conditions was determined by amino acid analysis of the digestion mixture. It was found that 40% of the protein could be accounted for as free amino acids. All the methionine, leucine, tyrosine, and phenylalanine had been released, whereas no free asparatic acid, proline, or cysteine could be detected.

Separation of the Ninhydrin-negative Fraction.—The lyophilized sample was dissolved in 3 ml of water, the *p*H was adjusted to 4 with 1 M HCl, and undissolved material was removed by centrifugation. The digest was placed on a column of purified Dowex-50-X2 (AG 50 w-X2, Bio-Rad Laboratories, hydrogen form, 200–400 mesh, 1.7 × 15 cm) and washed with glass-distilled water. The eluate fractions (25 ml each) were concentrated to a small volume on a flash evaporator. Aliquots of the concentrated solution were spotted on paper and tested with ninhydrin spray reagent (Sigma) and were also subjected to quantitative ninhydrin test (Moore and Stein, 1954). Approximately 125–200 ml was collected before any ninhydrin-positive material appeared. The ninhydrin-negative fractions were pooled, and samples were removed for hydrolysis (20 hours at 110° with 6 N HCl in sealed evacuated tubes) and hydrazinolysis (*vide supra*).

Identification of Amino Acids and Hydrazides.—This was accomplished by paper chromatography. Control samples of acetyl hydrazide and alanine hydrazide were prepared by hydrazinolysis of *N*-acetylalanine and alanylleucine, respectively. Solvent systems and color reagents are given in the tables. Quantitative amino acid analyses were performed with the Beckman/Spinco amino acid analyzer according to the method described by Moore *et al.* (1958).

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Glycine Transport by Hemolyzed and Restored Pigeon Red Cells*

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Pigeon red cells can be lysed and restored by a modification of the method used for mammalian red cells. During lysis, glycine is equally distributed, and K^+ nearly so, between cell and lysing-fluid phases. Such preparations retain glycine and K^+ at 0–7° but lose about 32% of the K^+ in 40 minutes at 39°. The lysed and restored cells can build up glycine concentration gradients only if a Na^+ gradient exists across the cell membrane. If the normal gradient is reversed (Na^+ inside, K^+ outside) lysed and restored cells pump glycine out to an extent comparable to the accumulation occurring with a normal gradient. The Na^+ dependence is discussed in relation to Christensen's hypothesis that the energy for active transport of glycine arises from a cation gradient. This hypothesis is supported by the data, although others are not excluded.

Hypotonic hemolysis of mammalian red cells is a "reversible" process in that much of the impermeability of the cell membrane, lost during hemolysis, can be restored by restoring the ionic strength and then holding at 37° (Hoffman *et al.*, 1960; Whittam, 1962). Mammalian erythrocytes lysed and restored in this way can transport Na^+ actively (Hoffman, 1962). By use of this phenomenon the composition of the red-cell interior can be varied within wide limits.

Since mammalian red cells concentrate glycine very poorly in contrast to avian cells (Christensen *et al.*, 1952a) the technique of lysis and restoration was adapted to pigeon red cells. The procedure, some

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Table I

Composition of Lysed and Restored Cells and Distribution of Glycine and K^+ between Cell and Lysate Fluid Phases[a]

Glycine/ml Pellet H_2O / Glycine/ml Lysate Supernatant H_2O	K^+/ml Pellet H_2O / K^+/ml Lysate Supernatant H_2O	Retention of [^{14}C]Glycine by Cells at 0–7° (%)	Extracellular Space (as % of pellet H_2O)	Loss of Dry Wt. on Lysis and Restoration (%)	Gain of Pellet H_2O on Lysis and Restoration (%)
0.98[b]	1.12[b]	70.0	23.0	52.3	38.9
0.95	1.18	71.6	18.4	55.0	29.0
1.01		75.1	17.5	58.9	55.8
1.02			24.8	51.9	29.7
				56.8	25.6
Mean 0.99	1.15	72.2	20.9	55.0	35.8

