discuss this point. So far, we do not possess a sufficient number of facts about the α and β components to advance any definite hypothesis as to their position in the structure of procollagen—that is, to speak about the latter's structure.

We hope that the further study of procollagen will enable us to answer many questions concerning the structure and transformation of this protein (*18*).

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# Hemoglobin Standard

## This is the second and final report on a proposal to establish a certified standard for general use.


Division of Medical Sciences,
National Academy of Sciences–National Research Council


In 1953 the Hematology Study Section of the National Institutes of Health requested the Division of Medical Sciences of the National Academy of Sciences–National Research Council to explore the possibility of establishing a hemoglobin standard for general use throughout the country. In response to this request, the Division, in 1954, organized an Ad Hoc Panel for the Establishment of a Hemoglobin Standard under its Subcommittee on Blood and Related Problems.

George Cartwright (School of Medicine of the University of Utah) accepted the chairmanship of the panel. Its members were David L. Drabkin (University of Pennsylvania Graduate School of Medicine); William H. Crosby, Jr. (Walter Reed Army Institute of Research); George Brecher (National Institutes of Health); Wallace Brode (National Bureau of Standards); Israel Davidsohn (Mt. Sinai Hospital, Chicago), representing both the College of American Pathologists and the American Society of Clinical Pathologists; and A. H. Neufeld, representing the National Research Council of Canada. In 1956 John Gould succeeded Brode, and in May 1957, Bradley E. Copeland (New England Deaconess Hospital, Boston) and Donald Brown (Hackensack General Hospital) succeeded Davidsohn as representatives of the College of American Pathologists and the American Society of Clinical Pathologists, respectively. The panel was also fortunate in having the cooperation of E. J. King of the Postgraduate Medical School of London, who has given his full support to this undertaking.

### Routine Methods

Most routine methods of clinical hemoglobinometry depend upon the photometric measurement of a blood sample after quantitative conversion of the hemoglobin which it contains into one or another of its derivatives. For the standardization of such a procedure there is needed a color standard which, when measured in the photometer and cuvette in routine use, will establish the relation between instrument reading and concentration of the particular hemoglobin derivative. This relation can then be used in converting instrument readings for unknown samples of blood to concentration of hemoglobin by the use of Beer's law or by construction of a calibration curve.

The problem, however, is complicated by the fact that several methods of analysis involving conversion to different forms of hemoglobin are in common use. Each of these methods should have its own standard. Since a multiplicity of standards is neither desirable nor practicable, the panel decided, early in its deliberations, to select only one method of analysis, for which a suitable direct standard would be developed. It should, however, be pointed out that this procedure does not preclude the use of the standard for the indirect calibration of another method. All that need be done is to construct a calibration curve based on the measurement of a small series of normal blood samples by the standardized method and also by the method which the analyst prefers to adopt for routine use.

In the opinion of the panel, the most significant contribution which could be made to the refinement of clinical hemoglobinometry would be the widespread adoption of a single method of analysis. Failing this, the indirect calibration of other methods with the chosen method offers the only simple photometric means for the comparison of data. The procedure, however, is subject to error if the blood sample contains significant amounts of certain of the abnormal forms of hemoglobin. For example, methemoglobin and carbon monoxide-hemoglobin are


This article was prepared by the staff of the Division of Medical Sciences, National Academy of Sciences–National Research Council. R. Keith Cannan is chairman.

quantitatively convertible to cyanmethemoglobin but not to oxyhemoglobin.

The panel reviewed the several photometric methods in current use and came to the conclusion that the procedure involving the measurement of hemoglobin as cyanmethemoglobin was the most promising. It offered the following advantages:

1) A simple and accurate procedure has been devised by Drabkin (*1*) involving the addition of a single reagent to the sample of blood.

2) The method has been adopted by the U.S. Army after extensive field trials (*2*).

3) All forms of hemoglobin likely to occur in circulating blood, with the exception of sulfhemoglobin, are determined by the method.

4) The color is suitable for measurement in filter-type photometers as well as in narrow band spectrophotometers because its absorption band in the region of 540 mμ is broad and relatively flat.

5) The U.S. Army has had extensive experience in the use of solutions of cyanmethemoglobin as direct standards and has found these to be satisfactory (*2*). The U.S. Army standards have remained unchanged in optical density for extended periods when stored at refrigerator temperatures, provided bacterial contamination was avoided.

