

This Week's Citation Classic

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Allen W M. A simple method for analyzing complicated absorption curves, of use in the colorimetric determination of urinary steroids. *J. Clin. Endocrinol. Metab.* 10:71-83, 1950.
[Dept. Obstet. & Gynecol., Washington Univ. Sch. Med., St. Louis, MO]

This paper gives the rigorous mathematical proof for a simple formula which makes possible the analysis of contaminated absorption curves in both the visible and ultraviolet regions of the spectrum. The only requisite is that the 'background' absorption be linear in the region analyzed. [The SC® indicates that this paper has been cited over 485 times since 1961.]

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"My first exposure to absorption spectra came in 1933 when Oskar Wintersteiner reported to me that the pure corpus luteum hormone which I had isolated had an absorption maximum at 240 mu. This fact, together with other analytic data he had obtained, virtually established the structural formula of progesterone.

"My next exposure came in 1941 when I was using a Coleman photoelectric spectrophotometer to measure estriol in extracts of human pregnancy urine. This instrument gave excellent absorption spectra of the colors produced by the Kober Reagent (mostly concentrated sulfuric acid). Good results were obtained when the estriol content was high, but abnormally high results were always obtained when the estriol content was low. By 1942, I was fully aware of the problem. I had looked at hundreds of absorption curves and had seen the 'estriol hump' sitting, as it were, on a high baseline due to contaminating brown colors.

"These background colors plagued everyone doing steroid analyses for years and no one, including me, was able to achieve a perfect blank so that the colorimeter would automatically subtract the background color. Consequently, I turned to mathematics. Sometime during 1942 I succumbed to the important conclusion that a mathematical solution was possible if the slight curve of the absorption spectrum of the background was considered to be a straight line.

"By 1943, I had a workable formula which used the optical densities at 420-450-480 mu, and two constants which were derived from the separate absorption spectra of pure estriol and estriol-free 'background.' Apparently, I was not satisfied with this one because I soon developed a closely related formula which was devoid of arbitrary constants. Actually, I think this new one came from the top of my head without mathematical proof.

"In 1947, after we had discovered the Allen-Blue Test for adrenal tumors I decided to develop the mathematical proof for this formula which we had used for about three years. This proved to be an ordeal in logic and analytical geometry which consumed me for several months. Success finally came when I saw that the addition of a simple expression to both sides of the equation would break the impasse. Only then did my cherished formula emerge.

"This formula soon became known as 'The Allen Correction' in steroid laboratories around the world. The beauty of the formula lies in its simplicity. Nothing I have ever done has given me more pleasure and satisfaction."