LX. NOTE ON ISOCHOLESTEROL, COPRO- STEROL AND THE CLASSIFICATION OF THE STEROLS.

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Isocholesterol was discovered by Schulze [1873] in the fat extracted from raw sheep's wool with ether. The fat is derived from the secretion of the fatty glands surrounding the hair follicle in the skin. For many years the individuality, and even the existence of ischolesterol, were questioned, as many workers, including the present writer, were unable to obtain it. In reply to criticisms of Darmstädter and Lifschütz [1898], Schulze [1898] repeated his original experiments and subsequently Moreschi [1910] obtained the substance from wool fat and fully confirmed the observations of Schulze. In 1910 additional interest was given to the question by the discovery of ischolesterol in a plant product, the so-called South African rubber, which consists of the coagulated latex of various species of Euphorbiaceae. The latex contains about 6 per cent. of rubber and 70 per cent. of resin. The ischolesterol was found as a constituent of the latter (excretory) product [Cohen, 1908]. A careful comparison of the substance with a specimen of the original ischolesterol supplied by Schulze was made by Cohen, and in my opinion no doubt can be entertained as to the identity of the two products and consequently of the recognition of ischolesterol as a definite member of the sterol group.

Coprosterol occurs normally in the faeces of men on ordinary diets, and in those of animals, in place of cholesterol, when diets rich in cholesterol, e.g. raw brain, are given [Dorée and Gardner, 1908]. It is no doubt produced from the cholesterol of the food by changes taking place in the intestine. König and Schluckebier [1908] have stated also that it appears in the excrement of animals which have been kept on diets rich in phytosterol such as peas, maize, coco-nut cake, etc. [Cp., however, Kusumoto, 1908; Dorée and Gardner, 1909.]

The recognition of ischolesterol as a definite member of the sterol group leads me again to point out that while ischolesterol and coprosterol, in their

mode of occurrence and properties, closely resemble one another, they differ in these respects markedly from all the other sterols [Dorée, 1909, 1]. Hitherto the sterols have been classified roughly as zoo- or phyto-sterols according as they were obtained from animal or vegetable sources respectively. But in order to emphasise the special properties and relationships of isocholesterol and coprosterol it is now suggested that a third class should be formed to include them and any other similar substances that may be discovered, and that the terms zoo- and phyto-sterols should be limited to a somewhat more exact definition. Seeing that coprosterol and isocholesterol undoubtedly stand in a close relationship to cholesterol and phytosterol, and that they appear to form a connecting link between these characteristic constituents of animal and vegetable protoplasm, they may be classified as "natural derived sterols" or *metasterols*.

The metasterols occur in the excretions and secretions of animals (intestinal canal, glands of the skin) and of plants (resin). They are never found as constituents of animal or vegetable protoplasm. The connection between them and the other sterols from a biochemical standpoint is shown in the following scheme.

The metasterols are monatomic alcohols of high molecular weight. Unlike the other sterols they (a) are saturated towards bromine and hydrogen [Moreschi, 1910], (b) do not crystallise from alcohol in plates, (c) give the colour tests of Liebermann and Salkowski in a modified way, (d) have practically no anti-toxic action towards haemolytic poisons [Hausman, 1905], (e) are dextro-rotatory.

It is probable that spongosterol [Henze, 1908], which has no anti-toxic power and apparently is a saturated alcohol, will fall into this class. The position of stigmasterol, $C_{30}H_{48}O$ [Windaus and Hauth, 1906], and brassicasterol, $C_{28}H_{46}O$ [Windaus and Welsch, 1909], found, together with large proportions of ordinary phytosterol in Calabar beans and rape seed respectively, is uncertain.

With the formation of this class it is now proposed to limit the terms zoo- and phyto-sterol to sterols which are found as tissue constituents of

animals and plants respectively. Cohen [1908], in the light of his discovery of ischolesterol as a plant product, has stated that the distinction between zoo- and phyto-sterols can no longer be maintained. But with the proper recognition of ischolesterol as a derivative produced from zoo- or phyto-sterols by metabolic changes, there is now every reason for maintaining it. For in spite of a great number of researches (Hauth [1907] quotes some 50 papers and many more have since appeared), no substance resembling cholesterol has ever been obtained from vegetable protoplasm or one resembling phytosterol from animal protoplasm; and this in spite of the fact that herbivorous animals take in phytosterol with their food and the carnivorous plants ingest a cholesterol. If phytosterol taken with the food is used by the animal in building up its tissues, it must first be converted into cholesterol. Furthermore a zoosterol is an invariable constituent of all animal organs and tissues so far examined from Chordata to Coelenterata [Dorée, 1909, 1; Welsch, 1909], and a phytosterol seems similarly to be contained in the tissues of plants of all orders. So fundamental is this distinction considered to be that it has become an important criterion in chemical technology for deciding the animal or vegetable origin of various natural substances. Thus Lewkowitsch [1913] defines these products to be of animal or vegetable origin respectively according to the presence of a zoo- or phyto-sterol in the unsaponifiable portion. [Cf. also the 'phytosterol acetate test.']

