CHOLESTERIN METABOLISM:

AN ACCOUNT OF PHYSIOLOGICAL, PATHOLOGICAL, AND EXPERIMENTAL INVESTIGATIONS.

THESIS

FOR THE DEGREE OF "DOCTOR OF SCIENCE"

BY

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APPENDIX - four reprints -

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3. J.W.McNEE.

"Die Cholesteringehalt der Galle während der Schwangerschaft".
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"Recent work on the Etiology of Gall-stones".
Glasgow Medical Journal.

INTRODUCTION.

Within recent times the so-called "lipoidal" substances have been recognised to play an important part in the metabolism of the body, both in health and in disease. They have been found to cross very many lines of investigation and research which, at first sight, seem far apart. It is evident that a knowledge of the facts in connection with the properties and biological reactions of these substances in the living body, may be of great value in the investigation of many problems in physiology and pathology which are still unsolved.

Among the various lipoidal substances which have been described and named, only cholesterin has hitherto been obtained in a chemically pure state. The lecithins, phosphatide, cerebroside etc. are at present to be regarded as mixtures, exhibiting more or less definite properties, and obtained from various organs by the action of certain organic solvents.

By reason of the ready isolation of cholesterin in a state of purity, much accurate observation of its properties and metabolism has been made possible during the period 1909 to 1914. In such investigations the writer has taken an active interest,

and has made a few contributions to the already extensive literature on the subject. As has occurred in the study of many imperfectly known processes of normal metabolism (e.g.the ductless glands) much physiological information of value has accrued from a study of the pathological conditions with which cholesterin is concerned, and the present thesis is to be regarded as an essay in both Chemical Physiology and Chemical Pathology, normal and abnormal elements being considered together.

The subject being so large, and the problems involved so varied, it is necessary to give a brief account of the known facts, and some historic theories, in order that the writer's own work may be placed in its proper perspective. The literature is scattered widely through British, French, German, and Russian scientific journals, and acknowledgment must be made to Dr. N.Anitschkow, Assistant to Professor Maximow in Petrograd, for a very complete account of some important experimental investigations published only in the Russian language.

The writer's personal investigations have concerned the following problems.-

1. Investigations of the general physiology and metabolism of cholesterin in the body.

2. The physiological alterations in cholesterin metabolism which occur during pregnancy.

3. Cholesterin metabolism in relation to the formation of gall-stones.

4. Experimental work on the relations of cholesterin to the

production of atheroma and arterio-sclerosis.

Much of this work has been already published in contributions to.-

1. The Quarterly Journal of Medicine.
2. The Glasgow Medical Journal.
3. Deutsche med. Wochenschrift.
4. Ziegler's Beiträge Z. path. Anatomie u. allgemein. Pathologie.

Some parts of the work are as yet incomplete and unpublished.

The investigations have been carried out in three Laboratories:-

1. The Pathological Institute of the University of Glasgow. (Professor Robert Muir, F.R.S.)
2. The Pathological Institute of the University of Freiburg-i-B. (Professor L.Aschoff.)
3. The Chemical Institute of the University of Freiburg-i-B. (Professor A.Windaus.)

CHOLESTERIN.

HISTORICAL.

As long ago as 1775, Conradi[1], while examining gall-stones, observed a peculiar substance which from its physical characters and action towards solvents, he considered to be a fat, and he gave it the name "gall-stone fat".

In 1815, Chevreul[2] showed that certain properties distinguished the substance from ordinary fats, and to this writer is due the name "cholesterin".

For a long time all work in connection with cholesterin was confined to the province of gall-stones, and it was not until comparatively recent times that cholesterin was recognised to be a normal constituent of every cell, of all tissue fluids, of the bile, etc.

Before the universal presence of cholesterin in the cells was recognised, many speculations had been made to account for its presence in gall-stones etc.

Labbé and Besancon[3], for instance, believed that the cholesterin was a product of leucocytic destruction.

Flint (1862)[4] offered the first real theory supported by

some experimental evidence. His conclusions were briefly as follows.- "Cholesterin is an excretal product formed chiefly by the breaking down of nerve cells, sep-rated from the blood by the liver, shed normally into the upper intestine along with the bile, and converted into stercobilin during its passage through the remainder of the bowel". Flint found-d his views on experiments which seemed to show that cholesterin was present in greater amount in blood taken from the jugular vein than in that taken from the carotid artery. Later work, however, showed that Flint's experiments, carried out perforce in somewhat primitive fashion, were inaccurate, newer methods making it clear that the cholesterin content of the blood in all vessels of the body is the same in any individual. There are, nevertheless, some parts of Flint's original work which stand today, namely, that in jaundice the cholesterin content of the blood is generally increased, and that in the intestine cholesterin is converted into stercobilin. The stercobilin of Flint has been identified with the substance studied and described at a later date by Bondzynski and Humnicki[5] (1896) under the name "coprostearin".

Duval and Gley[6], from their observations that less cholesterin was present in bile obtained from the bile ducts than in that taken from the gall bladder, first formulated the theory of production of cholesterin by the wall of the gall bladder.

Naunyn[7] (1892) developed this theory further, in his classi-
cal

work on "Cholelithiasis", holding that cholesterin was not an excretion through the liver at all, but was a direct <u>secretory</u> product of the cells lining the bile passages, and especially of the gall-bladder epithelium. Naunyn's researches showed in his view that cholesterin was first produced in the form of myelin droplets in the cells of the gall-bladder wall, and was shed into the gall-bladder by desquamation of these cells, being only then liberated as cholesterin. His theory of gall-stone formation was built up on these observations.

