ESTIMATION OF GLUCOSE IN THE BLOOD AND CEREBRO-SPINAL FLUID.

BY

FRANCIS TEMPLE GREY, M.A. OXON.,
M.B., CH.M. SYDNEY,
PATHOLOGIST, EAST LONDON HOSPITAL FOR CHILDREN.

HAVING had excellent results with Maclean's method¹ in a large number of determinations of glucose in the blood and cerebro-spinal fluid, it has occurred to me that my experience might be of interest. To ensure success certain points must be attended to which, although doubtless very elementary, have been found to be neglected in some cases where I have been consulted. The difficulties appear to be of three orders: personal, physical, and chemical.

The most frequent mistakes common to all methods are those of arithmetic, careless reading of the burette, and the use of odd slips of paper instead of a properly kept notebook. Not the least advantage of the well kept notebook is that the titrator may leave his entries to be afterwards dealt with by another; indeed, one might go so far as to say that a notebook of which another worker cannot make head or tail has not been properly kept.

Next to mistakes in arithmetic come errors which arise from an absence of the proper physical conditions. On one occasion a flask awaiting titration, the iodine having been liberated, was left standing in the sun; in others the proper heating conditions had not been obtained; thus on one occasion a wire gauze of another mesh than that with which the flame had been standardized was used.

Last, but not least, the reagents may be at fault. In one laboratory, for instance, after exhausting every other possible source of error, the sodium sulphate supplied by a reputable firm was found to contain sulphite. More often the "dialysed" iron is found to be at fault and to contain free hydrochloric acid. If the worker does not make up his own volumetric solutions it is as well to have the titre of the thio—supplied by commercial houses as decinormal—checked.

Professor Maclean's method may be divided into stages as follows:

Stage I (Deproteinization).—Into a 50 c.cm. Erlenmeyer flask take 23.8 c.cm. of 15 per cent. sodium sulphate acidulated to the extent of 0.1 per cent. with glacial acetic acid. (Sodium sulphate solution which has lost its acidity may be reacidulated.) Add 0.2 c.cm. blood; raise just to the boil; add 1 c.cm. dialysed iron; filter through starch-free filter paper, whose diameter is about 4 inches, into a small Erlenmeyer flask.

Stage II (Reduction). The filtrate, or 20 c.cm. of it, is transferred to a larger flask and allowed to cool either automatically as it waits its turn, or under the tap; add 2 c.cm. standard copper solution and reduce under standard conditions—that is, raise to the boil in one and a half to two minutes and keep boiling for another six minutes.

Stage III (Titration).—Cool as above, and when ready to titrate, but not before, liberate the iodine with 2 c.cm. 75 per cent. HCl. Add a few drops of starch solution and titrate against N/400 sodium thiosulphate.

Modifications of methods are in general to be deprecated unless they make for simplicity and give constant results. To take these modifications in turn.

I. There is no need for a reflux condenser at this stage, as scarcely any fluid is lost by bringing the lysed blood just to the boil. The dialysed iron may be added immediately; if a good sample—and poor samples of dialysed iron must on no account be used—its effect is instantaneous and the fluid may be filtered hot; in fact, it filters more quickly hot. The filtrate, measured before any evaporation has taken place on standing, is very constantly 21 c.cm., so that, if one likes, the whole filtrate may be taken, multiplying the result by 0.95. Obviously, if evaporation has occurred to any extent, as may happen when large numbers are being dealt with, it is even more accurate to take the whole filtrate than to imagine that 20 c.cm. obtained with difficulty represent 4/5ths of the sugar in the blood taken, when in reality it represents slightly more—that is, up to 21/25ths.

II. Reduction must take place under the standard conditions laid down by Professor Maclean. I find it

convenient, but not essential, to use a reflux condenser at this stage in order to avoid over-concentration. The condenser need not even be reflux. Clamp a large test tube filled with cold water, and adjust it immediately over the flask; the column of water will eventually become heated up, but not before it has prevented the over-concentration in the flask. The standardization of the flame by a manometer is unnecessary and unreliable—unnecessary because the observer's ear soon becomes accustomed to the sound from a given Bunsen burner at a given gas pressure; unreliable because heat supplied does not vary exactly as the pressure, but depends on the gas quality (thermic value). It is better, therefore, to find by trial the size of flame which will bring materials to the boil in from one and a half to two minutes, and reckon six minutes more (preferably by an interval alarm clock). This procedure gives more freedom as to the kind of flask used, for if the manometer is depended on it will make a difference whether, for instance, Bohemian or Swedish flasks are used.

III. It is not necessary, when liberating the iodine with strong acid, to wait for the effervescence to pass off, as this will occur during the shaking in the titration. A blank titration of the standard should be done with every batch of blood sugars—say twice a day. This is especially necessary if the standard is made up in bulk, for the solution ripens on keeping, and its value may rise so that 2 c.cm. are equivalent to 13 c.cm. of N/400 thio. When filling up the bench bottle (which is preferably of brown glass) titrate and correct, if necessary, as follows:

Suppose the value to be 11.7, take 110 c.cm. of the stock solution and add 7 c.cm. H₂O, and so on; or the correction may be made arithmetically. Make up the N/400 sodium thiosulphate as follows: take 2½ c.cm. N/10 sodium thiosulphate into a 100 c.cm. volumetric flask—a graduated glass cylinder is not accurate enough—add distilled water to the mark; empty out, shaking the while, into a larger flask and shake thoroughly. Volumetric flasks are for measuring, not for mixing.

Finally, the technique should be checked by seeing that reasonable results are obtained with normal bloods—that is to say, a figure around 0.085 per cent. in the case of a healthy person during the last hour before lunch—or with a known solution of glucose, say 1/4 per cent., which will keep if a little toluol be added.

Until everything is going well one should be prepared to do two or three titrations on any one particular case. For this purpose 1 or 2 c.cm. of blood should be taken either from the ear or from a vein, and the blood collected in a small tube which has been rinsed out with 20 per cent. potassium oxalate solution and dried.

When everything is going well, 0.2 c.cm. of blood is drawn into a pipette having preferably a capillary mouth subdivided, if possible, to 0.05 c.cm. A small length of rubber tubing (1/8 to 1/4 in. bore) makes a useful tourniquet; wind one turn just past the last joint of the thumb, leaving a small space on the dorsal aspect of the thumb between the joint and the nail. The advantage of this little plateau is that the blood is not so likely to run off it. Stab boldly; have a watch-glass (with a trace of potassium oxalate on it) in readiness to catch any overflow in case the bleeding is unexpectedly energetic. The pipette after use is washed out with water and then in potassium oxalate solution and set aside to dry; more than one should be at hand.

The time at which the blood is taken should be noted. If many hours elapse between the taking of the blood and its titration a misleadingly low reading may be obtained, even if the blood has been kept on ice. This seems to be due to ferment action, and may be inhibited by adding a trace of formalin. It is often convenient, however, to do Stage I (which destroys the blood enzymes) in the consulting room, the filtrate being reserved for reduction and titration at leisure.

Cerebro-Spinal Fluid.

Here the estimation is as for blood, except that where globulin is not increased the first stage (deproteinization) may safely be omitted, as the cerebro-spinal fluid contains normally a hundred times as little protein as the blood. When, however, globulin is increased, 0.1 c.cm. dialysed iron may be used—that is, ten times as little as for blood. The normal glucose content of the cerebro-spinal fluid is somewhat lower than that of the blood, and is fairly constantly about 60 mg. per 100 c.cm. There may be none in acute meningitis.

REFERENCE.

¹ Modern Methods in the Diagnosis and Treatment of Glycosuria and Diabetes. Constable.