[a] Cells were lysed and restored as described under Methods. Glycine-distribution figures were obtained by determination of glycine or ^{14}C in the pellets and supernatants from annealed cell suspensions. For the first experiment glycine was determined chemically; for the others, [^{14}C]glycine was added to the lysing solution and ^{14}C in pellet and supernatant was determined by counting. Thick-sample plates were used (Vidaver, 1964). The K^+ distribution figures were obtained by flame-photometric analyses of picric acid extracts of pellets and supernatants from cells lysed and restored with solutions containing only Na^+. Cells lose 55% of their dry weight on lysis; therefore 0.55×0.325 g solid is dissolved in the *ca.* 3.3-ml lysate fluid. This is 5.4% (w/v); assuming a volume of 0.75 ml/g, 1 ml lysate fluid contains $1 - (0.054 \times 0.75)$ or 0.96 ml H_2O. Glycine and K^+ distribution figures were calculated on the basis of measured H_2O contents of the pellets and a value of 96% for H_2O/ml lysate fluid. "Retention of [^{14}C]glycine" is the amount of ^{14}C in the cells (cpm/ml pellet H_2O) after annealing but before washing, divided by the ^{14}C in the cells after washing and resuspension in the incubation media but before incubation, times 100. Most of the ^{14}C loss of 28% can be accounted for as ^{14}C washed out of the extracellular space (*ca.* 21%). Extracellular space was determined by diluting duplicate cell suspensions with high and low Na^+ diluents in the cold, immediately centrifuging, and determining Na^+ in pellets and supernatants, or by the equivalent procedure with ^{14}C-containing and ^{14}C-free diluents. Penetration of glycine or Na^+ is very slow in the cold. Loss and gain of dry weight and H_2O were determined by weighing. Values for different quantities on the same line are not necessarily data from the same cell samples. [b] An average value for two different samples obtained in the same experiment.

properties of lysed and restored cells, and some features of glycine transport by them are described. A glycine concentration gradient can be built up by such cells if there is a Na^+ gradient across the cell membrane. The direction of the Na^+ gradient determines the direction of net glycine transport.

The observations support the hypothesis of Christensen (Christensen *et al.*, 1952b; Riggs *et al.*, 1958) that the energy source for glycine-active transport is the cation gradient.

Materials and Methods

Fresh cells were prepared, and glycine, Na^+, K^+, radioactivity, and extracellular space were all determined as in earlier work (Vidaver, 1964).

Cells were lysed and restored in the following way. Each aliquot of cell suspension containing 1–3 g of cells was centrifuged in the cold at 11,000 rpm for 15 minutes in a 15-ml cellulose nitrate Servall tube in a Servall SS1 head (Ivan Sorvall Inc., Norwalk, Conn.). The supernatant was removed and the pellet was distributed over the bottom of the tube with a stirring rod. For each gram of cells, 3 ml of lysing solution was quickly added and the tube contents was vigorously beaten with a stirring rod for 1 minute. For each gram of cells, 0.34 ml of restoring solution was then quickly added and the tube contents was vigorously mixed for 1 minute. The solutions and tube contents were kept at 5–6° during these operations. The tube was then immediately placed in a bath at 37–39° for 17 minutes ("annealed"). (This time is arbitrary; in a single trial, 8 and 34 minutes were equally effective.) The annealing step completes the restoration process. After the cells were chilled, centrifuged, and washed, they were ready for use. When several aliquots had been lysed and restored, they were held at 0° after the annealing step until all were ready for the remaining operations. The lysing solution contained 5 mM Na_2HPO_4 and/or K_2HPO_4, 5 mM NaH_2PO_4 and/or KH_2PO_4, 2 mM $MgSO_4$, 1 mM $CaCl_2$, and 1 mg/ml glucose. The restoring solution contained 1.38 M NaCl and/or KCl, 0.020 M $MgSO_4$, and 0.010 M $CaCl_2$.

