

AN AMISH DEME - A NONSPHEIROCYTIC HEMOLYTIC ANEMIA

A review of the English Literature on Congenital  
Nonspherocytic Hemolytic Anemia Due to Erythrocyte  
Pyruvate Kinase Deficiency.

by

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In 1963 Bowman and Protopic<sup>1</sup> reported five cases of hereditary non-spherocytic hemolytic anemia of the pyruvate kinase deficient type among children of Amish extraction, whose ancestry had settled principally in Mifflin and Juniata Counties of Pennsylvania about 1770, and who farmed the Kishacaquillas Valley. One year later it was reported by Bowman, McKusick, and Dronamrajn<sup>2</sup> after more extensive study of these few cases that there were at least twenty-one cases of pyruvate kinase deficient hemolytic anemia among this Amish kindred. The parents of the affected siblings, which involved ten families, could tract their genealogy back to a common ancestor, "Strong" Jacob Yoder (immigrated 1742) and his wife. (See Fig. 1). This was the only immigrant couple ancestral to all twenty parents of the affected children and, therefore, one of this couple was presumably a heterozygote of the disease. The pedigree of these twenty-one affected children was rather confirmatory evidence of a previously suspected autosomal recessive mode of inheritance based primarily on biochemical evidence to be elucidated later in the paper.

Having lived among the Lancaster County Amish most of his life, the present writer became interested in knowing more about this congenital hemolytic anemia which genetically was among the Amish kindred and as to whether or not his neighbors may contain this genetic material. In answer to the latter curiosity, to date this disorder has been identified only in the Mifflin County (Pennsylvania) Amish deme. The Mifflin County Amish constituted a deme (endogamous local communities or consanguineous kin groups (Murdock, 1949), partially distinct from the Lancaster County Amish with regards to geographic factors (See Fig. 2), observations of family names (See Tab. 1), and customs. For example, a custom of the Mifflin County group is that "the hair is worn longer in the men, who, furthermore, use only one suspender--more is considered unnecessary and therefore mere adornment". Both groups do have one thing in common: the founders of both settlements were descendants from pre-Revolutionary immigrants of Swiss-German origin. Otherwise, they continue to thrive as two distinct socio-economic groups with very little, if any, inter-marriage or cultural exchange. It can be concluded, therefore, that this disorder will probably not appear among the Lancaster County Amish kindred.

The pyruvate kinase deficient hemolytic anemia found among the Mifflin County Amish is a rather specific type of "congenital non-spherocytic anemia", a term that has been applied to a rather heterogeneous group of congenital hemolytic anemias. In 1954 Selwyn and Dacie<sup>3</sup> purported a classification (Type I and Type II) of this group of anemias on the basis of in vitro tests. The most significant differences in the two types was the presence of a markedly increased autohemolysis, not corrected by glucose, on the incubation of sterile defibrinated blood in type II cases (2). In contrast, type I cases (2)

