J. exp. Biol. (1979), **78**, 105–120
Printed in Great Britain

TRANSPORT OF THALLIUM IONS ACROSS THE ISOLATED MIDGUT OF *HYALOPHORA CECROPIA*

BY KARL ZERAHN AND BODIL KOEFOED
Institute of Biological Chemistry A, University of Copenhagen, Denmark and University Institute of Pathology, Hospital of Odense

(*Received* 31 *March* 1978)

SUMMARY

Tl^+ permeates the *Hyalophora cecropia* midgut equally in both directions, but the flux of Tl^+ per mM is up to 50 times higher than the passive flux for potassium, rubidium and caesium. This is explained by assuming that the Tl^+ flux is a carrier-mediated facilitated diffusion or a passage through channels similar to gramicidin channels in black membranes. We have found this special way for Tl^+ to pass through membranes in the *Hyalophora cecropia* midgut; some other membranes, such as red blood cells, seem to show the same properties, but others, such as frog muscle and skin, do not.

INTRODUCTION

Permeation of the alkali metal ions across biological membranes has been studied extensively, especially for Na^+ and K^+. Movements of Tl^+ have been studied much less, although the Tl^+ ion resembles the alkali metal ions in having a charge of one and an ionic radius obtained from crystal data of 1·44 Å, between the radius for Rb^+ of 1·48 Å and that of K^+ of 1·33 Å.

Thallium is poisonous (and this has been well known for a long time), but if care is taken, the permeation of Tl^+ can be measured as was done by Mullins & Moore (1960), who studied the flux of Tl^+ into and out of frog muscle. They drew the conclusion that the muscle fibre membrane cannot distinguish between the toxic heavy metal Tl^+ and K^+, provided that the concentrations of the former ion are kept low. Skulskii, Manninen & Järnefelt (1973) studied the uptake of Tl^+ by red blood cells and found that the cell/medium distribution was only 1·8–2·0, close to the passive distribution of small anions, but they also found that the rate constants for Tl^+ flux were much larger than for K^+.

In *Chlorella* Tl^+ can be accumulated by a K pump (Solt, Paschinger & Broda, 1971). Maslova, Natochin & Skulskii (1971) found that in the frog skin Tl^+ ions will activate the K^+-dependent step of the Na^+, K^+-dependent ATPase, and are significantly more effective than K^+. But this activation does not result in active Na-transport, which, as measured by the short-circuit current, is inhibited by Tl^+ (50% for 1 mM-Tl), an inhibition reminiscent of the action of ouabain.

Koefoed (1975) compared the transfer of Tl^+ and K^+ in the rectal complex of the

mealworm (*Tenebrio molitor*). She found that the two ions are treated similarly by the tissue, possibly indicating an active transport of both ions.

We have therefore performed a comparative study of the transport of Tl^+ and K^+ across the midgut from *Hyalophora cecropia* (L), which is very well characterized with respect to K transport (see review, Harvey & Zerahn, 1972).

The result of the study does not support the former views of active Tl^+ transport. Tl^+ ions are transported across the midgut many times faster than K^+, but the transport is not active. In the short-circuited gut the flux of Tl^+ is the same in both directions and is independent of the metabolism.

It is concluded that Tl^+ may cross the gut wall by facilitated diffusion, but other models are also possible.

METHODS

The larvae of the American silkworm, *Hyalophora cecropia*, were reared on either leaves of willow, *Salix babylonica*, or on artificial diet (Riddiford, 1968). The larvae were chilled on ice for an hour or longer before the gut was removed and put up in the apparatus described by Harvey, Haskell & Zerahn (1967) (plastic tubing, no rubber). The advantage of this arrangement is that the midgut can be kept as a sphere and maintains the same thickness throughout the experiment. The composition of the bathing solution 32-K was: 32 mM-K^+, 2 mM-HCO_3^-, 30 mM-Cl^-, and sucrose to keep the total osmolarity to 260 m-osmol (Harvey & Zerahn, 1971). Analytical reagents were used and the solution contained no Ca or Mg ions (see page 114). At low concentration thallium was present in the form of TlCl, and at high concentration either in the form of Tl_2SO_4 or $TlNO_3$. There was no difference in behaviour if the Tl was accompanied by different anions. When cecropia larvae were not available some other saturniid larvae were used as indicated in the legends to the tables. The contents of ions in the guts were determined as usual (Zerahn, 1973). Tl^+ was determined by absorption flame spectrometry. The radio-thallium was determined in a Packard beta spectrometer (Tricarb) usually in 1 ml samples plus 10 ml Instagel with an accuracy of $\pm 2\%$ or better. The experiments were made by the procedure given by Harvey & Zerahn (1972). Radio-tracers were added to one side at the start and samples taken from the other side at times given in the tables. This will allow the delay of the flux through the gut (the lag time) to be determined (see Harvey & Zerahn, 1969). The short circuit current for distended guts will be equal to the net K flux with an accuracy of 10% or less (Harvey & Zerahn, 1972); it is assumed to be similar for undistended guts, but accurate measurements are not available. The values given for the short-circuit current are mean values for the period. Washing the gut out between the different periods causes a change in potential with time, but the influence of these changes was cancelled by alternating the sequence; for example first starting with an influx period, and the next time with an efflux period.

RESULTS

Flux measurements

In a short-circuited cecropia midgut the current is derived from the active transport of potassium (Harvey & Nedergaard, 1964). $TlNO_3$ was added to the bathing solution

Table 1. *Tl^+ flux from blood-side to lumen across the isolated short-circuited cecropia midgut*

(Bathing solution 1 mM-$TlNO_3$ in 32-K. Flux in μequiv/h. Short-circuit current (I_{sc}) compared to Tl^+ flux by multiplying by 33.)