When glycine-concentration ratios were determined chemically, incubations were done in shaken (*ca.* 80/minute) flasks for 40 or 100 minutes. For glycine entry and exit measurements with [^{14}C]glycine, incubation was for 10 minutes in centrifuge tubes. Incubation media (modified Krebs Ringer Phosphate, *p*H 7.2) and subsequent handling were as described for intact cells (Vidaver, 1964). Solutions and cells were not gassed. No color change (deoxygenation of hemoglobin) was observed in incubated cells.

Results

Pellets of intact cells are the color of black cherries. Those of lysed and restored cells are a nearly uniform red-cherry color. If intact cells are mixed with lysed and restored ones and the mixture is centrifuged, the intact cells are deposited as a dark-red button at the bottom of the bright-red pellet. Three per cent of intact cells are easily seen. Incomplete lysis can therefore be determined visually.

As shown in Table I, glycine in the lysing solution distributes itself equally between intra- and extracellular H_2O; cell K^+ is nearly equally distributed. The cells lose about 55% of their dry weight and gain somewhat more than an equal weight of H_2O. The extracellular space is somewhat greater and more variable than that (10%) of intact cells.

The observed [^{14}C]glycine retention (Table I) by lysed and restored cells and the agreement between the calculated and observed values for K_i^+ (Table II) show that the bulk of the cells become relatively impermeable to glycine and K^+ during restoration.[1]

Lysed and restored cells have a limited capacity to retain K^+ at 39°. During 40 minutes of incubation ($39 \pm 0.5°$), $K_i^+ - K_o^+$ decreased by 32% (Table II). The per cent loss was independent of K_i^+, K_o^+, and $(K_i^+ - K_o^+)$.

[1] The subscripts "*o*" or "*i*" after a symbol for or name of a substance mean that the substance represented is present in the medium or the cell, respectively. K_m is the glycine concentration giving half-maximal entry rate, as estimated from a Lineweaver-Burk plot.

TABLE II

POTASSIUM RETENTION AT 0–7° AND LOSS AT 39°[a]

Experiment Number	K_i^+, Calcd, before Incubation (μmoles/ml pellet H_2O)	K_i^+, Determined, before Incubation (μmoles/ml pellet H_2O)	K_i^+, Determined, after 40-min Incubation at 39° (μmoles/ml pellet H_2O)	K_o^+ (mM)	Loss of K_i^+ in 40 min at 39° (%)
1	76.6	68.7	51.0	6	28
	76.6	69.8	50.2	6	31
2	22	20	15	6	36
3	30.5	29.2	22.2	6	30
	56.2	51.8	36.5	11.7	38
	105.9	90.8	72.3	23	27
					Avg. 32

[a] The "determined" K_i^+ values were obtained by analysis of pellet extracts. The "K_o^+" values are K^+ added to media. "Per cent loss of K^+ at 39°" is 100 × (K_i^+, determined, of pellets before incubation − K_i^+, determined, of pellets after 40-min incubation at 39°) ÷ (K_i^+, determined, of pellets before incubation − K_o^+). The K_i^+ decrease is divided by $K_i^+ - K_o^+$ rather than K_i^+ because the net loss of K_i^+ should depend on the difference. Cell K^+ was also calculated from the equation, $K_i^+ = 147 \times$ fraction $K^+ + 5.4$. "Fraction K^+" is the sum of K^+ in cell H_2O (*ca.* 130 mM; Vidaver, 1964), lysing solution, and restoring solution, divided by the total Na^+ plus K^+ in these solutions. The figure 5.4 is the excess K^+ in cells lysed and restored with solutions containing only Na^+, over the theoretical amount for free distribution. The figure 147 is the total $Na_i^+ + K_i^+$ (152 μmoles/ml cell H_2O) in lysed and restored cells minus excess K^+. The numerical values used here (5.4 and 152) were obtained from other experiments. The determined values average 91% of the calculated values.

