ABNORMALITIES IN THE SODIUM PUMP OF ERYTHROCYTES FROM PATIENTS WITH HYPERTHYROIDISM

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SUMMARY

1. Intracellular cation composition has been measured in the red cells from twenty patients with hyperthyroidism. The mean concentration of sodium was 11.18 m-mole/l red cells; in sixty normal control subjects the mean red cell sodium level was 7.04 m-mole/l. The difference between these two groups was highly significant. There was no measurable difference between the potassium concentration and water content of red cells from thyrotoxic and control groups.

2. Measurements of active sodium efflux were carried out in red cells from ten hyperthyroid subjects and their matched controls. The rate constant for active sodium efflux was significantly lower in the patients than the control group.

3. The total amount of sodium actively pumped from red cells in 1 hr was significantly higher in the patients than the controls.

4. The total amount of sodium moving out of the red cells, both actively and by exchange diffusion, matched the total influx of sodium. This was true for control subjects and those with hyperthyroidism and this would support the view that the intracellular sodium concentration is constant and represents the result of a balance between influx and efflux. In hyperthyroidism this balance persists, but with an abnormally high intracellular sodium concentration.

5. There was a linear relationship between the cell sodium content and the active transport of sodium from the cell in control and hyperthyroid subjects.

6. Triiodothyronine did not produce any change in sodium transport by normal red cells in vitro.

7. It is concluded that there is a depression of the activity of the sodium pump in the red cells of hyperthyroid subjects. This allows the resting intracellular sodium concentration to rise until a new steady state is reached. Evidence is given that these changes are reversed when hyperthyroidism is corrected.
The human red blood cell has proved to be a useful model in which to study the mechanisms which control the ionic composition of the intracellular space. It is now evident that the diffusion of sodium into, and potassium out of the cell down their concentration gradients is balanced by the active transport of these cations against the same concentration gradient. This active process requires metabolic energy which can be derived from glucose (Harris, 1941) via the anaerobic glycolytic pathways which are available to the non-nucleated human erythrocyte. The energy stores of the cell are primarily in the form of the high energy phosphate bonds of adenosinetriphosphate (ATP), and the work of Post et al. (1960) and Dunham & Glynn (1961) has shown that the release of energy from ATP could be brought about by the action of an enzyme system (an ATPase) located within the transporting membrane itself. Further evidence has amply confirmed that this enzyme is, indeed, the main energy transducer concerned with sodium transport across cell membranes (Skou, 1965).

A great deal has now been learnt about cation transport in normal red cells and many of the factors which influence this are now known. By contrast, however, changes that occur in this fundamental activity of the cell membrane in pathological states have only recently been studied closely. Among such abnormal states is thyrotoxicosis, and it was in a small group of hyperthyroid patients that Boekelman (1958) showed a higher amount of sodium in red cells than in a group of control subjects. We have taken this observation further by confirming Boekelman's findings and characterizing the defect of sodium transport with which it is associated.

**METHODS**

Most of the thyrotoxic patients studied were seen in the Department of Medicine at Fulham Hospital; some were seen at the Hammersmith Hospital. All had an unequivocal elevation of radio-iodine neck uptake, as measured by standard techniques, and clinical evidence of hyperthyroidism. Their ages ranged from 22 to 65 years and three-quarters of them were women. The control subjects were hospital staff and medical students who had no known disease, and were taking no medication.

**The measurement of red cell cation content**

Determinations were made on the red cells from 10 ml of whole blood. It was drawn, without stasis, into a plastic syringe moistened with heparin (1000 units/ml). A wide-bore needle was used to prevent undue trauma to the cells. The blood was separated in a refrigerated centrifuge (Sorvall RC-2B) by spinning it momentarily to 15 000 g. Plasma,uffy coat, and the very topmost layer of red cells were removed by aspiration and the remaining cells were washed three times in 285 mOsmole MgCl₂ containing 25 ml/l of an isotonic glycylglycine–MgCO₃ buffer at pH 7.4. Washing was performed using a 'Rotamixer' and the cells were spun down by centrifuging them momentarily to 15 000 g and then aspirating the supernatant fluid. After the third wash the cells were suspended in sufficient wash solution to give a haematocrit of about 50%, and the exact haematocrit was then determined in triplicate. The cell suspension was then diluted for estimations of sodium and potassium by flame photometry. This dilution was at least one in fifty, sufficient to produce complete haemolysis. Measurements were made on a standard Eppendorf flame photometer and the cation content was expressed as m-mole/l of red cells by reference to the haematocrit of the cell suspension used. The sodium concentra-
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The measurement of cell water content

