Red Cell Glucose-6-Phosphate and 6-Phosphogluconic Dehydrogenases and Nucleoside Phosphorylase

Glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and purine nucleoside phosphorylase are known to be present in mature mammalian erythrocytes (*1–3*). These cells, however, lack many of the enzymes of the tricarboxylic acid cycle (*4*). Accordingly, glucose-6-phosphate and 6-phosphogluconic dehydrogenases, which catalyze reactions providing a mechanism for glucose oxidation to CO_2 with generation of reduced triphosphopyridine nucleotides, may be of particular importance in the metabolic processes of erythrocytes. Purine nucleoside phosphorylase may play a role in the synthesis and degradation of nucleosides in these cells.

Studies of erythrocytes of patients with an increased percentage of reticulocytes have revealed that the levels of cholinesterase, glyoxylase, and carbonic anhydrase, but not purine nucleoside phosphorylase, are elevated (*5*). There are no previous reports of the activity of erythrocyte glucose-6-phosphate or 6-phosphogluconic dehydrogenases in human adult subjects (*6*). In studies in rabbits, Rubinstein *et al.* (*4*) found that glucose-6-phosphate dehydrogenase was about equally active in the reticulocyte and in the mature erythrocyte.

The investigations described in this report (*7*) were designed (i) to measure the levels of these enzymes in erythrocytes of healthy adult human subjects and (ii) to determine whether alterations in the activities of these enzymes occur in erythrocytes of patients with an increased proportion of young red blood cells.

Heparinized venous blood was centrifuged (1500 *g* for 10 min), and the plasma and buffy layer were removed. The erythrocytes were washed twice with isotonic potassium chloride buffered at *p*H 7.4 and diluted to three times the sedimented volume with this solution. This procedure was performed at 0°C. The cells were hemolyzed by freezing and thawing twice. Prior to hemolysis, aliquots of the resuspended cells were removed for counting of erythrocytes (RBC), leukocytes (WBC), and reticulocytes and determination of hemoglobin concentration and hematocrit (*8*). Glucose-6-phosphate dehydrogenase was assayed by a method based on that of Kornberg and Horecker (*9*), 6-phosphogluconic dehydrogenase by a technique similar to that of Horecker and Smyrniotis (*2*), and purine nucleoside phosphorylase by the method of Price *et al.* (*10*) (Table 1). Erythrocytes completely free of leukocytes could not be obtained. Nevertheless, the activities of the enzymes per gram of hemoglobin were not affected by the leukocytes present provided that removal of the buffy layer increased the RBC/WBC ratio to greater than 1000. The presence of ghosts in the hemolysates did not affect the activity of these enzymes.

Individuals with reticulocytosis, compared with normal subjects, had significantly greater activities of glucose-6-phosphate and 6-phosphogluconic dehydrogenases, but not purine nucleoside phosphorylase referred to a unit of hemoglobin, a unit number of erythrocytes, or a volume of packed erythrocytes (Table 1) (*11*). Among the normal individuals, no significant differences were found between the mean values for these enzymes in erythrocytes of males compared with females, Caucasians compared with Negroes, or when age groups were compared by decades from 20 to 70 years. The patients with reticulocytosis included eight with hemolytic anemia of undetermined etiology, six with pernicious anemia, two with sprue, three with sickle-cell anemia, eleven with neoplastic disease, nine with bleeding peptic ulcer, and nine with other conditions associated with anemia and reticulocytosis. All but three individuals with reticulocytosis had elevations in the dehydrogenases beyond the range of the normal values. There was, however, no significant correlation between the degree of reticulocytosis and the activity of either dehydrogenase. In five individuals with macrocytic anemia, a sharp rise occurred in the activities of erythrocyte glucose-6-phosphate and 6-phosphogluconic dehydrogenases, but not in the activity of purine nucleoside phosphorylase, coincident with the reticulocytosis induced by vitamin B_{12} therapy. Although the reticulocyte count returned to normal by the 20th to 25th day, the dehydrogenases remained elevated for 58 to 81 days following institution of therapy.

These findings suggest that elevated levels of glucose-6-phosphate and 6-phosphogluconic dehydrogenases are sensitive indices of erythrocyte populations with a younger than normal mean cell age. In addition, the present data are compatible with the conclusion (*7*) that young, nonreticulated erythrocytes have high levels of these enzymes and that these levels decrease with the aging of the red blood cell in vivo.

