LETTERS TO THE EDITORS

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Pure *All-trans* Vitamin A Acetate and the Assessment of Vitamin A Potency by Spectrophotometry

The international unit of vitamin A activity corresponds with 0·3 μgm. of vitamin A alcohol or 0·345 μgm. of vitamin A acetate[1]. The International Standard Preparation is a solution of the *all-trans* acetate in cottonseed oil, and it serves a dual purpose as a biological and a spectrophotometric standard of reference. The properties of the acetate are therefore important.

The absorption spectra of many organic solutes vary to some extent in different solvents, even when there is no evidence of equilibria between isomeric forms. In the case of vitamin A such variations have been known to occur since 1935[2]; but they are second-order effects which have hitherto been largely ignored.

Advances in spectrophotometric technique, together with the advent of pure vitamin A and its esters, have made it possible to ascertain the solvent effects with considerable precision, and it is no longer appropriate to neglect them. Further, the spectrum of vitamin A alcohol (expressed in terms of molar extinction coefficient plotted against wave-length) is not necessarily identical with that shown by its esters in the same solvent. Granted that solvent effects occur, it is necessary to establish their magnitude in relation to the degree of accuracy which is both attainable and worth while. The present report is the result of work in five laboratories on synthetic samples (supplied by Dr. O. Isler of Hoffmann-La Roche, Basle). We are satisfied, however, that purified samples of *all-trans* vitamin A acetate obtained from natural products (and supplied by Dr. J. Matet of Alimentation Equilibree, Commentry) are identical with the synthetic acetate by chemical and physical tests[3,4].

PROPERTIES OF *All-trans* VITAMIN A
(Each figure is the mean of the results from the five laboratories)

Solvent	Alcohol $\lambda_{max.}$ (mμ)	Alcohol ($\varepsilon_{max.}$)	Acetate $\lambda_{max.}$ (mμ)	Acetate ($\varepsilon_{max.}$)
*Iso*propanol	324–326	52,300*	324–326	50,280†
Ethanol	324–325	52,480*	325–326	51,180
*Cyclo*hexane	326–327	49,760†	327–328	50,000†
Light petroleum (40°–60°)	324–326	52,000*	324–326	52,200*

Analysis of variance of the forty individual results indicates that: (1) the coefficient of variation between laboratories is 1·3; (2) the residual coefficient of variation of the one determination is 1·1; (3) the four means marked * are not significantly different; (4) the three means marked † are not significantly different; and (5) the remaining mean (51,180) falls between the other two groups and cannot confidently be regarded as a normal sampling variant of either. The two coefficients of variation given in (1) and (2) are normal—perhaps, indeed, rather below (that is, better than) normal. With the possible exception of the *cyclo*hexane result, the given mean values are generally consistent with those in the literature. Most published figures, however, favour a 1–2 per cent lower value for vitamin A esters in *cyclo*hexane than in *iso*propanol.

It follows from the above, and from the definition[1] of the International Unit of vitamin A so as to give a potency of $3 \cdot 3 \times 10^6$ I.U./gm. to the *alcohol*, that the appropriate factors for conversion of values at $\lambda_{max.}$ to I.U. per gram are most likely to be those given below:

CONVERSION FACTORS

Solvent	Vitamin A alcohol	Vitamin A acetate
*Iso*propanol	1,825	1,906
Ethanol	1,825	1,850
*Cyclo*hexane	1,906	
Light petroleum (40°–60°)	1,825	

The value of 1,906 for vitamin A acetate in *iso*propanol corresponds to an E(1 per cent, 1 cm.) value of 1,525, identical with the figure accepted by the Expert Committee on Biological Standardization of the World Health Organization[1]; and that body recommended the rounded-off factor 1,900 for practical purposes.

Now, in practice, vitamin A is often estimated in the form of the alcohol (the U.S. Pharmacopœia assay[5] stipulates saponification of vitamin A-containing oils, and chromatographic methods may involve saponification) for which the factor 1,900 would seem to be about 4 per cent too high if the final solvent is *iso*propanol. *Cyclo*hexane, on the other hand, would take the 1,900 factor under all conditions. In light petroleum $\varepsilon_{max.}$ is apparently identical with $\varepsilon_{max.}$ in *iso*propanol for the alcoholic form of the vitamin. This has certain practical advantages.

It should be emphasized that use of the conversion factors here accorded would not cut across the recommendation of the World Health Organization Committee, because the primary definition of the International Unit of activity is a weight of the vitamin, and the conversion factor is derivative. The presence of some neo-vitamin A (an isomer) in natural products is an additional complication. This point has been discussed by several authors[4,6] and will not be considered here.

J. BOLDINGH (Lever Bros. and Unilever N.V., Zwijndrecht).

H. R. CAMA, F. D. COLLINS and R. A. MORTON (University of Liverpool).

N. T. GRIDGEMAN (Lever Bros. and Unilever, Ltd., Bedford).

O. ISLER and M. KOFLER (F. Hoffmann-La Roche and Co., Ltd., Basle).

R. J. TAYLOR (Lever Bros. and Unilever, Ltd., Port Sunlight).

A. S. WELLAND and T. BRADBURY (Van den Berghs and Jurgens, Ltd., Purfleet).

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