Washed red cells, at a known haematocrit, were weighed in thin aluminium tares on an electro-balance (Cahn Instrument Co.). They were then dried to constant weight in an oven at 100° and the water content expressed as the percentage by weight.

The measurement of sodium efflux

The technique was similar to that used by Sachs & Welt (1967). Blood was drawn into a heparinized plastic syringe and immediately separated by a momentary spin to 15,000 g in the refrigerated centrifuge. 2.5 ml of cells were then incubated in 0.5 ml of isosmotic sodium phosphate buffer solution at pH 7.4, to which was added dextrose (250 mg/100 ml) and 25 µCi of 22Na. After a 3 hr loading period at 37° the cells were washed six times in isotonic MgCl2 to remove residual extracellular radioactivity. The washed and loaded red cells were then suspended in incubation medium at a haematocrit of between 0.1 and 0.2%; the basic composition of the incubation medium was: NaCl 140 mM, KCl 5 mM, phosphate buffer (pH 7.4) 1.4 mM. Isotonic glycylglycine–MgCO3 buffer (pH 7.4) was added at a concentration of 10% and dextrose at a concentration of 150 mg/100 ml. The incubation was carried out in 125 ml capacity neoprene flasks at 37.5 ± 0.5° and in duplicate. When ouabain was added its concentration was 10−6 M, and it was added as an aqueous solution, an equal amount of water being added to control flasks.

A 15 min period was allowed for temperature equilibration and then a sample of the suspension from each flask was decanted into tubes containing one drop of 1% saponin. These haemolysed samples were used to assess the total number of counts in each flask. At a time arbitrarily designated as ‘zero’, 10 ml samples were poured from each flask into iced plastic centrifuge tubes and immediately separated by centrifuging them to 15,000 g at 0°. Similar samples were taken at 20 min intervals for 60 min, and 5 ml samples of each supernatant together with 5 ml of each haemolysed sample were counted in a Packard Autogamma scintillation counter. The haemoglobin content of each sample was measured by reading the optical density at 420 mp and the fractional haemolysis was determined for each supernatant as compared with the haemoglobin content of the totally haemolysed sample.

Sodium efflux (kNa) was expressed as a rate constant using the equation of Hoffman (1967) which states that:

\[ a k_{Na}.t = \log \frac{1 - Na^*_h/Na^*_s}{1 - \text{fractional haemolysis}} \]

where \( Na^*_h \) is the activity of the supernatant in counts ml⁻¹ min⁻¹,

\( Na^*_s \) is the activity of the haemolysed sample in counts ml⁻¹ min⁻¹.

This formulation is only valid at haematocrits below about 0.3%, a situation which was ensured in all our experiments.

The rate constant for efflux (which is the fraction of intracellular sodium extruded per hr) can then be described by the slope of the line which relates the right hand side of this equation to the time ‘t’. This slope can be calculated by the method of least squares. The rate constant can then be divided into ‘pump’ (\( k_{Na}^p \)) and ‘residual’ (\( k_{Na}^r \)) components, on the assumption
that $k_{Na}$ was totally inhibited by ouabain. This assumes that the component of active transport which has been called 'pump II' (Hoffman & Kregenow, 1966) is small enough to be neglected in these circumstances. Total sodium pumped per hour ($M_{Na}$) was determined by multiplying $k_{Na}$ by the intracellular sodium concentration.

**The measurement of sodium influx**

Red cells were separated from plasma and the buffy coat aspirated; after three washes in isotonic magnesium chloride they were suspended in an incubation medium similar to that used in the efflux studies. The haematocrit was about 10% and the temperature 37.5°.

