notes on methodology

Measurement of arachidonic acid in the plasma by gas–liquid chromatography–flame ionization using dihomo-γ-linolenic acid as an internal standard

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Summary A gas–liquid chromatography–flame ionization method is described for measuring arachidonic acid in plasma using dihomo-γ-linolenic acid as an internal standard. We found this technique to be reproducible, and quicker and superior to previously described techniques because of the similar physico-chemical properties of the unsaturated fatty acid internal standard and arachidonic acid. In addition, we observed that the use of the saturated fatty acid, *n*-tricosanoic acid, was unsatisfactory as an internal standard because of its poor extractability from plasma as compared to arachidonic acid.—**Gerber, J. G., J. S. Barnes, and A. S. Nies.** Measurement of arachidonic acid in the plasma by gas–liquid chromatography–flame ionization using dihomo-γ-linolenic acid as an internal standard. *J. Lipid Res.* 1979. **20:** 912–914.

Supplementary key words *n*-tricosanoic acid

Arachidonic acid is the fatty acid precursor for the prostaglandins of the "2" series. Since the liberation of arachidonic acid is reported to be the rate-limiting step in the production of prostaglandins (1), an increase in plasma level of arachidonic acid could represent an overall stimulation of prostaglandin production (2). Accurate measurement of prostaglandins in the plasma is not feasible with the present methodologies; consequently, measurement of arachidonic acid in the plasma across an organ may give an accurate assessment of overall activity of prostaglandin synthesis in that organ.

The most common way to measure arachidonic acid in the plasma is by gas–liquid chromatography–flame ionization (GLC–FI) after a lipid extraction step involving protein denaturation. Since there is an extraction step, the usual procedure is to add an internal standard that has physico-chemical properties sufficiently similar to the compound of interest so that both would behave the same during the work-up procedure. Past researchers have used saturated fatty acids of different carbon chain lengths as internal standards for the measurement of arachidonic acid (3–6).

We initially attempted to measure arachidonic acid in the plasma using *n*-tricosanoic acid (23:0) as an internal standard but found it unsatisfactory. When added to the plasma, the saturated fatty acid was poorly extracted compared to the arachidonic acid (**Fig. 1**). If the internal standard was added after the extraction step, we had no assurance that the recovery of arachidonic acid was quantitative in all the samples measured; in fact, using the extraction solvent described in the procedure, we found that arachidonic acid extraction from plasma varied from 65 to 85%.

We have therefore developed a sensitive and simple assay for arachidonic acid using dihomo-γ-linolenic acid (20:3) as the internal standard. Although this fatty acid does occur normally in the plasma in low nanogram quantities, by adding microgram quantities of dihomo-γ-linolenic acid to plasma samples, we diluted out any effect the endogenous fatty acid would have on our assay (**Fig. 2**). The advantage of using dihomo-γ-linolenic acid as the internal standard is that it is also an unsaturated fatty acid and consequently its extraction properties from the plasma are very similar to those of arachidonic acid (Fig. 1). Also, since the retention times on most GLC columns for these fatty acids are very close to one another, we do not have to worry about separating out the more abundant shorter chained saturated fatty acids.

PROCEDURE AND RESULTS

Dihomo-γ-linolenic acid (2.5 μg) (NuCheck, Elysian, MN, <99% purity) was added to 1 ml of plasma. A stock solution of dihomo-γ-linolenic acid dissolved in hexane and sealed under nitrogen was stored in the dark at −60°C. This unsaturated fatty acid is stable for at least a year under anaerobic conditions at −60°C. The plasma was then mixed with 20 ml of chloroform–*n*-heptane–methanol 56:42:2, vortexed for several minutes, and then centrifuged for 10 min at 300 *g*. The organic layer was evaporated to dryness under nitrogen after which the fatty acids were methylated using diazomethane at room temperature for 10 min. The sample was then reevaporated to dryness under nitrogen and dissolved in 10 μl of isooctane.