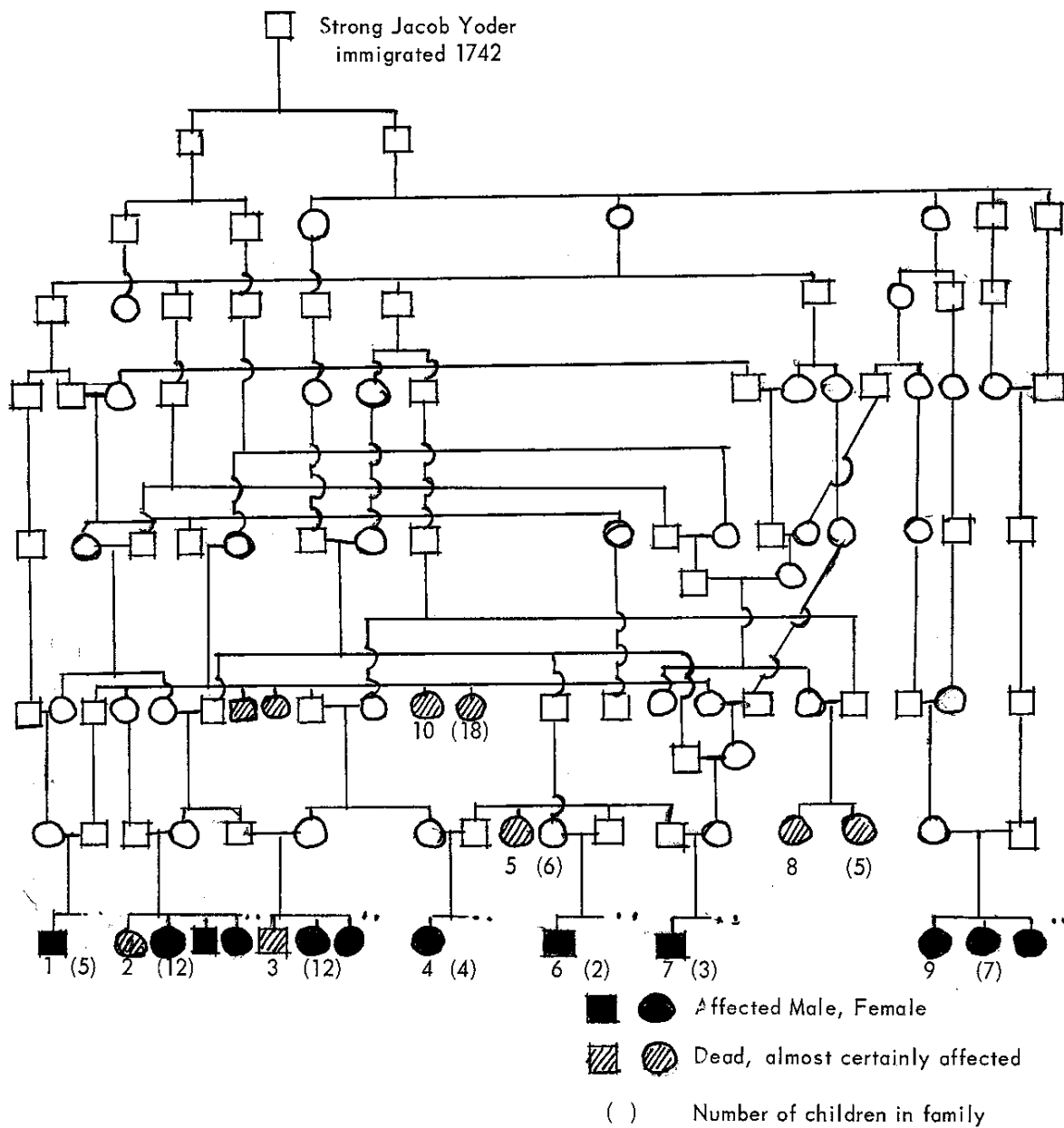


Fig. C. The pedigree of cases of pyruvate kinase deficiency congenital nonspherocytic hemolytic anemia among the Mifflin County Amish. (Taken from Bowman, McKusick, and Dronamraju. 2 Modifications present.)

Table 1. Old Order Amish Family Names\*

Lancaster County, Penna.			Mifflin County, Penna.	
	(a)	(b)		
Stoltzfus	23%	24%	Yoder	28%
King	12%	14%	Peachey	19%
Fisher	12%	14%	Hostetler	13%
Beiler	12%	11%	Byler	6%
Lapp	7%	7%	Zook	6%
Zook	6%	7%	Speicher	5%
Esh ***	6%	4%	Kanagy	4%
Glick	<u>3%</u>	<u>3%</u>	Swarey	<u>4%</u>
	81%	84%		85%
Totals: (a) 1106 families, 1957			238 families, 1951	
(b) 760 households, 1960				

\*\* Including Stoltzfoos.

\*\*\* Including Esch and Eash.

\* Taken from Bowman, McKusick, and Dronamraju. 2

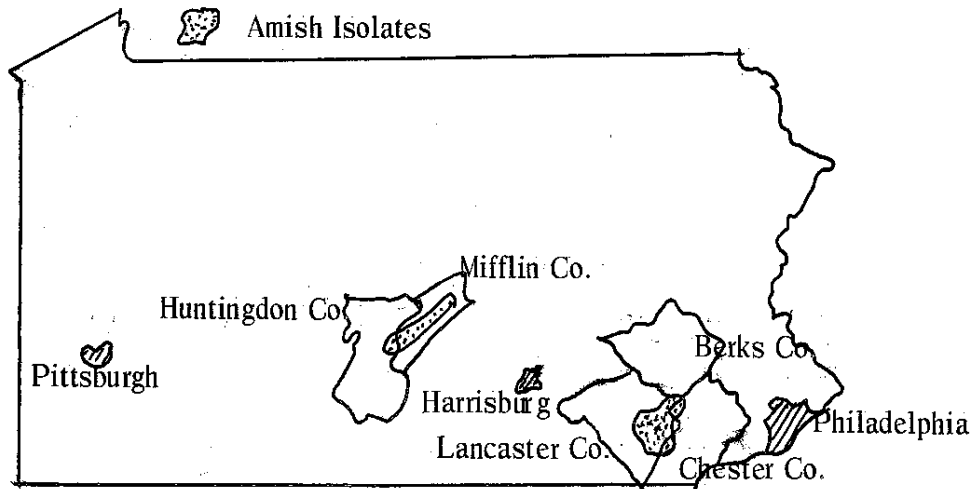


Fig. 2. Location of the Old Order Amish groups in Mifflin County and in Lancaster County. (Taken from Bowman, McKusick, and Dronamraju. 2)

demonstrated normal autohemolysis of white blood, decreased by the addition of glucose, but to a lesser degree than normal blood.

