the NAG index thus seems to have a high specificity.

Moreover, the clinical significance of NAG isoenzymes has also been examined and expectations of their future usefulness are good (7–10). Severini et al. (7) showed that the proportion of the B form in urine of diabetics with vascular changes was significantly higher than in control.

In conclusion, the present method is easily applicable to various types of automated analyzers and allows fast determination with good reproducibility. It should be useful in view of the prospect that NAG activity will be increasingly determined in the future.

References

Hyponatremia in Patients Admitted to a Coronary Care Unit
John R. Evans,† John P. McIntosh,‡ H. John McIntosh,§ and Peter E. G. Mitchell‖

Calculation of osmolal gap in plasma and urine samples from patients after acute myocardial infarction was carried out to see whether changes in these variables support the concept of a "sick-cell" mechanism being responsible for the hyponatremia associated with acute myocardial infarction. Some supportive evidence was found on the first day after admission but, overall, the evidence was not convincing.

Additional Keyphrases: acute myocardial infarction • osmolal gap • urine

Systemic metabolic changes follow acute myocardial infarction including increased plasma concentrations of catecholamines, cortisol, renin, angiotensin II, and arginine vasopressin (AVP) (1–6).

Flear and Hilton (7) showed that hyponatremia is common in patients with confirmed AMI and that the degree of illness correlates roughly with the decrease in plasma sodium. The hyponatremia is transient, being greatest on day 3 or 4 after admission, then returning towards first-day plasma sodium concentrations by day 6 or 7. The average decrease in plasma sodium is about 5 mmol/L in the most severely affected group, with smaller changes in the less-affected patients. Flear and Hilton (7) thought this hyponatremia could be caused by a redistribution of body water and solutes, due to a widespread increase in cell-membrane permeability (i.e., the "sick-cell syndrome") or to a net gain of water caused by a stress-related sustained secretion of AVP.

We undertook this study to seek evidence for the involvement of the sick-cell syndrome mechanisms in the pathogenesis of hyponatremia in AMI, in particular by measurement of osmolal gaps in plasma and urine.

Patients and Methods

Patients

We studied 57 patients who were admitted with chest pain and suspected AMI; patients diagnosed as having other serious illnesses were not included. The patients studied received therapy as indicated clinically. There were no restrictions on oral fluid intake. Each patient was assessed clinically and placed into one of three groups according to the following criteria:

- Group 1 (24 patients)—no definite evidence of infarct, as assessed clinically, and without electrocardiograph changes or increased plasma concentrations of aspartate aminotransferase (AST) and a-hydroxybutyrate dehydrogenase (HBD) activities.
- Group 2 (18 patients)—definite evidence of infarct, with electrocardiograph changes and increases in plasma concentrations of AST and HBD activities, but without any of the additional criteria specified for Group 3.
- Group 3 (15 patients)—definite evidence of infarct, with electrocardiographic changes and increases in the concentrations of AST and HBD activities in plasma, plus any of the following specific criteria: HBD >1050 U/L (3 × upper reference limit), systolic blood pressure <100 mmHg, left or right ventricular failure, cardiogenic shock, worsening grade of failure, ventricular arrhythmias requiring therapy, or cardiac arrest.
Heparinized blood samples and an untimed sample of urine were obtained on the morning of days 1, 2, and 3 after admission. The first available morning urine sample was collected. Normally, this was the overnight pre-breakfast urine, but if this specimen was not obtained, then the next available specimen was sent to the laboratory. A preliminary study of 26 patients showed that there were no significant differences in urine sodium, potassium, chloride, urea, creatinine, osmolality, or osmolar gap between the preprandial overnight urine and the second morning urine specimens. All urine samples were tested for glucose with Clinistix—any patients with a positive urine glucose were not included in the study, because of an effect of urine glucose on urine osmolal gap comparisons (8–10).

Methods

Plasma sodium and potassium were measured by flame emission spectrophotometry (IL 543 spectrometer; Instrumentation Laboratory, Lexington, MA). Total CO₂ was measured by generation of carbon dioxide with acid and diffusion into sodium bicarbonate with phenolphthalein as a pH-change indicator (11). Urea was measured by reaction with diacetyl monoxime in the presence of thiosemicarbazide under acid conditions (12), and creatinine was measured by the Jaffe reaction (13). Chloride was measured electrometrically with a Corning 925 chloride meter (Corning Medical, Medfield, MA). Osmolality was measured by freezing-point osmometry (Roebeling osmometer). Phosphate was measured by reaction with molybdate and reduction of the resulting phosphomolybdc acid to molybdenum blue (14). Plasma glucose was measured with a glucose-selective electrode (Model 23AM glucose analyzer; Yellow Springs Instrument Co., Yellow Springs, OH). Sodium, potassium, chloride, osmolality, urea, creatinine, and phosphate were measured in urine by the same methods as used for plasma, with prior dilution with distilled water where appropriate. Urine ammonia was measured by the Berthelot indophenol method (15).

