Elevated Frequency of Carriers for Triosephosphate Isomerase Deficiency in Newborn Infants

H. W. MOHRENWEISER[40] AND S. FIELEK

Department of Human Genetics, University of Michigan Medical School, Ann Arbor, Michigan, USA

Summary

Seven newborns with erythrocyte triosephosphate isomerase (TPI) activity levels consistent with the existence of a "null" allele in heterozygous form were identified among 146 Black infants studied. This allele frequency of 0.024 is ten times the frequency of 0.0024 observed in White newborns (5 heterozygotes/1048 infants). Each carrier infant has one parent with a level of TPI activity expected of a heterozygous deficient (carrier) adult, whereas the other parent has a normal level of TPI activity, as would be expected for an autosomally inherited genetic trait. All probands as well as affected parents, are asymptomatic as anticipated for heterozygotes having 50% of the expected enzymatic activity. The data are consistent with the existence of a "null" allele, designated TPI 1°, at the structural locus for TPI, although no immunologic or other direct evidence of inactive or altered subunits was obtained. This high allele frequency implies one of every 2000 Black newborns should be homozygous deficient; this is in conflict with the low number of homozygous deficient afflicted individuals which have been actually identified to date.

Triosephosphate isomerase (TPI: EC 5.3.1.1), an enzyme widely distributed in all tissues of a wide variety of organisms (33, 36), catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. This dimeric enzyme (MW = 53,000) of identical subunits (8, 38) is involved in both glycolysis and gluconeogenesis as well as in glyceride synthesis. TPI is characterized by a high cellular level of enzyme activity (13, 17, 23), a high catalytic efficiency (1), and a similarity in the kinetic properties of the enzyme isolated from many sources (8, 13, 21, 30). A significant degree of sequence homology is observed in enzymes from divergent species (2, 4, 21, 38), especially in the active site region (18), indicating a slow rate of evolution (9). Few electrophoretic variants have been identified in surveys of human populations (25, 28, 29).

An autosomal recessive disorder involving TPI deficiency has been described in 17 individuals in six different families (16, 31, 32, 35, 37). Affected individuals exhibit severe neurologic and muscular disorders, retarded growth, chronic nonspherocytic hemolytic anemia, and increased susceptibility to infection (32, 35, 37). Symptoms usually do not appear until the infant is several months of age but they are progressive and the condition is fatal, usually before an age of 6 years. Although some residual activity (5–20%) is detected in erythrocytes of homozygous deficient individuals, the 50-fold increase in dihydroxyacetone phosphate concentration would suggest an almost complete metabolic block at this step (32). The metabolic basis for the symptomatology in other tissues in the homozygous deficient individuals is not immediately obvious as glycolysis should be maintained at significant levels and dihydroxyacetone phosphate should not accumulate to high concentrations in tissues that have glycerophosphate dehydrogenase activity.

Recent reports by Mohrenweiser (23) and Eber *et al.* (11, 12) indicate that the frequency of TPI deficient carriers (heterozygous individuals with one normal and one "null" allele) is much higher than expected from the number of reported homozygous deficient individuals. This report will extend the earlier report of Mohrenweiser (23), present the frequency within ethnic groups and examine the basis for the deficiency.

MATERIALS AND METHODS

The study population consisted of all infants born at the University of Michigan Women's Hospital maternity service between August, 1977 and January, 1981 whose parents agreed to donate blood samples (23). The sample from newborn infants was obtained from the umbilical cord at delivery, whereas parent samples were obtained by venipuncture. All samples were collected with acid citrate dextrose (ACD) as the anticoagulant. The red cells were washed three times with saline and then stored as packed cells in liquid nitrogen. Hemolysates for enzyme activity studies were prepared by diluting 1 vol of packed cells, after freezing and thawing, with 7 vol of buffer (50 mM N-2-hydroxyethylpiperazine N-2-ethanesulfonic acid, pH 7.0, 1 mM EDTA, 2 mM dithiothreitol and 0.06% Triton X-100) and centrifuging at $40,000 \times g$ for 20 min. Hemolysates for the electrophoretic studies were prepared by diluting 1 vol of packed cells with 1 vol of H_2O and then extracting with 0.5 vol of toluene (10).

