A Note on the Cerimetric Determination of Blood Glucose

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It has been found that the method developed by Levvy (1946) for the determination of reducing sugars, with particular reference to glucuronic acid, has many advantages over the cerimetric procedure previously used (Giragossintz, Davidson & Kirk, 1936) for routine determination of blood glucose.

METHOD

When the blood sample was limited in size, 0·1 ml. was delivered into a clean 10 ml. centrifuge tube containing 0·9 ml. water. After laking was complete, 0·5 ml. of each of the protein precipitants specified by Levvy was added, followed by solid $BaCO_3$. With larger samples available, 0·5 ml. blood was laked with approximately 2 ml. water, and 2 ml. of each of the protein precipitants were added. After the addition of a pinch of solid $BaCO_3$ and allowing to stand for 10 min., the volume was adjusted to 10 ml. Whichever scale of precipitation was used, the mixture was centrifuged for 5 min. at 3800 r.p.m. and 1 ml. of the supernatant used for treatment with alkaline ferricyanide, etc., as described by Levvy for glucuronic acid.

A Krogh-Keys syringe pipette (1931) was found of great value in making rapid and accurate deliveries of 1 ml. or less. It was found that the precipitated protein which adhered to the glass vessels could readily be removed by soaking in approximately 3% sodium metasilicate for a few hours, followed by thorough rinsing.

Setopaline C was, during this investigation, in short supply. To meet this, MacFadyen & Van Slyke (1943) recommended the use of *o*-phenanthroline-ferrous complex. An even better substitute was found in the form of Lissamine green (British Drug Houses Ltd.) in 0·05% solution; this indicator gives rise to a smaller blank (similar to setopaline C) and is considerably cheaper than *o*-phenanthroline. The colour change is almost indistinguishable from that of setopaline C, and is best seen in a 'daylight-blue' light.

RESULTS

1 μg. glucose was found to be equivalent to 0·002 ml. 0·0140 N-$Ce(SO_4)_2$ (calculated from titres of 0·0110 N-solution corresponding to many levels of glucose). As in the case of glucuronic acid, the factor was unaltered by changing the amount of glucose within the prescribed range or by lowering the concentration of Na_2CO_3 in the alkaline ferricyanide solution from 10 to 1%. Glucose has a slightly greater reducing power than glucuronic acid, even when allowance is made for the difference in molecular weight. 0·25 ml. alkaline ferricyanide solution, under the conditions used, was completely reduced by 135 μg. glucose. The range of the method was similar for glucose and glucuronic acid.

The precision of the method was tested on samples of heparinized blood from various species and with varying glucose content. In a series of 24 determinations using 1 ml. of supernatant from the small-scale protein precipitation, found to contain 27·0 μg./ml. supernatant, a standard deviation of $\pm$ 0·76 μg. ($\pm$ 1·25 mg./100 ml. original blood; S.E. $\pm$ 2·8%) was found. In a similar series where 1 ml. supernatant contained 100 μg. glucose the standard deviation was almost the same ($\pm$ 0·79 μg.), giving a standard error of $\pm$ 0·8%.

In recovery experiments, varying amounts of glucose (recrystallized according to Hudson & Dale, 1917) were added to blood which had been allowed to glycolyze at 38° for 24 hr. When 25·2 mg./100 ml. were added the mean recovery (corrected for 'blank') was 23·4 mg./100 ml. (93·5%; 27 exps.; S.D. $\pm$ 0·7 mg.); when the amount added was increased to 100·8 mg./100 ml. the mean recovery was 98·5 mg. (18 exps.; S.D. $\pm$ 0·8 mg.). The glycolyzed blood itself (ox) gave a value corresponding to 17·8 mg. glucose/100 ml. (8 exps.). These results suggest a small constant loss of glucose, presumably during protein precipitation, and possibly through removal by $CuCO_3$.

The use of $BaCO_3$, making possible a filtrate free of copper, was found greatly to improve the sharpness of the end-point. The excellent keeping qualities of approximately 0·01 N-$Ce(SO_4)_2$ in an all-glass reservoir, shielded from light, have been confirmed; during nine months no significant fall in titre was detected.

SUMMARY

Levvy's method for the cerimetric determination of reducing sugars has been applied successfully to blood glucose.

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