MUSCLE BIOPSY WATER AND ELECTROLYTE CONTENTS IN CHRONIC RENAL FAILURE

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SUMMARY

1. Water and electrolyte status in chronic renal failure has been measured by skeletal muscle biopsy. These results have been compared with a series of muscle biopsy specimens taken from the same muscle (vastus lateralis) of normal subjects.

2. The water content, especially of the extracellular phase, is increased in chronic renal failure: the extracellular sodium and chloride contents are also increased. These changes tend to be more marked as the severity of renal failure increases.

3. The intracellular water and potassium contents in chronic renal failure are little different from those in normal patients.

4. A significant correlation between diastolic blood pressure and extracellular water content is shown and this is especially marked in more severe degrees of renal impairment.

5. These results support the belief that hypertension in chronic renal failure is salt and water dependent, and that hypertension in these patients can be controlled by removal of salt and water.

Measurement of body water and intracellular electrolyte content by muscle biopsy is now widely used (Bergström, 1962; Flear, Carpenter & Florence, 1965; Graham, Lamb & Linton, 1967; Flear, Florence & Williams, 1968). This method has been used to study changes in water and electrolyte status in patients with varying degrees of chronic renal failure, excluding those on regular dialysis treatment. Previous studies have suggested that most patients with chronic renal failure show an increase in extracellular water and total body sodium (Moore et al., 1963; Comty, Rottka & Shaldon, 1964; Bittar et al., 1962; Blumberg et al., 1967; Bergström & Hultman, 1969); the effect of renal impairment on intracellular water and potassium is more difficult to evaluate. Moore et al. (1963), using isotope dilution techniques, found low total exchangeable potassium values, but this may in part be due to loss of lean body mass in these patients. Using muscle biopsy techniques, Villamil et al. (1963) and Campanacci...
et al. (1967) found low cell potassium contents, while Bergström & Hultman (1969) found the reverse. Bittar et al. (1962) obtained variable results. In the present study, we have attempted to elucidate this problem by relating changes in intracellular water and electrolytes to the degree of renal impairment and have investigated the relationship between blood pressure and water and electrolyte levels.

PATIENTS AND METHODS

Twenty-two patients with chronic renal failure were studied. All had elevated serum creatinine concentrations (2.5–16.3 mg/100 ml), and endogenous creatinine clearances ranging from 3 ml/min to 20 ml/min. All patients were taking a modified Giordano-Giovannetti diet providing either 18 g or 40 g of protein. Two were receiving anti-hypertensive drugs and eight diuretics at the time of the study. Blood pressures were taken regularly under steady state conditions.

Twenty-nine patients with normal renal function who had been admitted to hospital for minor elective surgical procedures were studied as controls. All had normal blood urea and serum electrolyte values, and patients with any disorder likely to produce electrolyte imbalance were excluded. The details of the investigation were explained to each patient and free consent obtained.

All biopsy specimens were taken from the vastus lateralis, since Flear et al. (1965) showed small variations in the water and electrolyte content of different muscle groups in the body. Most biopsies were obtained under local anaesthesia, but occasionally under general anaesthesia; general anaesthesia has been shown not to affect water and electrolyte content if the muscle is obtained soon after induction of anaesthesia (J. A. Graham, unpublished observations).

The chloride content of the biopsy specimens was used as an indicator of the extracellular volume. The chloride content of the cells is easy to determine since chloride is passively distributed between the cells and the extracellular water according to the membrane potential of the cells (Conway, 1957; Hodgkin & Horowicz, 1960). For a constant membrane potential, the extracellular concentration of chloride bears a linear relationship to the internal concentration, which can therefore be calculated from the Nernst equation, assuming a constant membrane potential. The membrane potential in this investigation was taken as $-85 \text{ mV}$ (Elmqvist et al., 1964). Once the internal chloride concentration is calculated, the extracellular water in the specimen can be calculated from the equation:

$$W_e = \frac{Cl_i - W_e(Cl)_i}{(Cl)_e - (Cl)_i}$$

$W_t$, $W_e$ = total and extracellular biopsy specimen water
$Cl_i$ = total chloride content of biopsy
$(Cl)_e$, $(Cl)_i$ = extracellular and intracellular chloride concentrations.