After a 10 min equilibration period 1.25 μCi $^{22}$Na were added to each flask which was well shaken; 1 hr later a 10 ml sample was poured from each flask into iced plastic centrifuge tubes and centrifuged momentarily to 15,000 g. Duplicate 1 ml samples of the supernatant fluid were transferred to counting tubes and made up to a total volume of 5.0 ml. The residual button of red cells was washed rapidly, three times, with isotonic MgCl₂, to remove external $^{22}$Na and then suspended at a haematocrit of about 30% in isotonic MgCl₂. This haematocrit was measured in triplicate. 1.0 ml samples of the red cell suspension were transferred to counting tubes and made up to a total volume of 5.0 ml with 0.1% aqueous saponin—this ensured complete haemolysis. All samples were then counted in a Packard Autogamma scintillation counter.

Influx was expressed in terms of the percentage of counts in the supernatant fluid which entered the cells per hour. Sodium efflux was measured simultaneously and allowed a correction to be made for sodium pumped out of the cell during the influx study, according to the method of Sachs & Conrad (1968). Without such correction major errors of influx measurement will occur.

**RESULTS**

**Red cell cation composition**

Measurements of intracellular sodium concentration were made in a group of sixty healthy control subjects. Their ages ranged from 18 to 57 years and there was a slight female preponderance. Similar measurements were made in a group of twenty patients with hyperthyroidism fourteen of whom were women. The distribution of red cell sodium content, expressed as m-mole/l of red cells, for these two groups is shown in Fig. 1. The mean sodium concentration in controls was 7.04±1.24 (SD) m-mole/l and in hyperthyroid subjects 11.18±2.45 (SD). The difference between these two groups was highly significant ($P<0.001$). Amongst the hyperthyroid patients studied 65% had sodium levels above the highest seen in any control.

In both normal and thyrotoxic subjects duplicate measurements made on the same occasion did not vary by more than 2%. Repeated measurements of cell sodium in the same control subject showed close agreement over a period of weeks or months, findings matching those of Beilin et al. (1966) who used an extremely accurate technique to correct for trapped plasma.

Measurement of cell potassium showed a wider range in normal subjects but there was no significant difference between normal and hyperthyroid ($P>0.45$). Similarly there was no significant difference between the cell water content in the two groups ($P>0.15$). If the difference between the red cell sodium in the two groups had simply been a result of a change in water content, then a very considerable difference in cell water would have occurred and would have
been of the order of 50–100%. Details of our findings are shown in Table 1; those in normal subjects agree well with data from Beilin *et al.* (1966), Awwad & Goolden (1960) and Hutt (1952).

![Graph](image)

**FIG. 1.** The distribution of red cell sodium concentration in m-mole/l red cells for a group of sixty control subjects (●) and twenty patients with hyperthyroidism (○).

**Table 1.** The sodium, potassium and water content of red cells from control subjects and patients with hyperthyroidism (sodium and potassium are expressed as m-mole/l of red cells; water content is expressed as a percentage by weight)

<table>
<thead>
<tr>
<th></th>
<th>Cell sodium</th>
<th>Cell potassium</th>
<th>Cell water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Controls</td>
<td>60</td>
<td>7.04</td>
<td>1.36</td>
</tr>
<tr>
<td>Patients</td>
<td>20</td>
<td>11.18</td>
<td>2.45</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sodium efflux studies**

In all experiments red cells from a hyperthyroid patient were compared with cells from a control subject matched for age and sex. Both were incubated over the same time period and in
the same water bath; both were suspended in incubation medium which had been made up as a single batch. This was done to allow for the variations of efflux which can occur due to minor variation of temperature, pH and purity of such components of the medium as glycyl-glycine.

