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Further Observations on the Inhibitory Effect of Extracellular Potassium Ions on Glycine Uptake by Mouse Ascites-Tumour Cells

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1. The initial rate of uptake of glycine by the tumour cells was measured as a function of the Na^+ and K^+ concentrations in the solution in which the cells were suspended. When [Gly] was 1mM or 12mM, the rate in the absence of Na^+ was independent of [K^+] and about 3% or 10% respectively of the rate when [Na^+] was 150m-equiv./l. 2. The Na^+-dependent glycine entry rate, v, at a given value of [Na^+] was successively lowered when [K^+] was increased from 8 to 47 to 96m-equiv./l. A kinetic analysis indicated that K^+ competitively inhibited the action of Na^+. The results were in fair agreement with previous determinations of the kinetic parameters. 3. The presence of 2mM-sodium cyanide and 10mM-2-deoxyglucose lowered the cellular ATP content to less than 3% of the value in the respiring cells. Although v was then about 50% smaller, the relative effects of K^+ and Na^+ on the system were similar to those observed during respiration. 4. A theoretical analysis indicated that the variation of v with [K^+] is not a reliable guide to the extent to which the K^+ gradient between the cells and their environment may contribute to the net transport of glycine.

There is good evidence that extracellular Na^+ markedly stimulates the initial rate of uptake of glycine, v, by mouse ascites-tumour cells (Kromphardt, Grobecker, Ring & Heinz, 1963; Inui & Christensen, 1966; Wheeler, Inui, Hollenberg, Eavenson & Christensen, 1965; Eddy, Mulcahy & Thomson, 1967) whereas the effect of K^+ on the rate is less striking and might even seem unimportant. Kromphardt *et al.* (1963) indicated, without giving details, that v was almost independent of extracellular [K^+] except in the range up to 5m-equiv./l. On the other hand Eddy *et al.* (1967) found v to be distinctly smaller when both [Na^+] was small and [K^+] relatively large than when choline ions replaced K^+ as the principal extracellular cation. The importance of K^+ was also shown when the tumour cells were first depleted of ATP and then exposed to glycine. Almost 1equiv. of Na^+ appeared to accompany the glycine into the cells and about 0·6equiv. of K^+ left them, a circumstance that is difficult to explain except in terms of the ion-gradient hypothesis of amino acid transport, with both Na^+ and K^+ playing a part in the transport of glycine (Eddy, 1968*a*). The present work was undertaken to study the relations between v and both [Na^+] and [K^+] in greater detail. The results confirm that there are circumstances where increasing [K^+] systematically lowers the Na^+-dependent component of v, probably because K^+ competitively inhibits the action of extracellular Na^+. This effect of K^+ appears not to involve cellular ATP.

MATERIALS AND METHODS

In general these followed the procedures used in earlier work (Eddy *et al.* 1967; Eddy, 1968*a*,*b*), except that the tumour cells were transplanted without the addition of penicillin and streptomycin, which appeared to serve no useful purpose. The standard Ringer solution (Umbreit, Burris & Stauffer, 1957) contained sodium phosphate buffer, pH 7·4, 155m-equiv. of Na^+/l., 8m-equiv. of K^+/l., 1·2mM-$MgSO_4$ and no added Ca^{2+}. A similar solution contained 50mM-tris base, 1·2mM-$MgSO_4$ and selected amounts of NaCl, KCl and choline chloride, the sum of the concentrations of the last three compounds being 163mM. Each solution was brought to pH 7·4 by the addition of 2M-HCl. The use of tris buffer lowered the Na^+-dependent component of the glycine entry rate by about 30% without affecting the amount of glycine eventually absorbed.

To measure v the tumour cells were freed from erythrocytes by differential centrifugation at 300*g* during the preliminary manipulations described by Eddy *et al.* (1967). Usually the cells were then suspended in the buffered tris solution containing 158mM-choline chloride at 37°, separated by centrifugation and resuspended twice further in a similar solution, to remove superfluous Na^+ and K^+. The cell

preparations were next suspended in a series of solutions at 37° containing [^{14}C]glycine, the tris buffer and selected concentrations of Na^+, K^+ and choline ions. Alternative washing and assay procedures are mentioned in the text. The treatments with the buffered choline chloride solution, which occupied about 20min., appeared not to affect the ability of the cells to absorb glycine from the standard Ringer solution. Sampling the various cell suspensions and assay of the cellular [^{14}C]glycine content was carried out as described by Eddy *et al.* (1967). The samples were timed so as to reveal the initial rate of uptake of glycine, v, during the first minute (see Fig. 1). In computing v a correction was made for the glycine content of the extracellular phase by using method 2 of Eddy *et al.* (1967).