Once the extracellular water content is known the total biopsy specimen electrolyte contents can be easily partitioned into extracellular and intracellular phases.

RESULTS

The mean values for muscle water and electrolyte content in normal patients are shown in Table 1. Each biopsy specimen result is the mean of triplicate estimations, but analysis of
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variance showed that the standard deviation found is largely due to a real difference between individual subjects. Table 2 shows the results obtained in each of the patients with chronic renal failure and, in addition, gives details of the patients' serum creatinine concentration and diastolic blood pressure. It can be seen that there is a tendency towards an increase in both total water and extracellular water, probably becoming more marked in the patients in the later stages of the disease. The intracellular water is not significantly increased in any patient, but is decreased in four patients. Likewise the sodium content of the biopsy is significantly increased in eight cases, and the chloride content in eleven. Despite this, all patients had normal

<table>
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<th>Value</th>
<th>Total</th>
<th>Extracellular</th>
<th>Intracellular</th>
<th>Sodium</th>
<th>Potassium</th>
<th>Chloride</th>
<th>Sodium</th>
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<td>2443</td>
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<tr>
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<td>159</td>
<td>190</td>
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<td>36</td>
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or low serum sodium concentrations, suggesting that extracellular water was increased to a greater degree than sodium. The total potassium contents of each biopsy specimen were normal in all but three patients who had reduced potassium contents. These patients had reduced intracellular water, and none was on diuretics.

Figs. 1 and 2 demonstrate significant correlations between extracellular water and sodium content, and between intracellular water and potassium content. These relationships have already been shown in normal subjects (Graham et al., 1967; Moore et al., 1963) and regression lines for the normal series are not significantly different from those in the present series of patients with chronic renal failure.

A significant correlation was found between diastolic blood pressure and biopsy extracellular water content (Fig. 3), and this relationship became even more significant in the thirteen patients with serum creatinine concentrations greater than 8 mg/100 ml (Fig. 4). No correlation could be found between blood pressure and biopsy sodium content.

The mean serum sodium concentrations in the patients with diastolic blood pressures greater than 90 mmHg was $137 \pm 6$ mEq/l, and for those with diastolic blood pressures less than 90 mmHg was $143 \pm 4$ mEq/l ($t = 2.78; P < 0.02$).

Comparison of the results found in patients receiving diuretics with those not receiving such therapy did not reveal any significant differences.

**DISCUSSION**

The significance of these observations depends on the validity of the methods. Dow & Irvine (1967) have suggested that analysis of chloride in acid extract of muscle tissue gives an overestimate due to the presence of sulphydryl groups, but Flear, Pickering & McNeill (1969) have repeated this work and have found no evidence for this. Dow & Irvine (1967) claimed that the
<table>
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<tr>
<th>Patient</th>
<th>Biopsy specimen water (ml/kg dry weight)</th>
<th>Total biopsy specimen electrolytes (mEq/kg dry weight)</th>
<th>Intracellular electrolytes (mEq/l)</th>
<th>Serum creatinine concentration (mg/100 ml)</th>
<th>Diastolic blood pressure (mmHg)</th>
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</table>
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**Fig. 1.** Correlation between sodium content and extracellular water in biopsy specimens of patients with chronic renal failure (CRF). Regression line for the normal series also shown.

\[ y = 6.8x - 179.7 \]
\[ r = 0.75, p < 0.001 \]

**Fig. 2.** Correlation between potassium content and intracellular water in biopsy specimens of patients with chronic renal failure. Regression line for the normal series also shown.

\[ y = 5.6x + 19.6 \]
\[ r = 0.77, p < 0.001 \]
percentage error in chloride estimation in muscle increased as the sample size decreased; our own observations do not support this view.

