HEREDITARY METHEMOGLOBINEMIA is a very rare condition characterized by cyanosis, a variable amount of hemoglobin in the oxidized form methemoglobin, a compensatory polycythemia, and no other pathologic changes. According to a recent review by Gibson fewer than 50 cases have been reported, although Dines suggests that there are more than 100. Available evidence indicates that the condition is inherited as a recessive trait.

In air, hemoglobin undergoes a slow spontaneous oxidation to methemoglobin and, as shown by Warburg, is kept in the reduced state in the red cell by an enzymatic system which utilizes glucose as substrate. The oxidation of glucose generates reduced pyridine nucleotides which in turn reduce methemoglobin by an electron transfer mechanism that is not fully understood. Huennekens et al. have attempted to isolate a methemoglobin reductase from red cells.

Barcroft et al. and Sievers and Byron showed that the rate of reduction of methemoglobin in cells of persons with hereditary methemoglobinemia is lower than in normal cells. Gibson, largely because of the marked effectiveness of methylene blue in reducing methemoglobin in methemoglobinemic cells, suggested that the enzyme diaphorase was missing in methemoglobinemic cells. Eder, Finch and McKee, however, showed that flavin adenine dinucleotide, a co-enzyme of the known diaphorases, was present in normal amounts in methemoglobinemic cells.

The present report describes an epidemiologic study of hereditary methemoglobinemia in Alaskan Eskimos and Indians, where its prevalence is comparatively high. Evidence will be presented to show that the level of methemoglobin in this condition varies with some environmental factor which may be dietary intake of ascorbic acid.

METHODS

Hemoglobin was determined as oxyhemoglobin. Methemoglobin was determined by the method of Evelyn and Malloy, using absorption at 635 μm with the Beckman spectrophotometer or a No. 62 filter with the Klett-Summerson photoelectric colorimeter. Where methemoglobin was found, an appropriate correction was applied to the hemoglobin levels.

Rate of methemoglobin reduction was determined in both whole cells and hemolyzates. Washed red cells were treated with an excess of sodium nitrite (2 mg. per ml. cells). After 2 hours, the cells were washed repeatedly with phosphate-buffered saline (9 vols.)
0.9% sodium chloride plus 1 vol. 0.1 M phosphate buffer, pH 7.35). To the oxidized whole cells, 200 mg. glucose per ml. cells were added as reducing substrate and the rate of reduction was followed at 38° by periodic withdrawal of samples for methemoglobin determination. Hemolyzates of the oxidized cells were made by freezing or by addition of distilled water. The reducing system with the hemolyzates contained per ml.: 3 μmoles methemoglobin, 20 μmoles of glucose-6-phosphate and 0.7 μmoles triphosphopyridine nucleotide. Serum ascorbic acid was determined by the method of Bessey, Lowry and Brock, modified for larger volumes of serum.

**RESULTS**

**Occurrence of Methemoglobinemia**

Methemoglobin was demonstrated in 15 persons in 9 Eskimo or Indian families as shown in table 1. In addition, two newborn infants in these families were reported to be cyanotic, but have not yet been tested. Of these 15, eight persons in six families were discovered because they presented cyanosis without any other symptoms. The others were found by testing all available relatives of the known cases.

The geographic locations of these families in Alaska are shown in figure 1. Because of the great distances involved, and because Eskimos and Indians tend to stay in a given area, it is extremely unlikely that all of these families are related. In fact, the condition appears to occur in four distinct areas in Alaska. Definite relationships could be found between four families; these are shown in figure 2. Because of geographic proximity, a distant relationship is also possible between families III and V and between families VII and VIII, although none was found.

**Effects of Methylene Blue and Ascorbic Acid**

When child 1–3 was hospitalized, 25 per cent of his total hemoglobin was methemoglobin. He was given methylene blue by injection, and after this treatment no methemoglobin could be detected. After another week in the hospital, 8.4 per cent of his hemoglobin had reverted to methemoglobin.

