Letters to the Editor.

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The Activation of Cholesterol at Liquid Oxygen Temperature.[1]

AT the temperature of liquid oxygen (− 183°), most bimolecular chemical reactions are largely inhibited. The rates of these reactions, in general, decrease rapidly with decreasing temperature. At very low temperatures the rates become too small to be measured, and for practical purposes it may be said that reaction ceases. This is especially true of oxidations, since, for example, neither sodium nor phosphorus immersed in liquid oxygen undergoes any apparent oxidation. However, the rates at which photo-molecular changes take place decrease more slowly with temperature. With this in mind, the irradiation of activatable cholesterol was carried out in liquid oxygen with the object of obtaining evidence as to the nature of the change involved. It was found that cholesterol of ordinary purity even at liquid oxygen temperature becomes antiricketic upon exposure to ultra-violet light.

The cholesterol used in these experiments melted clear at 149°. An investigation of its absorption spectrum by Dr. W. A. MacNair demonstrated that it contained 1·2 parts per thousand of ergosterol. Preliminary biological tests proved it to be highly activatable, although not antiricketic prior to irradiation. Our biological technique (line test) is described elsewhere.[2]

Irradiation was performed as follows: A cylindrical brass tube 42·5 cm. long and 4·5 cm. in diameter, closed at the lower end, was immersed in liquid oxygen contained in a silvered Dewar flask. The depth of immersion varied between 25 cm. and 15 cm. on account of evaporation of the oxygen. To prevent the condensation of water vapour in the tube and the settling of frost on the sample to be irradiated, the open, upper end of the tube was covered with a quartz plate thermally insulated from the metal by a ring made from asbestos board. A thin disc 4·2 cm. in diameter bearing 200 mgm. of pulverised cholesterol was lowered to rest on the bottom of the tube, and allowed to cool to liquid oxygen temperature. A quartz mercury vapour arc was adjusted a few centimetres above the tube and operated at 120 volts with about 30 ohms resistance in series. After irradiation for 105 minutes, the cholesterol was allowed to warm to room temperature, then dissolved in ether and evaporated on to McCollum's Diet 3143. Before and after irradiation the cholesterol was carefully protected from ultra-violet light. A similar experiment was made with the same apparatus, but at room temperature. On account of transportation, etc., a week elapsed between the preparation and administration of the modified diets.

The cholesterol irradiated at room temperature induced advanced healing of rickets when administered at 1/10 per cent., or even at 1/100 per cent. in the diet. The cholesterol irradiated at liquid oxygen temperature induced advanced healing at 1/10 per cent., but failed at 1/100 per cent. Thus it is evident that cholesterol is readily activated at liquid oxygen temperature, although the product obtained under the conditions of this experiment was not so potent as the product of irradiation at room temperature.

We regard these data as a strong confirmation of the evidence which has been accumulated recently by Rosenheim and Webster,[3] Windaus and Hess,[4] and Bills and McDonald,[5] that the activation of cholesterol (or ergosterol) consists not in an oxidation, but in an isomerisation—a rearrangement at the double bond or an *Elektronenverlagerung*.

In the activation of sterols by ultra-violet rays, it is important to consider the temperature coefficients of both the vitamin formation and accompanying destruction. For the formation the coefficient is evidently low. We find, however, that the spontaneous deterioration of (unactivated) ergosterol has a high temperature coefficient. If activated ergosterol also exhibits a high coefficient in its decomposition, then the way is clear for the preparation of a vitamin D of greater potency than has hitherto been attained.

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Cholesterol and Vitamin D.

BILLS, Honeywell and MacNair have recently demonstrated (*Jour. Biol. Chem.*, **76**, 251; 1928) that both ergosterol and ordinary purified cholesterol, in addition to the previously observed well-defined absorption bands at 293·5, 281·5, and 270 $\mu\mu$ (Morton, Heilbron and Kamm, *Biochem. Jour.*, **21**, 78; 1927), exhibit a faint but distinct band at 260 $\mu\mu$. They have also discovered that cholesterol purified by a thrice repeated conversion into the dibromide and recovery from same by treatment in boiling alcohol with zinc dust (so-called cholesterol E) can, contrary to the contentions of Windaus and Hess (*Nach. Ges. Wiss. Göttingen, math.-physik. Klasse*, 175; 1927) and Rosenheim and Webster (*Biochem. Jour.*, **21**, 389; 1927), still be activated by ultra-violet rays. According to the American workers, this activatability is associated with faint absorption bands at 315 and 304 $\mu\mu$ as well as the three characteristic ergosterol bands.

We are able to confirm the existence of a faint band at 260 $\mu\mu$ in ergosterol, but this is only detected with certainty when a continuous light source is used. As regards the newly observed bands, however, we desire to direct attention to the bands found by us in cholesterilene, which is characterised by selective absorption with maxima at 294, 304, and 321 $\mu\mu$ (*Jour. Chem. Soc.*, p. 47; 1928). The first of these coincides with the 293·5 $\mu\mu$ band of ergosterol, whilst the others are in close agreement with the two found by Bills, Honeywell and MacNair in their specially purified cholesterol. Bearing in mind the known instability of cholesterol dibromide (Lifschütz, *Zeit. physiol. Chem.*, **106**, 271; 1919), and also the complex nature of its decomposition, we suggest that the two bands observed in cholesterol E may well be due to traces of cholesterilene formed during the purification process. The amount of this hydrocarbon which would be necessary to show the selective absorption

[1] Publication approved by the Director of the Bureau of Standards of the U.S. Department of Commerce.
[2] Bills, C. E., Honeywell, E. M., and MacNair, W. A., *Jour. Biol. Chem.*, **76**, 251; 1928.
[3] Rosenheim, O., and Webster, T. A., *Biochem. Jour.*, **20**, 537; 1926.
[4] Windaus, A., and Hess, A., *Nachr. Ges. Wiss. Göttingen, math.-physik. Klasse*, 175; 1927.
[5] Bills, C. E., and McDonald, F. G., *Jour. Biol. Chem.*, **72**, 13; 1927.