10 min, period from 5 to 10 min: guts almost undistended.
20 min, period from 10 to 20 min: guts almost undistended.
45 min, period from 35 to 45 min: guts distended.
Experiment 15.5.75, gut undistended in all periods.

Date	Period (min) 4	8	12	16	24	
(A) 15. 5. 75	0·93	0·92	0·89	1·02	0·91	μequiv Tl^+/h
	31	30	29	34	30	33 × μequiv Tl^+/h
	25	24	23	24	24	I_{sc} μequiv/h

Date	Period (min) 10	20	45	
(B) 2. 10. 75	0·88	0·83	0·98	μequiv Tl^+/h
	29	27	32	33 × μequiv Tl^+/h
	16	15	16	I_{sc} μequiv/h
3. 10. 75	0·59	0·71	0·76	μequiv Tl^+/h
	19	23	25	33 × μequiv Tl/h
	13	8	8	I_{sc} μequiv/h
6. 10. 75	0·90	0·93	1·01	μequiv Tl^+/h
	30	31	33	33 × μequiv Tl^+/h
	19	19	19	I_{sc} μequiv/h
6. 10. 75 (b)	0·51	0·54	0·83	μequiv Tl^+/h
	17	18	27	33 × μequiv/h
	26	25	28	I_{sc} μequiv/h
Mean value				μequiv Tl^+/h 0·82
				μequiv Tl^+/h × 33 27
				I_{sc} μequiv/h 19

to make it 1 mM in Tl^+. The Tl^+ was labelled with ^{204}Tl on the blood-side and the flux of Tl^+ was measured and compared to the short-circuit current. The results are given in Table 1.

Since the concentration of thallium was 1 mM, it had to be multiplied by 33 in order to compare it with the K flux. When this correction for molarity is made, the magnitude of the Tl^+ flux is then comparable to the flux of K, which is about equivalent to the short-circuit current. Thus at first sight it looks as if the gut could not discriminate between K^+ and Tl^+, and there is a possibility that Tl^+ is actively transported in the same way as K^+. However, the flux of Tl^+ from lumen to blood-side is not significantly different from the flux in the opposite direction (Table 2). Thus it is only the unidirectional flux of Tl^+ which resembles the K flux and not the net flux, which is almost nil. Therefore there is no indication of an active transport of Tl. However, these fluxes are from different guts and the variation is quite large, so it would be better to measure the Tl^+ flux in both directions on the same gut. Preliminary experiments showed that when the guts were labelled with ^{204}Tl from one side followed by washing with unlabelled saline, the activity could be washed away sufficiently in half an hour, after which the Tl^+ flux in the opposite direction could be determined. The animals used for the experiments were *Actia selene* (Hübner [1810]) and not *Hyalophora cecropia*,

Table 2. *Tl^+ flux from lumen to blood-side across the isolated short-circuited cecropia midgut*

(Bathing solution 1 mM-$TlNO_3$ in 32-K.)

10 min, period from 5 to 10 min: guts almost undistended.
20 min. period from 10 to 20 min: guts almost undistended.
35 min. period from 25 to 35 min: guts distended.
45 min. period from 35 to 45 min: guts distended.

	Period (min)				
Date	10	20	35	45	
20. 9. 76 (B)	1·21	1·46	2·12	2·13	μequiv Tl^+/h
	28	28	34	34	I_{sc} μequiv/h
21. 9. 76 (A)	0·82	0·80	0·91	0·89	μequiv/Tl^+h
	20	19	18	17	I_{sc}
21. 9. 76 (C)	0·31	0·35	0·68	0·75	μequiv Tl^+/h
	47	46	55	71	I_{sc}
Mean values	0·78	0·87	1·24	1·26	μequiv Tl^+/h
	32	31	37	41	I_{sc}
	26	29	41	42	μequiv Tl^+/h × 33

Table 3. *Tl^+ flux (μequiv/h) across short-circuited midguts of* Actia selene *larvae in both directions*

(The midgut was washed free of ^{204}Tl between the determinations (sandwich experiments). B–L flux from blood-side to lumen, L–B flux from lumen to blood-side.)

Date	(1) B–L	(2) L–B	(3) B–L	Ratio	mM-Tl^+
7 June	0·078	0·103	0·079	0·76	0·1
	0·085	0·107	0·086	0·80	0·1
8 June	0·058	0·072	0·104	1·12	0·1
	0·086	0·080	0·116	1·26	0·1
13 June*	0·078	0·040	0·107	2·30	0·1
14 June	1·20	0·94	1·48	1·42	1·0
	0·72	0·76	1·30	1·32	1·0
18 June*	0·61	0·67	0·75	1·01	1·0
Mean value (± S.D.), June 13 omitted				1·10 ± 0·1	
Mean value (± S.D.), all values				1·25 ± 0·2	

(1). 5–10 min and 10–20 min. (2). 65–70 min and 70–80 min. (3). 125–130 min and 130–140 min.

* Only one 10 min period.

but the relation between short-circuit current and Tl^+ flux seems to be similar for the two species. Table 3 shows the results and indicates that the mean ratio between Tl^+ fluxes in opposite directions is 1·10. The large variation of the flux ratios for different experiments may be explained as biological, the guts are alive and have a muscle layer which may change the available surface for permeation. Since the expected ratio for a passive flux is 1·00, and for active K flux the ratio is probably 10 or more, we must again conclude that the Tl^+ flux is passive.

From Tables 1, 2, and 3 we concluded that the large flux is a passive one and no active transport of Tl^+ takes place. Thus we also must assume that no energy is needed for the permeation of Tl^+

Table 4. *Tl^+ flux from blood-side to lumen across the isolated cecropia midgut*

(Bathing solution 1 mM-Tl^+ in 32-K. Aeration with O_2 and N_2. The flux is in μequiv/h in the periods 5–10 and 10–20 min.

