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SATURATION BEHAVIOR OF ASCITES TUMOR CELL CHLORIDE EXCHANGE IN THE PRESENCE OF GLUCONATE

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Summary

Steady state Cl^- flux across the Ehrlich mouse ascites cell membrane was studied when gluconate replaced Cl^- in the external medium. Saturation behavior was observed; $K_{1/2}$ was 23.9 mM Cl^- and V was 758 $\mu mol \cdot g^{-1}$ dry weight $\cdot h^{-1}$. The cells lost K^+, Cl^- and H_2O, consistent with relative impermeability to gluconate, and the Cl^- efflux rate coefficient was elevated. The results indicate that a major portion of Cl^- exchange occurs as a membrane transport process and suggest that the process is sensitive to intracellular Cl^- levels.

A number of studies have provided evidence for a transport mechanism which exchanges Cl^- across the ascites cell membrane [1,2,3]. Investigation of the kinetic characteristics of the transfer process has been hampered, however, by the difficulty of finding an appropriate substitute for environment Cl^-. Thus, anions such as NO_3^- and Br^- can compete with Cl^- for the transfer process [2,3] while acetate enters the cell rapidly, causing swelling [2,3]. As a result of the work reported here it is suggested that gluconate is a useful anionic replacement for Cl^-. With gluconate it was possible to demonstrate directly a maximum rate of Cl^- transfer.

Steady state Cl^- flux as a function of environment Cl^- concentration was determined when sodium gluconate replaced NaCl in isosmotic amounts; the results are shown in Fig. 1. The curve was drawn from a Michaelis-Menten equation whose constants were determined by statistical analysis of a double reciprocal plot [4]; V = 758 $\mu mol \cdot g^{-1}$ dry weight $\cdot h^{-1}$ (S.E. = 0.22) and $K_{1/2}$ = 23.9 mM external Cl^- (S.E. = 0.02). From these data it is reasonable to

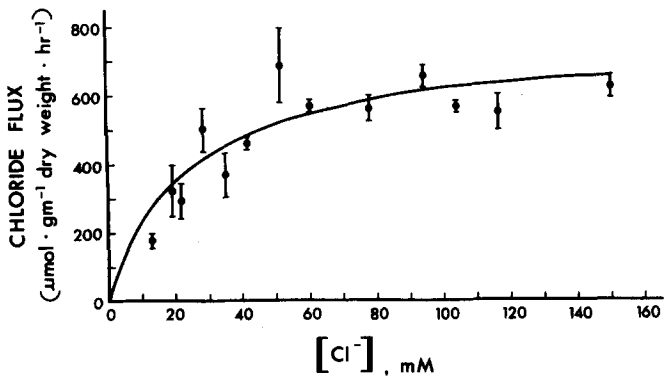

Fig. 1. Steady state Cl^- exchange flux as a function of extracellular Cl^- concentration. Sodium gluconate replaced NaCl in isosmotic amounts. Cells were grown in mice, harvested, and washed once at pH 7 as described [3]. Aliquots were washed 3 times in the different gluconate-Cl^- Ringer solutions at pH 7, temp. 21—23° C, and incubated for 2 hr, so that cell Cl^- was in a steady state. Cell concentration was $9 \cdot 10^7$—10^8 cells/ml. A sampling procedure for rapid uptake of ^{36}Cl [5,6] was used in which 1 ml of cell suspension was pipeted into 3 ml of Ringer containing ^{36}Cl. The Cl^- efflux coefficient [5] and cell Cl^- content were measured [3], from which the Cl^- flux was calculated [3,5]. Results from 4 different experiments were pooled; data points at each external Cl^- concentration are the mean ± S.E. of at least 2 separate experiments. The point at 150 mM Cl^- is the mean for 4 different control groups without gluconate.

conclude that Cl^- self exchange saturates as external Cl^- is raised, behavior which would be expected for transport that involved binding to a membrane site or carrier.

