spike, a miniature potential remains. This suggests that rhythmical spike activity is triggered by rhythmical local events in the dendrites or soma of this nerve cell (see *5*).

It seems to be of general interest that the time courses of activity in peripheral nerve cells and in central nervous tissue are similar after a change in temperature. This, together with the finding that the isolated nerve cell already is stabilized against changes of excitability by means of two interacting processes of opposite sign (see *6*), shows that there is a strong compensation for effects of temperature in cold-blooded animals (*7*).

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19 September 1958

## Second Spectroscopically Abnormal Methemoglobin Associated with Hereditary Cyanosis

*Abstract.* Isolation of an abnormal methemoglobin from two families exhibiting dominantly transmitted cyanosis have permitted the recognition of two different pigments of the hemoglobin M type. It is possible that the abnormal properties which characterize the acidic methemoglobin derivatives result from a crevice configuration of the heme, with two Fe-protein bonds.

In a previous article (*1*) an account was given of the electrophoretic isolation of both a normal and an abnormal hemoglobin from an affected member of a family exhibiting dominantly transmitted cyanosis. The abnormal component was designated hemoglobin M because the absorption spectrum of the acidic methemoglobin showed the same anomalous features as the oxidized whole hemolysates from the patients examined by Hörlein and Weber (*2*), by Kiese, Kurz, and Schneider (*3*) and by Heck and Wolf (*4*). A second family, living in Canada, with a comparable genetic transmission of cyanosis (*5*) has now been studied, and the presence of a similar spectroscopically abnormal methemoglobin has been demonstrated (*6*). However, upon isolation the latter methemoglobin was found to differ from that present in the first family in several fundamental respects. In the following brief account, the clinical, spectroscopic and chemical features characterizing and distinguishing these two abnormal hemoglobins will be given. To facilitate discussion, the pigment described previously (*1*) will be identified as hemoglobin M, Boston type (symbolized as Hgb $M_B$), and that described here for the first time will be identified as hemoglobin M, Saskatoon type (Hgb $M_S$).

Clinically, the presence of either variety of hemoglobin M is accompanied by cyanosis. With Hgb $M_B$, an increased level of methemoglobin could not be demonstrated by the method of Evelyn and Malloy (*7*). The cyanosis occurring with Hgb $M_S$, on the other hand, was associated with an increased amount of methemoglobin as determined by this technique (*8*).

Separation of the hemoglobin into two fractions was effected in both types of patients by starch block electrophoresis of the hemolysates after conversion into methemoglobin by treatment with potassium ferricyanide (Fig. 1). Optimum resolution occurred under the conditions of cathodic migration (sodium phosphate buffer, *p*H 7.0, ionic strength 0.1). Each electrophoretic band was distinctively colored: methemoglobin A was brown, $M_B$ was gray, and $M_S$ was green. The several pigments were recovered in pure form by elution after careful excision of each colored band from the starch block. The eluates were then examined spectroscopically by one of us (P. G.).

The absorption spectra of the acidic forms of methemoglobins $M_B$ and $M_S$ from 450 to 700 mμ are shown in Fig. 2 (*B* and *C*). In both, the 632 mμ peak of acidic methemoglobin A (see Fig. 2*A*) is absent, and both are characterized by a new peak at 602 mμ. The intensity of this new maximum relative to the common maximum near 500 mμ is appreciably greater for methemoglobin $M_S$ than for methemoglobin $M_B$, namely 0.72 compared with 0.61. Furthermore, in the spectrum of methemoglobin $M_S$ there is a poorly resolved band at about 540 mμ which is scarcely perceptible in methemoglobin $M_B$. This same band is a little more pronounced in methemoglobin A. In the Soret region the maxima for acidic methemoglobin $M_B$ and $M_S$ are at about 406 mμ (like that of methemoglobin A), but both have lower intensities. Relative to the intensity of the Soret band for the corresponding carbonmonoxyhemoglobins, the values are 0.92 for Hgb A, 0.76 for Hgb $M_S$ and 0.62 for Hgb $M_B$.

In the reactivity of their hemes there is an even more marked contrast: methemoglobin $M_S$ resembles methemoglobin A, whereas methemoglobin $M_B$ is in a class apart.