Date	Condition	5–10 min	10–20 min
1. 11. 76 (A)	O_2	1·58	1·52
	N_2	1·86	1·74
1. 11. 76 (B)	O_2	1·61	1·55
	N_2	2·26	2·46
	O_2	1·76	1·90
2. 11. 76	O_2	1·01	0·82
	N_2	0·90	0·64
	O_2	0·61	0·69
Mean value	O_2	1·31	1·30
	N_2	1·67	1·61

Table 5. *Simultaneous ^{42}K and ^{204}Tl fluxes in μequiv/h through the short-circuited midgut of cecropia from lumen to blood-side*

(Lag times of both ions. Solution 1 mM-$TlNO_3$ in 32-K.)

					Flux in a 1 mM solution	
Date	0–5 min	5–10 min	10–20 min	Lag time	5–10 min	10–20 min
20. 9. 76 (A)	0·13	0·25	0·27	2·9	0·25	0·27 Tl
	0·07	0·53	0·70	7·6	0·017	0·022 K
20. 9. 76 (B)	0·79	1·21	1·46	3·1	1·21	1·46 Tl
	1·45	2·69	3·28	5·6	0·084	0·102 K
21. 9. 76 (A)	0·58	0·82	0·80	2·0	0·82	0·80 Tl
	0·70	1·63	2·30	5·0	0·051	0·072 K
21. 9. 76 (B)	0·12	0·18	0·28	4·5	0·18	0·28 Tl
	0·95	0·70	1·32	3·5	0·022	0·041 K
21. 9. 76 (C)	0·20	0·31	0·35	2·4	0·31	0·35 Tl
	0·79	0·74	1·79	5·6	0·023	0·056 K
Mean value		0·55	0·63	3·0	0·55	0·63 Tl
		1·26	1·88	5·5	0·040	0·059 K
		Ratio Tl^+/K^+			14	11

Influence of metabolism

We can stop the supply of energy by stopping the metabolism and the best way is to replace the oxygen supply by stirring the solutions with nitrogen. A sign of the efficiency of this procedure is that the transmural *pd* drops to almost zero.

From Table 4, it is obvious that there is no decrease in the Tl^+ flux from blood-side to lumen when the K transport is abolished by quenching the metabolism. Thus we have a further indication that the large Tl^+ flux is passive.

Tl^+ flux compared to the flux of the alkali metal ions

It was not practical to compare the behaviour of Tl^+ ions with all alkali metal ions, but K^+, Rb^+ and Cs^+ were used as representatives.

As seen in Table 5 the flux of Tl^+ from lumen to blood-side is 11–14 times as high per mM as that of K^+. The delay between addition of the tracer and its appearance in the solution on the other side of the gut (see Harvey & Zerahn, 1969) is about twice as long for $42K^+$ as for $^{204}Tl^+$. The reason for this delay will be discussed later.

Table 6. *^{86}Rb and ^{137}Cs flux (μequiv/h) from lumen to blood-side of the isolated cecropia midgut*

(Rb and Cs concentration 1 mM in 32-K. Guts cylindrical to diminish effect of leak.)

	Period (min)						
	0–5	5–10	10–20	20–40	48–80	80–120	Lag time
			Z 10. 11. 76				
Rb	0·030	0·102	0·127	0·128	0·124	0·123	5·0
Cs	0·104	0·133	0·129	0·122	0·103	0·099	1·2
Cs/Rb	3·5	1·30	1·02	0·95	0·83	0·80	
			Z 11. 11. 76				
Rb	0·036	0·053	0·089	0·102	0·122	0·135	5·4
Cs	0·073	0·112	0·124	0·133	0·129	0·129	2·2
Cs/Rb	2·03	2·11	1·39	1·30	1·06	0·96	
			H 10. 11. 76				
Rb	0·071	0·031	0·121	0·094	—	—	5·8
Cs	0·079	0·114	0·131	0·141	—	—	2·8
Cs/Rb	1·11	3·67	1·08	1·50			
			H 11. 11. 76				
Rb	0·032	0·082	0·099	0·119	0·160	0·172	4·0
Cs	0·065	0·095	0·117	0·134	0·125	0·107	3·4
Cs/Rb	2·03	1·16	1·18	1·13	0·78	0·62	
			H 11. 11. 76 (B)				
Rb	0·052	0·095	0·110	0·119	0·156	0·155	3·9
Cs	0·083	0·139	0·150	0·171	0·208	0·201	2·7
Cs/Rb	1·60	1·46	1·36	1·44	1·33	1·30	
Mean values							
Rb	0·044	0·073	0·109	0·112	0·143	0·146	4·8
Cs	0·081	0·119	0·130	0·140	0·141	0·134	2·5
Cs/Rb	1·8	1·6	1·2	1·3	0·99	0·92	

As a control, we compared the flux of ^{86}Rb and ^{137}Cs from the lumen-side to blood-side on the same gut simultaneously. These experiments were performed on guts which were only slightly distended to minimize the effect of small leaks in the gut, which would influence the ratio between Rb and Cs fluxes; for this reason the guts were not short-circuited. Rb^+ is expected to follow K^+ to within 10–20% (Nedergaard & Harvey, 1968). On the other hand Cs in a concentration of 1 mM will not be actively transported. Since the measured fluxes are, however, from lumen to blood-side, where we expect an ordinary passive diffusion for these ions, it is possible to compare the fluxes of Rb^+ and Cs^+ directly. The results from Table 6 are derived from guts which are not short-circuited, but as seen later (Table 10) this will only change the Tl^+ flux insignificantly. Table 6 shows the results of the measurements.

After 10 min the two ions have a ratio for the passive fluxes from lumen to blood-side of around one. Even if Rb^+ is actively transported from the blood-side to lumen, and takes more time than Cs^+ to come to equilibrium, the two ions will behave very similarly when steady state is reached.

