CC/NUMBER 39
SEPTEMBER 29, 1980

This Week's Citation Classic

Huisman T H J & Dozy A M. Studies on the heterogeneity of hemoglobin. IX. The use of tris(hydroxymethyl)aminomcthane-HCl buffers in the anion-exchange chromatography of hemoglobins. *J. Chromatography* **19**:160-9, 1965. [Dept. Biochem., Medical College of Georgia, Augusta, GA]

A modified procedure for the separation of various hemoglobin types by anion exchange chromatography has been presented. DEAE-Sephadex, A-50 medium, was preferred over DEAE-cellulose as chromatographic medium. Complete separations of many hemoglobin fractions were obtained by applying a single pH gradient to the columns using TRIS-HCl buffers of reasonably high concentrations (0.05 M). The method was applicable both for analytical and preparative purposes. [The *SCI*® indicates that this paper has been cited over 460 times since 1965.]

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July 15,1980

"This paper was one in a long series devoted to the development of chromatographic procedures to quantitate and isolate variants of human and animal hemoglobins (Hb). My technical assistant, Andrée Dozy, played a key role in these studies. She worked with me on these and related problems for some ten years, and is presently a key member of the research team of Y. W. Kan in San Francisco, California.

"Numerous human Hb variants had been discovered, mainly after 1960, and a great need existed for a reliable procedure to isolate these proteins for further structural analyses. The DEAE-Sephadex procedure is ideally suited for this purpose; it is an easy method which provides a pure isolated hemoglobin variant. The method has been modified by various investigators. Dozy, Enno Kleihauer (presently professor of pediatrics at the University of Ulm, Germany), and I introduced the use of the beaded DEAE-Sephadex anion exchanger which gave sharp Hb zones.[1] Presently the method has been replaced in several laboratories (including my own) by one which uses DEAE-cellulose and glycine-KCN-NaCl developers and was developed by Edathara C. Abraham, Alice Reese, Mamie Stallings, and myself.[2] Over the years I had numerous discussions and conducted collaborative studies with my friend Walter Schroeder, who has worked at Caltech in Pasadena for over 30 years. Together we developed and modified techniques for Hb separations which are presently used all over the world. We summarized our cobined efforts in a book which just came off the press.[3]

"Anion exchange chromatography similar to the one described in this '*Citation Classic*' will continue to be important in the analyses of Hb and its variants. Important new developments are modifications allowing analyses of micro quantities of Hbs in mixtures (numerous examples are listed in ref. 3). In the very near future we will observe a major advance when these anion exchangers are used in high performance liquid chromatographic methods. It seems likely that the long lasting (2-4 days) Hb analyses of the mid-1960s will be replaced by highly automated analyses which will be completed in less than 2 hours. It is gratifying to know that Dozy, Kleihauer, and I were able to develop methodology which forms the basis for so many approaches in column chromatography of Hbs."

1. **Dozy A M, Kleihauer E F & Huisman T H J.** Studies on the heterogeneity of hemoglobin. XIII. Chromatography of various human and animal hemoglobin types on DEAE-Sephadex. *J. Chromatography* **32**:723-7, 1968.
2. **Abraham E C, Reese A, Stallings M & Huisman T H J.** Separation of human hemoglobins by DEAE- cellulose chromatography using glycine-KCN-NaCI developers. *Hemoglobin* **1**:27-44, 1976.
3. **Schroeder W A & Huisman T H J.** *Chromatography of hemoglobin.* New York: Marcel Dekker, 1980. 272 p.