Aschoff and Bacmeister[8] (1901), in a study of cholelithiasis, criticised Naunyn's work, considering that the fat found in the gall bladder epithelium was due to direct <u>absorption</u> from the bile, and was not in any way a secretory product of the cells.

This concludes a brief summary of work carried out in the days before any good chemical method of isolating cholesterin in the pure state had been described, and the publication of the Digitonin method by Windaus[9] in 1909 marks the beginning of a new epoch in the study of cholesterin.

CHEMICAL AFFINITIES and PHYSICAL CHARACTERS.

Cholesterin, as obtained pure, (from a cholesterin gall-stone, for example) is an odourless, rather greasy, colourless, crystalline substance, and is insoluble in water. It dissolves readily at ordinary temperatures in chloroform, acetone, ether, xylol, and similar substances. It is only slightly soluble in ethyl alcohol in the cold, but dissolves readily on boiling.

Its reference to the chemical
constitution of the natural
(a) Its alcohol-like structure
(a. OH group
Its double linkage at the end
of the chain.
2to relationship to terpenes
— constitutional formula
5 benzene nuclei

From solution in ether or chloroform it crystallises out as silky needles. From boiling alcohol it crystallises out, on cooling, as transparent rhombic plates, often showing the typical appearance of "a corner cut out". The chemical formula accepted by Windaus, Abderhalden, etc., is $C_{27}H_{46}O$, but other workers such as Abel[10] regard the formula as $C_{27}H_{44}O$. Cholesterin is soluble in fatty acids, and neutral fats (glyceryl esters), and in the body is indeed commonly found as esters of the fatty acids. In the laboratory cholesterin esters can be prepared, by various methods, from a number of the fatty and aromatic acids. Lehmann was the first to point out, while working with artifically prepared cholesterin-esters, that during their passage from the solid to the molten isotropic state, they passed through an intermediate anisotropic phase in which the subatance, while possessing all the physical characters of a fluid, is turbid abd doubly refractive to light. In the animal body the commonest cholesterin-esters are those of oleic, palmitic, and stearic acids.

Coprostearin, the substance isolated by Bondzynski and Humnacki[5] in 1896, and identical with the stercobilin of Flint, would appear to be a reduction product of cholesterin. Its empirical formula, if cholesterin be taken as $C_{27}H_{44}O$, may be regarded as $C_{27}H_{45}OH$ (dihydrocholesterin). All attempts to reproduce this substance in the laboratory by reduction of cholesterin have so far failed (Craven Moore)[12].

CHEMICAL TESTS for CHOLESTERIN.

The Liebermann[13] test, is the one commonly employed, and is exceedingly delicate. It is used in most of the colorimetric methods of estimating cholesterin quantitatively. The test is as follows.-

"If sulphuric acid and acetic anhydride be added to a solution of cholesterin in chloroform, the fluid immediately becomes reddish, then quickly changes to dark green."

Various other tests are available for qualitative work, but are limited in their scope.

QUANTITATIVE ESTIMATION.

Great strides in the investigation of cholesterin at once began to be made after the publication of the researches of Windaus (1909)[9], describing the method of cholesterin estimation by means of the purified alkaloid Digitonin. This method is exceedingly simple in application and accurate in its results. It depends on the fact, found by Windaus, that free cholesterin unites with digitonin, in alcoholic solution of the two substances, to form a staple compound digitonin-cholesteride, which precipitates as transparent crystals. The union, moreover, takes place in a definite quantative proportion, the ratio of cholesterin to digitonin-cholesteride being 1 to 4. By weighing the precipitate the amount of cholesterin in any given substance can be readily obtained. Cholesterin-ester cannot be directly estimated as such, but must first be saponified to set free

the cholesterin. If it is desired to estimate the amounts of cholesterin and cholesterin-ester separately, the material must be divided into two parts; in one part the free cholesterin is estimated, and in the other the total cholesterin after setting free by saponification that bound as ester. The amount of cholesterin-ester present is then readily found by simple subtraction. This method is extremely reliable, and easily carried out with the ordinary apparatus in a Laboratory.

We owe to clinicians, especially those of the French School, various colorimetric methods applicable particularly to small samples of cholesterin-containing material, e.g. blood-serum. With practice, however, these have been found to give, within limits, fairly accurate results, and the writer has controlled such methods many times by Windaus' Digitonin method. The best known of the colorimetric methods are.-

1. Grigaut's method. (France)[14]
2. Iscovesco's method. (France)[15]
3. Weston and Kent's method. (America)[16]
4. Autenrieth and Funk's method. (Germany)[17]

All of the above may be summarised as methods of extracting small amounts of cholesterin in chloroform, ant then applying the Liebermann or other similar tests. The green colour resulting is then compared and titrated, either with a Liebermann test put up with a standardised solution of pure cholesterin, or (as in the Autenrieth-Funk method) with a standardised permanent artifically-prepared green solution.

The Weston-Kent colorimetric method.