Enzyme activities were assayed as described by Fielek and Mohrenweiser (15) and Mohrenweiser (23) except for enolase (EC 4.2.1.11) and phosphoglyceromutase (EC 2.7.5.3), which were assayed by methods described by Bergmeyer (5). The Centrifugal Fast Analyzer was utilized for all assays (7). The units of enzyme activity are μmole of product formed/g hemoglobin/h at 30°C. The variation associated with the date of assay and grouping within date is removed by a nested-analysis of variance (23). Polyacrylamide gel electrophoresis was as described by Decker and Mohrenweiser (10). Thermostability studies were conducted according to methods described by Asakawa and Mohrenweiser (3). Antiserum against rabbit muscle TPI was raised in roosters according to procedures described by Eber *et al.* (12). Antiserum against human TPI was kindly supplied by Dr's. Krietsch and Eber of the University of Munich, Munich, West Germany. Immunoinactivation experiments were conducted by the procedures described by Asakawa and Mohrenweiser (3). All incubations for the immunoinactivation experiments were adjusted to a TPI activity of 35 units/50 μliters of total incubation.

RESULTS

The distribution of TPI activity in a group of 828 newborns is presented in Figure 1. This includes 300 newborns which were previously reported (7). The remaining 432 newborns from the previous study were not displayed in this distribution because it was not possible to remove variation associated with grouping within date although low activity individuals could be scored in this initial group. The coefficient of variation for erythrocyte TPI activity among the 828 newborns, after adjustments for assay variation, was 8.7%. Infants with reduced levels of erythrocyte TPI activity were divided into two groups. The first group, consisting

of 12 newborns with a level of TPI activity of less than 65% of the population mean for newborns–a level of activity which is more than 4.0 S.D. below the mean–was defined as heterozygotes for TPI deficiency (Fig. 1). The TPI activity in erythrocytes from seven of these individuals and their parents has been previously reported (23). The level of TPI activity in erythrocytes from the five additional probands and their affected and normal parents is similar to the activities presented for the original seven families. The activity in this group of 12 newborns ranged from 48–65% of normal and averaged 56.7% of the mean activity in erythrocytes of normal newborn infants (Table 1). This average activity is 4.9 S.D. (range 4.0–5.9) below that expected in normal newborns. In each family, one parent had a level of TPI activity which was less than 65% of normal (average 57%), whereas the other parent had a normal level (average 97%) of activity. Two of the probands are half siblings, born to a heterozygous mother. In seven of the 11 families, the affected parent is the father. The variant allele associated with the loss of TPI activity is designated TPI 1°; the genotype of these heterozygous individuals is TPI 1/1°. The alteration of enzyme activity apparently involves only TPI, as the activities of 12 other erythrocyte enzymes (adenylate kinase, glutamic oxaloacetic transaminase, phosphoglyceromutase, enolase, glyceraldehyde 3-phosphate dehydrogenase, glucose 6-phosphate
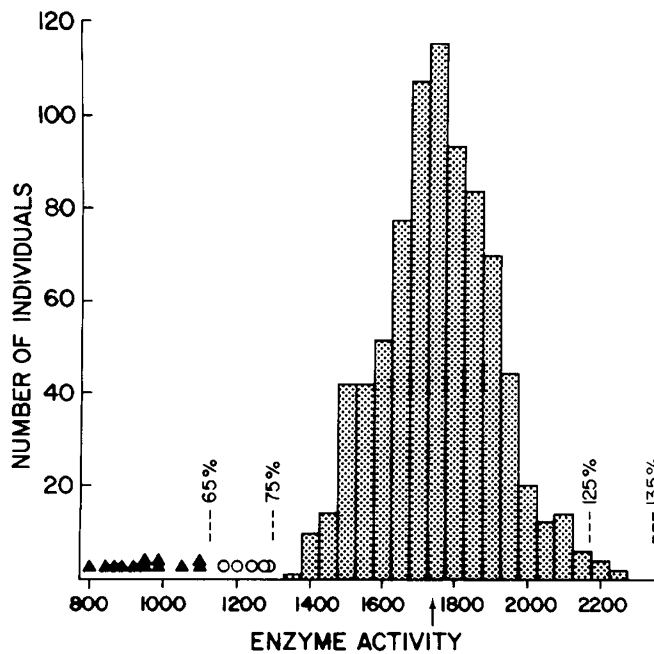

Fig. 1. Distribution of triosephosphate isomerase activity in erythrocytes of newborn infants. Activities are adjusted to remove variation associated with date of assay and grouping within date. Enzyme activity is expressed as μmole product/g hemoglobin/h. (↑), population mean; (▲), heterozygous deficiency variants (1/1°); (○), heterozygous "low activity" variants (1/1⁻).

