Short Articles

HISTORY OF THE MEASUREMENT OF GLUCOSE IN URINE: A CAUTIONARY TALE

by

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In a recent study[1] of glucose reabsorption by the kidney it became clear that enzymatic methods of determining glucose in blood and urine produced a picture totally at variance with classical teaching, but traditional results could be obtained with the same material if the traditional copper reduction methods of estimation were employed. The major difference lay in the gross underestimation of glucose in urine by copper reduction techniques, the converse being true for plasma but to a lesser extent; the compound effects of the errors exaggerating the kidney's ability to reabsorb glucose.

Glucose determinations based on the reduction of copper have been used since the nineteenth century and are still commonly employed in clinical laboratories. They were used in the classical work on renal physiology undertaken thirty to forty years ago, and which is only now being questioned. It seemed worth going back to the original biochemical literature to find out whether doubts had been expressed previously and, if so, why they nevertheless failed to cause persistent misgivings and subsequent search for better methods. Our review of the literature has shown that doubts indeed arose but for some reason they only led to a polemical situation which eventually died away during the 1920s without resolution. Despite the biochemists' failure to agree, clinicians and physiologists appear to have accepted "authoritative" methods without recognizing their limitations. As a result, edifices of clinical and physiological theory have persisted despite their weak technical foundations.

THE LITERATURE

During the first quarter of the present century a stream of papers described, first, the use of copper reduction as a qualitative test of glucose in urine and, subsequently, refinements and modifications designed to make the test quantitative. The two main protagonists were Benedict and Folin, and although these two pioneers undoubtedly made great contributions, many of their later publications on the present subject were polemical, rather than objective contributions to clinical chemistry. It is understandable that the physiologists working on the renal handling of glucose became confused, and more often than not quoted references to methods which had originally been described for blood and not for urine.

In 1907 Benedict,[2] introducing a copper reagent with a carbonate base as being less destructive of sugar than Fehling's hydroxide base, recognized that there were interfering substances in urine which could either partially reduce, or else inhibit reduction

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of copper. It is curious that having recognized or suspected them he does not appear to have made any attempt to measure their effects, merely stating that ". . . In order to test the accuracy and reliability of this method a solution of dextrose in distilled water was prepared . . .". A slightly modified form of the same method for use with urine was unaffected by creatinine and uric acid[3] and ". . . repeated determinations and comparisons with results by the polariscope and by the gravimetric process have shown the method to be considerably more exact than any other titration method suitable for sugar work". But the critical test, recovery from urine, was always lacking.

Folin took the stage in 1915[4] with a paper describing a test for small amounts of glucose he recognized to be present in normal urine ". . . a fact which is not adequately shown or recognised by the current qualitative 'tests for sugars' in urine".

There followed in 1918 a series of three papers by Benedict and his colleagues [5,6,7] for determination of glucose in blood and urine in which copper reduction was abandoned in favour of a modified picrate-picric acid method based on an earlier reaction described by Lewis and Benedict (1915)[8] for determination of sugar in blood. Benedict and his colleagues[7] also recognized that all normal urine contained some glucose and believed that ". . . Progress in the study of carbohydrate metabolism will probably be more rapid if the term 'glycosuria' can be abolished. This word was not created in the mind of man, but by the inefficiency of the copper tests . . .". For the new method it was claimed, without presenting the data ". . . that glucose added to urine is quantitatively recovered within a few thousandths of one per cent . . .".

Folin and his colleagues took over the lead from 1919, with a massive paper, part of which was concerned with glucose determination in blood. This was to form the basis of most glucose estimations in blood and urine thereafter using a new copper reduction method.[9,10] In passing, the picrate-picric acid method of Benedict is dismissed as subject to many sources of error. The same year at a meeting of the American Association of Biological Chemists, Benedict (quoted by Folin and Wu, 1920[10]) condemned the Folin and Wu method ". . . on the ground of excessive, inevitable and uncontrollable reoxidation of cuprous oxide . . ." which led Folin and Wu[10] to introduce the Folin-Wu tube in an attempt to avoid the problem ". . . and we gladly give Benedict credit for having compelled us to re-examine our method . . .".

