ESTIMATION OF BLOOD SUGAR BY THE PRACTITIONER

A MODIFICATION OF FOLIN AND WU'S METHOD

BY

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The need for a quick, easy, and inexpensive method of blood-sugar estimation has long been felt by a large number of doctors in their own practices and at the smaller hospitals where no laboratory facilities are present. Usually the difficulty has been either the expense of a colorimeter or the tedious checking of solutions and the liability to error in the titration methods.

Folin and Wu's method is a colorimetric estimation which for greatest accuracy demands the use of a colorimeter, but I have found that a simple dilution of the final colours in specially calibrated tubes and comparison by the naked eye gives results within ± 10 mg. of the more precise colorimeter reading; this is accurate enough for all clinical purposes. The results shown below are duplicated estimations, one being obtained by the original Folin-Wu method, using the colorimeter, and the other by the means here described.

Folin and Wu's Mg.	Dilution Method Mg.	Folin and Wu's Mg.	Dilution Method Mg.
172	180	126	130
98	100	178	180
208	220	200	190
169	180	325	320
250	240	189	200
88	90	187	180
86	95	194	190
149	160	111	120
73	80	400	400
303	280	260	250
152	160	72	80
104	120	95	97
170	160	317	320
136	145	212	205
261	260	303	300
175	170	303	300
196	200	130	130
106	107	104	100
95	95	168	167
143	147	114	115
87	85	246	238
237	230	253	260
172	158	176	167
200	198	168	175
144	147	206	205

It will be seen that of these fifty duplicated estimations twenty-three have a difference of less than 5 mg., sixteen of 5 to 10 mg., nine of 10 to 15 mg., one of 15 to 20 mg., and one exceeding 20 mg. Twenty of these estimations were also duplicated entirely by the modified method; of these eight have no difference, eight have a difference of 5 mg., and four a difference of 10 mg.

Description of Apparatus

The method has been designed to enable estimations to be done with the minimum of technical knowledge and laboratory experience. Thus the solutions, which are the same as those used in the original method, are placed in order of use from left to right in a specially arranged box which has a handle on top and is easily portable. Each bottle contains its own pipette of automatic design, and the necessary tubes are calibrated to requirements. The solutions remain stable for many months, and accuracy with the standard glucose is ensured by putting the required amount in a small sealed ampoule. The comparison tubes in which the dilution of the final colour is made are calibrated to show the actual blood-sugar reading in milligrammes per 100 c.cm. at the point where the two colours match after dilution. The complete

apparatus, with solutions sufficient for thirty-six separate estimations, can be obtained from Messrs. Baird and Tatlock (London), Ltd., 14-17, St. Cross Street, Hatton Garden, E.C.1.

Directions for Use

Blood.—Into a tube marked A place water (see Note 8) up to the mark (3.5 c.cm.). Collect blood into the 0.1 c.cm. blood pipette up to the mark by pricking the ear or finger or from venous blood, and wash this into the water in tube A by sucking the water up and down until the pipette is quite clean. Add to this 0.2 c.cm. sulphuric acid solution, shake, and add 0.2 c.cm. sodium tungstate solution. Mix thoroughly by gentle shaking. Place a filter paper and funnel in tube B and filter into this the solution from tube A. When filtration is complete take 2 c.cm. of the filtrate by means of the pipette marked F and put into tube C (marked at 12.5 c.cm.). To this add 2 c.cm. Folin copper solution, shake gently to mix, and place a capillary stopper in the top of the tube.

Standard.—Into tube S1 put a standard ampoule. Break this on the bottom of the test-tube by means of the glass rod provided. Do not remove the rod, but hold it above the water-line and add water up to the mark on the test-tube, and to this add 0.4 c.cm. sulphuric acid and 0.4 c.cm. sodium tungstate (0.2 c.cm. twice). Shake thoroughly to mix, holding the glass rod on to the side of the test-tube.

When thoroughly mixed, wash the filtrate pipette by taking 2 c.cm. of the solution and discarding. Take another 2 c.cm. and put into tube S2 together with 2 c.cm. of Folin copper solution. Shake to mix, and place a capillary stopper in the top of the tube. Place both tubes (C and S2) in a water bath or small saucepan of *boiling* water and leave for six minutes. The water bath or saucepan must be small enough to allow the tubes to stand so that their tops are well out of the water. Cool the tubes for two minutes in cold water and then add to each 2 c.cm. of phosphomolybdic acid solution. Shake gently until there is no more frothing. Add water up to the mark and mix by inverting on the *clean* thumb. Into the comparison tube marked S pour the standard solution from tube S2 up to the 100 mg. mark, and into the comparison tube marked U pour the solution from tube C also up to the 100 mg. mark. Dilute the stronger coloured solution with water until the colours match. Mix by inverting on the thumb after each addition of water. The result of the estimation is marked on the tube at the point where the dilution is completed.

Notes

1. Background for Matching Colours.—A good method is to stand with the light coming from behind, the tubes held up in front of the face with one hand and a piece of white paper held at an angle behind the tubes with the other.

2. A space of about ten seconds should be allowed to elapse after each addition of water before comparing the colours.

3. When the unknown colour is very much stronger than the standard, a first dilution may be made large enough to take the comparison to within 60 mg. or so of the standard colour. Thereafter it is advisable to add water at the rate of 10 to 20 mg. at a time.

