SODIUM TRANSPORT ACROSS THE SURFACE MEMBRANE OF RED BLOOD CELLS IN HEREDITARY SPHEROCYTOSIS [1]

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The erythrocyte of hereditary spherocytosis (HS), compared with the normal human erythrocyte, is characterized by an increased thickness: diameter ratio, a greater tendency to hemolyze in hypotonic solutions of sodium chloride (osmotic fragility), and a higher rate of hemolysis when incubated at body temperature (spontaneous autohemolysis) (1). Evidence of abnormalities in the glycolytic cycle of the HS cell has recently been presented (2) and corroborated (3). The *in vitro* movement of sodium ion (Na) across the surface membrane of washed erythrocytes from one patient with HS has been quantitated (4): the rate of extrusion was abnormally high both before and after splenectomy. A normally functioning glycolytic cycle has become an experimentally well established requisite for normal cation transport [3] across the surface membrane of erythrocytes (6). Cation transport is required to maintain the low Na and high potassium (K) concentrations in human red cells, as they contrast to plasma Na and K concentrations. Consequently this study was planned to determine:

1) whether HS red cells demonstrate *in vitro* Na transport rates that vary from normal to the same degree for each patient; and
2) how the variation from normal relates to other characteristics of HS cells.

Studies on a total of 14 individuals are presented: Cases 1 through 10 are splenectomized patients, with complete hematologic recovery by routine testing (hemoglobin, hematocrit, and reticulocyte count), whose erythrocytes continue to demonstrate the characteristics of HS. Cases A and B are hematologically normal controls; duplicate Na transport studies were done on their red cells to determine reproducibility. Cases C and D are hematologically normal controls splenectomized in the past for traumatic splenic rupture and idiopathic thrombocytopenic purpura, respectively.

Na transport measurements were done on red cells from all cases presented. Determinations of osmotic fragility and rate of autohemolysis were done on all cases except C and D, and were performed on the same day as the transport study unless otherwise noted in Table I. Correlations were sought among the values for rate of Na transport, osmotic fragility, and rate of autohemolysis.

METHODS

Design of system for in vitro measurement of rate of Na transport. Related experimental schemes have been described by Sheppard and Martin (7), Raker, Taylor, Weller, and Hastings (8), Harris and Maizels (9), Solomon (10), and Love and Burch (11). The method used in this study was a composite, striving for minimal cell manipulation prior to addition of isotope. In each case, 40 ml. of venous blood were drawn through a No. 18 needle into a 50-ml. syringe containing 8 mg. heparin sodium in 0.8 ml. of isotonic sodium chloride solution. The subjects were under no dietary restrictions. Blood was transferred immediately to a siliconed 250-ml. Erlenmeyer flask containing 100 mg. glucose (more than sufficient to maintain an adequate concentration of glucose in the blood in the presence of leukocytes throughout the experiment [12]). The flask was immediately fixed to a shaker platform enclosed in an air incubator with temperature controlled to 37.5 ± 0.5°C. Agitation of the system was horizontal

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[3] "Transport" has been defined as movement against a gradient, implying "the use of an energy supply other than, or additional to, thermal agitation" (5). Since it remains to be shown precisely how energy from glycolysis relates to cation carrier mechanisms, in this paper the more general application of the term "transport" is used: movement against gradients of concentration and electrochemical potential. "Transfer" indicates movement in any direction.

