

This Week's Citation Classic

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Chen R F. Removal of fatty acids from serum albumin by charcoal treatment.
J. Biol. Chem. 242:173-81, 1967.
[Laboratory of Technical Development, National Heart Institute,
Bethesda, MD]

The paper described a new and simple method for removing fatty acids and other impurities from serum albumin; namely, treatment with activated charcoal at low pH. Physical tests showed that the method did not denature the protein. [The SCI® indicates that this paper has been cited over 1,070 times since 1967.]

Raymond F. Chen
National Heart, Lung, and
Blood Institute
National Institutes of Health
Bethesda, MD 20205

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"This work was a by-product of studies on protein fluorescence. In the early-1960s, as a result of work by Gregorio Weber,¹ there was great interest in fluorescence spectroscopy as a new tool for studying proteins. Unfortunately, most laboratories did not have the expertise to construct the requisite fluorescence instrumentation. To remedy this situation, the chief of my laboratory at the National Heart Institute (NHI), Robert L. Bowman, encouraged me to see how commercial instruments could be adapted to measure quantum yields, corrected spectra, polarization, and lifetimes. Eventually, I was able to show how these data could be obtained with available instruments, thus contributing to making fluorescence a generally accessible technique. Some measurements, such as quantum yields, required standards, and I was led to examine the suitability of many compounds. For protein quantum yields, I naively thought that the best standard would be another protein, and what better choice could there be than common serum albumin?

"However, I soon discovered that because of impurities, no two samples of

serum albumin had the same emission properties. It turns out that albumin binds nearly everything and is more stable when complexed with fatty acids, certain alcohols, or N-acetyltryptophan. These or similar additives are often deliberately incorporated into commercial samples. Impurity removal required prolonged solvent extraction or other tedious procedures.

"The idea of using charcoal to purify serum albumin probably came to me for two reasons. First, our storeroom at the NHI was chronically obstructed with an enormous amount of activated charcoal. Theodor Kolobow, in another section of Bowman's multifaceted laboratory, had been studying charcoal facilitation of metabolite transport across silicone rubber membranes used in artificial lungs and kidneys. In response to his request for a free sample, Atlas Chemical Industries had shipped him (to his horror) several hundred pounds of charcoal. Second, while working on my dissertation a few years earlier under Gerhard W.E. Plaut at the University of Utah, I was studying the stereochemistry of the reaction catalyzed by the DPN-linked isocitrate dehydrogenase of cardiac mitochondria. In order to see if tritium was transferred to the α - or β -side of DPN⁺, Plaut suggested isolating the tritiated DPNH by charcoal adsorption, a technique which I found worked despite the presence of protein. (Incidentally, the enzyme uses the α -side of DPNH.²)

"As perhaps the most studied protein, serum albumin serves as a model for other proteins and has great importance and interest in its own right. There are many clinical, commercial, and laboratory applications which require purified serum albumin. These factors contribute to the frequency of citation of my paper.

"Incidentally, serum albumin turns out not to be a particularly good fluorescence standard, because even after charcoal treatment, there are minor differences between samples³ for reasons which continue to be obscure."

1. **Weber G.** Rotational Brownian motion and polarization of the fluorescence of solutions. *Advan. Prot. Chem.* 8:415-59, 1953.
2. **Chen R F & Plaut G W E.** Studies on the hydrogen transfer mediated by the DPN-linked isocitric dehydrogenase of heart. *Biochemistry* 2:752-6, 1963.
3. **Chen R F & Koester V J.** Fluorescence properties of human serum albumin: effect of dialysis and charcoal treatment. *Anal. Biochem.* 105:348-53, 1980.