Hemoglobin M\textsubscript{Kankakee}, A New Variant of Hemoglobin M

By Paul Heller, Hyman G. Weinstein, Vincent J. Yakulis, and Ira M. Rosenthal

The presence of an abnormal hemoglobin in one of the types of hereditary methemoglobinemia has been well established. Hoerlein and Weber\textsuperscript{2,3} showed clearly that in the family which they studied, an abnormality in the globin portion of the hemoglobin molecule was responsible for the methemoglobinemia. This disorder, therefore, was considered to be a hemoglobinopathy by Singer\textsuperscript{4} who suggested the designation “Hemoglobin M” for this abnormal hemoglobin.

“Hemoglobin M-disease,” which is transmitted as an autosomal dominant trait, must be distinguished from the congenital methemoglobinemia caused by a deficiency of the enzyme diaphorase in the erythrocyte.\textsuperscript{5,6} This enzyme mediates the reduction of ferrihemoglobin to ferrohemoglobin and thus prevents the accumulation of ferrihemoglobin in the erythrocyte. Diaphorase deficiency is transmitted as an autosomal recessive trait. Another type of hereditary methemoglobinemia with dominant transmission has recently been described. It has been attributed to an inadequacy of the synthesis of glutathione,\textsuperscript{7} but the exact mechanism has not been established.

Recent studies by several investigators\textsuperscript{8-11} have shown that hemoglobin M, isolated from different families affected by hemoglobin M-disease, is heterogeneous. These variants have been designated as M\textsubscript{Kankakee} (M\textsubscript{K}),\textsuperscript{8,9} M\textsubscript{Milwaukee} (M\textsubscript{M}),\textsuperscript{10,14} M\textsubscript{Saskatoon} (M\textsubscript{S}),\textsuperscript{9} M\textsubscript{Leipzig} (M\textsubscript{L}),\textsuperscript{11} M\textsubscript{Wanata} (M\textsubscript{W}),\textsuperscript{12} and M\textsubscript{Chicago} (M\textsubscript{CH}).\textsuperscript{13} Differences between these various hemoglobins M were first demonstrated by spectroscopic and electrophoretic techniques. Gerald and Efron\textsuperscript{14} have recently studied the polypeptide and amino acid structure of five variants of hemoglobin M and have detected different amino acid substitutions in four of these abnormal hemoglobins.

It is the purpose of this paper to report findings based upon a study of a family with five members affected by hemoglobin M-disease (fig. 1). The ferrihemoglobin found in the hemolysate of affected members of this family has spectroscopic and electrophoretic properties which appear to differ from other types of hemoglobin M. Since the family lives near Kankakee, Illinois, we propose to call this apparently previously undescribed hemoglobin by the name “Hemoglobin M\textsubscript{Kankakee} (M\textsubscript{K}).”

Clinical Features

The affected members of the family are the propositus R. H. and four of his five children (fig. 1, table 1).

The propositus, 35 years old, has been cyanotic since birth. He has never had any
complaints except for the cosmetic effect of the persistent cyanosis. His activity has never been limited by his condition. While in school he engaged in sports without difficulty. Nevertheless, his parents were warned on several occasions that his life expectancy was limited. Following graduation from high school he worked as a farmer. He was rejected from military service because of the cyanosis. At present he operates his own farm and does heavy labor for long hours without difficulty.

The parents of the propositus are of German and Scotch ancestry. Neither they nor their two daughters and other grandchildren have cyanosis (fig. 1).

The propositus was first seen in the clinics of the University of Illinois Research and Educational Hospital in July 1954. Physical examination revealed moderate cyanosis of the lips, nails and skin. There was no clubbing of the fingers or toes and no evidence of cardiac or pulmonary disease. Muscular development and strength were better than average. The hematologic findings are listed in table 1. The diagnosis of methemoglobinemia of unknown cause was made.

In July 1961, he was re-examined at the request of the clinic. He had remained well and presented the same appearance except for some premature graying of his hair. There was again no evidence of cardiovascular or pulmonary disease and the hematologic findings were normal (table 1).

