of knowledge. Medical research is increasing in different parts of the world, and new journals continue to appear. The difficulty of assessing the present state of knowledge is perhaps nowhere greater than in therapeutics, largely on account of the number of publications in which the information must be sought. For instance, there is scarcely any kind of medical publication in which it would not be possible to find some reference to quinine. If a person were to begin to make a complete study of the chemistry, pharmacology, toxicology, and therapeutics of quinine he might have to spend a good part of a lifetime in reading the literature on this single alkaloid. Anyone who begins now to research on such a subject is confronted with the heroic task of ascertaining what has already been done. With the rapid growth of medical research this task becomes more formidable from year to year. It is a realization of this difficulty that has prompted in recent years the creation of special journals devoted to "abstracts" and "reviews." The difficulty of coping with even abstracted literature is becoming increasingly great. These methods of dissemination of knowledge, though a present aid, will before long break down under pressure, and some better method will have to be devised.

I have often wondered whether some such plan as I am about to sketch might not be feasible, though the time may not yet be ripe for it. Every country which engages in medical research should establish a bureau of pharmacology and therapeutics to which would be sent separate reprints of every paper dealing with these subjects. At present, certain privileged libraries in this country receive a copy of every book published in this country. It would be a problem differing rather in degree than in kind to have a similar system internationalized. It would require arrangement with publishers, for it could not be left to authors. Each national bureau would receive roughly as much as it gave. The advantages would be: (1) that the future literature on the subject would be complete in one special library in each country; (2) that it is better to have one complete library on a subject than several incomplete ones; and (3) that it is far easier to extract the desired information from a library consisting of separated pamphlets on one subject than from an ordinary library of volumes of journals, even if the latter are complete. May one go further and prophesy that the time may come when we shall have an institute which will combine laboratories for pharmacology and chemotherapy, a hospital for therapeutic research, and a bureau for sifting and promulgating the advances in knowledge? We have an elaborate organization, with a Ministry, for the prevention of disease; we have no such organization as yet for the treatment of it.

SUMMARY

The past century has been in the main an era of aetiology and diagnosis; the coming century bids fair to be an era of therapeutics. For taking a worthy part in the advances that are certain to be made in the treatment of disease we are in this country none too well organized. We need more laboratories adequately equipped for research in pharmacology and chemotherapy, subjects which will play an increasingly important part in the advance of therapeutics proper. We need more researchers and more careers open to them.

Both in teaching and in investigation more attention must be paid to the assessment of the remedial value of drugs in man. Advance in the treatment of disease might be facilitated by having at least one hospital definitely devoted to research in therapeutics and by the creation of a specialized bureau and library for collecting and disseminating the knowledge of these subjects.

CHOLESTEROL METABOLISM IN DISEASE*

BY

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Cholesterol is an integral constituent of all cells and organs of the animal body. All body fluids contain some cholesterol; even urine contains traces, much augmented in the urine of patients suffering from nephrosis. It exists in the body in the free or alcoholic condition, and also in the form of its esters with the higher fatty acids. These two forms are not equally distributed.

The importance of cholesterol from the point of view of the pathologist was increased by the discovery that under certain physiological conditions—for example, pregnancy—the cholesterol content of the blood might be increased above normal; that in infective conditions at the height of fever the cholesterol of the blood was subnormal—hypcholesterolaemia; that in morbid conditions, such as in various forms of nephritis, cholelithiasis, diabetes, etc., the sterol content of the blood was increased often far above normal value—hypercholesterolaemia. The interest of pathologists was further stimulated by the discovery, I think in Professor Aschoff's laboratory, that the doubly refracting crystals or droplets found histologically in the normal suprarenal cortex, in atheromatous intima, in inflamed kidney, and in other organs, consisted of cholesterol esters; by the observations of Ransom that cholesterol has the property of inhibiting the haemolytic action of saponins; and by the work of the Russian observers, Anitschkow and Chalatow, showing that prolonged feeding to rabbits leads to cholesterol infiltration of the various tissues, the changes in which have been considered to resemble human pathological processes—for example, atherosclerosis.