TABLE I

Summary of hematological and biochemical data

Case (Age in years)	Date of splenectomy	Date of experiment	$k_{ab}\frac{1-H}{H}$	Average $[Na]_{plasma}$ *mEq./L.*	Na Transport as mEq. / L. cells × hrs.	Na Transport as mEq. × 10^{15} / μ^3 × hrs.	Initial total $[Na]_{cell}$ *mEq./L.*	Final total $[Na]_{cell}$ *mEq./L.*	Rapidly exchangeable $[Na]_{cell}$ *mEq./L.*	Rapidly exchangeable $[Na]_{cell}$ / Average total $[Na]_{cell}$	Thickness / Diameter	50% O.F. % *NaCl*	A.H. at 48 hrs. % *hemolysis*
1 (58)	1953	2/27/56	.044	138.6	6.1	4.1	6.6	6.4	7.1	1+	.32	.67	37.4
2 (33)	1955	5/28/56	.040	142.5	5.7	4.0	5.7	5.2	5.3	.97	.34	.60	14.2
3 (36)	1945	2/9/56	.039	133.3	5.2	3.4	8.8	8.8	7.0	.80	.34	.64 5/23/52	22.4
4 (43)	1950	3/16/56	.038	130.0	4.9	3.2	9.6	8.8	7.7	.84	.33	.63 8/4/54	19.0 2/21/56
5 (7)	1954	5/31/56	.032	140.8	4.5	3.1	6.1	6.9	6.8	1+	.33	.66	23.7
6 (19)	1944	6/9/56	.032	142.7	4.6	3.2	8.3	7.8	6.9	.86	.33	.68 8/21/52	21.3
7 (25)	1941	5/22/56	.029	139.8	4.0	2.6	11.1	10.4	8.2	.76	.30	.61 8/26/54	21.8
8 (58)	1946	5/29/56	.029	140.2	4.1	2.8	7.0	6.9	5.8	.83	.33	.58 4/15/47	6.7
9 (16)	1952	2/14/56	.028	144.5	4.0	2.7	8.3	8.0	6.8	.83	.33	.66 2/6/54	14.3
10 (17)	1940	4/27/56	.026	135.7	3.5	2.4	9.5	9.0	6.9	.74	.33	.66 2/23/54	20.1 4/12/55
A (30)		2/8/56	.027	140.5	3.8	2.3	8.0	8.0	6.4	.80	.28	.53 6/13/56	3.5 6/13/56
A′		4/5/56	.027	141.1	3.8	2.6	7.0	7.0	5.8	.83	.30		3.2 6/14/56
B (30)		3/21/56	.025	137.8	3.4	2.3	8.0	7.4	6.4	.83	.31	.56 6/19/56	4.4 6/20/56
B′		4/20/56	.023	138.5	3.2	2.1	7.4	7.2	5.8	.79	.28		3.7 6/20/56
C (20)	1954	4/17/56	.020	137.5	2.8	1.8	10.9	9.9	7.9	.76	.25		
D (51)	1955	3/29/56	.021	135.3	2.8	1.8	10.1	9.2	7.1	.74	.23		

at 150 strokes per minute over a 2-cm. traverse. A constant atmosphere of 5 per cent CO_2 and 95 per cent O_2 was maintained in the flask. When constant pH[4] was reached, approximately 0.3 microcurie of Na^{22} [5] contained in 0.4 ml. of isotonic sodium chloride solution was added to the blood, the flask was returned to the shaker, and the experiment timed from the moment of addition of isotope.

Sampling. About 4.5 ml. of whole blood for determinations of cell and plasma radioactivity and total Na ($Na^{22}+Na^{23}$) concentrations were pipetted from the flask at 12 minutes and at hourly intervals thereafter until the concentration of radioactivity in the cell fraction reached a plateau (Figure 1). Duration of experiments ranged from 6 to 7 hours, with plateau generally not being reached until after 5 hours. Duplicate microhematocrit (13) determinations were done on each sample, and then duplicate aliquots of whole blood were immediately pipetted into 10 by 60 mm. glass tubes with a pipette recalibrated to deliver 2.00 ml. of blood. The aliquots were centrifuged at 1,710 R. C. F. for 30 min., following which the supernatant plasmas were pipetted off and combined for subsequent measurements of total Na and of radioactivity. The surface of each packed cell mass, plus the tube interior above the surface, were carefully washed free of plasma with four changes of isosmotic (approximately 5.2 per cent) glucose solution, using a Pasteur pipette and exercising great care not to disturb the red cell mass. The subsequent determination of radioactivity remaining in the packed cell mass was not significantly altered when three to six such changes of wash solution were employed. Four changes were arbitrarily chosen.

Radioactivity determinations. Duplicate 0.5-ml. aliquots of plasma and the duplicate aliquots of packed cells were counted at constant volume in a well-type scintillation counter to within 1 per cent probable error. In more than 100 sets of duplicate packed cell counts, all but 3 duplicates agreed within 2 per cent, and those 3 agreed within 5 per cent.

