A competitive protein-binding assay for 25-hydroxyvitamin D

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In a recent issue of Clinical Science and Molecular Medicine (1976), 51, 605-607, Dr J. M. Pettifor and his colleagues reported their studies using a competitive protein-binding assay for 25-hydroxyvitamin D$_3$ (25-OHD$_3$). These authors (Pettifor, Ross & Wang, 1976) suggested that the chromatographic separation of vitamin D$_3$ from 25-OHD$_3$ before measurement of the metabolite was unnecessary as normal concentrations of vitamin D$_3$ do not interfere in the assay. Whilst they failed to demonstrate any difference in results of competitive protein-binding between samples subjected to chromatography or not so treated, this has not been the experience of some other groups.

It has been shown that if the same samples are assayed with and without prior chromatography, consistently higher values are obtained for the samples assayed without chromatography (Graham, Preece & O'Riordan, 1977). The same group and Haddad, Min, Walgate & Hahn (1976) have also demonstrated that some of the dihydroxy forms of vitamin D$_3$ are of high cross-reactivity in the competitive protein-binding assay. Both these groups have shown that 24,25-(OH)$_2$D$_3$ is at least as active as 25-OHD$_3$ in displacing $^3$H-25-OHD$_3$ from rat serum binding sites. Haddad et al. (1976) investigated four normal subjects and reported values for 24,25-(OH)$_2$D$_3$ which were 25-48% of the total 25-OHD$_3$ and 24,25-(OH)$_2$D$_3$ concentrations. Graham et al. (1977) have also shown that significant concentrations of 24,25-(OH)$_2$D$_3$ are present in normal subjects although at a lower relative concentration: 5-6% of the total 25-OHD$_3$ and 24,25-(OH)$_2$D$_3$ concentrations. They also demonstrated that 25,26-(OH)$_3$D$_3$ was highly active in displacing $^3$H-25-OHD$_3$ from the rat serum binding protein. As it has already been shown (Preece, O'Riordan, Lawson & Kodicek, 1974) that the 25-hydroxy forms of vitamin D$_3$ and vitamin D$_2$ behave identically in assays using the rat serum protein, it is likely that similar remarks would apply to metabolites of vitamin D$_3$ in sera from patients receiving the vitamin therapeutically.

Besides the paper cited above (Pettifor et al., 1976), other workers have advocated the use of the rapid 25-OHD$_3$ assay without prior chromatography (Belsey, DeLuca & Potts, 1974; García-Pascual, Peytremann, Courvoisier & Lawson, 1976; Offermann & Dittmar, 1974). We would agree with Haddad et al. (1976) that the higher values generally obtained in assays not preceded by chromatography, are probably due to measurement of other hydroxylated forms of vitamin D$_3$ as well as 25-OHD$_3$. Some of the differences may be due to environmental factors also, although this will remain uncertain unless specific assays are used. These shortened assays may be useful as a preliminary screening test for vitamin D deficiency or intoxication. However, for more detailed studies of the physiology and pathology of mineral metabolism, it is necessary to use assays for vitamin D metabolites which are more specific and therefore use prior chromatography. This may be most relevant to vitamin D-deficiency states where the total values will be reduced and the effects of other hydroxylated forms of vitamin D may be more pronounced.

References

AUTHORS' REPLY

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In reply to the letter of Dr G. N. Hendy and colleagues, which is directed to the inaccuracy of the non-chromatographic methods for measuring serum concentrations of 25-hydroxyvitamin D (25-OHD), the following comments may be made.

Although we agree that the non-chromatographic assay system most probably measures other metabolites of vitamin D besides 25-OHD, particularly 24,25-(OH)2D, the concentration of these compounds in serum over a wide range of 25-OHD values is probably such as not to interfere seriously with the results obtained. Thus Taylor, Hughes & da Silva (1976) found concentrations of 24,25-(OH)2D in serum to be 10% or less of the particular 25-OHD value over a range of 25-OHD concentrations from 8.8 ng/ml to 186 ng/ml. These results agree well with the figure of 5–6% obtained by Graham and his colleagues and quoted in the above letter. The higher values obtained by Haddad, Min, Walgate & Hahn (1976) are based on very few cases and are well outside the estimates for 24,25-(OH)2D concentrations obtained by Mawer, Backhouse, Hill, Lumb, da Silva, Taylor & Stanbury (1975) in radioactively labelled vitamin D turnover studies. The relative accuracy of the non-chromatographic method is also confirmed by our studies, which, for the same serum either chromatographed or not before assay, gave a mean concentration of 25-OHD of 21 and 23 ng/ml respectively.

We believe that our simple, non-chromatographic assay is very useful for the screening of large numbers of patients and that the error so introduced is probably not more than 10%. We do agree, however, that for the very accurate measurement of serum 25-OHD concentrations, it may be necessary to chromatograph the sera before analysis.

References


Correction


Page 34, second column, line 15 below 'Methods': sentence should read Platelet-rich plasma was obtained as the supernatant from centrifuging blood, anticoagulated (9:1, v/v) with sodium citrate (38g/l) adjusted with citric acid (100 g/l) to pH 7.3, at 200 g for 8 min.