One or two cubic centimetres of the material to be examined are placed in a wide-necked stoppered bottle, and covered with 20 cubic centimetres of absolute alcohol. This is allowed to stand in an oven at 55° Centigrade for a least 24 hours, after which the alcohol is decanted from the precipitate left at the bottom, and replaced by ether. After a further 24 hours the ether and alcohol extracts are mixed. The precipitate in the bottle is then thoroughly washed with boiling absolute alcohol, and the alcoholic extract so obtained added to the original alcohol-ether extract until a volume of 80 to 90 cubic centimetres is reached. A stick of caustic soda is then added to the extradt,and the whole is saponified for 2 hours on the water-bath. In this way any cholesterin-ester present is liberated as free cholesterin. At the end of 2 hours the extract has as a rule evaporated to a volume of about 10 cubic centimetres, and to this is thereupon added 50 cubic centimetres, or even rather more, of a saturated solution of calcium chloride. At once a white flocculent precipitate falls,which contains the cholesterin. The precipitate is obtained by filtration and thoroughly well washed. A new supply of calcium chloride must again be added to the filtrate, in case some further precipitation may be obtained. The whole precipitate is dried on the filter paper,which is then extracted with 100 cubic centimetres of ether. The ether is then evaporated, and the residue dissolved in 5 cubic centimetres of cloroform. This solution is now ready for the application of the colorimetric test, which is that of Liebermann, previously

described. The green colouration is compared with the colour given by applying the same test to various standardised solutions of pure cholesterin. All dilution required to match the colours is carried out with a solution made up as follows - chloroform 5, anhydrous acetic acid 2, concentrated sulphuric acid 0.1.

The Autenrieth-Funk Colorimetric Method.

One or two cubic centimetres of serum, or a suitable small quantity of other material, are placed in a beaker to which is added 20 cubic centimetres of a 25 % watery solution of caustic soda. The mixture is saponified for 2 or 3 hours on a water-bath to liberate free cholesterin from the ester. On cooling, the fluid is placed without dilution in a shaking-funnel and shaken thoroughly for 5 minutes with three or four times its volume of ordinary ether. The ether is allowed to separate, and is then carefully decanted. This process of shaking with ether is repeated several times. The total ether-extract is filtered, and evaporated to dryness. The residue, which contains the cholesterin, is now dissolved in a known quantity of chloroform, and is ready for the colorimetric test.

There is an alternative method of extraction, applicable only to blood-serum and bile. It is slightly less accurate than the other technique, but more sparing in reagents. After saponification with caustic soda, the ehter stage may be omitted and extraction carried out direct with chloroform. At the first shaking 25 cubic centimetres of chloroform should be employed, and thereafter a further 15 cubic centimetres. The chloroform

extract, which is always opaque from admixture with water, is cleared by adding about 10 % of solid anhydrous sodium sulphate, and shaking. The extract is then filtered to get rid of the solid sodium salt, and sufficient chloroform is washed through the filter paper to make up the bulk of the extract to 100 cubic centimetres. For the colorimetric test 5 cubic centimetres of this extract are employed.

In the case of bile, the shaking out with anhydrous sodium sulphate gets rid of the bile pigments from the solution entirely.

For the colorimetric test the "Autenrieth-Funk colorimeter" is employed. This apparatus was cheap and well made, and has been much used by the writer. For the test, 5 cubic centimetres of the chloroform extract are placed in a graduated glass cylinder, to which is then added 2 cubic centimetres of anhydrous acetic acid and 0.1 of a cubic centimetre of concentrated sulphuric acid. After shaking, the cylinder is kept in the dark for 15 minutes, by which time the solution is of a deep green colour. The small receptacle of the colorimeter is filled with part of this green solution, and the "standard" green-coloured wedge moved about until the colours are equal. The reading on the scale, which moves with the standard wedge, is then compared with a cholesterin curve or graph made up for the apparatus by testing known quantities of pure cholesterin. In this way a direct estimation of the amount of cholesterin in the 5 cubic centimetres of chloroform extract is arrived at. Thereafter the figure must be multiplied to give the result for the total cholesterin extract (50, or, usually, 100 c.c.)

THE BIOLOGY of CHOLESTERIN.

It is impossible, in the limits of a thesis, to attempt a complete survey of this part of the subject. All the main points, however, and especially those which concern the writer's own work, will receive notice.

General Biology.

Cholesterin has now been shown to be a constant constituent of every vegetable and animal cell. The general occurrence of a cholesterin-like substance in the vegetable cell was drawn attention to first by Beneke[18], who isolated from various seeds a body identified by Kolve[19] as cholesterin. Later, however, this substance was differentiated by Hesse[20] as a isomeric compound, and named by him Phytosterin. It has since been shown that isomers of cholesterin exist in members of the vegetable series, from bacteria upwards. Thus they have been isolated from the fatty constituents of bacteria, myxomycetes, seeds, and stems and leaves of plants.

In the animal body, the cholesterin, which forms part of all normal cells and all tissue fluids, is present in a microscopically invisible form.

In what state does it exist?

We know now that in the blood serum it exists chiefly as esters of the fatty acids. The condition in which it is present in the cells themselves (e.g. the nervous tissues) is much less certain. Normally it does not exist as esters, although Mair[21] and others have shown that in Coptic mummies the brains have often been largely converted into cholesterin-ester. It seems most likely that in the normal brain, and other tissues as well, the cholesterin is simply present in colloidal solution, i.e. in association with substances which render it miscible with water. Now this property of colloidal solution or emulsion is not confined to cholesterin, but is shared by other bodies as well. It is, for example, a very marked property of mixtures of lecithin and cholesterin (c.f. the Browning-Cruikshank-Mackenzie[22] antigen in the Wassermann test for syphilis). It can, in short, be safely premised that in the normal cell, cholesterin is physically associated with lecithin, the mixture existing in the colloidal state. The esterising of cholesterin with fatty acids, as found in the blood serum, has no doubt the similar object of maintaining a colloidal solution there. On this view, the deposit of free cholesterin crystals in various pathological conditions may be regarded as simply due, in principle, to the cholesterin having fallen out of the colloidal state.

