

## Functionally Abnormal Na<sup>+</sup>-K<sup>+</sup> Pump in Erythrocytes of a Morbidly Obese Patient

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### Research Article

The Na<sup>+</sup>-K<sup>+</sup> pump in the erythrocytes of a morbidly obese patient shows a unique constellation of functional abnormalities. The number of pump units, measured by [<sup>3</sup>H]ouabain binding to intact cells, as well as the enzymatic activity of the (Na<sup>+</sup>-K<sup>+</sup>)-dependent ATPase in erythrocyte membranes were found to be markedly increased compared with control cells (18-fold and 14-fold, respectively). There was a concomitant fivefold increase in the rate of pump-mediated uptake of <sup>86</sup>Rubidium (a K analogue); this was balanced by an increased rate of <sup>86</sup>Rb efflux. In striking contrast to normal cells, however, a major portion of this efflux (80%) was inhibited by ouabain, and thus appeared to be mediated by the Na<sup>+</sup>-K<sup>+</sup> pump.

Erythrocytes from this patient had elevated levels of intracellular K<sup>+</sup> and reduced levels of intracellular Na<sup>+</sup>. This finding, taken together with the ouabain inhibition of K<sup>+</sup> efflux and the absence of associated abnormalities, argues against the possibility that the increased number of Na<sup>+</sup>-K<sup>+</sup> pump units was a compensation for a primary increase in the permeability of the erythrocyte membrane to monovalent cations, as is seen in a variety of erythrocyte disorders. Further evidence for a primary abnormality of the enzyme was our observation that the cardiac glycoside ouabain bound to these cells with reduced affinity and had a right shifted dose response for pump inhibition. The markedly increased [...]

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# Functionally Abnormal $\text{Na}^+\text{-K}^+$ Pump in Erythrocytes of a Morbidly Obese Patient

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**ABSTRACT** The  $\text{Na}^+\text{-K}^+$  pump in the erythrocytes of a morbidly obese patient shows a unique constellation of functional abnormalities. The number of pump units, measured by [ $^3\text{H}$ ]ouabain binding to intact cells, as well as the enzymatic activity of the ( $\text{Na}^+\text{-K}^+$ )-dependent ATPase in erythrocyte membranes were found to be markedly increased compared with control cells (18-fold and 14-fold, respectively). There was a concomitant fivefold increase in the rate of pump-mediated uptake of  $^{86}\text{Rb}$  (a K analogue); this was balanced by an increased rate of  $^{86}\text{Rb}$  efflux. In striking contrast to normal cells, however, a major portion of this efflux (80%) was inhibited by ouabain, and thus appeared to be mediated by the  $\text{Na}^+\text{-K}^+$  pump.

Erythrocytes from this patient had elevated levels of intracellular  $\text{K}^+$  and reduced levels of intracellular  $\text{Na}^+$ . This finding, taken together with the ouabain inhibition of  $\text{K}^+$  efflux and the absence of associated abnormalities, argues against the possibility that the increased number of  $\text{Na}^+\text{-K}^+$  pump units was a compensation for a primary increase in the permeability of the erythrocyte membrane to monovalent cations, as is seen in a variety of erythrocyte disorders. Further evidence for a primary abnormality of the enzyme was our observation that the cardiac glycoside ouabain bound to these cells with reduced affinity and had a right shifted dose response for pump inhibition. The markedly increased number of  $\text{Na}^+\text{-K}^+$  pump units in these cells did not appear to extend to mononuclear leukocytes.

In conclusion, the erythrocytes from this patient have a very large number of functionally abnormal

$\text{Na}^+\text{-K}^+$  ATPase units. A unique abnormality of the erythrocyte  $\text{Na}^+\text{-K}^+$  ATPase of these cells is the most likely explanation for these findings.

