# Pyruvate Kinase Deficient Hemolytic Anemia in an Amish Isolate

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In 1954 Selwyn and Dacie first classified the nonspherocytic hemolytic anemias into two types, in each of which abnormal erythrocyte autohemolysis occurs on *in vitro* incubation. The basis for classification was the behavior with added glucose. When glucose is provided, autohemolysis of Type I cells is diminished, although not reduced to normal. Autohemolysis of Type II cells is not affected by addition of glucose. These findings suggested that a specific defect of red cell glycolysis exists in each class of hemolytic disorder.

In 1961, Tanaka, Valentine and Miwa (1961, 1962) reported that red cell pyruvate kinase deficiency is present in some cases of Type II nonspherocytic hemolytic anemia. They presented strong biochemical evidence of autosomal recessive inheritance; both parents of affected cases showed intermediate levels of red cell pyruvate kinase activity (1.73 units or less, as compared with the normal value of 2.0–3.4 units).

In 1963, Bowman and Procopio reported pyruvate kinase deficient hemolytic anemia (PK deficiency) in an Amish kindred living mainly in Mifflin County, Pennsylvania. Again, the parents of affected children exhibited intermediate enzyme levels ranging from 1.0–1.49 units. Clinically the disorder was somewhat different from that described by Tanaka, Valentine, and Miwa in the first report of this type of enzyme deficiency and from children in the later study of Oski and Diamond (1963). In the untreated Amish cases, death occurred between the ages of one and three years. Splenectomy in the affected Amish children was unequivocally beneficial.

Here we shall report on successful efforts to trace all parents of affected Amish children back to a single common ancestral pair, one of whom can be presumed to have carried the gene. The pedigree has been brought up to date and revised in minor respects, with addition of newly studied cases, including three additional affected sibships. Furthermore, the Amish isolate in which PK deficiency was found will be described.

## THE DISEASE

In the Amish cases, hemolytic disease has its onset in the first two years of life, and without treatment death probably occurs before the age of three or four years. Pallor and transient icterus are features of all cases. Splenomegaly

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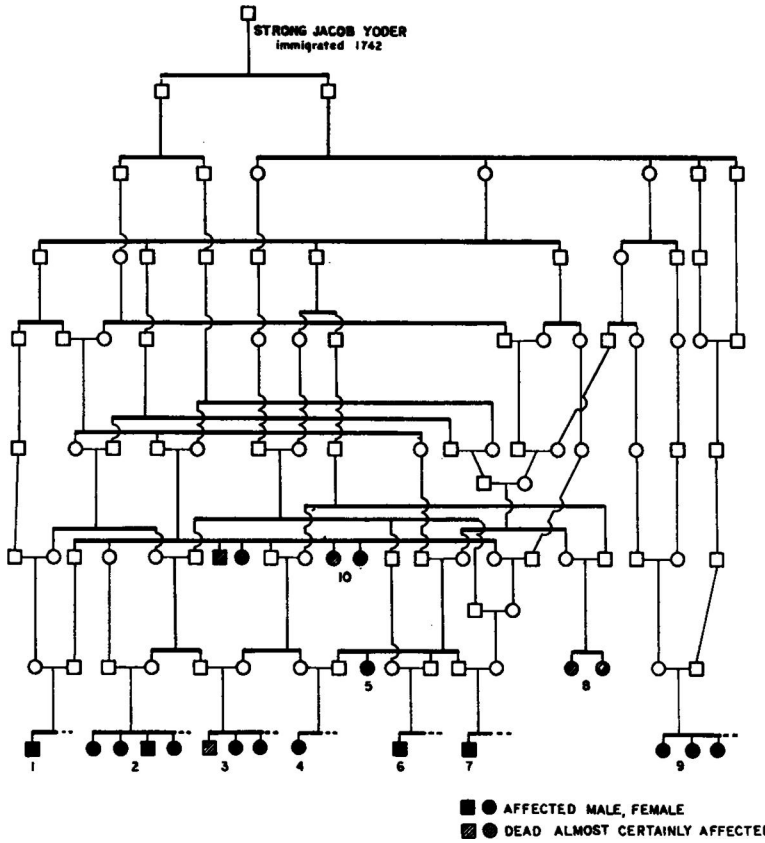