In all thyrotoxic patients that were studied, the rate constant for active sodium efflux ($k_{\text{Na}}^\text{p}$) was less than in their individual matched control, the difference being greatest when the difference between red cell sodium content in patient and control was greatest. When all patients and all controls were considered together there was a significant difference between the two groups and this is detailed in Table 2. For ten patients, mean active sodium efflux rate constant was 0.215±0.038 (SD) and for ten controls it was 0.284±0.051 (SD). This difference is significant ($P<0.0025$). The rate constant is, of course, the fraction of intracellular sodium extruded per hour; the amount of sodium actually pumped ($M_{\text{Na}}^\text{p}$) can be calculated by multiplying the rate constant by the cell sodium concentration. When this was done the values (expressed as m-mole of sodium pumped litre red cells$^{-1}$ hr$^{-1}$) were for patients 2.55±0.32 (SD) and for controls 2.07±0.18 (SD). This difference was significant ($P<0.001$) but here the hyperthyroid cells were pumping a larger total amount of sodium than controls even though the rate constant was lower.

These differences between normal and thyrotoxic subjects were significant even though three of the patients had cell sodium concentration that was not above the highest level seen in the control group. It seemed as though there was likely to be some sort of correlation between the cell sodium content and the activity of the pump in all the studies we had done. When the rate constant for sodium efflux was plotted against cell sodium concentration there was a striking linear correlation showing an inverse relationship (Fig. 2). A straight line fitted both

<p>| Table 2. Data on sodium efflux for ten patients and their controls (cell sodium is in m-mole/l of red cells; efflux rate constant is the fraction of intracellular sodium extruded per hour, total active efflux is in m-mole sodium pumped per litre of red cells per hour) |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Cell sodium</th>
<th>Efflux rate constant</th>
<th>Total active efflux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Control</td>
<td>Patient</td>
</tr>
<tr>
<td>16.49</td>
<td>7.04</td>
<td>0.178</td>
</tr>
<tr>
<td>13.86</td>
<td>6.83</td>
<td>0.220</td>
</tr>
<tr>
<td>9.45</td>
<td>7.56</td>
<td>0.272</td>
</tr>
<tr>
<td>11.65</td>
<td>4.94</td>
<td>0.219</td>
</tr>
<tr>
<td>12.08</td>
<td>6.93</td>
<td>0.178</td>
</tr>
<tr>
<td>13.23</td>
<td>9.98</td>
<td>0.172</td>
</tr>
<tr>
<td>10.41</td>
<td>10.33</td>
<td>0.191</td>
</tr>
<tr>
<td>12.76</td>
<td>7.63</td>
<td>0.205</td>
</tr>
<tr>
<td>9.71</td>
<td>7.08</td>
<td>0.292</td>
</tr>
<tr>
<td>11.39</td>
<td>7.07</td>
<td>0.220</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.215±0.038</td>
<td>0.284±0.051</td>
</tr>
<tr>
<td>$P$</td>
<td>&lt;0.0025</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
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Fig. 2. The relationship between the red cell sodium concentration and the rate constant for active sodium efflux from the cells in ten patients with hyperthyroidism (■) and their matched controls (●). The line is \( y = 0.413 - 0.0167x; r = -0.81; P < 0.001. \)

Fig. 3. The relationship between red cell sodium concentration and the total amount of sodium actively pumped from the cells; ten hyperthyroid subjects (■) and their matched controls (●) are shown. The line is \( y = 0.0841x + 1.484; r = 0.68; P < 0.01. \)
normal and hyperthyroid subjects, and the higher the rate constant, the lower was the cell sodium concentration. A similar linear correlation was seen for the relationship between total sodium pumped and cell sodium level as is shown in Fig. 3, though on this occasion the relationship was a positive one.

By contrast to the studies of active (ouabain inhibited) transport, the residual efflux was not significantly different in the two groups. Total residual efflux for controls was $0.656 \pm 0.255$ (SD) m-mole l$^{-1}$ hr$^{-1}$ and for patients was $0.799 \pm 0.306$ (SD) m-mole l$^{-1}$ hr$^{-1}$ ($P > 0.45$).