The zoo- and phyto-sterols will therefore include such sterols as are found entering into the composition of animal and vegetable protoplasm respectively. Speaking only of those whose properties have been carefully examined (for zoosterols see Dorée [1909, 1] and Welsch [1909]; for phytosterols see Hauth [1907]) the zoo- and phyto-sterols may be held to include "a number of secondary, monatomic alcohols, chiefly $C_{27}H_{46}O$, containing one ethylene linking in the molecule. They crystallise from alcohol in plates. Their benzoates melt at about 145° and show the phenomena of liquid crystals. They are all laevo-rotatory and have a powerful anti-haemolytic action towards saponine, etc." Their constant presence in the tissues is in part due to the necessity for the protection of the cells from such toxic substances: their occurrence in the fluids and secretions shows that they are continually being metabolised and, it is believed, conserved by the organism [Dorée and Gardner, 1909].

A summary of the properties of the best known sterols and their derivatives is given in the following table:

(a) Zoosterols:

	M.p.	$[\alpha]_D$	Acetate m.p.	Benzoate m.p.	Dibromide m.p.	Occurrence
Cholesterol	147°	-37°	114°	145°	123°	Universally distributed in the animal kingdom.
Bombicestrol	148°	-35°	129°	146°	111°	In <i>Bombyx mori</i> [Menozzi and Moreschi, 1908].
Clionasterol	138°	-37°	133°	143°	114°	In <i>Cliona celata</i> [Dorée, 1909, 1].

(b) Phytosterols:

Phytosterol	137°	-34° ¹	127°	146°	98°	Universally distributed in the Phanerogams.
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(c) Metasterols:

Isocholesterol	137°	+60°	134°	194°	None	Wool fat; resin of Euphorbiaceae.
Coprosterol	100°	+24°	88°	122°	None	Excrement of animals.

¹ In ether solution; others in chloroform.

Of the chemical relationship existing between cholesterol, phytosterol and the metasterols little is known. On a milk diet, during which bacterial change in the intestine is reduced to a minimum, cholesterol [Müller, 1900] and phytosterol [Windaus, 1908] are not converted to coprosterol. Normally in the case of men, and on diets rich in sterols in the case of animals, coprosterol is produced. Analyses, to which however no great weight can be assigned, seemed to indicate that the coprosterol molecule contained two atoms of hydrogen more than that of cholesterol, and it has been thought that coprosterol was dihydrocholesterol produced by bacterial reduction. Dihydrocholesterol artificially produced [Willstätter and Mayer, 1908] is not however identical with coprosterol, although very similar in its properties as will be seen from the following table:

	M.p.	$[\alpha]_D$	Acetate m.p.	Benzoate m.p.	Ketone m.p.	Observer
Dihydrocholesterol	142°	+29°	111°	155°	128°	Willstätter.
Coprosterol	100°	+24°	88°	122°	63°	Dorée.
Isocholesterol	137°	+60°	134°	194°	?	Cohen.

Observations on a large number of cholesterol and phytosterol derivatives have shown that any modification of the ethylene linking causes a change in the optical rotatory power from negative to positive and at the same time the antihæmolytic function is abolished or reduced to a minimum [Hausman, 1905]. The presumption therefore is strong that it is the side chain containing the double linking in cholesterol that is modified to produce coprosterol. The change however is more than one of simple reduction. The only other explanation that has been offered, based upon the behaviour of a peculiar carbazole derivative of coprostanone [Dorée, 1909, 2], is that the

bacterial action might simultaneously bring about reduction and rearrangement of the unsaturated side chain, producing two methyl groupings, thus:

Attempts have been made to solve the problem by studying the oxidation of coprosterol and cholesterol in the hope of obtaining, among the products, a derivative common to both. Coprosterol is difficult to obtain in any quantity, and considering the similarity between coprosterol and ischolesterol and the fact that the raw material from which ischolesterol is obtained is a commercial product, I decided two years ago to work up a large quantity of wool fat in the hope of preparing sufficient ischolesterol to enable a thorough examination of that substance to be made. Quantities of 2 to 3 kilos of wool fat from various sources were saponified under pressure by the method of Lewkowitsch and the mass extracted with ether in the usual way. The unsaponifiable residue was then fractionated in alcohol, acetone, methyl alcohol, etc. None of the fractions appeared to contain ischolesterol and they were therefore severally benzoylated. A number of crystalline benzoates were obtained, but none melting higher than 145°. A quantity of wool fat treated by the method of Darmstädter and Lifschütz also gave a negative result. An explanation of these results may be found in the observation of Cohen [1908] that ischolesterol undergoes a change of the nature of autoxidation on keeping. The somewhat drastic treatments to which commercial wool fat is subjected may therefore have destroyed the ischolesterol. The best method of obtaining it would seem to be to extract fresh clipped raw wool with ether, or to employ the resin of South African rubber.

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