Table 1. *Triosephosphate isomerase (TPI) activity in carrier and normal individuals*

	TPI deficient families[1]		
	Carrier newborn	Carrier parent	Normal parent
Activity[2]	102,280 ± 14,690	78,460 ± 11,440	136,560 ± 19,170
% of expected activity	56.7	57	97
	TPI activity in normal families[3]		
	Newborns	Mothers	Fathers
Activity[2]	173,400 ± 15,048	146,800 ± 19,860	141,650 ± 18,980

[1] n = 11.

[2] μmole product/g hemoglobin/h.

[3] n = 828 for newborns and 140 for adults.

Table 2. *Ethnic distribution of allele(s) associated with reduced triosephosphate isomerase activity in newborn infants*

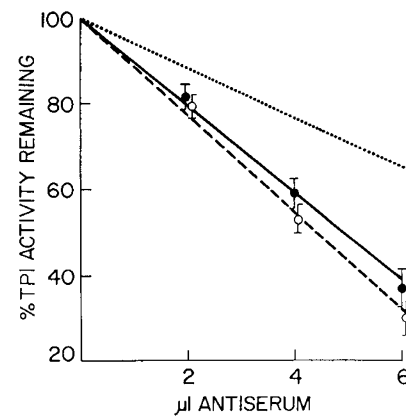|	Frequency of 1°		
Ethnic group	Newborns studied	No. of heterozygote deficients	Allele frequency
White	1048	5	0.0024
Black	146	7	0.0240
	Frequency of 1⁻		
White	1048	4	0.0019
Black	146	1	0.0034

Fig. 2. Immunoinactivation of triosephosphate isomerase from erythrocytes of control and carrier individuals. Procedures for immunoinactivation of triosephosphate isomerase are described in "Materials and Methods." (●), control; (○), affected; bars indicate ± S.D., and (...), theoretical curve expected if immunologically reactive, inactive enzyme were present.

dehydrogenase, glucosephosphate isomerase, glutathione reductase, lactate dehydrogenase, malate dehydrogenase, phosphoglycerate kinase and pyruvate kinase) were as expected in both the proband and affected parents.

The ethnic distribution of the TPI deficiency carriers is presented in Table 2. In this unselected group of newborn infants, the frequency in the Black sample of 4.6 heterozygous TPI deficient newborns per 100 infants is ten times the frequency of 0.5 heterozygous individuals/100 White newborns. Glucose 6-phosphate dehydrogenase deficiency associated with the A⁻ allele occurred with a frequency of 0.08 in the Black group. Excluding the two polymorphic deficiencies, the frequency of enzyme deficiency variant alleles at seven other loci does not differ between the ethnic groups, being 0.007 in both groups (23).

This high frequency of an allele(s) associated with TPI deficiency, especially in the Black population, is in marked contrast to the low frequency of electrophoretic variants at this locus in both ethnic groups in this same population. Only a single TPI electrophoretic variant occurring in a White infant, has been observed among 2224 newborns (frequency = 0.004). The average frequency of electrophoretic variants at 29 loci encoding other erythrocyte enzymes is 0.013 in this population (28).

The molecular etiology and possible heterogeneity of the TPI deficiency was studied in several experiments. First, utilizing antirabbit-TPI sera from roosters, the relationship between the amount of enzyme inactivated and antisera added was examined. As seen in Figure 2, no significant differences in the inactivation profile were detected between samples from carrier deficient and control individuals. Because all samples were adjusted to similar levels of enzyme activity, it is concluded that the specific activity of TPI is identical in normal and heterozygous deficient individuals. Similar results were obtained when antihuman-TPI sera was used; thus, no evidence of cross reactive material (CRM) was observed in any deficient individual. The dotted line represents (Fig. 2) the immunoinactivation profile expected if a normal

quantity of antigenically reactive, catalytically inactive enzyme were present in erythrocytes of carrier individuals.

