CXXIX. STUDIES ON THE CHOLESTEROL CONTENT OF NORMAL HUMAN PLASMA.

PART III. ON THE SO-CALLED ALIMENTARY HYPERCHOLESTEROLAEMIA.

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As far as the exogenous portion of the blood cholesterol is concerned, the work of the past 20 years has made it perfectly clear that in animals most of the cholesterol which is absorbed from the food reaches the blood stream *via* the lymphatic channels and the thoracic duct [Müller, 1915, 1916]; some, however, is absorbed directly from the intestines. It would appear probable that, if the cholesterol content of the blood is increased by this process, the increase would be mainly if not entirely in the plasma. The red blood corpuscles contain their cholesterol almost wholly in the free condition and probably as an integral part of the cell structure. Experiments which have been made on pure isolated red blood cells indicate that their cholesterol content is remarkably constant in a given animal in health or disease. The experimental data are not, however, numerous, and the conclusion has been denied by observers who have determined the cholesterol content of these cells indirectly by estimating the cholesterol, free and ester, of the whole blood and also of the plasma, using the haematocrit to ascertain the proportion of red cells in the whole blood. Indirect methods of this type lead to results which are open to serious doubt owing to inherent errors in the estimation of the true volume of the red cells by the haematocrit or other methods. On the other hand, the isolation of pure red blood corpuscles perfectly free from plasma constituents is a tedious and difficult operation.

In the human subject the data in the literature on the question of alimentary hypercholesterolaemia are very conflicting. Widal, Weill and Laudat [1912] stated that in healthy subjects, who had fasted for 15 hours, a meal consisting of meat, potatoes, bread and 50 g. of butter caused a marked increase in the cholesterol of the blood. Grigaut [1913], commenting on these results, pointed out that the actual total increase of cholesterol in the whole of the blood was many times greater than the amount of cholesterol ingested. Hence he suggested, without other evidence, that a certain quantity of cholesterol is formed at the expense of the ingested fat. He also pointed out that, in contrast

to pathological hypercholesterolaemia, alimentary hypercholesterolaemia is transitory and there is a return to normal soon after the cessation of the special feeding. Rouzaud and Cabanis [1913] fed 11 healthy individuals with a meal containing soup, bread, meat, green vegetables, two eggs and wine, and found no difference in the cholesterol of the blood taken before and 4 to 5 hours after this meal. There was one case which showed an increase of 5 %. Luden [1917], in experiments on herself, found that, whereas a light meal of bread and butter and tea had no effect on the blood cholesterol, a somewhat heavier meal containing bread, butter, tea, one egg and half a cantaloup produced an increase of blood cholesterol of about 25–30 %. Here again, if we calculate from the figures given, the increase in blood cholesterol is greater than could be accounted for by the cholesterol ingested. She also states that on prolonged feeding with a vegetable diet, blood cholesterol is decreased, and with prolonged feeding with meat, blood cholesterol is increased. Denis [1917] fed three men with a heavy fat meal and found that no increase of blood cholesterol followed.

In all these experiments, however, the total cholesterol only was estimated, as though free cholesterol and ester cholesterol had an identical significance, an assumption by no means proven. Further, the estimations were made by colorimetric methods against which serious objections have been raised.

Bürger and Habs [1927] reported the results of feeding "starving" patients, suffering from various complaints, though presumed to be metabolically sound, on a breakfast consisting of 100 g. of olive oil in which had been dissolved 5 g. of cholesterol or its equivalent of cholesterol esters. They found in the serum a marked hypercholesterolaemia, reaching a maximum about 4 hours after the meal. This more or less rapidly decreased again during 24 hours to normal. The ratio of free to ester cholesterol during these experiments was stated to remain constant, though the figures in some of the cases scarcely bear out this conclusion. Although they used Windaus's method of estimating the extracted sterols, a consideration of their methods and the rather low results they obtained suggests to us that the mode of extraction used was probably inefficient. There is however the further difficulty of drawing conclusions, which are applicable to normal states, from experiments in which a meal of such an unphysiological nature is fed. In these experiments the excess of fat has clearly increased the rate of absorption and quantity of the ingested sterols absorbed.