In contrast to these findings, an earlier study using acetate as the replacing anion showed that 60% of the total Cl^- exchange varied linearly with outside Cl^- at the higher Cl^- concentrations [2]; it was inferred that the linear component represented simple diffusion and that the remaining flux saturated. It is interesting that $K_{1/2}$ in the presence of acetate was 21.9 mM which is similar to the value in gluconate, and that the Cl^- fluxes in control Cl^- media are within 15% of each other in the two studies. Acetate, which entered the cells, resulting in water uptake [2,3], may therefore have altered the distribution of the total flux between transport and 'leak' pathways. Gluconate, on the other hand, appears to be relatively non-penetrating, as shown by the data in Table I. Cells equilibrated in sodium gluconate solutions lost K^+ and Cl^- in approximately equivalent amounts and H_2O was lost as well. This response is consistent with relative impermeability to gluconate.

An additional point of interest concerns the fractional exchange rate or turnover of cell Cl^-, determined as the efflux rate coefficient, when gluconate is substituted for Cl^- in the medium. As shown in Table II, steady state Cl^- turnover accelerates in low Cl^- solutions, an effect which was also observed when cell Cl^- was lowered in the presence of other relatively non-penetrating solutes such as SO_4^{2-} and sucrose [5]. In Levinson's recent study of anion transfer in the ascites cell there is evidence for the same effect although it was not remarked upon [6]. Indeed, this phenomenon explains why inhibition of Cl^- exchange by SO_4^{2-} could be demonstrated only when external and internal Cl^- were kept constant [5,6].

The mechanism responsible for increased Cl^- turnover in low Cl^- solutions is not clear. Cell shrinkage could only account for it to a limited extent

TABLE I

CELL COMPOSITION AFTER EQUILIBRATION IN LOW Cl^-, GLUCONATE SOLUTIONS FOR ONE REPRESENTATIVE EXPERIMENT

Control environment without gluconate is at 151 mM Cl^-. Cell Cl^-, K^+, Na^+ and H_2O were analyzed as described [3].

Environment Cl^- (mM)	Cell			
	Cl^- (μmol/g*)	K^+ (μmol/g*)	Na^+ (μmol/g*)	H_2O (ml/g*)
151	180	408	208	3.99
117	169	398	193	3.90
104	156	375	206	3.98
78	115	310	223	3.81
61	99	313	220	3.68
41	69	288	216	3.39
34	64	274	230	3.32

*Dry weight.

TABLE II

EFFECT OF LOW Cl^- ON Cl^- TURNOVER IN ONE REPRESENTATIVE EXPERIMENT

Experimental details are described under Fig. 1.

Environment Cl^- (mM)	Cl^- efflux coefficient (h^{-1})
117	3.44
103	3.52
79	3.97
60	6.17
41	6.87
34	9.01
21	8.29

since comparable shrinkage resulting from equilibration in hypertonic NaCl resulted in much smaller increases of Cl^- turnover (unpublished). Further, it is not a non-specific effect because SO_4^{2-} turnover does not increase in cells incubated in low Cl^-/SO_4^{2-}/sucrose solutions (unpublished). Also, permeability to Na^+ does not appear to change since there are only minimal alterations in cell Na^+ content (Table I). It is tempting to speculate that the cell can sense a falling intracellular Cl^- content or concentration and that it then attempts to maintain Cl^- at a constant level by accelerating the rate at which its transport mechanism turns over.

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References

1 Heinz, E., Geck, P. and Pietrzyk, C. (1975) Ann. N.Y. Acad. Sci. 264, 428—441
2 Levinson, C. and Villereal, M.L. (1976) J. Cell Physiol. 88, 181—192
3 Aull, F., Nachbar, M.S. and Oppenheim, J.D. (1977) Biochim. Biophys. Acta 471, 341—347
4 Wilkinson, G.N. (1961) Biochem. J. 80, 324—332
5 Aull, F. (1972) J. Physiol. Lond. 221, 755—771
6 Levinson, C. (1978) J. Cell Physiol. 95, 23—32