His wife has always been in good health. Neither of her parents are cyanotic. She has no siblings.

When seen in 1954, the propositus had four children, one son 7 years old and three daughters, aged 6, 4 and 1. The three older children had been cyanotic since birth. Examination of these children revealed no other abnormalities. Physical growth and development were normal. Electrocardiograms and roentgenograms of the chest and long bones were within normal limits. Results of blood counts are listed in table 1. Treatment with oral ascorbic acid did not produce any evident change in the cyanosis. The youngest daughter was not cyanotic and had no abnormalities.

When the family was seen again in 1961, there was one more son, 3 years old. He also had been cyanotic since birth, without any other abnormalities. The other three cyanotic children had continued to develop normally, were doing well in school and participated in sports without any restriction and with no discomfort. The hematologic findings remained normal (table 1).

**Special Studies**

Blood was collected with 5 per cent sodium EDTA as anticoagulant (0.15 ml./10 ml. blood). Following centrifugation, the packed cells were washed three times in 0.15 M NaCl buffered to pH 7.3 with 0.01 M potassium phosphate. An aliquot of washed packed cells was hemolyzed with 9 volumes of distilled water and the stroma removed by centrifuga-
HEMOGLOBIN $M_{Kanakee}$

Table 1.—Hematologic Data

<table>
<thead>
<tr>
<th></th>
<th>RBC (10$^9$/mm$^3$)</th>
<th>Hb (Gm.%)</th>
<th>Ht (%)</th>
<th>WBC (10$^9$/mm$^3$)</th>
<th>Neutrophils (%)</th>
<th>Eos. (%)</th>
<th>Ly. Mon. (%)</th>
<th>Reticulocytes %</th>
<th>RBC</th>
<th>Blood Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>1964</td>
<td>1954</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.2</td>
<td>5.3</td>
<td>73</td>
<td>63</td>
<td>35</td>
<td>2</td>
<td>0.7</td>
<td></td>
<td></td>
<td>B Rh neg.</td>
</tr>
<tr>
<td>Child 1</td>
<td>1964</td>
<td>1954</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>45.6</td>
<td>4.7</td>
<td>6.5</td>
<td>55</td>
<td>1</td>
<td>43</td>
<td>1</td>
<td></td>
<td></td>
<td>A Rh neg.</td>
</tr>
<tr>
<td>Child 2</td>
<td>1964</td>
<td>1954</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.2</td>
<td>4.8</td>
<td>6.2</td>
<td>65</td>
<td>3</td>
<td>27</td>
<td>1</td>
<td></td>
<td></td>
<td>A Rh neg.</td>
</tr>
<tr>
<td>Child 3</td>
<td>1964</td>
<td>1954</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.7</td>
<td>5.2</td>
<td>6.8</td>
<td>59</td>
<td>2</td>
<td>39</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child 4</td>
<td>1964</td>
<td>1961</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.9</td>
<td>11.1</td>
<td>6.1</td>
<td>57</td>
<td>2</td>
<td>39</td>
<td>2</td>
<td>0.9</td>
<td></td>
<td>B Rh neg.</td>
</tr>
</tbody>
</table>

For 20 minutes at approximately 10,000 g. These samples were diluted with M/10 sodium phosphate buffer (pH 6.5 or pH 8.0) for spectroscopy. The final hemoglobin concentration was 0.2-0.6 mg./ml. Spectroscopy was performed with a Beckman DK$_1$ Spectrophotometer.

