Letter to the Editor

Evaluation of the Aldononitrile Peracetate Method for Measuring Arabinitol in Serum

Several findings in an article by de Repentigny and colleagues (2) appeared to be internally inconsistent or to contradict our previous results (1, 4). For example: (i) 2.5 to 5.0 μg of arabinitol per ml was found in uninoculated yeast nitrogen base plus 0.3% glucose, although this chemically defined medium is known not to contain arabinitol; (ii) *Candida albicans* 3181A did not produce arabinitol in yeast nitrogen base plus 0.3% glucose, although every *C. albicans* strain we have studied produces arabinitol in this medium; (iii) two groups of normal rabbits had substantially different baseline arabinitol/creatinine ratios (see Fig. 2B and 5A of reference 2); and (iv) the serum arabinitol/creatinine ratios in normal rabbits rose significantly over time, although nothing was done to these animals except that they were bled at 4-day intervals.

Because of these unexpected findings, we evaluated the authors' aldononitrile peracetate method for measuring arabinitol (3). Solutions of arabinitol or glucose in water and normal human serum to which glucose was added were studied. Aldononitrile peracetate derivatives were prepared as described by the authors (3), peracetate derivatives were prepared by the same method except that hydroxylamine HCl was not added, and trimethylsilyl ether derivatives were prepared as previously described (1, 5). Specimens were analyzed by gas chromatography and by combined gas chromatography and chemical ionization mass spectrometry.

When glucose was derivatized by the aldononitrile peracetate procedure, considerable amounts of several reaction byproducts were formed. One of these was a pentitol peracetate with the same chromatographic retention time and mass spectrum as arabinitol peracetate (Fig. 1). Whether the compound was arabinitol or ribitol was not determined because the peracetates of these pentitols cannot be separated on OV-225 columns (3). The same byproduct was formed from glucose in serum; addition of increasing amounts of glucose resulted in correspondingly increased amounts of the pentitol peracetate. No pentitols were found by gas chromatography or mass spectrometry in reagent blanks or when the peracetate or trimethylsilyl derivatives of glucose were prepared, so contamination cannot explain these findings.

Further experiments showed that the same pentitol peracetate was a byproduct of forming the aldononitrile peracetate derivative of mannose and that a tetritol indistinguishable from erythritol was a byproduct of forming the aldononitrile peracetate derivative of arabinose. No polyols were found when the peracetate or trimethylsilyl ether derivatives of mannose or arabinose were prepared. Although we did not study the mechanism by which polyol peracetates were formed during derivatization of glucose, mannose, and arabinose, reductive cleavage of the nitrile in position C-1 of each aldononitrile or its peracetate would be expected to yield the polyol peracetates we found.

We conclude that the aldononitrile peracetate method can give falsely high arabinitol results because the derivatization procedure itself can convert glucose to a pentitol indistinguishable from arabinitol. The potential for error is considerable because of the large and variable amounts of glucose in serum.

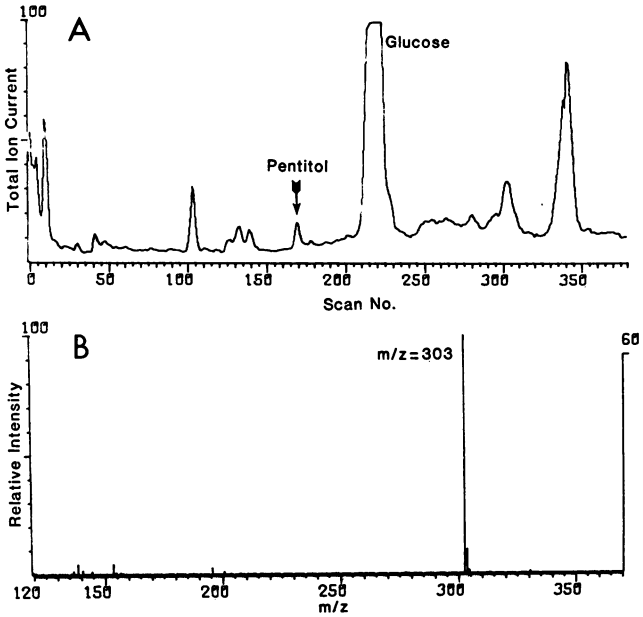

FIG. 1. When 1,000 μg of glucose per ml in water was derivatized by the aldononitrile peracetate procedure, approximately 4 μg of a pentitol peracetate per ml was found by gas chromatography-chemical ionization mass spectrometry. The peak indicated by the arrow in the total ion monitoring mode chromatogram (A) had the same retention time (8.56 min) as arabinitol peracetate. The mass spectrum of the compound (B) was identical to that of arabinitol peracetate. Analytical conditions: glass column (1.8 m by 2.0 mm) packed with OV-225, oven temperature 160 to 275°C at 10°C per min; methane was the carrier and reagent gas.