## INTRODUCTION

The  $\text{Na}^+\text{-K}^+$  ATPase is the enzymatic equivalent of the membrane  $\text{Na}^+$  pump (1, 2). Investigations of the normal physiology and biochemistry of the pump have often used the erythrocyte as a model system, because of easy availability and simplicity of the study of this cell type. In addition, erythrocytes have been used in experiments seeking to define abnormalities of this important membrane enzyme in disease. Alterations in the numbers of erythrocyte pump units and pump activity have been described in a variety of abnormal states. Thus, groups of obese (3), or hyperthyroid (4)<sup>1</sup> humans have fewer erythrocyte  $\text{Na}^+\text{-K}^+$  ATPase units than their thin, euthyroid controls, and patients with such varied conditions as protein-calorie malnutrition (5) and Huntington's disease (6) have increased activity of the erythrocyte  $\text{Na}^+\text{-K}^+$  ATPase. Hemolytic syndromes such as hereditary spherocytosis (7), and hereditary stomatocytosis (8, 9) are also characterized by an increase in the erythrocyte complement and activity of the sodium-potassium pump, and these changes appear to be compensatory for an unknown primary defect causing increased cellular permeability to monovalent cations. Both, uremia (10), and liver disease (11) have been associated with changes in the sodium pump activity of erythrocytes. In these various syndromes the  $\text{Na}^+\text{-K}^+$  pump itself has been shown (or assumed) to function normally, except possibly in myotonic muscular dystrophy (12), where an altered stoichiometry of  $\text{Na}^+$  and  $\text{K}^+$  pumping may exist. We

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wish to report a patient with morbid obesity whose erythrocytes display grossly abnormal cation pumping activity in the absence of other erythrocyte abnormality. Erythrocytes from this patient have a very large (18-fold) increase in the number of  $\text{Na}^+\text{-K}^+$  ATPase units, and those pump units that are present demonstrate a unique functional abnormality.

## METHODS

**Case report.** The subject (M.A.J.) is a 45-yr-old woman whose presenting medical complaint was marked obesity. The patient had been heavier than her peers from age 8 yr onwards, and at the time of the initial presentation she weighed 147 kg. With her height of 165 cm, she was considered to be 152% above her ideal body weight (Metropolitan Life Insurance Company tables).

Her previous history included three pregnancies, one of which resulted in a stillbirth. She had an ovarian cystectomy and a cholecystectomy 12 and 10 yr, respectively, before presentation. Apart from her obesity, she was otherwise well, and in particular showed no carbohydrate intolerance or hypertension. Because her obesity had repeatedly proven refractory to dietary treatment, she underwent a gastric stapling operation in December 1979 and subsequently lost 54 kg over a 12-mo period. Apart from this weight loss, there appeared to be no other effects of the surgery and specifically she showed no electrolyte or hematologic abnormalities and there was no clinical or biochemical evidence of hepatic dysfunction. Her drug intake at the time of the studies consisted of multivitamin supplements.

Family history was remarkable for the fact that both her parents and two sisters were of normal weight. There was no history of any hematologic disorder in any member of the family.

Studies on the  $\text{Na}^+\text{-K}^+$  pump of her erythrocytes were performed on four separate occasions over a 12-mo period subsequent to the gastric bypass surgery.

Limited studies of the  $\text{Na}^+\text{-K}^+$  pump of erythrocytes from two sons of the patient were also carried out. Both were in normal health. The elder of the two (J.A.S.) was 18 yr of age, 173 cm tall and weighed 81.7 kg, which was 21% above his ideal body weight. The younger son (J.E.R.), was 16 yr old, 173 cm in height and his weight of 90.8 kg caused him to be 49% above ideal body weight.

### *Erythrocyte and mononuclear leukocyte $\text{Na}^+\text{-K}^+$ pump status*

Heparinized blood was drawn in the fasting state. After separation of the plasma, the cells were centrifuged through Ficoll-Hypaque (13) to give preparations of mononuclear leukocytes and erythrocytes essentially free of polymorphonuclear leukocytes contamination.

After repeated washes of both cell suspensions with isotonic ice-cold choline chloride solution, the following studies were performed.

**Ouabain binding to erythrocytes and mononuclear leukocytes.** Erythrocytes ( $5\text{--}10 \times 10^9$  cells/ml), were incubated with [ $^3\text{H}$ ]ouabain sp act 19.5 Ci/mmol (New England Nuclear, Boston, Mass.) and increasing concentrations of unlabeled ouabain. The buffer used contained 140 mM NaCl, 30 mM Hepes, 10 mM dextrose, pH 7.40. After a 60-min incubation at 37°C the cells were washed three times with ice-cold 140 mM choline chloride and bound radio-

activity was eluted with 5% TCA and counted in a Packard liquid scintillation spectrometer (Packard Instrument Co. Inc., Downers Grove, Ill.).

