able for proximal ligation, trapping, or exclusion of the sac directly, a preliminary note on the method seems justifiable. Later complications arising directly from this material itself are unlikely, but only time will show whether, for instance, the parent artery rebels at encasement for part of its course in a rigid tube, though that would seem to be an unlikely danger.

I wish to acknowledge my indebtedness to the consultant neurosurgeons of the unit for their encouragement to proceed with the method in their cases after experiments under general anaesthesia, provided by Dr. P. L. F. Mortimer, had been successfully concluded at the Field Station of the Veterinary School at Langford, by courtesy of Professor Messervy, of the University of Bristol.

ENZYME TESTS FOR THE DETECTION OF GLUCOSE*

BY

J. A. HUNT, M.B., B.S.

Registrar, Department of Diabetes, King's College Hospital

C. H. GRAY, M.D., D.Sc., M.R.C.P., F.R.I.C.

Professor of Chemical Pathology, King's College Hospital Medical School

AND

D. E. THOROGOOD, B.Sc., A.R.I.C.

Research Assistant, King's College Hospital Medical School

The availability of highly purified glucose oxidase has prompted the commercial development of two tests highly specific for the detection of glucose in urine. Each consists of a strip of filter paper impregnated not only with this enzyme but also with *o*-toluidine, and with a second enzyme, a vegetable peroxidase. When the strip is dipped in urine containing glucose and withdrawn, the glucose in the minute amount of urine absorbed on the strip is oxidized by atmospheric oxygen in the presence of the oxidase. Gluconic acid and hydrogen peroxide are formed, and the latter then reacts with *o*-toluidine in the presence of the peroxidase to produce a blue pigment.

"Tes-tape" is provided as a roll of test paper in a convenient container. As well as the substances indicated above, the tape is impregnated with a yellow dye which gives colour changes ranging from light green through dark green to dark blue according to the concentration of glucose in the urine. A colour scale (which is different for different batches of tes-tape) is provided on the side of the container and is stated to indicate colours corresponding to about 0, 0.1, 0.25, 0.5, and 2 g. of glucose per 100 ml. of urine.

"Clinistix" is provided in the form of separate strips of thick paper resembling a book-match; only the last half-inch is impregnated with the reagents. No colour scale is provided, and the manufacturers state that the test should not be used quantitatively.

In view of the potential importance of these two tests in the detection of glucose in urine and in the treatment of diabetes a critical examination of their behaviour seemed important.

Methods and Clinical Material

Tes-tape and clinistix were used as recommended by the manufacturers. For the purposes of this investigation only,

*Based upon a paper presented at the Banting meetings at King's College Hospital Medical School on July 6 and 7.

a colour scale was constructed for clinistix to give an arbitrary classification of intensity of colour—+, ++, +++. The urines were also examined by the "clinistix" technique and by the Benedict qualitative method (Harrison, 1947). Reducing substances were estimated by the potentiometric method of Quilley (1955), the results being expressed as glucose. True glucose was estimated by the method of Froesch and Renold (1956).

The following specimens were available for the investigation: 46 from non-diabetic patients; 43 from diabetic patients excreting no or trace amounts of glucose; and 174 from diabetic patients excreting glucose.

Detection of Glucose

The results given by the 46 normal urines and the 43 urines from diabetics are summarized in Table I. Of the normal urines, only four showed traces of reducing substances by Benedict's test, and with these tes-tape gave

TABLE I.—Results of Tests on Urine Containing Little or No Reducing Substances

No. of Specimens	Benedict's	Clinistest	Clinistix	Tes-tape
<i>Normal Urines</i>				
41	0	0	0	0
1	0	0	0	Less than 1/10*
4	V. sl. trace	0	0	" "
<i>Diabetic Urines</i>				
4	0	0	0	0
4	0	0	0	1/10
9	V. sl. trace	0	0	0
1	0-3%	0	0	0
5	V. sl. trace	0	0	1/10
10	" "	0	Trace	1/10-1/4
5	" "	0	+	1/10
2	" "	1/4	0	0
2	" "	1/4	Trace	1/10
1	" "	1/4	+	0

* These reactions were only just recognizably positive.

only a just recognizably positive result. With one urine tes-tape was the only test giving a positive result. Tes-tape also detected a small quantity of glucose in some of the urines from diabetics, sometimes when Benedict's test was negative and sometimes when this test indicated a very slight trace of reducing substances.