Methemoglobin $M_S$ reacts rapidly with the ligands $F^-$, $CN^-$ and $N_3^-$ to give the usual complexes. It is reduced very rapidly by $Na_2S_2O_4$ and is oxidized by $H_2O_2$ to the higher oxidation state. All these reactions proceed smoothly to completion, and in this respect methemoglobin $M_S$ is indistinguishable from methemoglobin A, although there may be differences between the rate constants which would become apparent in a detailed kinetic study. In addition, although acidic methemoglobin $M_S$ has its own characteristic spectrum, in solutions of *p*H > 9 an alkaline form predominates

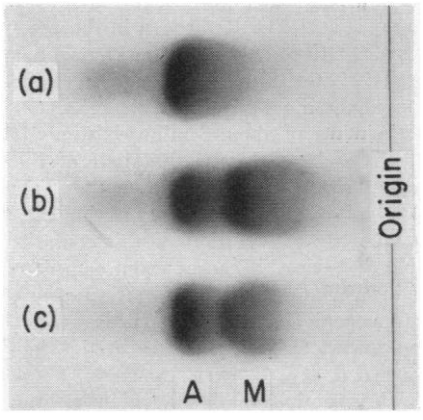


Fig. 1. Starch block electrophoresis of oxidized hemolysates (that is, the methemoglobins) at *p*H 7.0: *a*, normal blood; *b*, Hgb $M_S$ trait; *c*, Hgb $M_B$ trait. Migration is toward the cathode.

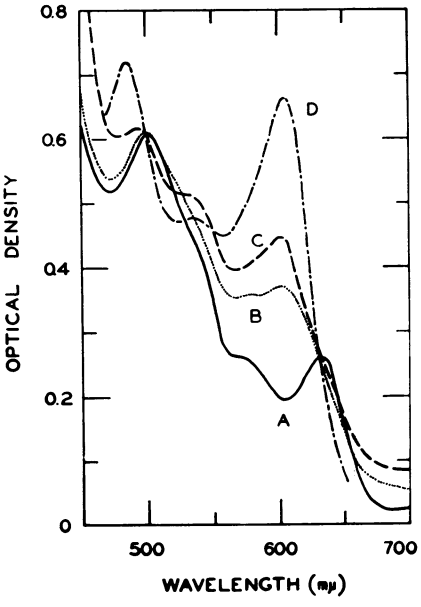


Fig. 2. Absorption Spectra at *p*H 7.0: *A*, methemoglobin A; *B*, methemoglobin $M_B$; *C*, methemoglobin $M_S$; *D*, methemoglobin A fluoride complex. For purposes of comparison all the optical densities have been made equal to 0.61 at 500 mμ.

with a spectrum very similar to that of alkaline methemoglobin A.

On the other hand, spectrophotometric measurements in the visible and Soret regions show that only a fraction of the hemes in methemoglobin $M_B$ give an alkaline form or react rapidly with $F^-$, $CN^-$, and $N_3^-$ to give the normal complexes, even though the same concentrations are used that give rapid and complete formation with methemoglobins $M_S$ and A (*9*). Treatment with $H_2O_2$ gives about the same fraction of the higher oxidation state. Likewise, on addition of excess $Na_2S_2O_4$, about the same fraction undergoes rapid reduction. The remaining fraction is then reduced very slowly at *p*H 7, and a little faster at *p*H 10 with a half-reaction time of about 20 minutes at 25°C. Reduction in the presence of CO follows a similar course, but at *p*H 7 the remaining fraction reacts somewhat more rapidly with a half-reaction time of about 3 minutes. Analysis of these data suggests that half of the hemes react rapidly and the other half slowly, if at all. Partial denaturation cannot be the explanation of this remarkable behavior, because upon complete reduction at *p*H 10 the Soret spectrum has a single band with its peak at 430 mμ, characteristic of a true native hemoglobin.

The reactivity and absorption spectra of the hemolysates studied by Hörlein and Weber (*2*) suggest that their methemoglobin M component resembles methemoglobin $M_S$. With the exception of the spectrum in alkaline solution (*4*, Fig. 3*b*), the same inference would appear to hold for the single case described both by Kiese, Kurz, and Schneider (*3*) and by Heck and Wolf (*4*). However, until data on the isolated components are available no definite conclusion can be drawn, especially since other types of methemoglobin M may exist, different from both methemoglobin $M_S$ and methemoglobin $M_B$ (*9a*).