Therefore it is sufficient to compare one of the ions with Tl^+, and Cs was chosen. Table 7 shows the flux of Cs^+ and Tl^+ from lumen to blood-side simultaneously across the same midgut.

Table 7. *^{204}Tl and ^{137}Cs flux (μequiv/h) through the isolated cecropia midgut from lumen to blood-side*

(Tl and Cs 1 mM in 32-K. Guts cylindrical to minimize effect of leak.)

	Period (min)						
	0–5	5–10	10–20	20–40	40–80	80–120	Lag time
			Z 12. 11. 76				
Tl^+	0·73	0·76	0·82	0·91	0·79	0·74	1·1
Cs^+	0·018	0·012	0·023	0·020	0·028	0·029	3·6
Tl^+/Cs^+	41	63	36	46	28	26	
			H 12. 11. 76 (B)				
Tl^+	0·74	1·29	1·61	1·63	1·43	1·08	3·8
Cs^+	0·014	0·020	0·021	0·027	0·033	0·029	6·0
Tl^+/Cs^+	52·7	64·6	76·8	60·2	43·4	37·4	
			H 17. 11. 76				
Tl^+	1·04	1·23	1·34	1·22	1·29	1·21	1·0
Cs^+	0·025	0·040	0·046	0·051	0·055	0·071	3·7
Tl^+/Cs^+	41·8	30·7	29·1	23·9	23·4	17·0	
			H 17. 11. 76 (B)				
Tl^+	0·37	0·52	0·85	0·93	0·98	0·86	5·8
Cs^+	0·024	0·044	0·057	0·072	0·088	0·095	7·4
Tl^+/Cs^+	15·5	11·9	15·0	12·9	11·2	9·1	
			H 19. 11. 76				
Tl^+	1·31	1·58	1·68	1·77	1·55	1·84	2·2
Cs^+	0·020	0·023	0·025	0·033	0·030	0·041	5·4
Tl^+/Cs^+	65·7	68·9	67·4	53·5	51·6	45·0	
Mean Tl^+/Cs^+	44	48	45	39	31	27	

A large difference is observed, with Tl^+ showing a flux which is 9–70 times as high as the flux of Cs, when the concentration of both ions is 1 mM.

The dependency on concentration

The potential difference and short-circuit current of the midgut decrease with increasing concentration of Tl^+. This indicates that the active K transport is decreasing and that the Tl^+ ion is an inhibitor for the K transport, possibly a rather specific one. This inhibition of the K transport is often reversible, but sometimes irreversible. The inhibition was effective on the midguts from the species *Hyalophora cecropia*, *Samia cynthia* (Drury), *Antherea* pernyi and *Actia selene*. Preliminary experiments on cecropia midguts have shown that 2 mM-Tl^+ on the lumen-side inhibits 95% of the active K flux, but 10% only of the passive K flux. In contrast, passive Tl^+ flux is proportional to the concentration when measured within the range between 0·1 and 6 mM-Tl^+ (Table 8) independent of inhibition of the active K flux.

As seen from Table 8 the Tl^+ flux is proportional to the concentration and no saturation of the flux at high concentration was observed.

Effect of temperature on the Tl flux

In general we would expect a passive diffusion to have a low temperature coefficient in contrast to a rather large one for an active transport. The temperature dependence of the Tl^+ flux is shown in Table 9.

Table 8. *Tl flux (μequiv/h) measured with ^{204}Tl on midguts from different species*

(First column is flux measured from 5–10 min, second from 10–20 min. Tl^+ concentration given in third and flux per mM in last column.)

	Tl^+ flux (μequiv/h)		Tl^+ conc.	
	5–10	10–20	(mM)	Tl^+ flux per mM
	Date 22. 7. 77			
Antherea pernyi	2·69	2·62	5	0·53
	0·46	0·53	1	0·50
	Date 21. 7. 77			
	—	0·028	0·1	0·28
Antherea pernyi	0·35	0·34	1·0	0·35
	2·09	2·01	6·0	0·34
Actia selene	—	0·084	0·1	0·84
Mean value for all Tl^+ fluxes from Table 3	—	0·88	1·0	0·88
Hyalophora cecropia	—	0·050	0·1	0·50
Mean value of 5 determinations	—	0·63	1·0	0·63

Table 9. *Effect of temperature on Tl^+ flux from blood-side to lumen of the isolated cecropia midgut*

(Flux μequiv/h, solution 32-K + 1 mM-Tl^+ or 0·1 mM-Tl^+.)

			Undistended		Distended	
Date	Solution (mM-Tl^+)	°C	10 min	20 min	10 min	20 min
5. 11. 76	1	24	0·56	0·62	1·04	1·10
		4	0·31	0·29	0·35	0·25
Ratio			1·86	2·14	2·97	4·4
4. 11. 76 (B)	1	24	0·49	0·49	—	—
		4	0·35	0·28	—	—
Ratio			1·40	1·75	—	—
8. 11. 76	1	24	0·56	0·60	1·58	1·67
		4	0·36	0·32	0·28	0·24
Ratio			1·56	1·88	5·64	6.96
9. 11. 76	0·1	23	0·039	0·041	0·102	0·104
		4	0·019	0·017	0·037	0·033
Ratio			2·05	2·41	2·76	3.15
30. 1. 78	0·1	24	0·021	0·021	0·030	0·026
		4	0·0047	0·0050	0·0133	0·0128
Ratio			4.47	4·20	2·26	2·03
1. 2. 78	0·1	24	0·043	0·039	0·052	0·059
		4	0·0119	0·0112	0·0146	0·0122
Ratio			3·61	3·48	3·56	4·84
Mean ratio			2·48	2·64	3·43	4·28

We found that the flux of Tl^+ in undistended guts is reduced by a factor of 2·5 on cooling the gut by 20 °C, but by 3·5 times in distended guts. The measurements show that distension may have a larger effect on the Tl penetration of the gut at room temperature than at lower temperatures.