Corrections for trapped plasma. Using I^{131} human serum albumin as a plasma tracer, we confirmed the finding (14) that the volume of plasma trapped among HS cells spun at 10,000 RPM in a capillary tube (13) is about 2.5 times that trapped with normal cells. The true value of hematocrit,[6] within the error of the method and within the hematocrit range of this study (from 36 to 46 per cent), may be obtained by subtracting 1 from the capillary hematocrit reading for normal cells, and subtracting 2.5 from the reading for HS cells. In similar fashion the corrections for plasma trapped in the packed cell mass in 10 by 60 mm. tubes under the conditions of the experiment were obtained: 0.032 ml. plasma per ml. normal cells, and 0.038 ml. plasma per ml. HS cells. These corrections were applied when calculating counts per minute (CPM) per ml. cells, and when determining total Na concentrations of cells and mean corpuscular volume (MCV).

Total Na ($Na^{22}+Na^{23}$) determinations. A flame photometer (lithium internal standard) of the type described by Berry, Chappell, and Barnes (15) was used. Aliquots of plasma were diluted 1:500, and the packed cell masses after counting were diluted to 100 ml. The feasibility of employing red cell hemolysate for flame photometry is recognized (16–18). Small downward corrections of cell Na values in the presence of K were required. The agreement between determinations of cell Na concentration done on the duplicate aliquots was, to 2 standard deviations (2 S.D.), within 0.3 mEq. per L. cells. Variability of plasma Na concentration over the course of each experiment was never over 2.5 per cent, and was generally much less.

Hemolysis during Na transport experiments. Determined as oxyhemoglobin, this was slightly over 0.5 per cent of the cell mass in four instances. The per cent hemolysis bore no constant relation to cell Na transport rate.

Na transport rate calculations for red cells. Solomon's notation (10) is employed. Let the movement of isotope be described:

$$\frac{dq}{dt} = k_{ab}\, p \frac{v_p}{v_q} - k_{ba}\, q \frac{v_p}{v_q} \qquad (1)$$

where $q = [Na^{22}]_{cells}$ and $p = [Na^{22}]_{plasma}$

t = time

v_q = cell mass volume = H = hematocrit

v_p = plasma volume = 1 − H.

Letting $q_\infty = [Na^{22}]_{cells}$ at plateau, the plot of $\ln\left(1 - \frac{q}{q_\infty}\right)$ against time (Figure 2), thus minimizing errors involved in single determinations of q, should yield a straight line if the assumptions leading to construction of Equation 1 are correct. If slope equals T, then:

$$k_{ab}\frac{1-H}{H} = \frac{-Tq_\infty}{p_0} \qquad (2)$$

where $p_0 = [Na^{22}]_{plasma}$ at time zero.

$k_{ab}\frac{1-H}{H}$ represents the fraction of the total Na concentration of plasma ($Na^{22}+Na^{23}$) which transfers into one liter of cells in 1 hour. If a steady state obtains, this is also the quantity of Na transported out of 1 liter of cells per hour.

p drops less than 4 per cent during the course of an experiment, and since p_0 is a critical value in computing k_{ab}, we have employed the average value of p during each experiment to represent p_0. We have also used average p instead of p_∞ in the calculation of "rapidly exchangeable"

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[4] Beckman model G glass electrode pH meter, open cup. Readings by this method are slightly higher than those obtained in a closed system. True pH of 7.6 was not exceeded (9). pH dropped about 0.2 unit during each experiment.

[5] Identity and purity of the radioactive isotope were confirmed through the courtesy of H. Mermagen and D. H. Maillie, Atomic Energy Project, University of Rochester.

[6] The term "hematocrit" is used by some authors to indicate the calibrated tube in which blood is centrifuged in order to determine the fractional volume of blood occupied by red cells. "Hematocrit" in general clinical usage means this fraction expressed as per cent, and this meaning is intended in this paper.

cell Na (10):

$$[Na]_{exch.} = \frac{q_\infty}{p_\infty/[Na]_{plasma}} \quad (3)$$

where $p_\infty = [Na^{22}]_{plasma}$ at plateau.