Space forbids a detailed history of progress in this subject, but I should like, before going on to an account of some recent work, to acknowledge how much we owe to Chauffard and his co-workers in France, and more especially to Professor Aschoff and his school in Germany.

During the past twenty years progress on the chemical side has been more rapid than on the physiological side. Thanks largely to the brilliant work of A. Windaus and of H. Wieland on the oxidative breakdown of cholesterol, and of the bile acids, it has been possible to assign structural formulae, having a high degree of probability, to these substances, and to show that there is an intimate chemical relation between cholesterol and the bile acids.

Cholesterol is an unsaturated secondary alcohol of the molecular formula $C_{27}H_{46}O$, derived from the parent saturated hydrocarbon cholestane $C_{27}H_{48}$. By catalytic reduction the double link is saturated with the formation of bihydrocholesterol or β cholestanol, a saturated secondary alcohol. Coprosterol, which occurs in human faeces, is a stereo-isomer of β cholestanol, the difference depending on a steric rearrangement of the substituent groups at one of the carbon atoms. The parent hydrocarbon, coprostane, is a stereo-isomer of cholestane.

Various artificial isomers of cholesterol have been prepared in the laboratory, the most interesting being the very labile "allocholesterol," which very readily changes into ordinary cholesterol, particularly in acid media. This allocholesterol is readily reduced *in vitro* to coprosterol.

The specific bile acids and cholesterol possess the same carbon structure, except that in the bile acids an isopropyl group $(CH_3)_2CH$ has been eliminated from the side chain.

The bile acids which have been most studied are those of ox bile—namely, lithocholic acid (3 oxycholanic acid) deoxycholic acid (3.7 dioxycholanic acid) and cholic acid

* Read in opening a discussion in the Section of Pathology at the Centenary Meeting of the British Medical Association, London, 1932.

(3.7.12 trioxycholanic acid), which are derivatives of a parent acid, cholanic acid ($C_{24}H_{46}O_2$). In goose, ox, and human bile another deoxycholic acid, with the hydroxyl group in position 3 and 12, is also found—namely, cheno- or anthro- deoxycholic acid. These acids, though they do occur as such in the bile, are usually coupled with glycine or with taurine. They belong to the same stereo-isomeric series as coprosterol. However, from pig's bile a hyodeoxycholic acid (3 : 13 deoxycholanic acid) has been isolated which belongs to the same stereo-chemical series as cholesterol. The following scheme shows these relations:

The above-mentioned constitutional formulae, though probably correct in the main, must still be regarded as tentative, as they are based on a study of analytical reactions. The formulae have yet to be confirmed by synthetic methods.

It is important to note that in vertebrate animals the only unsaturated sterol isolated from tissue substance is common cholesterol. Schönheimer has, however, recently found that this cholesterol is generally accompanied by small quantities of β cholestanol, varying in different tissues from 1 to 5 per cent. of the total sterol. In contrast to the sterols, many different bile acids, with considerably divergent properties, have been isolated from the bile of different species of animals. Furthermore, in the different animals the acids, even though the same, are present in very different proportions. Consequently in this domain the results of animal experiment are not necessarily applicable in human physiology.

At the present time we have little or no precise information as to the functions of cholesterol in the economy of the cells or of the whole organism, nor have we the data on which to base a sound comprehensive theory of the sterol metabolism (in the wide sense) or even effectively to criticize hypotheses propounded from time to time.

In the study of sterol metabolism in the human subject attention has been mainly directed to the blood and excreta. Other organs can, as a rule, be dealt with only post mortem. In this paper I propose to draw your attention briefly to some phenomena in pregnancy and to some diseases marked by disturbance of the sterol metabolism. I have selected mainly those on which I have had opportunity of working.

Before dealing with these subjects I must mention certain biochemical facts, as to the origin and fate of the cholesterol in the body, which I think must be taken into account in any interpretation of the results obtained in disease.

