The Origin and Destiny of Cholesterol in the Animal Organism. Part X.—*On the Excretion of Cholesterol by Man, when fed on Various Diets.*

By G. W. Ellis and J. A. Gardner.

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(From the Physiological Laboratory of the University of London, South Kensington.)

In earlier papers of this series we have shown that cholesterol is never excreted in the normal fæces of herbivorous animals such as horses, cattle, sheep, and rabbits. In the case of carnivora such as dogs and cats, provided the body weight remains constant, the cholesterol excreted in the fæces can be all accounted for by that naturally ingested with the food. Klein in his experiments also arrived at a similar conclusion. Evidence was also brought forward which rendered probable the view that, in herbivora, at any rate, cholesterol is a substance which is strictly conserved in the animal economy, that when the destruction of the red blood corpuscles and possibly other cells takes place in the liver, their cholesterol is excreted in the bile, and that the cholesterol of the bile is re-absorbed in the intestine along with the bile salts, finding its way into the blood stream to be used in cell anabolism; further, that any waste of cholesterol might be made up from that taken in with the food. This latter process would be limited in herbivorous animals by the fact that their normal food does not contain cholesterol, but isomeric substances such as phytosterol, which have to be converted into cholesterol before utilisation, and in carnivorous animals by the partial, or even complete, change of cholesterol into coprosterol which takes place under certain dietetic conditions. In man, under normal conditions, cholesterol is never excreted as such in the fæces, but always in the form of coprosterol. It seemed therefore desirable to estimate the amounts of coprosterol found in the fæces of man under various dietetic conditions. The opportunity of making such investigations was very kindly afforded us by Dr. R. H. A. Plimmer, who handed over to us the dried fæces collected during a series of experiments carried out in the Physiological Institute, University College, London, and published in the 'Journal of Physiology,' August 26, 1909, under the title of "A Metabolism Experiment, with Special Reference to the Origin of Uric Acid," by R. H. Aders Plimmer, Maxwell Dick, and Charles C. Lieb.

The subject of the experiment was a healthy man, aged 39. The three

diets selected were chosen so that each yielded 110 grm. protein, 240 grm. carbohydrate, and 100 grm. fat per diem. The carbohydrate and fat constituents consisted of potato and butter, and the protein constituents of (1) beefsteak, (2) egg-white, or (3) herring-roe.

The experiment was commenced with an ordinary mixed diet for one week. After this the beefsteak diet was administered, and this was followed by the egg-white. After one week the subject unfortunately suffered from an attack of influenza, so that the experiment had to be discontinued for about 10 days, though analysis of urine was continued except for a period of four days. The egg-white diet was then taken for a period of 35 days. The herring-roe diet concluded the experiment, but this diet was taken only for three days owing to its bad effect on the patient. The fæces in this last period were not kept separate, but included with those obtained during the 35 days of egg-white diet. For further details as to the experiment the reader is referred to the original paper.

Cholesterol Contents of Various Diets.

The cholesterol content of the constituents of the various diets was determined by the digitonin method in the usual manner.

Diet I.—Total per diem :—

Beefsteak	500 grm.
Potato	800 „
Butter	100 „
Sugar.....	80 „

Meat, according to our own observations and those of others, may be taken as containing not less than 0·0685 per cent. of cholesterol—free and combined.

Butter, according to Magnus-Levy, contains 0·19 per cent. A sample of " best dairy butter " obtained from Harrods' Stores was found, by the digitonin method, to contain 0·1744 per cent. of cholesterol—free and combined.

The total cholesterol ingested in Diet I would, therefore, be at least $0{\cdot}3425 + 0{\cdot}1744 = 0{\cdot}5169$ grm. per diem.

Diet II.—Total per diem :—

Egg-white	800 grm.
Potato	800 „
Butter	130 „
Sugar.....	40 „

It was found that neither potato nor egg-white contains any trace of cholesterol. The total cholesterol ingested was, therefore, 0·227 grm. per diem.