FIG. 1. The pedigree. Several changes from the pedigree given as Fig. 2 by Bowman and Procopio (1963) have been made because of new information or new births.

is detected in the first year or two in all fully studied cases. Hepatomegaly is more variable. Children who have not received repeated transfusions before splenectomy show prominence of the frontal cranial eminences producing altered facies and accompanied by radiographic changes in the skull. Splenectomy has ended the need for transfusion in all affected children and permitted their osseous defects to regress. However, it has palliated rather than completely arrested the hemolysis.

Increased neonatal jaundice was encountered in three of five children in the report of Bowman and Procopio (1963), and in one instance exchange transfusion was performed. Neonatal icterus occurred in three recently detected cases. In an infant of sibship 6, jaundice was noted the second day of life and progressed rapidly to a total bilirubin level of 47 mg/100 ml. The infant died in the first week despite exchange transfusion. Autopsy showed marked kernicterus and extensive extramedullary hematopoiesis in the liver and spleen. In an infant of sibship 9, icterus and pallor developed a few hours after birth, the total bilirubin level was 8.6 mg/100 ml, and four separate exchange trans-

TABLE 1. ANALYSIS OF FAMILIES WITH PYRUVATE KINASE DEFICIENCY HEMOLYTIC ANEMIA BY *a priori* METHOD

| Family size | Number of families | Number affected | | Variance |
|---|---|---|---|---|
| | | Observed | Expected | |
| 2 | 1 | 1 | 1.1428 | 0.122 |
| 3 | 1 | 1 | 1.2973 | 0.263 |
| 4 | 1 | 1 | 1.4625 | 0.420 |
| 5 | 2 | 3 | 3.2778 | 1.184 |
| 6 | 1 | 1 | 1.8248 | 0.776 |
| 7 | 1 | 3 | 2.0196 | 0.970 |
| 12 | 2 | 7 | 6.1960 | 4.040 |
| 18 | 1 | 4 | 4.5034 | 3.341 |
| Total | 10 | 21 | 21.7242 | 11.116 |
| | | | | SD = 3.334 |

fusions were required. An infant of sibship 3 was admitted to hospital at the age of 20 hours because of jaundice. The total bilirubin level was 19.4 mg/100 ml, there were 254 erythroblasts and normoblasts per 100 leukocytes, and the hematocrit fell gradually to 31%. Transfusion was not required, however. In all three cases materno-fetal blood group incompatibility was excluded.

### THE PEDIGREE

Figure 1 presents the pertinent portions of the pedigree. Affected children born since the publication of Bowman and Procopio (1963) have been added. Several cases considered only very questionably affected have been indicated as "almost certainly affected," because of further information from the family. Three "new" sibships with five affected members (indicated as sibships 6, 7 and 9) have been added.

The ten sibships presented in full in Fig. 2 contain a total of 21 affected persons. As indicated in Table 1, segregation analysis by the Lenz-Hogben technique shows good agreement of "observed" with "expected," assuming autosomal recessive inheritance. Biochemical identification of homozygotes and heterozygotes has been accomplished in some members of the ten sibships and their parents; although these data are incomplete, they are also consistent with autosomal recessive inheritance.

As is indicated in Table 2 and Fig. 1, all parents are traced back to "Strong" Jacob Yoder (immigrated 1742) and his wife. This is the only immigrant couple ancestral to all twenty parents of affected children. Therefore, Strong Jacob or his wife may have been heterozygous for the pyruvate kinase deficiency gene. That Yoder or his wife is the carrier ancestor is further supported by the fact that parents of affected children are related to them through many more lines of descent than to any other immigrant ancestor. Three parents, brothers, are descended from Jacob Yoder through no fewer than seven pathways. In Table 3, the coefficients of relevant consanguinity ($F'$) are given for each affected sibship. These figures do not represent total consanguinity,