Many years ago, from the examination of very large quantities of the excreta of herbivorous animals "at grass"—horse, cow, sheep, rabbit—I found that the material contained no recognizable trace of cholesterol. The main crystalline product isolated was a paraffin alcohol contained in grass, and feeding experiments showed that it passed through the intestine of the rabbit unchanged. (We altered the name of this substance to chortosterol under the impression that it was a saturated sterol, since it had the composition $C_{27}H_{54}O$ or $C_{27}H_{56}O$. I now know that it was really an open chain saturated paraffin alcohol.) Small quantities of phytosterol were also isolated, and, later on, a few milligrams of a similar substance, which from my notes I now recognize was β cholestanol.

Now the bile of these animals contains quite readily estimable amounts of cholesterol, 0.02 to 0.07 per cent., so that had this cholesterol, poured into the intestine with the bile, been excreted, we could scarcely have failed to isolate some. Dorée and I were therefore led, from this and other facts then available, to formulate the following working hypothesis:

1. Cholesterol is a constant constituent of all cells, and when these cells are broken down in the life process the cholesterol is not excreted as a waste product, but is utilized in the formation of new cells.

2. A function of the liver is to break down dead cells—for example, blood corpuscles—and eliminate their cholesterol in the bile.

3. After the bile has been poured into the intestines in the process of digestion the cholesterol is reabsorbed, probably in the form of esters, along with the bile salts, and carried by the blood to the various centres and tissues for reincorporation into the constitution of new cells.

I believe the destruction of red corpuscles is now attributed to the reticulo-endothelial system in general.

This hypothesis received considerable support from feeding experiments, and from the careful investigations of Mueller (1915), who showed by analyses of chyle, obtained from thoracic duct fistulae in dogs, that cholesterol is readily absorbed from the intestinal tract through the chyle, and may be recognized and measured in the contents of the thoracic duct. This work of Mueller has been amply confirmed and extended, most recently by Schönheimer. Since then I have found no reason to doubt the reality of this cholesterol cycle, though I admit, and shall show presently, that it is by no means the whole story.

Some explanation of the mechanism of the cycle is afforded by the work of Wieland and Sorge (1916), who found that deoxycholic acid formed stable molecular compounds with the higher fatty acids—for example, $8C_{24}H_{48}O_4$ and $C_{18}H_{36}O_2$ with stearic acid. The choleic acids isolated from bile were a mixture of such stearic, palmitic, and oleic acid compounds. These compounds are very stable, crystallize well, and their salts are easily soluble in water. Besides the fatty acids, many other substances form somewhat similar compounds. Materials which are insoluble in water, such as naphthalene, xylene, alkaloids, cholesterol, camphor, etc., are brought into solution by an aqueous solution of deoxycholate. This power of uniting with insoluble substances to form soluble products Wieland called the "choleic acid principle," and concluded that it had an important function in transporting insoluble substances, such as fat and fatty acids and cholesterol, through the intestinal wall.

It has recently been shown by Schönheimer that sterols of the vegetable kingdom are not absorbed by the animal organism from food, or when administered as such, and, indeed, in tissues behave as foreign bodies; consequently any waste of cholesterol in these herbivora must be made up by synthesis. That cholesterol is synthesized in the body clearly follows from balance experiments by Gardner and Fox, Thannhauser and others, and the more direct experiments of Beumer, Channon, Randles and Knudsen, and others. We have no real evidence as to the seat of such synthesis, though various hypotheses have been advanced and have led to much discussion.

In carnivora and omnivora an excretory mechanism appears to exist, so that there is always a quantity of sterol excreted in the faeces: this is mainly in the form of cholesterol or coprosterol, or both. In man this excretion has been shown by balance experiments to be always greater in quantity than the amount ingested with the food. Fox and I, making use of very reliable material obtained by a subcommittee of the Royal Society Food (War) Committee, in experiments on the digestibility of bread, investigated the sterol balance. In the experiments the diets were consumed for periods of ten days, the six significant days being the fourth to the ninth inclusive. The daily intake and output of sterols precipitable by digitonin have been estimated, and, taking an average of all subjects, there is a negative balance of 0.31 gram per day. It has long been known that the sterol of adult faeces consists largely of coprosterol, accompanied by smaller quantities of β cholestanol and sometimes cholesterol. Since Voit's classical experiment on the production of a faeces-like material, containing fat, in an isolated loop of small intestine, evidence has gradually accumulated that sterols may be excreted in the intestinal lumen otherwise than with the bile.