**Table 3.** The rate constant for active sodium efflux measured in normal human red cells loaded and incubated under standard conditions and also in the presence of triiodothyronine 20 $\mu$g/100 ml; four experiments, each on red cells from a different subject

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control</th>
<th>With triiodothyronine</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.287</td>
<td>0.278</td>
<td>-3.1%</td>
</tr>
<tr>
<td>2</td>
<td>0.271</td>
<td>0.267</td>
<td>-1.5%</td>
</tr>
<tr>
<td>3</td>
<td>0.330</td>
<td>0.309</td>
<td>-6.0%</td>
</tr>
<tr>
<td>4</td>
<td>0.230</td>
<td>0.260</td>
<td>+13.0%</td>
</tr>
<tr>
<td>Mean</td>
<td>0.279</td>
<td>0.278</td>
<td>-</td>
</tr>
</tbody>
</table>

**Table 4.** The effect of 22 hr incubation in the presence of ouabain (at $10^{-6}$ M) upon the sodium content of red cells from three normal subjects. In each case incubation was carried out with, and without triiodothyronine (T3) at a concentration of 20 $\mu$g/100 ml. Cell sodium is expressed as m-mole/l of red cells

<table>
<thead>
<tr>
<th>Subject</th>
<th>Resting red cell (Na$^+$)</th>
<th>Cell (Na$^+$) after 22 hr incubation</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without T3</td>
<td>With T3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.0</td>
<td>20.0</td>
<td>20.4</td>
</tr>
<tr>
<td>2</td>
<td>6.7</td>
<td>28.8</td>
<td>27.8</td>
</tr>
<tr>
<td>3</td>
<td>9.2</td>
<td>25.6</td>
<td>24.2</td>
</tr>
</tbody>
</table>

**Sodium influx studies**

Our finding that the resting cell sodium level was very constant over a period of days or weeks led us to assume that such a steady state must imply an even balance between total influx and total efflux of sodium. This assumption was confirmed by a number of experiments in which simultaneous measurements of total influx and efflux were made on cells from the same subject. Ten such experiments were carried out on seven control and three hyperthyroid subjects. Total sodium efflux (i.e. pump and residual efflux) had a mean value of $2.94 \pm 0.58$ (SD) m-mole l$^{-1}$ hr$^{-1}$ whilst mean total sodium influx was $3.05 \pm 0.57$ (SD) m-mole l$^{-1}$ hr$^{-1}$,
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This implied that the cells from patients with hyperthyroidism should have an increase in total influx to match their increase in total pump efflux. This was tested and proved to be the case. In a group of nine control subjects total influx was $2.82 \pm 0.46$ (SD) m-mole $1^{-1} \text{hr}^{-1}$ and for hyperthyroid patients it was $3.40 \pm 0.35$ (SD) m-mole $1^{-1} \text{hr}^{-1}$. The increased level in the hyperthyroid groups was significantly different from the controls ($P < 0.01$).

The effects of thyroid hormone in vitro

Preliminary studies have been carried out to see if tri-iodothyronine (T3) had any effects upon the handling of sodium by normal red cells in vitro. In these studies a concentration of T3 of 20 $\mu$g/100 ml was used; this is a level above the range said to be present in the plasma of thyrotoxic patients.

In one group of experiments it was shown that the rate constant for active sodium efflux was not altered by exposure to T3 for a brief period of 4 hr. These data are detailed in Table 3. In a second group of experiments the net accumulation of sodium within normal red cells in which the sodium pump had been inhibited by ouabain was studied. Sodium accumulated to the same extent whether T3 was present or not (Table 4), and from these we have inferred that T3, in this experimental situation, does not significantly alter the membrane permeability of normal red cells.

The effect of reversing hyperthyroidism

Seven patients whose red cell sodium had been elevated in association with hyperthyroidism were reviewed when they had become clinically euthyroid. In all of them the red cell sodium had fallen to within the normal range, but the rate of this change was not determined. In two patients, serial measurements of sodium efflux confirmed that the rate constant rose to normal as the red cell sodium fell. Attempts to determine the rate of these changes were difficult, since all the patients had a period of antithyroid during therapy and therefore became clinically euthyroid relatively slowly; for this reason the point at which one can start timing the rate of reversal is uncertain. From these preliminary observations it is not possible to determine whether the affected cells recovered or whether they remained abnormal until replaced by new cells from the bone marrow.