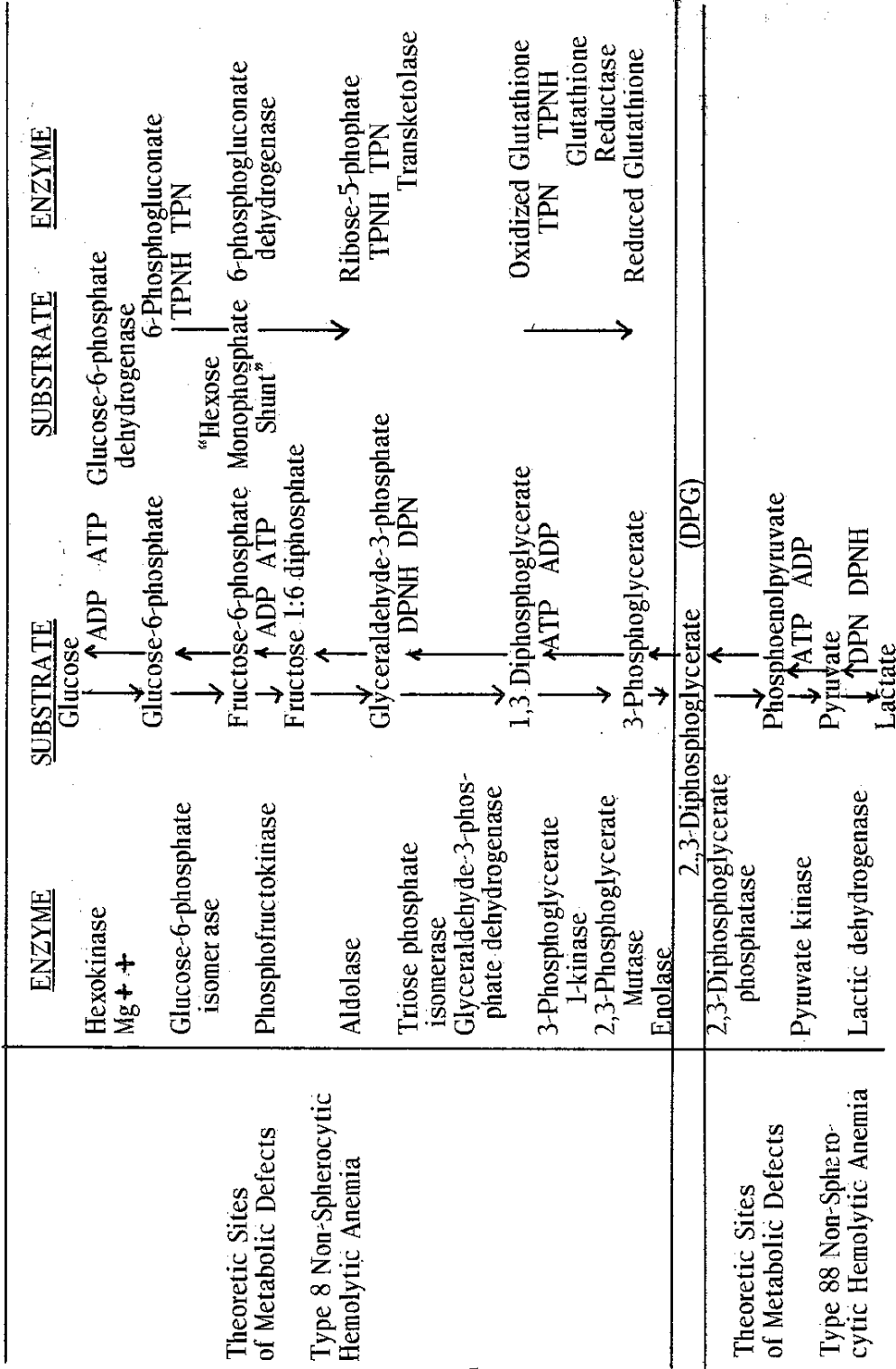
In 1960 DeGrunchy and his associates<sup>4</sup> studied three patients with type II congenital non-spherocytic hemolytic anemia and demonstrated that the increased autohemolysis, although uncorrectible by glucose, was correctible by the addition of adenosine triphosphate (ATP). He observed that the red cells were low in ATP content. With this information, in addition to the fact that Selwyn and Dacie<sup>3</sup> had found that their two cases of type II were unable to utilize glucose at a normal rate, he postulated a defect in glycolysis of the erythrocyte as the cause of anemia. Later, in 1961, Robinson, Loder, and DeGrouchy<sup>5</sup> also demonstrated that patients with this type anemia had a reduced red cell ATP content. It was observed that this ATP content fell more markedly and rapidly than normal red upon incubation. These investigators also demonstrated an accumulation of large amounts of the glycolytic intermediate, 2,3 diphosphoglycerate (DPG) during incubation. These observations suggested a block in the glycolytic path beyond the site of DPG. It has subsequently been shown that this stop in erythrocyte metabolism may form a biochemical division between types I and II congenital non-spherocytic hemolytic anemia as illustrated in Fig 3.<sup>1</sup> It is noted that the theoretical sites for metabolic defects in these anemias are above or below the location of DPG in the sequence of glycolysis.