Diaphorase activity was determined according to the technic of Scott.$^6$

Electrophoresis (E. P.) of the hemolysate containing approximately 10 Gm. per cent of hemoglobin was performed in the following ways:

(a) On agar gel in barbital buffer (pH 8.8) for 90 minutes, as previously described.$^{15}$

(b) On agar gel in Tris-EDTA-boric acid buffer (TEB) at pH 8.9 for one hour (20 V/cm.). The buffer composition was as follows: Tris (Hydroxymethylaminomethane) 80.7 Gm., disodium ethylene diamine tetraacetate 10.5 Gm., boric acid 6.3 Gm., distilled water 8.1. The concentration of the buffer in the gel was half that in the electrolyte vessels.$^{13}$

Another aliquot of the hemolysate was incubated with one-half of its volume of 5 per cent potassium ferricyanide at 25 C. for approximately 10 minutes$^8$ and then electrophoresed in agar gel with TEB buffer adjusted to pH 7.1 for 30 minutes at 20 V/cm.$^{15}$ Following E.P. the fractions were eluted from the agar gel by freezing and thawing and dialyzed against M/10 phosphate buffer (pH 6.5).

The eluted ferrihemoglobin fractions were adjusted to a concentration of 0.65 mg. of hemoglobin per ml., and the spectroscopic pattern determined. Two and six-tenths ml. of these ferrihemoglobin solutions were placed into a 1-cm. cuvette and mixed with 0.05 ml. of 5 per cent KCN.$^{11}$ The rate of formation of cyanferrihemoglobin was measured by recording the diminution of the absorbance at 580 m.$
u$ which is the characteristic peak of ferrihemoglobin $M_K$. The half-reaction time$^{11}$—that is, the time required for conversion of half of the available ferrihemoglobin to cyanferrihemoglobin—was measured graphically from the recorded trace. Similar rate studies were also performed on other available methemoglobin samples ($M_{Chicago}, M_{Milwaukee}$).

For the estimation of the relative amounts of hemoglobin $M_K$ in the hemolysates, they were oxidized with potassium ferricyanide and electrophoresed in small starch blocks (10 x 7.5 x 0.3 cm.) containing 1/20 M phosphate buffer (pH 7.0) for 3 hours at 15 V/cm.

The buffer concentration in the electrolyte vessels was twice that in the starch. The starch block was divided into two parts, one containing only the greenish hemoglobin $M_K$ fraction, the other having the remainder of the hemoglobin. The sections were eluted separately with five successive aliquots of distilled water. Following the addition of 1 ml. 2 per cent KCN to each of the two pooled samples, they were adjusted to equal volumes of 15 ml. and the absorbance measured at 540 m.$
u$. Since the molecular extinction coefficient of cyanferrihemoglobin $M_K$ is not known, spectroscopic measurements were also made at 280 m.$
u$ to determine the relative amount of protein in each fraction. These measurements agreed within 3 per cent.

For the determination of the polypeptide chain abnormality, "hybridization" of the
hemoglobin fractions with canine hemoglobin was performed. This procedure was based on the observation of Itano, Singer and Robinson,\textsuperscript{16} that asymmetrical dissociation of the hemoglobin molecule into $\alpha$ and $\beta$ double-chains occurs on incubation in buffer below pH 6 and that recombination of the separated chains takes place following neutralization. In case of a mixture of hemoglobins, reassociation of the chains occurs at random, but $\alpha$ chains recombine always with $\beta$ chains. Hemoglobin mixtures from different species, e.g., man and dog, also reassociate at random following dissociation. This technic can be utilized for the identification of the chain abnormality.\textsuperscript{17}

For the performance of these hybridization studies, the method of Huehns and Shooter\textsuperscript{18} was chosen, with modifications. The ferrihemoglobin $NI$ fraction was eluted from agar gel by freezing and thawing and dialyzed against M/10 sodium phosphate buffer (pH 6.8) and reduced with crystalline sodium dithionite. This material, which showed the characteristic spectroscopic pattern of normal ferrohemoglobin (single absorption band at 556 nm), was converted to carbonyl hemoglobin, and dialyzed (two changes) against phosphate buffer (pH 6.8) saturated with carbon monoxide. The hemoglobin solution was mixed in equal amounts and concentration with canine hemoglobin. Ten ml. of this mixture and 1 ml. of M/1 sodium acetate buffer (pH 4.7) were dialyzed against 2 L. of the same buffer (M/10) for 24 hours at 4 C. The dialysis sacs were then placed into M/25 phosphate buffer (pH 6.8) for 24 hours (two changes). Simultaneous concentration and dialysis was then performed according to a recommendation by Huehns and Shooter.\textsuperscript{18} A mixture of $A$ and canine hemoglobin and another mixture of Hb S and canine hemoglobin were subjected to the same procedure.