Patient IV–1 was in the hospital for three weeks and received 75 mg. of ascorbic acid daily, in addition to that in her hospital diet. During this period her methemoglobin level fell from 19.3 to 11.7 per cent. At this time her serum ascorbic level was 1.4 mg./100 ml. She was then given 400 mg. of ascorbic acid per day for a week, and her methemoglobin level fell to 9.7 per cent while her serum ascorbic acid level was 1.6 mg./100 ml.

At the time of admission of patient VII–2 to the hospital, her methemoglobin level was 37.3 per cent of total hemoglobin and there was no detectable ascorbic acid in her serum. After two weeks on 400 mg. of ascorbic acid per day, only 8.8 per cent of total hemoglobin was methemoglobin; this did not change during four more weeks of therapy. After two weeks of therapy, serum ascorbic acid was 1.6 mg./100 ml. One month after release from the hospital, over 30 per cent of her hemoglobin was again methemoglobin.

**Enzymatic Studies**

The capacity of red cells to reduce methemoglobin was determined in the case of three methemoglobinemic persons as shown in table 2. In all three cases, the reducing capacity was below normal. As reducing substrates in hemolyzates, glucose-6-phosphate plus either di- or triphosphopyridine nu-
### Table 1.—Cases of Methemoglobinemia in Alaskan Eskimos and Indians

<table>
<thead>
<tr>
<th>Family</th>
<th>Siblings</th>
<th>Present Age</th>
<th>Sex</th>
<th>Hemoglobin Gm./100 ml.</th>
<th>Methemoglobin Gm./100 ml.</th>
<th>Race</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>M</td>
<td>10</td>
<td>13.8,14.6</td>
<td>0.0</td>
<td>Ingalik</td>
<td>Measured</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>7</td>
<td>13.9,12.9</td>
<td>0.0</td>
<td>Indian</td>
<td>in</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>5</td>
<td>16.0,15.4</td>
<td>4.0,7.0</td>
<td>Sept. '55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M</td>
<td>4</td>
<td>14.0,13.1</td>
<td>1.9,5.2</td>
<td>Dec. '56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>F</td>
<td>3</td>
<td>12.0,14.5</td>
<td>1.0,4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>F</td>
<td>2</td>
<td>11.8, 9.2</td>
<td>2.6,3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>?</td>
<td>&lt;1</td>
<td>——</td>
<td>——</td>
<td></td>
<td>——</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>F</td>
<td>10</td>
<td>13.7,13.1</td>
<td>0.0</td>
<td>Ingalik</td>
<td>Measured</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>8</td>
<td>14.1,14.6</td>
<td>2.2,1.0</td>
<td>Indian</td>
<td>in</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>7</td>
<td>12.3,12.9</td>
<td>0.0</td>
<td>Sept. '55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M</td>
<td>5</td>
<td>12.9,13.1</td>
<td>0.0</td>
<td>Dec. '56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>M</td>
<td>3</td>
<td>12.9,12.9</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>——</td>
<td>——</td>
<td></td>
<td>——</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>M</td>
<td>7</td>
<td>11.4</td>
<td>0</td>
<td>1/2 5 normal half-siblings; measured</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>6</td>
<td>12.5</td>
<td>0</td>
<td>Ingalik</td>
<td>in</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>4</td>
<td>12.3</td>
<td>0</td>
<td>Indian, 5/16 measured</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M</td>
<td>3</td>
<td>12.5</td>
<td>3.8</td>
<td>Koyokuk</td>
<td>Dec. '56</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>F</td>
<td>1</td>
<td>10.7</td>
<td>0</td>
<td>Indian, 3/16 measured</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>M</td>
<td>&lt;1</td>
<td>——</td>
<td>——</td>
<td></td>
<td>——</td>
</tr>
<tr>
<td>IV</td>
<td>1</td>
<td>F</td>
<td>16</td>
<td>15.1,16.1</td>
<td>2.8,3.1</td>
<td>Ingalik</td>
<td>3 normal half-siblings; measured Oct. '55, Jan. '57</td>
</tr>
<tr>
<td>V</td>
<td>1</td>
<td>M</td>
<td>7</td>
<td>13.7</td>
<td>0</td>
<td>7/8 Measured</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>5</td>
<td>13.5</td>
<td>1.0</td>
<td>Koyokuk</td>
<td>April '56, after ascorbic acid therapy</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>F</td>
<td>4</td>
<td>13.2</td>
<td>1.0</td>
<td>Indian, 1/6 White</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>1</td>
<td>F</td>
<td>6</td>
<td>12.9</td>
<td>2.8</td>
<td>Eskimo</td>
<td>Measured</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>4</td>
<td>12.8</td>
<td>0</td>
<td>Eskimo</td>
<td>Sept. '55</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>3</td>
<td>11.2</td>
<td>3.5</td>
<td>Eskimo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>?</td>
<td>&lt;1</td>
<td>——</td>
<td>——</td>
<td></td>
<td>——</td>
</tr>
<tr>
<td>VII</td>
<td>1</td>
<td>F</td>
<td>22</td>
<td>11.6</td>
<td>0</td>
<td>Eskimo</td>
<td>Measured</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>15</td>
<td>17.2</td>
<td>6.2</td>
<td>Eskimo</td>
<td>June '57</td>
</tr>
<tr>
<td>VIII</td>
<td>1</td>
<td>F</td>
<td>17</td>
<td>16.2,11.4</td>
<td>3.6,0.5</td>
<td>Eskimo</td>
<td>1 normal half-sibling; VIII-1 measured</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M</td>
<td>14</td>
<td>14.1</td>
<td>0</td>
<td>Eskimo</td>
<td>in</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>F</td>
<td>12</td>
<td>13.8</td>
<td>0</td>
<td>Eskimo</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M</td>
<td>9</td>
<td>11.9</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>M</td>
<td>7</td>
<td>11.3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>M</td>
<td>5</td>
<td>11.9</td>
<td>0</td>
<td>'48; others measured in Dec. '56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>F</td>
<td>1</td>
<td>11.3</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>1</td>
<td>F</td>
<td>9</td>
<td>13.5</td>
<td>0</td>
<td>Eskimo</td>
<td>Measured</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>7</td>
<td>14.5</td>
<td>5.4</td>
<td>Eskimo</td>
<td>in</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M</td>
<td>3</td>
<td>9.5</td>
<td>0</td>
<td>Eskimo</td>
<td></td>
</tr>
</tbody>
</table>