Some aspects of glycine transport are shown in Tables III and IV and Figure 1. The cases where Na_i^+ was not equal to Na_o^+ are illustrated by Table III and Figure 1. With Na_i^+ low and Na_o^+ high (the Na^+ gradient in the "normal" direction), glycine was accumulated (Table III). With Na_i^+ low and Na_o^+ low, but with an appreciable gradient in the normal direction, glycine was still accumulated (Fig. 1). With Na_i^+ high and Na_o^+ high, but still with a normal Na^+ gradient, glycine was also accumulated (experiment 5, Table III; Fig. 1). Note that this case shows that high K_i^+ is not necessary for glycine accumulation. With Na_i^+ high and Na_o^+ low, i.e., the reverse of the normal gradient, glycine is not accumulated, but is instead pumped out about as efficiently as it would be pumped in with a similar Na^+ gradient in the normal direction (experiments 3 and 4, Table III).

The cases where Na_i^+ equals Na_o^+ are illustrated by Table IV. Table IV shows that if the glycine concentrations are equal inside and out, and the Na^+ concentrations are equal inside and out, then glycine exit and entry rates are equal. This is independent of the numerical values of the Na^+ and glycine concentrations. That is, no pumping occurs in either direction in the absence of an Na^+ gradient.

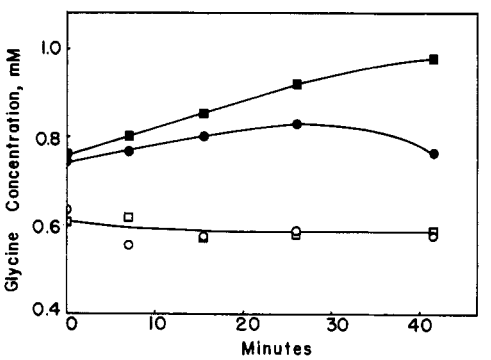

FIG. 1.—Glycine uptake by lysed and restored cells is plotted against time. One portion of the cells was lysed and restored with solutions containing only Na^+, the other with solutions containing only K^+. The calculated values for Na_i^+ prior to incubation were 126 and 3 μmoles/ml cell H_2O, respectively. Calculations were done as for the data of Table II. Aliquots of the Na^+-rich sample were incubated at 39° for varying periods of time in a slightly modified Krebs Ringer phosphate glucose medium (Vidaver, 1964) with Na_o^+ = 140 mM (the initial $Na_o^+ - Na_i^+$ was therefore 14 mM) and the aliquots of the Na^+-poor sample were incubated in a medium with Na_o^+ = 40 mM (initial $Na_o^+ - Na_i^+$ = 37 mM). The glycine values used were obtained by (duplicate) chemical analysis of media and pellets. Open symbols refer to glycine in the medium, solid ones to glycine in the cells. ○, ● $Na_o^+ - Na_i^+$ = 14 mM; □, ■ $Na_o^+ - Na_i^+$ = 37 mM.

DISCUSSION

The free distribution of solutes during lysis and the capacity of lysed and restored pigeon cells to pump glycine are analogous to the free solute distribution during lysis (Hoffman, 1958) and the capacity to pump Na^+ (Hoffman, 1962) previously reported for mammalian red cells. The avian and mammalian systems probably differ in their Ca^{2+} and/or Mg^{2+} requirement for restoration. In experiments prior to the introduction of the annealing step, it was observed that hemoglobin retention was very poor in the absence of Ca^{2+} and Mg^{2+} in the lysing and restoring solutions. Even in their presence it was poorer than with the annealing step included. Since the capacity for glycine-active transport in pigeon cells is absolutely dependent on the annealing step, the pigeon-cell system seems analogous to Hoffman's (1962) group II cells rather than group I cells.