Second, the TPI electrophoretic pattern was examined for all of the deficiency variants. A variant TPI subunit, although catalytically inactive may still hybridize with normal subunits and alter the electrophoretic pattern of TPI. Such an altered electrophoretic pattern has been observed for a "null" activity variant of lactate dehydrogenase (LDH) (26); however, as seen in Figure 3, the banding patterns, detected by activity staining, for TPI in hemolysates from two families are normal. Each family consists of an affected and normal parent and a deficiency carrier child, and all individuals possess identical electrophoretic patterns. A normal electrophoretic pattern was also observed in hemolysates from the other 10 deficiency variant newborns (not shown). The electrophoretic pattern for a previously described electrophoretic variant, TPI 1/Manchester (3, 10) is included in the outer lanes for comparison. The normal electrophoretic patterns in the samples from heterozygous deficient individuals are consistent with the results of the immunoinactivation studies.

The thermostability of the TPI enzyme in erythrocytes of heterozygous deficiency variant individuals was compared with the stability of the enzyme from control individuals. The $t_{1/2}$ at 57°C for enzyme from control individuals was 30.5 ± 1.2 min. The $t_{1/2}$ for erythrocyte TPI from heterozygous deficient individuals ranged from 28–32 min and thus did not differ from that observed in control individuals. These results are again consistent with the immunoinactivation and electrophoretic studies described above and further support the conclusion that inactive TPI subunits are not present in erythrocytes of the heterozygous deficient individuals.

A second group of five newborns (one Black and four White) were classified as heterozygous for a "low activity" variant, the putative variant allele being designated 1^-. The TPI activity in erythrocytes from these individuals (both newborn and adult) with a phenotype of TPI $1/1^-$, is between 66–72% of expected and 3.0–4.0 S.D. below the population mean (Fig. 1). The results of immunoinactivation experiments, the electrophoretic pattern and the thermostability profile of TPI from erythrocytes of these individuals, both parent and child, were identical to the results obtained in studies of TPI in erythrocytes from both control and heterozygous null individuals. No evidence for the existence of either inactive or unstable enzyme molecules was observed in the erythrocytes of these heterozygous, low activity variant individuals either.
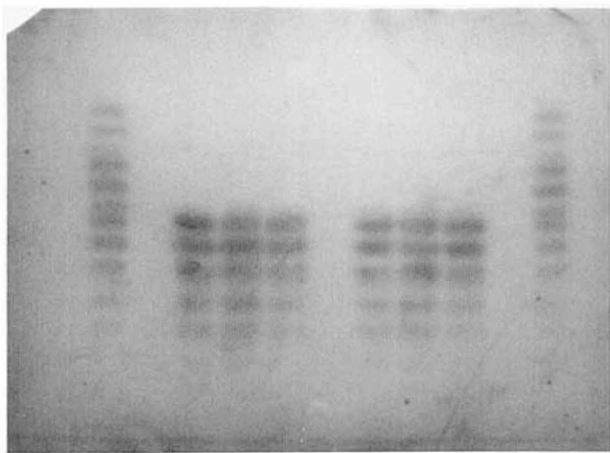

Fig. 3. Electrophoretic pattern of erythrocyte triosephosphate isomerase from control and carrier individuals. Electrophoresis conditions are described in "Materials and Methods." All samples were adjusted to equal levels (10 units) of enzyme activity. Samples were applied at the top of the gel with the anode being at the bottom. Samples, from the left are: *wells 1 and 11*, 1/Manchester variant individual; *wells 3 and 7*, control; *wells 4 and 8*, carrier parents, (1/1° phenotype); *wells 5 and 9*, carrier newborns (1/1° phenotype); and *wells 2, 6 and 10* are blank.

DISCUSSION

This significant reduction in TPI activity is not the type of generalized alteration involving many enzymes caused by changes in mean cell age or % reticulocytes (24), exposure to cytostatic drugs (6, 20), or other dyserythropoetic conditions (6, 14). The activities of glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12) and the B locus of lactate dehydrogenase, both of which are located adjacent to TPI on chromosome 12 (22) are normal in all individuals that carry the presumptive TPI null allele. Although TPI is located more distally than the other two enzymes, it seems apparent that the deficiency should not involve loss of a large segment of the chromosome. This evidence of specificity, *i.e.*, involvement of only a single enzyme per proband and genetic segregation, is consistent with involvement of a structural locus, this locus presumably being the single structural locus for TPI (10) on chromosome 12 (22) or possibly the intervening or flanking regions.