Abbreviations: GLC–FI, gas–liquid chromatography–flame ionization

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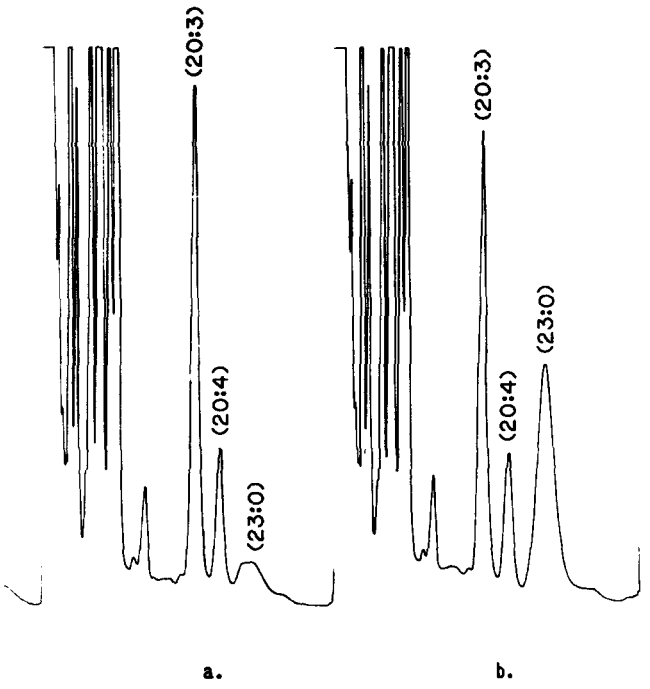

Fig. 1. Use of 23:0 internal standard yielded a very poor peak when added to the plasma (*a*) and a better peak when added to the organic extract of the plasma (*b*), indicating that a significant amount of this fatty acid was not extracted from the plasma. A similar situation did not occur with dihomo-γ-linolenic acid (20:3).

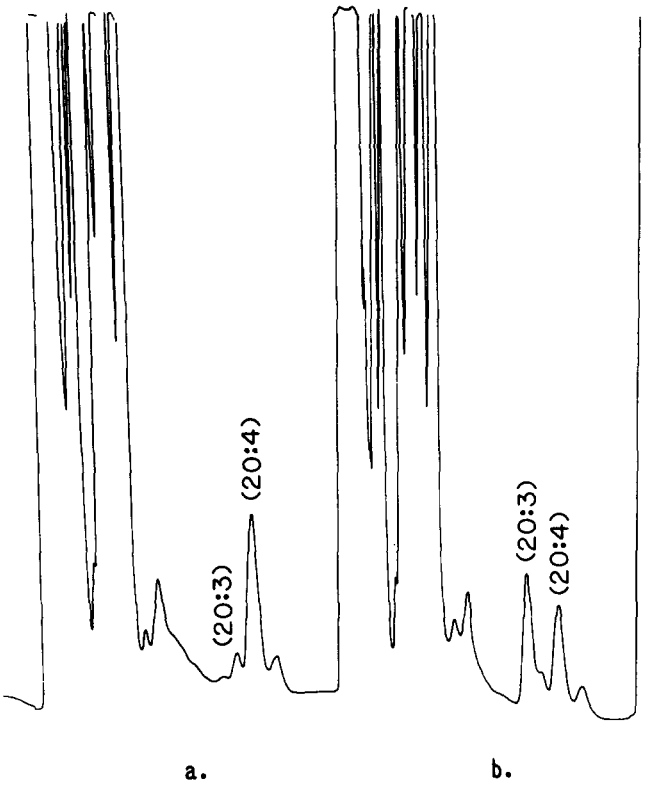

Fig. 2. Plasma without an internal standard gave an insignificant peak for dihomo-γ-linolenic acid (20:3) as compared to the arachidonic acid (20:4) peak (*a*). The same plasma with 2.5 μg of dihomo-γ-linolenic acid is shown in *b*. This figure illustrates that the added fatty acid is so much more abundant than the endogenous amount that its use as an internal standard is appropriate. Also, it can be seen that the separation of arachidonic acid from the more abundant, volatile saturated fatty acids is very good. The endogenous level of arachidonic acid in canine arterial blood is 1.7 μg/ml.