Assay of cellular ATP. The cell sample (about 5mg.), suspended in an appropriate Ringer solution (2·0ml.), was mixed with ice-cold 30% (w/v) $HClO_4$ (0·5ml.) and kept at 0° for 10min. before neutralization with 1M-KOH (1·5ml.). Cell debris was separated by centrifugation at 0° and portions of the supernatant solution containing from 10 to 100pmoles of ATP were assayed for ATP by a modification of the firefly-lantern technique (Cole, Wimpenny & Hughes, 1967). The light flashes produced during the interval from 10 to 20 sec. after mixing the reagents with the cellular extract were counted in a photomultiplier unit attached to a scaler with an automatic print-out (Panax Ltd., Redhill, Surrey). The number of such flashes varied linearly with the amount of ATP added to the assay system in the range up to 100 pmoles.

Chemicals. A.R.-grade compounds were used when these were available. ATP was obtained from Boehringer Corp. (London) Ltd., London W.5. 2-Deoxyglucose and a freeze-dried firefly-lantern extract (stock no. FLE 250) were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.

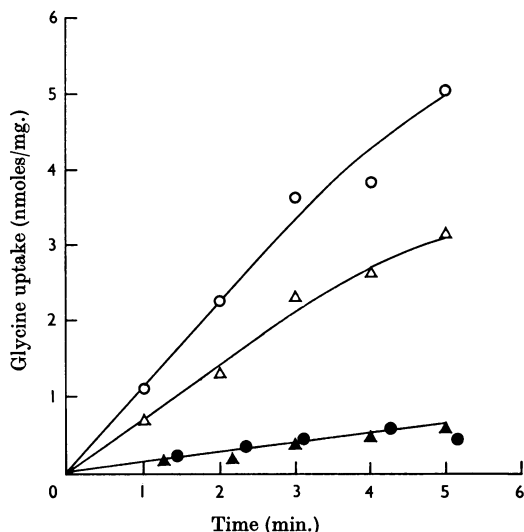

Fig. 1. Inhibition of the Na^+-dependent mode of glycine uptake by K^+. The tumour cells (4mg./ml.) were suspended at 37° in the tris buffer, pH 7·4, containing 1mM-[^{14}C]glycine (0·3μc/ml.) and selected concentrations of NaCl, KCl and choline chloride totalling 158mM. Samples were withdrawn at intervals and the cellular [^{14}C]glycine content was assayed (see the Materials and Methods section). Ion concentrations (m-equiv./l.) in the extracellular phase were: ○, [Na^+] 12, [K^+] 0; △, [Na^+] 12, [K^+] 96; ●, [Na^+] 0, [K^+] 0; ▲, [Na^+] 0, [K^+] 158.

RESULTS

The two upper lines of Fig. 1 show that, when [Na^+] was 12m-equiv./l., glycine was taken up more slowly in the presence of 96mM-potassium chloride than when the latter was replaced by choline chloride. As v was little affected by the replacement of choline chloride by sucrose, with both [Na^+] and [K^+] constant (Inui & Christensen, 1966; P. J. Thomson, unpublished work), the presence of K^+, rather than the omission of choline ions, appeared to be the important factor in Fig. 1. The two lower lines of Fig. 1, together with the results in Table 1, demonstrate that K^+ failed to influence v in the virtual absence of Na^+ ([Na^+] $\simeq$ 0·4m-equiv./l.). Hence K^+ does not appear to substitute for Na^+ in this sytem.

The effect of altering [Na^+] was studied in a series of experiments in which [K^+] was varied independently. This was in contrast with earlier work (Eddy *et al.* 1967) where either (1) [K^+] was 8m-equiv./l. and [Na^+] varied, or (2) [Na^+] was varied with the sum [Na^+] + [K^+] constant. Fig. 2 is concerned with the Na^+-dependent component of v, i.e. v less the rate when [Na^+] was zero. The observations from 12 experiments were combined to show how the reciprocal of the Na^+-dependent rate varied with 1/[Na^+] at 0, 47 and 94m-equiv. of K^+/l. The corresponding lines in Fig. 2 were computed by using the logarithmic transformation described by Barber, Welch & Mackay (1967). The method makes use of the circumstance that the transport parameter corresponding to $V_{max.}$ is more likely to vary than K_m when v is determined with a series of independent preparations of the tumour cells. Barber *et al.* (1967) showed how the variance associated with $V_{max.}$ can then be separated from that due to other causes. Their procedure enables K_m to be estimated more precisely than do conventional methods ignoring the distribution of variance. An important aspect of the analysis is illustrated in the inset to Fig. 2 for one representative group of observations. Here the standard deviation of $1/v$ varied linearly with 1/[Na^+], whereas the corresponding quantity for $\log(1/v)$ was almost constant. This situation is expected when most of the variance is associated with $V_{max.}$ (Barber *et al.* 1967).