Erythrocyte enzyme deficiencies can occur via at least three different mechanisms, namely an unstable variant enzyme molecule, an enzymatically inactive variant molecule or the absence of enzyme protein. The lack of evidence for an immunologically reactive, enzymatically inactive protein in erythrocytes of the 12 probands and their respective affected parents is strong evidence for the absence of inactive enzyme molecules. Similarly, Eber *et al.* (11) reported that in only one of 17 families with reduced TPI activity was any CRM + TPI detected. No evidence for the presence of inactive or unstable TPI enzyme molecules was obtained in electrophoretic or thermostability studies. At this time it is not possible to further define the molecular basis for the TPI deficiency although it seems probable that the TPI deficiencies reported here are associated with a lack of synthesis of TPI protein, which is consistent with the previous observations that TPI deficiency is expressed in nonerythroid cells (32, 37). Thus, the characteristics of the heterozygous individuals, either 1/1° or 1/1⁻ genotype, including the level of TPI activity as well as the activity of other enzymes in erythrocytes, the normal electrophoretic profile and the apparent absence of enzymatically inactive enzyme molecules, are identical to those observed in parents of homozygous TPI deficient individuals (16, 31, 32, 35, 37).

Our total allele frequency for nulls plus low activity variants, of 0.004 for Whites sampled in Ann Arbor, MI is similar to the frequency reported by Eber *et al.* (11, 12) from a group of some 3000 individuals studied in Germany. The much higher allele frequency (0.024) for the putative TPI null allele, TPI 1°, in the Black population sample is similar to the frequency of alleles associated with several of the common genetic diseases. For example, it is more than twice the allele frequency of phenylalanine hydroxylase deficiency (phenylketonuria) (34). It is similar to the allele frequency for hexosaminidase A deficiency (Tay Sachs Disease) in American Jews (19) and to the frequency of allele(s) for cystic fibrosis in the United States White population (27).

An apparent discrepancy exists between the frequency of allele(s) for TPI deficiency (1°) reported during surveys of human populations (this paper, 11, 12, 23) and the number of homozygous deficient individuals observed in various clinics. This discrepancy is most extreme in the Black population where homozygous affected newborns are expected with a frequency of one in every 2000 births, although even the lower allele frequency in Whites is not reflected in the number of clinical cases reported. Given these allele frequencies, it would be expected that approximately 200 homozygous TPI deficient infants (1°/1° genotype) would be born each year in the United States, whereas to the best of our knowledge, only two TPI deficient individuals have ever been identified in the United States. Similarly, Eber *et al.* (11) have suggested that given the frequency of heterozygotes, some 500 cases of TPI deficiency should have been identified in Germany. None of the 17 reported cases that we could identify (16, 31, 32, 35, 37) is from Germany. It is unlikely that a condition with such severe consequences would go undetected, although it is possible

that the syndrome is not identified as TPI deficiency. It is also possible that total TPI deficiency results in a metabolic block which is incompatible with normal embryo/fetal development and therefore only individuals retaining some low residual level of TPI activity, possibly infants with the $1°/1^-$ genotype, survive to term and are subsequently identified in hospital clinics. The biochemical and/or molecular basis for the TPI deficiency, and the associated metabolic consequences, as well as the apparent discrepancy between the heterozygote and homozygote frequencies, each require further investigation.