Clinistix usually gave results corresponding to those given by tes-tape, but this was not always the case, and in one urine clinistix gave a positive result when tes-tape was unaffected.

Quantitative Aspects

Eighty-one urines contained reducing substances corresponding to 2 g. or more glucose per 100 ml., as measured by the potentiometric method. Table II shows that in these specimens tes-tape indicated 2 g. or more of glucose per 100 ml. in all but five, and clinistix gave a +++ result in all but four.

TABLE II.—Results of Enzyme Tests with Urines Containing More Than 2 g. of Glucose per 100 ml.

Glucose* Content (g./100 ml.)	No. of Urines	Clinistix			Tes-tape (g./100 ml.)		
		+	++	+++	0.5	0.5-2	2
2-4	33	1	0	32	4	1	28
4-6	25	0	3	22	0	0	25
6-8	16	0	0	16	0	0	16
8	7	0	0	7	0	0	7

* Measured potentiometrically.

Fig. 1 presents the results in a series of 92 urines all containing 2 g. or less of true glucose per 100 ml. The results have been separated into four groups corresponding to the values on the tes-tape colour scale. About half of the urines examined were accurately measured by tes-tape, but considerable errors occurred in the remainder. Thus 19 of the 36 urines containing about 0.5 g. of true glucose per 100 ml. were found to contain this by tes-tape, but the

remainder of the results by tes-tape were either too high or too low (one of the urines appeared to contain 2 g. or more per 100 ml., two of them 0.1 g. per 100 ml.). With the arbitrary colour scale, clinistix revealed a similar range of readings.

The observations reported so far were made jointly by all three of us, but in 42 of these specimens the tests were also carried out independently by us three as well as by

FIG. 1.—Results in 92 urines containing 0.1 to 2 g. of true glucose per 100 ml. The height of the columns shows the number of readings at the particular values on the colour scale.

two trained technicians. Each urine was thus examined independently by five different observers. The results are summarized in Fig. 2 A-E, from which it will be seen that there is often a significant difference reported by different observers on the same specimen. Fig. 2 F shows similar discrepancies in reading when eight untrained observers (six patients and two nurses) examined seven urines containing 0.4 g. of true glucose per 100 ml.

Stability.—Under laboratory conditions in Britain tes-tape and the most recent supplies of clinistix appear to be reasonably stable. It is possible that they might not be so under tropical conditions, or even in the hot humid atmosphere of the bathroom.

Discussion

The sensitivity desirable for the qualitative detection of glucose in urine has never been accurately defined. It has been reported that Benedict's qualitative technique is slightly more sensitive than clinistest (Gray and Millar, 1953), but the significance of such small traces has never been assessed.

The precise sensitivity of the enzymic tests will be affected by the presence or absence of interfering substances in the urine, but the claim by the two manufacturers that about 0.1 g. per 100 ml. is the limit of sensitivity is well supported by the present observations. This sensitivity is affected by pH, ascorbic acid, and other unknown urinary constituents, but variations in the pH and in the amount of ascorbic acid, etc., in the urine are insufficient to be of significance. It is probable that the weakly positive reactions reported in five of the normal urines with tes-tape are of no significance.