*Newborn child reported to be cyanotic by medical personnel.

*Newborn child, not cyanotic.
FIG. 1.—Geographic location of methemoglobinemic families. Each circle represents the present residence of one family. The arrows indicate birthplace of the parents of methemoglobinemic families, where this differs from the present residence.

cleotides, glucose plus adenosine triphosphate and triphosphopyridine nucleotide, and the reduced forms of di- and triphosphopyridine nucleotides were about equally effective.

In this hemolyzate system, a number of substances will speed up the reduction of methemoglobin in the presence of reducing substrate but not in its absence, and in both methemoglobinemic and normal cells. These include methylene blue, ferricyanide, D- or L-cystine, riboflavin, riboflavin phosphate, and flavin adenine dinucleotide. The last four must be present in much higher than physiologic concentration to be effective. Homocystine, glutathione, ergothioneine, co-enzyme A and lipoic acid were ineffective.

Patient IV–1 had normal levels of pyridine nucleotides, riboflavin nucleotides and sulfhydryl compounds in her cells. The hemoglobin of this patient and that of I–3 had the same electrophoretic mobility as normal hemoglobin A, and no indication of an abnormal absorption spectrum of the methemoglobin was obtained.

DISCUSSION

The principal conclusion from this study is that methemoglobinemia in Alaskan Eskimos and Indians is hereditary in origin, but that the level of methemoglobin is modified by some environmental factor. The relationships in figure 2 are consistent with the inheritance of methemoglobinemia as a recessive trait.
Fig. 2.—Relationship of four families with methemoglobinemia. Squares = males; circles = females; diamond = sex unknown. Open symbols = normal; solid symbols = methemoglobinemic; crossed symbols = deceased. All living persons shown were tested for methemoglobin.