Among the 112 healthy individuals (64 Caucasians and 48 Negroes) studied, six (1 Caucasian and 5 Negroes) had levels of erythrocyte glucose-6-phosphate dehydrogenase below three standard deviations of the mean for the remainder of the group. Family studies in these individuals, which have been described in detail elsewhere (*12*), indicate that this erythrocyte enzyme deficiency is genetically determined. The observed decrease in glucose-6-phosphate dehydrogenase in certain healthy subjects is of interest with regard to the demonstration that susceptibility to increased hemolysis following primaquine (*13*) naphthalene or fava bean (*12*) ingestion is associated with an erythrocyte defect in this enzyme.

The finding that glucose-6-phosphate dehydrogenase is deficient in old erythrocytes and in red blood cells particularly

Table 1. Activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and purine nucleoside phosphorylase in erythrocytes from normal individuals and from subjects with reticulocytosis. The enzyme assays were performed as follows: (i) Glucose-6-phosphate dehydrogenase: 0.1*M* $MgCl_2$, 0.5 ml; 0.25*M* glycylglycine buffer, *p*H 7.6, 0.5 ml; $2.3 \times 10^{-3}M$ triphosphopyridine nucleotide, 0.1 ml; 0.5*M* glucose-6-phosphate, 0.5 ml; hemolysate plus water to 2.5 ml. (ii) 6-Phosphogluconic dehydrogenase: 0.1*M* $MgCl_2$, 0.5 ml; 0.25*M* glycylglycine buffer, *p*H 9.0, 0.5 ml; $2.3 \times 10^{-3}M$ triphosphopyridine nucleotide, 0.1 ml; 0.05*M* 6-phosphogluconate, 0.5 ml; hemolysate plus water to 2.5 ml. Read at 340 mμ. (iii) Purine nucleoside phosphorylase: 0.05*M* phosphate buffer, *p*H 7.4, 2.6 ml; 0.0075*M* inosine, 0.2 ml; xanthine oxidase in excess; hemolysate plus water to 3.0 ml. Read at 293 mμ. Assays were performed in cuvettes with a light path of 1.0 cm. The level of activity of the enzyme is expressed as the change in optical density (ΔO.D.) per minute per gram of hemoglobin, or per 10^6 erythrocytes, or per milliliter of packed erythrocytes, as indicated.

Normal subjects		Subjects with reticulocytosis	
No.	ΔO.D./min (mean ± S.D.)	No.	ΔO.D./min (mean ± S.D.)
	Glucose-6-phosphate dehydrogenase		
112	15.9 ± 2.4/g of hemoglobin	47	29.7 ± 2.4/g of hemoglobin
94	0.54 ± 0.12/10^6 erythrocytes	36	1.09 ± 0.48/10^6 erythrocytes
76	4.95 ± 0.65/ml of erythrocytes	29	8.86 ± 1.24/ml of erythrocytes
	6-Phosphogluconic dehydrogenase		
66	17.3 ± 3.90/g of hemoglobin	31	36.2 ± 8.70/g of hemoglobin
54	0.66 ± 0.15/10^6 erythrocytes	26	1.29 ± 0.51/10^6 erythrocytes
46	6.41 ± 1.04/ml of erythrocytes	21	11.41 ± 8.10/ml of erythrocytes
	Purine nucleoside phosphorylase		
52	145 ± 21.0/g of hemoglobin	28	165 ± 38/g of hemoglobin
44	5.51 ± 0.87/10^6 erythrocytes	19	6.48 ± 2.01/10^6 erythrocytes
37	43.6 ± 3.10/ml erythrocytes	16	53.9 ± 7.9/ml erythrocytes

susceptible to hemolysis by certain drugs suggests that the activity of this enzyme may be an important factor in the maintenance of the integrity of these cells.

PAUL A. MARKS

Department of Medicine,
College of Physicians and Surgeons,
Columbia University, New York

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Three Chromosome Numbers in Whites and Japanese

Since Painter (*1*) reported in 1923 that the chromosome number in man is 48, this has been confirmed by a number of authors (*2*). This number (48) has had general acceptance except by the Japanese school, which has followed de Winiwarter and Oguma's report (*3*) that the Y-chromosome does not exist in man and that the total number of chromosomes in the male is 47. Recently a different number, 46, has been found by Tjio and Levan (*4*) in cultured tissues of four Swedish embryos. The same number of chromosomes (46) was discovered by Ford and Hamerton (*5*) in the testes of three Whites in England and by me and my coworkers (*6*) in the testes of Japanese. There is no doubt that this number (46) exists in man, but this is not the only possible number in the species; we (*6*) found, besides individuals with 46 chromosomes, some with 47 and others with 48 among Japanese.

