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RESPONSE TO NANCE LETTER

To the Editor: The pioneering work done by Dr. Nance on the quantitative expression of the Gd^B and Gd^A alleles in the Nigerian population was so much present to our mind when we wrote our recent article [1] that it was the first item mentioned under Results (reference [16] in the paper). Unfortunately, the detailed data were not before us, and it appears they could have only been obtained by direct inquiry. Therefore, we are indeed very happy if we have prompted Dr. Nance to make them available now through the *Journal*. Regarding the information in Nance's letter, we would like to offer the following comments. For the sake of simplicity we shall refer to the subjects studied by Nance as series *N* and to the subjects studied by us as series *B*.

1. Both series conclude that the quantitative expression of Gd^B in red cells is higher than that of Gd^A .

2. We have reported results on male subjects only. The results on females shown in Nance's figure 3B [2] do not seem to contribute significantly to the problem of variation of G6PD activity specified by Gd^A and Gd^B . Besides, the Gd genotype was not positively established in a number of the female subjects. Therefore, we shall not discuss the results on females further.

3. We are, of course, aware of significant difference in G6PD levels associated with hemoglobinopathies [3]. All of our samples were typed by hemoglobin electrophoresis, and no cases of SS or SC were included. The difference between G6PD levels in AA, AS, and AC subjects is not statistically significant.

4. There are some differences in methodology. First, we have assayed G6PD both by the regular assay and by adding G6P and 6PG as substrates. However, we have rejected the choice of expressing G6PD as a difference between the two assays for the following reasons. (1) It can be calculated that the amount of 6-phosphogluconate produced during the initial 5 min of the reaction (a time sufficient to determine the slope) cannot lead to an overestimate of G6PD activity of greater than 5%. (2) 6-Phosphogluconate is an inhibitor of G6PD. Therefore not only are values obtained by subtraction quite significantly and systematically lower than those obtained by the regular assay, but on several occasions, the NADPH production in the presence of the two substrates was found to be even lower than with G6P alone.

Second, our samples were not glycerolized and frozen; rather, they were processed within 24 hr of collection and assayed on the spot. We do not mean to suggest that transport, freezing, and storage have necessarily affected Nance's results, but we note that the mean activity for normal G6PD B in males according to his table 1 is 1.92 IU/G of hemoglobin, whereas currently accepted normal values are between 6 and 9 [4].

Third, we carried out all G6PD assays at constant Hb concentration because we noticed that there was not a perfect linearity between hemolysate concentration and observed G6PD activity.

5. Although our data were obtained in the course of family studies for a different purpose (to be published elsewhere), we have included in our paper data from only one male subject per family. All subjects were children between 2 and 10 years of age. By contrast, the age of Nance's subjects is not indicated, but presumably the range was much wider. In addition, it appears that many subjects per family were included (on the average four to five). Thus, pooling of unrelated and related subjects might make the interpretation of the skewness and kurtosis data in table 1 rather difficult.

6. If we take as 1.00 the quantitative expression of gene Gd^A , we find that the quantitative expression of Gd^B is approximately 1.11 in series N and 1.21 in series B. From the analysis of data in series B, we have estimated that the genetic contribution to the overall variance of G6PD levels in all Gd^+ males, which is due to the existence of the two distinct alleles Gd^A and Gd^B , is 8%.

The same type of analysis carried out on the data in series N yields an estimate of about 2%–4%. For the reasons outlined in the previous paragraphs, we believe that the estimates based on series B are probably more accurate.

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