Improved Technique of O-Aminobiphenyl Method for the Determination of Glucose in Serum

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The ultramicro direct colorimetric method for the determination of serum glucose with o-aminobiphenyl reagent[1] is one of the simplest procedures of clinical chemistry. It is particularly suited for routine work because it enables us an accurate and precise estimation of glucose with an amount of blood serum as small as 50λ (0.05 ml) circumventing the tedious procedure of deproteinization. In our experience for these two years this method compared quite well with the conventional Somogyi-Nelson method.

However, a defective feature of the o-aminobiphenyl method was pointed out recently at the Chûgoku-Shikoku Local Meeting of the Japan Society of Clinical Pathologists (Ube) by SHIRAKATA[2], who stated that it gave a faulty high glucose estimation when it was applied to the plasma of a blood sample which had been treated with sodium fluoride to stop spontaneous glycolysis.

This defect was overcome by the use of an o-aminobiphenyl reagent containing an appropriate amount of boric acid and sodium fluoride. His study was re-examined and its validity was confirmed in our laboratory.

The purpose of the present paper is to describe the result obtained in our study.

METHOD

Reagent.

1. O-aminobiphenyl reagent: In 120 ml of saturated aqueous solution of boric acid dissolve 360 mg of sodium fluoride, and add to this solution 880 ml of 0.5 g/dl o-aminobiphenyl solution in glacial acetic acid.

2. Standard glucose solutions (50, 100, 200, 300 and 400 mg/dl) which were described in the original method.[1]

Procedure

Introduce 50λ of blood serum (or plasma) into a small test tube (1.8 cm × 12 cm) S with an ultramicropipet. Rinse the ultramicropipet with distilled water and measure with it 50λ aliquots of 50, 100, 200, 300 and 400 mg/dl aqueous glucose solutions to put them into the test tubes (of the same size as tube S) 1, 2, 3, 4 and 5,

respectively. Add 5.0 ml of o-aminobiphenyl reagent to each of all the tubes, mix, and heat in a boiling water bath for 30 minutes. The solutions in the tubes develop greenish color.

Measure the optical densities of the colored solutions in a photoelectric colorimeter (Klett-Summerson) with a red filter (660 mμ) and glacial acetic acid as blank solution of colorimetry.

Construct the calibration curve with optical densities of tubes 1, 2, 3, 4 and 5, regarding them to be equivalent to 50, 100, 200, 300 and 400 mg/dl of serum glucose concentration, respectively.

Collate the optical density of the solution of tube S to the calibration curve to read the glucose concentration of the serum sample.

RESULTS AND DISCUSSION

The original o-aminobiphenyl method[1] gave significantly higher glucose estimations with fluoride-added plasma than with the ordinary plasma obtained from the oxalated blood by centrifugation shortly after its withdrawal from the veins (Table I). The deviation amounted to 6 mg/dl on an average for normal blood glucose level. In the hyperglycemic plasmas the shift to higher estimation became as large as 40 mg/dl with increase in the amount of fluoride added. This is accounted for by the excess coloration in the sample tubes of fluoride-added plasma as compared with that appearing in the standard glucose and sample tubes which have no fluoride. The intensification of the coloration of glucose with o-aminobiphenyl in the presence of fluoride is clearly seen by the comparison of the calibration curve of the present procedure with that of the original (Figure 1).

Table I. The interfering effect of fluoride added to serums on the glucose estimation by the original o-aminobiphenyl method

Sample	(A) Without addition of NaF	(B) NaF added in 5mg/ml	(B)−(A)
1	72	80	+ 8
2	75	83	+ 8
3	78	89	+11
4	84	88	+ 4
5	88	94	+ 6
6	88	96	+ 8
7	90	94	+ 4
8	90	96	+ 6
9	94	100	+ 6
10	94	103	+ 9
11	94	92	− 2
12	95	101	+ 6
13	105	114	+ 9
14	107	111	+ 4

(mg/dl)

