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CC/NUMBER 13
MARCH 31, 1986

Ho R J. Radiochemical assay of long-chain fatty acids using ^{63}Ni as tracer. *Anal. Biochem.* **36**:105-13, 1970.
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A simple and sensitive method is described for the quantitative determination of long-chain free fatty acid (FFA) as ^{63}Ni-FA complex. The sensitivity ranges from 1 to 40 nmoles/assay and its linearity can be maintained by increasing or decreasing the radiospecific activity of $^{63}Ni(NO_3)_2$ used. [The SCI® indicates that this paper has been cited in over 265 publications.]

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December 2, 1985

Long-chain free fatty acid (FFA) is one of the most important contents of blood. Turnover of this component is rapid and both regulated and influenced by nutritional, hormonal, physiological, and pathological states such as exercise, obesity, starvation, diabetes, and atherosclerosis. It is not surprising, therefore, that there has been and still is great interest in methods of measurement of FFA.

We were all familiar with the titration method of Dole,[1] which was tedious and very subjective in its endpoint. The performer had to rest every 20 minutes to refresh his or her eyes. A colorimetric method was later introduced by Duncombe.[2] It was based on FFA reactivity with Cu^{++} and this salt's distinct solubility in chloroform. CU^{++} content in $CHCl_3$ became a measure of FFA; unfortunately it was heavier than water, and sampling of the $CHCl_3$ layer was technically difficult. The aqueous layer can be made heavier than $CHCl_3$, but vaporization of chloroform was still a problem for reproducibility. A radiochemical assay at this point appeared to be a reasonable alternative. FFA salts of Co and Ni all have solubilities in $CHCl_3$ similar to that of Cu. Both ^{60}Co (and also ^{57}Co) and ^{63}Ni have been introduced as tracers for this purpose, which takes advantage of their workable, long half-lives. This work was carried out at the time that I was in the laboratories of Raymond H.C. Meng and Earl W. Sutherland in the department of Rollo Park, Vanderbilt University.

My memories of research concerning FFA are beautiful. It is my thread of connection to my colleagues, friends, and professional acquaintances. Looking back during the 15 years before 1970, we are all appreciative of the availability of Dole's method and the attempted improvements made by others. I am happy to have been able to develop the ^{63}Ni-method as a simple and sensitive choice.

In the 15 years following the publication of the ^{63}Ni-method, investigators have not only cited it as a method for FFA determination, but also have extended its application in measurement of phospholipids,[3] triacylglycerol,[4] and lipase reactions.[4] In conjunction with determinations of other metabolites in relation to FFA, the radioenzymic methods for glycerol,[5] glucose, and triacylglycerol were developed by C.C. Fan and three other graduate students (L.A. Barrera, J.W. Brown, and J.A. Ruiz) in my laboratory in the department of William J. Whelan, after we moved to the University of Miami. They determined the content of glucose, glycerol, and FFA in a single sample of mouse serum obtained from its tail.[6] Furthermore, a time course for such a study was performed with a single mouse.

The simplicity, sensitivity, reproducibility, and adaptability of this method to determine esterified fatty acids in addition to the free form are the reasons behind its continued usefulness in research.

1. **Dole V P.** A relation between non-esterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35**:150-4, 1956. (Cited 3,765 times.)
2. **Duncombe W G.** The colorimetric micro-determination of long-chain fatty acids. *Biochemical J.* **88**:7-10, 1963. [See also: **Duncombe W G.** Citation Classic. *Current Contents/Life Sciences* **23**(36):16, 8 September 1980.]
3. **DePierre J W.** Extraction and assay of small quantities of free fatty acids produced by hydrolysis of microsomal membranes. *Anal. Biochem.* **83**:82-90, 1978.
4. **Ho R J, Ruiz J A & Fan C C.** New sensitive assay for lipases and esterified long-chain fatty acids. (Abstract) *Fed. Proc.* **37**:445, 1978.
5. **Barrera L A & Ho R J.** Radioenzymic assay of glycerol using ion-exchange column chromatography. *Biochem. Biophys. Res. Commun.* **80**:750-8, 1978.
6. **Fan C C, Barrera L A, Brown J W & Ho R J.** Three radiochemical methods for nanomoly quantity of serum glucose, glycerol and long-chain free fatty acids from surviving mice. (Abstract) *The Endocrine Society program and abstracts. 60th Annual Meeting*, June 14-16, 1977, Miami, Florida. Bethesda, MD: Endocrine Society, 1978. p. 359.

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