Method 1. Method 2 is, however, faster and more convenient.

The secondary advantage of either JET method is the decreased technician working time required for the extraction as compared to XAD-2, charcoal, or liquid–liquid extraction protocols. Although any time-study is subject to significant error, our subjective impression of greater technologist efficiency is reinforced by the observation that, after the change from XAD-2 extraction to the longer JET (Method 1) procedure, one technologist, in 6 h, could perform the work previously done by two technologists in 13 h. This more than compensates for the higher material cost of the JET tubes over the XAD-2 resin. Based on these factors, we now use the faster Method 2 for our large-volume routine urine drug-abuse assays and reserve Method 1 for those cases that require extraction at low or very high pH.

We thank Thomas Evans of Har-len Associates and Dr. Phillip Harris of Manhattan Instruments for providing the radiolabeled drugs.

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Posture and the Composition of Plasma

Marian Dixon and Colin R. Paterson

For a group of normal medical students, we examined the effect of posture on the concentration of a number of constituents in plasma. On standing, there is a significant increase in plasma total protein, albumin, calcium, alkaline phosphatase, bilirubin, and cholesterol—all proteins or substances bound to protein. Although it is possible to make an allowance for postural variations in plasma calcium, no correction is possible for changes in protein concentrations and care is needed whenever the precise values are important, as in the follow-up of patients with the nephrotic syndrome.

Additional Keyphrases: plasma calcium • corrected plasma calcium • nephrotic syndrome • variation, source of

As long recognized, there are substantial physiological changes when a person lies down or stands up. When one stands, the venous pressure in the lower parts of the body rises, the capillary pressure increases, and plasma is ultrafiltered into the interstitial space. One consequence is an increase in the concentration of those constituents in plasma, such as cells and protein molecules, which do not readily pass the capillary endothelium. In addition, concentrations of substances that are wholly or partly bound to protein in the plasma increase. Earlier work (1–4) demonstrated the considerable changes in plasma calcium, plasma protein, and plasma cholesterol that result from a change in posture. We have now examined the effect of change in posture on a wider range of normal plasma constituents and also used the results to determine a factor for the "correction" of plasma calcium.

Methods

The subjects were 12 healthy students, eight men and four women, ages between 20 and 25 years. A plastic butterfly needle was inserted into a vein in the cubital fossa, and 10 ml of blood was drawn at 10-min intervals through a three-way tap; at other times the needle was filled with a dilute solution of heparin (1000 int. units/ml).

The subjects first stood for 30 min with as little movement as possible. For the next 60 min they lay supine, without a pillow, and for a final 30-min period they again stood up. The first blood sample was obtained 10 min after the start of the initial period of standing; in all, 12 samples were obtained from each subject.

Total calcium, total protein, albumin, alkaline phosphatase, inorganic phosphate, bilirubin, and creatinine were measured by standard automated techniques with the Vickers M300 (5). The alkaline phosphatase method was based on that of Kind and King (6). Cholesterol was determined by an automated continuous-flow method (7) and plasma ultrafiltrable calcium by cone centrifugation followed by atomic absorption spectroscopy (8).

References

5. Johnsen, V. J., Group extraction of organic compounds present in liquid samples. U.S. Pat. no. 3966140 (1976).
Results

Figure 1 shows the mean values for total calcium, albumin, and total protein in plasma. All the values change together, declining when the subject lies down, increasing on standing up. The changes in each are almost complete at 10 min and complete at 20 min. In four subjects ultrafiltrable calcium was also measured; there was no change.

We compared, for each subject and each plasma constituent, the mean of the four values after at least 20 min of standing up (10, 20, 100, and 110 min) with that of the five values after at least 20 min of recumbency (40, 50, 60, 70, and 80 min). The results are shown in Table 1. The changes in the concentrations in plasma of total protein, albumin, total calcium, alkaline phosphatase, bilirubin, and cholesterol are significant. Changes in the direction indicated were seen in every subject for total protein, total calcium, alkaline phosphatase, and cholesterol. All but one of the subjects had changes in albumin and bilirubin values.

Discussion

Although the effect of posture on plasma calcium has been amply demonstrated (1-4) it seldom is considered in assessing the results of plasma calcium estimations. Most clinicians take care to obtain blood samples from fasting subjects, and without the use of tourniquet, but neither restriction influences the result of the assay as much as posture (9, 10). Indeed, one of our subjects had, while upright, three results for plasma calcium that exceeded the reference range used in this department. Recognition of the effect of posture is of importance when a patient is having regular plasma calcium estimations, for example in a steroid suppression test, or when an inpatient is subsequently followed as an outpatient.

In this study, values were not determined for subjects in a sitting position. Husan et al. (3) found that plasma calcium and total protein values in seated subjects were intermediate between the values obtained while upright and while recumbent.

