Acid-base determinations are an important and established aid to modern clinical medicine. Severinghaus stated that the Astrup's ultra-micro method 'This approach greatly simplifies this entire problem of acid-base balance for clinical medicine and as far as I can see, for research work too'. Serial acid-base determinations are particularly important in the management of newborn infants suffering from the idiopathic respiratory distress syndrome. The Neonatal Respiratory Unit at Groote Schuur Hospital recently acquired an Astrup apparatus and this method has now replaced the older technique of Van Slyke blood gas analysis, which we had used in conjunction with a Metrohm pH meter. The Astrup method has the advantage that arterialized capillary blood can be used. After warming the extremity, collection of blood samples and acid-base determination take only 10 minutes.

A comparative study was carried out between the Astrup micro-method and our previous method. The aim was to determine our experimental error when using the Astrup apparatus and to assess the comparability of the 2 methods. The Astrup micro-method will be used exclusively in the cases to be studied in future.

Determinations were carried out as a part of routine laboratory procedure. No attempt was made to improve the accuracy by taking special precautions which would not be practicable under normal laboratory conditions. A wide range of acid-base disturbances was chosen such as would be encountered in our investigation of newborn infants.

**APPARATUS**

**Method 1**

(a) The Metrohm pH meter E300 (scale divisions = 0.02 pH units) with the Metrohm suction microglass electrode using about 100 μl of blood. The pH meter and electrode jacket were kept at a constant temperature by a circulating water bath with built-in thermostat set at 38°C.

(b) The Van Slyke manometric apparatus for determination of whole blood total CO₂ content using 0.2 ml of whole blood.²

Plasma total CO₂ content corrected for pH and oxygen saturation was calculated with the Van Slyke and Sendroy line chart.³ Oxygen saturation was determined from the oxygen content and oxygen capacity (Hb. x 1.34 volumes % in adults and 1.26 volumes % in neonates).² The plasma bicarbonate, P.CO₂ and base excess were read from a nomogram.⁴

**Method 2**

(a) The Radiometer pH meter 27 (scale divisions = 0.01 pH units) with the Radiometer suction microglass electrode using about 20 μl of blood.

(b) The Astrup micro-tonometer for equilibration by the Astrup method.²,⁵

A circulating water thermostat kept both the electrode and tonometer at 38°C and Centigrade thermometers inserted into the system at both these points checked the temperature. Corrections were made for unsaturation and results were plotted on the nomogram provided to obtain P.CO₂ actual bicarbonate and base excess. The CO₂ content of the gases used for equilibration were analysed with the Scholander micro-gas analyser (SE = 0.047%).

**Method**

Blood samples of 3 ml were collected anaerobically after filling the dead space of a syringe with heparin. The syringe was then capped and kept on ice. Samples were either umbilical cord blood, venous blood or arterial blood. All determinations were carried out in duplicate and were completed within 1-hour of collection.

**Results**

A wide variation in acid-base status was found in the patients studied. The range was as follows:

- pH = 7.105 to 7.547;
- O₂ = 4.9 to 23.0 volumes %;
- HCO₃⁻ = 14.6 to 25.2 mEq./l;
- P.CO₂ = 20 to 75 mm.Hg;
- Base excess = -9.8 to +1.9 mEq/l.

The standard error of all determinations was within acceptable limits. No significant difference was found between the 2 methods for any of the values, with the exception of the pH (Table I and Figures 1, 2 and 3).

**Discussion**

On examination of the data it was found that in 13 of the 14 readings the Radiometer pH was higher than that of the Metrohm. The mean difference was 0.009 units. Sendroy et al.¹¹ found that the glass electrode read 0.01 - 0.02 pH units lower than the hydrogen electrode when using whole blood. This difference did not appear when plasma was used. Severinghaus et al.¹² resolved the problem by adding 0.01 to all whole blood pH readings when the P.CO₂ was to be calculated, and Siggaard-Andersen¹³ came to the same conclusion.

Jenny et al.¹⁴ reported that solid particles in pastes and suspensions have a lowering effect on the observed pH and ascribed it to the effect these particles (in our case the blood cells) have on the diffusion of ions at the boundary between the KCl and the unknown suspension. Siggaard-Andersen, however, ascribed the lower pH readings to the precipitation and haemolysis of the red blood cells at the junction between the blood and the saturated KCl.¹³

Although both electrodes used in the study were glass, these results raise the suspicion that the observed difference of 0.009 pH units may have been due to the following structural differences in the 2 systems:

(a) The Radiometer micro-electrode lies horizontally, thus reducing the danger of error due to sedimentation and diffusion whereas the Metrohm has a vertical electrode.

(b) The inner bore of the Metrohm pH meter electrode capillary is more than 3 times that of the Radiometer. A larger surface of blood will, therefore, be presented to the saturated KCl and more haemolysis will take place.

