CXXIII. A NEW METHOD FOR THE DETERMINATION OF FRUCTOSE IN BLOOD.

By LESLIE DOUGLAS SCOTT.

*From the Department of Biochemistry, Charing Cross Hospital,
Institute of Pathology.*

(Received March 6th, 1935.)

A CONSIDERABLE experience in the routine practice of the fructose tolerance test has amply demonstrated the inadequacy and unsatisfactory nature of the test in determining liver insufficiency when the response is measured by the changes in the level of the total reducing sugar of the blood, resulting from the ingestion of fructose. The defects of the test in its present form will be fully discussed in a subsequent paper from this laboratory and need not be elaborated here except to say that they led to the recognition that it was eminently desirable to attempt to determine the actual fructose in the blood following the ingestion of this sugar in place of the ordinary values for the total reducing sugar content. The methods most generally adopted for the purpose of estimating the true fructose content of the blood are those based on the Van Creveld principle using the colour developed in the reaction with dimethylamine [Radt, 1928], whilst an entirely different principle, biological in character, has recently been used by Harding *et al.* [1933].

An alternative colorimetric method emerged from a previous study of the interaction of bile salts and fructose. It was first suggested by Dr J. Patterson that an attempt should be made to adapt the bile salt reaction described by Scott [1934] in order to apply it for the determination of fructose in blood, that is, instead of an excess of fructose being utilised for the estimation of minute amounts of bile salts, the latter might, it was thought, be employed in a similar manner to react with minute quantities of fructose. On trial it was found that a similar reddish purple colour still resulted as in the original reaction, so that it was subjected to a detailed study and has been made the basis of the method for fructose determination which is outlined below.

Reagents and apparatus:

- (1) 10 % zinc sulphate solution.
- (2) 0.5*N* sodium hydroxide.
- (3) 1 % (by volume) acetic acid.
- (4) 1 % stock fructose (Merck or B.D.H. glucose-free) in a saturated solution of benzoic acid.
- (5) Absolute alcohol.
- (6) 2.25 % alcoholic sodium tauroglycocholate (B.D.H.).
- (7) Concentrated hydrochloric acid (B.D.H. "Analar").
- (8) Filter-papers: No. 41, 9 cm., and No. 50, 7 cm. (Whatman).
- (9) Monax boiling-tubes, 25 × 150 mm.

METHOD.

Into a boiling-tube (25 × 150 mm.), graduated at 40 ml., place 5 ml. of whole blood. Then add about 30 ml. of water, followed by 5 ml. of 10 % ZnSO_4 and 5 ml. of 0.5*N* NaOH. Mix well after the addition of each. Dilute both to the 40 ml. mark and again mix thoroughly. Place in the water-bath at 80–85° for

5 minutes, noting that the water in the bath completely covers the volume of the blood mixture in the tube. At the end of 5 minutes cool very thoroughly in running water and mix well. Filter through a No. 41, 9 cm. paper, stirring gently the remaining gelatinous precipitate on the filter-paper to free the last few ml. of filtrate. Transfer 20 ml. of clear filtrate to one of the monax boiling-tubes, add 3 drops (0.15 ml.) of 1 % acetic acid and a few silica chips to prevent bumping in the subsequent evaporation and carefully boil down to about 1.5 ml. When the fluid decreases in volume avoid excessive heat so as not to char the solution. Prepare the standard by adding to a similar monax tube 2.5 ml. 0.01 % fructose solution (by dilution of the 1 % stock fructose). Carefully boil off the excess water until the volume is about 1.5 ml. To the standard and unknown add 3 ml. 2.25 % alcoholic sodium tauroglycocholate. After the addition of an extra 7–10 ml. absolute alcohol and a few more silica chips to each tube, evaporate to complete dryness on the boiling water-bath. If bumping should occur in the blood filtrate tube owing to the precipitate which forms on the addition of the alcoholic bile salts, gently rotate the tube until all the alcohol has been evaporated. After the last drops of alcohol have disappeared allow the tubes to remain in the water-bath an extra minute or two to ensure the complete removal of the last traces of alcohol, which should be accomplished without causing the precipitate to adhere too firmly to the bottom and sides of the boiling-tube. When cool add 10 ml. concentrated HCl to both tubes, and after mixing well, securely stopper the tubes. Place in the water-bath or a beaker of water at 40° for half an hour with occasional agitation to ensure an even emulsion and production of colour. A reddish purple colour, proportional to the amount of fructose present, slowly develops, reaching a maximum intensity in 30 minutes. Remove the tubes from the bath and cool very thoroughly in running water. Filter the coloured solutions through No. 50, 7 cm. papers, refiltering the first 2 or 3 ml. as these are usually slightly cloudy. Compare in the colorimeter in the usual way. Calculation:

