A NEW INHERITED ABNORMALITY OF HUMAN ERYTHROCYTES - ELEVATED ERYTHROCYTIC ADENOSINE TRIPHOSPHATE*

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Received December 14, 1964

During a comparative study of the content of adenosine triphosphate (ATP) in the erythrocytes of glucose-6-phosphate dehydrogenase (G-6-PD) deficient and normal subjects, a G-6-PD deficient Negro man who exhibited twice the normal level of ATP in his erythrocytes was observed (Brewer, June, 1964). His level of ATP was 6-7 standard deviations above the normal mean. The family of this individual has been studied and the results indicate that this abnormality of ATP metabolism is inherited. The study of the family, in addition to the results of a survey of the Negro population carried out while looking for other examples of the abnormality, form the basis of this report.

Materials and Methods

Subjects: The propositus of the family with elevated erythrocytic ATP and most of the other affected members of the kindred are clinically and hematologically normal. The unrelated Negro male and female subjects forming the control group were primarily individuals from whom blood samples were drawn in the outpatient laboratory, University Hospital, University of Michigan Medical School, Ann Arbor, Michigan, either at the time of routine pre-employment physical examination, or while pre-

* This work was supported by U. S. Public Health Service Fellowship 1-F3-AM-7959-01 and U. S. Public Health Service Grant AM-07752-01, both from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland (U.S.A.).

senting with one of a variety of minor illnesses. Patients with major systemic disease or hematologic disorders were excluded.

Methods: Venous blood was anticoagulated immediately after removal from the vein with 0.15 ml. of acid-citrate-dextrose solution (NIH Formula A) per 1.0 ml. of blood and kept at 4° C. until used. Assay of ATP content of the erythrocytes was performed within 24 hours of collection of the blood. Protein-free extracts of the erythrocytes were prepared by the trichloroacetic acid extraction method of Bartlett (1959).

TABLE 1

Erythrocytic ATP Levels, Blood Types, Hemoglobin Types, and Identifiable G-6-PD Deficiency in Tested Members of the Kindred

Pedigree No.*	Sex	ATP Levels** (μmoles ATP/Gm Hgb)	Blood Types ABO	MN	Rh	Hemoglobin Types	G-6-PD Activity
I-6	M	3.12	B	MN	ccDEe	-	Normal
II-1	M	3.55	B	MN	ccDee	AA	Normal
II-4	M	3.20	O	M	ccDee	AA	Normal
II-5	F	4.63h	O	M	ccDee	AA	Normal
II-6	M	4.66h	O	M	ccDee	AS	Normal
II-7	F	3.78	B	MN	ccdee	-	Intermed.
II-9	M	5.90h	O	N	CcDee	AA	Normal
II-11	F	3.88	B	MN	ccDee	AA	Normal
II-13	M	6.08h	O	M	ccDEe	AA	Deficient
II-15	M	3.75	A	MN	CcDee	-	Normal
III-1	F	3.68	O	MN	ccDEE	AA	Normal
III-2	M	4.01h	O	M	ccDee	AS	Normal
III-3	M	3.30	O	MN	ccDee	AS	Normal
III-4	M	6.04h	B	M	ccdee	AS	Normal
III-6	F	3.34	O	N	ccDee	-	Normal
III-7	M	4.02h	AB	M	ccDee	-	Normal
III-8	M	4.25h	O	MN	ccDee	AS	Normal
III-9	M	3.68	O	N	CcDee***	-	Deficient
III-10	F	3.51	B	N	ccDEe***	-	Normal

Control Erythrocytic ATP Values -

119 Negro Males 3.14 ± 0.42 (Mean ± one S.D.)
130 Negro Females 3.25 ± 0.56 (Mean ± one S.D.)

* See Figure 1.
** An h sign indicates that the ATP value is considered abnormally high.
*** Blood types reveal exclusion of II-13 as the biological father.

Adenosine triphosphate was assayed spectrophotometrically by a modification of the enzymatic method of Kornberg (1950) using the hexokinase and G-6-PD reactions to reduce triphosphopyridine nucleotide. Details of the assay method will be published. Hemoglobin types were determined by starch gel electrophoresis. Deficiency of G-6-PD activity was determined both by assay of hemolysates for G-6-PD activity by the method of Zinkham and Lenhard (1959) and by the methemoglobin reduction test (Brewer, Tarlov, and Alving, 1960 and 1962).