In 1911 Böhm, in a paper the importance of which was perhaps not realized at the time, reported on the contents of a completely closed loop, of some fourteen years' standing, in the intestine of a human subject. The contents were sterile, and the sterol present was found to consist of β cholestanol; this could only have got there by excretion through the walls of the loop.

Recently Schönheimer and von Behring, working on dogs, examined the excretion of a large intestine which had been isolated by the performance of a complete ileostomy. The sterol isolated was β cholestanol, but no coprosterol could be detected. The appearance of the material was similar to that described by Böhm, and an examination of the mucous membrane of the large intestine after killing the animal showed no histological changes. Clearly there is another way of excretion of sterols in the faeces besides that of the bile. Schönheimer and his co-workers have also shown by thoracic duct experiments that neither β cholestanol nor coprosterol is absorbed through the chyle.

It is generally believed that coprosterol is produced by the reduction of cholesterol in the intestine by bacterial action. The evidence for this is not positively strong, as nobody has succeeded in producing this change *in vitro* by the action of organisms. It is significant, however, that in the faeces of infants in the milk stage no coprosterol is formed. The sterol I have found to consist almost entirely of pure cholesterol, though traces of β cholestanol, such as have been found by Schönheimer in the cholesterol of most organs, are not excluded. Indeed he has detected this in the intestinal contents of a foetus.

Fox and I made a number of balance experiments on breast-fed infants. During the first week of life there was a negative balance of 0.14 gram per day. During the second and third weeks the average balance was 0.002. Out of the seven cases five showed a virtual balance, any difference being within the errors of experiment. One case showed a marked positive balance. In the case of children between 7 weeks and 10 months an average positive balance—that is, excess of intake over output—of +0.069 gram per day was obtained. Six of the seven cases showed a positive balance. At what stage of life, or alimentation, the process of reduction to coprosterol begins we do not know. Clearly, in the light of recent work the production of coprosterol and bihydro-cholesterol in the intestines would limit the absorption of cholesterol.

There appears to be some difference in degree, if not in type, between herbivora and carnivora and omnivora with regard to the effects of absorption of cholesterol. It has been long known from the work of Ignatowski, Starokadomski, Stuke, and Chalataw that feeding rabbits with various animal foods, above all yolk of eggs, produces intimal changes in the aorta and accumulation of anisotropic fats in the liver and other organs. This led Chalataw and Anitschkow to try the effect of administering cholesterol dissolved in sunflower oil: they produced thereby accumulation of lipoids, chiefly doubly refracting esters in the suprarenal cortex, in the liver, spleen, and lymph glands, and later a deposit in the intima of the aorta, together with hyperplasia of the elastica—similar changes to those of atherosclerosis in man. This work was criticized by Wachter and Hueck because of the use of a stomach tube and the feeding of unaccustomed food. They fed rabbits by the mouth with oats mixed with cholesterol, and found an increase in the blood compared with normal of both free and ester cholesterol, as well as an accumulation of doubly refracting fats in the suprarenals, liver, and other places, and also aortic changes of the kind mentioned. They were unable to produce aortic changes in either dogs or cats, though feeding was prolonged for two months. Anitschkow also failed to find arterial change in rats fed with cholesterol for five months. Yuasa, by very prolonged feeding with diets containing added cholesterol, produced the so-called cholesterol disease in mice, rats, and cats, but only in half of the rats and mice was

atherosclerosis observed, and in none of the cats. He thinks that his results do not point to any essential difference in principle between the metabolism of herbivora and carnivora, but that there is a difference in the sensitiveness of the intima of the arteries of different kinds of animals towards excess of cholesterol.

In all these observations it is tacitly assumed that cholesterol deposition in the arterial intima is identical with the process of the laying down of cholesterol in the liver, suprarenal, and other organs. From my own work on the feeding of rabbits and cats with cholesterol, in as physiological a manner as possible, for periods of about ten to twelve days, the variation produced in the cholesterol ester content of the liver, at any rate, is hardly pathological; within fairly wide limits it is clearly a physiological process. On the other hand, the deposition of cholesterol in the arteries, when it occurs, can scarcely be considered to be physiological. In man there is no evidence whatever to associate the occurrence of atherosclerosis with the exogenous cholesterol metabolism.

