CXLIII. THE DISTRIBUTION OF STEROLS IN HUMAN FAECES.

AN EXAMINATION OF ILEAL CONTENTS.

By JOHN ADDYMAN GARDNER, HUGH GAINSBOROUGH
AND RUTH MONA MURRAY.

From the Biochemical Department, St George's Hospital.

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LONG ago Flint [1862; 1868] observed that cholesterol undergoes a modification in the human intestine, and that it is found in the faeces as "stercorine." This substance, rediscovered by Bondzynski [1896] who gave it the name of "coprosterine," was more fully described by Bondzynski and v. Humnicki [1896]. The formula $C_{27}H_{48}O$ was ascribed to it and confirmed by the analysis of derivatives, and its discoverers regarded it as a dihydrocholesterol formed by bacterial reduction in the intestine. Support was lent to this hypothesis by the observation of Müller [1900] that in a patient kept for a long time on a milk diet, which presumably altered the intestinal flora, the sterol of the faeces was excreted as unchanged cholesterol. Later, Gardner [1921] isolated from human faeces, in addition to coprosterol, small quantities of β -cholestanol and cholesterol. These substances were found not only in the mixed faeces of a village community but also in the excreta of soldiers in training. Recently, Schoenheimer [1934] has perfected an improved method suitable for small quantities of material for the isolation of cholesterol from mixtures of β -cholestanol and coprosterol and has confirmed the presence of cholesterol in human faeces.

Fox and Gardner [1925] published the result of a long series of experiments on the cholesterol metabolism of breast-fed infants and found that the crystalline sterol of the faeces consisted only of cholesterol. At what stage of life the change of the faecal sterols, from the cholesterol of infant's faeces to the mixture of coprosterol, β -cholestanol and cholesterol characteristic of adult human faeces, takes place has yet to be determined.

Some evidence has accumulated as to the origin of the different forms of faecal sterol. The observation of Boehm [1911] that an isolated loop of intestine, accidentally produced in a woman under operation and left *in situ* for years, contained sterile material, the sterol content of which was solely β -cholestanol, suggests a secretion of this sterol through the wall of the intestine into its lumen. Beumer and Hepner [1929] found cholesterol in the colonic contents of a bile fistula dog fed on a lipid-free diet and concluded that cholesterol was secreted by the large intestine. Schoenheimer and v. Behring [1930] examined the secretions of the isolated large intestines of a series of dogs. After suitable washing out of the secretions and sealing of the intestinal tube for a length of time, he finally obtained a cheesy material, similar to that obtained by Boehm, containing sterol of which 52 % was saturated, and showed it to be mainly β -cholestanol, the remainder being cholesterol. Sperry [1926-27] fed dogs which had had a bile fistula made, on a lipid-free diet, and the unsaponifiable matter obtained from the faeces was afterwards closely examined by Schoenheimer and Sperry [1934]. They concluded that, despite a sterol-free diet and the total exclusion of bile

from the intestine, coprosterol was the principal sterol excreted by their dogs; only 11 % of the total sterols excreted was unhydrogenated (probably cholesterol); β -cholestanol was also present. They considered that in such dogs coprosterol must be formed from cholesterol which is secreted into the intestine through the intestinal wall. It is to be noted that in Boehm's and Schoenheimer's experiments, where β -cholestanol appeared to be the predominant sterol, the intestinal contents were sterile.

The question arose as to how and where the faecal coprosterol was produced. It is to be remembered that coprosterol is not of the same stereoisomeric series as cholesterol, the direct hydrogenation product of which is β -cholestanol. So far the only explanation offered is that coprosterol is produced from cholesterol by bacterial action, and the only evidence available here is that of Müller mentioned above and that of Dam [1934] who thought he could show an increased formation of coprosterol in faecal putrefaction experiments in which cholesterol had been added, though the production of coprosterol here was only inferred from the solubility curve of the digitonide and the increase measured by Schoenheimer's method, which is possibly scarcely accurate enough to permit such conclusions to be drawn.