It is assumed that the glycine-pump mechanism acting in lysed and restored cells is, or is part of, that acting in the intact-cell system, and that observations made on the lysed and restored system are at least qualitatively applicable to the intact-cell system. It is also assumed that Na^+, rather than K^+, is the active ion for lysed and restored cells as it is for intact ones (Kromphardt *et al.*, 1963; Vidaver, 1964).

Figure 1 and Tables III and IV show that neither internal Na^+ (or K^+) alone nor external Na^+ (or K^+) alone controls glycine pumping, but that the Na^+ difference between inside and outside is the critical factor. Figure 1 shows that glycine accumulation can occur from a fairly low Na^+ medium if Na_i^+ is low enough to give a Na^+ gradient. Experiment 5 of Table III shows that considerable accumulation occurs even with fairly high Na_i^+ (and low K_i^+), again, provided a Na^+ gradient exists. Table IV shows that no

TABLE III

GLYCINE ACCUMULATION AND EXPULSION BY LYSED AND RESTORED CELLS[a]

Experiment Number	Sample	Cell Type	Initial Cell Na^+, Value Calculated (mM)	Na^+ in Medium (mM)	Incubation Time (min at 39°)	Initial Glycine in Medium (mM)	Initial Ratio, $Glycine_i/Glycine_o$	Final Ratio, $Glycine_i/Glycine_o$
1	a	Lysed and restored	24	140	40	0.46	1.45	2.76
	b	Lysed and restored	24	140	100	0.46	1.45	3.02
2	c	Lysed and restored	24	140	103	0.63	1.17	2.31
	d	Intact	17.5	140	103	0.63	6.06	8.22
3	e	Lysed and restored	24	140	40	0.60	1.59	2.39
	f	Lysed and restored	24	0	40	0.60	1.57	1/1.08
	g	Lysed and restored	115	0	40	0.60	1.43	1/2.06
	h	Lysed and restored	126	0	40	0.60	1.24	1/2.34
4	i	Lysed and restored	24	140	40	0.57	1.12	1.95
	j	Lysed and restored	126	0	40	0.57	1.07	1/1.94
5	k	Lysed and restored	115	140	40	0.62	1.63	1.73
	l	Lysed and restored	84	134	40	0.62	1.67	1.91
	m	Lysed and restored	24	125	40	0.62	1.68	2.57

[a] Cells were lysed and restored as described under Methods. For samples a–c, e, f, i, and m, the lysing solution contained only K^+ and the restoring solution K^+/Na^+ ratio was 4.31. For samples h and j, both solutions contained only Na^+. For samples g, k, and l, intermediate proportions of Na^+ were used. The initial cell Na^+ values for lysed and restored cells, in μmoles Na^+/ml cell H_2O before incubation, are calculated from the equation $Na_i^+ = 152 - K_i^+$. The value for K_i^+ was calculated as for Table II. The total Na^+ plus K^+ in lysed and restored cells is 152 μmoles/ml cell H_2O. The numerical value used here (152) was obtained from other experiments. Comparison of calculated and analytical values in other experiments gave reasonable agreement, e.g., the observed value for Na_i^+, third line, Table IV, is 37 mM. The calculated value is 35.5 mM. The K_i^+ data in experiment 3, Table II, are from experiment 4 in this table. Since lysed and restored cells are leaky at 39° with respect to K^+, the calculated values are taken to be, here, as adequate as analytical ones would be. The Na_i^+ value listed for the intact-cell sample (sample d) is simply the average Na_i^+ determined in other experiments (Vidaver, 1964). The glycine values used for the $glycine_i/glycine_o$ ratios were obtained by (duplicate) chemical analysis of media and pellets. (Second-decimal-place digits are given because, for some values, rounding off overstates the error.)

