

Chernoff A I & Pettit N M, Jr. The amino acid composition of hemoglobin. III. A qualitative method for identifying abnormalities of the polypeptide chains of hemoglobin. *Blood* 24:750-6, 1964.
[Memorial Research Center, University of Tennessee, Knoxville, TN]

A reliable method for separating globin chains from samples of purified hemoglobin was described in this publication. Its major advantage over available techniques lay in the degree and nature of the separation that resulted and in its applicability to the large-scale preparation of specimens. [The SCJ⁹ indicates that this paper has been cited in over 130 publications since 1964.]

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Following the demonstration in the 1950s that hemoglobin variants exist in considerable number; that they could be identified by a variety of electrophoretic, chromatographic, and physical techniques; and that the precise abnormalities could often be identified by an analysis of enzymatic digests of the purified protein, a major effort was directed toward developing techniques that would allow investigators to determine with some degree of ease and certainty that a hemoglobin molecule was, in fact, different from the normal variety. The same methods could often be applied to the large-scale preparation of pure peptide chains in the quantities required for analysis by the techniques of the day.

My own work on the abnormal hemoglobins had expanded from clinical descriptions of the various hemoglobinopathies into efforts to generate clinically useful techniques for the laboratory diagnosis of these disorders. Thus, after developing the one-minute alkali denaturation test for fetal hemoglobin while working in Karl Singer's laboratory¹ and introducing several modifica-

tions of electrophoretic and chromatographic techniques,^{2,3} it was but a small step for me to initiate the amino acid structure phase of our research.

The available practical methods for isolating "pure" globin chains were largely limited to column and countercurrent methods. Working in a small laboratory, we were eager to have a simple method to use in the qualitative analysis of suspect hemoglobin specimens that would help identify the site of an amino acid substitution; electrophoresis seemed to offer such a capability. The literature contained descriptions of such separations at an acid pH using urea buffers, but the results were less than consistent or definitive. My assistant, Nelson Pettit (then a graduate student at the University of Tennessee, Knoxville), and I decided to try working at a basic pH, using the chain-dissociating capabilities of concentrated urea buffers to achieve our objectives. After considerable manipulation of the conditions of electrophoresis, we obtained sharp, precise separations, the alpha and non-alpha chains usually moving in opposite directions from the point of origin. The method was quick and reproducible, and the results were easy to interpret. A qualitative technique, which could also be scaled up to provide quantitative and preparatory capabilities, became available in laboratories that lacked much of the highly sophisticated machinery being used for these purposes.

While this technology worked well, carrying out the procedure was often far from simple. Preparing the concentrated urea buffers was messy, and the need to degas the mixture resulted in many an unexpected implosion of the Erlenmeyer flasks used in the procedure. Fortunately, other techniques⁴ came along to improve and simplify the procedure further and few use our method at present. Nonetheless, in its time it provided a significant advance in the identification of abnormal globin chains, their purification, and their ultimate isolation and analysis, which undoubtedly explains why investigators have had the occasion to refer to this article so frequently.

1. Singer K, Chernoff A I & Singer L. Studies on abnormal hemoglobins. I. Their demonstration in sickle cell anemia and other hematologic disorders by means of alkali denaturation. *Blood* 6:413-28, 1951. (Cited 1,160 times since 1955.)
2. Chernoff A I. The amino acid composition of hemoglobin. I. An improved method for separating the peptide chains of human hemoglobin. *J. Chromatography* 6:252-7, 1961.
3. Chernoff A I & Pettit N M. Some notes on the starch-gel electrophoresis of hemoglobins. *J. Lab. Clin. Med.* 63:290-6, 1964.
4. Huisman T H J & Ionxis J H P. *The hemoglobinopathies: techniques of identification.* New York: Dekker, 1977. 449 p. (Cited 320 times.)

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