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Author's Reply

The following is in reply to the comments of Wong et al. about the segment of our paper (2) concerning measurement of arabinitol.

(i) The lot of Difco yeast nitrogen base (YNB) (no. 645292) did not contain arabinitol. We made a 0.3% solution of the glucose used in our medium (Mallinckrodt lot KPTN) and derivatized it as the peracetylated aldononitrile (PAAN), and it contained 3.4 μg of arabinitol per ml (0.11%).

(ii) Bernard et al. (1) showed that arabinitol production by *Candida albicans* is variable in YNB plus 0.3% glucose (0.36 to 51.9 μg/ml). Thus, some strains of this yeast produce minute concentrations close to the limit of sensitivity of the assay. Furthermore, we showed that *C. albicans* 3181A produced arabinitol (35 to 76 μg/ml) after incubation in normal rabbit serum. Thus, YNB plus 0.3% glucose is not an ideal medium for demonstrating in vitro arabinitol production.

(iii) There is some variation between the two groups of identical normal rabbits (see Fig. 2B and Fig. 5 of reference 2), but in no case did the arabinitol/creatinine ratios approach those in uninfected rabbits receiving cortisone. In addition, the mean ± standard deviation in 50 normal human blood donors was 0.45 ± 0.37 μg/ml (manuscript submitted for publication). These results agree with those of Roboz et al. (3), who found a mean arabinitol level of 0.52 ± 0.34 μg/ml in 39 normal subjects. We interpret this close agreement with other laboratories as evidence for the adequacy of our analytical method.

(iv) We noted that serum arabinitol/creatinine ratios of control rabbits increased during the course of the experiment. This is not the result of analytical error, because none of 22 rabbits studied before the experiment had such a high arabinitol/creatinine ratio. It did not result from upward drift in the gas-liquid chromatography (GLC) quantitation, because the sera of 22 normal rabbits (day −2) were interspersed during the assay among serum samples drawn later. The trauma of repeated bleedings may produce stress and increased endogenous corticosteroids. Wong et al. have not introduced new data on this point, and we consider it an open question. There is little doubt that cortisone acetate given to uninfected rabbits elevated the arabinitol/creatinine ratios, because the identity of arabinitol was confirmed by mass spectrometry.

The observation by Wong et al. that a pentitol with the retention time of arabinitol penetaacetate is produced as a by-product of the PAAN derivatization of glucose prompted us to check this by derivatizing reagent-grade D-glucose. We also found that 0.4% of the sample generates an artifact with the same retention time and mass spectrum as arabinitol. We followed this observation by dialyzing normal rabbit serum and making a PAAN derivative of the lyophilized dialyzate. This preparation was chromatographed by capillary GLC at a concentration equivalent to or higher than that of the reagent-grade glucose. We could not find any by-product corresponding to D-arabinitol by flame ionization detection, nor could we find a mass ion 303 having the retention time expected for D-arabinitol by GLC-mass spectrometry and chemical ionization detection. However, in the much more sensitive multiple ion mode (*m/e* 303, 363), we were able to detect these ions at the retention time expected for D-arabinitol. This finding suggests that in normal capillary GLC analysis of rabbit serum with flame ionization detection it would not be possible to detect the by-product. It may be of some significance that β-D-glucose dissolved directly in pyridine gave rise to 25% as much D-arabinitol in the PAAN derivatization procedure than did reagent-grade D-glucose. The GLC procedure for arabinitol must make a distinction between endogenous arabinitol and microbial arabinitol which complicates the interpretation of serum arabinitol. This is done by using the arabinitol/creatinine ratio recommended by Wong et al. (4). Our research suggests that conditions unrelated to renal failure can elevate arabinitol, i.e., administration of systemic corticosteroids. If that is the case, as our data suggest, the arabinitol/creatinine ratio will not suffice to differentiate endogenous from microbial arabinitol.

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