Tes-tape may be slightly more sensitive than clinistix, but for the qualitative detection of glucose there is little to choose between them, and it is clear that they will play an important part in hospitals and general medical practice. The techniques are simple, no apparatus or boiling is required, and their specificity is beyond doubt. If they are to be used by nurses it is important to remember that they are essentially ultra-micro-methods in the strictest sense and require all the standards of chemical cleanliness such methods demand. Provided these rigid standards are maintained, these enzyme tests must be of the greatest value in the detection of glycosuria, especially in the antenatal and post-natal

clinics, where urines may often contain lactose, a reducing sugar affecting all the simple copper-reduction tests. It is fortunate that any errors in the enzyme test will give positive results which could be checked rather than negative results which can be accepted without question. Whether the reduction methods are used for screening purposes and the enzyme test for confirming the findings, or whether the enzyme test is used as a screening test and some reduction method to confirm the quantity of glucose present in the urine, will be dictated by the personal preference of the clinician.

The accurate quantitative estimation of glucose in urine presents considerable difficulty. The inaccuracies of Benedict's qualitative estimation, especially when the urinary content is low, has already been recognized (Gray and Millar, 1953). The present studies have emphasized that the potentiometric method and the polarimetric technique (unpublished observations) are accurate only when the glucose concentrations are higher than 1 or 2 g. per 100 ml. For this reason the highly specific and accurate estimation of Froesch and Renold (1956) has been used in a substantial number of the present tests.

On theoretical grounds a test depending on two distinct enzyme reactions would be expected to be influenced by factors interfering with both of these reactions. It is probably for this reason that the manufacturers of clinistix take the view that accurate quantitation is impracticable at the present time. On the other hand, the manufacturers of tes-

FIG. 2.—Observer errors in the detection of glucose.

tape have provided a colour scale and claim that accurate quantitation is possible. This colour chart varies from batch to batch, and the present results show that reasonable accuracy is possible in only about half the urines examined; moreover, the distinction between the colours given by concentrations of glucose above 0.5 g. per 100 ml. is difficult and above 2 g. per 100 ml. impossible.

In the control of diabetic treatment the presence of small amounts of glucose in the urine is of little importance. A test of practical use must distinguish accurately between low and high concentrations of glucose. Neither test can do this, and the relative difficulty in distinguishing with certainty between 0.5 g. per 100 ml. and concentrations around 5 g. per 100 ml. render these tests unsuitable for use by diabetic patients themselves. A test which does not readily distinguish between a glucose concentration corresponding to a loss of 5 g. of glucose a day from a glucose concentration corresponding to a loss perhaps of 100 g. or more a day is likely to mislead gravely both the patient and his clinician. The existing tests may be little better for the quantitative determination of low concentrations of glucose, but the bright orange colour of complete reduction in Benedict's test or the clinitest is striking enough and indicative of a significant level of glycosuria.

Summary

A comparison has been made between two new enzyme tests for the detection of glucose and existing methods. Both tests are equally good in this respect. They are highly specific for glucose and may be of great value either to supplement existing tests or perhaps to take their place. The claim that one of these tests permits accurate estimation of glucose is not confirmed.

Thanks are due to Ames Company (London) Ltd. and Eli Lilly and Co. Ltd. for supplies of clinitix and tes-tape respectively. We also thank the former for seconding one of us (D. E. T.) to the Department of Clinical Pathology at King's College Hospital Medical School during the whole period of this study. We thank Mrs. B. Rigby and Miss M. T. Pickett for assistance with the estimation of large numbers of true urine glucose.

REFERENCES

- Froesch, E. R., and Renold, A. E. (1956). *Diabetes*, 5, 1.
Gray, C. H., and Millar, H. R. (1955). *British Medical Journal*, 1, 1361.
Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*. Churchill, London.
Quilley, E. (1955). *J. Lab. med. Tech.*, 13, 158.

Medical Memorandum

Enzyme Test for Glycosuria

Recently an enzymatic test has been developed which is specific for detecting glucose in urine. "Tes-tape" contains such an enzyme system, and also incorporated in the paper is a yellow dye. When the paper tape is dipped in urine containing glucose the paper changes colour from light green to dark green and finally to blue, depending upon the amount of glucose in the urine. A colour chart is supplied with each batch of tes-tape, and yellow, greenish yellow, light green, dark green, and dark blue are claimed to correspond respectively to 0, 0.1, 0.25, 0.5, and 2 g. of glucose per 100 ml. of urine.