At the end of dialysis the hemoglobin mixtures were electrophoresed on agar gel in barbital buffer (pH 8.8). For these hybridization studies, ferrihemoglobin itself could not be used because it precipitated at the low pH required for dissociation.

**RESULTS**

The hemolysates of four of the five affected members contained 22.4–27.8 per cent hemoglobin $M_K$. The blood of the youngest child was not examined. Diaphorase activity performed according to the technic of Scott\textsuperscript{6} was normal. Electrophoresis of the hemolysate on agar gel in barbital buffer (pH 8.8) did not show an abnormal pattern (fig. 2b). E.P. on agar gel in TEB buffer (pH 8.9) revealed three fractions: The main component $A_1$, $A_2$, and a fraction between $A_1$ and $A_2$ (fig. 3b). E.P. of the oxidized hemolysates at pH 7.1 on agar gel revealed a greenish component of slower cathodic mobility in addition to the normal main fraction (fig. 4b).

These electrophoretic features were distinct from those of other variants of Hb M of which only Hb M$_{\text{Chicago}}$ and M$_{\text{Milwaukeee}}$ were available for study: E.P. on agar gel in barbital buffer (pH 8.8) showed Hb M$_{\text{ch}}$ to be slightly faster than Hb A$_S$ (fig. 2c), while Hb M$_{\text{m}}$ did not separate from the main fraction (fig. 2d). On agar gel in TEB buffer (pH 8.9), Hb M$_{\text{ch}}$ was slightly faster than Hb M$_{\text{K}}$ (fig. 3c). Hb M$_{\text{m}}$ again could not be separated from the main fraction (fig. 3d).

The ferri form of Hb M$_{\text{ch}}$ and Hb M$_{\text{m}}$ had greater cathodic mobility than Hb M$_{\text{K}}$, when electrophoresed at pH 7.1 (figs. 4c and 4d).

The absorption spectrum of whole hemolysate of Hb M$_{\text{K}}$ diluted in phosphate buffer of pH 8.0 revealed the bands of normal oxyhemoglobin and an

\*In our report on Hb M$_{\text{chicago}}$\textsuperscript{13} it was stated that Hb M$_{\text{ch}}$ could not be separated from Hb A$_2$ in agar gel at pH 8.8. This statement applies to Noble Agar (Difco). On Ionagar No. 2 (Consolidated Laboratories), Hb M$_{\text{ch}}$ and Hb A$_2$ separate.
additional inflection at 610 (fig. 5a). The ferricyanide treated hemolysate at pH 6.5 showed a complex pattern having bands around 495, 580 μ and inflections around 539, 565, 610 and 628 μ (fig. 5b), suggesting a mixture of normal and abnormal ferrihemoglobin.

Spectroscopy of the isolated ferrihemoglobin fraction in phosphate buffer (pH 6.5) revealed a pattern distinct from previously described patterns of Hb M variants. The absorption band maxima were around 580 and 490 with inflections at 540 and 610 μ (fig. 6a). The isolated A fraction from the Mk containing hemolysate was spectroscopically normal.

Eluates of the abnormal slow fraction from electrophoregrams (TEB-buffer, pH 8.9) of the unoxidized hemolysates were examined spectroscopically at pH 8.0. The pattern was that of normal oxyhemoglobin with an additional inflection around 615 (fig. 7a). Following treatment of this eluate with potassium ferricyanide the spectroscopic pattern was identical with that of the isolated ferrihemoglobin fraction (fig. 7b).