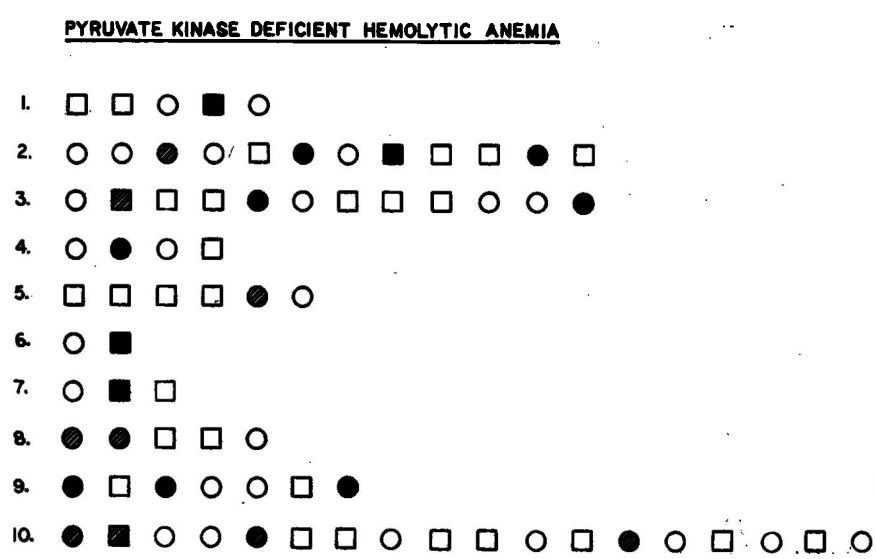


FIG. 2. The sibships. Symbols are the same as in Fig. 1.

since each parental pair is related through other common immigrant ancestors as indicated by Table 2. The average coefficient for the ten sibships is the equivalent of all parents being related as about second cousins, and the highest coefficient (that for sibship 4) is the equivalent of almost first cousins.

## THE ISOLATE

The characteristics of Amish society which make the group useful for genetic studies of certain types have been outlined elsewhere (McKusick, Hostetler, and Egeland, 1964). The existence of subisolates, or *demes* (endogamous local communities or consanguineous kin groups [Murdock, 1949]), comprising, for example, the Lancaster County (Pennsylvania) Amish as distinct from the Holmes County (Ohio) Amish, has been documented by historical and genealogic information, by observations on family names, and by differences in the frequency of certain recessive disorders such as the Ellis-van Creveld syndrome (McKusick, Eldridge, Egeland, and Krusen, 1964).

The Amish settlement of Mifflin County occupies a beautiful valley called the Kishacoquillas, or more simply, Big Valley. The founders of the settlement were, like those of Lancaster County, descendants from pre-Revolutionary immigrants. The first Amish settler was Christian Zook, who in 1792 settled on land which has since been continuously tilled by his descendants (Hostetler, 1948).

The Mifflin County Amish constitute another deme partially distinct from the Lancaster County and Holmes County groups. Religious factors have kept them separate from the non-Amish of their vicinity. Amish who marry non-Amish leave the group. Geographic factors, illustrated in Fig. 3, have kept them separate from other Amish groups. Big Valley is long and narrow, hemmed in by mountains—Stone Mountain on the northwest and Jack Moun-

TABLE 2. IMMIGRANT ANCESTORS

| Parents of Affected Sibships | Strong Jacob Yoder imm. 1742* | Peter Bitsche (Peachey) imm. 1767 | Widow Barbara Yoder imm. ca. 1714 | Moritz Zug (Zook) imm. 1742* | Jacob Hertzler imm. 1749 |
|---|---|---|---|---|---|
| 1. Father | + | + | + | − | − |
| Mother | + | + | + | + | + |
| 2. Father | + | + | + | − | − |
| Mother | + | + | + | + | + |
| 3. Father | + | + | + | + | + |
| Mother | + | + | + | + | − |
| 4. Father | + | + | + | + | + |
| Mother | + | + | + | + | − |
| 5. Father | + | + | + | − | − |
| Mother | + | + | + | + | + |
| 6. Father | + | + | + | + | + |
| Mother | + | − | + | + | + |
| 7. Father | + | + | + | + | + |
| Mother | + | + | + | + | + |
| 8. Father | + | + | − | + | − |
| Mother | + | + | + | + | + |
| 9. Father | + | − | + | + | + |
| Mother | + | − | + | + | + |
| 10. Father | + | + | + | − | − |
| Mother | + | − | + | − | − |

*Jacob Yoder and Moritz Zug immigrated on the same ship.