It seemed to us desirable to investigate the subject of alimentary cholesterol-aemia more closely by means of the digitonin method of estimation, which permits of separate estimation of the free cholesterol and that in form of esters. The details of the methods used have been fully discussed in Part I of this series [1927, 1].

EXPERIMENTAL.

In experiments dealing with the effect of a single meal, it is not surprising that the results should be somewhat inconclusive, for simple calculation will

show that a meal containing, say, 0.2 g. of cholesterol, even making the improbable assumption that the latter is completely absorbed, could only raise the average content of the plasma of a man weighing 70 kg. by less than 0.007 %, which is scarcely beyond the limits of the error of experiment.

However, our experiments show that it is possible to vary the cholesterol level of the plasma in man, just as in animals, by sufficiently prolonged feeding with diets of high or low sterol content, the blood specimens for analysis being *always* taken when the subject is in a *fasting condition* (e.g. before breakfast) so as to eliminate, as far as possible, any disturbing factors, such as digestion or muscular work, which might affect the general composition of the plasma. The following experiment illustrates this.

The subject of the experiment was a man, aged 40, weighing about 8 stone, who had been in hospital for some time with a mild colitis and was convalescing. He had been fed for 24 days on a light hospital diet (A) consisting of bread, butter, milk and puddings, of approximately 1900–2000 calories per day; the cholesterol content of the diet was calculated to be approximately 0.32 g. per day. During the following 7 days he was put on a diet (B) which was almost sterol-free and consisted largely of white of egg, potato and bread, supplemented by porridge and treacle, and an apple or orange. For drink he had bovril, tea and beer. The daily calorie value was about 1500 and the sterol content approximately 0.06 g. Care was taken that this diet was cooked in as appetising a manner as possible, with the result that the patient ate it readily and made no complaint. During this period he lost 2–3 lb. in weight. He was then, for a further period of 7 days, given an ordinary full hospital diet (C), enriched in cholesterol by the addition of about 200 g. of calves' brain daily. The calorie value was about 2500 and the sterol content about 4.0 g. per day. His weight remained unchanged. For the following period of 11 days he was given a full diet (D) consisting of fish, two eggs, bread, butter and potatoes and fresh milk; the calorie value was about 2200 and the sterol content about 0.94 g. daily. During the whole of the experiment the patient remained in bed and the samples of blood for analysis, of about 20–25 cc., were taken at the end of each experimental period at about 11.30 a.m., the patient having only had a cup of tea at 6.0 a.m. As far as possible physiological requirements were carefully considered in constructing the diets, but of necessity the low cholesterol diet was deficient in fat and the brain diet had an excess of ether-extractable matter.

The results are given in Table I.

Table I.

Diet	Approximate sterol intake per day (g.)	Duration of diet (days)	% of cholesterol in plasma taken at end of feeding period and "starving"			% of total cholesterol as ester
			free	ester	total	
A	0.32	24	0.0500	0.0653	0.1153	56.6
B	0.06	7	0.0400	0.0319	0.0719	44.3
C	4.0	7	0.0413	0.0808	0.1221	66.1
D	0.94	11	0.0504	0.1044	0.1548	67.5

It will be noticed that the free cholesterol of the plasma maintains approximately the same level even after such totally different diets as B and C, but on the other hand the amount of ester cholesterol in the plasma appears definitely to follow the cholesterol content of the food. It will be noticed, however, that the increase of ester cholesterol with the brain diet is not so much as with the more normal diet D. Possibly the explanation of this is that brain is not so easily absorbed as other forms of food, or that the brain cholesterol is more readily reduced in the intestine to coprosterol and so not absorbed. In this connection we may recall a similar effect obtained by Dorée and Gardner [1908] in experiments on dogs. Also if Wieland and Sorge's "choleic acid principle" [1916] be valid, the amount of cholesterol absorbed from food might perhaps be limited by the quantity of deoxycholic acid derivatives in the bile passing into the intestines.

Experiments on the effect of a single full meal.

The next series of experiments was designed to investigate the effect of a single full meal on a fasting individual.

The first specimen of blood (20–30 cc.), from a vein in the arm, was taken about 1 hour before the meal was given, the subject having fasted since the previous evening (referred to in the table as "fasting"); the later specimens of about the same volume were taken during the course of digestion. The meals were substantial and, except those of very low sterol content, of more or less normal dinner character. They contained varying amounts of sterol, some being nearly free and others enriched, as regards sterol, by adding such constituents as eggs or calves' brain.

