XIX. THE RELATION OF CHOLESTEROL TO VITAMIN D.

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In continuation of our work on the molecular groupings essential for the successful activation of sterols by ultraviolet light [Rosenheim and Webster, 1926] we had the privilege of collaborating with Prof. Windaus of Göttingen in the examination of various isomers of cholesterol, recently prepared by him, and of other sterols. The details of this work which gave entirely negative results [see also Hess and Windaus, 1926] we hope to publish shortly in conjunction with Prof. Windaus.

Our failure to find any derivatives of sterols which were either active by themselves or could be rendered antirachitic by irradiation, led us to a reconsideration of the problem. The following facts emerge from previous work.

1. At least 99·9 % cholesterol is recovered unchanged when the active substance is separated from cholesterol, irradiated in a nitrogen atmosphere, by precipitation with digitonin. Further, no appreciable difference in activity is observed when the time of irradiation is varied from only a few minutes to many hours. Apparently only a small amount of cholesterol can be converted into the active condition.

2. Changes produced in the cholesterol molecule by oxidation of the hydroxyl group, or replacement of this group by chlorine, or its complete removal, deprive the derivatives so formed (cholestenone, cholesteryl chloride, cholestene) of the power to be rendered antirachitic by ultra-violet light. The presence of the OH group appears therefore essential, but the fact that the esters of cholesterol (acetate, palmitate) can be activated is contrary to this conclusion, unless the assumption is made that the esters are slightly hydrolysed during irradiation.

3. Phytosterols prepared from a sample of corn oil, which had been kept many years, cannot be activated [Steenbock and Black, 1925]. Further, another phytosterol, stigmasterol, prepared by Windaus and Hauth [1906] by way of the tetrabromide, has been found by Windaus to be incapable of being activated, a fact which we were able to confirm.

4. Allo-cholesterol, a new isomer of cholesterol recently discovered by Prof. Windaus, cannot be activated although it easily changes into cholesterol. We have recently been informed by Prof. Windaus that the actual specimen of allo-cholesterol examined by us contained some free cholesterol.

Arising out of these considerations, it seemed to us essential to investigate the question, how far the purity of the cholesterol employed is related to its capacity for activation by ultra-violet light. Although we ourselves and others had used specimens of cholesterol which had been purified with special care by the usual methods (saponification and recrystallisation, etc.) we decided to purify it still further by a chemical method. For this purpose a many times recrystallised specimen of cholesterol (M.P. 148–9°) was converted into the dibromide, and the latter again reduced to cholesterol by means of sodium amalgam in the presence of acetic acid. The melting point of the purified substance remained unchanged (148–9°) and it gave the usual colour reactions with great intensity.

This product was irradiated for 1 hour and tested biologically by the methods previously used [Rosenheim and Webster, 1926].

Details of the animal experiments are given in the following table:

Rat	Daily dose mg.	Inorganic blood phosphate mg. %	X ray result
1	2	2·3	Rickets
2	4	2·4	,,
3	8	2·4	,,
4	Control	2·2	,,
5	,,	2·3	,,

The result of this series of experiments is striking, especially when we consider that the original cholesterol preparation, when irradiated, prevented rickets in rats even when administered in such small doses as 0·5 mg. *per diem* [Rosenheim and Webster, 1925]. Purification by way of the dibromide completely deprived this cholesterol, which would previously have been considered as "chemically pure," of its power to become antirachitic by irradiation with ultra-violet light.

This observation throws new light on the photo-chemical formation of vitamin D. It is evident that the precursor of vitamin D is not cholesterol itself, but a substance which is associated with and follows "chemically pure" cholesterol in all its stages of purification by the usual methods (esterification, saponification, recrystallisation).

The possibility that the absorption spectrum of cholesterol may be due to a small amount of an impurity had indeed already been suggested by Schlutz and Morse [1925] in their careful study of the absorption spectra of cholesterol. The precursor of vitamin D[1] need not necessarily be an extraneous impurity of ordinary cholesterol. When we consider the ease with which sterols form stable complex additive compounds such as the "phytosterol" of calabar beans [Windaus and Hauth, 1906] and the complex of β-cholestanol with pseudocoprosterol [Windaus and Uibrig, 1915], the possibility must be kept

[1] In our first communication (*J. Soc. Chem. Ind.* **45**, 932) we proposed the name "Vitasterol" for the precursor of vitamin D, but as this term might lead to confusion we have adopted the more expressive name "Provitamin," suggested by Prof. Windaus, for the parent substance of vitamin D.

in mind that the provitamin may be a substance allied to cholesterol in character. The amount present in ordinary "pure" cholesterol may be judged from the yield of active substance obtained by us [Rosenheim and Webster, 1926] in the digitonin experiment, to be of the order of $\frac{1}{10}$ %. Preliminary experiments seem to justify the statement that provitamin D is precipitated by digitonin and that its separation from cholesterol may be possible by fractional precipitation or extraction of the digitonin complex.

It is obvious that the nature of this unidentified substance, its relation to cholesterol, and its separation from it can now be investigated by experimental means.

In view of the spectrographic work of Hess and Weinstock [1925], and of Schlutz and Morse [1925] it is suggestive that, according to information kindly supplied by Prof. Windaus, a specimen of cholesterol, prepared by him at our suggestion by way of the dibromide, no longer showed the absorption spectrum in the ultra-violet region characteristic of ordinary "pure" cholesterol. This specimen had been converted into the dibromide, which was recrystallised twice and then reduced with zinc dust in the presence of acetic acid, the whole series of operations being repeated.

It is interesting to note that Heilbron, Kamm and Morton [1926] as the result of an analysis of the ultra-violet absorption spectra of cholesterol make the suggestion that cholesterol may not be the precursor of vitamin D, but that the activation of ordinary cholesterol may be due to the presence in it of traces of an unknown substance[1].

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[1] Our biological test of two preparations of these authors is referred to in their paper in this number of the Journal.

[Addendum: Jan. 31st, 1927.] Since the above communication went to Press, it has been found that ergosterol, or a sterol of similar constitution, is the parent substance of vitamin D. A preliminary communication by ourselves on this work is in the Press (see *Lancet*, Feb. 4th, 1927) and a similar one will be made in Germany by Prof. Windaus.