TABLE 3. COEFFICIENTS OF RELEVANT CONSANGUINITY ($F'$)

| Sibship number | $F'$ | Number of relationships of parents |
|---|---|---|
| 1 | .038695 | 12 |
| 2 | .034728 | 15 |
| 3 | .040770 | 20 |
| 4 | .050973 | 28 |
| 5 | .015807 | 10 |
| 6 | .003600 | 14 |
| 7 | .027096 | 42 |
| 8 | .006713 | 5 |
| 9 | .001464 | 4 |
| 10 | .009765 | 2 |
| Average | .022961 | |

tain on the southeast. The main approach to the valley is at its northeastern end.

The family names in three Old Order Amish groups are indicated in Table 4. The differences support the view that the Amish of each county constitute a separate deme.

Customs in the Mifflin County group differ in some respects from those of the

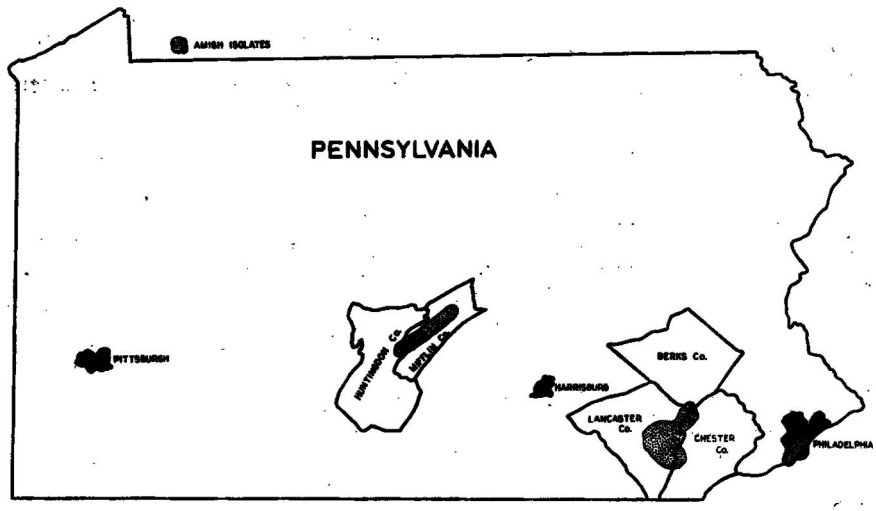


FIG. 3. Location of the Old Order Amish groups in Mifflin County and in Lancaster County.

Lancaster County group. For example, the hair is worn longer in the men, who, furthermore, use only one suspender—more is considered unnecessary and therefore mere adornment. (See Fig. 4.)

The distribution of pyruvate kinase deficient hemolytic anemia is further evidence that the Mifflin County Amish comprise a distinct deme. Questionnaires to physicians practicing in Amish areas of Pennsylvania, Ohio, Indiana,

TABLE 4. OLD ORDER AMISH FAMILY NAMES

| Lancaster County, Pennsylvania (a) | | (b) | Holmes County, Ohio | | Mifflin County, Pennsylvania | |
|---|---|---|---|---|---|---|
| Stoltzfus* | 23% | 24% | Miller | 26% | Yoder | 28% |
| King | 12% | 14% | Yoder | 17% | Peachey | 19% |
| Fisher | 12% | 14% | Troyer | 11% | Hostetler | 13% |
| Beiler | 12% | 11% | Hershberger | 5% | Byler | 6% |
| Lapp | 7% | 7% | Raber | 5% | Zook | 6% |
| Zook | 6% | 7% | Schlabach | 5% | Speicher | 5% |
| Esh† | 6% | 4% | Weaver | 4% | Kanagy | 4% |
| Glick | 3% | 3% | Mast | 4% | Swarey | 4% |
| | 81% | 84% | | 77% | | 85% |
| Totals: (a) 1106 families, 1957<br>(b) 760 households, 1960 | | | 1611 families, 1960 | | 238 families, 1951 | |

*Including Stoltzfoos.