Kinetic relations between [Na^+] *and* [K^+]. Application of the logarithmic transformation gave the estimates of both $V_{max.}$ and the K_m for Na^+ that are shown in Table 2 in relation to [K^+]. Previous

Table 1. *Failure of* K^+ *to replace the need for* Na^+ *during the absorption of glycine in the presence or absence of* 2mM-*potassium cyanide*

The initial glycine entry rate (±S.E.M.) was determined under the various conditions specified below, the osmotic pressure being kept constant by appropriate additions of choline chloride. The phosphate buffer (P) was that used in the standard Ringer solution, whereas the tris buffer (T) was that described in the Materials and Methods section, where the other experimental procedures are also outlined. The measurements involving 2mM-KCN were carried out after starving the cells for 25min. in a Ringer–cyanide solution lacking Na^+ (Eddy *et al.* 1967).

[Gly] (mM)	KCN	[Na^+] (m-equiv./l.)	[K^+] (m-equiv./l.)	Buffer	Glycine entry rate (nmoles/mg./min.)
1	−	0	0	T	0·19±0·02 (5)
1	−	0	158	T	0·16±0·02 (3)
1	+	0	0	T	0·16±0·01 (7)
1	+	0	158	P	0·18±0·01 (7)
1	−	0	158	P	0·18±0·03 (3)
1	+	150	8	P	6·21±0·70 (7)
12	−	0	0	T	2·24±0·11 (5)
12	−	0	158	T	2·17±0·23 (3)
12	+	0	0	T	1·61±0·06 (7)
12	+	150	8	P	24·4±1·5 (7)

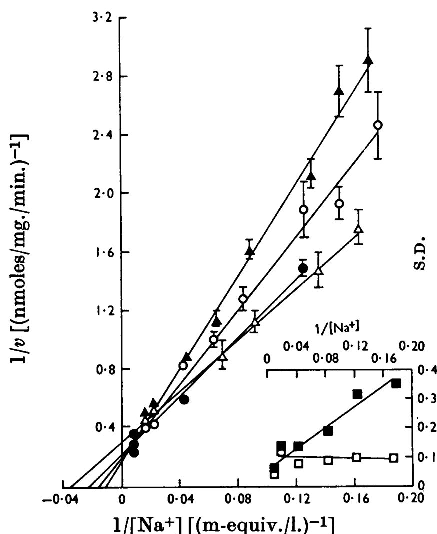

Fig. 2. Plots of $1/v$ against $1/[Na^+]$ at four values of [K^+]. These were, in m-equiv./l.: △, 0; ●, 8; ○, 47·4; ▲, 93·6. The Na^+-dependent component of the initial rate of glycine uptake, v, from a 1mM solution was measured as a function of [Na^+] and [K^+]. Each point represents the mean of usually six observations. The corresponding bar represents the standard error. The absence of a bar at the larger values of [Na^+] means that the error was smaller than the symbol itself. The pooled results were collected in 21 experiments. The positions of the lines were computed by using the method explained in the text. Inset: comparison of the standard deviations of $\log(1/v)$ (□) and $1/v$ (■) at various values of $1/[Na^+]$ for one set of observations.

work was consistent with the view that K^+ acted as a competitive inhibitor of the action of Na^+ in this system (Eddy *et al.* 1967). If this were so, $V_{max.}$ would be independent of [K^+] and the three lines drawn through the data for 0, 47 and 94m-equiv. of K^+/l. in Fig. 2 would intersect on the $1/v$ axis. The data in Table 2 are just consistent with that possibility and with the K_m values computed from previous estimates of the kinetic parameters of the system (Table 2 of Eddy *et al.* 1967). According to the F-test for equality of variance, a significantly better fit ($P < 0·1$) was obtained, however, with a point of intersection near the co-ordinates $1/[Na^+] = 0·018$, $1/v = 0·44$. The scatter of the observations in Fig. 2 precludes firmer conclusions, especially when the fluctuations in $V_{max.}$ represent the main component of variance.