Analysis of the binding data was performed as previously described (3) to determine maximal ouabain binding to the cells.

Mononuclear leukocytes ( $1\text{--}10 \times 10^6$  cells/ml) were treated in identical fashion to the erythrocytes, with the exception that [ $^3\text{H}$ ]ouabain binding was measured at a single ouabain concentration only (12.7 nM). Nonspecific binding of labeled ouabain to both erythrocytes and leukocytes was measured in the presence of 0.1 mM unlabeled ouabain and was always <5% of [ $^3\text{H}$ ]ouabain binding measured in the absence of unlabeled ligand.

**$^{86}\text{Rb}$  transport in erythrocytes.** For the measurement of  $^{86}\text{Rb}$  uptake by erythrocytes, a suspension of erythrocytes ( $5\text{--}10 \times 10^9$  cells/ml) was incubated with  $^{86}\text{Rb}$  (sp act 3.5 mCi/mg, New England Nuclear) and increasing concentrations of ouabain (0–0.1 mM). The buffer used contained 140 mM NaCl, 10 mM Tris, 1.6 mM RbCl, 10 mM dextrose, pH 7.40. We have shown that in normal cells the rate of Rb uptake mediated by the  $\text{Na}^+\text{-K}^+$  pump is linear with time over a period of 4 h (data not shown) and the experiments were routinely stopped at 1 h of incubation at 37°C. The cells were washed three times in 140 mM ice-cold choline chloride and intracellular radioactivity was then measured after precipitation of protein by 5% TCA. To measure  $^{86}\text{Rb}$  efflux from erythrocytes, cell were preincubated with  $^{86}\text{Rb}$  for 5 hs at 37°C, washed repeatedly with 140 mM choline chloride and then resuspended in tracer-free buffer of the same composition as stated above. When indicated, ouabain was present at a final concentration of 0.1 mM. The appearance of  $^{86}\text{Rb}$  in the supernatant was measured at 20-min intervals for 60 min and related to total intracellular radioactivity at time zero of the efflux period.

**$\text{Na}^+\text{-K}^+$  ATPase activity of erythrocyte membranes.** 5 ml of packed erythrocytes were osmotically lysed in 10 vol of ice-cold 5 mM Tris/0.1 mM disodium EDTA, pH 7.6. The membranes were centrifuged at 20,000 g for 20 min at 4°C. They were then washed three times in 0.017 M NaCl/5 mM Tris, pH 7.6 and three times with 10 mM Tris (pH 7.45) and the hemoglobin-free membrane suspension was stored in this last buffer at –20°C until assay of  $\text{Na}^+\text{-K}^+$  ATPase activity (3–4 d). This was performed by incubating 50  $\mu\text{l}$  of membrane suspension (representing ~0.2 mg of membrane protein) with 5 mM Tris-ATP, 25 mM KCl, 75 mM NaCl, 5 mM  $\text{MgCl}_2$ , 0.1 mM EGTA, 25 mM Tris, pH 7.45 in a total volume of 0.5 ml. After 90 min at 37°C in a shaking water bath, the reaction was stopped by the addition of TCA to give a final concentration of 5% (wt/vol). After centrifugation for 20 min at 1,500 g, an aliquot of the supernatant was used to measure total inorganic phosphate by the method of Miller et al. (14). The results are expressed as micromole of inorganic phosphate per milligram membrane protein per hour. Protein was measured using the Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, Calif.) (15).

**$^{125}\text{I}$ -Insulin binding to erythrocytes.** The procedure was carried out as described by Gambhir (16). Intracellular electrolytes were measured by flame photometry (Instrumentation Laboratory, Inc., Lexington, Mass.) of an appropriately diluted lysate of erythrocytes, previously washed at least four times in 10 vol of ice-cold 140 mM choline chloride to eliminate extracellular  $\text{Na}^+$  and  $\text{K}^+$  contamination. The results are expressed in terms of the volume of packed cells used to prepare the lysate.