Table 10. *Influence of the cecropia gut potential on the Tl^+ flux from blood-side (B) to lumen (L) and vice versa.*

Tl^+ flux given in μequiv/h, potential in mV, lumen positive. First period, 0–5 min, omitted. Solution is 0·1 mM-$TlNO_3$ in 32-K. It is seen that the flux from B to L is increased only about 1·2 times for a change from 100 to 50 mV, the Tl^+ flux from L to B increases with decreasing potential on the lumen.)

		B to L		B to L	
Min		Flux Tl^+	mV	Flux Tl^+	mV
5–10		0·042	87	0·095	113
10–20		0·065	76	0·088	105
	Mean value period 1	0·054	—	0·092	—
125–130		0·069	49	0·121	45
130–140		0·061	46	0·104	41
	Mean value period 3	0·065	—	0·113	—
	Period 3/period 1	1·20	—	1·23	—
		L to B		L to B	
Min		Flux Tl^+	mV	Flux Tl^+	mV
5–10		0·306	106	0·234	109
10–20		0·329	90	0·276	101
	Mean value	0·318	—	0·255	—
125–130		0·309	41	0·319	44
130–140		0·310	37	0·292	42
	Mean value	0·310	—	0·306	—
	Period 1/period 3	1·03	—	0·84	—

Flux ratio of Tl^+ fluxes

As shown by Ussing (1949) a passive diffusion process is characterized by the following equation, where M_1 is the flux measured in one direction and M_2 in the opposite direction. R is the gas constant, F is the Faraday, T the absolute temperature and $\psi_1-\psi_2$ is the potential difference.

$$M_1/M_2 = \exp\left[-F(\psi_1-\psi_2)/RT\right]$$

or transformed to $\log M_1/M_2 = pd/59$.

Since we had only one isotope of Tl (^{204}Tl) at our disposal, we used the 'sandwich technique' described by Harvey & Nedergaard (1964) in order to obtain the fluxes in both directions.

Four experiments were carried out, and samples for flux determinations were taken after 5 min, 10 min, and 20 min. The washing out of labelled Tl^+ took about 30 min, after which the flux in the opposite direction was measured. This was followed by a new washing after which the flux in the first direction was measured again. Two experiments started by measuring the flux from blood-side to lumen and two by measuring fluxes from lumen to blood-side. The potential difference of the gut changes from about 90 mV for the first period of measurements to about 40 mV for the last. However the influence of this *pd* change on the Tl^+ flux was only about 10–20% (Table 10), so that only a small error is introduced by taking the mean value.

Thus we could calculate the flux ratios for all four experiments and compare the

Table 11. *Determination of flux ratio from sandwich experiments on cecropia midgut*

(Solution 0·1 mM-$TlNO_3$ in 32-K, Tl^+ labelled with ^{204}Tl. Flux determinations made at steady state for periods of 10 min. The values found were compared to the values calculated from the potential difference after Ussing (1949): $\log M_1/M_2 = pd/59$.)

Ratio found	mV	Ratio calculated	Date
4·3	76	19	
4·4	46	6	2. 8. 77
4·5	46	6	
5·4	90	34	
5·2	55	9	3. 8. 77
5·1	37	4	
3·9	101	51	
4·1	55	9	4. 8. 77
4·2	42	5	
3·5	105	60	
3·2	53	8	5. 8. 77
3·0	41	5	

ratios actually found with the ratios calculated from the potential difference for each period (Table 11).

In the case of high potential differences there was a pronounced difference between the calculated and observed values, indicating that much of the Tl flux is not passing the midgut by simple passive diffusion. For the low potential differences the flux ratios were rather close to the values expected for a passive diffusion.

Influence of other ions

Because we know so little of the process of Tl permeation we could imagine that Tl penetrates the gut by dissolving in the membrane phase as a salt. This could, however, be expected to be very different for different salts like the thallous chloride and the sulphate. This was tested by measuring the flux first with one and later with the other anion, and then back to the first again. It seems clear that changing the anion has very little influence on the Tl^+ flux through the midgut (Table 12). Similarly, the change from K to Na as the main cation in the solution has no significant effect (see below).

Effect of different cations in the solutions

Most of the results are obtained with K^+ as the main cation in the solution. It is possible that Tl^+ is passing the midgut by exchanging with K^+, in which case the rapid transport will not continue when only other cations are available. We tested this possibility by replacing K^+ in 32-K with Na^+ (32-Na). Table 13 shows that Tl^+ will penetrate the cecropia midgut equally well, whether Na^+ or K^+ is the main cation.

Generally the solution we used was that called 32-K, with 32 mM-K^+, 30 mM-Cl^-, 2 mM-HCO_3^-, and sucrose to give 260 m-osmol/l. If, however, the solution also contained 5 mM-$CaCl_2$ and 5 mM-$MgCl_2$ the findings were the same. Three experiments performed with this solution + 1 mM-$TlNO_3$ gave a mean value of short-circuit current of 16·5 μequiv/h and a Tl^+ flux ($\times 33$) of 18·8 μequiv/h. It is obvious therefore that Tl^+ still behaves in the same way, even when Ca and Mg are also present.

Table 12. *Experiments comparing Tl^+ flux (μequiv/h) in solutions of* 30 mM-*Cl^- with solutions where all Cl^- was replaced with* 15 mM-*SO_4^{2-}*

(Tl concentration 0·1 mM. Tl flux from blood-side to lumen. Cecropia larvae – unshorted guts. There was a SO_4^{2-} period before and after a Cl^- period. The SO_4^{2-} values are mean values for the two intervals which bracket the Cl interval.)