It must be pointed out that cell Na concentration dropped slightly over the course of most of the experiments (see Table I). This is a recognized phenomenon (9–11) and detracts from the accuracy of the transport calculations, but does not invalidate them.

Expression of transport rate in terms of surface area of cells rather than volume of cells. Movement of Na across the red cell membrane in terms of cell surface area may permit a more valid comparison of one cell type with another when area:volume ratios differ (9). Convenient rather than highly accurate devices were employed in this conversion. We used Emmons' formula (19) for cell surface area, which assumes the cell is a truncated cylinder:

$$\text{Area} = 2\pi r(r + h) \quad (4)$$

where r = cell transverse diameter/2

h = cell thickness = $MCV/\pi r^2$.

It is assumed that the error introduced by using this formula to calculate surface area is no greater when applied to slightly spheroidal (*i.e.*, biconvex) HS red cells than when applied to biconcave normal red cells.

The following expression then may be derived:

Na transport as mEq./μ^2 of surface area × hrs.

$$= \frac{\left(k_{ab}\frac{1-H}{H}\right)([Na]_{plasma})(MCV)}{(10^{15})(Area)}. \quad (5)$$

Erythrocyte diameter was measured by the Haden-Hausser erythrocytometer[7] with a reproducibility of ±0.1μ. The hematocrit figure employed to obtain MCV was the average true hematocrit of the experiment, and remained constant to within ±3 per cent (2 S.D.). Red cell counts as performed yielded the greatest source of possible error, being accurate only to within ±9 per cent (2 S.D.) as computed by the formula of Berkson, Magath, and Hurn (20). Obviously these errors could yield considerable variation in the rates calculated using Equation 5, but in practice the reproducibility was good (see Table I).

Determination of osmotic fragility (O.F.) and rate of autohemolysis (A.H.). The procedures used are described by Young and his associates (21, 22). The "50% O.F." figure in Table I is the concentration of sodium chloride solution in which 50 per cent of a cell population lyses after the cells have incubated for 24 hrs. at 37°C. in autogenous serum. The figure for "A.H. at 48 hrs." is per cent hemolysis occurring in a cell population during 48 hrs. incubation at 37°C. in autogenous serum.

RESULTS

The pertinent data are summarized in Table I. Na transport rates for normal erythrocytes (2.8 to 3.8 mEq. per L. cells × hrs.) (cases A, A', B, B', C, and D) fit exactly with previously reported ranges (10, 11). Transport rates for the HS erythrocytes (cases 1 through 10) vary from high normal to considerably elevated figures. No advantage is gained in expressing Na transport in terms of surface area rather than volume: Both methods demonstrate equally well the difference between HS and normal red cells. Case histories, response to splenectomy, and the values reported here for cell thickness: diameter ratio, autohemolysis, and osmotic fragility leave no doubt that cases 1 through 10 correspond to the syndrome of hereditary spherocytosis; although patients 7, 9, and 10 have no similarly affected relatives, including both parents of each, among those tested.

Inspection of the data reveals no significant correlation between Na transport rates and total Na concentrations in cells, nor between Na transport rates and ratios of "rapidly exchangeable" Na: total Na concentrations in cells. The following scattergrams were constructed using the data from cases 1 through 10, but are not shown here:

- Na transport rate versus plasma Na concentration;
- Na transport rate versus osmotic fragility;
- Na transport rate versus autohemolysis rate;
- Total Na concentration in cells versus osmotic fragility;
- Total Na concentration in cells versus autohemolysis rate;
- Osmotic fragility versus autohemolysis rate;
- Ratio of "rapidly exchangeable" Na: total Na concentration in cells versus osmotic fragility;
- Ratio of "rapidly exchangeable" Na: total Na concentration in cells versus autohemolysis rate.

None of these scattergrams revealed significant correlation; but it must be noted that the number of cases studied was small. In this laboratory (22), determinations on a much larger number of HS patients have demonstrated a positive correlation between osmotic fragility and rate of autohemolysis. HS erythrocytes cannot here be distinguished statistically from normal erythrocytes either in terms of total Na concentration or in terms of the "rapidly exchangeable" Na: total Na concentration ratio. Routine hematologic data (hemoglobin, hematocrit, and reticulocyte count)

[7] Distributed by Clay-Adams, New York, New York.