Table 2. *Effect of varying* [K^+] *on* $V_{max.}$ *and on the* K_m *for* Na^+, *as deduced from the data in Fig.* 2 (*see the text*)

The mean values of K_m and $V_{max.}$ and their 95% confidence limits are shown. K_m was also computed from the carrier parameters given in Table 2 of Eddy *et al.* (1967) on the basis of the assumptions used in estimating these parameters.

[K^+] (m-equiv./l.)	K_m for Na^+ (m-equiv./l.)	$V_{max.}$ (nmoles/mg./min.)	Calculated K_m (m-equiv./l.)
0	29·9 (19·8–40·0)	3·40 (2·65–4·15)	34·0
8	43·4 (33·7–53·1)	4·46 (4·00–5·00)	36·8
47·4	66·0 (34·8–97·2)	5·23 (4·04–6·77)	52·0
93·6	83·0 (49·9–116·1)	5·31 (3·80–7·44)	70·3

In a series of paired measurements at 120, 23 and 8m-equiv. of Na^+/l. the effect of changing [K^+] from 0 to 8m-equiv./l. was examined. The mean percentage effect at each value of [Na^+] was used to

determine where the line for 8m-equiv. of K^+/l. would be expected to lie in relation to the lines for 0, 47 and 94m-equiv. of K^+/l. (Fig. 2). Inspection of Fig. 2 shows that raising [K^+] to 8m-equiv./l. increased the mean value of v when [Na^+] was large and lowered it when [Na^+] was small. As $P \geqslant 0.1$, however, in both cases, the significance of these observations is uncertain. Nevertheless, Fig. 2 shows that the lines for 8, 47 and 94m-equiv. of K^+/l. intersect quite near the $1/v$ axis, as though $V_{max.}$ were almost constant. Hence additional evidence was sought to confirm the relative position of the line for 8m-equiv. of K^+/l. In the further measurements [Na^+] was 128m-equiv./l. and [K^+] was either 8 or 64m-equiv./l. The mean rate at 64m-equiv. of K^+/l. was 86% ($P < 0.05$) of that at 8m-equiv./l. In the light of Fig. 2 this result appears consistent with a constant value of $V_{max.}$ at the three larger values of [K^+].

Table 3. *Effect of starvation on cellular ATP content*

The ATP content of the respiring tumour cells was constant during the 30min. in which samples were taken. The other preparations were kept with 2mM-KCN for 30min. before the addition of 1mM-glycine, L-phenylalanine or L-leucine; 10mM-2-deoxyglucose (DOG) was added 15min. before the amino acid. The samples for assay of ATP were taken from 1 to 15min. later, when the ATP content appeared to be constant. The mean values ± S.E.M. for the stated numbers of independent cellular preparations are shown.

Conditions	ATP content (nmoles/mg.)
During respiration + Gly	11·10 ± 2·98 (5)
KCN + Gly	0·297 ± 0·043 (8)
KCN + DOG + Gly	0·176 ± 0·019 (8)
KCN + DOG + Phe	0·146 ± 0·022 (4)
KCN + DOG + Leu	0·170 ± 0·020 (4)

The above observations can perhaps be understood qualitatively if K^+ has two additive effects on the Na^+-dependent glycine transport system: (1) a competitive inhibition of the action of extracellular Na^+, revealed at relatively large values of [K^+]; (2) a small stimulatory effect manifest at 8m-equiv. of K^+/l. when [Na^+] is large.

Dependence on ATP. (*a*) Cellular ATP content. Table 3 summarizes a series of measurements of the ATP content of the tumour cells in three situations in which v was measured in other independent experiments: (1) during respiration; (2) after starvation in the presence of cyanide for about 3min. (Eddy *et al.* 1967); (3) after starvation for 15min. in the presence of cyanide, followed by 15min. in the same solution plus 10mM-2-deoxyglucose. The hexokinase of the tumour cells converts some of the latter compound into deoxyglucose 6-phosphate (Letnansky, 1964), less ATP thereby being available, presumably, for the amino acid-transport systems. Table 3 shows that both modes of starvation lowered the cellular ATP content to less than 3% of the value in the respiring cells.

(*b*) Inhibition by K^+. Table 4 shows that K^+ inhibited the Na^+-dependent component of glycine uptake by the starved cells independently of the presence of deoxyglucose and probably of ATP (cf. Fig. 1). The values of v obtained in the absence of Na^+ show that K^+ was not able to replace the need for Na^+.