Min	SO_4^{2-}	Cl^-	SO_4^{2-}	Cl^-	SO_4^{2-}	Cl^-
5	0·048	0·041	0·053	0·070	0·031	0·045
10	0·031	0·048	0·077	0·082	0·053	0·053
20	0·053	0·051	0·087	0·065	0·057	0·040
	8. 8. 77		9. 8. 77		10. 8. 77	

	Min	SO_4^{2-}	Cl^-
Mean value of 3 experiments	5	0·044	0·052
	10	0·054	0·061
	20	0·066	0·052
Mean value		0·055	0·055

Table 13. *Tl^+ flux (μequiv/h) from blood-side to lumen across the short-circuited cecropia midgut*

(Solution; 1 mM-$TlNO_3$ in 32-Na. Flux measured in the periods 5–10 and 10–20 min.)

Date	5–10 min	10–20 min	mV
10 Oct. (B)	1·15	1·33	0
12 Nov.	1·32	1·42	0
12 Nov. (B)	0·98	1·13	0
13 Nov.	2·20	2·09	0
14 Nov.	1·74	1·88	0
30 May	1·79	1·76	78
Mean value	1·53	1·60	
Mean value Table 1		0·82	
Mean value Table 2		1·26	

DISCUSSION

Although Table 1 and 2 show that Tl^+ penetrates the midgut from blood-side to lumen almost as if Tl^+ was hardly discriminated from K^+, the flux from lumen to blood-side is of the same magnitude. This is confirmed in Table 3 where it is shown that the net Tl^+ flux is not significantly different from zero when the sandwich technique is used and the fluxes are measured on the same midgut. Thus the conclusion is that Tl^+ is not actively transported by the cecropia midgut. This was further confirmed by Table 4 which shows that the Tl^+ transport from blood-side to lumen does not depend on the metabolism of the midgut.

These results do not, however, imply that the flux of Tl^+ is a simple passive diffusion for the following reasons:

(*a*) The ratio of the two fluxes through the gut as seen in Table 11 does not follow the flux ratio equation given by Ussing (1949) for a simple passive diffusion.

(*b*) The flux of Tl^+ through the midgut as seen from Tables 5–7 is much faster than could be expected for an ion of a size similar to K or Cs or Rb passing the gut by passive diffusion.

So we must conclude that neither diffusion nor active transport are the means for

the relatively fast penetration of Tl through the midgut of *Hyalophora cecropia* and some of the other saturniids.

Exchange diffusion

But there is another form of transport that has not been considered: the process defined by Ussing (1947) as exchange diffusion, where an ion can penetrate the tissue or membrane only by exchanging with another identical ion on the other side of membrane. Experiments with different concentrations of Tl^+ (Table 8) show that the flux is proportional to the concentration even at very low concentrations, such as 0·1 mM. Therefore there would be few Tl^+ ions on the opposite side to exchange with at these low concentration; as a result the flux should be proportionally less which is contrary to our findings.

We therefore measured the flux of Tl^+ across the gut with and without Tl^+ on the opposite side. With Tl^+ in the solution on both sides, the flux from blood-side to lumen was 0·121 μequiv/h, but with a Tl-free solution on the lumen-side the flux from blood-side to lumen was 0·101 μequiv/h. In the opposite direction (lumen to blood-side) the flux was 0·108 μequiv/h with 0·1 mM-Tl on both sides, but 0·091 μequiv/h with a Tl-free solution on the blood-side. The mean flux value for all determinations was 0·116 μequiv/h in 0·1 mM-Tl solutions and 0·096 μequiv/h for the experiments in which the solution in the direction of the flux contained no Tl. Exchange diffusion therefore seems to play no important role in the flux of Tl if any.

Facilitated diffusion

This process was first proposed as an explanation for the large flux of glucose and amino acids across the membrane of the red cell where the penetration is much larger than expected for a simple diffusion (see Wilbrand, 1960). Whether the Tl^+ permeates the gut by a carrier-mediated transport or a specific pore system like that suggested by Mullins (1959) requires a closer study. But pore systems do fit into the picture with a flux which is without saturation kinetics and also independent of the flux of other ions. A comparison with the specific permeable glasses used for glass electrodes may also be considered (Eisenmann, 1962). Studies have shown that Tl^+ can replace K^+ in, for example, muscle (found by comparing flux or cell concentration). In most tissues where it has been studied, K^+ can be replaced by Tl^+ for stimulating the ATPase, even if high Tl^+ concentrations inhibit the process. Whether Tl^+ can replace K in a particular chemical or physical process is not well established.

How can the high rate of permeation of Tl^+ be explained? From the values of the ion radius given from crystal data (see Mullins & Moore, 1960) it seems clear that the differences in size of the ions should be too small for explaining the large permeation rate for Tl^+. The passive permeation rates of K, Rb, Cs (see Tables 5, 6, 7) are rather similar. Tl^+ is the exception. The chemistry of Tl^+ seems to provide no explanation for this. Tl^+ forms many chemical compounds that are only slightly soluble, namely chloride, bromide, iodide, sulphide, etc., so one might have thought that formation of insoluble compounds might delay the permeation. But actually, Tl^+ ions pass faster than the other ions through the gut, and even if small amounts are found to be bound to the membrane this does not seem to delay the transport, which if anything is too fast. Tl^+ seems to behave similarly in several membranes, as judged from the literature.

We have considered possible effects of different solubility of Tl compounds, but two different anions make no difference (see Table 12) and Tl^+ does not have any compounds which are especially lipoid soluble. So we return to the assumption of facilitated diffusion, which in one respect is clearly supported: Tl^+ permeation is much more rapid than expected.

Certain findings do not agree with this hypothesis: (*a*) the Tl^+ flux is not saturated at high concentrations and (*b*) we know no compound which inhibits the flux, because it has not been investigated.

But to deal with point (a), it is possible that the concentrations used were too low and that a saturation may in fact occur at higher concentrations. An inhibitor may be found, but none except Tl^+ seems to be known at the moment. At this point the only way to decrease the Tl^+ flux seems to be to decrease the surface of the gut.