The results are set out in Table II.

DISCUSSION.

The results of these single meal experiments are conflicting and we cannot yet account for all the phenomena presented. At first sight it might be thought that some of the variations were due to errors of experiment, since, for obvious reasons, the analyses were not done in duplicate. The analyses, however, were made on quite adequate weights of plasma—10 to 14 g.—and our long experience of the method rules out errors of manipulation, beyond such slight errors as are inevitable in all gravimetric procedure.

It might well be, however, that the samples of blood drawn from an arm vein did not accurately represent the average composition of the whole blood, more especially during active digestion. Such error would, however, we think, tend to smooth out variations rather than accentuate them.

The first three experiments tabulated were done with meals of very low sterol content, and consequently of sub-normal fat content. The results are concordant in showing a small but definite lowering of the total cholesterol content of the plasma.

In Exp. 1, on a quite normal individual, the lowering is practically confined

Table II. *Normal healthy subjects.*

Exp. no.	Date of experiment	Subject	Composition of meal	Approx. sterol intake (g.)	Time of taking plasma after meal	Cholesterol content of plasma (g. %)			Appearance of plasma	Remarks
						free	ester	total		
1	17. iii. 1927	J. A. G.	Potatoes 450 g., whites of 5 eggs, 14 g. butter, slice of brown bread, 1 pint beer	— 0.04	Fasting 3½ hours	0.0778 0.0753	0.1302 0.1024	0.2080 0.1782	Clear yellow, no haemolysis Slight opalescence, no haemolysis	
2	21. xii. 1927	X.	—	—	Fasting 3½ hours	0.1027 0.0768	0.1908 0.1744	0.2935 0.2512	Clear brown, no haemolysis Clear brown, no haemolysis	Non-sterol unsap. matter 0.2466 % 0.1870 %
3	24. xii. 1927	W.	—	—	Fasting 3½ hours	—	—	0.2295 0.1858	Plasma clear, very slight haemolysis Plasma clear, very slight haemolysis	Non-sterol unsap. matter 0.3291 % 0.3787 %
4	19. v. 1925	J. A. G.	Turtle soup, saddle of mutton, peas, potatoes, bread, butter, cheddar cheese, radishes, ½ pint Chamberlain and coffee	— 0.25-0.35	Fasting 2½ hours	0.0514 0.0404	0.1386 0.1210	0.1900 0.1614	Plasma yellow, clear, no haemolysis Plasma darker and greenish, milky, no haemolysis	
5	19. v. 1925	H. G.	—	—	Fasting 2½ hours	0.0418 0.0375	0.1142 0.0809	0.1560 0.1184	Plasma yellow, clear, no haemolysis Plasma milky, greenish colour, no haemolysis	
6	20. iv. 1927	S.	Double helping lean meat, 1 small potato, green vegetables, 80 g. cheese, 1 bottle beer	— 0.25-0.3	Fasting 2½ hours	0.0514 0.0547	0.0797 0.0899	0.1311 0.1446	Plasma clear, slight haemolysis Plasma opalescent, slight haemolysis	20 grains of Fel bovinum 1 hour before meal
7	15. v. 1928	J. A. G.	Turtle soup ½ pint, 252 g. steak (182 g. after cooking), 1 tomato, peas, fried potatoes, fresh raspberries, 15 g. cream, toast, 14 g. butter, 1 pint claret	— 0.2-0.3	Fasting 2½ hours 4½ hours	0.0613 0.1135 0.1715	0.1388 0.0974 0.0488	0.2001 0.2109 0.2203	Clear yellow, no haemolysis Clear darker, greenish, fluorescence Faintly opalescent, no haemolysis	Non-sterol unsap. matter 0.2558 % 0.4243 % 0.4942 %
8	15. v. 1928	G. E.	Ditto, but no butter or turtle fat	— 0.2	Fasting 2½ hours	0.0860 0.1186	0.0889 0.0412	0.1749 0.1598	Clear, bright, no haemolysis Very slightly opalescent	Non-sterol unsap. matter 0.2554 % 0.1380 %