†Including Esch and Eash.

The Lancaster County families are (a) those listed in the *Fisher Family Record* (1957), which included all but four of the Old Order Amish families in that county at the time of publication, and (b) 760 households in a one-third sample of the Amish church districts in 1960 (Janice A. Egeland, personal communication). The Holmes County data are from the Ohio Amish Directory assembled and privately circulated in 1960 by Ervin Gingerich, Star Route, Millersburg, Ohio. The Mifflin County listing was given by Hostetler (1951).

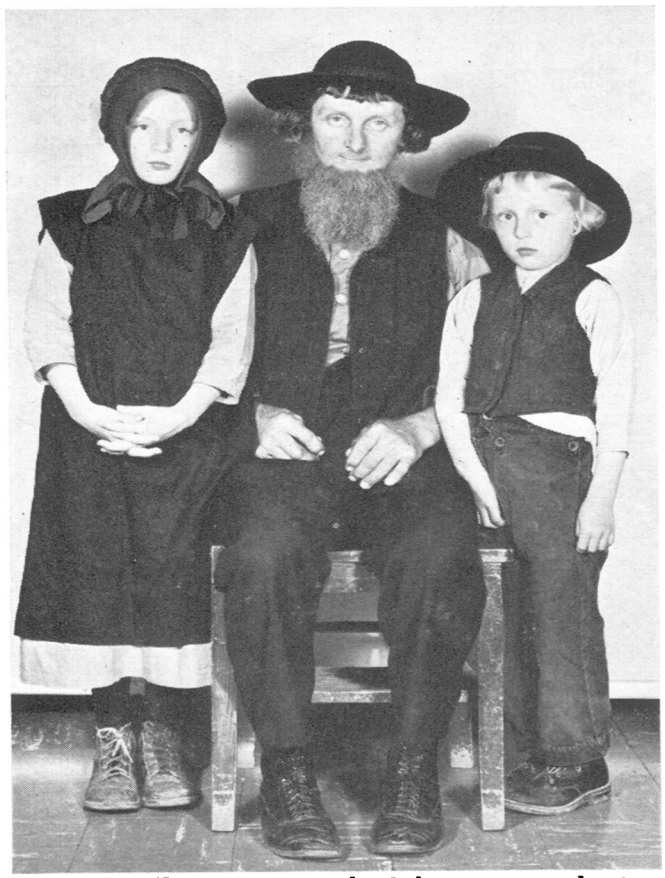

FIG. 4. Dress in the Mifflin County Amish. Only one suspender is worn, and in the men the hair is kept longer than in some other Amish. Both children have erythrocyte pyruvate kinase deficiency and have been essentially well since splenectomy. The girl is pictured in Fig. 1 of Bowman and Procopio (1963). Before splenectomy, skull changes were prominent. Since splenectomy, the skull has returned to normal and growth has also proceeded well so that the weight and height of both children are within normal limits. The father has an intermediate level of erythrocyte pyruvate kinase activity in his erythrocytes.

and Ontario and inquiry of Amish and other informants in many communities have uncovered no cases in other Amish groups.

Whereas the Old Order Amish ("House Amish") of Mifflin County have become splintered into four groups in the last 100 years, and other more liberal factions have become "Church Amish" (Hostetler, 1951), all but one of the sibships with erythrocyte pyruvate kinase deficiency have been in the most conservative of the groups of House Amish. Thus, there is further isolate formation even in the Old Order Amish of Mifflin County. The exceptional sibship (number 9) is in a Mennonite family in which both parents have Old Order Amish grandparents.

## SUMMARY AND CONCLUSIONS

An Amish kindred containing at least 21 cases of pyruvate kinase deficient

hemolytic anemia has been described. Both parents of all affected sibships can be traced to only one immigrant couple, one of whom was presumably a heterozygote. This disorder has been identified only in the Mifflin County (Pennsylvania) Amish deme. The characteristics of the disease and of the deme in which it occurs have been described briefly.

ACKNOWLEDGMENTS

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