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Rossi Fanelli A, Antonini E & Caputo A. Studies on the structure of hemoglobin. I. Physicochemical properties of human globin. *Biochim. Biophys. Acta* **30**:608-15, 1958.
[Inst. Biochemistry, Univ. Rome, and "Regina Elena" Inst. for Cancer Res., Rome, Italy]

A method for preparing globin from human hemoglobin is reported. The protein so obtained was submitted to different purity analyses, such as electrophoresis, ultracentrifugation, diffusion, and heat stability behavior. The data confirmed the high degree of purity of the protein. The most important physicochemical constants of human globin and its capacity for hematin were reported. [The *SCI*® indicates that this paper has been cited in over 450 publications since 1958.]

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One of the most fascinating problems in protein chemistry is represented by the correlations between structure and function in hemoglobin and myoglobin. I became interested in this area of research in 1938 when I was an assistant professor at the Institute of Biological Chemistry at the University of Naples, and I continued these investigations when I became a full professor at the University of Pavia.[1] However, my most exciting experiments were carried out at the Biochemistry Institute of the University of Rome. I started working there in 1949 and had put together a group of very talented pupils and collaborators who contributed new ideas and developments to this area of research.[2-4] Among them, I vividly remember E. Antonini for his scientific capabilities and ingenuity. He is a coauthor on this paper (together with A. Caputo).

During the late 1950s, while investigating the relationships between structure and function in hemoglobin, we realized that a very promising approach was represented by the study of the chemical, physicochemical, and functional properties of hemoglobin obtained from the recombination of heme with the apoprotein (called globin). We also realized that previous attempts had been unsuccessful because the biochemical method for preparing globin was incapable of yielding an apoprotein that was homogeneous and in its native state.

Performing a series of accurate physicochemical measurements, which included analytical ultracentrifugation, diffusion, and electrophoretic mobility over a wide pH range, we arrived at a method to dissociate the heme from human hemoglobin and to obtain a preparation of globin that, in the light of several controls, proved to be absolutely homogeneous, pure, and in the native configuration. Indeed, when this globin was exposed (under precise experimental conditions) to an equivalent amount of protoheme, we obtained reconstituted hemoglobin that, on the basis of its chemical, physicochemical, and functional properties, was indistinguishable from native hemoglobin.

An attempt to analyze the reasons why this paper has become a *Citation Classic* leads to the following considerations. 1) The paper described, for the first time, an original and simple procedure for preparing native and homogeneous human globin whose properties were accurately described employing sophisticated (for that time) physicochemical methods. 2) The biochemical preparation provided a method to reconstitute reproducibly *in vitro* native hemoglobin starting from the isolated components, i.e., protoheme and globin. This result represented a very clear example of the general principle whereby the stable quaternary assembly of a native protein was reached rapidly and spontaneously, as demanded by thermodynamics. 3) The paper opened a new era in the investigation of the structure-function relationships in hemoglobin because it allowed probing of the role of the chemical nature of the prosthetic group in controlling the phenomena of heme-heme interactions and the Bohr effect, which has become a prototype of molecular control in functioning macromolecules.

1. **Rossi Fanelli A.** Crystallization of human myoglobin: some physicochemical properties and chemical composition. *Science* **108**:15-16, 1948.
2. **Rossi Fanelli A & Antonini E.** The oxygen equilibrium of reconstituted hemoglobins. I. Reconstituted human protohemoglobin. *Arch. Biochem. Biophys.* **80**:299-307, 1959.
3. ----------. Dissociation of hematin from hemoproteins at neutral pH. *J. Biol. Chem.* **235**:PC4-5, 1960.
4. **Rossi Fanelli A, Antonini E & Caputo A.** Hemoglobin and myoglobin. *Advan. Prot. Chem.* **19**:73-222, 1964. (Cited 175 times.)