CXV. THE FORMATION OF COPROSTEROL IN THE INTESTINE¹.

I. POSSIBLE RÔLE OF DIHYDROCHOLESTEROL, AND A METHOD OF DETERMINING DIHYDROCHOLESTEROL IN PRESENCE OF COPROSTEROL.

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CHOLESTEROL of exogenous or endogenous origin is eliminated with the faeces mainly in the form of coprosterol. Soon after the discovery of this compound by Bondzynski and Humnicki [1896-97] it was suggested that coprosterol is formed by bacterial hydrogenation of cholesterol in the intestine, but no definite proof of this theory has as yet been forthcoming. All attempts to convert cholesterol into coprosterol by bacteria *in vitro* have been unsuccessful.

Human faeces contain, besides coprosterol, small quantities of unchanged cholesterol and dihydrocholesterol [Windaus and Uibrig, 1915]. Schönheimer *et al.* [1930] have shown that dihydrocholesterol is formed from cholesterol in the body tissues (or synthesised there) and is continuously secreted into the intestine, but intensive search for coprosterol in the organs has failed to reveal it even in traces. It is possible that the intestinal bacteria effect the stereochemical inversion of the secreted dihydrocholesterol at carbon atom 5 rather than the hydrogenation of the double bond in cholesterol. Were this the case, ingested dihydrocholesterol should, at least in part, reappear as coprosterol

in the faeces. Feeding experiments designed to test this view can be carried out approximately quantitatively because neither coprosterol nor dihydrocholesterol can be absorbed from the intestine [von Behring and Schönheimer, 1930].

To this end a method for the quantitative determination of dihydrocholesterol in presence of coprosterol is necessary. As these two isomerides closely

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resemble each other in physical and chemical properties, few ways of doing this offer themselves. At first an attempt was made to estimate the amount of both sterols in a mixture with the aid of the fact that they are converted by sodium ethylate (or sodium in xylene) into the non-precipitable isomerides (epicholesterol and epicoprosterol) to a different extent. This method proved to be impracticable for analytical purposes as the values were not well reproducible. The method finally adopted involves the differential solubility of the digitonides of the two substances [Schönheimer and Dam, 1933]; coprosterol digitonide dissolves to the extent of 2 g. in 100 ml. of methyl alcohol, while the corresponding solubility of dihydrocholesterol digitonide is only 0.35 g. A curve can, therefore, be constructed whereby the composition of a mixture of the two can be determined directly from its solubility in methyl alcohol.

With the aid of this method it has been possible to show that dihydrocholesterol given by mouth is excreted unchanged and is not converted into coprosterol.

EXPERIMENTAL.

1. *Curves for the solubility of mixtures of the two sterol digitonides in methyl alcohol.*

The digitonides were prepared by precipitation of 0.5 % alcoholic solutions of the sterol mixtures with digitonin (50 % excess, time of precipitation 24 hours). On account of the solubility of coprosterol digitonide in ethyl alcohol, the precipitates were washed with 20 ml. of a saturated solution of coprosterol digitonide in 96 % alcohol and finally with 1 ml. of ether. For the solubility determination, quantities of about 135 mg. of the digitonide (dried at 110°) were weighed out in small flat-bottomed glass vessels and again dried; a loss of a few mg. was observed owing to the hygroscopic character of the substance. Then enough of the product was taken out to leave 127 ± 0.5 mg. in the vessel, which was placed in a narrow, stoppered glass cylinder and covered with a rubber cap, and the cylinder shaken overnight in a thermostat at $25 \pm 0.1^\circ$. The solution was then filtered and the dry residue determined in 2 ml. of the filtrate. Great care must be taken that the methyl alcohol is dry, for the least trace of moisture depresses the solubility of the digitonide. The methyl alcohol for these experiments was dried by boiling for 20 minutes with calcium turnings and distilled with the exclusion of moisture by means of a calcium chloride tube. Extreme precautions were also taken to avoid contamination by moisture during storage.

Since the separation of the saturated sterols from cholesterol by the method of Schönheimer involves preliminary treatment with bromine, a solubility curve was also constructed of the digitonides which had been precipitated in alcohol containing sufficient bromine to give it a yellow colour. The presence of bromine slightly raised the solubility of the digitonides (curve 2, Fig. 1). All the values were obtained with the same digitonin preparation, for digitonins of different sources are known to possess slightly different properties.

An attempt was made by this method to estimate dihydrocholesterol added to the mixture of saturated sterols isolated from faeces. The saturated sterols from the faeces of a human subject whose diet contained very little plant sterol were isolated after bromination by the method of Schönheimer [1930]. The resulting digitonide had a solubility of 43.8 mg. in 2 ml. Since pure coprosterol digitonide has a solubility of 41.7 mg. (when precipitated in presence of bromine) this finding indicates that traces of an unknown more soluble substance were present in the mixture. Attempts to isolate a more soluble saturated fraction

by fractional precipitation with digitonin of the mother-liquor from the preparation of coprosterol led only to a digitonide with a maximum solubility of 46.7 mg. in 2 ml. of methyl alcohol. In the feeding experiments a special curve (No. 3 in Fig. 1) was therefore employed which related not to the content of dihydrocholesterol in a mixture with pure coprosterol but to the composition of mixtures of dihydrocholesterol and the saturated sterol of faecal origin.

Fig. 1. Solubility of digitonides from coprosterol and dihydrocholesterol in absolute methyl alcohol at 25°, 127 mg. digitonide + 5 ml. methyl alcohol. 1. Digitonides precipitated without bromine. 2. Digitonides precipitated in presence of bromine. 3. Digitonide of sterol from human faeces + dihydrocholesterol, precipitated in presence of bromine.