Concerning further delineation of the enzyme defects involved in the type I anemia, several specific enzyme deficiencies have been found. These enzymes include glucose-6-phosphate dehydrogenase,<sup>6,7,8</sup> glutathione reductase,<sup>9</sup> disphosphoglycerate mutase,<sup>9</sup> and triosephosphate isomerase.<sup>9</sup> Thus, type I represents an entity which may be the result of one of several erythrocyte enzyme deficiencies. It should be noted that these enzymatic defects occur above the location of DPG in glycolysis as outlined in the previous paragraph. In contrast, the type II non-spherocytic hemolytic anemia represents a fairly homogeneous entity. Only one enzyme--pyruvate kinase--has been found to be the erythrocyte enzyme deficiency responsible for this type of anemia.

Valentine, Tanaka, and Miwa<sup>10</sup> in 1962 were the first to identify pyruvate kinase enzyme as being deficient in erythrocytes of type II congenital non-spherocytic hemolytic anemia. Hence, this collaborated the previous biochemical observations of a possible metabolic block in the glycolytic path beyond the site of DPG. Since these investigators demonstrated the specific enzyme deficiency in seven cases of the type II anemia, approximately twenty-nine cases of unequivocal pyruvate kinase deficiency congenital

EMBDEN-MEYERHOF PATHWAY

AEROBIC PATHWAY



Theoretic Sites of Metabolic Defects  
Type 8 Non-Spherocytic Hemolytic Anemia

Theoretic Sites of Metabolic Defects  
Type 88 Non-Spherocytic Hemolytic Anemia

Fig. 3. Diagrammatic outline of the 2 pathways of intraerythrocyte glycolysis and theoretic sites of metabolic aberration in type 8 and type 88 non-spherocytic hemolytic anemia. (Taken from Bowman and Procopio. 1)

non-spherocytic hemolytic anemia have been reported<sup>1,2,11,12,13,14,</sup>

## MODE OF INHERITANCE AND INCIDENCE

This disease is inherited as an autosomal recessive characteristic as illustrated by the pedigree of the twenty-one cases found among the Mifflin County Amish.<sup>2</sup> No indication of clinical disease has been found in the heterozygotes. Characteristically, the disease has been found in a single individual within a family or multiple cases in siblings, but not in parents nor offsprings of those patients affected.<sup>2,4,10</sup> Tanaka et. al. who first observed pyruvate kinase as the deficient enzyme in this disease originally showed that it was possible to differentiate the heterozygous individuals from the homozygous individuals on the basis of erythrocyte pyruvate kinase (PK) assays. The mean value of PK activity in the heterozygote was found to be about half that of normal subjects.<sup>10</sup> Subsequent studies have confirmed this observation.<sup>1,11,13,14</sup> The heterozygote has also been found to have a positive autohemolysis test but this is corrected by the addition of glucose.<sup>10,13</sup>

It is interesting to note that the disease may express itself quite differently within the same family. One child may have a severe hemolytic process whereas another sibling may be found to have the enzyme deficiency only when the entire family is screened for the disease. Hence, the expression of the genetic disorder seems to be quite variable.<sup>13</sup>

The incidence of this disease is rare to be sure. However, since the initial studies have been done distinguishing this specific disease entity from the rather heterogeneous group of congenital non-spherocytic hemolytic anemias, the relative rapidity of case reporting has suggested that the disease "may not be extremely rare."<sup>13</sup>

## PATHOGENESIS

The pathogenesis of this disorder related quite directly to erythrocyte metabolism. The question is how the disturbance in the metabolism of the erythrocyte contributes to the mechanisms of hemolysis in the disorder. Obviously the entire picture is not clear at this point, but laboratory investigations have resulted in a working hypothesis and increased understanding concerning the realities of erythrocyte metabolism.