The rôle of cholesterin in the cell.

The peculiar chemical inertness and stability of cholesterin do not suggest that it can be much concerned with the complex

chemical processes of living protoplasm. Its physical characters, however, are such as to favour the idea that it might have an important rôle in cell economy, especially in relation to absorption. It was demonstrated by Overton[23] that substances which can penetrate into the living cell all have the common property of being soluble in fats and lipoids, such as lecithin and cholesterin. On the other hand, substances known to be unable to penetrate into cells do not possess this solubility. Overton accordingly formulated the theory that cholesterin and other lipoids constitute, by peripheral concentration, a semi-permeable colloidal membrane enveloping the cell. In favour of this theory a number of facts can be cited. It is known, for instance that monohydric alcohols, aldehydes, ketones, and substances like aniline, all readily penetrate cells. Moreover aniline dyes which have the property of intra-vital staining (e.g. neutral red methylene blue, Nile blue, saffranin etc.) are all lipoid-soluble, whereas other dyes found useless for intra-vital colouring are insoluble in lipoids.

In passing it may simply be noted here that this theory of Overton has attained great prominence in connection with attempts to give a physiological explanation of narcosis.

There are two questions concerning cholesterin which are so dependant on Overton's work that they may be suitably considered here.

1. <u>The relations of cholesterin to the phenomena of haemolysis.</u>

In 1901, Ransome[24], while investigating the mode of action of saponin on the blood, found that if red corpuscles were washed

entirely free from serum, they were thereby rendered much more susceptible to the action of saponin. He found further that the constituent of the serum, which interfered with the haemolytic action of saponin, was contained in the ethereal extract, and was in fact, cholesterin. Lecithin was shown to be without any effect. He concluded that the toxic effect of saponin depended on the solubility reaction which existed between the saponin and the lipoid portion of the cell. He concluded further that the existence of extra-cellular cholesterin in the serum would, within limits, be a defence against the entrance of saponin into the cells. Nogouchi[25] extended this work, and found that by adding cholesterin he could neutralise the haemolytic action of agaricin and tetanolysin as well as saponin, lecithin being without such effect. Later Flexner and Nogouchi[26] working with cobra-venom, found that red corpuscles washed thoroughly free from serum were agglutinated by the venom, but did not haemolyse until serum was added. They concluded, therefore, that the venom-haemolysin is an amboceptor, activated by some substance in the serum. Keyes[27] confirmed these observations and showed that the substance contained in the serum which activated the venom is lecithin. He regarded the union between the venom and the lecithin as of a chemical nature, and actually prepared a substance cobra-lecithid, which has been used by the writer to produce an experimental anaemia in rabbits. Koeppe,[28] Peskind[29], van der Weld[30], and others, have pointed out that the haemolytic activity of such substances as chloroform, ether,

bile-salts, probably depends on the fact of their solubility in the lipoids of the cell.

2. The effects of cholesterin in the Wassermann reaction.

With the introduction into practical medicine of the Wassermann reaction in syphilis, the lipoids, especially lecithin and cholesterin, have come in for renewed researches. The reaction was introduced as an ordinary immunity phenomenon, a saline extract of a congenital syphilitic liver being employed on the supposition that such an extract would contain the specific receptors of the spirochaete pallida, which, uniting with corresponding anti-bodies in a syphilitic serum, would fix complement. It was soon found, however, that alcoholic extracts of normal non-syphilitic liver had also the property of fixing or deviating complement in presence of a syphilitic serum; hence it was obvious that the reaction did not correspond to the ordinary union of antigen and antibody. A search has been made to find the substances in the alcoholic extract of liver on which this reaction depends. Much of this work has been carried out in the Glasgow School, by Browning, Cruikshank, and Mackenzie.(22) From the fact that the extract used is an alcoholic one, it was at once surmised that the lipoidal substances lecithin and cholesterin would play a part. A number of these lipoids have been investigated to ascertain if they can be used as antigens in the Wassermann reaction. It is not proposed to enter fully into this work here, full references being given in the publications of Browning and his colleagues. It may be noted, however, that these authors found that

that if cholesterin be dissolved to saturation in an alcoholic solution of lecithin, this solution acts as a very efficient substitute for the crude organ-extract generally employed. It was further found that much depends on the method of making the solution, turbid emulsions in saline giving better complement deviation than colloidal solutions.

THE ORIGIN of the CHOLESTERIN of the BODY.

Prior to the introduction of Windaus' Digitonin method of estimation, nothing was really known of the origin and destiny of cholesterin in the organism. Since 1909, however, many workers have studied the problem, with the result that two sharply divided opinions are now held.

The first view is dependent on the work of Chauffard and his colleagues (Grigaut, La Roche etc.) in France. This School claims that the cholesterin for the needs of the organism is produced within the body itself by certain "cholesterinogenic" organs, namely, the suprarenal bodies, and, in the female, the corpora lutea of the ovaries.