The lack of change in plasma ultrafiltrable calcium values with posture is consistent with the mechanism of the postural changes described earlier. Methods for plasma ionized and ultrafiltrable calcium are not yet widely available and in the meantime the plasma total calcium had to be "corrected" for plasma protein. The best method for doing this remains controversial (11-14), but clearly depends on the methods used by a laboratory for both calcium and albumin estimations. The present study provides an opportunity for examining the relationship between plasma albumin and plasma calcium under physiological conditions. Table 1 shows that a change of 0.1 mmol/liter in total plasma calcium corresponds to a change of 6.0 g/liter in albumin or that 17 μmol of calcium per liter is equivalent to 1 g of albumin per liter. This is very similar to the figure of 18 μmol/liter obtained by a quite different method by Orrell (11).

While it is possible to make an allowance for postural changes in plasma calcium, no correction is possible for the changes in other measurements. The changes in plasma bilirubin and alkaline phosphatase are small relative to the normal range. The postural changes in total protein and albumin

![Figure 1. Mean values for total protein, albumin, and total calcium in the plasma of 12 subjects, and plasma ultrafiltrable calcium in four subjects](image)

Table 1. Composition of Plasma After at Least 20 Min Upright and After at Least 20 Min Supine

<table>
<thead>
<tr>
<th></th>
<th>Mean upright</th>
<th>Mean supine</th>
<th>Difference</th>
<th>t</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/l)</td>
<td>74.9</td>
<td>67.7</td>
<td>7.2</td>
<td>12.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>48.6</td>
<td>43.5</td>
<td>5.1</td>
<td>1.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>2.44</td>
<td>2.33</td>
<td>0.011</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alkaline phosphatase (KA units)</td>
<td>7.51</td>
<td>6.73</td>
<td>0.78</td>
<td>10.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Inorganic phosphate (mmol/l)</td>
<td>0.97</td>
<td>1.02</td>
<td>-0.05</td>
<td>0.14</td>
<td>1.23</td>
</tr>
<tr>
<td>Bilirubin (μmol/l)</td>
<td>10.10</td>
<td>8.61</td>
<td>1.49</td>
<td>2.59</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.74</td>
<td>4.88</td>
<td>0.86</td>
<td>4.82</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>104.9</td>
<td>104.6</td>
<td>0.3</td>
<td>22.7</td>
<td>&gt;0.5</td>
</tr>
</tbody>
</table>

* The significance of the difference was determined using the paired t-test.
concentration are large as compared with their normal range of values. Errors in the interpretation of these results are potentially important, for example in the followup of patients with the nephrotic syndrome or cirrhosis. We recommend that, when the precise result is of importance, the patient's posture on each occasion should be the same for the 20 min before venepuncture.

We are greatly indebted to our subjects for their patience and support, to Mrs. Joan Merry and Mr. Neil Wyllie for their technical help, and to Professor G. H. Bell for comments on the manuscript.

References


N-Ethylmaleimide Prevents Destruction of Corticotropin (ACTH) in Plasma

William Jubiz and George Nolan

Addition of N-ethylnaleimide, an inhibitor of proteolytic enzymes, to samples for plasma corticotropin determinations prevents destruction of the hormone at room temperature for at least 72 h. A concentration of 125 mg per liter of blood is effective. N-Ethylmaleimide is not so effective in preventing corticotropin degradation in whole blood. Use of the inhibitor should make plasma corticotropin determinations more practical and reliable.

Additional Keyphrases: sample handling • conditions for shipping and storage • hormones

Availability of plasma corticotropin (ACTH) radioimmunoassays has contributed enormously to our understanding of the pituitary–adrenal axis under physiological and pathological conditions (1). However, proper collection and preservation of the plasma samples constitute a major drawback to accuracy. To minimize destruction by proteolytic enzymes, blood specimens must be centrifuged at 4 °C and the plasma separated immediately and kept frozen. Small hospitals, clinics, and physicians working in rural communities may lack the facilities for proper processing of the specimens. Moreover, there are obvious difficulties when samples must be shipped elsewhere for assay. A way to obviate these difficulties would greatly facilitate plasma corticotropin radioimmunoassays.

Here, we demonstrate that N-ethylmaleimide, an enzyme inhibitor (2), preserves corticotropin in human plasma for as long as 72 h.

Methods

Blood was collected from five normal subjects (three men and two women, 39–45 and 23–44 years old, respectively) at 0800 hours, after a 3-g oral dose of metyrapone on the previous midnight. From each subject, 10-ml blood samples were drawn into evacuated blood-collection tubes (Vacutainer Tubes) containing 286 USP units of lithium heparin. N-Ethylmaleimide (Eastman Kodak Co., Rochester, N.Y. 14650) dissolved in ethanol, 1.25 mg in 10 μl, had been added to half of the tubes before blood was drawn. Final concentration of the inhibitor was 1.25 mg/10 ml. Sample pairs with or without the N-ethylmaleimide were handled as follows: (a) immediate