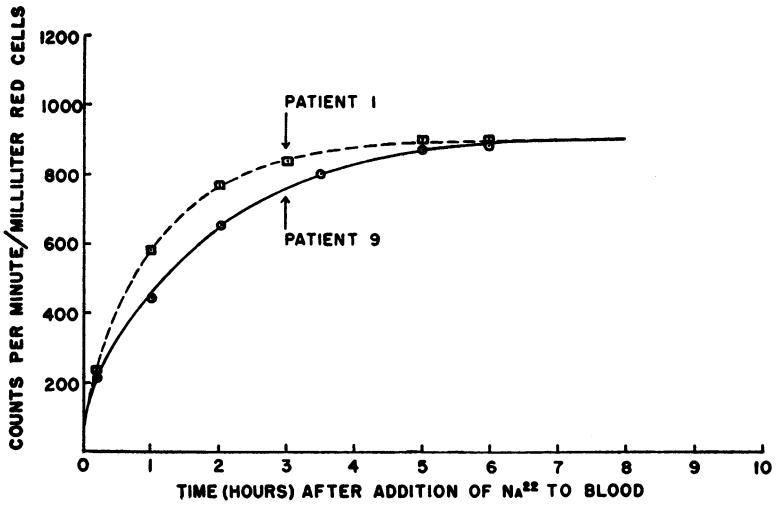

FIG. 1. IN VITRO RISE OF RADIOACTIVITY IN RED CELLS FROM TWO HS PATIENTS

These two patients represent opposite extremes of the scale of Na transport rate, patient 9 being at the upper limit of normal. The superimposition of plateaus is coincidental.

obtained before splenectomy were available on cases 4 through 10; no correlation exists between the apparent severity of the disease prior to splenectomy and the data reported here.

The average total initial Na concentration in cells, for the 10 HS patients, is 8.1 mEq. per L. cells; for the 6 determinations on the controls (cases A through D) the average concentration is 8.6. The average "rapidly exchangeable" Na concentration in cells for the 10 HS patients is 6.8; for the 6 controls the figure is 6.6. Combining these two sets of non-dissimilar averages one obtains an overall (16 determinations) average "slowly exchangeable" Na fraction of 19.3 per cent. Love and Burch's comparable figure (as calculated in [23]) is 17.8 per cent, and Gold and Solomon's figure (23) is 20.1 per cent.

DISCUSSION

There is satisfactory evidence that the defects of HS cells basically responsible for their shortened life span in the untreated (non-splenectomized) patient arise intrinsically (24). There is no evidence directly relating the known abnormalities of the HS cell to its premature *in vivo* destruction. The characteristically delayed passage of these spheroidal cells through the spleen of either a normal or an HS patient results in an accentuation of the cells' susceptibility to hypotonic hemolysis *in vitro* (25), but such low levels of tonicity are not realized *in vivo*. Selwyn and Dacie (26) have demonstrated that the hemolysis produced by the incubation of sterile HS blood is not attendant upon progressive erythrocyte swelling, and Crosby (27) cites evidence against the concept of hypotonic hemolysis being the result of "bursting" of the red cell from intracellular pressure.

The premature demise of the HS cell may be related to abnormal glycolysis (2, 3, 28). There exists sufficient evidence (2, 3) to conclude that the incorporation of extracellular phosphate into ATP (adenosine triphosphate) in the HS red cell deviates significantly from normal. The present study indicates that the rate of transfer of Na across the HS cell surface membrane is higher by a variable amount than the rate across the membrane of the normal red cell; however, the total Na concentration (26, and this paper) of the HS cell is essentially normal. Transmembrane concentration gradients of both Na and K are reduced in the cold-stored normal erythrocyte (29), in HS or normal red cells incubated over 24 hrs. at 37°C. (26), in sickled cells (30, 31), in red cells exposed to inhibitors of glycolysis (32), and in mammalian red cells exposed to a variety of nox-

ious stimuli (33). Disordered phosphate turnover rates are not peculiar to the HS cell alone (2, 34), and it may well be that pathologically altered glycolytic pathways and abnormal cation transport rates (4) characterize erythrocytes destined for early destruction by a variety of forces (28). It is reasonable to imagine that specific enzyme defects or deficiencies in specific disease states might lead to this "final common pathway." A more general example of this is the aging normal erythrocyte, whose total enzyme activity level, it has been suggested (35), decreases exponentially with time. Pyruvic phosphoferase, required in the phosphorylation of ADP (adenosine diphosphate) to ATP, is activated by potassium ion, but the typically low K concentration of HS red cells probably does not contribute to their abnormal phosphate turnover (36).