The alternative theory has been supported by the majority of other workers, chiefly of the British, German, and Russian Schools. It denies that the suprarenals and corpora lutea are to be regarded in any way as "factories" of cholesterin, but are to be considered merely as intermediate "depôts" necessary in some way for metabolism. On the other hand, it affirms that the cholesterin of the body is derived from without, being absorbed from the food, either as cholesterin itself in the case of animal diets, or as one or other of the various isomers present in vegetables. Further, a corollary of this theory

maintains that the metabolism of cholesterin in the body is of a strictly conservative nature, the amount lost through the bile being normally reabsorbed from the intestine. Any permanent loss is at once made up by absorption from the food.

The evidence on which these two divergent theories have been built up is important, and will be discussed in some detail.

The work of the French School has been ably set forth in a monograph by A.Grigaut (31) - "Le Cycle de la Cholesterinaemie", Paris, G.Steinheil,1913 - and the following summary is chiefly derived from that work.

Grigaut clears the way to develop the theory of his School by discussing in the first place work carried out to estimate the rôle of the liver in cholesterin metabolism. Chauffard, Grigaut, and La Roche have repeated the old observations of Naunyn, studying the occurrence and localisation of fat droplets in cells of the bile passages in normal dogs,and in dogs in which the hepatic duct had been ligatured. From the results of these experiments the conclusion is arrived at that the criticism of Naunyn's results by Aschoff and Bacmeister is unwarrented, the fat droplets not being due to simple resorption from the bile as the latter authors declared. This decision against resorption is arrived at chiefly because, in the animals where the hepatic duct was ligatured, no great increase in fat droplets occurred in the cells lining the bile passages, although under such experimental circumstances resorption would be maximal in degree. In Grigaut's opinion, speaking for the French School, the rôle of the liver may be summed up in the following sentence

"In the present state of scientific knowledge, it appears probable that the cholesterin of the bile is a secretion of the epithelium of the bile passages, and that this secretory activity has its seat of election in the area of the bile capillaries and in the wall of the gall bladder itself."

Grigaut next passes in review the possible origins of the cholesterin of the blood serum. His first assumption is that a large proportion of this cholesterin must be formed within the organism, when the enormous disproportion between the content of the serum in various pathological states, and the small amount of cholesterin in the food, is considered. He then proceeds to bring forward the view that the suprarenals and corpora lutea are the actual factories of cholesterin for the organism. With regard to the corpus luteum, he points out that it is only within the last ten years that the important functions of this ductless gland in relation to menstruation and pregnancy have been recognised. The activity of this gland is periodic and temporary, but the lipoids found in the glandular cells are formed during the period of growth, and not during the recessive stage of the gland. In the initial haemorrhagic state of the gland, the cholesterin content differs little from that of the blood serum, Grigaut's analyses varying between 1.27 and 2.27 grms. per litre (normal serum 1.5 grms. per litre). At the stage of maturity, when the corpus luteum is still soft but entirely cellular, the average analyses shows a cholesterin content of 5.85 grms. per litre. During the stage of retrogression, when the body has shrunk so as to be not more than a few centigrammes in weight,

the average cholesterin content of ten examinations was 10.92 gms per litre. Here, of course, the great shrinkage in size of the body accounts largely for the high figure. The interpretation of the above facts allows of only two hypothesis; either the corpus luteum is a simple depôt, or else the cholesterin is an active glandular secretion. Grigaut, from the histological appearances of the active gland, is certain that the latter is the true explanation, because the lipoids appear at an early stage coincident with the body becoming cellular, and disappear when the corpus luteum is completely ended. In his view, therefore, the corpus luteum is to be regarded as a temporary factory of cholesterin, important for cholesterin production during the pregnant state.

It is evident, as Grigaut points out, that the corpus luteum, being confined to one sex, could not have an equal importance with the suprarenal capsules in relation to cholesterin metabolism. These glands contain normally abundant cholesterin in three of the four layers into which the cortex is divided by histologists. The suprarenals are richer in cholesterin than any other tissue in the body, the average cholesterin content being, according to Grigaut's analyses, as follows:-

45 grammes per 1000, in the male. } per 1000 grammes
55 grammes per 1000, in the female. } of fresh substance

On these figures the suprarenals contain proportionally more cholesterin than the brain, which gives an average analysis of 25 grms per 1000 of fresh substance. (Grigaut's figures for normal suprarenals, however, require some explanation. They refer to analyses made in accidental cases of sudden death, and no doubt represent the true normal. In the usual [illegible]

at autopsy in our hospitals the average cholesterin content is considerably lower - about 20 grammes per 1000 of fresh substance- since during the agonal period before death the lipoids tend to disappear. See Landau and McNee - appendix.)

Grigaut next notes that in all diseases shown to be associated with hypercholesterinaemia, the suprarenal glands are enlarged, hypertrophied, and laden with fat, whereas the converse holds in infections, etc, where hypocholesterinaemia supervenes. This fact was first observed by Guieysse[32], and has been amply confirmed by Albrecht and Meltzmann[33], Landau and McNee[34] etc. In passing it is also of interest to mention that numerous writers - Pilliet[35], Vaquez[36], Josué[37] etc. - have pointed out the frequent association of Adrenalinaemia with the hypercholesterinaemia of Bright's disease.