The arachidonic acid in the samples was quantitated by GLC–FI. Three μl of the extract was injected into the GLC apparatus through a silanized glass column (6 ft × 3 mm) of 10% SP 216 PS on Supelcoport 100/120 (Supelco Inc., Bellefont, PA) maintained isothermally at an oven temperature of 180°C. Helium at a flow rate of 30 ml/min was employed as a carrier gas. The retention time for the methyl ester of arachidonic acid was 6.5 min and the retention time for the methyl ester of dihomo-γ-linolenic acid was 5.6 min. There were no interfering substances with these retention times extracted from the plasma. A five-point standard curve was constructed using peak height ratios of dihomo-γ-linolenic acid to arachidonic acid with various concentrations of arachidonic acid. The concentration of arachidonic acid in the plasma was estimated by fitting the peak height ratios of the fatty acids on the standard curve. By this technique the coefficient of variation within-assay at $n = 11$ was 2.25% and between-assay was 13.8%.

COMMENTS

Our described method of measuring arachidonic acid in the plasma is a quick, sensitive method that requires very little preparation. By the use of dihomo-γ-linolenic acid as the internal standard, which is added to the plasma, we do not need to worry about quantitative recovery after extraction or extensive separation from other fatty acids, because the physico-chemical properties of these two unsaturated fatty acids are very similar to each other. Under conditions where the dietary intake of dihomo-γ-linolenic acid is high, endogenous levels of free dihomo-γ-linolenic acid will be high, and our method of measurement of arachidonic acid plasma levels may not be useful. Routinely a plasma sample without added internal standard should be analyzed with each group of plasma samples to be sure that there are no interfering chromatographic peaks in the plasma at the arachidonic acid peak and that the endogenous level of dihomo-γ-linolenic acid is indeed very low. In over a hundred sets of plasma that we have analyzed for free arachidonic acid levels by our technique, we have not encountered a high endogenous dihomo-γ-linolenic acid level or an inter-

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fering peak. We also want to emphasize that the use of a saturated fatty acid as an internal standard for the measurement of arachidonic acid may not be appropriate because of its poorer extractability from plasma.

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REFERENCES

1. Kunze, W., and W. Vogt. 1971. Significance of phospholipase A for prostaglandin formation. *Ann. N.Y. Acad. Sci.* **180:** 123–125.
2. Weber, P. C., B. Scherer, and C. Larsson. 1977. Increase of free arachidonic acid by furosemide in man as the cause of prostaglandin and renin release. *Eur. J. Pharmacol.* **41:** 329.
3. Hagenfeldt, L. 1968. The concentrations of individual free fatty acids in human plasma and their interrelationships. *Arkiv. Kemi.* **29:** 57–62.
4. Ogburn, P. L., W. E. Brenner, R. C. Reitz, F. P. Zuspan, L. G. Staurovsky, and J. R. Dingfelder. 1978. Arachidonic acid and other free fatty acid changes during abortion induced by prostaglandin $F_{2\alpha}$. *Am. J. Obstet. Gynecol.* **130:** 188–193.
5. Schwartz, B., M. Schultz, P. C. MacDonald, and J. M. Johnston. 1975. Initiation of human parturition III. Fetal membrane content of prostaglandin E_2 and $F_{2\alpha}$ precursor. *Obstet. Gynecol.* **46:** 564–568.
6. Beare-Rogers, J. L. 1971. Liver phospholipids of rats fed a choline deficient diet supplemented with choline or methionine. *Lipids.* **6:** 649–651.

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