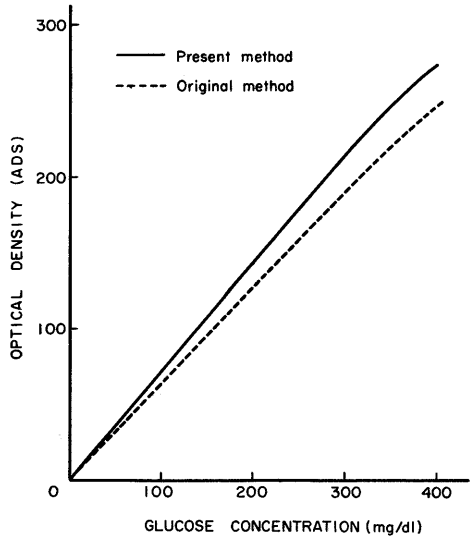

Fig. 1. Calibration curves of the original and the present method

Essentially the same glucose estimations were obtained by the present and the original methods when they were applied to serums or plasmas to which fluoride had not been added (Table II). Furthermore, it is evident from Table III, which is the summary of the glucose estimations of the serums having various degrees of addition of fluoride, that the values obtained by the present procedure are independent of the amount of fluoride preliminarily added to the serums. It is obvious that the

Table II. Comparison of the original and the present method.
(with the same serum samples without addition of NaF)

Samples	(A) Original method	(B) Present method	(B)−(A)
1	71	70	−1
2	85	84	−1
3	85	84	−1
4	86	84	−2
5	88	88	0
6	88	84	−4
7	90	87	−3
8	94	94	0
9	95	95	0
10	116	116	0
11	161	165	+4
12	161	157	−4
13	325	325	0
14	389	387	−2
15*	76	74	−2
16*	63	63	0

* These are the samples of spinal fluid. (mg/dl)

Table III. Accurate determination of glucose by the present method: There is no interfering effect of fluoride on the estimation irrespective of the amount of fluoride added to serum.

addition of NaF to serum / Sample	In 0 mg/ml	In 5 mg/ml	In 10 mg/ml	In 15 mg/ml	In 20 mg/ml	In 25 mg/ml
1	70	72		70		
2	70		69			69
3	79		79		79	
4	84	85		86	86	86
5	84		84			
6	94		94			94
7	116		116			
8	157		157			
9	165		165			
10	266	269	266	266		
11	347	350	349	352		

interference of pre-existing fluoride in serums or plasmas upon the coloration has been entirely eliminated in the present procedure.

The glucose concentration decreases relatively rapidly when a blood sample is allowed to stand at the room temperature of hot season. The glucose in serum is consumed by the glycolysis taking place in erythrocytes. The blood is, therefore, usually withdrawn from the vein to a tube containing sodium fluoride which is an inhibitor of glycolysis, and this makes a habit particularly in the case of glucose tolerance test, in which blood samples are forced to be left in the ward for a few hours before they are sent to the laboratory. The fact that the present procedure gives accurate estimation of glucose with the serums or plasmas obtained from the fluoride-added blood samples is a remarkable improvement which enhances its utility in routine clinical chemistry.

The original o-aminobiphenyl method requires three pipettings for color development: (1) serum, (2) saturated aqueous boric acid solution and then (3) o-aminobiphenyl reagent. In contrast, the present procedure needs only two pipettings: (1) serum and then (2) o-aminobiphenyl reagent containing boric acid and fluoride. This is a notable simplification of manipulation, which contributes to the shortening of the time consumed for the determination. The present method is therefore more convenient for routine work than the original procedure.

SUMMARY

The o-aminobiphenyl colorimetric method for the determination of glucose in serum which was developed by SHIBATA was improved by the use of color reagent

recommended by SHIRAKATA, which is a mixture of 880 ml of 0.5 g/dl o-aminobiphenyl solution (dissolved in glacial acetic acid) with 120 ml saturated apueous solution of boric acid containing 360 mg of sodium fluoride. In the improved method 50λ of blood serums is added to 5.0 ml of the color reagent, heated in a boiling water bath for 30 minutes, and the greenish color thus developed in the solution is subjected to photoelectric colorimeter with a red filter (660 mμ). This enables us an accurate glucose determination of serum or plasma in the presence of fluoride as well as in its absence.

REFERENCES

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