

APPENDIX 11

Reagents used for blood lactic acid determination by the method of Barker and Summerson, 1941 (Oser, 1976).

- 1 Copper sulfate, 20% solution. Dissolved 400 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in about a litre of water with the aid of heat, cooled, diluted to 2 litres and mixed. It was stable indefinitely.
- 2 CuSO_4 , 4% solution of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Diluted 1 volume of 20% CuSO_4 solution to 5 volumes with water and mixed. The solution was stored in a bottle fitted with a stopper, carrying a 1 ml pipette, which delivered approximately 20 drops per ml. If this is achieved, then 1 drop of the solution could be used as 0.05 ml as specified in the method.
- 3 Calcium hydroxide powder. Either USP or CP grades are satisfactory. It was conveniently dispensed with a spoon spatula known to hold approximately one gram, since exact measurement was unimportant.
- 4 Sulfuric acid, concentrated. Reagent grade iron-free sulfuric acid was used. It was dispensed from a buret, suitably protected against absorption of atmospheric moisture. Precautions should be taken against error due to slow drainage of the viscous acid. Nitrites and

nitrate in the acid interfere; only grades low in nitrate content should be selected.

- 5 p-hydroxydiphenyl reagent. Dissolved 1.5 g of p-hydroxydiphenyl in 10 ml of 5% NaOH solution, plus a little water, by warming and stirring, and then diluted to 100 ml. with water. Stored in a brown bottle fitted with a stopper and a pipette capable of delivering 20 drops per ml. Two drops of this was equivalent to 0.1 ml as required in the method. The reagent was stable for many months; deterioration was evidenced by a high blank reading.
- 6 Standard lactic acid solution. This was prepared from lithium lactate, which was anhydrous. For the stock standard, 0.213 g of pure dry lithium lactate was dissolved in about 100 ml of water in a one litre volumetric flask. To this, added 1 ml of conc. H_2SO_4 and diluted to the mark with water and mixed well. This solution contained 1 mg lactic acid in 5 ml and was stable indefinitely, if refrigerated. To prepare working standard, diluted 5 ml of stock standard to 100 ml in a glass stoppered volumetric flask with water and mixed well. This solution contained 0.01 mg lactic acid per ml, and was best freshly prepared.

Calculations

The blank duplicate readings were averaged to obtain the blank density. This value was subtracted from the average of the standard and unknown to obtain their true densities. Since the 1 ml portion of the copper-lime supernatant used for color development contained 0.005 mg lactic acid in the case of the standard and represented 0.02 ml of blood in the unknown.

The calculation was as follows :

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times \frac{0.005}{0.002} \times 100$$

= mg lactic acid per 100 ml blood.