$$\frac{\text{Standard}}{\text{Unknown}} \times \text{Strength of standard (0.25 mg.)} \times \frac{100}{2.5} = \text{mg. fructose per 100 ml. blood.}$$

Table I. *Showing the quantitative recovery of various quantities of fructose added to normal whole blood.*

Standard employed	Amount of fructose added per 100 ml. normal blood	Amount of fructose recovered mg. per 100 ml.
1 ml. (1 mg.) of 1 in 10 dilution of 1 % fructose	50 25	50 27
2.5 ml. (0.25 mg.) of 1 in 100 dilution of 1 % fructose	15 10 5	15 10 6
Using as standard 20 ml. (equiv. 2.5 ml. blood) of normal blood-filtrate to which had been added the requisite amount of fructose (see above)	50 25 15 10 5	48 24 14 10 5

When for various reasons it is impossible to obtain venous samples, the test can be carried out on 2 ml. whole blood obtained by finger puncture with only slight modification of the original technique. The essential difference can be summarised as follows:

Into a test-tube, graduated at 20 ml., place 10 ml. water, 2 ml. blood and corresponding amounts of precipitating reagents. After diluting to 20 ml. with

water place in the water-bath at 80° for 5 minutes. Cool and filter through a No. 41, 9 cm. paper. Measure 10 ml. filtrate into a monax tube, add 2 drops of 1 % acetic acid and boil down to about 1 ml. After the addition of 1 ml. alcoholic bile salts *plus* 7–10 ml. alcohol, evaporate to dryness on the boiling water-bath. The standard is prepared by taking 1 ml. of a 1 in 100 dilution of 1 % fructose *plus* 1 ml. bile salt solution and 7–10 ml. alcohol. Take to dryness on water-bath. 5 ml. concentrated HCl are added to each tube, after which the tubes are heated at 40° for 30 minutes. Cool and filter through No. 50, 7 cm. papers. Compare the colours in a micro-colorimeter or by small graduated test-tubes suitable for dilution colorimetry. Calculation:

$$\frac{S}{U} \times 10 = \text{mg. fructose/100 ml. blood.}$$

Quantitative figures again show a complete recovery of added fructose.

Comparison of the capillary blood with venous samples shows that the actual fructose content is a shade higher in the former than in the latter, the difference being about 2 mg. per 100 ml. (Table II). The fructose curve thus shows the

Table II. *Showing the difference between the venous and capillary fructose in a normal subject; also a case of liver disease complicated by diabetes after the ingestion of 50 g. pure fructose.*

Case	Venous		Capillary	
	Actual estimated fructose mg./100 ml.	Total reduction (MacLean's method) mg./100 ml.	Actual estimated fructose mg./100 ml.	Total reduction (MacLean's method) mg./100 ml.
Normal subject:				
Before fructose	0	102	0	105
1 hour after	8	102	9	121
2 hours after	5	97	3	92
Subacute yellow atrophy and diabetes:				
Before fructose	0	184	—	—
1 hour after	16	232	—	—
2 hours after	13	221	—	—
After 2 months when the jaundice had entirely disappeared:				
Before fructose	0	176	—	—
1 hour after	12	240	—	—
2 hours after	7	217	—	—

Note. For the sake of convenience it might be mentioned that the average normal response in terms of actual fructose is 10 mg. and 5 mg. per 100 ml. blood in the 1 and 2-hour samples after fructose respectively.

same tendency, though not nearly so marked, as does the total reducing sugar curve, in rising to a slightly higher level in the capillary than in the venous blood. Different standards of normality must therefore be accepted for the different types of blood used. In the single normal case studied in detail where the fructose contents of venous and capillary blood show close correspondence, the blood sugar levels in the two display much greater divergence.