We feel by no means certain that the bacterial hydrogenation of cholesterol to produce coprosterol, if true, is the whole story. We have been amazed at the rapidity with which large quantities of coprosterol can be produced under certain circumstances. Dorée and Gardner [1908] made an extensive series of feeding experiments on a trained dog, using a number of different diets for long periods. It has been necessary to reinterpret the findings in that paper in the light of the greater knowledge of to-day, and we can safely conclude that the sterol matter isolated from the dog's faeces when the animal was fed on diets containing cholesterol in what might be considered normal amounts consisted of a mixture of cholesterol and β -cholestanol. Coprosterol was not isolated. However, when this same animal was fed on a diet of cooked sheep's brains, it excreted quantities of coprosterol. The actual quantity isolated in apparently pure condition was 1.2 g. per day, whereas approximately only 1 % of the total sterols isolated consisted of a mixture of cholesterol and β -cholestanol. Similar experiments by Ellis and Gardner [1909] with cats produced similar results, except that the cat, on a normal diet, produced coprosterol more easily than the dog. The contrast is very marked in the case of the herbivora [Dorée and Gardner, 1908] with which in no case could any trace of animal sterol be found in the faeces. It is curious that the normally fed dog should excrete cholesterol and β -cholestanol but no isolable coprosterol, and yet when fed on sheep's brains a mechanism should be available, of which there was no previous sign, which could produce such large quantities of coprosterol. If the mechanism is bacterial then why is there so little evidence of coprosterol formation with an ordinary diet? It is conceivable that on the brain diet a different intestinal flora appeared, but this explanation does not seem very likely, because the dog after feeding on sheep's brains showed a return to normal faecal sterol distribution on the withdrawal of that diet. In other words, if the reduction of cholesterol to coprosterol can be so extremely efficient in the brain-fed animal, why should the normally fed dog excrete so much cholesterol and β -cholestanol? At the same time (1908) it was shown that the total sterol excreted was much less than the sum of the total intake *plus* the biliary cholesterol, whence it was inferred that, as it seemed unlikely that cholesterol was destroyed in the gut, cholesterol was being absorbed in the alimentary canal. This has been confirmed, though coprosterol and β -cholestanol have been shown by Schoenheimer *et al.* [1930] not to be so

absorbed from the intestine. We suggest that the mechanism of coprosterol formation shown by the dog and the cat protects the animals from the effects of over-absorption of cholesterol which can be so readily observed in the case of rabbits, where cholesterol is avidly taken up from the intestine, eventually producing a lipoid infiltration of the organs and tissues.

The question as to the site of coprosterol formation in man seemed to us easy of solution, but there have been many difficulties in obtaining uncontaminated ileal faeces from surgical cases. However, the opportunity finally presented itself of examining two cases in which ileostomy had been performed. In 1930 we had analysed some caecostomy faeces from a case of ulcerative colitis and obtained a clear-cut result, but as the faeces had already entered the colon we did not consider the unsupported result sufficiently significant. In this case 0.1 g. of crystalline material was obtained directly by crystallisation from the unsaponified matter of the ether extract and a further 0.12 g. after treating the residues with digitonin and subsequently splitting the digitonide in the usual way. The former portion melted at 146–147°, had $[\alpha]_D^{20} - 40.7^\circ$ and was clearly cholesterol; the portion obtained by digitonin had melting point 142–143°, and $[\alpha]_D^{20} - 31.6^\circ$ and was found to contain about 10 % of saturated sterol, probably β -cholestanol. No trace of coprosterol was isolated.

The ileal contents examined were from the following two cases:

M. C. Female, aet. 32. Ulcerative colitis. A caecostomy had been performed in August 1933 and, as only temporary benefit was obtained, a transverse ileostomy was made in July 1934 about 6 in. from the ileal termination.

L. H. Female, aet. 30. Ulcerative colitis. A caecostomy had been performed in May 1928; this was remade in November 1930, but as symptoms recurred later a transverse ileostomy was made in November 1933. This case unfortunately had a small ileo-caecal fistula, but it is unlikely that caecal contents could have flowed back into the ileum, hence the ileostomy discharge was considered to be uncontaminated ileal contents.

In both cases no grease of any kind was used to protect the skin of the abdomen; a kaolin paste was used for this purpose and great care was taken that no caecostomy discharges were included in the ileal excreta.