## Field Trials

On the basis of this evidence, the panel decided to develop a certified solution of cyanmethemoglobin as a standard and to promote an extensive field trial of its suitability (*3*). A preliminary report of its recommendations and plans appeared in a number of scientific and technical journals in 1955 (*4*). This report outlined recommendations of the panel, described arrangements for the preparation and distribution of certified standard solutions of cyanmethemoglobin, and invited cooperation in an extensive field trial of the use of the standards and of the recommended method of analysis.

More than a thousand laboratories volunteered to cooperate in the trial sponsored by the National Research Council. Distribution of the standards was made with the assistance of the College of American Pathologists, the National Association of Clinical Laboratories, the Walter Reed Army Institute of Research, and the National Research Council of Canada. The laboratories received not only descriptions of the procedures for the use of the standards to calibrate photometers but also directions for the routine determination of hemoglobin in the form of cyanmethemoglobin.

The results of the study were most gratifying. The need for, and the ready and grateful acceptance of, a simple method for the standardization of hemoglobinometers was apparent. The recommended method of analysis was likewise well received. At the onset of the field trial study, only 7 percent of the cooperating laboratories had been determining hemoglobin as cyanmethemoglobin. At the time of the last report, two-thirds of the cooperating laboratories were using this method.

In the first field trial, the standard solutions were prepared from crystalline hemoglobin by David Drabkin. Three solutions in carefully determined concentrations of approximately 60, 40, and 20 mg, respectively, of hemoglobin per 100 milliliters were distributed. The optical densities of the final solutions were independently confirmed, and a continuing control on stability was maintained in the laboratories of Brecher, Gould, Crosby, King, and Neufeld. Agreement having been reached on the optical density values, the hemoglobin concentrations of the standards in milligrams per 100 milliliters were computed from the optical densities; it was assumed that the extinction coefficient of cyanmethemoglobin per milligram atom of iron (55.85 mg) per liter is 11.5 and that the pigment contains 0.335 percent of iron.

Two problems were encountered during the course of the field trial study. The first of these was the growth of certain microorganisms observed in some samples in spite of the presence of cyanide. This made it necessary to prepare and to maintain the solutions under sterile conditions. The second problem was a change, unpredictable in degree and not reported by all checking laboratories, of 2 to 6 percent in the optical density six to nine months after distribution of the standard. Samples from each lot were found to have undergone varying degrees of change—mostly fading, which was compensated for in some samples by a comparable increase in turbidity. The standards prepared and distributed by the U.S. Army in its earlier field trial had remained unchanged in optical density for three years. Inasmuch as these solutions had been prepared directly from whole blood or from washed red cells, it was suspected that the manipulation involved in the preparation of the crystalline hemoglobin for the National Research Council standards might have reduced the stability of the pigment. Therefore, a new standard was prepared from washed cells. The new standard was further modified by increasing the concentration of cyanide, since some previous preparations with such higher concentrations had shown greater stability and since the growth of most organisms would be limited by the higher concentration of cyanide.

Since the percentage change in optical density of the first standards was greater with increasing dilution of the hemoglobin pigment, and since cyanmethemoglobin solutions follow Beer's law, the second standard was distributed in only the most concentrated (60 mg of hemoglobin per 100 milliliters) of the three dilutions.

The second group of standards, modified as outlined above, was distributed in July and August of 1956. Their stability was determined in the laboratories of Drabkin, Brecher, Gould, and Crosby. The stability was satisfactory for at least nine months from the time of preparation, no change of more than 2 percent in optical density being observed.

The members of the panel have concluded that solutions of cyanmethemoglobin, when prepared, calibrated, and handled properly, are acceptable as standards for hemoglobinometry. They recognize that such standards are not ideal in all respects. However, until better standards can be developed, they are of the opinion that the availability of this reagent will greatly simplify the calibration of hemoglobinometers and will greatly increase the accuracy of hemoglobinometry.

Finally, they encourage further independent investigation in the hope that an even better standard may be developed, particularly one with improved stability and more certain maintenance of sterility.