9	28. iii. 1927	J. A. G.	—	—	Fasting 4½ hours	0.0671 0.0732	0.1590 0.1437	0.2261 0.2169	Plasma orange and very slightly opalescent Opalescent and opaque, very slight haemolysis	Non-sterol unsap. matter 0.3504 % 0.3698 %
10	9. ii. 1928	J. A. G.	330 g. lean beefsteak, 230 g. potato, 110 g. brown bread, 42 g. butter, 56 g. cheese, 1 green artichoke, 1 pint beer	—	0.4-0.5	—	—	—	—	—
11	22. ii. 1927	J. A. G.	220 g. mutton chop, 2 eggs, Brussels sprouts, 10 g. brown bread, 1 small pancake, 1 pint beer	—	0.7-0.8	0.0654 0.0671	0.1405 0.1328	0.2059 0.2000	Plasma clear, no haemolysis Milky opalescence, no haemolysis	—
12	29. vii. 1926	H. G.	Bovril, roll, butter, chop, potatoes, Brussels sprouts, 2 scrambled eggs on toast, coffee	—	0.75-0.8	0.0707 0.0776 0.0772	0.0991 0.1234 0.1344	0.1698 0.2010 0.2116	Brownish red, very slight haemolysis Very milky, very slight haemolysis Milky, no haemolysis	Total solids of plasma 9.086 % — 9.088 % Heparin used as anticoagulant
13	22. iii. 1927	J. A. G.	330 g. veal cutlet, potatoes, bread, 56 g. butter, mushroom omelette (3 eggs), coffee with milk	—	0.9-1.0	0.0601 0.0612	0.1420 0.1318	0.2021 0.1930	Clear yellow, no haemolysis Opalescent, no haemolysis	—
			450 g. potatoes, 180 g. calves' brains, 60 g. beefsteak, 1 egg, 2 slices brown bread and butter, 1 pint beer	—	3.8-4.0	0.0708 0.0675	0.1057 0.1325	0.1765 0.2000	Clear brownish, no haemolysis More opalescent, no haemolysis	Heparin used as anticoagulant

Some pathological cases.

14	21. vi. 1925	A. T.	Minced meat, vegetable, 1 egg, bread and butter	—	0.35-0.4	0.0445 0.0410	0.0996 0.0766	0.1441 0.1176	—	Case of Graves' disease
15	25. vii. 1925	A. P.	Ordinary hospital dinner	—	0.25	0.0652 0.0695	0.1071 0.0667	0.1723 0.1562	—	Secondary contracted kidney Red corpuscles 2,720,000 before 2,240,000 after

to the ester cholesterol; but in Exp. 2 there was also a decrease in free cholesterol. In the next six experiments the meals contained between 0.2 and 0.5 g. of sterol. Here the results are markedly varied. Exps. 4 and 5 show a definite decrease of total cholesterol $2\frac{1}{2}$ to $2\frac{3}{4}$ hours after the meal, and this is shared by both free and ester cholesterol. In Exp. 6 there is a small increase of total cholesterol during digestion, mainly due to ester, and in Exp. 9 a decrease, also chiefly in the ester cholesterol.

In Exps. 7 and 8 there is a small change in total cholesterol, in opposite directions in the two experiments, but in both there is a very marked disturbance in the ratio of free to ester cholesterol during the progress of digestion. In both experiments there is a marked increase in the free cholesterol and a corresponding and almost proportional decrease in the ester cholesterol. The two subjects of this experiment lunched together and had similar food, the weights of the various items being as nearly equal as the *chef* could manage. The meal differed from the others of the group in containing very little fat beyond that naturally contained in the lean meat and vegetables. G. E. omitted even the small quantity of butter and left the turtle fat. It seems probable that this deficiency of fat relatively to protein and carbohydrates may be the cause—direct or indirect—of the disturbance in the normal free cholesterol to ester cholesterol ratio.

The meals in Exps. 10–13 were characterised by a relatively high content of sterol. This was effected in Exps. 10, 11 and 12 by the use of eggs and butter, and in Exp. 13 by the addition of calves' brain. In these experiments there was evidently little or no difference between the values for free cholesterol in fasting conditions and during the third to fourth hour of digestion. Any small changes occur chiefly in the ester and are in some cases positive and in others negative.