That the condition was inborn was indicated by the fact that parents and neighbors of these children knew that the children's appearance was unusual and that they had had this appearance since birth. For example, the parents of family VI knew and were concerned that their children, VI-1 and VI-3, were distinctly blue, just as they knew that child VI-2 looked like other Eskimo children. The parents of family II likewise recognized that their child, II-2, was different from the others, knew this condition existed in family I, and suggested its occurrence in IV–1.

One of these patients was hospitalized for 13 months with persistence of the methemoglobinemia throughout. This, and its recurrence after methylene blue treatment, appears to rule out a toxic cause. Finally, the difference in rate of methemoglobin reduction between methemoglobinemic and normal subjects, shown in table 2, appears to show that methemoglobinemia results from the

Table 2.—Rate of Reduction of Methemoglobin by Cells and Hemolyzates of Methemoglobinemic and Normal Subjects

<table>
<thead>
<tr>
<th>Methemoglobinemic Patient</th>
<th>Whole Cells + Glucose</th>
<th>Hemolyzates + G-6-P and TPN</th>
<th>Control</th>
<th>Whole Cells + Glucose</th>
<th>Hemolyzates + G-6-P and TPN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>percent per hour</td>
<td>percent per hour</td>
<td></td>
<td>percent per hour</td>
<td>percent per hour</td>
</tr>
<tr>
<td>IV–1</td>
<td>3.4 (4)¹</td>
<td>2.5 (10)</td>
<td>13.4 (4)</td>
<td>6.5 (10)</td>
<td></td>
</tr>
<tr>
<td>VII-2</td>
<td>2.9 (1)²</td>
<td>2.9 (1)</td>
<td></td>
<td>5.3 (1)</td>
<td></td>
</tr>
<tr>
<td>VII-2</td>
<td>3.0 (1)²</td>
<td>2.4 (1)</td>
<td>6.2 (1)</td>
<td>8.0 (1)</td>
<td></td>
</tr>
<tr>
<td>IX–2</td>
<td>4.4 (1)²</td>
<td>4.4 (1)</td>
<td>5.8 (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Glucose-6-phosphate and triphosphopyridine nucleotide.
²Control was the same age, race, and sex as patient.
³Figures in parenthesis are number of determinations.
⁴30 μmoles reduced triphosphopyridine nucleotide used in place of G-6-P and TPN.
absence of an enzyme or other factor necessary for complete reduction of
hemoglobin, as suggested by Sievers and Ryan and by Barcroft.

Gibson, on the basis of a differential effect of methylene blue on methemo-
globin reduction by glucose and lactate, has inferred that the normal reduction
of methemoglobin is mediated by a diphosphopyridine nucleotide-linked di-
aphorase and that this diaphorase is missing in hereditary methemoglobinemia.
A triphosphopyridine nucleotide-linked diaphorase is postulated as present in
both normal and methemoglobinemic cells, but it is inactive in methemoglobin
reduction unless methylene blue is added. This explanation is inadequate,
since the reduced forms of both pyridine nucleotides are nearly equally active
in reducing methemoglobin in normal cell hemolyzates. However, the enzym-
atic studies above show that the missing component in hereditary methemo-
globinemia must be some substance that mediates the reduction of methemo-
globin by reduced pyridine nucleotides.

Our observations indicate that methemoglobinemia in our subjects is not
due to the presence of the abnormal hemoglobin M, which Hörlein and Web-
er first observed in a German family. Besides the direct evidence in two sub-
jects cited above, it has been shown that the methemoglobinemia associated
with hemoglobin M is due to abnormal sensitivity of hemoglobin M to oxidation
and not to a slow rate of methemoglobin reduction. Further, it has been sug-
gested that hemoglobin M, like other abnormal hemoglobins, is inherited as
dominant characteristic.

That an environmental factor affects methemoglobin level in these persons
is evident from the data in table 1. Thus the amount of methemoglobin found
in members of family I was definitely less in September than in December,
15 months later. Only two children were evidently cyanotic in September,
while four were markedly cyanotic in December.