A case of liver disease complicated by diabetes has been included in Table II for the purpose of illustrating the influence of diabetes on the actual fructose curve. The disturbance from the normal due to diabetes appears to be of the order of 2 mg. per 100 ml. in the 1 and 2-hour samples of venous blood after the administration of 50 g. pure fructose.

Table III shows a comparison of the fructose content of the blood following the ingestion of 50 g. Merck's pure fructose in normal subjects with that of

Table III.

Series No.	Type of case	Venous samples		
		New fructose method mg. per 100 ml.		Old fructose method. Total reduction (MacLean) mg. per 100 ml.
1	Normal	B.L.	0	53
		$\frac{1}{2}$ hour	10	70
		1 hour	10	70
		$1\frac{1}{2}$ hours	7	85
		2 hours	4	76
		3 hours	1	69
2	—	B.L.	0	92
		$\frac{1}{2}$ hour	12	92
		1 hour	12	95
		$1\frac{1}{2}$ hours	9	90
		2 hours	5	92
3	—	B.L.	0	85
		$\frac{1}{2}$ hour	8	88
		1 hour	9	86
		$1\frac{1}{2}$ hours	8	86
		2 hours	6	87
4	Acute alcoholic intoxication of liver	B.L.	0	109
		$\frac{1}{2}$ hour	12	135
		1 hour	12	143
		$1\frac{1}{2}$ hours	11	141
		2 hours	11	139
5	N.A.B. jaundice	B.L.	0	96
		1 hour	18	110
		2 hours	15	104
6	Subacute yellow atrophy and diabetes	B.L.	0	184
		1 hour	14	232
		2 hours	11	221

patients suffering from certain forms of liver disease. The alteration in the total reducing sugar as estimated by MacLean's method is also included for purposes of comparison.

A full survey of the results obtained by this method and their relation to the carbohydrate metabolism and liver function will, it is hoped, be ready for publication shortly.

If the amounts of fructose shown here as appearing in normal blood after the ingestion of 50 g. fructose be compared with the corresponding values found by the biological method of Harding *et al.*, they are seen to be of about the same order (actual figures are not given there but have to be arrived at by inspection of the recorded chart). On the whole the biological values are a little smaller and show the disappearance of fructose from the blood at an earlier stage than does the colorimetric method. On the other hand almost exact agreement has been obtained between the present method and a modification of the original Radt diphenylamine method [Patterson, unpublished results].

SUMMARY.

1. A new method is described for the estimation of fructose in blood based on the reaction between bile salts and fructose. The delicacy of the reaction is such that fructose contents of the order of 5–20 mg. per 100 ml. can be estimated with an error of approximately ± 1 mg. Using the method comparisons have been made between venous and capillary blood and also between normal and diabetic blood following the ingestion of fructose.

2. Preliminary results also show that a more satisfactory fructose tolerance test for liver function can be made on the basis of specific fructose estimations, than by taking account of the total blood sugar values.

I wish to thank Dr J. Patterson for his encouragement and advice throughout the course of this work, and also Dr R. A. Hickling, Physician to Charing Cross Hospital for his kindness in allowing access to his wards for the liver cases.

REFERENCES.

- Harding, Nicholson and Armstrong (1933). *Biochem. J.* **27**, 2035.
Radt (1928). *Biochem. Z.* **198**, 195.
Scott (1934). *J. Lab. Clin. Med.* **19**, 523.