The conversion rate of ferrihemoglobin to cyanferrihemoglobin, as determined by the quantitative technic of Betke, was normal, in contrast to Hb Meh which showed slow reactivity under the conditions of the test. The resultant spectroscopic curve showed the single peak centered around 540 μ. However, the shape of the curve differed from the normal since there was some broadening of the peak and a slight asymmetry (figs. 6b and 7c).

The hybridization studies suggest that the structural abnormality of hemoglobin M is in the α chain. For the purpose of this determination Hb M as well as Hb A and Hb S were hybridized with canine hemoglobin. Canine hemoglobin has similar E.P. mobility as Hb S. The four end products of the hybridization of Hb A (α2Aβ2A) with canine hemoglobin (α2canβ2can) were demonstrated by E.P. on agar gel (barbital buffer, pH 8.8) (fig. 8a). The two hybrids α2Aβ2can and α2canβ2A were present in addition to the two original hemoglobins. Because the human α chain has a higher positive charge than the β chain, it was likely that the slow moving hybrid was α2Aβ2can. This assumption was strengthened by the fact that this hybrid was in the same position as the slow hybridization product of the mixture of canine hemoglobin (α2canβ2can) with hemoglobin S, a known β chain abnormality (α2canβ2S). On the other hand, the hybrid α2Sβ2S had a slower mobility than the hybrid α2canβ2A (fig. 8b). The mobility of the new hybridization products of canine hemoglobin and hemoglobin M was the same as those between canine and normal hemoglobin (fig. 8c). However, the hybrid α2Mβ2can had a distinct grey-greenish color. After elution and oxidation with potassium ferricyanide, the hybrid α2Mβ2can had spectral characteristics typical of hemoglobin M, while the spectra of the other hybrid α2canβ2A resembled normal methemoglobin (fig. 9).

**DISCUSSION**

The reports on variants of hemoglobin M are steadily increasing. At this time at least seven variants have been described which differ from one another by the spectroscopic pattern and other physical-chemical criteria (table 2).

The deviations from the normal spectroscopic pattern of hemoglobin are
Fig. 2.—Agar gel electrophoresis, barbital buffer, pH 8.8. a. Hb A: near the cathode is a non-hemoglobin fraction; the other fractions are $A_2$ and the main component $A_1$. b. Hb $M_K$: similar to a. c. Hb $M_{ch}$: $M_{ch}$ is on the cathodic side of A. d. Hb $M_{Me}$: similar to a and b.

caulsed by an abnormal heme-globin relationship in one of the two polypeptide double chains, while the electrophoretic abnormalities, as in the case of the other hemoglobinopathies, indicate substitutions of amino acids by others with a different electrical charge.

The abnormal electrophoretic mobility of Hb M is best demonstrated—as Gerald has first shown with Hb $M_{Boston}$—with the oxidized hemolysate. The charge difference between the ferri-form of the Hb $M_B$ and Hb A is sufficiently large at neutral pH to permit good separation by starch block electrophoresis. On the other hand, when E.P. is performed in agar gel with TEB buffer, Hb $M_{Chicago}^{13}$ and Hb $M_{Kankakee}^{12}$ have previously been shown to be separable from Hb A in the unoxidized form. The same applies to Hb $M_{Kankakee}$. Agar gel also has the advantage of easy preparation, and the elution by freezing and thawing is simple.

Complete elution, however, from agar gel is rarely possible and, therefore,
Fig. 3.—Agar gel electrophoresis, TEB buffer, pH 8.9. Similar to fig. 2 except (b) MK is between A2 and A1.

it cannot replace starch when quantification of the fractions is required. In the present study the relative amounts of hemoglobin MK have been determined by starch block electrophoresis of completely oxidized hemolysates. Quantification of the abnormal fraction by determination of the ratio of absorbancies at different wavelengths8,10,13 necessitates the assumption that the spectral constants of the normal and abnormal hemoglobins are identical and that the relationship of the absorbancy ratios to the concentration of the abnormal hemoglobin fraction is linear. These assumptions are not justified and, therefore, such calculated values are highly approximate.

