CXXXIX. THE COLOUR REACTIONS OF SUBSTANCES CONTAINING VITAMIN D.

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(Received May 22nd, 1928.)

SHEAR and KRAMER [1926] have found that on boiling oils containing the antirachitic vitamin with a solution of aniline hydrochloride in excess of aniline a characteristic red colour is produced, and suggest that this reaction may be employed in the detection of vitamin D. The empirical nature of such a test casts some doubt on its reliability as a sure method of detecting the vitamin, especially as it has recently been demonstrated that two biologically inactive substances, ergosterol peroxide and ergopinacone, give the blue colour with antimony trichloride which is a characteristic property of vitamin A [Windaus, Borgeaud and Brunken, 1927].

With a view therefore to investigating the specificity of this reaction, the effect of the reagent upon a number of sterol derivatives has been studied. The aniline hydrochloride solution, which was almost colourless, was prepared exactly as described by Shear and Kramer by mixing five parts of redistilled aniline with one part by volume of concentrated hydrochloric acid. Cod-liver oil was found to respond to the test exactly as described by these authors. On shaking together the oil and the reagent a green emulsion was first formed. Heat soon destroyed the green colour and after boiling for half a minute and allowing the two layers to separate, the lower layer appeared intensely red. In the case of "essogen," a highly concentrated preparation of vitamins A and D, for a sample of which I am indebted to Messrs Lever Bros., Ltd., the initial green colour was not noted, though on heating the oil behaved in a similar manner to cod-liver oil.

In the case of the solid substances, about 0.05 g. was introduced into a test-tube and 3 cc. of the aniline preparation were added. The liquid was heated to boiling and boiled for 30 seconds. The results are indicated in the table. A red colour was produced by irradiated ergosterol, while the unirradiated sterol caused only a slight darkening of the liquid. The hydrocarbons were without effect as also were cholesterol, cholesteryl acetate and dicholesteryl ether. The compounds which proved most sensitive were the ketones, and of these the singly unsaturated ketones alone gave the red colour to any marked degree. The saturated ketones brought about slight darkening, while the doubly unsaturated oxycholesterylene gave a deep brown. For the purpose of comparison two terpene ketones, camphor and carvone, were examined. These

behaved in a similar manner to their sterol analogues, the saturated camphor causing slight darkening while the doubly unsaturated carvone produced a brown colour.

These results show clearly that the test is by no means specific for vitamin D. On the other hand, the positive reaction given by irradiated ergosterol and by the ketones is in harmony with the view expressed by Heilbron, Morton and Sexton [1928] that the antirachitic vitamin is possibly ketonic in character. It must be borne in mind however that the photochemical formation of vitamin D from ergosterol is by no means a quantitative process. It is not impossible therefore that the ketone which may be produced during the irradiation of ergosterol is not the vitamin.

Bezssonoff [1924] has described a preparation of phosphomolybdotungstic acid which gives a colour with oils which contain the antirachitic vitamin. Cod-liver oil and "essogen" were found to respond to the test as described by Bezssonoff, but irradiated ergosterol gave no colour at all. The colour produced by the oils must therefore be due to some constituent other than vitamin D.

Substance	Type	Colour
Cholestane	Hydrocarbon, saturated	Nil
Cholestene	Hydrocarbon, 1 double bond	Nil
ψ -Cholestene	Hydrocarbon, 1 double bond	Nil
Cholesterylene	Hydrocarbon, 2 double bonds	Slight darkening
Cholesterol	Alcohol, 1 double bond	Nil
Cholesteryl acetate	Ester, 1 double bond	Nil
Dicholesteryl ether	Ether, 2 double bonds	Nil
Ergosterol	Alcohol, 3 double bonds	Slight darkening
Irradiated ergosterol	—	Reddish brown which becomes deeper and redder after 20 minutes
Cholestane-4-one	Saturated ketone	Slight darkening. Prolonged standing (24 hours) in air gives red
Cholestane-7-one	Saturated ketone	Slight darkening. Prolonged standing (24 hours) in air gives red
Cholestenone	Ketone, 1 double bond	Orange red, becoming blood red in 10 minutes
β -Oxycholestenol acetate	Ketone, 1 double bond	Orange red, becoming blood red in 10 minutes
Oxycholestenone	Diketone, 1 double bond	Blood red, rapidly deepening
Camphor	Saturated ketone	Slight darkening
Oxycholesterylene	Ketone, 2 double bonds	Brown, darkening on standing
Carvone	Ketone, 2 double bonds	Brown, darkening on standing

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