(*c*) Accumulation of cellular glycine in the presence of deoxyglucose. Deoxyglucose failed to inhibit the accumulation of glycine in preparations of starved tumour cells that were depleted of Na^+ and then transferred to the standard Ringer solution containing the metabolic inhibitors and 1mM-glycine (Table 4). This behaviour reinforces the

Table 4. *Effect of 10mM-2-deoxyglucose on glycine uptake from a 1mM solution in the presence of 2mM-potassium cyanide in various ionic conditions*

Two series of measurements were carried out, each involving four preparations of cells on which the stated numbers of independent observations were made. The mean values of v ± S.E.M. are shown. All the cells were starved for 25min. with KCN before v was measured in the presence of KCN. 2-Deoxyglucose (DOG) was added 10min. before the starvation was completed. It was then also present when v was measured.

Series	[Na^+] (m-equiv./l.)	[K^+] (m-equiv./l.)	DOG	Initial rate (nmoles/mg./min.)	Cellular concn. of [^{14}C]glycine at 10min. (mM)
1	150	8	+	7·81 ± 0·47 (4)	5·30 ± 0·62 (4)
1	150	8	−	8·63 ± 0·72 (4)	5·48 ± 0·48 (4)
1	0	158	+	0·14 ± 0·02 (4)	
1	0	158	−	0·14 ± 0·02 (4)	
1	24	0	+	2·05 ± 0·10 (4)	
1	24	134	+	1·24 ± 0·12 (4)	
2	24	0	−	3·32 ± 0·19 (6)	
2	24	134	−	1·83 ± 0·03 (6)	

Table 5. *Rate of glycine uptake as a function of cellular* [Na+], *with and without* 2mM-*sodium cyanide present*

For one series, the tumour cells were starved with NaCN in the presence of 150m-equiv. of either Na+ or K+/l. Then v was determined in the standard Ringer solution containing NaCN. For the other series, the respiring tumour cells were initially kept either in the standard Ringer solution (150m-equiv. of Na+/l.), when cellular [Na+] was relatively large during the subsequent measurement of v in the standard Ringer solution, or, alternatively, they were kept in a Ringer solution containing 158m-equiv. of K+/l., when cellular [Na+] was subsequently smaller when v was measured. The glycine concentration was 1mM.

Range of cellular [Na+] (m-equiv./l.)	Glycine uptake rate (nmoles/mg./min.)	
	NaCN absent	NaCN present
40–60	13·2±1·0 (10)	6·4±1·3 (4)
70–90		6·0±0·5 (11)
100–120	7·6±0·5 (8)	4·2±0·5 (3)

view (Eddy *et al.* 1967; Eddy, 1968*a*,*b*) that the net movement of glycine in these circumstances, leading in this instance to the amino acid being concentrated about fivefold with respect to the extracellular phase, depends on some source of energy other than ATP.

(*d*) Effect of cyanide on v. Eddy *et al.* (1967) concluded that the rate of uptake of glycine was almost independent of the cellular ATP content provided that cellular [Na+] was roughly the same as during respiration (about 30m-equiv./l.). The earlier work compared (1) respiring cells from the standard Ringer solution containing 150m-equiv. of Na+/l. with (2) preparations kept for 30min. with cyanide and about 150m-equiv. of K+/l. Now Table 5 shows that when the respiring cells were initially kept in the presence of K+ instead of Na+ they absorbed glycine more rapidly ($P < 0{\cdot}01$) from a solution containing 150m-equiv. of Na+/l., probably because cellular [Na+] was smaller. Table 5 also shows that the presence of cyanide lowered v in cell preparations taken either from the K+ or the Na+ solutions ($P < 0{\cdot}01$). In the earlier experiments cyanide presumably cancelled out the effect of replacing Na+ by K+ during the preliminary treatments.

Prolonged exposure to K+. Tumour-cell preparations were kept for 25, 40 or 55min. with cyanide at 158m-equiv. of K+/l. The rate at which they took up glycine from a 1mM or 12mM solution, either with or without 150m-equiv. of Na+/l. and in the presence of cyanide, was independent of the duration of the initial treatment. These observations provide no evidence that the cellular permeability to glycine was altered irreversibly during the exposure to K+ under these conditions. Robinson (1967) detected such changes in preparations of rat intestine.