In the cecropia midgut Tl^+ acts as an inhibitor for the active K flux and causes the potential across the midgut to fall. But Tl^+ is in many cases a potent inhibitor of K transport. A Tl concentration as low as 0·1 mM on the lumen-side alone would sometimes depress the potential to less than 30% of the starting value. The action of Tl as an inhibitor, although not investigated sufficiently, makes Tl an important tool for studying the midgut, because in contrast to the study of Na transport in many other membranes, there is in this case no specific inhibitor available. But even if Tl is not actively transported in the cecropia midgut it may behave quite differently in other membranes, like the frog skin, where it has been found that potassium and thallous ions are showing the same behaviour and where both are actively transported from inside to outside (private communication).

Black membranes

Neher (1975) reported that typical gramicidin channels in black membranes lack the high specificity for certain cations which is common in biological systems, but the thallous ion is an exception. Eisenmann, Krasne & Ciani (1974) found that the permeability ratio for Tl^+ versus K^+ is approximately 50 as compared to ratios between 0·1 and 2 for most other monovalent cations. Neher found that in black membranes Tl^+ blocks Na^+ currents at concentrations which are two orders of magnitude lower than the sodium concentration even though the ion itself carries current through the channels better than Na^+ and K^+. In the cecropia midgut, Tl^+ penetrates the gut much faster than Cs ions (see Tables 6 and 7), but this was not found for K ions (or Rb^+). Preliminary experiments showed that 95% inhibition of the active K flux with 2 mM-Tl^+ on the lumen side produced an insignificant inhibition of 10% of the passive K flux.

Studies on other tissues

A careful study of the movement of Tl^+ across the membrane of frog sartorius muscle fibres in both directions was made by Mullins & Moore (1960). The flux of Tl^+ was measured directly by use of the isotope ^{204}Tl. For example, with an external Tl^+ concentration of 2·5 mM, the Tl^+ influx was 8·3 pmol/cm^2 s and the influx of K was estimated to be 6 pmol/cm^2 s. Thallous ions reach a steady-state distribution between fibre water and solution that is very close to the corresponding ratio for K^+: Tl_i/Tl_o was about 40, whereas the ratio for K was 38. Also during stimulation it

seems clear that the permeability of the muscle membrane to Tl^+ is as large as to K^+.

It is therefore convincing when Mullins & Moore (1960) conclude (p. 772) as follows. 'It seems difficult to escape the conclusion that neither the mechanism that determines the selectivity of the resting membrane toward ions, nor the mechanism that determines the selectivity of the active membrane, can discriminate between K^+ and Tl^+ in spite of the obvious chemical differences in these ions.'

Effect of Tl on ATPase

Gehring & Hammond (1967) report that the (Na + K) ATPase from rat erythrocytes is activated by Tl^+ (Na present). Skulskii *et al.* (1973) found that in human or rabbit erythrocytes, Tl is as effective as K in this activation at only one-tenth of the concentration of K. This was also found by Lishko, Kolchynska & Parkhomenko (1977) in human red cells. ATPase from rabbit kidney is also stimulated by Tl^+ (Britten & Blank, 1968), and in addition, Tl^+ is 10 times as effective as K^+. The same was found in beef brain by Inturrisi (1969), and by Maslova *et al.* (1971) for frog skin, where the affinity of the (Na + K) ATPase for Tl is also 10 times higher than for K. It seems that not only can Tl replace K at low Tl concentration with respect to the activation of (Na + K) ATPase, but does so with an even higher affinity.

Cavieres & Ellory (1974) found that Tl^+ can replace K^+ with a higher affinity in the activation of the ouabain sensitive Na^+ efflux. This is also found for squid nerve by Landowne (1975).

Tl^+ flux

Skulskii *et al.* (1973) found that the Tl^+ and K^+ flux rate constants for fragmented red cells were 9·36 h^{-1} for Tl^+ and 0·153 h^{-1} for K^+ inward, and 4·68 h^{-1} for Tl^+ and 0·0066 h^{-1} for K^+ outward.

The permeability of K channels to various cations was studied in myelinated frog nerve by Hille (1973). The ratios $P_{Tl}:P_K:P_{Rb}:P_{NH_4}$ are 2·3:1·00:0·92:0·13. No other ions are found to be measurably permeant, including Li^+, Na^+, or Cs^+.

Skulskii *et al.* (1973) studied the snail brain and found that the inward rate constants had the following sequence $Tl^+ > Rb^+ > Cs^+$. The passive permeability of Tl^+ in the snail brain was 50–100 times less than the Tl^+ permeability of red cell membrane.

Inhibition by Tl^+

For the (Na + K) ATPase it was found that low concentrations of Tl^+ may stimulate the ATPase, but high concentrations will inhibit the enzyme. The active Na transport in blood cells will also be inhibited by high concentrations of Tl^+ (Cavieres & Ellory, 1974). Natochin & Skulskii (1971) found that active transport of Na in the frog skin was inhibited by T^+ and explained that Tl^+ inactivates the ability of the pump (and possibly this particular enzyme) to translocate ions. This may, however, be different for the cecropia midgut, because here the inhibition is often reversible, which must imply that the Tl^+ cannot be firmly bound.

Rectal complex of Tenebrio

Koefoed (1975) found that Tl^+ follows K^+ in the uptake by the rectal complex of the mealworm, *Tenebrio molitor*. This could be explained as an active transport of Tl^+, but other possibilities were also taken into account. Whether the transport is a kind of facilitated diffusion, as we seem to have in the *Hyalophora cecropia* midgut, cannot be decided, because different tissues behave differently with respect to Tl^+.