REFERENCES AND NOTES

1. Albery, W. J. and Knowles, J. F.: Evolution of enzyme function and the development of catalytic efficiency. Biochemistry, 15: 5631 (1976).
2. Artavanis-Tsakonas, S. and Harris, J. I.: Primary structure of triosephosphate isomerase from Bacillus stearothermophilus. Eur. J. Biochem., 108: 599 (1980).
3. Asakawa, J. and Mohrenweiser, H. W.: Characterization of two new electrophoretic variants of human triosephosphate isomerase: stability, kinetic and immunological properties. Biochem. Genet., 20: 59 (1982).
4. Banner, D. W., Bloomer. A. C., Petsko. G. A., Phillips, D. C., Pogson, C. I., Wilson, I. S., Corran, P. H., Furth, A. J., Milman, J. D., Offord, R. E., Priddle, J. D., and Waley, S. G.: Structure of chicken muscle triosephosphate isomerase determined crystallographically at 2.5 A resolution using amino acid sequence data. Nature (London), 255: 609 (1975).
5. Bergmeyer, H. U.: Methods of Enzymatic Analysis Vol. I. p. 449 (Academic Press, New York, New York 1974).
6. Boivin, P.: Red cell enzyme abnormalities. In: Dyserythropoietic Anaemias edited by Lewis, S. M., Verwilghan, R. L., p. 221 (Academic Press, New York, New York 1977).
7. Burtis. C. A., Mailen, J. C., Johnson, W. F., Scott, D. D., and Tiffany, T. O.: Development of a miniature fast analyzer. Clin. Chem., 18: 753 (1972).
8. Corran. P. H. and Waley, S. G.: The tryptic peptides of rabbit muscle triosephosphate isomerase. Biochem. J., 139: 1 (1974).
9. Dayhoff, M. O.: Survey of new data and computer methods of analysis in Atlas of Protein Sequence and Structure Vol. 5 Silver Spring. MD, National Biomedical Research Foundation, p. 3 (1978).
10. Decker, R. S. and Mohrenweiser, H. W.: Origin of the triosephosphate isomerase isozymes in humans: Genetic evidence for the expression of a single structural locus. Am. J. Hum. Genet., 33: 683 (1981).
11. Eber, S. E., Belohradsky, B. H., and Krietsch, W. K. G.: A case for triosephosphate isomerase testing in congenital non-spherocytic hemolytic anemia. J. Pediatr. in press (1982).
12. Eber, S. E., Dunnwald, M., Belohradsky, B. H., Bidlingmaier, F., Schievelbein, H., Weinmann, H. M., and Krietsch, W. K. G.: Hereditary deficiency of triosephosphate isomerase in four unrelated families. Eur. J. Clin. Invest., 9: 195 (1979).
13. Eber, S. W. and Krietsch. W. K. G.: The isolation and characterization of the multiple forms of human skeletal muscle triosephosphate isomerase. Biochem. Biophys. Acta, 614: 173 (1980).
14. Etremble, J., Bernard, J. F., Picat, C., Belpomme, D., and Boivin, P.: Red blood cell enzyme abnormalities in patients treated with chermotherapy. Brit. J. Haemat., 42: 391 (1979).
15. Fielek, S., and Mohrenweiser, H. W.: Erythrocyte enzyme deficiencies assessed with a miniature centrifugal analyzer. Clin. Chem., 25: 384 (1979).
16. Freycon, E., Lauras, E., Bovier-Lapierre, F., Corche, C. L., and Goddon, R.: Anemie hemolytique congenitale par deficit en triosephosphate isomerase. Pediatrie, 30: 55 (1975).
17. Gracy, R. W.: Triosephosphate isomerase from human erythrocytes. Methods Enzymol., 41: 442 (1975).
18. Hartman, F. C. and Gracy, R. W.: An active site peptide from human triosephosphate isomerase. Biochem. Biophys. Res. Comm., 52: 388 (1973).
19. Kaback, M. M. and Zeiger, R. S.: Heterozygote detection in Tay-Sachs disease: a prototype community screening program for the prevention of recessive genetic disorders. In: Sphingolipids, Sphingolipidoses and Allied Disease edited by Volk, B. W., Aronson, S. M., p. 613–632. (Plenum Press, New York, New York 1972).
20. Kahn, A., Cottreau, D., Boyer, C., Marie, J., Galand, C., and Boivin, P.: Causal mechanisms of multiple acquired red cell enzyme defects in a patient with acquired dyserythropoiesis. Blood, 48: 653 (1976).