TABLE IV

EQUALITY OF GLYCINE EXIT AND ENTRY FROM EQUAL GLYCINE CONCENTRATIONS WHEN NO Na^+ GRADIENT EXISTS[a]

Glycine Inside (μmoles/ml cell H_2O)	Na^+ Inside (μmoles/ml cell H_2O)	Glycine Exit (μmoles/ml cell H_2O in 10 min, 39°)	Glycine Entry (μmoles/ml cell H_2O in 10 min, 39°)	Na^+ Outside (μmoles/ml medium)	Glycine Outside (μmoles/ml medium)
0.82	125	0.24	0.27	125	0.90
2.75	125	0.55	0.61	125	3.00
0.82	37	0.16	0.15	35	0.90
0.82	3.4	0.060	0.066	3.3	0.90

[a] Cells were lysed and restored as described under Methods. The desired cell-Na^+ concentration was obtained by use of the appropriate proportion of Na^+ in the lysing and restoring solutions. Either [^{14}C]- or [^{12}C]glycine was added to the lysing solution to produce the desired glycine and ^{14}C contents. Glycine entry and exit was measured to and from pairs of samples. One member of each pair had ^{14}C inside and ^{12}C outside; the other had ^{14}C outside and ^{12}C inside; otherwise the composition and incubation media of the two members of the pair were identical. Incubation was done in centrifuge tubes (see Methods). Radioactivity was determined from thick-sample plates. Exit and entry rates approximating initial rates were calculated as previously described (Vidaver, 1964), with the modification that the entry rates given here include a "re-exit" correction. A fraction of the internal glycine leaves the cells during incubation. The re-exit correction used was 0.5 times this fraction, as estimated from ^{14}C loss from the internally labeled member of the pair, times the measured ^{14}C entry into the other member. Values for concentrations are given in μmoles/average ml cell H_2O, using the average of the H_2O/pellet values for incubated and unincubated samples (corrected for extracellular space). Entry and exit rates are given in μmoles/unincubated ml cell H_2O. The glycine values used were obtained by (duplicate) chemical analysis of media and pellets.

pumping occurs in either direction in the absence of a Na^+ gradient regardless of the Na^+ and K^+ concentrations inside and outside. The data of Table III also show that the Na^+ gradient so far controls glycine transport that, on merely inverting the normal gradient, glycine is pumped out to an extent comparable to the accumulation occurring with a normal gradient.

It may be noted that K_m for glycine entry into intact cells depends on $(Na^+)^2$ rather than Na^+ (Vidaver, 1964). At $Na_o^+ = 40$ mM, K_m for intact cells is 0.7 mM, thus the considerable glycine accumulation with $Na_o^+ = 40$ mM (and $Na_i^+ = 3$ mM) (Fig. 1) is not unexpected.

The equality of glycine entry and exit rates from equal Na^+ and glycine concentrations implies that compartmentalization of Na_i^+ is not significant in lysed and restored cells.

The limited ability of lysed and restored cells to maintain internal K^+ (and presumably to exclude Na^+) probably explains in part the poor glycine accumulation by lysed and restored cells compared with intact ones. This leakiness also limits the significance of the numerical values for glycine concentration ratios.

One aim of the present work was to test Christensen's hypothesis that the energy for amino acid accumulation comes from the alkali metal-ion gradient (Christensen *et al.*, 1952b). This hypothesis has two forms. One is that internal K^+ exchanges for external amino acid. Hempling and Hare (1961), using the Ehrlich ascites system, concluded that the energy

expenditure by K^+ efflux down the K^+ gradient was insufficient to account for the glycine influx at the glycine-concentration ratio maintained. The other form of the hypothesis is that there is a linked entry of Na^+ and amino acid, with the energy from Na^+ influx down its chemical-activity gradient furnishing the energy for the transport of glycine against its gradient (Riggs *et al.*, 1958). The dependence of glycine entry in Na_o^+ (Kromphardt *et al.*, 1963; Vidaver, 1964) and the apparent involvement of a complex containing two Na ions and one glycine in the glycine-entry process (Vidaver, 1964) made this form of the hypothesis attractive.