The Folin and Wu method was then applied to the measurement of glucose in urine[11,12] but the main purpose of the papers seems to have been to attack Benedict's clinical concepts, and no evidence was given that the Folin and Wu process was a good method for measuring glucose in urine. Indeed, it was acknowledged that the method should be useful ". . . provided that a suitable process could be found for removing substances which can interfere".

In the same year (1922), Smith[13] revived the Benedict copper reduction method for urine claiming that the technique in current use (i.e. Benedict, 1911[3]) gave a variable error of 15–30 per cent and suggesting a modification to reduce the error.

Three years later, Benedict[14] had reluctantly accepted that his picrate-picric acid method was inaccurate though he doubted ". . . whether a clinician has ever been misled in his interpretation of a diabetic case by the figures obtained from these analyses when properly carried out . . .". That paper marked Benedict's re-entry into the copper reduction field and there is an aura of resentment that Folin had, in the meantime,

overtaken him: ". . . The greater delicacy and specificity of copper reagents containing carbonate in place of hydroxide were pointed out by the present writer many years ago . . .". Benedict went on to introduce yet another modification to the method for determination of blood sugar, the addition of sodium bisulphite, which he claimed gave lower blood glucose values than the Folin and Wu method and when applied directly to diluted normal urine ". . . the new procedure consistently yields figures about one-third as great as those given by the Folin-Wu modified tartrate reagent . . .". In passing Benedict observes that the Folin-Wu tube is of no value in solving the problem of reoxidation of copper.

The following year, Folin[15] replied with another modified copper reagent for use with blood and urine, and was "rather surprised" that Benedict found the Folin-Wu tube unhelpful stating that ". . . I now believe that Benedict's conclusion is based on compensating experimental errors". In a series of comparative tests on blood he could not confirm that Benedict's new reagent gave lower blood glucose estimates and concluded that the bisulphite was unstable. Folin claimed that his new reagent gave good results in urine but no recovery experiments were described.

Benedict[16] acknowledged Folin's observation regarding the instability of the bisulphite stating that ". . . criticism is fully justified, and we much regret the error which at this point appeared in the published form of the method". Benedict then returned to the attack pointing out that ". . . Folin has himself apparently fallen into serious error in assuming that his new copper reagent can be applied to the determination of sugar in urine" and goes on to show that ". . . recovery of sugar added to urine by the new Folin method showed a loss of about 25 per cent of the 40 mg added. Obviously the new Folin procedure is not adapted to the determination of sugar in normal urine".

Folin finally capitulated[17] and accepted Benedict's criticism: ". . . one most unfortunate oversight occurred. No attempt was made to ascertain whether known amounts of glucose added to urine were quantitatively recovered. There was no tangible reason to believe that the new method might be less dependable . . ."; and ". . . the flaw is sufficiently serious so that the method would necessarily have to be abandoned unless the errors involved could be eliminated."

Some papers on copper reduction methods for blood sugar continued to appear for several years[18,19,20] before attention turned to ferricyanide reagents, but in retrospect it is clear that the bell had tolled for estimation of sugar in urine.

CONCLUSION

That reducing substances other than glucose can reduce the copper reagents designed for glucose estimation has always been appreciated and the relative effects of many of the substances are known. What was also known, at least since Benedict's 1907 paper,[2] but not generally appreciated, was that urine contains substances which in some way inhibit the copper reduction reaction and therefore cause underestimation of any glucose present.

Estimation of glucose in blood was always the primary objective in the development of the copper reduction methods and although attempts to apply them to urine occurred persistently, it can be discerned through the fog of claims and counter claims that they were never satisfactory. And yet those methods formed the basis of investigations into

glucose excretion by the kidney which gave rise to the classical concepts of the renal handling of glucose,[21,22,23,24] and which are still enshrined in the physiological literature.

Thirty years later we still do not know what those interfering substances might be, but the problem has been finally removed by the development of modern enzymatic techniques.

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