Several of the abnormal features can be accounted for by the hypothesis that in acidic methemoglobin M some of the hemes are bound in a crevice so that a group from the protein occupies the sixth coordination position of the iron instead of a water molecule, which is generally accepted for the structure of acidic methemoglobin A. This would explain why only the spectrum of the acidic form is different, since the atoms directly bonded to the iron would be the same in all other derivatives. For example, if $L^-$ is the ligand and $Fe^+$ the ferriprotoporphyrin iron atom, complex formation and reduction of methemoglobin A would occur:

$$\text{Globin-Fe}^+(H_2O) + L^- \leftrightarrows \text{Globin-FeL} + H_2O$$

$$\text{Globin-Fe}^+(H_2O) + e^- \leftrightarrows \text{Globin-Fe}(H_2O)$$

whereas, if a group Y, which may be neutral or negatively charged, is bonded to the iron originally, the reactions would proceed:

$$\text{Globin-Fe}^+ - Y + L^- \leftrightarrows Y\text{-Globin-FeL}$$

$$\text{Globin-Fe}^+ - Y + e^- + H_2O \leftrightarrows Y\text{-Globin-Fe}(H_2O)$$

According to this hypothesis some similarity might be expected between the spectra of the acidic forms of methemoglobins $M_B$ and $M_S$ and one of the typical complexes of methemoglobin A. It is therefore interesting to note that the band maxima for methemoglobin A fluoride, which are characteristic of a class of complexes with high magnetic susceptibilities, occur at almost identical wavelengths (see Fig. 2*B*, *C*, and *D*).

But even if the participation of crevice bonding is accepted in principle, the contrast between methemoglobins $M_B$ and $M_S$ is an indication of other fundamental structural differences. In methemoglobin $M_B$ the fraction of the hemes that react rapidly may be bound normally as in methemoglobin A, while the fraction that reacts very slowly may be bound in a crevice deep within the polypeptide chains. With methemoglobin $M_S$, the more abnormal spectrum suggests as one possibility that a greater fraction, if not all of the hemes, are bound in a crevice configuration: yet the rapidity with which they react would require the bonding to be far more labile.

The hypothesis that the heme is situated in a crevice in normal hemoglobin has also been widely discussed, and is supported by indications of steric hindrance in the formation of its isocyanide complexes (*10*), and by more recent physical studies employing nuclear magnetic resonance (*11*). Other evidence, however, which has been surveyed in a recent review (*12*), would suggest that any such crevice configuration in normal hemoglobin enfolds the heme to a far lesser extent than the crevice present in cytochrome *c*. Furthermore, apart from the possibility of linkage via the porphyrin side chains, there is no evidence that the heme is held by more than one Fe-protein bond. X-ray studies favor a structure of this kind for myoglobin (*13*). In contrast, as proposed above, the distinguishing feature of acidic methemoglobin M, especially with the type designated Hgb $M_B$, may be a crevice configuration with two Fe-protein bonds (*14*).

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9a. *Note added in proof*: Hemoglobin Ms has now been found in another family here; and a third, and probably a fourth, type of Hbg M has been discovered in other families. The former has the acidic methemoglobin band at 623 mμ, and its heme reactions are rapid. The latter, in its acidic methemoglobin form, resembles MetHgb $M_B$ spectroscopically, but differs in that the heme reactions are rapid.

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## Field Observations on Effects of Alaska Earthquake of 10 July 1958

*Abstract.* The Alaska earthquake of 10 July 1958 was caused by movement on the Fairweather fault amounting to at least 21½ feet horizontally and 3½ feet vertically. Effects of strong shaking were evident over a large area in southeastern Alaska. In Lituya Bay an enormous wave, possibly resulting from a rockslide, reached a maximum height of more than 1700 feet.

Late on the evening of 9 July 1958, local time, a major earthquake was felt at most of the principal communities in southeastern Alaska and in adjoining parts of British Columbia and Yukon Territory, Canada. The U.S. Coast and Geodetic Survey has made the following determinations: instrumental epicenter, at 58.6°N, 137.1°W [in the Fairweather Range of the Saint Elias Mountains, about 100 miles west of Juneau (*1*)]; origin time, $06^h15^m51^s$ Greenwich Civil Time, 10 July 1958 (*2*). Pasadena reports Richter magnitude $M = 8$; Gutenberg unified magnitude $m = 7½$ (*3*). On Khantaak Island near Yakutat, a