From a consideration of the whole figures in this table, it is clear that there is no direct connection between the intake of sterol with the food and the sterol content of the blood plasma at the height of digestion. There is therefore no such thing as alimentary hypercholesterolaemia in the usually accepted sense of the term. Since the free and ester cholesterol respectively of the plasma undergo changes during digestion differing both in degree and in direction, it is obvious that these variations cannot be accounted for by any alteration in the plasma dilution.

Our data, which in general confirm the conflicting observations of other workers quoted above, whatever the precise explanation may be of individual cases, seem to indicate that, during digestion, there is an active endogenous metabolism going on, in which the sterols play a part, so that at any moment the cholesterol and cholesterol ester percentages in the blood are the resultant of various factors, just as in the case of any other metabolic products, for example, the purines. As to what these factors are which govern the resultant percentage we have little or no information at present.

In Part II [1927, 2] of this series we brought forward evidence that in the

living plasma cholesterol and its esters are not merely present in simple colloidal solution or suspension, but are in intimate association with the proteins, particularly the euglobulins.

In some of the experiments in the present paper we attempted to correlate changes in the albumin-globulin ratio during digestion with the changes in sterol content, using the refractometric method of Robertson. This method is, of course, open to the objection that a complete separation of albumin and globulin cannot be obtained by a single precipitation with ammonium sulphate, but, apart from this, the method, in our hands at any rate, proved unreliable in the case of some of the milky plasmas taken during digestion.

In recent years the experiments of Gardner and Fox [1921, 1925], Thannhauser [1923], Beumer and Lehmann [1923], Channon [1925] and Randles and Knudson [1925] have proved that cholesterol can be synthesised in the animal organism, but from what source there is no evidence to form any opinion. On the other hand, no properly characterised degradation products of the sterols have, to our knowledge, ever been isolated from the tissues. The close relationship between cholesterol and the bile acids, conclusively proved from different points of view by Windaus and Neukirchen [1919], and by Wieland and Jacobi [1926], is significant, but this similarity of the carbon structure of the molecule in itself scarcely warrants the conclusion that has been drawn, that the bile acids originate through some oxidative breakdown of the sterol molecule. Conclusive evidence on this point can scarcely be obtained until some reliable quantitative method has been discovered for estimating the bile acids.

The unsaponifiable matter of the "lipoid" extract of plasma contains a non-sterol portion, often much greater in amount than the sterols. This non-sterol portion does not give the sterol colour reactions. In chloroform solution it gives with acetic anhydride and a few drops of sulphuric acid only a brownish coloration. This affords a satisfactory test that precipitation of the sterol digitonide has been complete in the estimation of sterol esters, and in the analyses in this paper was always applied. In some cases the non-sterol residue was weighed and the percentage is given in Table II. We make no comment on the figures at this stage, as this residue is not a single substance but a more or less complex mixture. Its composition is under investigation and we hope to be able to communicate the results of our work in the near future.

There is a further point of interest in the figures of Table II which is perhaps worth mention, namely, the constancy of the fasting values for J. A. G., which extend over a period of 3 years. The free cholesterol figures are of much the same order, the lowest being that obtained 3 years ago. Some variations occur in the ester cholesterol, but these are small compared with those observed during digestion.

From our results it would appear advisable, in making comparative determinations of sterols in pathological cases, to work with blood from patients in a fasting condition, or at any rate after only a very light meal.

SUMMARY.

1. The level of the cholesterol content of human plasma, taken while fasting, can be raised or lowered by sufficiently prolonged feeding with diets of high or low sterol content. Such changes are most marked as regards the cholesterol in ester form; the free cholesterol remains practically constant.

2. As the result of a single meal, alimentary hypercholesterolaemia does not occur, and there is no connection between the amount of sterol ingested and the cholesterol level of the plasma during digestion.

3. During the process of digestion changes in the cholesterol content of the plasma frequently occur, sometimes an increase, sometimes a decrease compared with fasting values, and under certain conditions a marked disturbance of the free to ester cholesterol ratio is observed.

We consider these changes evidence of an active endogenous metabolism, in which cholesterol takes part, during the process of digestion.

4. It is important, in comparing the cholesterol content of plasma under normal and pathological conditions, to use bloods drawn while the subject is in a fasting condition.

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