Data for the patient are compared to similar measurements made in normal weight controls who have been used in other studies of  $\text{Na}^+\text{-K}^+$  pump status carried out in our

laboratory. All data are expressed as mean $\pm$ SD, except where otherwise indicated.

## RESULTS

Hematologic data are presented in Table I. Erythrocyte indices are within normal limits, and there are no findings that might suggest reduced erythrocyte lifespan or subtle tendency to hemolysis, such as reticulocytosis, stomatocytosis (9), or altered osmotic fragility.

A saturation curve of ouabain binding to erythrocytes from the patient and from control individuals demonstrates that the total amount of ouabain specifically bound to M.A.J. erythrocytes is much higher than control values at all concentrations of ouabain (Fig. 1). By extrapolation of these curves to saturating concentrations of ouabain, the maximal ligand binding capacity of M.A.J. cells can be calculated to be  $\sim 18$ -fold higher than controls. The curve shown represents one individual study; this and three other studies in this patient gave a mean value for binding capacity of  $10.44 \pm 1.23$  pmol of ouabain/ $10^9$  cells (compared with a control value of  $0.596 \pm 0.109$  pmol/ $10^9$  cells that corresponds to  $\sim 360$  pump units/cell). The ouabain binding curve also shows that the affinity of the patient's cells for ouabain is reduced, since the concentration of the ligand at which 50% of the available sites are saturated under the experimental conditions used is  $15.4 \pm 2.0$  nM for the patient and  $6.6 \pm 1.3$  nM for controls.

The uptake of rubidium by patient and control cells is shown in Fig. 2. Both the total hourly uptake and the uptake inhibited by 0.1 mM ouabain are markedly increased in the patient's cells, whereas the uptake in the presence of 0.1 mM ouabain (i.e. nonpump-mediated) is even lower in her cells, although the lowering is not significant. Again, these data represent one particular study and the mean value for  $\text{Na}^+$ - $\text{K}^+$  pump-

## Erythrocyte Ouabain Binding

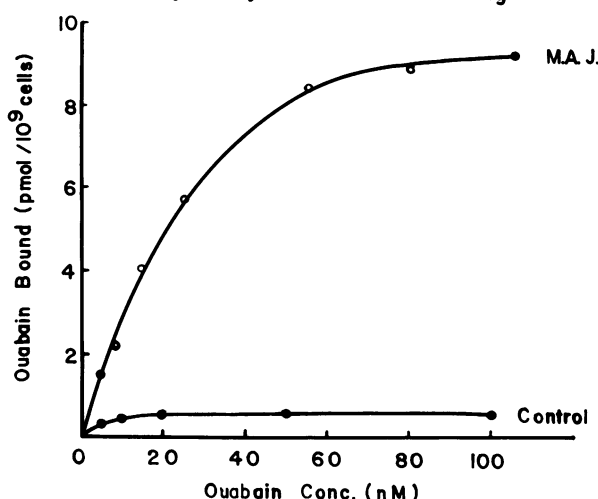


FIGURE 1 Specific ouabain binding to erythrocytes of M.A.J. and controls. The curve shown for the patient represents one individual study. The control values are the mean of 40 individual measurements in normal individuals. Interrupted vertical lines indicate the concentration of ouabain required for 50% saturation of binding sites. No standard error bars are shown for the controls since they do not exceed the thickness of the line on the particular scale used in this figure.

mediated Rb uptake by the patient's cells in the four studies performed was  $451 \pm 55$  nmol/ $10^9$  cells per h compared with a control value of  $83.5 \pm 10.2$  nmol/ $10^9$  cells per h. The uptake data also provides further indication of a decrease in the affinity of the pump for ouabain, since the concentration of ouabain at which uptake of Rb is inhibited by 50% is  $\sim$ fourfold higher with M.A.J. cells (380 vs. 86 nM) (Fig. 2).

