Detection of Carriers in Hereditary Galactosæmia

Attempts are being made[1] to detect the heterozygous state for galactosæmia by means of the uridine diphosphoglucose consumption test on erythrocytes—a test devised by us for the detection of galactosæmia[2]. This test was designed so as to give a maximum of difference between normal persons and persons having congenital galactosæmia, in which no galactose-1-phosphate uridyl transferase is discernible in erythrocytes[3,4]. In order to magnify this difference and hence provide an unambiguous proof for the clinician[2,3] this reaction mixture is over-incubated. With near-normal activities, the reaction is brought close to the equilibrium point (cf. refs. 2, 4, 5), and the rates measured are obviously not maximum rates. Experiments in which aliquots for uridine diphosphoglucose determination were taken at 5, 10 and 20 min. incubation time designed to give a quantitative expression of maximum rates have also been carried out[5]. In this work we used the consumption as well as the combined epimerase test[2,3], and yet, only in about two-thirds of the specimens did we succeed in obtaining linearity between incubation time and conversion. Accordingly, we believe it even less likely that the unmodified clinical consumption test (in which only one aliquot at 30 min. incubation is drawn) should be able to detect heterozygous carriers. Therefore, as stated previously[2,6], we do not recommend the consumption test for the latter purpose.

This is not to deny the high degree of reliability of the consumption test and especially our combined test[2,3] for detecting homozygous individuals afflicted with galactosæmia. Not only has the test proved to be reliable and specific, but it also circumvents the loading of afflicted infants with galactose. Moreover, as suggested by Dr. H. N. Kirkman[7], a slight modification of the test may prove useful in spotting galactose-1-phosphate in blood and hence turn attention toward possible faulty diets in case of galactosæmia[7].

We have pointed out on earlier occasions[2,6] that in order to develop reliable techniques for detecting heterozygous carriers in galactosæmia it is necessary to develop methods in which it is possible to obtain accurate and abundant results under conditions in which one can expect maximum rates for galactose-1-phosphate uridyl transferase. Such methods, which were developed more than a year ago by Dr. Kirkman[8], permit the recording of 20–25 points during the period in which the reaction is proceeding at maximum rate. The first results, which were reported some time ago[9], showed that the parents of galactosæmic children frequently manifest partial defects in the above-mentioned enzyme. In a more detailed publication[8] Kirkman evaluates the method and the results. It seems that this method permits the detection of an enzyme defect in the great majority of carriers of hereditary galactosæmia.

Note added in proof: A paper dealing with galactosæmia carriers has recently appeared (Brettauer *et al.*, *Proc. U.S. Nat. Acad. Sci.*, **45**, 328; 1959) which describes preliminary results obtained with a similar modification of our diagnostic methods[2], namely, shorter incubation time and higher substrate concentrations. Values for transferase activity in hæmolysates ranging between 4·8 and 8·1 μmoles/hr./gm. cells are reported. In a smaller survey I found values ranging between 4·0 and 5·8 μmoles/hr./gm. (unpublished work).

We also agree with Brettauer *et al.* that the modified diagnostic method makes it possible to find markedly lower enzyme values in hæmolysates from parents of galactosæmic children. I have found values as slow as 1·2–2·2 μmoles/hr./gm. cells. In spite of these differences we cannot recommend the "one point method" for use in the diagnosis of individual carriers. The manometric method as described by Kirkman gives a guarantee that one is really dealing with zero-order kinetics and we believe that at the present time it is the only test which can be used in the diagnosis of carriers in families in which galactosæmia has not been observed before. A detailed discussion of these problems will be given elsewhere.

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Gene Action in Erythrocyte Deficiency of Glucose-6-phosphate Dehydrogenase: Tissue Enzyme-Levels

The susceptibility to increased hæmolysis following ingestion of such agents as primaquine, naphthaline, nitrofurans, other drugs and the fava bean is associated with intrinsic defects in erythrocyte metabolism. These defects include a lower concentration of reduced glutathione[1], instability of reduced glutathione during incubation of the cells with acetylphenylhydrazine[2], and a deficiency in glucose-6-phosphate dehydrogenase[3]. Recent studies have suggested that this erythrocyte enzymatic deficiency is genetically determined, and inherited as a sex-linked characteristic of intermediate dominance[4–6].

In this investigation, it has been found that subjects with an erythrocyte deficiency of glucose-6-phosphate dehydrogenase have normal levels of this enzyme in their leucocytes. In addition, two subjects with markedly deficient erythrocyte glucose-6-phosphate dehydrogenase did not have a similarly decreased level of this enzyme in their liver.

Leucocytes were separated from venous blood by a method of dextran flotation which yields a white blood cell preparation contaminated with fewer than 5 red cells for each leucocyte[7]. This degree of contamination by red cells does not affect the assays of leucocyte enzyme. The leucocytes were suspended in approximately 20 vol. of isotonic potassium chloride buffered at *p*H 7·4 and disrupted by freezing and thawing three times. Leucocyte counts were performed prior to, and following, this procedure to ensure that assays were performed only on samples in which more than 90 per cent of the white blood cells were destroyed. Liver tissue was obtained by surgical biopsy through an abdominal incision. This tissue was homogenized and resuspended in isotonic