4. Where doubt is felt as to the final colour match, take a reading, add another 10 mg. of water, and compare again. If the first reading was correct the second will be obviously weaker.

5. Automatic Pipettes.—These are designed to "deliver" the required volume, and as each solution has its own pipette the excess which pours over into the bulb may be put back into the bottle. Those contained in the bottles need not be cleaned. The filtrate pipette, however, should be washed with water and allowed to drain. It

should never be used wet, except when enough solution is available to allow of a first wash.

6. The Folin copper solution has a tendency to deposit cuprous oxide. This can be seen on the bottom of the bottle when present, and is orange-red in colour. The solution is kept in a dark bottle to prevent this; but if it occurs the solution must be decanted and the bottle washed and dried before the solution is returned.

7. Cleaning.—A good wash under running cold water, and draining, is all the cleaning necessary. Wash tube A before it has time to dry.

8. Ordinary tap-water is perfectly safe to use in making the estimations.

In evolving this method I am greatly indebted to Dr. R. D. Lawrence for his invaluable criticism and suggestions and for his kind permission to carry out the work in the diabetic clinic at King's College Hospital. I owe thanks to the whole staff of the clinic for their encouragement.

THE SEROLOGICAL GROUPS AND TYPES OF STREPTOCOCCI ISOLATED FROM EXCISED TONSILS

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A survey of the serological types of haemolytic streptococci in Australia has been proceeding at these laboratories for the past year. The progress findings in respect of the streptococci isolated from scarlet fever patients in Melbourne and Adelaide, from puerperal and surgical infections, and from normal throats in Melbourne have already been reported (Keogh and Kelsey, 1939; Keogh, Macdonald, Battle, and Puckey, 1939). The strains found in scarlet fever patients were rarely isolated from normal throats or from patients with puerperal or surgical infections. This present study was undertaken primarily to discover whether the predominant serological types of streptococci encountered in the tonsils would be those commonly associated with scarlet fever or those commonly found in surgical infections and in the throats of healthy adults and children. The results, however, will be discussed not so much from that particular viewpoint as from their bearing on the much-debated question of the indications for tonsillectomy.

There have been numerous previous investigations of the bacterial flora of tonsils, most of them, however, undertaken before the introduction by Lancefield of the precipitin methods, which enable a distinction to be made between probable pathogens (Group A) and probable non-pathogens (not Group A) among strains of haemolytic streptococci. So far as we are aware, no study of the tonsillar flora using the finer analysis of identification of the type of strains by the slide agglutination method of Griffith has been carried out, though a similar line of inquiry is being pursued in Sydney at the present time.

Methods

The tonsils examined were removed at routine operations at the Children's Hospital, Melbourne, during last summer and autumn (November, 1938, to March, 1939). Immediately after excision they were put in sterile containers and placed in a refrigerator until the following morning. Both tonsils of each patient were minced up in a single tube of sterile broth, a drop of which was plated on neo-peptone blood agar. Subcultures into tryptic digest broth were made from the various colonial types of beta-haemolytic streptococci present on the plates. The broth tubes were incubated overnight at 30° C. and the deposit used for typing by Griffith's technique. Strains which failed to show specific agglutination with the type sera at our disposal were then allotted to their respective Lancefield groups, using the formamide method of Fuller (1938) for extraction of the group polysaccharide. Any strains which agglutinated with our Type 4 or Type 28 sera were also checked as to group, since we find that these sera may give apparently specific agglutination with strains belonging to Group B, C, or G. The sera used had already been employed in the classification of large numbers of strains, and included samples of the following Australian types not appearing in the Griffith series, provisionally named "Wade," "Stewart," "Hale," "Woodbury," "John," and "Alli," which had been isolated from surgical and puerperal infections.

Results

Haemolytic streptococci were isolated from 259 of 378 pairs of tonsils (68.5 per cent.). Sawers and Barrett (1933) found haemolytic streptococci in the crypts of 61 per cent. of tonsils examined by them in Sydney. The strains which we isolated belonged to the following groups (Lancefield): Group A, 189 (50 per cent.); Group B, 21 (5.5 per cent.); Group C, 33 (8.7 per cent.); Group G, 28 (7.4 per cent.). It will be noted that 271 strains were isolated from 259 tonsils; in twelve cases two different strains were present. In eleven instances one strain belonged to Group A, the other to either Group B, C, or G; in the remaining case two different serological types of Group A streptococci were isolated (Types 12 and "Alli"). This incidence of Group A strains in the tonsils is ten times higher than in the throats of healthy children and adults. The details are set out in Table I.

TABLE I

Source of Strains	Percentage from which Haemolytic Streptococci were Isolated	
	Group A	Groups B, C, G
378 tonsils removed at operation	50.0	21.7
466 throats of healthy adults and children	4.5	13.1

This comparative incidence of Group A and non-Group A strains in the throats of healthy subjects agrees with the findings of workers in other countries. Hare (1935) estimated that in England about 7 per cent. of the general population carry Group A streptococci and 13 per cent. other groups. Davis and Guzdar (1936) found a carrier rate of 3 per cent. of Group A streptococci and 6.4 per cent. of non-Group A streptococci in healthy Chinese. Our figures in the healthy group for Groups B, C, G (13.1 per cent.) may be high, owing to the inclusion of a number of children from an institution many of whom were carriers of a Group C strain. The objection may be raised that this very high incidence of the potentially pathogenic