Our evidence indicates that this environmental factor may be ascorbic acid.
Thus, in September, persons in the village where family I lives were actively
engaged in picking wild berries. On the other hand, we have found that over
a third of all Eskimos and Indians tested show no detectable serum ascorbic
acid during the winter months, and indeed it is difficult to see from what
dietary source they could obtain the vitamin at that time. The results on
patient IV–1 presented above suggest that moderate intakes of ascorbic acid
may be almost as effective as larger doses in lowering methemoglobin level.

Hereditary methemoglobinemia appears to occur in Alaskan Eskimos and
Indians with a frequency not found in other populations. Thus, these cases
occur in a total Eskimo and Indian population of about 20,000. All the cases
in Indians are in Athabaskan Indians, of which there are many subdivisions,
including the Apache, the Navajo, and several Canadian and Alaskan tribes.
The Eskimo cases are all in the southern group of Eskimos who speak a
language distinctly different from that spoken by other Eskimos, including
Alaskan Eskimos living north of Norton Sound and Siberian, Canadian, and
Greenlandic Eskimos.

This apparent high frequency of occurrence in Indians and Eskimos may
be in part the result of the environmental factor. Recognition of methemo-
globinemia normally depends on observation of cyanosis, and in our experi-
ence, at least 20 per cent of the hemoglobin must be oxidized before cyanosis is evident. Ample ascorbic acid can prevent cyanosis by reducing the methemoglobin present to below this level. Consequently, methemoglobinemia might be evident only in persons with the genetic factor who also have low ascorbic acid intakes. In six other reports where serum ascorbic acid was measured, low levels (0.2 to 0.5 mg./100 ml.) were found.

All of our cases are in children, of which the oldest is now 17 years of age. 520 Eskimo men from all parts of Alaska, as well as over 1,200 other Eskimos and Indians including all available living relatives and near neighbors of the known cases, have been screened for methemoglobinemia with negative results. The observed exclusive occurrence in children may be a result of chance, or it may indicate an association of methemoglobinemia with early mortality. However, case VIII–1 was first discovered at the University of Chicago Hospital when the child was eight years old. At that time, 22.2 per cent of the hemoglobin was methemoglobin, and eight years later only 4.8 per cent was methemoglobin. This suggests the possibility that methemoglobinemia tends to disappear with age. If it does, this might explain the large number of cases in family I. In this family, five of seven children have the condition while both parents do not. If the character is recessive, its occurrence in five out of seven children would be expected to occur in only one out of 70 families. If however the condition tends to disappear with age, one parent may be homozygous and may have been methemoglobinemic as a child. Gibson and Harrison also found the condition in five of seven children in a family.

**Summary**

Methemoglobinemia is unusually frequent in Alaskan Eskimos and Indians in whom 15 confirmed cases in a population of about 20,000 are known. It appears to be due to the absence of a factor in red cells which mediates the reduction of methemoglobin by reduced pyridine nucleotides. This anomaly is probably inherited as a recessive trait.

The amount of methemoglobin present at any one time in these persons is variable, depending on some environmental influence. The evidence suggests that this environmental influence is the dietary intake of ascorbic acid.

**Summary in Interlingua**

Methemoglobinemia es inusualmente frequente in eschimos e indians de Alaska. Dece-cinque casos confirmate es cognoscite in un population de 20.000. Illo pare resultar del absentia de un factor erythrocytic que debereca mediar le reduction de methemoglobina per reducite nucleotidos pyridinic. Iste anormalitate es probabilemente hereditabile como un tracto recessive.

Le quantitate de methemoglobina presente a un momento particular in iste personas es variabile. Illo depende de un influentia ab le ambiente. Le datos suggere que iste influentia es le ingestion dietari de acido ascorbic.

**REFERENCES**

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Children 92:15, 1956.


Hereditary Methemoglobinemia in Alaskan Eskimos and Indians

E. M. SCOTT and DALE D. HOSKINS