The enzyme pyruvate kinase catalyses the conversion of phosphoenolpyruvate to pyruvate (See Fig. 4). In this sequence of glycolysis, ADP is converted to ATP. For every mole of glucose entering the Embden-Meyerhof cycle two

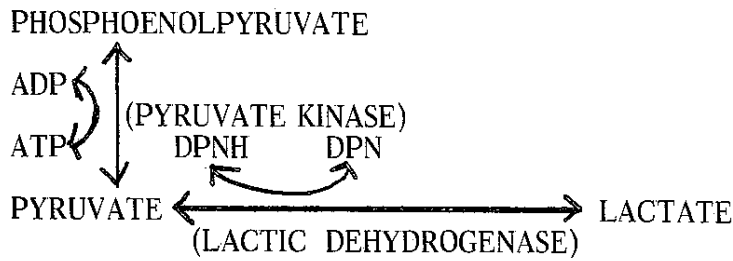


Fig. 4: Diagrammatic representation of glycolytic enzyme steps responsible for the conversion of phosphoenolpyruvate to lactate. (Taken from Tanaka et. al. 10)

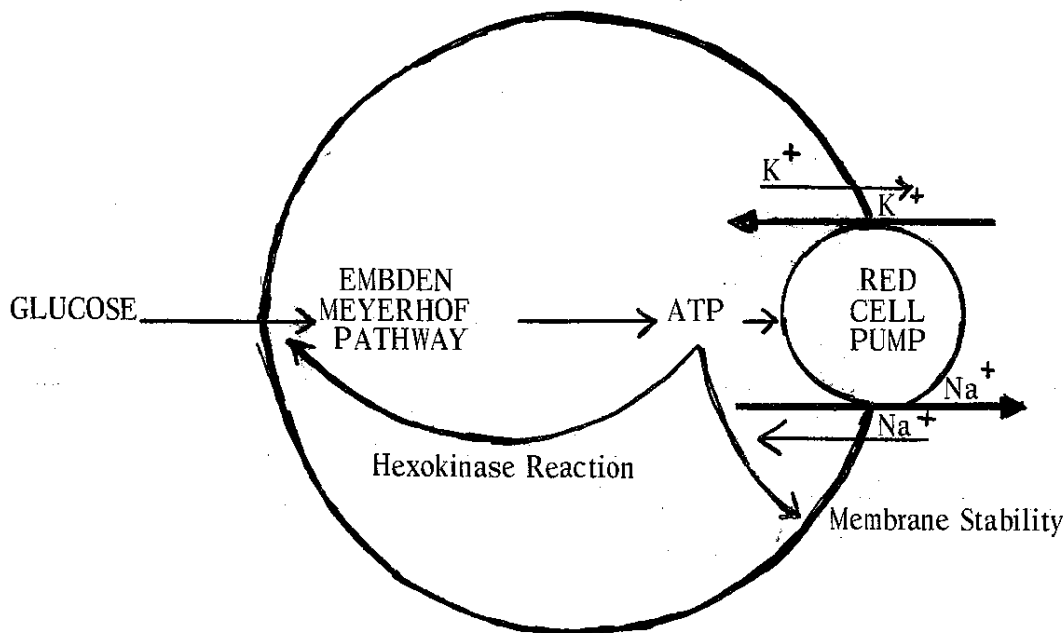


Fig. 5. Schematic Model of ATP Economy in the Normal Human Red Cell

ATP is expended at the hexokinase reaction and as the source of energy required for the active transport (heavy arrow) of potassium into the cell and sodium out of the cell. This pump activity balances the downhill leak (light arrow) of potassium out of the cell and sodium into the cell. ATP is generated in the Embden-Meyerhof pathway. (Taken from Nathan et al. 15)



moles of ATP are formed. The high energy phosphate bond of ATP provides the cell with energy to utilize in maintaining electrolyte equilibrium across the cell membrane. Normally the red cell membrane is relatively impermeable to the free movement of cations. If the cations distributed themselves freely between the plasma and the cell, lysis of the cell would result because of the high oncotic intracellular pressure of the hemoglobin-laden erythrocyte. This would lead to cellular imbibition of water and resultant lysis. It is well known that the gradient between the concentration of sodium and potassium intracellularly and in the plasma is quite large. Potassium tends to leak out and sodium into the cell due to the concentration gradients of the respective electrolytes. In order to prevent the tendency to equalize the cation concentrations between the cell and plasma due to the tendency to maintain an osmotic equilibrium across the cell membrane, an active, energy-demanding "pump" operate in the cell to pull potassium in and push sodium out. Both of these cation movements are "uphill" against the high concentration. Energy is required in the form of ATP to accomplish this task (See Fig. 5).<sup>15</sup> Hence, it has been proposed that the biochemical defect responsible for premature lysis of the erythrocyte in hereditary nonspherocytic hemolytic anemia due to pyruvate kinase deficiency in deficient regeneration of ATP.<sup>5, 13, 15</sup>

In addition to the direct block in the conversion of phosphoenolpyruvate to pyruvate and subsequent reduction in regeneration of ATP, the accumulation of phosphorylated carbohydrate intermediates, eg., <sup>2,3</sup>DPG, causes marked reduction of hexokinase activity and further embarrases glucose consumption (both in red cells and heart muscle) according to recent studies.<sup>15</sup> This also contributes to deficient regeneration of ATP.