Experiments are then detailed by which Grigaut and Troisier thought to throw further light on the function of the suprarenals. They found in a series of dogs that removal of one suprarenal is quickly followed by an increase of the amount of cholesterin in the blood, reaching its maximum about the end of the second week, then gradually returning to normal. In animals killed at the height of the cholesterinaemia the remaining suprarenal is found enlarged and laden with fat. This, however, does not imply as much as Grigaut would suggest. It would seem to indicate merely that the single organ had expanded to take on the function of both, whatever that function may be, whether cholesterinogenic or quite otherwise. Nevertheless, on such evidence as has been summarised, Grigaut

concludes that in, for example, pregnancy and Bright's disease, the increase of cholesterin in the blood is the direct result of increased production in the suprarenals, and that such hypercholesterinaemia is truly "par hypergenèse". This is in contrast to Grigaut's cholesterinaemia "par rétention", found in hepatic diseases, for which a number of differences are pointed out. Grigaut states that in jaundice the suprarenals do not present the appearances of increased functional activity, despite the increase of cholesterin in the serum. His chemical examinations of the suprarenals in jaundice confirm this, the figures being rather below than above the normal (average of five cases 16.26 grammes per 1000 of fresh substance). It must be remarked however, that these findings in the suprarenals in jaundice are not supported by those of Landau and the writer, who always found much more doubly refracting fat present in the suprarenal cortex in cases of jaundice than could be considered normal.

One other point of difference, first pointed out by Widal, Weill, and Laudet[38], has been confirmed; namely, that whereas in Bright's disease etc. both the free and esterised cholesterin in the serum are increased, in jaundice it is only the free cholesterin which is augmented.

In the view of the French School, this hypercholesterinaemia "par rétention", seen in jaundice, is to be explained by a deranged secretory function of the liver, whereby the cholesterin is prevented from escaping into the bile.

The alternative view may now be considered, namely, that

the suprarenals are not to be regarded as factories, but merely as depôts, the cholesterin lost to the organism being made up from the food.

Chronologically, the most important early work on this question is contained in a series of papers in the Publications of the Royal Society (Biology), by Gardner, Dorée, Ellis, Fraser (39) & Their experiments seemed to show that :-

1. In rabbits, when cholesterin is given in the food, some is absorbed into the blood stream, leading to an increase of both free and esterised cholesterin in the serum.

2. In rabbits, fed on bran which had been extracted with ether to remove all the plant isomers of cholesterin, the total free and combined cholesterin in the organism remains very constant.

3. The liver is quite unaffected by cholesterin feeding, there being no increase in the cholesterin content of the tissue.

4. In rabbits kept in a state of inanition, and living thus on their own tissues, a storing up of cholesterin in the liver takes place. The blood of such animals contains, in addition, more free and combined cholesterin than the normal.

5. In a human experiment, where the diet was varied, it was found that the amount of coprostearin excreted in the faeces could all be accounted for by the cholesterin taken in from the food, provided the body weight remained constant. In an attack of mild influenza, however, which occurred during the experiment and was accompanied by rapid loss of weight, the output of cholesterin (as coprostearin) exceeded the intake.

6. These authors consider they have brought forward evidence to

show that cholesterin is strictly conserved in the animal body. Any waste is made up by absorption of cholesterin from the food, this substance not being manufactured within the body. Experiments with growing chicks confirm this view, showing that cholesterin cannot be readily synthesised in the animal body from proteins, carbohydrates, or fats.

Turning now to the work carried out by the writer, in association with Landau, and later with Rothschild. Part of this has been already published, but owing to the war the experimental portion remains incomplete and must be continued when opportunity offers. Having before us the opposing theories advanced by Grigaut on the one hand, and by Gardner, Ellis, etc. on the other, we have carried out observations with a view to amplyfying and controlling the conclusions of these writers on the position of the suprarenal glands in cholesterin metabolism. One series of observations was made on human suprarenals obtained at autopsies, while the remainder of the work was purely experimental, and carried out in rabbits. Landau (40), as a result of previous microchemical and morphological work, had taken up a position in opposition to the French view, concluding that the suprarenals were merely depôts of cholesterin, necessary in some way for the metabolism of this substance, while not being the actual factories of the cholesterin of the body. Our first enquiry was a control by chemical methods, of these morphological investigations. A number of suprarenal glands, taken at autopsies, were subjected at the same time both to histological examination in frozen

sections stained by Nile Blue, and to chemical examination by the digitonin method. The results of these chemical analyses are given in the reprints (Appendix No.), and need not be detailed here. The main points of interest which emerged from this work are briefly as follows:-

1. The results of chemical analyses show that the conclusions founded on simple histological examinations are reliable. All cases which on morphological grounds were regarded as containing cholesterin-ester in abundance, gave a high figure for this substance on chemical analysis, and vice versa.

2. The content of the suprarenal gland in free cholesterin undergoes moderate changes, which are little if at all influenced by alterations in the cholesterin content of the blood serum.

3. The labile element is the cholesterin-ester, which undergoes wide variations in amount, dependent on the cholesterin content of the blood serum. Further, it may be noted here that in rabbits fed on cholesterin to produce arterial changes (vide infra),and where the cholesterin content of the serum can be artificially raised to as high as 14 times the normal (McNee), the suprarenals enlarge and store up cholesterin-ester just as do various other organs of the body.