It would be expected that a marked increase in Rb uptake by M.A.J. cells should be balanced by an equivalent increase in the rate of Rb efflux from these cells. That such is indeed the case is shown in Fig. 3. The fractional rate of release of  $^{86}\text{Rb}$  from cells preloaded with the isotope is much higher in the patient's cells (rate constants of  $0.0719 \text{ h}^{-1}$  and  $0.0276 \text{ h}^{-1}$  in patient and controls, respectively). The effect of ouabain on  $^{86}\text{Rb}$  efflux was also assessed (Fig. 3). Whereas the rate of efflux of  $^{86}\text{Rb}$  from control cells is not detectably altered by the presence of 0.1 mM ouabain, the rate of  $^{86}\text{Rb}$  efflux from M.A.J. cells is markedly inhibited in ouabain containing buffer, suggesting that a considerable proportion of  $\text{K}^+$  efflux from M.A.J. cells is mediated by the  $\text{Na}^+$ - $\text{K}^+$  pump.

The values obtained for erythrocyte  $\text{Na}^+$  and  $\text{K}^+$  concentration in the patient are seen in Table II.  $\text{Na}^+$  concentration was significantly lower than control and the  $\text{K}^+$  level was higher than the mean of control. These measurements were obtained from replicate

TABLE I  
Hematologic Characteristics of Patient M.A.J. and Controls

Parameter measured	Patient values	Controls
Hemoglobin, g/dl	13.3	12–15
Hematocrit, %	39.9	36–44
Mean cellular volume, $\mu^3$	88.0	78–98
Mean cellular hemoglobin, pg	29.8	28–34
Mean cellular hemoglobin concentration, %	33.4	32–36
Reticulocytes, %	0.3	<1
Median osmotic fragility, g NaCl/dl	0.42	0.40–0.45
Blood group	0 Rh+	—
Morphology (wet preparations)	Normal	—

## Erythrocyte Rb Uptake

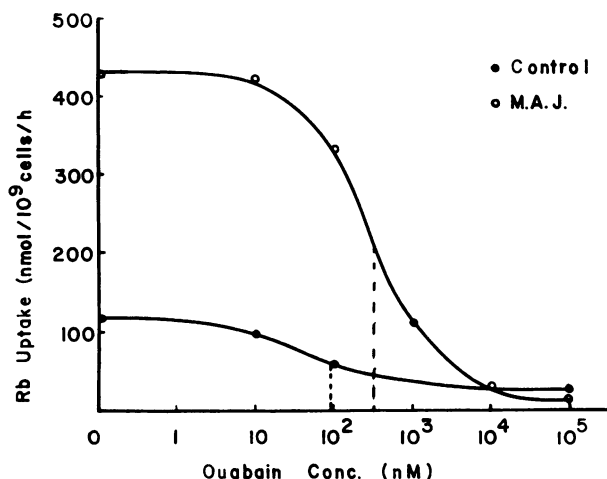


FIGURE 2 Rate of Rb uptake by M.A.J. and control cells. Each point shows the total hourly uptake of Rb by  $10^9$  cells at the stated concentration of ouabain. Patient values represent one individual study while the control curve is the mean of 40 separate normal determinations, with standard error bars too small to be adequately shown on the scale used. Interrupted vertical lines indicate the concentration of ouabain at which the maximal rate of Rb uptake is inhibited by 50%.

samples of blood obtained from M.A.J. and four control samples treated identically and processed simultaneously. It may be noted that the control values for erythrocyte  $\text{Na}^+$  and  $\text{K}^+$  differ from our previously published levels (3), as a result of an unavoidable change in methodology. However, the significance of relative differences seen both here and in our previous publication still obtain.

In addition to the lowered intracellular sodium levels in freshly obtained M.A.J. erythrocytes, ouabain blockade of the pump for 5 h resulted in only a 3 mM increase in the intracellular sodium concentration (compared with a mean increase of 3.3 mM in the cells from the four control subjects). In these respects, these cells differ from those seen in hemolytic syndromes associated with increased permeability of the erythrocyte membrane to  $\text{Na}^+$ , in which erythrocyte Na levels are consistently increased and rise dramatically when the activity of the  $\text{Na}^+\text{-K}^+$  pump is inhibited (9).