CONCLUSION

In the gut of *Hyalophora cecropia* Tl^+ permeates from blood-side to lumen as rapidly as if Tl^+ was a marker for K^+. Nevertheless Tl^+ is not actively transported. The high permeability is the same in both directions when the gut is short-circuited. The Tl^+ permeation does not depend on the metabolism of the gut, but is up to 50 times as high as the passive permeation of Cs or Rb. It is suggested that some special kind of diffusion may take place such as facilitated diffusion, or a specific diffusion like the Tl^+ diffusion through gramicidin channels in black membranes.

Tl^+ was found to be an inhibitor for the active K transport in the midgut even though it is not actively transported. This property may be useful in later work because no good inhibitor of active K transport in this tissue is known.

It should be noted that these conclusions have been shown to be valid only for the *Hyalophora cecropia* midgut. How far these properties of the Tl^+ flux apply in other membranes must be studied carefully. In other membranes, such as frog muscle and skin, Tl^+ and K^+ both behave in a similar way and both can either be actively transported or show a simple passive diffusion.

We thank Dr V. Koefoed-Johnsen, Professor S. O. Andersen, Professor J. Ringsted, and Professor H. H. Ussing for helpful criticism in preparing the manuscript and Dr Leon Pape for correcting the language.

REFERENCES

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

CAVIERES, J. D. & ELLORY, J. D. (1974). Thallium and the sodium pump in human red cells. *J. Physiol., Lond.* **243** (1), 243–266.

EISENMAN, G., KRASNE, S. & CIANI, S. (1974). International Workshop on Ion Selective Electrodes and on Enzyme Electrodes in Biology and in Medicine. Schloss Reisenburg, September 15–18.

EISENMAN, G. (1962). Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2**, 259.

GEHRING, P. J. & HAMMOND, P. B. (1967). The interrelation between thallium and potassium in animals. *J. Pharmacol. exp. Ther.* **155**, 187–201.

HARVEY, W. R. & NEDERGAARD, S. (1964). Sodium-independent active transport of potassium in the isolated midgut of the Cecropia silkworm. *Proc. natn. Acad. Sci., U.S.A.* **51**, 757–765.

HARVEY, W. R., HASKELL, J. A. & ZERAHN, K. (1967). Active transport of potassium and oxygen consumption in the isolated midgut of Hyalophora cecropia. *J. exp. Biol.* **46**, 235–248.

HARVEY, W. R. & ZERAHN, K. (1969). Kinetics and route of active K-transport in the isolated midgut of *Hyalophora cecropia*. *J. exp. Biol.* **50**, 297–306.

HARVEY, W. R. & ZERAHN, K. (1971). Active transport of sodium by the isolated midgut of *Hyalophora cecropia J. exp. Biol.* **54**, 269–274.

HARVEY, W. R. & ZERAHN, K. (1972). Active transport of potassium and other metals by the isolated midgut of the silkworm. *Current Topics in Membranes and Transport*, vol. 3. Academic Press.

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

INTURRISI, C. E. (1969). Thallium induced dephosphorylation of a phosphorylated intermidiate of the (sodium + thallium activated) ATPase. *Biochim biophys. Acta* **178**, 630–633.

KOEFOED, B. M. (1975). The Cryptonephridial system in the mealworm *Tenebrio molitor:* Transport of radioactive potassium, thallium and sodium; a functional and structural study. *Cell Tiss. Res.* **165**, 63–78.

LANDOWNE, D. (1975). A comparison of radioactive thallium and potassium. *J. Physiol., Lond.* **252**, 79–96.

LISHKO, V. K., KOLCHYNSKA, L. I. & PARKHOMENKO, M. T. (1973). Thallium and sodium pump of erythrocytes. *J. Biochim. Ukrain.* **1**, 42–54.

MASLOVA, M. N., NATOCHIN, YU. V. & SKULSKII, I. A. (1971). Inhibition of active sodium transport and activation of Na^+, K^+-dependent ATPase by thallium ions in frog skin. *Biochemistry, N.Y.* **36**, no. 4.

MULLINS, L. J. (1959). The penetration of some cations into muscle. *J. gen. Physiol.* **42** (4), 817–829.

MULLINS, L. J. & MOORE, R. D. (1960). The movement of thallium ions in muscle. *J. gen. Physiol.* **43** (4), 759–773.

NATOCHIN, YU, V. & SKULSKII, I. A. (1971). Difference of the influence of Tl^+ and Ba^{2+} as partial analogs of K^+ on the sodium pump in the frog skin. Academician E. M. Kreps. **203** (6), 1437–1440.

NEDERGAARD, S. & HARVEY, W. R. (1968). Active transport by the Cecropia midgut. IV. Specificity of the transport mechanism for potassium. *J. exp. Biol.* **48**, 13–24.

NEHER, E. (1975). Ionic specificitv of the gramicidin channel and the thallous ion. *Biochim biophys. Acta* **401**, 540–544.

RIDDIFORD, L.. (1968). Artificial diet for Cecropia and other saturniid silkworms. *Science, N.Y.* **160**, 1461–1462.

SKULSKII, J. A , MANNINEN, V. & JÆRNEFELT, J. (1973). Interactions of thallous ions with the cation transport mechanism in erythrocytes. *Biochim. biophys. Acta* **298**, 702–709.

SOLT, J., PASCHINGER, H. & BRODA, E. (1971). Die energieabhängige Aufnahme von thallium durch Chlorella. *Planta (Berl.)* **101** (3), 242–250. Springer-Verlag.

USSING, H. H. (1947). Interpretation of the exchange of radio-sodium in isolated muscle. *Nature, Lond.* **160**, 262–263.

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

WILBRAND, W. (1961). Membrane transport and metabolism. *Proc. Symposium. Prag.* (ed. A. Kleinzeller and A. Kotyk).

ZERAHN, K. (1973). Properties of the cation pump in the midgut of *Hyalophora cecropia*. *Alfred Benzon Symposium V* (ed. H. H. Ussing and N. A. Thorn), pp. 360–367.