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Technical methods

Modification of the method for the estimation of xylose in urine

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The method of Roe and Rice (1948) for the determination of xylose in urine has been used for some time in this laboratory. It became a cause for some concern that the standards, which were apparently treated in the same manner and contained equal concentrations of xylose, gave on separate occasions widely different readings on the colorimeter. It was, therefore, decided that the method should be examined systematically in an effort to determine the cause of this variation.

METHOD

Sixteen tubes containing 5 ml of 2% p-bromoaniline and 1 ml of 20 mg/100 ml xylose solution were heated at 70°C in a water bath. Duplicate tubes were removed from the bath at five-minute intervals from five to 20 minutes and also after 30, 60, 90, and 120 minutes. The tubes were quickly cooled, then placed in a dark cupboard for 70 minutes. The colour developed was then read at 530 nm in an EEL flow-through spectrophotometer. Unheated xylose solution and *p*-bromoaniline, in the same amounts as the test samples, were used as blanks. The results are shown in Figure 1.

Figure 1 shows that, when heated at 70°C, the intensity of the colour developed increases rapidly with time until it reaches a maximum at 15 to 20 minutes, and thereafter the colour decreases in intensity. It had been the practice in this laboratory to cool the tubes after 10 minutes of heating at 70°C. It can be seen that this time is on the steep part of the curve and that slight variations in the time of heating, or of cooling, about the 10-minute mark, will cause wide variations in the colour produced.

Increasing the temperature of the water bath causes more rapid development of colour and demands even greater precision timing to ensure reproducible results.

Lowering the temperature to 55°C results in a graph of a different shape and in particular the colour developed remains steady from 30 to 60 minutes (broken line Fig. 1).

Varying the time the solutions are allowed to develop in the dark shows that the increase in colour is linear with time, and varying the time from 60 to 80 minutes causes only a small difference in the EEL readings.

DETAIL OF FINAL METHOD To make up the dose, 5 g D(+) xylose (BDH) is dissolved in 250 ml of water; 1 ml of this is retained for the standard. The technique for the administration of the xylose to patients and for the collection of urine is as previously described by Sammons, Morgan, Frazer, Montgomery, Philip, and Phillips (1967).

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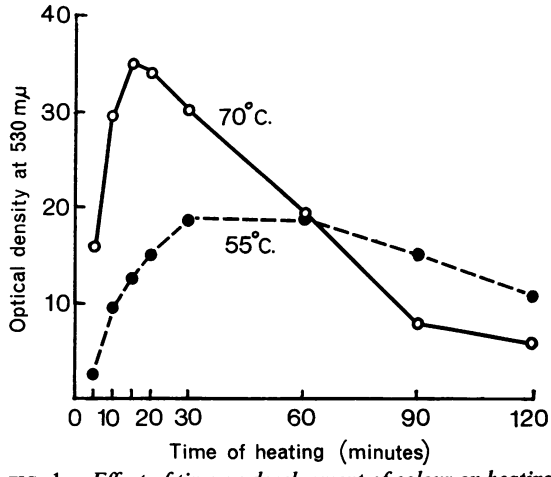

FIG. 1. *Effect of time on development of colour on heating 0·2 mg/ml xylose solution at 70°C (continuous line) and at 55°C (broken line).*

The standard (1 ml) is diluted to 50 ml (0·4 mg/ml). Urines are diluted 1:10, 1:25, or 1:100 according to the total volume of urine excreted and the expected excretion of xylose. Occasionally the urine is used undiluted.

To each of three tubes is added 1 ml of diluted urine or standard and 5 ml *p*-bromoanaline solution (2 g% in glacial acetic acid saturated with thiourea). Two of the three tubes are placed in a water bath at 55°C for 40 minutes and afterwards cooled. All the tubes are then left in a dark cupboard for 70 minutes. The solutions are read at 530 nm, each tube being read against its own unheated blank.

COMMENT

The above experiments suggest that heating solutions containing xylose and *p*-bromoaniline at 55°C for 40 minutes provides more reproducible results than heating at 70°C for 10 minutes. The advantages of this method are that the time of heating and the time taken to cool the tubes are not critical and this is particularly important when a large number of tests are being made together. This modified method has been used in this laboratory over the past six months and it gives results with a very high degree of reproducibility.

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