The  $\text{Na}^+\text{-K}^+$  ATPase activity of erythrocyte membranes is also markedly elevated in this patient (Table II). In contrast, another integral membrane protein, the insulin receptor, appears to be normal in erythrocytes from the affected subject (Table II).

The binding of [ $^3\text{H}$ ]ouabain to a population of circulating mononuclear leukocytes was also assessed (Table II). The finding that [ $^3\text{H}$ ]ouabain binding to mononuclear leukocytes from this patient is within

normal limits, suggests that the observed change in the erythrocyte  $\text{Na}^+\text{-K}^+$  pump is not expressed on all cells.

The results of ouabain binding to, and rubidium uptake by erythrocytes and the  $\text{Na}^+\text{-K}^+$  ATPase activity of erythrocyte membranes from the patient's two sons are shown in Table III. It may be seen that in the case of the younger son (J.E.R.) (who is also the more markedly obese of the two) all the parameters measured are abnormally elevated ( $>2$  SD from the mean control value), although obviously not to the same marked extent as seen in the mother.

## DISCUSSION

These data demonstrate the existence of a markedly increased number of functionally abnormal  $\text{Na}^+\text{-K}^+$  ATPase enzyme units in the erythrocytes of a morbidly obese patient. The greatly increased complement of  $\text{Na}^+\text{-K}^+$  ATPase pump units is reflected in both the 18-fold increase in ouabain binding capacity and in the 14-fold increase in the rate of ouabain-sensitive ATP hydrolysis by erythrocyte membranes. The cation transport activity of the pump, as measured by the rate of uptake of Rb by erythrocytes, is also markedly increased compared with controls, although to a lesser extent than would be expected from the number of pump units present. This would be the expected consequence of a lowered intracellular sodium level that would reduce the activity of the sodium pump in the

## Erythrocyte $^{86}\text{Rb}$ efflux

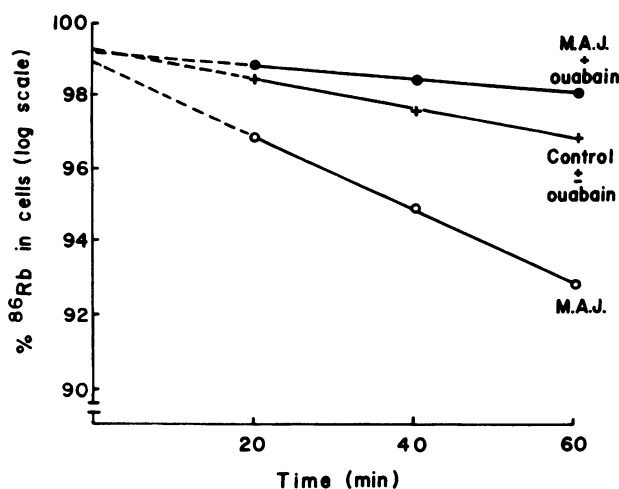


FIGURE 3 Rate of  $^{86}\text{Rb}$  efflux from control and M.A.J. cells. The control points are obtained from the mean of 25 separate measurements and there is no measurable difference in the efflux rate seen in the presence or absence of ouabain. The points for M.A.J. cells represent one individual study. The exponential fall in cellular  $^{86}\text{Rb}$  has been linearized by a log transformation and rate constants can be obtained directly from the figure and are quoted in the Results.

TABLE II  
Erythrocyte and Mononuclear Leukocyte Features  
in Patient M.A.J.

Parameter measured	Patient values	Controls
Erythrocyte Na <sup>+</sup> , mmol/liter cells	7.79±0.34*	10.83±0.80 (4)
Erythrocyte K <sup>+</sup> , mmol/liter cells	87.9±0.4*	77.3±3.0 (4)
Mononuclear leukocyte [ <sup>3</sup> H]ouabain binding, fmol/10 <sup>6</sup> cells†	74.43	66.46±2.23 (17)
<sup>125</sup> I-Insulin binding to erythrocytes, % tracer binding/10 <sup>9</sup> cells§	20.1	21±1.2% (47)

Numbers in parentheses below control values indicate the number of measurements made.