Exp. 1. Ileal faeces from M. C. were collected during the whole of May and June 1934. These were dried partly *in vacuo*, partly by heat, and as it was difficult to get rid of all the moisture the faeces were soaked in alcohol first cold, then warm. The alcoholic extracts were evaporated to dryness and the residue extracted with ether. The dried faeces were then exhaustively extracted with ether in a special Soxhlet apparatus without rubber connections, corks or taps. The two ether extracts were put together and made up to known volume. The solution was dark green and absorption bands indicated that this was due to chlorophyll. This extract after suitable concentration was hydrolysed with sodium ethoxide in alcoholic solution, the unsaponifiable matter extracted in the usual manner and this material crystallised from methyl alcohol. Successive crops of crystalline material, which very closely resembled cholesterol, were separated and 4.63 g. were obtained. The residual oil obtained from the mother liquors weighed 1.03 g.; this was precipitated by digitonin in the usual manner, and 0.13 g. of cream-coloured crystals was obtained by splitting the digitonide. The total crude sterol was altogether 4.76 g. This was brominated by the Windaus method and 4.67 g. of the dibromide were obtained, m.p. 112–113°. This was debrominated with zinc dust and acetic acid and after repeated crystallisation 1.77 g. of typical cholesterol crystals were obtained; m.p. 144–145°. This was again brominated, yielding a dibromide of m.p. 113–114°, and finally a sample of cholesterol was obtained (0.35 g.), of m.p. 146–147° and $[\alpha]_D^{19} - 36.8^\circ$. Further

examination was made of the second crops of dibromide that were precipitated by water in both brominations. These substances after debromination were converted into the acetates and crystalline substances obtained, m.p. 117–118°, which on recrystallisation yielded pure cholesteryl acetate. Clearly some phytosterol was present but in quantity insufficient for isolation by the method suggested by Windaus[1906]. The mother-liquors were similarly examined with identical results.

Thus the great bulk of the original crude sterol consisted of cholesterol, but after much search only traces of phytosterol were detected and no β -cholestanol or coprosterol could be isolated.

Exp. 2. 115 g. of dried ileal faeces were obtained from L. H. These were extracted with ether, and the total ether extract consisted of a brown oil weighing 48 g. This, after saponification, yielded 40 g. of unsaponifiable material and it was then discovered that instructions in the ward had been disregarded and the patient had been given paraffinum liquidum by mouth. A digitonin estimation on an aliquot part of the ether extract showed that it contained 0.796 g. sterol in the whole specimen. The unsaponifiable matter was then dissolved in alcohol, precipitated with digitonin, left overnight, the separated digitonide filtered off and washed free from "paraffin" with ether. The digitonide so obtained was decomposed by the pyridine-ether method and the recovered sterol crystallised from methyl alcohol in faintly pink plates. The first crop consisted of 0.6 g. cholesterol, m.p. 144–145°, and this, recrystallised, yielded 0.46 g. of colourless crystalline cholesterol, m.p. 146–147°, with $[\alpha]_D^{20} - 37.7^\circ$. Further portions from this recrystallisation gave material with m.p. 143–144°. The second crop (0.07 g.), the third crop consisting of brownish crystals in oil, some further material obtained from some digitonide which had been finally separated from "paraffin" with great difficulty and all the residues from the first crop were mixed together, weighing 0.22 g. in all. This was brominated by Schoenheimer's method, the dibromide filtered off, digitonin in alcohol added and after 18 hours 0.028 g. of digitonide was deposited, and the filtrate yielded after a further 24 hours 0.036 g. of digitonide. These were separately decomposed by pyridine and ether, the first yielding 0.007 g., and the second 0.005 g. These were added together and crystallised from methyl alcohol, yielding plates which gave a strong Burchardt-Liebermann reaction. A solution of the whole material in chloroform was compared by means of this reaction with standard solutions of cholesterol and it was found that over 75 % of the material was cholesterol.

Evidently the bulk of the material in this case was cholesterol and no β -cholestanol or coprosterol could actually be isolated, though it was very likely that some traces of β -cholestanol were present.

