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Letter to the Editor

Interference from Carotenoids in the Fluorometric Analysis of Serum Vitamin A in Cancer Studies

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THERE has been considerable interest in recent years in the possible associations between cancer, vitamin A and carotene [1]. Although much evidence has been obtained from animal experiments, few studies have more direct implications to public health than those relating cancer prevalence in humans to dietary intakes or serum levels of vitamin A and carotene. In view of the importance of such findings, it is imperative that the analytical methods on which they are based be carefully evaluated.

In a recent study by Basu *et al.* [2], vitamin A levels were found to be lower in patients with epithelial cancer than in those with myeloma or in controls. The mean control value, however, was reported to be 85 μg/100 ml, which is 1.4 times the value (60 μg/100 ml) considered to be typical in well-nourished adults [3, 4]. This should not be surprising as it was demonstrated many years ago that the simple fluorometric method [5] used in the study measures not only vitamin A but also the fluorescent carotenoid, phytofluene [6, 7]. Phytofluene occurs in common fruits and vegetables and the levels in blood vary considerably, reflecting the consumption of foods, such as tomatoes, that are rich sources. The error introduced into the determination of vitamin A varies erratically from 20 to 200% and the mean elevation has been reported to be 30 μg/100 ml [8].

Van Steveninck and De Goeij [9] described a modification to the early fluorometric procedures [5] to correct for the absorption of fluorescence by β-carotene. This correction was used by Basu *et al.* [2]. Both groups appear to have been unaware of the occurrence of phytofluene. The modification, involving a shift in the wavelength of the measurement from 480 to 550 nm, does not eliminate interference from the fluorescence of phytofluene, which overlaps that of vitamin A and extends above 600 nm [6].

If vitamin A is to be measured fluorometrically in serum or plasma, it is necessary to include either a correction formula [6, 8] or some form of column chromatography [7]. Both approaches require special care; most laboratories will find new procedures based on high-performance liquid chromatography (HPLC) more convenient and reliable [10].

The dramatic differences between the sera from patients and controls demonstrated by Basu *et al.* [2] suggest, therefore, several possibilities. Vitamin A, phytofluene or both substances could be reduced in cancer and the effects could be much larger or smaller than indicated. Phytofluene levels in blood do not correlate with those of vitamin A [8], but it is reasonable to assume that they are proportional to 'carotenoid' values. Although 'carotenoids' measured colorimetrically are often described as 'carotene', many pigments are present in blood, and the provitamin, β-carotene, is often a minor constituent of the mixture [11]. Separation of pigments by HPLC [12, 13] has confirmed that much of the colour in blood lipids often comes from lycopene, which, like phytofluene, is a constituent of tomatoes with no provitamin A activity (unpublished data).

In summary, the results on cancer patients reported by Basu *et al.* [2] should be confirmed with valid methods of analysis. Investigators contemplating similar research on cancer and

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blood carotenoids and vitamin A should review analytical methodology carefully before undertaking costly surveys. Procedures based on HPLC are now strongly recommended [10].

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