\* Erythrocyte Na<sup>+</sup> and K<sup>+</sup> concentration in M.A.J. cells obtained from three separate determinations.

† [<sup>3</sup>H]Ouabain binding to mononuclear leukocytes at a single, non-saturating ouabain concentration of 12.7 nM.

§ <sup>125</sup>I-Insulin present at a concentration of 0.1 ng/ml.

intact cell. The extent of the increase in the number of pump units is very large and is greater than previously reported in any condition. This finding is of particular note in the light of our previous observation that, as a group, obese humans have fewer Na<sup>+</sup>-K<sup>+</sup> pump units in their erythrocytes (3).

That the abnormality of this enzyme is qualitative as well as quantitative is suggested by the decrease in the apparent affinity of the enzyme for ouabain. Thus,

the concentration of ouabain at which 50% of the available binding sites are saturated under the experimental conditions used gives a measure of relative affinity of the Na<sup>+</sup>-K<sup>+</sup> ATPase for the ligand, and this concentration is almost three times higher in M.A.J. erythrocytes (15.4±2 nM vs. 6.6±1.3 nM for controls). Because steady-state has not been achieved after 60 min at 37°C, this value does not represent the true dissociation constant (*K<sub>d</sub>*) of the binding reaction. Preliminary experiments in which we have measured ouabain binding to isolated erythrocyte membranes confirm similar directional shifts in affinity of ouabain binding as are seen in intact cells. Independent confirmation that the affinity for ouabain is reduced in this patient's cells is provided by the data shown in Fig. 2 and Table III, where the rate of Rb uptake by cells is measured in the presence of increasing concentrations of the glycoside. Inhibition of total Rb uptake by 50% requires a fivefold higher concentration of ouabain in M.A.J. cells compared to normal erythrocytes.

A second abnormality of the cells, which may or may not be directly related to this alteration in their affinity for ouabain, is suggested by the results shown in Fig. 3. When the rate of efflux of <sup>86</sup>Rb from cells preloaded with the isotope is measured, it is seen that this rate is higher in M.A.J. cells than in normal cells. The total efflux of K<sup>+</sup> from cells is composed of at least two components: (a) a "leak" of K<sup>+</sup> down its concentration gradient and, (b) a Na<sup>+</sup>-K<sup>+</sup> pump-mediated (and therefore ouabain-sensitive) K<sup>+</sup> efflux which, although requiring the presence of intracellular ATP does not result in hydrolysis of the nucleotide phosphate (17). This has been termed sodium pump-mediated K<sup>+</sup>-K<sup>+</sup> exchange. Under normal conditions, this

TABLE III  
Na<sup>+</sup>-K<sup>+</sup> Pump Characteristics in Erythrocytes from M.A.J. and Two Children

Parameter measured	M.A.J.	JER J.	JAS J.	Controls
Maximal ouabain binding capacity, pmol/10 <sup>9</sup> cells	10.44±1.23	0.867	0.595	0.596±0.109 (40)
Ouabain concentration for 50% saturation of binding sites, nM	15.4±2			6.6±1.3 (40)
Na <sup>+</sup> -K <sup>+</sup> pump-mediated Rb uptake, nmol/10 <sup>9</sup> cells/h	451±55	125.1	104.1	83.5±10.2 (40)
Ouabain concentration for 50% inhibition of Rb uptake by erythrocytes, nM	380			86 (40)
Na <sup>+</sup> -K <sup>+</sup> ATPase activity, μmol phosphate released/h/mg protein	6.7	0.79	0.44	0.470±0.217 (5)

All values are given as the mean±SD with the number of separate determinations indicated in parentheses.