Diet III.—Total per diem:—

Herring-roe	500 grm.
Potato	800 „
Butter	130 „
Sugar.....	80 „

100 grm. of tinned herring-roe (soft) of the kind taken during the experiment was found to contain 0·595 per cent. of cholesterol—free and combined. The total cholesterol ingested was, therefore, 2·975 + 0·227 = 3·202 grm. per diem.

Treatment of the Fæces.

The fæces were supplied to us in a *dry*, finely powdered condition. Those from each series were thoroughly extracted with ether in a Soxhlet apparatus, and the fatty matter in the ethereal solution saponified with sodium ethylate. After separating the soap, the ethereal solution was thoroughly washed with water and evaporated to dryness. From the residues it was found possible to isolate a quantity of pure coprosterol by fractional crystallisation from alcohol. The mother-liquors were evaporated to dryness, and the residual coprosterol benzoated by means of benzoyl chloride in pyridine solution. The fæces from Series I, III, and IV, containing an excessive amount of oily impurity, rendered the isolation of pure coprosterol benzoate so difficult that the residues were further treated with digitonin, and in this way an amount of digitonin coprosteride was obtained. In order to be certain that the compound so formed was none other than the coprosteride, we recovered the coprosterol in combination by means of the xylene method.

Results of Analysis.

Series I.—In this series the subject was fed on an ordinary mixed diet for seven days. Five stools were passed, and yielded 167·7 grm. of dry material. The patient's weight was practically constant, varying from day to day from 75·8 to 76·2 kgrm.—average 75·99.

The fæces yielded on extraction 6·7445 grm. of unsaponifiable matter, from which 4·1669 grm. of coprosterol were obtained. This would correspond to a yield of 0·595 grm. per day. This daily output corresponds very closely to that found in the cases of one of us and another colleague on liberal diet from observations extending over a year. Fæces from a public latrine, however, yielded a smaller quantity.

Series II.—The subject was then fed for seven days on Diet I. His average weight was 75·3 kgrm., and varied on six of the days as follows: 76, 75·3, 75·3, 75, 75, 75·6. Four stools were passed, corresponding to

118·1 grm. of dry fæces. On extraction, this yielded 4·492 grm. of unsaponifiable matter, from which 3·3306 grm. of coprosterol were obtained. The balance sheet works out as follows:—

Total cholesterol (free and combined) ingested with food ...	3·6183	Total coprosterol excreted	3·3306
Intake per diem	0·5169	Output per diem	0·4758

Difference, 0·0411 of cholesterol per diem absorbed.

Series III.—Then followed seven days on Diet II. During this period the patient was sickening for an attack of influenza. His weight varied as follows: 75, 74·6, 74·2, 74·3, 74·3, 74·2—average 74·4 kgrm. The average loss in weight from the average of the previous period was thus 900 grm., or an actual loss during the week of 800 grm. Three stools were passed, containing 118 grm. of solid matter. This yielded 3·9605 grm. unsaponifiable matter, from which were obtained 2·6505 grm. of coprosterol. The balance sheet works out as follows:—

Total cholesterol ingested	1·587	Total coprosterol excreted	2·6505
Intake per diem.....	0·2267	Output per diem	0·3786
Excess of cholesterol excreted			1·0635
„ „ per diem			0·1519

If we reckon the loss in weight as due to fat and protein tissues the loss of cholesterol in this way would be from 0·45 to 0·8 grm., which would largely account for the excess excreted over intake.

Series IV.—During the next five days the patient suffered from influenza. Two meals of Diet II were taken and 35 grm. of dry fæces were obtained. The weights of the patient were as follows:—74·2, 72·6, 73·2, average 73·3. The fæces excreted during the first four days yielded 1·7638 grm. of unsaponifiable matter, from which 1·3109 grm. of coprosterol was obtained.