The exact circumstances surrounding the early death of the HS cell in non-splenectomized patients are still not known. Selwyn and Dacie's work (26) showed that changes in Na concentration in HS cells during *in vitro* incubation at 37°C. did not vary significantly from those occurring in normal human erythrocytes, even though incubation was carried out for 48 hours without added glucose. They suggested that "degenerative changes in the cell membranes" accounted for the increased rate of hemolysis of incubated HS cells, and linked this finding to prior speculation (37) on the role of the spleen in HS. The abnormal shape of the HS red cell certainly suggests a membrane defect, but further evidence exists: Prankerd, Altman, and Young found that the incorporation of radiophosphate into ATP and 2,3-diphosphoglycerate of the stroma of HS cells was lower than normal (2). Thus, a red cell membrane abnormality undoubtedly exists, but one must keep in mind that simplified mathematical analyses of isotope movement across cell surfaces, as in the present paper, are primitive approximations to probably complicated distributions involving total cell metabolism.

At present one cannot state with certainty how an accelerated movement of Na across the HS cell membrane is related to the aberrant behavior of these red cells *in vivo* and *in vitro*. The mechanism by which cations are transported across cell membranes is not known. Maizels has stated (6) that majority opinion favors "the view that Na and

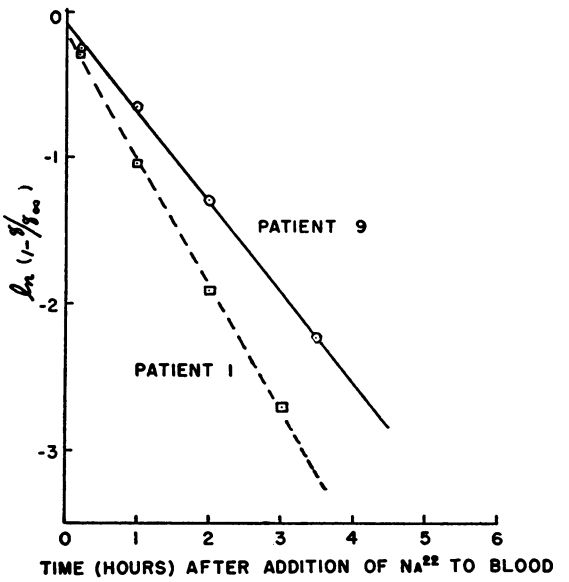

FIG. 2. PLOT OF THE LOGARITHMIC FUNCTION OF q (COUNTS PER MINUTE PER ML. OF RED CELLS) AGAINST TIME: THE TWO PATIENTS ARE THOSE OF FIGURE 1

The slopes (T) of these plots employed in Equation 3 are actually calculated by the method of least squares from the original data. The plots are visual checks on the validity of the slope calculation. q_∞ represents counts per minute per ml. of red cells at plateau.

K are transported in complex combination with a carrier, presumably lipoid or lipoid soluble." There is evidence (38) that active fluxes of red cell Na and K are linked in some manner. Also, recent work by Glynn (39) indicates that, under certain circumstances, the movement of Na *in vitro* out of washed erythrocytes from normal humans may have a passive component not dependent on glycolysis. Should this passive Na flux exist also under the more physiologic experimental conditions of this paper, an accelerated passive efflux from HS cells could explain our findings. This explanation would also be consistent with the demonstration (26) that the rate of disappearance *in vitro* of glucose from serum containing HS cells is normal.