The rate of the formation of cyanferrihemoglobin from ferrihemoglobin following exposure to cyanide has become an important differential characteristic of the various mutants of hemoglobin M. Betke18 has recommended that this reactivity of the hemoglobin M's be measured at the wavelength of 600 mλ since this is the isosbestic point for normal ferri- and cyanferrihemoglobin and, therefore, the absorbancy changes at this wavelength can be solely attributed to the cyanide reactivity of the M fraction. This appears to apply
Fig. 4.—Agar gel electrophoresis of ferricyanide oxidized hemolysate in phosphate buffer, pH 7.1.  

to all known M mutants, but the cyanide reactivity of hemoglobin M* cannot be measured at this wavelength since here, as in the case of hemoglobin A, ferri- and cyanferrihemoglobin M* have the same absorbancy. If measured at 585 m*, the reaction is prompt and complete, but the spectrum of cyanferrihemoglobin M* is not completely normal (figs. 6b and 7c). Thus hemoglobin M* differs from the other described variants of hemoglobin M: Hemoglobin M* has 8* reacts slowly and incompletely; hemoglobin M* has 13,19 and hemoglobin M* has 11,19 slowly, but completely; and hemoglobin M* and M* both β chain abnormalities, do not differ from Hb A.

The molecular abnormalities of the various hemoglobins M have recently been explored by Gerald and Efron14 by “fingerprinting” and in some cases by the determination of the amino acid sequence in the abnormal peptide. Hemoglobin M* has an abnormality in the α chain: Tyrosine has replaced histidine in position 58. Hb M* also has a substitution of tyrosine for histidine, but in the β chain. In hemoglobin M*, glutamic acid has replaced valine in position 67 of the β chain. Hemoglobin M* is probably a β chain abnormality, since it appears to be allelic with hemoglobin E. The amino acid alteration in Hb M* is in the α chain and again tyrosine is implicated.
Hybridization of hemoglobin MK with canine hemoglobin indicates that here also the α chains are abnormal. The hybrid consisting of the α chains of hemoglobin M and the β chains of canine hemoglobin had a greenish color on agar gel and spectroscopy of the eluate showed the abnormal pattern of Hb MK. The other hybrids were essentially normal.

The α chain abnormality has been confirmed by Gerald who recently found that peptide 3 of the tryptic digest of Hb ME is abnormal and there probably are other deviations from the normal pattern. These studies will be the subject of a separate publication. Hb MK resembles Hb Mivato with regard to this abnormality of peptide 3, but the electrophoretic migration and the spectroscopic curves (table 2) are different. These hemoglobins, therefore, are not likely to be identical.

Additional suggestive evidence for an α chain abnormality is the fact that the four children with Hb MK were cyanotic at birth, similar to the child with Hb Mivato. It can be surmised, therefore, that the fetal hemoglobin also had an abnormal α chain. Cyanosis at birth would not be expected if the abnormality were in the β chain because of the absence of this chain in fetal hemoglobin α2γ2).

It is of great interest that the molecular basis of a rare genetic disorder like hemoglobin M disease is not identical in each case. Gerald and George have speculated that in this disorder an internal complex forms between the heme iron and a reactive side chain of an amino acid. Normally such a complex does not form because the normal amino acid sequence along the helical structure of the polypeptide chains precludes such an “internal complexing.”
An aminoacid substitution in close vicinity of the iron atom, especially the replacement of histidine by tyrosine with its phenolic group, apparently provides a favorable condition for the formation of such a complex. This complex form is also an unsuitable substrate for the normal enzyme systems of the erythrocyte concerned with the reduction of ferrihemoglobin. The heterogeneity of hemoglobin M points to the manifold possibilities of spot mutations along the polypeptide chain which may lead to the formation of such an internal complex of poorly reducible ferrihemoglobin.