Such an easy quantitative test for glycosuria would be a tremendous saving of time in a busy diabetic clinic and a boon to diabetic patients. The findings from tes-tape were therefore compared with the results from routine testing of samples of urine obtained from 110 patients attending the Diabetic Clinic, Leeds. Each sample of urine was tested by one of us (D. C.) in a routine way by means of "clinitest"; the same numbered sample was tested by an independent worker with tes-tape. The results are shown in Tables I and II.

Table I shows very close agreement between the two tests when no sugar or only 0.1 g. per 100 ml. is present. The

TABLE I.—Comparison of Results from Tes-tape and Clinitest

	Glucose (G./100 ml.)						
	0	0.1	0.25	0.5	0.75	1	2
Tes-tape (%)	38	12	16	13			21
Clinitest (%)	52		11	2		13	22

TABLE II.—Percentage of Non-matching Results

Clinitest (2 g.)	..	7%	Clinitest (1 g.)	..	9%
Tes-tape (0.1-0.5 g.)	..		Tes-tape (Nil-0.5 g.)	..	
Clinitest (1 g.)	..	4%	Clinitest (Nil)	..	4%
Tes-tape (2 g.)	..		Tes-tape (0.5-2 g.)	..	

0.1 g. per 100 ml. results have been placed with the no sugar group, since the significance of such trace amounts of sugar is not known at the moment. Comparison of the two tests in increasing concentrations of urinary sugar does not show such close agreement, although a similar percentage is recorded by both tests for 2 g. or more glucose per 100 ml.

Table II, however, shows that percentages for a 2% result in Table I are misleading, as 24% of the tes-tape results did not match with the clinitest results. The 0.25 g. per 100 ml. differences between two tests were ignored in this analysis. For example, 7% of the tests showing 2 g. per 100 ml. by the clinitest revealed only 0.1 to 0.5 g. per 100 ml. by the tes-tape. Conversely, 4% were negative for glucose by the clinitest, but 0.5 to 2 g. per 100 ml. was recorded by the tes-tape.

From these results and previous work with "clinitix" (unpublished) it would appear that the enzyme tests may be useful in deciding whether glucose is present or not in a sample of urine. Such a test may be useful in a maternity out-patient department or casualty department of a general hospital.

These results show that the new enzyme test from urinary glucose is most unreliable when regarded as a quantitative test.

The findings with the enzyme test—namely, that blue reactions indicate a strongly positive result—are likely to be misinterpreted in view of previous experience with other methods. Diabetic patients have long been accustomed with Fehling's Benedict's, and more recently with clinitest, to interpret the blue reaction as a sign of absence of sugar, and confusion would therefore be inevitable. Furthermore, the new tests can be of no use to the physician in the management of diabetic patients when 11% of the tests cannot distinguish the presence of no sugar or over 2 g. per 100 ml.

R. E. TUNBRIDGE, M.D., M.Sc., F.R.C.P.

RONALD G. PALEY, M.D., Ph.D.

DOUGLAS COULSON.

Department of Medicine,
University of Leeds.

"Increasingly minds are at the mercy of central organs of education and propaganda. . . . There is deliberate commercial exploitation of the baser motives: envy, cupidity, acquisitiveness, vanity, rivalry and the desire for power. On the one hand sex is suppressed; on the other the sexual interest and appetite are ruthlessly titillated and inflamed by every possible instrument of communication. The phallic symbol of pagan tribes has been displaced by an out-thrusting female breast. The decreasing size and the narrowing functions of the family tend to make its remaining associations more intense, more self-contained and more self-centred, thus increasing the significance of the idiosyncratic nature of individual families as a cultural determinant, and favouring the development of solitary, or at most small in-group feelings, rather than social impulses. This tendency is accentuated by the debasement of family hospitality (at least in the middle classes) to its most superficial, sporadic and tangential forms."—Dr. ROBERT SUTHERLAND, National Association for Mental Health's Conference on Mental Health and Personal Responsibility, Harrogate, April 12-13.