Kinetic parameters. Eddy *et al.* (1967) measured v as a function of [Gly], [K+] and [Na+] and then estimated the seven dissociation constants characterizing the attachment of these ligands to the carrier system. The estimations involved a simplified version of the carrier equations in which a constant amount of the carrier E was assumed to be available at the outer cellular surface (eqn. 8 of Eddy *et al.* 1967). In the present work the reverse procedure was used to predict (*a*) the value of v at selected values of [Na+], [K+] and [Gly] (method 1) and (*b*) the K_m for Na+ as a function of [K+]. Table 2 shows that the predicted K_m values agreed fairly well with those observed. With a knowledge of the parameters k_1 to k_7, Eddy (1968*a*) estimated the magnitudes of the transfer coefficients (k^k, k^c, etc.) for the various carrier species. The present observations are consistent with the previous assumption that k^g and k^{kg}, the transfer coefficients for EGly and EKGly respectively, are usually negligible. Also k^n, relating to ENa, is probably small enough to be neglected (Eddy, 1968*a*). In method 2 used in the present work, the value of v was accordingly computed from the carrier equations describing v as a function of $[Na^+]_1$, $[K^+]_1$, $[Gly]_1$, $[Na^+]_2$, $[K^+]_2$ and $[Gly]_2$, the ligand concentrations on each side of the cell membrane, as follows:

$$v=\frac{k^{ng}e[Na^+]_1[Gly]_1}{k_1k_4}\left(1+\frac{[Na^+]_1}{k_1}+\frac{[K^+]_1}{k_2}+\frac{[Gly]_1}{k_3}+\frac{[Na^+]_1[Gly]_1}{k_1k_4}+\frac{[K^+]_1[Gly]_1}{k_2k_6}+\frac{\left(1+\frac{k^k}{k^ck_2}[K^+]_1+\frac{k^{ng}}{k^ck_1k_4}[Na^+]_1[Gly]_1\right)}{\left(1+\frac{k^k}{k^ck_2}[K^+]_2\right)}\left(1+\frac{[Na^+]_2}{k_1}+\frac{[K^+]_2}{k_2}\right)\right)^{-1} \quad (1)$$

The following data illustrate the two methods. The ratio of v at 8m-equiv. of K^+/l. to v at 96m-equiv. of K^+/l., when $[Na^+]$ was constant at 24m-equiv./l., was 1·56 by method 1 and 2·02 by method 2. On the other hand, with $[K^+]$ constant at 96m-equiv./l., the ratio of the rate at 24m-equiv. of Na^+/l. as opposed to 8m-equiv. of Na^+/l. was 2·50 by method 1 and 2·69 by method 2. Similarly, with $[K^+]$ constant at 8m-equiv./l., the ratio of the rates at 8 and 128m-equiv. of Na^+/l. was 0·230 by method 1 and 0·189 by method 2. The two methods of computing v thus gave similar results. Moreover, each set appeared to be in fair agreement with the experimental observations in Fig. 2. The scatter of the experimental points suggested there was little value in refining the original estimates, either of the dissociation constants or of the transfer coefficients, by an iterative procedure involving eqn. (1) above.

A further test of the parameters selected is provided by the observation, confirmed in the present work (Table 5), that the value of v measured in the presence of cyanide falls when cellular $[Na^+]$ increases (Eddy *et al.* 1967). Eddy *et al.* (1967) suggested two possible interpretations: (1) that the apparent value of v was artificially low, owing to recycling of glycine through the carrier system; (2) that v depended on the rate at which the carrier, which had initially crossed the cell membrane as ENaGly, returned from the inner to the outer surface, EK returning faster than ENa. Eqn. (1) also describes this effect. As Eddy (1968*a*,*b*) estimated that $k^{ng}/k^{c} \nless 1$, the effect of replacing cellular K^+ by Na^+ was studied as a function of k^{ng}/k^{c}. The observed inhibition was reproduced when k^{ng}/k^{c} was 4, a 50% smaller effect being expected when k^{ng}/k^{c} was 1. These calculations lead to a value of k^{ng}/k^{c} between 4 and 1, depending on the extent to which recycling of glycine is supposed to be involved in the phenomenon.

Significance of a relatively small effect of $[K^+]$ *on* v. In the earlier treatment of the ion-gradient hypothesis (Eddy, 1968*a*,*b*), based on the assumptions involved in method 2 above, the ratio of the cellular to extracellular concentrations of glycine was shown to be:

$$\frac{[Gly]_2}{[Gly]_1}=\frac{[Na^+]_1\left(1+\dfrac{k^k}{k_2k^c}[K^+]_2\right)}{[Na^+]_2\left(1+\dfrac{k^k}{k_2k^c}[K^+]_1\right)} \quad (2)$$