21. Krietsch, W. K. G., Pentcher, P. G., Klingenberg, H., Hofstatter, T., and Bucher, T.: The isolation and crystallization of yeast and rabbit liver triosephosphate isomerase and a comparative characterization with the rabbit muscle enzyme. Eur. J. Biochem., 14: 289 (1970).
22. Law, M. L. and Kao, F. T.: Regional assignment to human chromosome 12 of seven genes TP11, TP12, GAPD, LDHB, EN02, SHMT and PEPB. Cytogenet. Cell Genet., 25: 179 (1979).
23. Mohrenweiser, H. W.: Frequency of enzyme deficiency variants in erythrocytes of newborn infants. Proc. Natl. Acad. Sci. USA, 78: 5046 (1981).
24. Mohrenweiser, H. W., Fielek, S., and Wurzinger, K. H.: Characteristics of enzymes of erythrocytes from newborn infants and adults. Activity, thermostability and electrophoretic profile as a function of cell age. Am. J. Haematol., 11: 125 (1981).
25. Mohrenweiser, H. W. and Neel, J. V.: Frequency of thermostability variants: estimation of the total "rare" variant frequency in human populations. Proc. Natl. Acad. Sci. USA, 78: 5729 (1981).
26. Mohrenweiser, H. W. and Novotny, J. E.: Characterization of an enzymatically inactive variant of human lactate dehydrogenase–LDH GUA–1: study of subunit interactions. Biochem. Biophys. Acta, 702: 90 (1982).
27. Nadler, H. L., Tao, G. J., and Taussig, L. M.: Cystic fibrosis, in: The Metabolic Basis of Inherited Disease edited by Stanbury, J. B., Wyngaardern, J. B., Fredrickson, D. G., p. 1683–1710. (McGraw-Hill, New York, New York 1978).
28. Neel, J. V., Mohrenweiser, H. W., and Meisler, M. M.: Rate of spontaneous mutation at human loci encoding protein structure. Proc. Natl. Acad. Sci. USA, 77: 6037 (1980).
29. Neel. J. V., Satoh, C., Hamilton, H. B., Otake, M., Goriki, K., Kageoka, T., Fujita, M., Neriiski, S., and Asakawa, J.: Search for mutations affecting protein structure in children of atomic bomb survivors: preliminary Report. Proc. Natl. Acad. Sci. USA, 77: 4221 (1980).
30. Putman, S. J., Coulson, A. F. W., Farley, I. R. I., Riddleston. B., and Knowles. J. F.: Specificity and kinetics of triosephosphate isomerase from chicken muscle. Biochem. J., 129: 301 (1972).
31. Schneider, A. S., Dunn, I., Ibsen, K. H., and Weinstein, I. W.: Triosephosphate isomerase deficiency B. Inherited triosephosphate deficiency. Erythrocyte carbohydrate metabolism and preliminary studies of the erythrocyte enzyme. in: Hereditary Disorders of Erythrocyte Metabolism, edited by Beutler, E., City of Hope Symposium Series. Vol. 1, p. 273–287. (Grune and Stratton, New York, New York 1968).
32. Schneider, A. S., Valentine, W. N., Hattori, M., and Heins, H. L.: Hereditary hemolytic anemia with triosephosphate isomerase deficiency. N Engl. J. Med., 272: 229 (1965).
33. Scopes, R. K.: Methods for starch gel electrophoresis of sarcoplasmic proteins. Biochem. J., 107: 139 (1968).
34. Scriver, C. R. and Rosenberg, L. E.: Amino acid metabolism and it's disorders. p. 290–337. Saunders, Philadelphia, Pennsylvania 1973).
35. Skala, H., Dreyfus, J. C., Vives-Corrons, J. L., Matsunoto, F., and Beutler, E.: Triosephosphate isomerase deficiency. Biochem. Med., 18: 226 (1977).
36. Snapka, R. M., Sawyer, T. H., Barton, R. A., and Gracy, R. W.: Comparison of the electrophoretic properties of triosephosphate isomerase of various tissues and species. Comp. Biochem. Physiol., 49B: 733 (1974).
37. Vives-Corrons, J. L., Rubinson-Skala, H., Mateo, M., Estella, J., Feliu, E., and Dreyfus, J. C.: Triosephosphate isomerase deficiency with hemolytic anemia and severe neuromuscular disease. Familial and biochemical studies of a case found in Spain. Hum. Genet., 42: 171 (1978).
38. Yuan, P. M., Talent, J. M., and Gracy, R. W.: A tentative elucidation of the sequence of human triosephosphate isomerase by homology peptide mapping. Biochem. Biophys. Acta, 671: 211 (1981).