Another important difference is the lack of a built-in thermometer to check the thermostat setting in the
TABLE I. COMPARISON OF DIFFERENT METHODS OF STUDYING ACID-BASE STATUS

<table>
<thead>
<tr>
<th>Type blood</th>
<th>O₂ vol. %</th>
<th>Total CO₂ mEq/l.</th>
<th>pH</th>
<th>HCO₃⁻ mEq/l.</th>
<th>PCO₂ mmHg</th>
<th>Base excess mEq/l.</th>
</tr>
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<td>V.Slyke</td>
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<td>Metrohm</td>
<td>Radiometer</td>
<td>V.Slyke</td>
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<td>59</td>
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Mean difference between duplicate determinations
---
0.009

Standard error
---
0.009

Mean difference between the two methods
---
0.009

Standard error
---
0.009

p<0.001

Fig. 1. Scatter diagram showing comparison of pH between method 1 and method 2.
Fig. 2. Scatter diagram showing comparison of PCO₂ between method 1 and method 2.
Fig. 3. Scatter diagram showing comparison of [HCO₃⁻] between method 1 and method 2.

Metrohm pH system. An increase in temperature of 1°C will cause a decrease of 0.0147 in pH in whole blood. Indeed, the correction of pH and PCO₂ for the actual temperature of the patient has been stressed by various authors. An error of 0.01 in the pH will cause a 2.25% error in the PCO₂. Even after correction of the Metrohm pH readings our standard error in the PCO₂ remained unchanged. This is a reflection of the experimental error in the plasma bicarbonate measurement by the 2 methods, as well as the limitations inherent in the reading of nomograms. These and other sources of error have been fully discussed by Severinghaus et al. Berglund et al. and Siggaard-Andersen.

SUMMARY

Acid-base determinations were carried out on 14 blood samples, using 2 methods. The standard error was within acceptable limits and the results were comparable, despite the wide range in acid-base status. The Metrohm pH meter

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p refers to the probability that the difference between the two means is due to chance.

p* refers to the probability that the mean difference between the two methods is different from zero.
consistently gave a lower reading. Possible factors for this difference are discussed.

We consider that the Astrup micro-method fulfils all the requirements of acid-base determination stated by Wynn: "It is not entirely accuracy we want—it is reproducibility, reliability, speed and convenience.'

We wish to thank Professor F. J. Ford and Dr. E. B. Dowdle for their helpful criticism in the preparation of this paper: Dr. J. G. Burger, Medical Superintendent of Groote Schuur Hospital, for permission to publish; and the CSIR for financial assistance.

REFERENCES

Case Report

THYROID EYE-SIGNS IN NEPHROTIC SYNDROME: A SIDE-EFFECT OF STEROID THERAPY?

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In spite of the considerable laboratory and clinical evidence attesting to the inhibitory effect of adrenocortical steroids on thyroid function,

a recent report raises the possibility that hyperthyroidism may be related to steroid therapy. In the patient presented here, thyroid eye-signs and certain laboratory features of hyperthyroidism occurred during steroid therapy for the nephrotic syndrome and regressed upon steroid-withdrawal. It is therefore suggested that the thyroid signs could be related causally to the steroid therapy.

CASE REPORT

Sudden swelling of the abdomen and eyelids led to the admission to hospital of a Coloured boy, aged 7 years, in November 1960. The diagnosis of nephrotic syndrome was established by the finding of a solid proteinuria, a serum cholesterol of 500 mg./ml., and a plasma albumin of 0.70 G/100 ml. He was treated by 50 mg. of prednisone daily for 3 weeks with a good response, and discharged on a maintenance dose of 20 mg. daily.

His second admission on January 1961 followed a recurrence of symptoms. Two months of continuous or intermittent prednisone therapy at 25 - 60 mg. daily was not effective, but the oedema responded after 40 mg. of triamcinolone daily for 2 weeks. A moderate moon-face and slight hirsutes developed at this time. After discharge triamcinolone therapy was irregularly maintained until his third recurrence and hospital admission in May 1962. He was then treated with 24 mg. of methyl prednisolone daily.

About 12 days later he experienced a considerable diuresis following an intravenous infusion of plasma. Two days later prominent lid retraction, lid-lag and apparent proptosis were noted (Fig. 1). The following signs were all absent: ocular muscle palsies, goitre, frank tremor, and tachycardia. The serum protein-bound iodine was 1.2 µg./100 ml. Methyl prednisolone was maintained for 12 days, but because the eye-signs persisted it was then rapidly reduced to 20 mg. daily for 4 days per week, after which the eye-signs subsided within a few days.

During his fourth hospital admission in August 1962, methyl prednisolone was given as 24 mg. of methyl prednisolone capsules/day, but no eye-signs were noted. In October of the same year he was treated for about 6 weeks on 24 mg. of triamcinolone daily; the dosage was subsequently reduced to and maintained at 24 mg. 4 days/week, until his next hospital admission.

His sixth hospital admission was in May 1963; eye-signs were noted 1 week after commencement of 24 mg. of triamcinolone/day. The subsequent course and treatment are noted in Table I. From August 1963 to May 1964 he was maintained on intermittent steroid therapy, including 2 short courses, 11 and 7 days respectively, of triamcinolone and methyl prednisolone. At no stage was more than a slight lid-lag detectable.

In May 1964, he died unexpectedly while in hospital. Before this, lid-lag was the only detectable eye-sign. At necropsy the thyroid weighed 8 G (normal for his age) and histological examination demonstrated almost total absence of colloid, but autolysis of the follicular epithelium precluded any observations on the degree of epithelial cell activity. The kidneys were enlarged, the basement membranes thickened, and the tubular epithelium had undergone marked hydropic degeneration. The necropsy diagnosis was nephrosis with colloid depletion of the thyroid gland.

DISCUSSION

A boy suffering from a severe nephrotic syndrome twice developed prominent lid-lag, lid-retraction and apparent...