No pumping can occur in the absence of an energy source. If a Na^+ gradient is the energy source for the glycine pump, in its absence glycine exit and entry rates from equal glycine concentrations must be equal, regardless of what the glycine concentrations are, and regardless of what the Na^+ concentrations are. This appears to be the case (Table IV).

Some other type of energy source, such as ATP, should be unequally distributed between the inside and outside of the cell. Also, a pump mechanism adapted to operate between the different phases, cell interior and plasma, might be expected to have a polarity. In either case unequal entry and exit rates might be expected under the conditions of the experiment shown in Table IV. However, it is possible to devise a pump model using, e.g., internal ATP as an energy source which would operate equally effectively in the two directions, thus these data do not prove the Na^+ operated pump hypothesis.[2] Since the relationships found between glycine pumping and a Na^+ gradient, and the occurrence of a complex containing both glycine and Na^+ are required by any pump model with a Na^+ gradient as energy source, but only correspond to a special case of the (e.g.) ATP-powered-pump hypothesis, they are taken to support the former.

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[2] Such a model is represented by the sequence: $E_i + ATP \xrightarrow[\text{fast}]{} E_i^*$; $E_i^* \underset{\text{fast}}{\rightleftharpoons} E_o^*$; E_o^* (or E_i^*) + G_o (or G_i) + 2 Na_o^+ (or 2 Na_i^+) $\underset{\text{fast}}{\rightleftharpoons} E^*Na_2G_o$ (or $E^*Na_2G_i$); $E^*Na_2G_o$ (or $E^*Na_2G_i$) $\xrightarrow[\text{slow}]{} E^{**}G_o$ (or $E^{**}G_i$) + 2 Na_o^+ (or 2 Na_i^+); $E^{**}G_o$ (or $E^{**}G_i$) $\rightleftharpoons E^{**}G_i$ (or $E^{**}G_o$) (this is the translocation step); $E^{**}G_i$ (or $E^{**}G_o$) $\xrightarrow[\text{slow}]{} E_i$ (or E_o) + G_i (or G_o); $E_i \underset{\text{fast}}{\rightleftharpoons} E_o$. "E" is taken to be a mobile carrier in this model. The superscript asterisks represent "states" of E. "G" is glycine. All reactions except E_i + ATP → E_i^* might be catalyzed by the carrier ("E") itself and so be independent of location.

Mucate Inhibition of Glycine Entry into Pigeon Red Cells*

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A Donnan effect was produced in the pigeon-erythrocyte system when Cl^- was replaced by mucate ($COO^-(CHOH)_4COO^-$) in the incubation medium. The replacement of Cl^- by mucate caused a nearly complete inhibition of the Na^+-dependent component of glycine entry. The effect does not seem to be due to a specific "poisoning" by the mucate anion, but rather to the lack of external Cl^- and possibly also to some other concomitant (e.g., the electrical potential) of the Donnan effect. The inhibition is chiefly due to an increase in the glycine concentration giving half-maximal entry rate (K_m) of the entry process, although a moderate decrease in the maximum entry rate was also found. This effect of mucate is discussed in relation to Christensen's hypothesis that the Na^+ gradient may furnish the energy for amino acid–active transport.

Total glycine entry into pigeon red cells can be considered as consisting of two components, entry by a sodium-dependent route which obeys Michaelis-Menten kinetics with respect to both glycine and $(Na^+)^2$, and a small diffusionlike route. The Na^+ dependence implies the existence of a complex containing both Na^+ and glycine at some stage in the glycine-entry process (Vidaver, 1964a).

Experiments with hemolyzed and restored cells (Vidaver, 1964b) had supported Christensen's hypothesis that the difference in Na^+ concentration between the cell interior and the medium furnishes the energy for amino acid–active transport (Christensen *et al.*, 1952; Riggs *et al.*, 1958). Further tests, however, were necessary.

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