As to whether the actual hemolytic process is intra-vascular or extra-vascular is still a disputed question. Brunetti et. al.<sup>11</sup> states it is probably intra-vascular because histology study of the spleen in his two cases did not show any sign of erythrosthosis or erythrophagocytosis. Furthermore, he argues that splenectomy does not control the hemolytic process. Bowman and Procopic,<sup>1</sup> however, write that "the site of red cell distinction was apparently extravascular, as plasma hemoglobin values were normal and serum bilirubin was consistently increased". Thus, it is apparent that much confusion exists concerning the exact site of erythrocyte destruction.

## CLINICAL MANIFESTATIONS

The clinical manifestations of the disease vary greatly. Jaundice and icterus may occur in the neonatal period. Hyperbilirubinemia may be of

sufficient degree to require exchange transfusion. In other individuals icterus or jaundice may not occur until much later in life. For example, Tanaka et. al. had two cases discovered for the first time at ages 19 and 20 years.<sup>10</sup>

In addition to jaundice, varying degrees of anemia and splenomegaly at some time in life appear to be the rather constant features of the disease. Pallor, weakness, lethargy and other characteristic signs and symptoms of an anemia may present. Hapatomegaly is quite variable. Among the Amish cases a prominence of the frontal cranial eminences producing altered facies and accompanied by radiographic changes in the skull was seen in two patients, not siblings, and not needing repeated transfusions to maintain an effective red cell mass before splenectomy.<sup>1</sup> The roentgenographic changes consisted of a widening of the diploic space with an atrophic outer table. Fine radial lines were present at right angles to the inner table of the calvarium.

The enlarged spleens have shown no significant detail on histologic examination.<sup>1,4,10</sup> The enlargement appears to be the result of an increase in the reticulum itself rather than an excess of intrasplenic erythrocytes. Bowman and Procopio<sup>1</sup> had one spleen show erythrophagocytosis. In this case splenectomy was performed when the anemia was maximum.

## LABORATORY STUDIES

Morphologically the blood smear is characterized by the presence of a normochronic macrocytic type of anemia. The macrocytosis has been attributed to the presence of an increased number of reticulocytes. Maximum reticulocyte counts have ranged from 5.2 to 45 percent with one as high as 94 percent.<sup>14</sup> Spherocytes are typically absent, but may sometimes confuse this disorder with congenital spherocytic anemia in that there may be a small number present.<sup>3,13</sup> Furthermore, varying degrees of osmotic fragility on incubation of the cells may make the diagnosis even more confusing in attempting to differentiate between the two disorders. An occasional "tailed poikilocyte"<sup>3,13</sup> elongated oval forms<sup>1</sup> or irregularly contracted cells<sup>10,11,13</sup> have been observed. One case had approximately 80% of its cell population made up of an abnormal erythrocyte having a small spiculated configuration similar to that seen in the syndrome of acanthocytosis.<sup>14,15</sup> The mother of this child, however, was found to be a carrier of glucose-6-phosphate dehydrogenase deficiency trait in addition to the pyruvate kinase deficiency trait. Howell-Jolly bodies, target cells, Pappenheimer bodies, and siderocytes have been present in patients who have had splenectomies.<sup>10,14</sup>

As stated earlier, one criteria which separated this disease entity from type I congenital nonspherocytic hemolytic anemia was an increased autohemolysis.<sup>1,3,13</sup> This test remains quite useful in making the diagnosis, but caution must be exercised in that this test is not foolproof. Autohemolysis has been found to be normal in some cases.<sup>10,13</sup> Selwyn and Dacie<sup>3</sup> in their original classification stated that this increased autohemolysis was not corrected by glucose. Several investigations since then have noted minimal correction of the autohemolysis with the addition of glucose.<sup>1,10,13</sup> DeGrunchy et. al. first demonstrated that ATP did correct this increased autohemolysis.<sup>4</sup> There has been only one exception noted in the literature where no correction resulted.<sup>14</sup>