A new group of Japanese was studied recently to extend the earlier investigation (*7*). A group of Whites was also studied to determine whether the same chromosomal variation exists in this ethnic group (*8*). The material examined consisted of tissue from testes of 15 Japanese (epididymitis patients), secured by biopsy, and testes from eight American Whites (prostate cancer patients), secured by total orchidectomy. Immediately upon removal, the specimens were pretreated with a mixture of equal volumes of 1-percent chromic acid and 3-percent potassium bichromate solutions for about 1½ hours. Specimens were then fixed with a mixture of equal volumes of 4.5-percent chromic acid and 1.5-percent potassium bichromate solutions for 17 to 20 hours. After being washed thoroughly in running water, they were stained by Feulgen's method and squashed. The pretreatment in this procedure facilitates the dispersion of the chromosomes in squashed metaphase cells. Some of the White testes showed slight fibrosis, but otherwise no testes showed indications of pathologic changes. At least 15 first meiotic metaphases and three or four spermatogonial metaphases, in which the chromosomes were dispersed well in the cell and could be observed clearly, were selected in each specimen. The number of chromosomes as well as the structure of individual chromosomes was carefully studied.

In nine of the 15 Japanese testes the spermatogonial metaphases showed 46 chromosomes, and the first meiotic metaphases showed consistently the heteromorphic X-Y pair and 22 paired autosomes. While the autosomal pairs always formed tetrads, the sex chromosomes were separate from each other in 40 percent of the first metaphases. The pairing irregularity of the X-Y pair was found to occur also in the other six Japanese and the eight White testes.

In one of the 15 Japanese testes, the number of chromosomes was found to be consistently 47 in the spermatogonia and first meiotic metaphases. In the latter metaphases a small univalent was always present in addition to the X-Y and the 22 autosomal bivalents. There was no indication that the univalent chromosome was produced by fragmentation of one of the sex chromosomes or of one of the autosomes. It is an intact chromosome with its own centromere. The X-Y and the 22 autosomal bivalents in this testis were compared with those in the nine testes with 46 chromosomes, and all chromosomes were found to match well in size and shape. This indicated that the univalent chromosome in the testis with 47 chromosomes is an extra element present in addition to the 23 pairs that constitute the regular complement in 46- and 47-chromosome individuals.

In the remaining five of the 15 Japanese testes, 48 chromosomes were found in spermatogonial metaphases, and the heteromorphic X-Y pair and the 23 homomorphic bivalents were found in all first meiotic metaphases. The matching of these bivalents with those of the 47-chromosome individuals indicated that one of the 23 bivalents corresponds to the univalent chromosome of the latter individual. Evidently the extra chromosome present singly in the 47-chromosome individual is duplicated in individuals of the 48-chromosome type. Thus, the Japanese comprise individuals of three different chromosomal constitutions, and the differentiating factor is the chromosome which occurs singly in some individuals but as a duplicate in others. This chromosome appears to be a supernumerary chromosome.

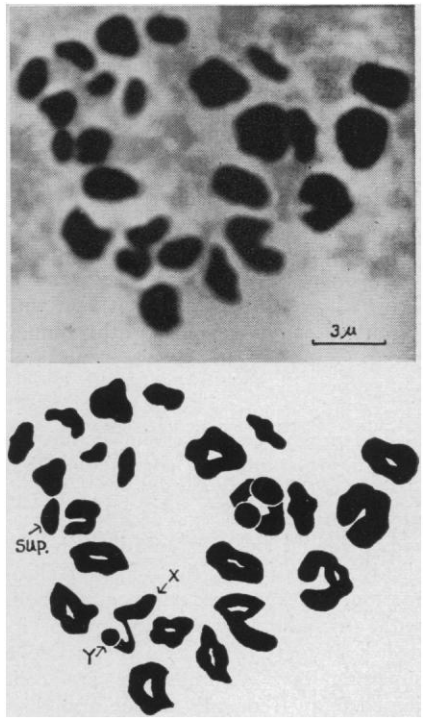

Fig. 1. A first meiotic metaphase of a White male with 48 chromosomes. Note the X-Y pair, the 22 autosomal pairs, and a bivalent supernumerary chromosome (*sup.*).

In seven of the eight White testes, 46 chromosomes were present consistently in all spermatogonial and primary spermatocyte metaphases. In size and shape the individual chromosomes were essentially the same as the 46 chromosomes in Japanese of the same type. In the remaining one of the White testes, 48 chromosomes were found in spermatogonial metaphases and the X-Y and the 23 bivalents were found in all first meiotic metaphases (Fig. 1). In Fig. 1 (top). the supernumerary bivalent appears to be connected to the adjacent bivalents. However, extensive observations of both Japanese and White materials have never indicated any tendency for the super-