As a result of Windaus's chemical work, it was suggested that the bile acids are derived in the organism from cholesterol. Unfortunately the methods available for the estimation of bile acids are only rough, and we have no means of making any quantitative estimation of the relative proportions of the different bile acids. Thannhauser and his co-workers, by injection of colloidal cholesterol solutions into the blood stream, found, using bile-fistula dogs, little or no change in the cholic acid content of the bile excreted over a definite period; but on injecting allocholesterol in a similar manner they obtained an increased output of bile acid. This result is interesting, and perhaps significant, but can scarcely be accepted as proving the conversion of allocholesterol into cholic acid, for even if we assume that the bile acid estimations are correct, the weight of bile acid formed was much greater in weight than the weight of the allocholesterol injected. However, nobody has yet succeeded in bringing forward any evidence of the existence of allocholesterol or coprosterol in tissues.

ALIMENTARY HYPERCHOLESTEROLAEMIA

The data given in the literature are very conflicting. Experiments made by myself and Gainsborough have shown that the level of the cholesterol content of the plasma, taken before breakfast, can be raised or lowered by sufficiently prolonged feeding with diets of high or low cholesterol content. Such changes are most marked as regards the cholesterol in ester form; the free cholesterol remains practically constant. We found that as a result of a single meal alimentary hypercholesterolaemia (in the ordinarily accepted sense) does not occur, and there is no obvious connexion between the amount of cholesterol ingested and the cholesterol level of the plasma during digestion. During the digestive process changes in the cholesterol content of the plasma frequently occur, sometimes as an increase, sometimes as a decrease, compared with fasting values. Often a marked temporary disturbance of the ester to total ratio is observed. These peculiar changes cannot be explained by the influx of the cholesterol absorbed from the alimentary canal, but must be considered as evidence of an active endogenous metabolism in which cholesterol takes part during digestion.

The cholesterol content of the "fasting" plasma varies markedly in different healthy individuals, but is fairly constant for the individual himself, even over long periods. The following table summarizes the results of our own observations on some fifty healthy individuals of both sexes; the figures represent grams per 100 grams of plasma.

	Total Cholesterol		Free Cholesterol		Ester Cholesterol	
	Average	Limits of Variation	Average	Limits of Variation	Average	Limits of Variation
Male ...	0.170	0.11-0.22	0.050	0.005-0.082	0.120	0.080-0.14
Female...	0.153	0.08-0.23	0.054	0.020-0.094	0.100	0.054-0.14

These values would, of course, include any traces of β cholestanol if present. The ester cholesterol usually forms from 60 to 70 per cent. of the total cholesterol. The cholesterol and cholesterol esters in the plasma are not simply present in colloidal solution, or suspension, as such, but, as Gainsborough and I have shown, are in intimate physical or perhaps loose chemical union with the proteins, particularly the globulins.

CHOLESTEROL METABOLISM DURING PREGNANCY

Blood analyses during pregnancy were first reported by Chauffard. Since then numerous papers have been published, but perhaps the most extensive data have been given by Tyler and Underhill (1925). The general conclusion drawn is that hypercholesterolaemia occurs in pregnancy and the early puerperium. Tyler and Underhill used whole blood, and estimated both free and ester cholesterol values. The use of whole blood tends to mask certain changes, since the cholesterol of the red cells is mainly, if not entirely, in the free state, and is fairly constant in percentage, whereas the cholesterol of the plasma is present in both free and ester forms, the latter usually in preponderating quantity. Hence an analysis of whole blood, including the almost constant sterol content of the red cell, tends to hide the value of quite large changes in the plasma cholesterol. Another disturbing factor is the anaemia of pregnancy, which varies markedly in different individuals and is often of considerable degree. In our own experiments we analysed the plasma. Blood specimens were taken at monthly intervals before parturition and once or twice during the period of confinement. We found: (1) that the free cholesterol of the plasma increases during pregnancy up to a maximum at about the thirtieth week; concurrently the ester cholesterol falls to a minimum at about the same time; (2) from the thirtieth week, more or less approximately, the amount of free cholesterol decreases, and the ester increases till an almost normal ratio of free to ester cholesterol is attained before or near parturition; (3) the plasma cholesterol values are generally normal in the first week or two of the puerperium; (4) some cases show a definite total hypercholesterolaemia in which the total cholesterol figures run roughly parallel to the free cholesterol values, a result in general agreement with those of earlier observers, and our actual figures are of much the same order as theirs.

