \pm 0.008$   | $1.07 \pm 0.08$   |
| $o \rightarrow i$ | $0.0080 \pm 0.0010$ | $0.0115 \pm 0.0017$ | $1.45 \pm 0.07$   |
| $\Delta$          | $0.101 \pm 0.010$   | $0.105 \pm 0.006$   | $1.05 \pm 0.08$   |

Mean value of four experiments.  $Tl^+/Rb^+$  ratio: mean value for 1 and 2 h periods.

Table 8. *Comparison of  $^{42}K$  and  $^{204}Tl$  fluxes from  $i \rightarrow o$  and from  $o \rightarrow i$ ,  $\Delta K^+$  and  $\Delta Tl^+$  across the short-circuited frog skin under stimulation with adrenalin*

|                           | Time (h) | $K^+$ flux ( $\mu\text{equiv h}^{-1}$ )                                   | $Tl^+$ flux ( $\mu\text{equiv h}^{-1}$ ) |
|---------------------------|----------|---|--|
| $i \rightarrow o$         | 0.25     | $0.112 \pm 0.091$   | $0.089 \pm 0.059$                        |
|                           | 0.5      | $0.180 \pm 0.095$   | $0.171 \pm 0.112$                        |
|                           | 1        | $0.178 \pm 0.075$   | $0.178 \pm 0.095$                        |
|                           | 2        | $0.223 \pm 0.091$   | $0.225 \pm 0.063$                        |
|                           |          | $\frac{Tl^+ \text{ flux}}{K^+ \text{ flux}}$ , mean value $0.95 \pm 0.15$ |  |
| $o \rightarrow i$         | 0.25     | $0.0030 \pm 0.0008$   | $0.0018 \pm 0.0012$                      |
|                           | 0.5      | $0.0098 \pm 0.0023$   | $0.0098 \pm 0.0049$                      |
|                           | 1        | $0.0254 \pm 0.015$  | $0.0143 \pm 0.0053$                      |
|                           | 2        | $0.0196 \pm 0.0038$   | $0.0257 \pm 0.0090$                      |
|                           |          | $\frac{Tl^+ \text{ flux}}{K^+ \text{ flux}}$ , mean value $0.96 \pm 0.09$ |  |
| $\Delta K^+, \Delta Tl^+$ | 0.25     | 0.109   | 0.088                                    |
|                           | 0.5      | 0.171   | 0.161                                    |
|                           | 1        | 0.152   | 0.163                                    |
|                           | 2        | 0.203   | 0.200                                    |
|                           |          | $\frac{Tl^+ \text{ flux}}{K^+ \text{ flux}}$ , mean value $0.97 \pm 0.17$ |  |

$Tl^+$  flux corrected to flux of the same concentration as  $K^+$  by multiplying by  $[K^+]/[Tl^+]$ . Ratio of  $Tl^+$  flux to  $K^+$  flux given as mean values from 1 h and 2 h periods. Standard deviation of  $\Delta K^+$  and  $\Delta Tl^+$  close to the value from  $i \rightarrow o$ .

Mean values of 4 experiments.

Adrenalin,  $50\ \mu\text{g}$  in 25 ml inside solution.

Levi & Ussing, 1952) and was found to stimulate  $\text{Tl}^+$ ,  $\text{K}^+$  and  $\text{Rb}^+$  transport about equally (Tables 7 and 8). Competition for transport by the glands is therefore about 1:1:1.  $10^{-6}$  M ouabain decreased the active  $\text{Tl}^+$  flux from  $0.0092 \pm 0.0010$  to  $0.0019 \pm 0.0010$   $\mu\text{equiv h}^{-1}$  ( $N = 3$ ) after 30 min. Amiloride at a concentration of  $10^{-6}$  M on the outside of the skin had no significant effect:  $\text{Tl}^+$  flux before amiloride treatment was  $0.0082 \pm 0.0006$   $\mu\text{equiv h}^{-1}$  and was  $0.0093 \pm 0.0002$   $\mu\text{equiv h}^{-1}$  ( $N = 3$ ) after 30 min of treatment.

## DISCUSSION

### *Active $\text{Tl}^+$ flux in relation to active $\text{Na}^+$ flux*

$\text{Tl}^+$ , like  $\text{K}^+$ , is transported actively in the outward direction across the frog skin (Table 1). This transport is increased by the action of antidiuretic hormone (Table 2) indicating that the transport is performed by the epithelium. The flux was about 100 times smaller than the  $\text{Na}^+$  flux (Table 1) and reduction of the  $\text{Na}^+$  flux by amiloride had no immediate effect on  $\text{Tl}^+$  flux (Table 5). However, after 1 h  $\text{Tl}^+$  flux was decreased, so the active transport of  $\text{Tl}^+$ ,  $\text{K}^+$  and  $\text{Rb}^+$  is apparently indirectly dependent on  $\text{Na}^+$  transport.

### *Effect of $\text{Ba}^{2+}$ on $\text{Tl}^+$ flux*

$\text{Tl}^+$  transport was increased (and the  $I_{sc}$  was decreased) when the skin was treated with 2 mM- $\text{Ba}^{2+}$  on the inside (Table 3). Barium reduces the  $\text{K}^+$  permeability of cell membranes (Sperelakis *et al.* 1967; Nagel, 1979), which may explain the reduction in  $I_{sc}$ , but cannot explain the altered  $\text{Tl}^+$  flux, since the flux remained high when the  $I_{sc}$  recovered. In the presence of  $\text{Ba}^{2+}$ , the exchange for  $\text{K}^+$  was from 0 to 20 % lower. In addition it was found that  $\text{Ba}^{2+}$  increased the flux of a skin pretreated with antidiuretic hormone (ADH) but the hormone had no effect on a 2 mM- $\text{Ba}^{2+}$ -treated skin, which suggests that either  $\text{Ba}^{2+}$  and ADH increase the permeability of the outside membrane of the skin, with  $\text{Ba}^{2+}$  having the strongest effect, or that ADH can have no effect in the presence of  $\text{Ba}^{2+}$ .

An alternative explanation for the effect of  $\text{Ba}^{2+}$  is that the reduction in membrane potential, from about  $-95$  to  $-40$  mV (Nagel, 1979) increases the leak of  $\text{Tl}^+$ ,  $\text{K}^+$  and  $\text{Rb}^+$  to the outside. Both explanations fit with the model given by Koefoed-Johnsen & Ussing (1958) and with the model of Ussing & Windhager (1964).

### *Competition between $\text{K}^+$ , $\text{Tl}^+$ and $\text{Rb}^+$ in epithelium and skin glands*

Competition between  $\text{Tl}^+$ ,  $\text{K}^+$  and  $\text{Rb}^+$  in the presence of  $\text{Ba}^{2+}$  has been shown to be in the ratio 1.7:1:0.9 in the epithelium (Table 6) and in the ratio 1:1:1 in the glands (Tables 7 and 8). The transport mechanism in the glands is probably a K,Na-ATPase driven system since the transport was inhibited by ouabain.

The  $\text{Na}^+$  transport of the epithelium is also considered to be driven by a Na,K-ATPase, so the different affinity of the three ions to the transport enzyme is not explained by a difference in the kind of enzyme. In contrast the enzyme used for  $\text{K}^+$  transport in the midgut of the American silkworm is different, not being inhibited by ouabain.  $\text{Tl}^+$  is not actively transported and the competition between active  $\text{K}^+$  and  $\text{Rb}^+$  flux averages 1.0:0.6 (Zerahn, 1980).

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