Exp. 3. It was thought wise to repeat the analysis of further material from M. C., taking strict precautions as regards eliminating paraffin administration, and eliminating green vegetables and fruit from the diet in order to reduce the intake of phytosterols as far as possible. 620 g. of dried ileal excreta, collected during November and December 1934, were thoroughly extracted with ether and 36.2 g. of ether-soluble matter obtained. The total sterol in this was estimated by digitonin in an aliquot part and amounted to 7.08 g. After saponification of the ether extract 6.6 g. of unsaponifiable matter were obtained and this was recrystallised from methyl alcohol. Crop I (5.015 g.) was further purified and crop I' (3.22 g.) of pure cholesterol was thus obtained with m.p. 147°, $[\alpha]_D^{20} - 35.3^\circ$, and from the mother-liquors of this 1.29 g. sterol were obtained, m.p. 139–140°, $[\alpha]_D^{20} - 37.6^\circ$. The pure sample (crop I') was brominated by the Windaus method and yielded an initial precipitate (A) of dibromide (3.8 g.),

m.p. 112–113°, and a further crop (B), by adding water (1.25 g.). From A, after reduction, 1.6 g. of pure cholesterol were finally obtained, m.p. 148°, clearing at 150°, and the material from the mother-liquors was evaporated to dryness and benzoylated by the pyridine method, yielding a solid melting at 147°, clearing at 173°; a further sample from the mother-liquors melted at 146° and cleared at 168°. Neither of these benzoates on melting and cooling showed any play of colours. The material B yielded no crystalline matter after reduction and it was therefore all benzoylated as before and a sterol benzoate obtained melting at 146° and clearing at 171° without any play of colours. Crop II from the crystallisation of the crude unsaponifiable matter consisted of 0.65 g. of orange stained crystals. From this 0.49 g. of a benzoate was obtained melting at 141° and clearing at 167° without any blue flash but on cooling there was a quick play of colours. This effect was matched by a sample of cholesteryl benzoate to which 5 % of β -cholestanyl benzoate had been added. Small additional crops from this crystallisation showed the same behaviour. Crop III from the unsaponifiable matter consisted of 0.65 g. of orange crystals mixed with oily matter. Some of this was unfortunately lost but 0.15 g. of a benzoate was obtained, m.p. 129–133°, clearing at 168°, showing similar colour changes to the material from crop II but weaker.

This experiment amply confirms the previous findings that only cholesterol can be isolated but that traces of β -cholestanol can be detected.

It may be noted here that cholesteryl benzoate made from pure cholesterol does not show any trace of play of colours on melting and cooling, whereas addition of β -cholestanol benzoate to cholesterol benzoate in increasing proportions produces such phenomena on melting. This can be used for a rough indication of the amount of β -cholestanol in a sample of cholesterol by comparison with artificial standard mixtures.

It had occurred to us some years ago that the sterol distribution in human faeces might be affected by the presence or absence of bile. We collected the clay-coloured faeces of two cases of complete biliary obstruction and recovered the sterols therefrom. The analyses were not carried out to the ultimate fractions but it became clear that the bulk of the faecal sterols in these cases consisted of coprosterol as in normal subjects.

DISCUSSION.

It is quite clear from these three analyses of ileostomy excreta and one of caecostomy excreta that the sterol content is quite different from that of normal human faeces. From the former only cholesterol can be isolated and traces of β -cholestanol detected, whereas the latter contain sterol mainly as coprosterol, smaller quantities of β -cholestanol and traces of cholesterol. It follows that the production of coprosterol must occur in the colon. The earlier experiments of Dorée and Gardner showed that the dog on a normal diet excreted mainly cholesterol in its faeces and the cat cholesterol and coprosterol. We realise now that as the apparently pure cholesterol isolated in these cases yielded benzoates which showed marked colour changes on melting there must have also been some β -cholestanol present. The mechanism of coprosterol formation was easily brought into evidence by feeding the animals on a diet of sheep's brains, and it is difficult to understand how such an action can be due solely to bacterial hydrogenation for, if this were the case, there is no obvious reason why this action should remain latent in normally fed animals, in which little or no coprosterol is formed.

SUMMARY.

1. Coprosterol could not be isolated from intestinal contents discharged from either a terminal ileostomy or a caecostomy.
2. It follows that coprosterol formation must occur in the colon.
3. We think that the hypothesis that coprosterol formation results from the bacterial hydrogenation of cholesterol is insufficient.

We wish to express our thanks to our colleagues at St George's Hospital for allowing us access to these cases.

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Note. In our last paper, *Biochem. J.* (1934), **28**, 1635, we regret that we inadvertently stated that Schoenheimer had found up to 10 % of β -cholestanol in gall stone cholesterol. This figure should have been 1.0 %. We thank Dr Schoenheimer for drawing our attention to this error.