The next work undertaken consisted in observing the effects of unilateral and bilateral extirpation of the suprarenal glands in rabbits. Similar experiments have been already recorded by Grigaut, and were referred to in the summary of his work. The main results arived at by us,before the experiments had to be broken off before completion, were as follows:-

In male rabbits which have survived excision of one suprarenal for some time, and have returned to normal as regards the cholesterin content of the serum, removal of the second gland leads to the rapid development of a high cholesterinaemia during the few hours that the animal survives. This is in contrast to the results of experiments on dogs recorded by Grigaut, who found no increase in cholesterin in the serum after extirpation of both glands. It may be that dogs and rabbits behave differently, but it is obvious that the experiments require repetition in both species of animals. Our results in rabbits, however, taken as they stand, are of great interest when considered along with the work of Stewart (41), summarised by McCallum at the International Congress, London, in 1913. Stewart found that pregnant cats survive double extirpation of the suprarenal glands longer than others, and further that any cat fed on cholesterin for some time survives the same operation for much longer than a normal animal. In this connection also it is important to remember the experiences of Hultgren and Andersson (42), who found a similar prolongation of life, after removal of both suprarenals, in male animals which had previously been castrated. It seems probable, therefore, that castration can lead not only to the well known increase in the glycerin-ester fats (general adiposity) but also to an increase in the cholesterin fats as well. Further, this experimental work lends undoubted support to the view that the essential organs of sex, including the corpus luteum, have some control over cholesterin metabolism.

This experimental work is admittedly incomplete, and much

information of value may yet be obtained when it becomes possible to renew and extend the experimental investigations. From what has already emerged however, it is the writer's opinion that the suprarenal glands, while not the actual producers of cholesterin must still have some very important intermediate function in cholesterin metabolism. What intermediate function can they possess? This question has been much debated, and it has been suggested that their function consists in esterising the free cholesterin obtained by absorption from the bowel. In this way a chemical disposal would be carried out somewhat akin to the glycogenic function of the liver. There is however, no real evidence available in support of this view. A second ingenious theory has been advanced by Landau, who was struck with the close relationship between the fat-laden cortex and the medulla composed of sympathetic nervous tissue. Might it not be, asks Landau, that if cholesterin is present so abundantly in the central nervous system generally, it might also be essential in some way for the functional needs of the sympathetic nervous system?

The position of the liver in cholesterin metabolism has also attracted our attention. The normal human liver shows on analysis an astonishingly low percentage content in cholesterin, although of course, when the actual bulk and weight of the organ is considered, the absolute amount is large. In a wide variety if diseases, including those known to associated with hypercholesterinaemia and the reverse, we have found on analysis no change whatever of note in the cholesterin content of the liver. It is evident therefore, that in man the cholesterin obtained from

the liver by analytical methods represents merely that contained in the essential structures of the organ. It appears that the human liver does not store up cholesterin even temporarily before passing it out in the bile, but merely acts as a filter.

In concluding this section, it may simply be pointed out that many of the questions raised are far from being completely settled. The problems are difficult, but of such obvious importance in the study of general metabolism, that no pains must be spared in trying to completely discover the origin and uses of cholesterin in the organism.

THE CHOLESTERIN CONTENT of the BLOOD-SERUM in HEALTH and DISEASE.

This has been, especially since the introduction of the various colorimetric methods, the subject of a great number of investigations, and our knowledge in this field is extraordinarily complete. Slow progress was made while the more technical Windaus method (requiring at least 10 c.c. of serum) was alone in use, but this method has been of great value in controlling the less complicated and less accurate colorimetric methods. It will be seen from the comparative figures given below, that a slightly higher figure is always obtained when the Windaus method is employed.

The normal content of the blood-serum in cholesterin.

Here it must be remembered that the greater part of the cholesterin present in the blood-serum is esterised, so that before any estimation of the total cholesterin can be made by any of the methods employed, saponification must be carried out to set free the ester.

The tabulated results of various writers are given below together with the methods employed.

Klinkert.(43)

Windaus method.	(average of 17 cases.)	1.822 grms per litre
Grigaut "		1.765 grms per litre

Schmidt.[44]

Weston & Kent method.	average of 17 cases.	1.398 grms per litre.

Bacmeister and Henes.[45]

Weston & Kent method.	average of 6 cases.	1.48 grms per litre.

Autenrieth and Funk.[17]

Their own colorimetric method.	average of 6 cases.	1.46 grms per litre.

From the above it will be seen that the normal average cholesterin content of the serum circles round 1.5 to 1.8 grms per litre. A figure below 1 gramme, or over 2 grammes, is certainly abnormal.

As most diseases have now been examined in this way, it will suffice to give a list of those in which the cholesterin in the blood is always increased (hypercholesterinaemia), and those in which it is diminished (hypocholesterinaemia). Thereafter a few special points can be dealt with.

Diseases associated with hypercholesterinaemia.

Chronic Nephritis

Arteriosclerosis

General Adiposity

Diabetes Mellitus (not always)

Xanthoma or Xanthelasma

Inanition and Starvation

Liver Diseases with Jaundice

Cholelithiasis

Enteric Fever (a notable exception to all other acute infections)

Diseases associated with hypocholesterinaemia.

Acute infections of all kinds (except enteric fever)

Chronic infections (tubercle, syphilis, etc.)

The severe Anaemias.

Of interest in these findings are the high cholesterin contents of the serum in the metabolic diseases - diabetes, adiposity, etc. The highest figures of all are generally obtained in cases of chronic nephritis, and of xanthoma, where figures of over 5 grammes per litre may be obtained. In diabetes 3 grammes is an average figure. In tubercle 0.8 or 0.9 are average figures.

The effects of these changes in the cholesterin content of the serum on the pathological findings in these diseases will be considered later.