pump-mediated  $K^+$  efflux is probably negligible and indeed we are unable to detect it in our control cells. Our data show that the greater part of  $^{86}Rb$  efflux from M.A.J. cells is inhibitable by ouabain and thus, unlike normal cells, presumably mediated by the  $Na^+-K^+$  pump. Indeed, the ouabain resistant "leak" of  $^{86}Rb$  from these cells is even lower than that measured in normal cells. The precise mechanistic explanation for these findings is not yet clear. One possible explanation for this phenomenon would be the existence of a greatly increased affinity of the intracellular "cation" site of the pump for  $K^+$ , such that during "normal" activity of the pump,  $K^+$  substitutes for  $Na^+$  and is transported to the outside of the cell in exchange for an equivalent amount of  $K^+$  coming into the cells. If such a scheme operates in M.A.J. erythrocytes, then it follows that the major fraction of  $Rb$  uptake by her cells takes place via the  $Na^+-K^+$  pump, but as part of a  $K^+-K^+$  exchange rather than the normal  $K^+-Na^+$  exchange. It would also follow that ouabain-sensitive sodium efflux from M.A.J. cells should be increased to a lesser degree than ouabain-sensitive  $K$  influx, a contention supported by preliminary data on  $Na^+$  fluxes (data not shown).

Abnormalities in erythrocyte monovalent cation transport have been described in a number of pathologic conditions (18, 19). Increased activity of the pump has been noted in situations where the permeability of the erythrocyte membrane to  $Na^+$  and/or  $K^+$  is increased leading to "downhill" movement of the two ions. The cell will then attempt to reestablish the normal gradient by increasing the activity of the  $Na^+-K^+$  pump, as well as by increasing the number of pump units per cell. This appears to be the case in various hemolytic syndromes such as stomatocytosis (9), where increased pump levels and activity are seen in cells with abnormally high sodium levels. That our patient does not represent a variant of these syndromes is indicated by the absence of any of the hematologic features seen in this group of patients (e.g. anemia due to accelerated hemolysis, reticulocytosis, abnormal osmotic fragility and cell morphology) and by the repeated observation that her erythrocyte sodium levels have always been significantly lower than normal, rather than increased, as is the case in the aforementioned conditions. Thus, a primary increase in the inward leak of  $Na^+$ , eventually leading to increased  $Na^+-K^+$  pump activity does not appear to be the defect in M.A.J. erythrocytes, since this would necessarily result in higher than normal intracellular sodium levels. Similarly, a primary increase in the "leak" of  $K^+$  from the cells could be envisaged as resulting in an increased pump activity to restore intracellular  $K^+$  levels towards normal. However, the data shown in Fig. 3 argue against this possibility since they show that the  $K^+$  "leak" (i.e. the rate of efflux of  $^{86}Rb$  from cells

with a blocked  $Na^+-K^+$  pump) is actually lower in this particular patient than in controls.

The biochemical basis for the cation transport abnormality in these cells is unknown. As discussed above, the available evidence argues against the increase in pump units and activity being a compensation for increased leakiness of the erythrocyte membrane for  $Na^+$  or  $K^+$ . In our view, the most likely explanation for the transport abnormality in this patient is a primary or secondary abnormality in the  $Na^+-K^+$  ATPase enzyme molecule, leading to altered affinities for ions and ouabain, and a secondary increase in pump units. Structural mutations in this important enzyme have not been previously described in man. Of great interest is the fact that the number of pump units on mononuclear leukocytes of this patient is in the normal range. This might serve as indirect evidence for separate genes encoding for the  $Na^+-K^+$  ATPase of different tissues. Other evidence favoring this concept already exists in that antigenic differences between enzymes from different tissues have been described (20).

The relationship of this finding to our previous observation (3) that obese individuals as a group have fewer pump units per erythrocyte than nonobese individuals is also unknown. Taken together with our previous data (3), we have now identified five obese individuals in whom the number of erythrocyte pump units exceeds that of any normal control and is at least 2 SD higher than the mean control value. One of these subjects is the younger son of our patient, data for whom are reported in Table III. It is very tempting to speculate that the abnormality described may have a genetic familial basis although obviously more extensive family studies will be needed to confirm such a hypothesis. Whether these individuals with high erythrocyte  $Na^+-K^+$  ATPase levels represent a subgroup of obese patients with a distinctly important abnormality of the enzyme, or whether the pump abnormality in these cases is unrelated to their obesity also remains to be determined.

In conclusion, we have demonstrated a unique functional abnormality in the erythrocyte  $Na^+-K^+$  ATPase of a morbidly obese patient. Further studies of this defective pump may provide important information on the normal biochemical mechanism underlying activity of this critical membrane system.

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