The clinical features of congenital methemoglobinemia consist mainly in cyanosis which is not associated with respiratory or cardiovascular difficulties. An interesting phenomenon in the propositus of this study is the slight increase in erythrocytes. Because of the fact that only approximately 75 per cent of the hemoglobin is functional in oxygen transport, this is possibly a compensatory effect stimulated by the great physical demands of farm work. Secondary polycythemia has to our knowledge not been observed in other people with this disorder.
Fig. 7.—Absorption spectra of the M<sub>K</sub> fraction isolated from agar gel at pH 8.9. 
a. Untreated; b. oxidized; c. cyanferrihemoglobin.

The occurrence of hemoglobin M in the propositus appears to be the result of a new mutation, since, similar to other patients with hereditary met-hemoglobinemia,<sup>10,13</sup> there is no known history of cyanosis in preceding generations.

Homozygosity of hemoglobin M disease has never been observed to our knowledge. It might not be compatible with life because the presence of only two normal chains per hemoglobin molecule would probably interfere with normal oxygen transport because of altered heme-heme interaction. In the heterozygous patients the proportion of the abnormal hemoglobin M is usually 20–30 per cent. This fortunate fact of greater expressivity of the genes for the polypeptide chains of normal hemoglobin probably is the reason for the relatively innocuous character of this disorder.

**SUMMARY**

1. A new variant of hemoglobin M (hemoglobin M<sub>Kankakee</sub>) has been detected in a family in Illinois.
2. The spectroscopic and electrophoretic features of this new mutant are described.
3. Hybridization with canine hemoglobin indicates the presence of abnormal α chains.

**SUMMARIO IN INTERLINGUA**

1. Un nove variante de hemoglobina M (hemoglobina M<sub>Kankakee</sub>) esseva detegite in un familia in Illinois.
Table 2.—Summary of Findings of Various Hemoglobin M Variants

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Spectroscopic Maxima of Acid Ferrihb</th>
<th>Cyanide Reaction 5 (Beke\textsuperscript{1})</th>
<th>Cyanide Spectrum</th>
<th>Electrophoresis</th>
<th>Oxidized Hemolysate pH 7.1</th>
<th>Chain Abnormalities\textsuperscript{11,19}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb A</td>
<td>502, 632</td>
<td>abnormal</td>
<td>normal</td>
<td>as A</td>
<td>as M\textsubscript{B}</td>
<td>peptide 20\textalpha (tyrosine for histidine)</td>
</tr>
<tr>
<td>Hb M\textsubscript{Boston}</td>
<td>495, 602</td>
<td>abnormal</td>
<td>normal</td>
<td>?</td>
<td>slower than A</td>
<td></td>
</tr>
<tr>
<td>Hb M\textsubscript{Saskatoon}</td>
<td>492, 602</td>
<td>normal</td>
<td>normal</td>
<td>as M\textsubscript{B}</td>
<td>peptide 20\textbeta (tyrosine for histidine)</td>
<td></td>
</tr>
<tr>
<td>Hb M\textsubscript{Milwaukee 1}</td>
<td>500, 622</td>
<td>normal</td>
<td>normal</td>
<td>as A</td>
<td>as M\textsubscript{B}</td>
<td>peptide 6\beta (glutamic acid for valine)</td>
</tr>
<tr>
<td>Hb M\textsubscript{Melpic}</td>
<td>500, 602</td>
<td>abnormal</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Hb M\textsubscript{Chicago}</td>
<td>494, 598</td>
<td>abnormal</td>
<td>normal</td>
<td>between A and A\textsubscript{2}</td>
<td>as M\textsubscript{B}</td>
<td>a peptide 3\alpha (tyrosine for ?)</td>
</tr>
<tr>
<td>Hb M\textsubscript{Iowa}</td>
<td>490, 610</td>
<td>?</td>
<td>?</td>
<td>“anodic”\textsuperscript{12}</td>
<td>“anodic”\textsuperscript{12}</td>
<td>a peptide 3\alpha and 23\alpha (? )</td>
</tr>
<tr>
<td>Hb M\textsubscript{Renske}</td>
<td>490, 580 (infl.)</td>
<td>normal</td>
<td>abnormal</td>
<td>Slower than M\textsubscript{CB}</td>
<td>slower than M\textsubscript{B}, M\textsubscript{MP}, and M\textsubscript{CB}</td>
<td></td>
</tr>
</tbody>
</table>
HEMOGLOBIN M<sub>Kankalu</sub>