The National Research Council's supplies of the standard cyanmethemoglobin solution are now exhausted, and no further production is planned under the auspices of the National Academy–Research Council. However, standards are now available from several commercial sources. The NAS–NRC has recommended the establishment of a program of certification of commercially produced cyanmethemoglobin standards for determining conformance with the specifications it has established. In response to the need for the establishment of such a program, as defined by the NAS–NRC, the College of American Pathologists has arranged for certification through the fa-

cilities of the laboratory of the American Medical Association in Chicago. On the basis of data obtained through this laboratory, the College of American Pathologists will certify whether commercially produced standards which have been submitted comply with the specifications established by the NAS–NRC. All users are urged to insist that the cyanmethemoglobin standards they purchase commercially carry the certification label of the College (*5*).

Detailed instructions for the preparation of the standards have been published by Crosby (*6*). Producers of the standard or instrument manufacturers may obtain technical details on the adaptation to and use of the standard in the various hemoglobinometers by writing to the Division of Medical Sciences of the National Research Council.

### Final Recommendations

The final recommendations of the National Research Council Ad Hoc Panel on the Establishment of a Hemoglobin Standard are as follows:

1) That cyanmethemoglobin be adopted as a standard in clinical hemoglobinometry.

2) That the standard be characterized spectrophotometrically on the basis that the extinction coefficient of 1 milligram atom of iron ($c = 1$ mg atom of iron per liter, $d = 1$ cm) in the form of cyanmethemoglobin at a wavelength of 540 mμ is 11.5.

3) That 0.338 percent (weight per weight) be accepted as the iron content of hemoglobin (molecular weight of 16,-$5\overline{20}$ per gram atom of iron) in accordance with the recent recommendation of the Protein Commission of the International Union of Pure and Applied Chemistry, and that a factor of $1{,}65\overline{2}$ be used in calculating hemoglobin in milligrams per 100 milliliters from millimoles per liter.

4) That the standard be distributed as a single concentration of not less than 55 mg of cyanmethemoglobin per 100 milliliters.

5) That solutions be distributed in brown glass containers and in sterile condition.

6) That, for the present, solutions be used as standards for a period not to exceed nine months from the time of preparation. This dating period is based upon the results of the National Research Council field trial. As experience accumulates with commercially prepared samples, an extension of the dating period may well be found to be justifiable.

7) That the standard be prepared from either crystalline hemoglobin or washed erythrocytes.

8) That commercial producers of the standards submit representative specimens from each lot to the College of American Pathologists, Prudential Plaza, Chicago 1, Illinois, for certification (i) that the concentration of cyanmethemoglobin is within ± 2 percent of the value stated on the label; (ii) that the solution is substantially optically clear; and (iii) that it is microbiologically sterile (*7*).

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# News of Science

## Research Based on Sputniks I and II Reported by Soviets

On 19 May the Soviet Embassy in Washington released an article on "Soviet artificial earth satellites" that presents in popular form some of the results of the experiments that Russian scientists are conducting in connection with the first two sputniks. The article, which is based on material published in *Pravda* on 27 April, states that fuller accounts of these results will soon appear "as scientific papers in various journals." Following are excerpts from the article.

"*Radio and optical observations of the sputniks.* Since an analysis of the changes in a sputnik's orbit as regards time makes it possible to estimate the density of the upper layers of the atmosphere, studies of the movements of sputniks are of great significance. The elements of a sputnik's orbit can be determined by tracking it by radiotechnical and optical methods.

"The radiotechnical methods included radio direction-finding and observations of the Doppler effect during the reception of radio signals from the sputniks. The Doppler effect is a result of the fact that the frequency of signals received increases as the object on which the radio transmitter is installed draws nearer to the receiving point. The changes in the frequency depend on the speed at which the object draws nearer or moves away. In the case of a sputnik the speed at which it draws nearer to, or moves away from, the receiving station on the ground is so great that the Doppler effect can not only be observed on an ordinary radio set, but can also be used for registering the moment the sputnik passes at the distance closest from the point of observation and also for measuring the distance to the sputnik and its velocity.

"During radio observations of the signals of Sputniks I and II, the frequencies of the signals received were measured by special radio equipment, including a recording chronograph.

"To obtain greater accuracy of measurement observations were conducted of signals at frequencies of 40 megacycles per second, which are less subject to the influence of the ionosphere. The power