As we know nothing whatever of the function of cholesterol in the cell, and very little of the part it plays in the general metabolism, I will not indulge in hypothetical explanations, but simply give the results of our observations. In order to explain the fact, if it be a fact, that 75 per cent. of cases of cholelithiasis occur in women, some writers have suggested that hypercholesterolaemia occurring in repeated pregnancies is of paramount importance in the formation of gall-stones. This suggestion, however, scarcely helps to explain the occurrence in men; also an examination of post-mortem records at St. George's Hospital showed that cholelithiasis had not occurred in cases of subacute parenchymatous nephritis, where hypercholesterolaemia rules much higher than in pregnancy.

BLOOD CHOLESTEROL IN BILIARY AND HEPATIC DISEASE

It is obvious from what I have said that the percentage of cholesterol in plasma is the resultant of many factors, the balance of which determines the plasma cholesterol level at the moment the blood is taken.

Gainsborough and I thought it possible that evidence as regards endogenous metabolism might be obtained by studying the variation of plasma cholesterol in disease of the liver and bile passages. It soon became evident to us that the metabolism was disturbed grossly and in a complex manner in such conditions, but that the one common factor which could be correlated with these changes was the occurrence of jaundice.

The earlier French workers generally believed hypercholesterolaemia to be a constant feature of cholelithiasis, and a causal factor of the disease. Their observations were added to by many workers, until Campbell showed

that hypercholesterolaemia only occurred when the disease was accompanied by jaundice. Such increase of blood cholesterol has been explained as a simple retention phenomenon, due to the failure of the excretion of the cholesterol of the bile.

Recently Thannhauser and Schaber found a disturbance of the percentage of the total cholesterol as ester, and noted a sudden fall of the ester values almost to the vanishing point, which they refer to as "estersturz." This they regard as due to a failure of the esterifying power of the liver cells, the result of parenchymatous damage.

Bürger and Habs, Stern and Suchantke, and Adler and Lemel criticized these authors, and were unable to confirm the occurrence of "estersturz." They agree, however, with Thannhauser and Schaber's finding of hypercholesterolaemia in biliary stasis, with simultaneous occurrence of comparatively low ester values. They regard the hypercholesterolaemia as due to the retention in the blood of the free cholesterol normally excreted with the bile, and believe that this suffices to account for the lowering of the ester to total ratio.

In our work we examined some thirty-five cases. In order to eliminate the disturbing factor of digestion the blood was taken while the patient was in the "fasting" condition—that is, before breakfast. It is rather important to stress the fact that jaundice from biliary obstruction and the actual biliary obstruction are not necessarily contemporaneous, and that the only plain clinical evidence of existing biliary obstruction is the absence of bile pigment from the faeces. For example, of two of our cases, both extremely jaundiced from the impaction of a gall-stone in the common bile duct, the one had had persistent colourless faeces for eighteen months, while the other, though even more deeply jaundiced, had occasionally, during ten months, periods of coloured stools, so that the obstruction must have been intermittent. The former showed marked hypercholesterolaemia while the latter did not, so that even though in this latter case most of the bile was probably retained, the cholesterol picture had not been so disturbed as the former. The analyses were:

Cholesterol per 100 grams of Plasma

	Free	Ester	Total	Percentage of Total Cholesterol as Ester
(1) Massive jaundice during 18 months. Stools clay-coloured	0.3584	0.2391	0.5975	40.6
(2) Massive jaundice for 10 months, but obstruction intermittent, with faeces occasionally coloured	0.0920	0.0671	0.1600	41.9

In seventeen cases of uncomplicated cholelithiasis without jaundice there was no hypercholesterolaemia and the percentage of the total as esters was within normal limits.