Total cholesterol ingested during period	0·4534	Total coprosterol excreted	1·3109
Excess of cholesterol excreted			0·8575
„ „ per diem			0·2144

The patient lost during illness 1·5 kgrm., so that about 1 grm. of cholesterol might be accounted for in this manner, and the excess of output over intake explained.

Series V.—During recovery from illness the patient was fed for six days on an ordinary mixed diet, after which the experiment was continued. He was fed for 33 days on Diet II, with addition of various salts, for one day on 100 grm. egg-white and 250 grm. boiled-out meat, for another day on 250 grm. of boiled-out meat, and for three days on a total of 400 grm. egg-white + 1250 grm. herring-roe.

The daily weights of the patient showed a steady decrease during this long period—from 73·8 to 70·7 kgrm.—a total loss of 3·1 kgrm. The total weight of dry fæces passed during the period was 665 grm. This yielded 20·2195 grm. of unsaponifiable matter, from which 14·7324 grm. of coprosterol were obtained.

Total cholesterol ingested with food	14·920	Total output of coprosterol	14·7324
Ditto intake per diem	0·393	Output per diem	0·3877

Excess of intake over output per diem, 0·0053.

The loss in weight during this experiment amounts to about 82 grm. per diem. This is considerably less than the loss in Series III and IV, which is about 114 grm. and 250 grm. per diem respectively; there must, therefore, have been an absorption of cholesterol going on in the intestines during the period.

Conclusion.

It would appear from these experiments that in man, as in the case of other animals, the excretion of cholesterol in the fæces can be accounted for by that taken in with the food, provided that the body weight remains constant. If, however, a *rapid* loss in weight takes place, as in illness, the output of cholesterol exceeds the intake. Further work will, however, be necessary before this view can be regarded as fully established.

Note on the Sterol Contents of Rabbit Fæces.—In Part VIII of this series of papers we described an experiment in which we succeeded in isolating cholesterol by the digitonin method from the fæces of a rabbit which had been fed on extracted bran, but into the peritoneal cavity of which olive oil had been injected. The animal in question had lost nearly a third of its weight during the experiment. In consequence of this result it was thought desirable to submit the fæces of rabbits fed on extracted bran, but which were not losing weight, to a more careful examination. For this purpose four rabbits were fed for about ten days on bran which had previously been roughly extracted with ether. The animals remained during the experiment perfectly constant in weight. About 1200 grm. of air-dried fæces were obtained. These fæces were extracted with ether and treated in the manner already described, and yielded about 3 grm. of unsaponifiable matter in the form of a stiff oil. This oil was dissolved in alcohol and mixed with excess of digitonin in alcoholic solution. The precipitate was filtered and washed with ether to get rid of oily matter, and repeatedly boiled out with methyl alcohol, in which it proved very insoluble. The oily matter separated from the compound did not give any sterol colour reaction in

chloroform solution with acetic anhydride and sulphuric acid. The digitonin precipitate, which the above treatment had not freed from traces of some fluorescent colouring matter, was finely powdered and decomposed by heating in xylene vapour. The clear xylene solution on evaporation gave a yellow, crystalline, oily solid. This was purified by repeated crystallisation from dilute alcohol, from which it separated when pure in microscopic hexagonal plates. It melted at 136°–137° C. and gave the usual sterol colour tests. The acetate, made in the usual manner with sodium acetate and acetic anhydride, crystallised from alcohol in glistening leaves. It was less soluble in alcohol than the original substance. It melted at 135°–136° C., but if heated very slowly, at about 130° C., the benzoate, made by the action of benzoyl chloride in pyridin solution, crystallised readily from strong alcohol, in which it was sparingly soluble. It melted at 142° C. to a clear liquid, which on cooling gave at the moment of solidification a brilliant green play of colours, gradually changing to brown. This substance was one of the phytosterols of the bran, which had not been eliminated in the rough extraction with ether. The same substance was obtained by Dorée and Gardner from horse dung.

No trace of cholesterol was discovered. This bears out our previous conclusion that cholesterol is not found in the normal fæces of herbivorous animals.

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