Fig. 8.—Hybrids of Hb A, S, M<sub>K</sub> with canine Hb. a.) Hb A<sub>1</sub>) α<sub>2</sub>Aβ<sub>2</sub>can. 2) α<sub>2</sub>can. β<sub>2</sub>can 3) α<sub>2</sub>α<sub>2</sub>A 4) α<sub>2</sub>α<sub>2</sub>can. b.) Hb S<sub>1</sub>) α<sub>2</sub>α<sub>2</sub>S 2) α<sub>2</sub>α<sub>2</sub>can. β<sub>2</sub>can 3) α<sub>2</sub>α<sub>2</sub>α<sub>2</sub>S 4) α<sub>2</sub>α<sub>2</sub>α<sub>2</sub>can. Fraction 2 and 3 have the same e.p. mobility c.) Hb M<sub>K</sub><sub>1</sub>) α<sub>2</sub>M<sub>2</sub>α<sub>2</sub>can. 2) α<sub>2</sub>α<sub>2</sub>M<sub>2</sub>can. 3) α<sub>2</sub>α<sub>2</sub>M<sub>2</sub>can. 4) α<sub>2</sub>α<sub>2</sub>α<sub>2</sub>M<sub>2</sub>can. Fraction 1 had a grey-green color. Fraction 3 for unknown reasons was always diminished.

2. Es descritbe le caracteristicas spectroscopic e electrophoretic de este nove mutante.

3. Hybridisation con hemoglobina canin indica le presentia de anormal catenas α.

ADDITIONAL

Just prior to receiving the galley proofs of this paper we became aware of several papers dealing with Hemoglobin M<sub>M<i>swat</i></sub> (Shibata, S., et al: Acta Haemat. Jap. 24:477, 1961; ibid. 24:486, 1961; Shibata, S.: Bull. Yamaguchi Med. Sch. 8:197, 1961) which describe the characteristics of Hemoglobin M<sub>M<i>swat</i></sub> in greater detail than ref. 12. Hemoglobin M<sub>M<i>swat</i></sub> is, indeed, very similar to Hemoglobin M<sub>Kankalu</sub>, but there appear to be differences of the spectroscopic pattern and of ligand reactivity. Therefore, the determination of the amino acid sequence in the abnormal peptides of the two variants will be of greatest interest.

ACKNOWLEDGMENTS

We are grateful to Mr. Lemuel Hall for technical assistance and to the Medical Illustration Service, VA West Side Hospital for the excellent illustrations. Dr. A. M. Josephson,
Michael Reese Hospital, Chicago, and Dr. A. V. Pisciotta, Marquette University School of Medicine, Milwaukee, Wis., generously supplied us with samples of Hb \text{M}_{\text{chicago}} and Hb \text{M}_{\text{Milwaukee}} respectively.

REFERENCES


HEMOGLOBIN MKankakee

Science 129:393, 1959.


Paul Heller, M.D., Associate Chief of Staff for Research, Veterans Administration West Side Hospital, and Associate Professor of Medicine, University of Illinois College of Medicine, Chicago, Ill.

Hyman G. Weinstein, M.S., Research Biochemist, Veterans Administration West Side Hospital, Chicago, Ill.

Vincent J. Yakulis, B.S., Research Immunologist, Veterans Administration West Side Hospital, Chicago, Ill.

Ira M. Rosenthal, M.D., Associate Professor of Pediatrics, University of Illinois College of Medicine, Chicago, Ill.
Hemoglobin $M_{K\text{ankakee}}$, A New Variant of Hemoglobin M

PAUL HELLER, HYMAN G. WEINSTEIN, VINCENT J. YAKULIS and IRA M. ROSENTHAL