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Citation Classics

Trout D L, Estes E H Jr. & Friedberg S J. Titration of free fatty acids of plasma: a study of current methods and a new modification. *Journal of Lipid Research* **1**:199-202, 1960.

This article identifies substances that interfere with Dole's titrimetric method for measuring plasma free fatty acids, and adds a step to remove them from the fatty acid extract. [The *SCI*® indicates that this paper was cited 922 times in the period 1961-1975.]

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"Our widely used method of measuring free fatty acids (FFA) in blood plasma was devised, tested and written up during my first 18 months after completing graduate work at Duke University. My dissertation had been in pharmacology, chiefly on the interaction between lysergic acid diethylamide (LSD) and serotonin in Siamese fighting fish. I then started research in lipid metabolism at the Veterans Administration hospital nearby.

"My boss was E. Harvey Estes, a clinician who also taught at Duke University (Medicine). He had written extensively on cardiology and was greatly interested in the possibility that plasma FFA played a role in atherosclerosis. My main cohort was Samuel J. Friedberg, who was also doing research during his residency training.

"Interest in plasma FFA was new when we started working on FFA methodology in 1958. Previously, biochemists thought plasma FFA were largely artifacts due to the lipolytic activity in blood. Studies in 1956 showed that plasma FFA exist in vivo in widely varying concentrations. It was soon clear that plasma FFA are largely derived from adipose tissues, released into the blood at rapid, highly controlled rates, and promptly extracted by various tissues. FFA proved to be a major transport form of lipid.

"Unfortunately, early methods of determining plasma FFA were either inaccurate or extremely laborious. Dr. V.P. Dole's method,[1] however, looked promising. FFA were extracted by a solvent mixture. When water and hydrocarbon were added, the system formed two phases. An aliquot of the relatively non-polar upper layer, which contained most of the FFA, was finally titrated. The method was admirably direct and simple. However, we observed that the upper layer also contained a fraction of the lactic acid present and some titratable phospholipid. We then found that, by shaking the FFA extract with very dilute sulfuric acid and centrifuging, the interfering substances were satisfactorily removed. This constituted our modification of the Dole procedure. Henry Kamin, who had taught me biochemistry, urged us to determine FFA values on the same blood plasmas by several methods. The validity of our modification was supported by our finding that it gave lower values than the original Dole method and values similar to those obtained by R.S. Gordon's standard method.

"Our paper was rejected as being too long by a first journal but was published promptly by the *Journal of Lipid Research*. Within 6 months, Dole and Hans Meinertz published an alternate method of washing out interfering substances from the Dole extract of plasma FFA.[2] Subsequently, colorimetric and automated procedures for FFA analysis have been developed, and some of these are far more sensitive than Dole's or our procedure. At present, the chief problem in determining the plasma FFA in a person or animal is to collect the blood without first stimulating lipolysis in adipocytes through physical or mental stress.

"Many scientists still use our FFA procedure, perhaps largely out of habit. The fact that we modified, in a simple way, a fairly satisfactory and highly ingenious method, does not detract from the usefulness of the resulting procedure. Of course, we were extremely lucky that the Dole-Meinertz paper or a similar one from another laboratory did not reach print ahead of ours."

1. **Dole V P.** A relation between nonesterified fatty acids in plasma and the metabolism of glucose. *Journal of Clinical Investigation* **35**:150-4, 1956.
2. **Dole V P & Meinertz H.** Microdetermination of long-chain fatty acids in plasma and tissues. *Journal of Biological Chemistry* **235**:2595-9, 1960.