DISCUSSION

The maintenance of concentration gradients for small cations across cell membranes is of importance to the integrity of the cell. The human red cell shares the same kind of internal ionic composition as the somatic cells in general, with a low sodium and a high potassium concentration. This means that the red cell may be used as a model for the behaviour of somatic cells which are less readily available for study. This is not true in some other mammals; the dog has a high sodium red cell (Spach & Streeten, 1964) and is thus of little use as a model for studying the sodium pump in the somatic cells in the dog. In the sheep, two genetic variants occur, one with a high and the other with a low sodium red cell (Tosteson, 1963) and this has been a useful situation in which to demonstrate that the amount of sodium in the cell is closely linked to the activity of its sodium pump.

The constancy of cation composition in the human red cell is very striking and is exemplified
by the cell sodium content. This is normally kept within an extremely narrow range of variation in any individual; in our experience the maximum range for a group of normal subjects is from 4.5 to 10.4 m-mole/l, and this closely matches the data of Beilin et al. (1966). A change of cell sodium of only a few m-mole/l of red cells will therefore be readily detectable, and for this reason a change in the activity of the cation pump can be detected when it produces a change in the resting sodium concentration of the cell.

Changes that may occur in disease, however, have only recently been well documented. D'Amico (1958) reported elevated red cell sodium levels in patients with heart failure and some patients with hypertension, but the sodium was measured by the difference between whole and haemolyzed blood, a method now considered to be unreliable. Kessler, Levy & Allen (1961) used a similar technique and reported changes with varying states of oedema and suggested that the red cell sodium fell in oedematous patients in association with hyponatraemia. Using more accurate methods, including the use of \^131I labelled albumin to correct for trapped plasma, Dowben & Holley (1959) reported elevation of red cell sodium in cases of pseudo-hypertrophic muscular dystrophy, but not in other types of myopathy. With a method similar to that described in the present studies, Welt, Sachs & McManus (1964) showed that some patients with severe renal failure had an abnormally high red cell sodium, and these findings were extended by Smith, Welt & Czerwinski (1967) who showed that this was associated with a defect in the sodium pump and a depression of the activity of membrane ATPase. In parallel studies Dunn (1968) has shown similar defects of sodium transport in red cells during the clinical course of malaria.

The first hint that something might be wrong with sodium transport in the red cells of thyrotoxic subjects was provided by Boekelman (1958) who showed that the cell sodium was higher than normal in a small group of hyperthyroid patients. No further studies were carried out at this time to determine the nature of the change in the red cell that brought this about, and no speculative explanation was offered. Later Losse, Wehmeyer & Zumkley (1966) also recorded significantly elevated red cell sodium concentrations in thyrotoxicosis and were able to induce the change in rats by the administration of thyroxine. Our findings would now appear to confirm these in a larger number of patients using a method known to produce results in normal subjects closely comparable to those determined by the most accurate methods worked out by Beilin et al. (1966). The increase of cell sodium was not accompanied by a detectable fall in the potassium concentration and could not be explained on the basis on a fall of cell water content. We therefore postulated that there must have been either a decrease in the ability of the cation pump to drive sodium out of the cell or an increase in the rate at which sodium diffused into the cell. Whichever mechanism was involved its result was to produce a new steady state, at least as far as the cell sodium level was concerned, for its sodium content, although high, remained constant at its new level.

Our data concerning sodium efflux showed that although the total amount of sodium pumped out of the cell in unit time was increased, this was done less efficiently as indicated by the reduced rate constant for efflux. At this point we wondered if the reduction in rate constant was merely a physiological response to an elevation of the sodium concentration inside the cell, and not an indication of a primary defect of the pump. This very question has been answered by Welt et al. (1967) who have shown that if the sodium concentration within red cells from the same subject is varied over a range up to 30 m-mole/l the rate constant for sodium efflux does not fall, and in fact it tends to rise between 10 and 15 m-mole/l. We are therefore justified
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in concluding that the fall in rate constant for sodium efflux seen in red cells from thyrotoxic patients is likely to be the cause and not the effect of a raised resting sodium concentration. Because a new steady state has developed it must be assumed that there is no asymmetry of sodium movement into and out of the cell; efflux and influx must be balanced, but with a greater amount of sodium moving in each direction per unit of time. With a reduced rate constant for active efflux the cell can only pump out as much sodium as diffuses in by accepting a higher resting sodium content. If the primary defect in hyperthyroidism was an increased permeability of the cells to sodium with no defect of the pump, one would not expect the rate constant for pump efflux to fall or the red cell sodium concentration to rise. Such an assumption is accurately borne out by studies in hereditary spherocytosis where an increased permeability occurs as a discrete lesion (Jacob & Jandl, 1964). This seems to offer further justification for our conclusion that the defect we have described is one of the pump.