Our method of calculating the extracellular water of the biopsy specimens is accurate only if it is assumed that the cell membrane potential does not vary by more than \( \pm 10 \) mV from our arbitrary figure of 85 mV. Flear et al. (1968) contend that membrane potential measurements in individual muscle fibres in normal subjects may vary by as much as 34–48 mV and
rightly make the point that variations of this magnitude will introduce serious error into
calculation of intracellular chloride concentrations. However, two important qualifications to
this must be made. Firstly, it is to be expected that in electrophysiological measurements there
is bound to be variation around the mean; obviously a muscle biopsy will sample a large num-
ber of individual fibres with variable membrane potentials and therefore different intracellular
electrolyte concentrations. The important finding will be the mean membrane potential of the
individual fibres and the mean intracellular electrolyte concentrations of these fibres. Secondly,
the variation in individual fibre membrane potential quoted by Flear et al. (1968) comes from
papers by Creutzfeldt et al. (1963) and Johns (1958). In these papers membrane potential
recordings were taken from muscle fibres in vivo and the authors stress the difficulties and
possible limitations of this method. Emlqvist et al. (1964) have produced an elegant and com-
pletely non-traumatized preparation of human intercostal muscle which they are able to
perfuse in vitro with oxygenated physiological solutions, during recording. As a result of this
the 95% confidence limits in the results are only 8.8 mV on either side of the mean. Finally, it
is possible that cell membrane potentials may be abnormal in uraemic subjects. Although it is
well known that many biochemical systems can be upset by uraemic plasma, it is not known
how many of these significantly affect the patient’s cells. With regard to membrane potential,
it has been shown that injection of uraemic plasma into the squid axon does not change the
membrane potential (Bittar, 1967). Furthermore, in uraemic subjects, there is no significant
change in intracellular pH (Maschio et al., 1969) nor in sodium flux across the jejunal wall
(Sraear & Ardaillou, 1969). Both these facts suggest that active transport systems are function-
ing normally in uraemia, and such mechanisms are important in the maintenance of normal
membrane potential.

Our results suggest that as chronic renal failure becomes more severe, there is increasing
retention of water, sodium and chloride, resulting in an expansion of the extracellular space.
Water retention tends to be greater than sodium retention, and serum sodium levels are usually
normal or low. Intracellular water and electrolyte contents remain almost normal except that
occasionally there may be a reduction in cell potassium content, which is compensated for by
an equivalent reduction in intracellular water, maintaining the intracellular potassium con-
centration within normal limits. This mechanism has also been shown in normal subjects
(Graham et al., 1967).

Despite doubt over the basic mechanisms, it is generally held that hypertension, at least in
the more severe degrees of chronic renal failure, is intimately related to retention of water and
salt (Lancet, 1969; Shaldon, 1966). Our findings of a good correlation between diastolic blood
pressure and extracellular water content in the biopsies support this view. The fact that this
relationship is more direct in the later stages of renal failure raises the possibility that factors
other than simple water retention are concerned in the aetiology of hypertension in mild to
moderate renal failure (Lancet, 1968). Although failure to demonstrate a relationship between
diastolic blood pressure and total biopsy sodium content seemed surprising, there is other
evidence to suggest that hypertension in severe degrees of chronic renal failure may depend
more on water retention than on sodium retention. Merrill, Giordano & Heetderks (1961)
noted that hypertension developed in renoprival patients when they became overhydrated,
and that these patients often developed hyponatraemia during the hypertensive episodes.
Shaldon (1966) found that although inter-dialytic weight gain (i.e. fluid retention) in patients
on regular dialysis was closely related to hypertension, increased salt intake was not necessary
for the rise in blood pressure to occur. He showed that hyponatraemia often occurred when excessive inter-dialytic fluid intake had produced a rise in blood pressure. Vorburger et al. (1969) using multiple isotope techniques demonstrated that in patients with chronic uraemia, hydration correlated well with changes in blood pressure. The evidence obtained from muscle biopsy in this paper suggests that in the later stages of chronic renal failure extracellular water retention predominates over salt retention, with the production of a dilutional hyponatraemia and that hyponatraemia is more common in the hypertensive patients. There is certainly little doubt that efficient removal of the retained water and salt by regular dialysis (Shaldon, 1966) or by renal transplantation (Papadimitriou, Chisholm & Shackman, 1969) will control the hypertension in the majority of these patients.

REFERENCES


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