An examination of the data of the other eighteen cases, in which failure of bile to flow into the intestine had been known to occur, and in which, with two exceptions, jaundice had been present, reveals two different types of changes in the blood cholesterol picture.

(a) On establishment of biliary obstruction there soon appears a lowering of the cholesterol ester content of the plasma, almost to the vanishing point. In some cases, and at the same time, the free cholesterol may be normal or slightly increased. If the biliary obstruction is relieved the picture returns quickly to normal.

(b) If absolute biliary obstruction persists a true hypercholesterolaemia ensues, with a low ester total ratio of 30 to 50 per cent.

Blood picture (a) was well illustrated in eight of our cases. I give one example. This case, of cholelithiasis, during a period of transient jaundice was treated with deoxycholic acid and dehydrocholic acid, with the result that in three days, with the establishment of the bile flow into the intestine, the cholesterol ester content of the plasma returned to normal.

Sex	Presence of Jaundice	Date	Cholesterol per 100 grams of Plasma			Per cent. of Total as Ester	Remarks
			Free	Ester	Total		
M.	+	29/12/27	0.0674	0.0048	0.0727	6.65	Gall stones visible clearly by x rays
	+	3/1/28	0.0846	0.0023	0.0869	2.65	Deoxycholic acid from 29/12/27 to 3/1/28. Faeces pale
	0	6/1/28	0.0817	0.1331	0.2148	62.6	Dehydrocholic acid intravenously 3/1/28 to 6/1/28. Faeces normal in colour
	0	6/2/28	0.0901	0.1728	0.2626	65	—

True hypercholesterolaemia of high degree, involving both forms of cholesterol, was well shown in three cases where the biliary obstruction had been complete for a considerable time. The figures in the case quoted above may serve in illustration.

A hint as to the significance of the first type of change was obtained from study of two cases with post-operative biliary fistulae. In one of these the fistula was apparently complete for six months, and the blood picture showed a remarkable "estersturz," the ester cholesterol falling to 0.009 per cent. Now the only common factor between these cases and those of jaundice showing the same blood picture is the absence of bile from the intestine. We see that by whatever means the bile is deviated from the intestine, whether by obstruction with biliary stasis or by obstruction without stasis (the bile flowing away freely and completely through an external fistula), the phenomenon of ester-vanishing tends to occur. Such absence of bile from the intestine leads, as we know, to a failure of fat and sterol absorption, which must be significant, and must be correlated closely with the diminution of cholesterol ester in the plasma. The re-establishment of normal bile flow and of fat absorption lead, in the case quoted, to an amazingly rapid return of cholesterol ester to the plasma to the extent of 4.3 grams in three days.

Consideration of the later-occurring hypercholesterolaemia presents certain difficulties, for, in spite of persistent failure of fat absorption, cholesterol in both forms piles up in the plasma, and though the ester form lags behind the free, its value can still reach a high figure. If the hypercholesterolaemia is due solely to the retention of the free cholesterol of the bile it might be suggested that some of the retained cholesterol is esterified by mobilization of fat from the fat depots. This utilization of the body stores of fat probably also liberates the associated cholesterol in these stores, and this possibility has been suggested as an explanation of the hypercholesterolaemia which occurs in starving animals. However, the hypercholesterolaemia which occurs in biliary obstruction is generally believed to be due to cholesterol retention; but when we consider that in bile stasis there is probably at the same time a failure of alimentary cholesterol absorption, it is by no means obvious that cholesterol retention is not balanced by this failure. It might be possible to obtain further evidence on this problem by performing balance experiments on patients with jaundice, or with complete biliary fistula, by feeding on a sterol-free diet. Such experiments would be rendered very difficult, however, by the constipation shown by these patients.