Results and Discussion

The erythrocytic ATP values of various members of the kindred are shown in Table 1, which is keyed to the pedigree shown in Fig. 1. Also

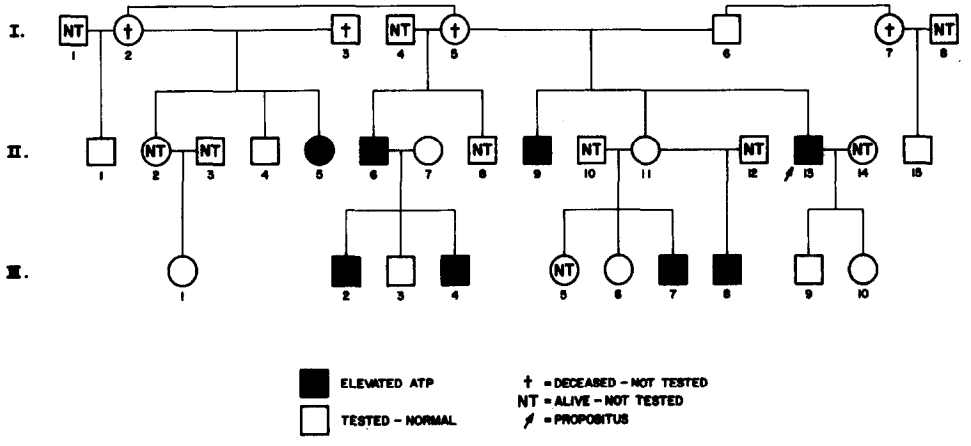

Fig. 1. Pedigree of the kindred with elevated erythrocytic ATP. Males and females whose red cell ATP values are above two standard deviations from the mean of their respective control distributions have arbitrarily been designated as affected.

included in Table 1 are results of tests for G-6-PD deficiency and sickle hemoglobin, and results of blood group studies. Individuals whose erythrocytic ATP values are above two standard deviations from the control mean (males and females compared separately) have arbitrarily been designated as affected with elevated erythrocytic ATP.

Two males in the family (II-9 and III-4) have levels of ATP in their red cells as high as that of the propositus. A number of other individuals, including one female (II-5), have intermediately elevated values. Considerable variation in expression of the trait, even within a sibship, is present. For example, a half-brother of the propositus (II-6), who has intermediately elevated levels, has one affected son with very high levels (III-4), and a second affected son with slightly elevated values (III-2). This, of course, represents male to male transmission of the trait and indicates an autosomal mode of inheritance. A full sister of the propositus (II-11) demonstrates lack of penetrance of the trait, i.e. she has two affected sons, but she has a normal level of erythrocytic ATP.

We interpret the data so far as being compatible with single factor autosomal inheritance of elevated erythrocytic ATP. All of the affected individuals are presumably heterozygous for the gene. The genes for sickle cell trait and G-6-PD deficiency segregate independently from the elevated ATP trait in the kindred.

In both the male and female control distributions there were two outlying values above two standard deviations from the control mean. The values were 4.11 and 4.42 for the male individuals and 4.56 and 4.57 for the female subjects. Studies of the families of these individuals will be necessary to determine if they represent additional examples of inherited elevated erythrocytic ATP. Even so, until we have identified the primary biochemical abnormality, it will not be possible to conclude that any two given families with elevated erythrocytic ATP have the same specific abnormality. If these individuals should eventually prove to have the same abnormality, the incidence of the condition in the Negro population would be significant, i.e. between one and two percent.

One of the more important aspects of this new genetic trait may be its use as a tool for evaluating the role of ATP in various cellular

functions. As two examples of such an application, we have used this trait in evaluation of the role of ATP in G-6-PD deficiency-type drug-induced hemolytic anemia, and in evaluation of the role of ATP in long term blood storage (Brewer and Powell, 1964).

Subsequent to our first report of elevated erythrocytic ATP (Brewer, June, 1964), Zürcher, Loos and Prins (September, 1964) reported another family with what they have termed "hereditary high ATP content of the erythrocytes". The family reported by these authors appears to represent an entity distinct from the one we have reported. In their family, the authors believe that they can fully explain the metabolic abnormalities present by an increased activity of pyruvic kinase in the erythrocytes of affected individuals. In our family, the activity of pyruvic kinase is normal in red cells of subjects with elevated erythrocytic ATP.

We have as yet not identified the primary biochemical abnormality in the erythrocytes of affected individuals from our family. Work in this area, as well as work on the tissue distribution of the abnormality, is in progress.

Acknowledgement

Blood typing and hemoglobin electrophoresis were performed through the courtesy of Dr. Henry Gershowitz and Dr. Donald Rucknagel, respectively

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