Osmolar gaps were calculated as measured osmolality (mmol/kg) minus a linear function of some of the measured concentrations of sodium, potassium, glucose, urea, and chloride (all measured in mmol/L). As in previous publications (8, 16), the units of osmolar gap were called mmol/kg. The osmolar gap in plasma was calculated as follows: measured osmolality − (2Na + 2K + glucose + urea) (16).

We determined the reference range to be +2 to −16 mmol/kg in pre-surgical blood samples from 30 patients undergoing minor surgery.

The osmolar gap in urine was measured as follows: measured osmolality − (Na(urate) × creatinine/(plasma)/creatinine(urine), where all the concentrations are in mmol/L. Potassium excretions were calculated in the same way.

Means of two sets of data were compared by a t-test in which one does not assume equal variances in the two sets of data (17). Correlations between sets of data were analyzed by the least-squares method of linear-regression analysis (18).

Results

Plasma and urine results. The plasma sodium decreased most markedly in Group 3 (Figure 1A), whereas the urine sodium concentrations were not significantly different between the groups (Figure 1B).

The plasma potassium values for Groups 2 and 3 tended to be higher than those for Group 1 on day 1, then decreased from day 1 to day 3 for these groups (Figure 1C). The urine potassium concentrations were higher in Groups 2 and 3 than in Group 1 on day 1; thereafter, they decreased to the same values as Group 1 (Figure 1D).

Although the plasma osmolar gaps were not increased, and were similar in all groups (Figure 1E), the urine osmolar gaps in groups 2 and 3 increased those in Group 1 and the upper reference limit on day 1 (Figure 1F). The urine osmolar gaps in Groups 2 and 3 decreased into the reference range on days 2 and 3, but the Group 3 mean value was still significantly higher than the Group 1 value on day 3.

The urine osmolalities were also higher in Group 2 and 3 than in Group 1 over the three days, but in all groups the urines were concentrated relative to plasma osmolalities (Figures 1G and 1H). The urine osmolar gap/osmolality ratios were significantly increased (P < 0.05) in Groups 2 and 3 relative to Group 1 on day 1, indicating that the greater osmolar gaps could not be attributed to the urines being more concentrated in Groups 2 and 3 relative to Group 1. This observation was true also for Group 3 relative to Group 1 on day 3.

For all patients, the decrease in plasma sodium from day 1 to day 3 and the value of the plasma sodium on day 3 (the dependent variables) were correlated against all the other variables measured for each of the three days of study. The relevant significant regressions (P of slope <0.05) are shown in Table 1.

Effect of diuretics and intravenous dextrose. Diuretics were given to some patients in all groups; intravenous dextrose (mean 2.6 L, SD 1.3 L, over three days) was given to some patients in group 3 only, to administer dobutamine. The effect of these treatments on the dependent variables in the regression analysis was assessed by t-tests. We found no significant differences in these variables between diuretic-treated and untreated patients in any group, nor between dextrose-treated and -untreated patients in Group 3.

Contribution of ammonia, phosphate, and creatinine to osmolar gaps in urine. We analyzed for these analytes in 50 urine samples (which had been stored at −20 °C) known to have increased urine osmolar gaps. For 22 samples with osmolar gaps between +2 and +3 SDs of the reference group (59–86 mmol/kg), the mean percentage contribution of the sum of the three analytes to the urine osmolar gaps was 82.4% (SD = 27.5). For 28 samples with osmolar gap greater than +3 SDs of the reference group (87 mmol/kg or greater), the mean percentage contribution of the sum of the three analytes was 63.8% (SD = 18.7). Student's t-test of the difference between the means gave t = 2.84 and P <0.01.

Discussion

The equation for plasma osmolar gap used in this work has been published previously (16), but the equation for urine osmolar gap was developed for this work (8). For both equations a calculated osmolality was subtracted from the measured osmolality.

For urine, the calculated osmolality was based on the
Fig. 1. Changes in sodium, potassium, osmolal gap, and osmolality in plasma and urine of patients on the three days after admission

- Group 1: A, Group 2: B, Group 3: A, plasma sodium; B, urine sodium; C, plasma potassium; D, urine potassium; E, plasma osmolal gap; F, urine osmolal gap; G, plasma osmolality; H, urine osmolality. Significant differences (P < 0.05) were as follows: A, Group 3 vs Group 1 on days 2 and 3; B, Group 2 vs Group 1 on day 1; Group 2, day 1 vs day 2; Group 3, day 1 vs day 2; Group 3, day 1 vs day 3; Group 1, day 1 vs day 3. F, Group 2 vs Group 1 on day 1; Group 3 vs Group 1 on day 3; Group 2, day 1 vs day 2. H, Group 2 vs Group 1 on day 1

Table 1. Significant Linear-Regression Data (P(slope) < 0.05) for the Change in Plasma Sodium (Na) from Day 1 to Day 3 and the Value of Plasma Sodium on Day 3