An explanation had to be advanced as to how ATP corrects the autohemolysis since it is known that it does not easily penetrate the erythrocyte membrane. It is thought that the ATP undergoes hydrolysis to ADP by the enzymatic action of the phosphatases of the plasma. The ADP thus formed then enters the membrane of the erythrocyte where it is converted to ATP and AMP by adenylate kinase. Some of these molecules enter the cell and reconstitute the energy requirements for maintenance of normal cell metabolism and thereby reduce autohemolysis.<sup>13</sup> The explanation would be consistent with the fact that ADP and AMP also correct the autohemolysis rate.<sup>10</sup>

Concerning the significance of the autohemolysis test, a recent writer has stated, "the classification of the congenital nonspherocytic hemolytic anemias into two types based on the results of autohemolysis tests serves a useful purpose and should be continued until the pathogenesis of these disorders is elucidated."<sup>13</sup>

Certainly the means of making an unequivocal diagnosis of a pyruvate kinase deficiency anemia is to perform enzyme assay studies of the erythrocytes. Values of pyruvate kinase (PK) activity are very low in deficient erythrocytes. Oski and Diamond<sup>13</sup> studied erythrocytes of thirty normal persons ranging from 8 months to 62 years of age. These persons had a PK activity of  $1.79 \pm 0.23$  S.D. unites per  $10^{10}$  erythrocytes (One unit of PK activity equals the activity resulting in the conversion of 1 micromole of DPNH to DPN per minute by  $10^{10}$  erythrocytes. These assay conditions were established by Tanaka et. al.<sup>10</sup>). The three patients with pyruvate kinase deficiency and congenital nonspherocytic hemolytic anemia which they studied, had PK activity values from 0.1 to 0.3 units. These values correlate well with those found by other investigators.<sup>1,10,11,</sup>

12, 14 Grimes<sup>12</sup> found levels of PK activity in 12 cases to be between 10 - 40% of the near normal value.

In heterozygote parents and siblings intermediate values of PK activity have been consistently observed. These values have been found to be midway between those of normal persons and those persons affected with the disease. In the study of Oski and Diamond<sup>13</sup> mentioned in the previous paragraph, they found three parents and two siblings to be heterozygous in the course of family surveys. Their pyruvate kinase values ranged from 0.53 to 0.96 units, being quite consistent with the observations of other investigators.

Bowman and Procopio<sup>1</sup> suggest that the pyruvate kinase found in either the heterozygote or homozygote may not be identical to that found in the normal erythrocyte. They point out, "enzymes determined by an abnormal gene might be defective in quality, being dissimilar, though acting upon the same substrate, as well as being decreased in quantity." This concept would account for the clinical variance previously pointed out in patients with this disorder.

Pyruvate kinase assays appear to be quite specific for this deficiency state. Tamaka et. al.<sup>10</sup> performed PK assays in over one hundred patients with a variety of hemolytic as well as non-hematologic disorders and in no case was a low PK activity value obtained. There was actually a moderate to marked elevation in patients with a hemolytic process such as congenital spherocytosis (presplenectomy), sickle cell anemia, acquired hemolytic anemia, etc. There appears to be no close correlation between the enzyme level and apparent severity of the hemolytic anemia.<sup>12</sup>

The adenosine triphosphate (ATP) content of the cell is reduced in this disorder. This is quite consistent with the deficiency in regeneration of ATP due to a metabolic block outlined in previous discussion (See Figs. 3 and 4). It is also noted that an incubation of red cells from patients with this hemolytic anemia, there is a rapid and marked fall in the ATP content of the erythrocyte.<sup>5, 13</sup> One case of a heterozygote showed a slightly increased fall in ATP content following incubate again suggesting some disturbance in red cell metabolism in these individuals but not of sufficient degree to be reflected in clinically apparent disease.<sup>13</sup>

Erythrocyte diphosphopyridine nucleotide (DPN) content is low and a marked fall in this coenzyme has also been noted during incubation of pyruvate kinase deficient red cells.<sup>12, 13</sup> This is undoubtedly the result of a decreased amount of pyruvate being converted to lactate in the presence of lactic dehydrogenase with its associated oxidation of DPNH to DPN (See Fig. 4).

The incubated osmotic fragility test is characteristically normal in this disorder. The incubated osmotic fragility test, however, may show varying degrees of abnormally fragile cells.<sup>4, 13</sup> As mentioned earlier, this may be confusing when attempting to differentiate this disorder from congenital spherocytic anemia with the presence of spherocytes in the peripheral blood smear.