Comparison of eqns. (1) and (2) shows the relative effect of varying $[K^+]_1$ on v and on $[Gly]_2/[Gly]_1$ respectively. Two hypothetical cases are of special interest. First, let k^k be zero, so that EK is not mobile. Then:

$$\frac{[Gly]_2}{[Gly]_1}=\frac{[Na^+]_1}{[Na^+]_2}$$

a quantity independent of $[K^+]_1$. Nevertheless v would still vary with $[K^+]_1$. As a second case, let k^c be zero, so that the carrier only moves either as ENaGly or as EK. Now:

$$\frac{[Gly]_2}{[Gly]_1}=\frac{[Na^+]_1[K^+]_2}{[Na^+]_2[K^+]_1}$$

a quantity that varies inversely with $[K^+]_1$. Keeping k^c zero, one may then ask how small the effect of $[K^+]_1$ on v may become when k^{ng} and k^k are systematically varied. Accordingly, $[Na^+]_1$, $[Na^+]_2$, $[K^+]_2$ and $[Gly]_1$ were assumed to be 24, 30, 170 and 1m-equiv./l. respectively, and v was computed from eqn. (1) when $[K^+]_1$ was either 100 or 8m-equiv./l. Increasing $[K^+]_1$ from 8 to 100m-equiv./l. lowered v by 25% when k^{ng}/k^{k} was 24, and by about 40% when k^{ng}/k^{k} was 10.

Table 6. *Effect of* Li^+ *on the rate of uptake of glycine by the respiring tumour cells at low* Na^+ *concentrations*

The sum of the concentrations of the various cations was kept at 158m-equiv./l. by the addition of the appropriate amount of choline chloride. Mean values of $v \pm$ S.E.M. are shown.

$[Na^+]$ (m-equiv./l.)	$[Li^+]$ (m-equiv./l.)	$[K^+]$ (m-equiv./l.)	Rate (nmoles/mg./min.)
0·1*	0	0	0·20±0·01 (6)
0·2*	153	0	1·68±0·17 (6)
15·1	0	0	2·08±0·10 (6)
16·4	138	0	2·56±0·17 (6)
0	83	0	1·35
0	83	75	0·90
0	97	0	1·26
0	97	61	1·06

* Determined by flame photometry.

Table 7. *Comparison of the glycine uptake rate in the presence of* K^+, Rb^+, NH_4^+, Tl^+ *or choline ions when small amounts of* Na^+ *were also present*

The behaviour of selected cations was compared in three experiments with cyanide absent. The Na^+ concentration plus that of the other cation was 158m-equiv./l. The action of Tl^+ was studied in a system containing NO_3^- instead of Cl^-, a substitution that in itself appeared not to affect the transport of glycine.

Expt. no.	$[Na^+]$ (m-equiv./l.)	Glycine uptake rate (nmoles/mg./min.) in the presence of: Choline+	K^+	Rb^+	NH_4^+	Tl^+
1	15·5	3·07	—	2·00	2·34	—
2	15·5	2·76	1·06	1·27	1·75	—
3	24·0	—	1·25	—	—	0·63

The above two hypothetical cases illustrate two important aspects of the ion gradient hypothesis: (1) a dependence of v on $[K^+]_1$ might be observed, in principle, even when the steady-state gradient of amino acid concentration was independent of $[K^+]_1$; (2) a relatively large effect of $[K^+]_1$ (and of $[K^+]_2$) on the amino acid gradient can occur in circumstances where the effect of $[K^+]$ on v may be relatively small and, quite incorrectly, might even seem unimportant (this possibility is relevant to discussions of the role of K^+ in the transport systems of the intestine; Goldner, Schultz & Curran, 1969).

Other alkali-metal cations. Table 6 shows that glycine influx was stimulated by the presence of Li^+ when $[Na^+]$ was very small ($P < 0{\cdot}01$). The effect of 150m-equiv. of Li^+/l., in the virtual absence of Na^+, was roughly the same as that of 10m-equiv. of Na^+/l. The smaller effect at 16m-equiv. of Na^+/l. may mean that Li^+ and Na^+ interacted competitively. Table 6 also shows that the effect of Li^+ was antagonized by K^+. Thus Li^+ produced effects on v that were qualitatively similar to those of Na^+. In contrast, Rb^+, NH_4^+ and Tl^+ each resembled K^+ in lowering the Na^+-dependent component of v (Table 7).

DISCUSSION

The initial aim of the present work was to test three assumptions made in earlier work that were indicated but not proved (Eddy *et al.* 1967; Eddy, 1968*a*,*b*): (1) that K^+ inhibited the Na^+-dependent uptake of glycine by the ascites-tumour cells; (2) that K^+ was unable to replace the need for Na^+ to a significant extent; (3) that ATP was not involved in the inhibitory effect of K^+. The present observations appear to show that each of these assumptions is valid. They also show that the inhibition by K^+ is not merely due to incipient cellular damage of the type other workers have encountered in certain intestinal preparations (Robinson, 1967; cf. Schultz, Curran, Chez & Fuisz, 1967).