NEPHRITIS

Hypercholesterolaemia is present in all those cases which possess the "nephrotic" syndrome, marked by diffuse parenchymatous degeneration, heavy albuminuria, changes in the plasma proteins, and with oedema as the chief symptom. Consequently, increases of plasma cholesterol are found in those cases of subacute and chronic nephritis of prolonged duration, where albuminuria is predominant and haematuria insignificant, and which range, as Gainsborough has stated, from cases of glomerulo-nephritis in which the initial nephrotic state is more or less rapidly followed by sclerosis and contraction of the kidneys, by gradual transition to those cases of so-called "pure nephrosis," where renal sclerosis and its accompanying

symptoms are not observed. The total cholesterol in the plasma may reach as much as 1 per cent., and the percentage of total in form of ester may assume abnormal value in either direction. This high level of cholesterol is present throughout the course of these cases of nephritis so long as there is any tendency to oedema, and it persists in cases of subacute parenchymatous nephritis for long periods, even though oedema is temporarily absent. After a more or less prolonged period the tendency to oedema is replaced by tendency to vascular changes, increase of blood pressure, and signs of nitrogen retention, and about the same time the hypercholesterolaemia diminishes.

The cause of this hypercholesterolaemia is not known, but may perhaps be related to the disturbance of the plasma proteins resulting from proteinuria. Gainsborough and I estimated the cholesterol of the carefully filtered urine of several patients showing hypercholesterolaemia, and found from 16 to 41 mg. per day were excreted, the excretion in the urine by normal persons being at most 1.7 to 4 mg. per day. As in the case of plasma, we found the same attraction between the sterols and the proteins of the urine, and the attraction is most marked as regards the globulin.

Hypercholesterolaemia is not found in cases of interstitial nephritis, in arteriosclerotic kidney, or in arteriosclerosis.

Many observers have reported on the chemical analyses of kidney tissue in nephritis. Windaus gives the normal amount of cholesterol in fresh kidney as: free cholesterol 0.22 per cent., ester cholesterol 0.01 to 0.03; but in large white kidney: free 0.22 to 0.33 per cent., and ester cholesterol 0.09 to 0.65 per cent. Fex obtained similar results, showing that in cases of glomerular nephritis and of nephrosis the free cholesterol is hardly if at all increased, but the ester is greatly increased. He also found the liver and suprarenals in these cases were normal.

In a case of advanced chronic glomerulo-nephritis we found that, though the blood cholesterol was normal, the kidney was abnormally rich in ester: free 0.27 per cent., ester 0.2 per cent. In another case in which high hypercholesterolaemia had been noted a few months before death the kidney contained free 0.39 per cent. and ester 0.36 per cent. The analysis of other organs—liver, lung, spleen—was approximately within normal limits.

MYXOEDEMA

The total cholesterol of plasma in this condition may be increased by nearly 50 per cent. above normal. Epstein and Lander thought that blood cholesterol was inversely proportional to the basal metabolic rate, and they suggested an analogy between myxoedema and pure "nephrosis," where the basal metabolic ratio is also below normal. This argument was then used to explain the occasional beneficial results of thyroid medication in "nephrosis." However, Gainsborough and I found that this relation of basal metabolic rate and blood cholesterol does not exist in states of hyperthyroidism where, as in exophthalmic goitre, the blood cholesterol is within normal limits.

It may be thought that I have laid too much stress on the chemical aspects of the problems of sterol metabolism. Another method of approach, however, to these problems—in some aspects just as important—is afforded by the histological study of pathological conditions in which deposits of cholesterol esters occur in the tissues, as in chronic nephritis, Niemann's disease, atherosclerosis and in xanthoma. In some of these the deposition of sterol is apparently not determined so much by a hypercholesterolaemia as by the local lesion, whatever may be its nature, as in atherosclerosis. In other cases the deposits seem definitely due to an increase in blood sterol. The contrast is well seen in cases of xanthoma. In xanthoma simplex the lesion is unassociated with hypercholesterolaemia. On the other hand, the hypercholesterolaemia in prolonged jaundice may lead to a widespread distribution of xanthomatous lesions, and that in diabetes may lead to a differently distributed but characteristic xanthoma eruption, whereas the high blood cholesterol of so-called nephrosis never, so far as I am aware, leads to such phenomena.