There appears to be no relationship between this disorder and blood groups or transferrin types.<sup>13</sup>

In the non-splenectomized subject, the haptoglobin, a hemoglobin-binding protein utilized as red cells are degraded intra-vascularly or extra-vascularly, has been found to be depleted. This suggests that the destructive process is evidently maximal in rate.<sup>1, 13</sup>

### TREATMENT AND PROGNOSIS

The treatment of this disease is rather not-specific since it is impossible to correct the basic erythrocyte enzyme deficiency and inherent metabolic defect. As mentioned earlier in the discussion, this disease may manifest itself in the neonatal period. The degree of hyperbilirubinemia may be of such extent to warrant exchange transfusions of blood in order to prevent the occurrence of kernicterus. And, as these patients continue through childhood and adult life, transfusions of blood may be required to correct the hemolytic anemia present. There seems to be considerable variation concerning the number and frequency of transfusions required to maintain a comfortable hemoglobin level. For example, one patient had received over 150 transfusions by age 33 years. On the other hand, the same investigator had four adult patients leading active lives without requiring any transfusions.<sup>10</sup> Thus, it is again readily apparent that there exists considerable clinical variability in the severity of the hemolytic process of the disease.

Splenectomy affords palliation rather than complete arrest of the hemolytic process differentiating it from the congenital spherocytic anemia. The major effect of splenectomy is to decrease red cell destruction, whether it be intra- or extra-vascular in origin. It is speculated that splenectomy may remove some humoral factor permitting red cell production to improve.<sup>1</sup> At any rate the transfusion requirements usually decrease following splenectomy may remove some humoral factor permitting red cell production to improve.<sup>1</sup> At any rate the transfusion requirements usually decrease following splenectomy and in some cases may do away with the need for any subsequent transfusion. This latter phenomenon is illustrated by the

Amish cases reported by Bowman and Procopic<sup>1</sup> where splenectomy ended the need for transfusions and permitted their osseous defects to regress.

Despite its nonspecificity, treatment of this disorder appears to be quite important, particularly the earlier the onset and the more severe the hemolytic process. In the case among the Mifflin County Amish kindred presented earlier in the discussion, where the onset of the disease was within the first two years of life, its therapy was not instituted in the form of transfusions and/or splenectomy, the child undoubtedly died before the age of three or four years.<sup>2</sup>

The prognosis of the disease varies considerably in relation to the severity of the disease. Among the Mifflin County Amish, Bowman et. al.<sup>2</sup> points out that the siblings in whom the disease was unrecognized and consequently not treated, died in infancy. Other investigators identified individuals with the disease who has already attained adulthood.<sup>10, 12</sup> This led Tanaka et. al.<sup>10</sup> to make the statement, "In spite of a moderately severe hemolytic anemia in most instances, survival to adulthood is apparently common". Hence, it is readily appreciated that prognosis of the disease is quite variable.

#### SUMMARY

Congenital nonspherocytic hemolytic anemia due to erythrocyte pyruvate kinase deficiency has been observed among children of the Mifflin County (Pennsylvania) Amish. The genealogy of all twenty-one affected children can be traced to a common ancestral couple providing rather confirmatory evidence to a previously suspected autosomal recessive mode of inheritance. Reasons are cited as to how the Amish deme differs from the Lancaster County Amish making the appearance of this genetic defect among the latter group unlikely.

The defect in the disorder relates to an enzymatic block in erythrocyte glycolysis with a resultant decrease in the regeneration of adenosine triphosphate (ATP). This block occurs with the conversion of phosphoenolpyruvate to pyruvate due to a deficiency of the enzyme pyruvate kinase necessary for this conversion. During this conversion adenosine diphosphate (ADP) is converted to ATP. The result is osmotic disequilibrium across the cell membrane leading to premature lysis of the cell, since the energy utilized by the cell to maintain this equilibrium is supplied by ATP.

This disease usually presents itself with varying degrees of a macrocytic normochromic anemia, reticulocytosis, jaundice, and splenomegaly. It may necessitate exchange transfusion in the neonatal period due to a hyperbilirubinemia. In other individuals the disease has been an incidental finding in the teens or adulthood. Transfusion, usually required less frequently (if at all) following splenectomy, has been the main emphasis of treatment to ameliorate the anemic process.

This congenital nonspherocytic hemolytic anemia is of the type II classification. Characteristically, the autohemolysis is markedly increased. This phenomenon is not corrected by the addition of glucose, but is corrected by the addition of ATP. Erythrocyte ATP content is low with a rapid fall in some during incubation of the cells. Diphosphopyridine nucleotide (DPN) content of cell is also low in keeping with the metabolic abnormality of the cell. Pyruvate kinase (PK) activity assays of the red cells reveal a low value. Heterozygous parents and offspring have an intermediate value of PK activity between those values of homozygotes and normal individuals. The unincubated osmotic fragility test is characteristically normal, but the incubated tests show varying degrees of abnormal cells.

The prognosis of the disorder varies considerably. In some cases death will ensue in infancy if treatment is not instituted. Other cases survive to adulthood with minimal or no treatment.

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