<table>
<thead>
<tr>
<th></th>
<th>y</th>
<th>x</th>
<th>r</th>
<th>P(slope)</th>
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<tr>
<td>Na(3) - Na(1)</td>
<td>Kp(1)</td>
<td>-0.273</td>
<td>0.040</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Kp(2)</td>
<td>-0.405</td>
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<tr>
<td></td>
<td>Kexc(2)</td>
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<td>0.012</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>K(2)</td>
<td>-0.390</td>
<td>0.012</td>
<td>0.011</td>
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<tr>
<td></td>
<td>Urea(1)</td>
<td>-0.313</td>
<td>0.018</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
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<td>0.0002</td>
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<tr>
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<td>Urea(3)</td>
<td>-0.396</td>
<td>0.011</td>
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<tr>
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<td>Osm(2)</td>
<td>0.425</td>
<td>0.001</td>
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</tr>
<tr>
<td></td>
<td>Osm(3)</td>
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<td>0.001</td>
<td>0.000001</td>
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<tr>
<td></td>
<td>OGp(2)</td>
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<td>Urea(2)</td>
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<td>0.0001</td>
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</table>

Kexc: mmol of K excreted; OGp: osmol gap in plasma.

The relative contribution of the sum of ammonia, creatinine, and phosphate concentrations decreased as the urine osmolar gap (as defined for this work) increased. Hence, because the relative contribution of unknown solutes to the osmolar gap increased with increasing osmolar gap, a new definition of osmolar gap that included ammonia, creatinine, and phosphate would give the same pattern of results shown in Figure 1, i.e., increased osmolar gaps in the more severely affected patients.

In this study we classified the patients into three groups according to clinical severity. The greatest decrease in plasma sodium occurred in the most severely affected patients (Group 3, Figure 1A) as observed previously (7). On day 1, Groups 2 and 3 had higher plasma potassium and higher urine osmolar gap values than did Group 1 (Figures 1A and 1B). The urine osmolar gap values in Groups 2 and 3 tended to remain higher than those in Group 1 on days 2 and 3, but only day 1 values in these two groups exceeded the upper reference limit.

The decrease in plasma sodium over the three days of study was correlated, weakly, to some potassium variables over days 1 and 2 (Table 1), so that the greater decreases in plasma sodium correlated with higher plasma potassium, potassium excretion, and urine potassium concentration on day 2. Thus, developing hyponatremia may be related to potassium movement from the extracellular fluid to the intracellular fluid, with subsequent excretion in the urine; this could be taken as evidence for the sick-cell concept.

summation of sodium, potassium, chloride, and urea concentrations. Others have pointed out that other analytes (e.g., ammonia, creatinine, and phosphate) could have been included in the calculated osmolality (9, 10); however, the study of urines with increased osmolar gaps showed the
However, the lack of correlation of the plasma sodium on day 3 with any of the potassium indices tends to invalidate the sick-cell concept.

Although we found no increased osmolal gaps in plasma (Figure 1E), Groups 2 and 3 on day 1 showed increased osmolal gaps in urine (Figure 1G), which could be taken as evidence for sick-cell concept. However, again, the concept was not supported by the lack of correlation of the value of the plasma sodium on day 3 with the osmolal gap in urine. Also, there was no correlation of the decrease in plasma sodium with the osmolal gap in urine or plasma, although the plasma sodium on day 3 did correlate with the osmolal gap in plasma on day 2 (Table 1).

In theory (19), the osmolal gap and potassium data should produce the strongest evidence for the sick-cell mechanism. On this basis, the data in this study have not produced convincing evidence for this mechanism operating in the development of hyponatraemia after AMI. If the sick-cell mechanism is operating at all, this study suggests the most likely time is during the first day of the illness.

The other mechanistic possibility is that there is a sustained release of AVP over the three days after AMI; some recent studies (4–6, 20) support this view.

We thank Dr. J. A. Tulloch for permission to study his patients, and Christine Bell, Lindsay Crowe, Joe Gibb, Graham Matthew, and Avril Nee for carrying out the majority of the assays. We thank the medical and nursing staff of the Coronary Care Unit, Strathclyde Hospital, for collecting blood and urine samples for this study.

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18. Ibid. 158–62.

Falsely Low Estimation of Triglycerides in Lipemic Plasma by the Enzymatic Triglyceride Method with Modified Trinder’s Chromogen

Mark D. S. Shephard and Malcolm J. Whitting

The enzymatic assay of triglyceride, based on the use of L-glycerol-3-phosphate oxidase (EC 1.1.3.21) and a modified Trinder's chromogen involving 4-chlorophenol, is subject to strong negative interference at concentrations of triglyceride >20 mmol/L, such as occur in grossly lipemic plasma. This interference is caused by the rapid utilization of oxygen, resulting in the reaction becoming transiently anaerobic. The dye product already formed may then be reduced ("bleached") by acting as an alternative electron acceptor for glycerol-3-phosphate oxidase. Reduction of the dye leads to a marked decrease in final absorbance at 505 nm. Grossly underestimated values for triglyceride concentrations, apparently within the linear range of the assay, may therefore be inadvertently obtained with equilibrium methods. We suggest that samples giving unexpectedly low results for lipemic plasma should be re-assayed after dilution or with use of a smaller volume of sample.

Additional Keyphrases: glycerol-3-phosphate oxidase • electron acceptor • indicator dye

Colorimetry of hydrogen peroxide by use of peroxidase-linked chromogenic systems is an indicator reaction used