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This Week's Citation Classic

Zak B, Dickenman R C, White E G, Burnett H & Cherney P J. Rapid estimation of free and total cholesterol. *Amer. J. Clin. Pathol.* **24**:1307-15, 1954. [Depts. Pathology, Wayne Univ. College of Medicine, Detroit Receiving Hosp., and Detroit Memorial Hosp., Detroit, MI]

The paper describes the determination of extracted total cholesterol and digitonide precipitated free cholesterol by ferric iron reaction in a glacial acetic-sulfuric acid milieu and discusses the several variables affecting the equilibrium reaction. [The *SCI*® indicates that this paper has been cited over 415 times since 1961.]

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"This paper came into being because a previously described direct reaction for serum cholesterol,[1] also a *Citation Classic*, resulted in interference problems with Hopkins-Cole reactants owing to glyoxal impurities in the acetic acid and tryptophane in the sample as the indicted offending compounds. In the original work of 1953, care was taken to inform readers that a high purity solvent was required, but some analysts ignored or overlooked the warning and unfortunately encountered the side reaction which resulted in overlapping spectral interference. A partial cleanup by means of simple extraction along with digitonide purification lengthened the procedure as an analytical modification. However, this then newer technique ensured that the results obtained would be more in harmony with true values. Later studies by others[2] on the nature of the milieu in which the reaction occurs proved that interferences could at least be partially or mostly eliminated by means of this analytical consideration. Subsequently, I have had the opportunity to emphasize the importance of the reaction medium more fully.[3] Several observations on the effect of the medium on the reaction as an optimization phenomenon were investigated and discussed here.

"Even though this procedure was more involved technically than the totally direct approach, it was still simple enough to popularize it somewhat over the extant use of techniques at least as complicated, ending up with variations of the much less sensitive and stable Liebermann-Burchard reaction as the concluding step of those procedures.

"Perhaps the most important realization that I gleaned from the application of an acceptable new reaction in the face of entrenched technology is the understanding that the older way is not necessarily the only way, and that when given the seed of an idea, curious investigators will modify and improve what appears in an attempt to hone it to perfection, while more innovative investigators, dissatisfied now with both the old as well as the new analytical devices, will visualize still newer reactions because such new reactions now seem to be a real possibility. The over-powering influence of a totally accepted equilibrium reaction which virtually everyone uses, partly from the belief that nothing else is available or partly from the belief that nothing else could be available, sometimes seems to program a slowing of the progression of inventiveness. In addition, total acceptance by most of the workers in an area even seems to cause resentment about what appears to them as a drastic change, and this in turn may impede acceptance of what might be a useful replacement of older technology, at least for the moment."

1. **Zlatkis A, Zak B & Boyle A J.** A new method for the direct determination of serum cholesterol.
J Lab. Clin. Med. **41**:468-92. 1953.
[Citation Classic. *Current Contents/Life Sciences* (I2):2O, 23 March 1981.]
2. **Wyrbenga D R. Plleggi V J, Dirstime P H & DiGioigio J.** Direct manual determination of serum total cholesterol with a single stable reagent. *Clin. Chem.* **16**:980-4. 1970.
3. **Zak B.** Cholesterol methodologies: a review. *Clin. Chem.* **23**:1201-14. 1977.