

# LV. NOTE ON SOME STEROL COLOUR RE-ACTIONS IN THEIR RELATION TO VITAMIN A.

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THE discovery of the brilliant blue colour reaction of cod-liver oil with  $\text{AsCl}_3$  and its suggested association with both vitamin A and cholesterol [Rosenheim and Drummond, 1925] led to a search for a similar reaction, obtainable from sterols or their derivatives under conditions which would throw light on the nature of the chromogen concerned in the cod-liver oil reaction. Starting from the assumption that oxidative processes are connected with the formation of the blue pigment in sterol reactions, the action of mild oxidising agents on sterols was investigated. It was found that treatment of cholesterol with benzoyl peroxide in chloroform solution produced a chromogenic substance which gave with  $\text{AsCl}_3$  a blue colour indistinguishable in appearance from that given by cod-liver oil with  $\text{AsCl}_3$ . The possibility of the formation of an aldehydic sterol derivative on mild oxidation suggested further a study of the influence of formaldehyde on the colour reactions of sterols. Again a change from the usual red colour to a clear blue was found to be produced when  $\text{AsCl}_3$  reacted with cholesterol in the presence of nascent formaldehyde. This reaction is conveniently carried out by adding  $\text{AsCl}_3$  to a solution of cholesterol in methylal. In this case, however, the chromogen cannot be isolated, as it is slowly converted into the pigment in the course of the reaction<sup>1</sup>.

Although the blue pigment formed from cholesterol resembles on superficial inspection that obtained in the cod-liver oil reaction, a more detailed investigation raised doubts as to their identity. This doubt was increased when it was found that cholic acid also gives rise to a similar blue colour reaction after heating with benzoyl peroxide in chloroform solution. The latter reaction again demonstrates the near relationship of the bile acids to cholesterol, the nature of which has been cleared up by the work of Windaus and Neukirchen [1919]. The similarity of these colour reactions to that of the chromogen of cod-liver oil suggests that there exists a similarity between the carbon ring

<sup>1</sup> Antimony trichloride, trichloroacetic acid, dimethylsulphate, etc., may be used as condensing agents in place of  $\text{AsCl}_3$  in all these reactions under slightly modified conditions. The various phytosterols and ergosterol react in a similar way. In view of the multiplicity of sterol reactions already available, a detailed description of the new reactions may be dispensed with.

system of the sterol molecule and that of the unknown chromogen and lends support to the view that oxidative changes of the sterol molecule may be concerned in the vitamin A formation from sterols.

Evidence is accumulating indicating that the blue colour reaction of cod-liver oil is specific for vitamin A [Rosenheim and Webster, 1926, 1927, 1]. As the new colour reactions of sterols are of interest only in their relation to this vitamin, a short description of one of them is sufficient in this respect. The chromogen is rapidly formed on evaporating to dryness a chloroform solution of cholesterol and benzoyl peroxide in equimolecular proportions. A quantitative colorimetric test in Lovibond's colorimeter showed that the maximum colour production is reached after boiling the solution for about two hours (1 mg. of the reaction product + 2 cc.  $\text{SbCl}_3$  solution equals 12 blue units). All attempts to isolate the chromogen in a pure condition from the complex mixture of amorphous oxidation products have so far failed.

The properties of the resinous product obtained resemble those described for a similar mixture called "oxycholesterol" by Lifschütz, and like the latter material it can also conveniently be prepared from cholesterol dibromide by debromination with sodium acetate in alcoholic solution [Lifschütz, 1919]. The substance to which Lifschütz ascribes, without justification, the formula  $\text{C}_{27}\text{H}_{46}\text{O}_2$ , is undoubtedly a complex mixture [see Windaus, 1908; Windaus and Lüders, 1921; Gardner, 1921], one constituent of which gives the green colour reaction, considered as characteristic for "oxycholesterol" by Lifschütz. The material also gives a blue reaction with  $\text{AsCl}_3$ <sup>1</sup>, but it appears doubtful whether the constituent giving rise to the green Lifschütz reaction is identical with the chromogen of the blue pigment. In any case there can be no doubt that Lifschütz's "oxycholesterol" has no bearing on the blue cod-liver oil reaction with  $\text{AsCl}_3$ , since, according to Lifschütz's and my own observations, the typical green colour reaction is not given by any liver fat (nor by cod-liver oil).

On the other hand, the fact that one constituent of the oxidation products of cholesterol gives a blue colour with  $\text{AsCl}_3$  suggested an investigation of its behaviour when added to such vegetable or animal oils as do not react with  $\text{AsCl}_3$  by themselves. It was found that in every case examined the production of the blue colour was completely inhibited when a purified preparation (from cholesterol dibromide) was dissolved in any natural oil or fat. This instability of the artificial chromogen in solution in a natural oil excludes its identity with the chromogen of cod-liver oil. The rapid destruction of the artificial chromogen appears to be connected with the presence of unsaturated linkages in the natural oils, for a freshly prepared solution in a neutral solvent, such as liquid paraffin, gives an intense positive reaction with  $\text{AsCl}_3$ . Even in this case, however, the chromogen gradually disappears on keeping under laboratory conditions.

<sup>1</sup> In the course of an examination of the sterols of echinoderms [Page, 1923], and of *Boletus granulatus* [Marston, 1924], the same observation is recorded incidentally.

Although in its relative instability the artificial chromogen resembles the chromogen of cod-liver oil, it may be pointed out that the blue pigment produced by  $\text{AsCl}_3$  from the cod-liver oil chromogen is typically unstable, the colour fading within a few minutes, whilst the blue colour given by the artificial product, when once formed, remains unchanged for many days. A characteristic difference in the absorption spectrum of the two pigments may be adduced as a further reason against their identity.

A few feeding experiments with the mixture of substances obtained on oxidation of cholesterol were kindly carried out by my colleague, Mr Webster. The results so far obtained, although not yet completely conclusive on account of the instability and impurity of the material, are against the view of its having the properties of vitamin A. In consideration of the relationship which has recently been shown to exist between ergosterol and the anti-rachitic vitamin D [Rosenheim and Webster, 1927, 2; Windaus and Hess, 1927], we are at present investigating the possible relation of vitamin A to the oxidation products of ergosterol.

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