The stimulatory effect on v of relatively low concentrations of K^+ at large values of extracellular $[Na^+]$ is not understood, nor is the inhibition of glycine efflux that takes place in similar circumstances (Eddy *et al.* 1967). The lowering of v in the presence of cyanide (Table 5) is also a puzzling phenomenon. As either the omission of K^+ or the presence of cyanide would inhibit the sodium pump, the three effects may possibly originate in an interaction between it and the glycine-carrier system.

Most of the other observations can be understood in terms of previous discussion (Eddy, 1968*a*,*b*) of the proposal, based on the classical concepts of carrier kinetics, that Na^+ is a co-substrate of a glycine-carrier system that does not itself interact with ATP. The K^+ competes with Na^+ for the same negative site on the carrier (E). On that supposition the Na^+-dependent component of glycine uptake would involve the carrier species ENaGly. This species serves to facilitate the movement together of both Na^+ and a glycine molecule across the permeability barrier at the cell surface. The present results are taken to mean that the uptake of glycine as EKGly can be disregarded. Except when $[Na^+]$ is small, or [Gly] relatively large, entry of glycine other than as ENaGly can be neglected.

We emphasized above that observations on the relation of v to $[Na^+]$ and $[K^+]$ do not show whether either type of ion crosses the membrane through the amino acid-carrier system. In terms of the ion-gradient hypothesis, K^+ might inhibit v without affecting the steady-state distribution of the amino acid. Conversely, a relatively small effect of K^+ on v might be associated with a relatively large one on the amino acid distribution. Thus the present observations merely show that the complex EK is formed in the tumour-cell system. The evidence that EK traverses the membrane is twofold: (1) when the tumour cells were depleted of ATP about 1equiv. of Na^+ entered the cells/mole of glycine absorbed, whereas about 0·6equiv. of K^+ left them (Eddy, 1968*a*); (2) the steady-state distribution of glycine between the cells and their environment was affected by the corresponding distribution of K^+ (Eddy, 1968*b*).

The Na^+-dependent uptake both of glycine and of alanine by rabbit reticulocytes (Wheeler & Christensen, 1967) and that of glycine by pigeon erythrocytes (Vidaver, 1964) was apparently not inhibited significantly by K^+ in conditions like those used for Fig. 1. The absence of an effect of K^+ would be important in relation to the ion-gradient hypothesis. Thus the systems like the glycine carrier in the tumour cells, through which we suggest both Na^+ and K^+ can pass, would be expected to form, under comparable conditions, larger gradients of amino acid concentration than those that move Na^+ but not K^+ [see eqn. (4) of Eddy (1968*a*), EKGly being immobile].

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REFERENCES

Barber, H. E., Welch, B. L. & Mackay, D. (1967). *Biochem. J.* **103**, 251.

Cole, H. A., Wimpenny, J. W. T. & Hughes, D. E. (1967). *Biochim. biophys. Acta*, **143**, 445.

Eddy, A. A. (1968*a*). *Biochem. J.* **108**, 195.

Eddy, A. A. (1968*b*). *Biochem. J.* **108**, 489.

Eddy, A. A., Mulcahy, M. F. & Thomson, P. J. (1967). *Biochem. J.* **103**, 863.

Goldner, A. M., Schultz, S. G. & Curran, P. F. (1969). *J. gen. Physiol.* **53**, 362.

Inui, Y. & Christensen, H. N. (1966). *J. gen. Physiol.* **50**, 203.

Kromphardt, H., Grobecker, H., Ring, K. & Heinz, E. (1963). *Biochim. biophys. Acta*, **74**, 549.

Letnansky, K. (1964). *Biochem. Z.* **341**, 74.

Robinson, J. W. L. (1967). *Pflüg. Arch. ges. Physiol.* **294**, 182.

Schultz, S. G., Curran, P. F., Chez, R. A. & Fuisz, R. E. (1967). *J. gen. Physiol.* **50**, 1241.

Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1957). *Manometric Techniques*, 3rd ed., p. 149. Minneapolis: Burgess Publishing Co.

Vidaver, G. A. (1964). *Biochemistry*, **3**, 662.

Wheeler, K. P., Inui, Y., Hollenberg, P. F., Eavenson, E. & Christensen, H. N. (1965). *Biochim. biophys. Acta*, **109**, 620.

Wheeler, K. P. & Christensen, H. N. (1967). *J. biol. Chem.* **242**, 1450.