XXI. VITAMIN D FROM STEROLS OF MUMMIFIED EGYPTIAN BRAIN.

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THE stability of ergosterol when present in minute amounts in ordinary cholesterol from all animal sources (brain, gallstones, blood, skin, eggs, etc.) is surprising in view of the relatively labile character of ergosterol itself. This unsuspected contamination of "pure" cholesterol was revealed in the first instance by its antirachitic activity acquired after irradiation, and is confirmed by spectrographic analysis as well as by a specific colour reaction. It is now well established that ergosterol is the specific parent substance of vitamin D [Rosenheim and Webster, 1928, 1, 2]. In explanation of the protective action of cholesterol towards ergosterol, it has been suggested that it possesses an antioxidant function similar to that of the sterols in natural crude rubber [Bills, Honeywell and MacNair, 1928].

There is evidence that this protective action is exerted not only in freshly prepared specimens of cholesterol, but that it remains unimpaired over a long period of years, for some of the specimens of cholesterol, in which the presence of ergosterol was originally discovered, had been prepared from brain 20 years previously [Rosenheim and Webster, 1926]. Further, in a sample of gallstone-cholesterol prepared by Prof. Abel 16 years ago, the presence of ergosterol has been demonstrated spectroscopically and biologically by Bills, Honeywell and MacNair [1928].

Cholesterol and fatty acids have been recovered unchanged from various organs of Egyptian mummies of the pre-dynastic and later periods by Schmidt [1907] and from the brain of a mummy of unknown date by Abderhalden [1911]. Whilst fresh brain contains the whole of its cholesterol in the free state [M. C. Rosenheim, 1914], it has been found by Mair [1913] that as much as 99 % of the cholesterol of ancient Egyptian brain is present in combination with fatty acids (palmitic and stearic) as esters.

Through the kindness of Dr W. Mair we received part of a mummified brain and a specimen of cholesterol prepared from it by him 16 years ago. The brain is of Coptic origin, dating from about 500 A.D., and one of the series described by Ruffer [1913], having been removed from bodies found in tombs in Antinoë in Upper Egypt. No embalming process had been used in this instance and the organs of the bodies were preserved *in situ* through 14 centuries, shrunk and mummified under the influence of the dry atmosphere of Egypt.

We had no difficulty in preparing the pure cholesteryl esters, in a yield of about 12 % from the brain, by employing a simple extraction method and avoiding the use of charcoal on account of its destructive effect on ergosterol. The dry, waxy brain was broken up under water, suspended in an equal volume of 2 % KOH and warmed for a short time on a water-bath. The esters collected as a yellowish oil on the surface of the brown solution and solidified on cooling, giving rise to the characteristic display of colour on passing through the liquid-crystalline phase. After extraction with ether, they crystallised on concentration of the extract. One recrystallisation from ethyl acetate and one from acetone yielded a perfectly white product, M.P. 78–80°, $[\alpha]_{5461}^{16^\circ}$ – 29° (cholesteryl palmitate, M.P. 78–80°, $[\alpha]_D$ – 24°, stearate, M.P. 82°).

The crude esters gave the Lifschütz “oxycholesterol” reaction and, when freed from this substance by recrystallisation, showed the typical blue colour with trichloroacetic acid, which is specific for ergosterol [Rosenheim, 1929]. The esters also gave an intense Tortelli-Jaffé reaction (green colour in chloroform solution with dilute bromine), which according to Häussler and Brauchli [1929] is characteristic for ergosterol and is not given by pure cholesterol. Spectroscopic analysis of a 5 % ethereal solution of the esters showed the absorption spectrum of ergosterol in the region 280–290 $\mu\mu$.

Feeding experiments employing our usual technique proved conclusively that daily doses of 2 mg. and 4 mg. of the esters after irradiation (equivalent to 1 mg. and 2 mg. cholesterol) exerted the same antirachitic action as 0.05 γ of irradiated ergosterol. The same result was obtained with 2 mg. doses of the irradiated cholesterol preparation of Dr Mair. We found previously [Rosenheim and Webster, 1926] that doses of from 1–4 mg. of irradiated brain-cholesterol were necessary to prevent rickets in rats kept on a rachitogenic diet and it is therefore evident that the ergosterol content of cholesterol, isolated from brain after about 1400 years, is still of the same order as that of cholesterol prepared from fresh brain.

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