The experimental findings are reproduced in Fig. 1. With the aid of these curves good recovery of added dihydrocholesterol was made from both coprosterol and the sterols of faeces. By means of curve 1 it was found that on hydrogenation of 0.2 g. allocholesterol with 0.2 g. of platinum oxide with hydrogen in cold ether, 55 % of coprosterol and 45 % of dihydrocholesterol were produced. The solubility of the digitonides of the reaction product was 23.8 mg. in 2 ml. of methyl alcohol.

2. Feeding experiments with dihydrocholesterol.

A healthy individual was kept on a constant diet of which the sterols were almost entirely of animal origin. The diet for each period consisted of 930 g. butter, 400 g. 30 % cream, 280 g. lean pork, 500 g. potatoes, 100 g. barley porridge, 732 g. zwieback and 40 g. Graham bread. During the experimental period he received a supplement of dihydrocholesterol approximately corresponding to the amount of total sterols excreted in the faeces. The total and saturated sterols were determined in the stools during a pre-experimental, an experimental and two post-experimental periods, and the solubility of the saturated sterol digitonides was measured. Each period occupied 5 days, of which the last was made a fast day in order to obtain a better differentiation of the periods (the day before the beginning of the experiment was also a fast day). The beginning of each period in the faeces was marked by a carmine tablet.

During the experimental period 3.45 g. of dihydrocholesterol, dissolved in butter, were taken, distributed through 12 meals. Immediately after defaecation the stools were ground with sodium sulphate and finely powdered, an accurately weighed sample (corresponding roughly to a 24-hour quantity) was extracted with ether and the amounts of total sterols and saturated sterols determined by Schönheimer's method. The precipitation of the digitonide of

the saturated sterols, employing 50 % excess of digitonin, was carried out after bromination in alcoholic solution. The solubility of the precipitates was determined, and the content of dihydrocholesterol calculated from curve 3, Fig. 1.

Table I. *Balance experiments with dihydrocholesterol.*

	Pre-experimental period	Experimental period 3.45 g. added dihydrocholesterol	1st post-experimental period	2nd post-experimental period
	g.	g.	g.	g.
Total unsaponifiable	5.35	9.91	7.22	5.71
Total sterol	4.23	8.42	5.88	4.26
Saturated sterol	3.65	7.41	5.20	3.42
Solubility of digitonides of saturated sterols in 2 ml. methyl alcohol:				
	mg.	mg.	mg.	mg.
Determination A	43.8	27.1	33.4	44.3
Determination B	43.9	27.6	35.0	43.6
Amount of dihydrocholesterol:				
Determination A	0 %	38 %	20 %	0 %
Absolute amount	0 g.	2.81 g.	1.04 g.	0 g.
Determination B	0 %	37 %	18 %	0 %
Absolute amount	0 g.	2.74 g.	0.94 g.	0 g.

From Table I it will be seen that dihydrocholesterol added to the diet was not excreted entirely during the experimental period, but a considerable portion appeared during the first post-experimental period. The whole amount of ingested dihydrocholesterol was recovered within these two periods. 3.85 g. dihydrocholesterol were found in the first determination and 3.68 g. in the second. The limit of error in the rather complicated experiment must however be considered relatively high, perhaps 10–20 %.

Under the influence of the relatively large quantities of ingested dihydrocholesterol the resorption of part of the cholesterol is apparently prevented, with resulting bacterial conversion into coprosterol (see the following paper), for during the pre-experimental period and the second post-experimental period 7.07 g. in all of saturated sterols were excreted. During the experimental and first post-experimental periods a total of 12.61 g. of saturated sterol was found, an increase of 5.54 g. Since 3.68–3.85 g. of this were found to consist of dihydrocholesterol, the missing 2 g. must have consisted of coprosterol. This cannot have been formed from the dihydrocholesterol inasmuch as the latter was recovered practically quantitatively.

The inhibition of the resorption of cholesterol by the ingestion of dihydrocholesterol can be explained on physical grounds, for cholesterol and dihydrocholesterol are known to form mixed crystals which cannot be separated by physical methods [Schönheimer, 1930; Lettré, 1932].

The amount of non-sterol unsaponifiable matter excreted in the experimental and first post-experimental periods is increased only to a small extent (10 %) over the corresponding excretion during the experimental and first post-experimental periods. This is barely beyond the limit of variation and thus supports the view that the non-sterol unsaponifiable matter does not originate from unresorbed cholesterol to more than a small extent.

SUMMARY.

A method is described for the determination of dihydrocholesterol in presence of coprosterol depending on the differential solubility of the corresponding digitonides in methyl alcohol.

With its aid it is shown that dihydrocholesterol added to the diet is not converted into coprosterol but reappears unchanged in the faeces.

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REFERENCES.

- von Behring and Schönheimer (1930). *Z. physiol. Chem.* **192**, 97.
Bondzynski and Humnicki (1896-97). *Z. physiol. Chem.* **22**, 396.
Lettré (1932). *Liebig's Ann.* **495**, 41.
Schönheimer (1930). *Z. physiol. Chem.* **192**, 77.
—— von Behring and Hummel (1930). *Z. physiol. Chem.* **192**, 73.
—— and von Behring (1930). *Z. physiol. Chem.* **192**, 102.
—— and Dam (1933). *Z. physiol. Chem.* **215**, 59.
Windaus and Uibrig (1915). *Ber. deutsch. chem. Ges.* **48**, 857.