Because the active efflux of sodium is linked to the active influx of potassium (Hoffman, 1966), one might expect to see a defect of active potassium transport in the red cells of thyrotoxic patients. This has in fact already been studied by Awwad & Goolden (1960) who were, like us, unable to demonstrate any change in resting intracellular potassium content, but did show that the rate constant for potassium influx was clearly reduced in the red cells of thyrotoxic subjects. This is entirely compatible with our findings concerning sodium efflux, and offers strong support for our view that there is a primary defect in the activity of the pump for cations in this situation.

It has been pointed out that, for the red cells of any individual subject, changes of cell sodium do not cause a fall in the rate constant for sodium efflux; however, when we examined all our data from normal and thyrotoxic subjects we found that there was a significant linear correlation between the rate constant for active sodium efflux and the resting sodium content of the cells studied. This would suggest that in a given subject the resting cell sodium level was determined by the innate rate constant of the sodium pump. The thyrotoxic subjects fell on the same line as the controls, indicating that the linear relationship between the pump and the cell sodium could still be maintained when some abnormal situation had depressed pump activity. When this depression was reversed by cure of the hyperthyroidism, the cell sodium fell as the rate constant rose, and the same linear correlation persisted.

The mechanism which results in this abnormality of the pump is not understood. In nucleated cells which contain the oxidative metabolic pathways of the Krebs cycle there is evidence that hyperthyroidism results in an increase of oxygen consumption and a running down of stores of energy rich substances such as ATP. This has been shown to occur in skeletal muscle (Satoyoshi, Murakami & Toni, 1963) and may be an explanation for the impairment of the membrane cation pump in that tissue which may well be a major factor in the development of the myopathy so frequently seen in thyrotoxicosis. Parsons & Ramsay (1968) have reviewed the several sites in the cell where thyroid hormone may exert an effect on metabolic processes and include amongst these a speculative suggestion that membrane ATPase might be regulated in some way. Since the red cell contains no Krebs cycle enzymes one could explain changes in this cell in the course of thyrotoxicosis on the basis of a direct change in ATPase activity in its role as part of the sodium pump; however, our preliminary studies have shown no effect of triiodothyronine upon ATPase in vitro and no effect due to this agent on the sodium pump in vitro either. Clearly this does not mean that it has no effect when acting in vivo over a prolonged period, as has been suggested by the work of Losse et al. (1966). Certainly there is no evidence in human red cells that the ATP stores become depleted and thus reduce the supply of energy to the pump;
a few observations of our own confirm the data of Matsuda (1966) in demonstrating normal levels of ATP in red cells from patients with hyperthyroidism.

Abnormalities of enzymes have been demonstrated in the red cells of thyrotoxic patients. Weatherly & McIntyre (1968) have shown a striking deficiency of carbonic anhydrase in this situation, an abnormality that was reversed when hyperthyroidism was cured. There is thus a precedent for the suggestion that changes in enzyme activity might underlie metabolic abnormalities concerning the sodium pump.

At present, therefore, we have no explanation for the defect we have shown to occur. We have not been able to reproduce it with thyroid hormone in vitro and it remains to be seen if we can induce it in vivo by the administration of thyroid hormones to intact animals. We consider that a continued study of the way in which a relatively simple cell such as the human erythrocyte handles small cations in abnormal metabolic states may be most rewarding in understanding more about the way in which the sodium pump is controlled. Hyperthyroidism may be a valuable model in this respect.

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REFERENCES


Erythrocyte sodium in hyperthyroidism