We chose splenectomized HS patients for the study presented in order that mean cell age might vary as little as possible from patient to patient. Harris and Prankerd (4) suggested that the younger cells in an erythrocyte population display a more active Na transport across their membranes. They also interpreted their data as sug-

gesting that erythrocytes with high Na transport rates are more sensitive to hypotonic hemolysis: Our figures show no such correlation among the 10 HS cell populations studied. Since studies on *in vivo* survival of red cells were not done on our patients, we cannot state with certainty that mean cell age was constant from experiment to experiment. Reports in the literature (25, 40) indicate that after splenectomy hereditary spherocytes have an essentially normal life span, although the conclusion is not unanimous (41). Furthermore, Harris and Prankerd (4) obtained a normal Na transport rate with the red cells of a patient with severe gastrointestinal bleeding and a reticulocyte count of 10 per cent. (In our series only case D, a splenectomized normal control, had a reticulocyte count over 2 per cent.) Thus it seems that mean red cell age differences alone cannot account for the range of Na transport values in Table I.

Young has commented previously (42) on the absence of hematologic abnormality in both parents and all tested relatives of our cases 7, 9, and 10 (his patients WE, EH, and VO). Splenomegaly, anemia, reticulocytosis, and hyperbilirubinemia were absent, and determinations of osmotic fragility were normal. This suggested either a minor degree of penetrance in a carrier parent (assuming HS is inherited as a Mendelian dominant), or the possibility that the patients represented gene mutations. Our Table I indicates that the cells of cases 7, 9, and 10 have the lowest transport constants of the HS group. Thus is strengthened the suspicion (2, 42) that the clinical syndrome of hereditary spherocytosis is a composite of genetically determined biochemical abnormalities in red cells, not all of which abnormalities need be present in one patient.

The values for total Na concentration in normal red cells are lower than some recently published (16, 23). The reason for this is not clear, since we too analyzed complete cell aliquots, not discarding the top Na-rich layer of cells. However, our figures serve chiefly to verify steady state conditions, and to provide comparative Na concentrations. Gold and Solomon (23) discovered that the red cells of two untreated (non-splenectomized) HS patients demonstrated a higher proportion than normal of "rapidly exchangeable" Na, thereby suggesting "that the fraction of slowly exchanging Na may increase with the age of the red cell." The significance of slowly and rapidly exchanging Na fractions in red cells remains to be determined. Their existence is demonstrated experimentally by the ratio of Na specific activity in red cells to that in plasma, this ratio reaching a sub-unity value at plateau and remaining there at least to the 30-hr. point (23). Our work confirms this existence, but reveals no statistical difference ($P > 0.1$) between normal red cells and post-splenectomy HS cells in regard to the magnitude of the fractions.

SUMMARY

1. *In vitro* Na transport rates were determined for the red cells of 10 splenectomized patients with hereditary spherocytosis (HS), and 4 normal controls. Osmotic fragility and rates of spontaneous autohemolysis were also determined on the red cells of these 14 individuals.

2. Na transport rates of HS cells ranged from high normal to considerably elevated values. The abnormal rates are consistent with the fact that glycolytic pathways deviate from normal in HS red cells; and erythrocyte cation transport is dependent on glycolysis. The possibility also exists that increased passive Na efflux, not linked to glycolysis, may be responsible for the abnormal rates.

3. Na transport rates of red cells of 3 HS patients, each of whom had hematologically normal parents, were the lowest of the 10 HS cases studied. This finding strengthens the suspicion that HS is a composite of genetically determined biochemical abnormalities.

4. The existence of slowly and rapidly exchanging Na fractions in red cells was confirmed. Normal red cells and post-splenectomy HS cells did not differ in regard to the magnitude of these fractions.

5. No significant correlations were found among Na transport rates, osmotic fragility, autohemolysis rates, total Na concentrations, and "rapidly exchangeable" Na concentrations of HS cells. Total Na concentrations and concentrations of "rapidly exchangeable" Na in HS cells were not distinguishable from the corresponding concentrations in normal erythrocytes.

6. Although experimental evidence indicates that the erythrocytes of HS patients have abnormally functioning surface membranes and altered intracellular glycolytic pathways, the precise mech-

anisms leading to the shortened survival of these cells in non-splenectomized patients are not known.

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