THE CHOLESTERIN CONTENT of the BILE in HEALTH and DISEASE

Evidence has been led to show that the liver is to be regarded as a cholesterin filter only, the actual content of the liver tissue itself varying little with alterations in the content of the blood-serum. It is interesting, however, to record the alteration in the cholesterin content of the bile filtered through the liver in cases associated with hypercholesterinaemia in the serum. It may be stated that experimentally anything which causes an increased amount of cholesterin in the serum leads at once to an increased output in the bile. The writer's own experiments with toluylendiamin, taken along with those of Kusumoto (46), bring this out very clearly. Toluylendiamin is a drug which, when injected into animals, causes blood destruction and jaundice. Kusumoto found that in dogs in which a biliary fistula had been made the injection of toluylendiamin brought about a rapid increase in the cholesterin of the bile. The writer (47) on the other hand complemented this work by showing that in dogs poisoned by toluylendiamin to produce jaundice the cholesterin content of the blood is increased. A table demonstrating this result is given below.

Experiment

The dog received in all 4.1 grms of toluylendiamin in 5 doses.

Day	Cholesterin in grms per litre
1	0.06
4	0.069
7	0.70
10	1.04
12	1.20
18	3.44
22	1.91

In this connection, also, the observations made by Bacmeister[48] on a case of diabetes are of interest. In this case a complete biliary fistula existed following an operation, and by the Windaus methos the cholesterin content of the bile was found to be increased. Moreover Pierce[49], who examined the gall-bladder bile in a number of pathological conditions, obtained the highest figures in cases of chronic nephritis. The writer's own observations on the cholesterin of the bile will be dealt with at length in the section describing the alterations in cholesterin metabolism which occur in pregnancy. To sum up, however it may be said that all facts go to show that any increase in the cholesterin content of the blood-serum means an increased cholesterin content of the bile as well.

THE CHANGES IN CHOLESTERIN METABOLISM DURING NORMAL PREGNANCY.

It has been recognised since 1911 that during the normal period of pregnancy the metabolism of cholesterin undergoes profound changes. There is a gradual increase in the cholesterin content of the blood-serum during the whole period, reaching its maximum during the 8th and 9th months, when a common average figure is 3 grammes per litre. This increase is to be regarded as a constant phenomenon. The following illustrative tables may be given.-

Klinkert (43)

7th to 9th month - blood removed direct from vein--
Windaus method - average of 17 cases 2.633 grms per litre -
Highest figure 4.255 grms, lowest 1.875 grms per litre.

Chauffard, LaRoche, and Grigaut (50)

8th and 9th month - 32 cases - average of all 2.45 grms per litre

Opportunity has also been taken of examining the blood of the new-born child, the samples being readily obtained from the ruptured umbilical vein at birth. It is interesting and somewhat astonishing to find that the cholesterin content of the serum here is much below even the normal adult figure, and therefore far below the cholesterin content of the maternal serum. The following table sufficiently indicates this.

Chauffard, LaRoche, and Grigaut.

13 cases - blood from umbilical vein - average 0.55 grms per litre highest figure 0.85, lowest 0.35 grms per litre - colorimetric method employed.

Klinkert

7 cases - Windaus method - average figure 1.19 grms per litre

Until investigated by the writer in 1912, nothing whatever was known of the cholesterin content of the bile during the pregnant state. From the experimental work with Toluylendiamin, however, which has been already referred to, an increased cholesterin content of the bile was to be expected in pregnancy also. The analyses of the writer's cases[51] are given below - see also Appendix No.3.

Table. (minee)

Case	month of pregnancy	Windaus method		Weston-Kent method
		free cholesterin	ester cholesterin	total cholesterin
1	4	6.04	0.84	5.89
2	5	6.80	0.40	6.14
3	6	-	-	6.60
4	9	-	-	9.50

Thus it is evident that during pregnancy the cholesterin content of the bile is much increased, up to six times the normal in the fourth case of the table, where death occurred from heart failure during parturition. This will be referred to again in discussing the relations of pregnancy to the etiology of gallstones.

After child-birth, as Hermann and Neumann[52] have shown, the hypercholesterinaemia in the mother gradually passes off. The

normal figure is reached by the beginning of the second week in women who suckle their children, but not for a longer period in those who do not. In an investigation of human milk the same authors were able to show that the milk is one of the principal routes by which the excess of cholesterin is eliminated.

The question naturally arises as to the reasons for the hypercholesterinaemia of pregnancy and the way in which it is brought about. The view of French writers in this connection has already been discussed, the explanation offered by them being the development of the corpus luteum. We have, however, no idea of what function the increased amount of cholesterin can possibly serve.

THE RELATIONS OF ABNORMAL CHOLESTERIN METABOLISM to the ETIOLOGY OF GALL-STONES

This will be very briefly dealth with here, since full details of the writer's views are given in a paper entitled "Recent work on the etiology of gall-stones", a copy of which forms part of the appendix to this thesis. (Appendix No 4.)

Suffice it to say that the notorious frequency of gall-stones in women who have borne children is universally recognised. Schröder's (53) large series of figures from v.Recklinghausen's laboratory show that gall-stones are five times as common in women as in men, and ten times more frequent in women who have borne children than in those who have not. What is the cause of this special proclivity of child-bearing women to gall-stone formation? We know now that in pregnancy there are two conditions which, on "a priore" grounds, would seem to favour the origin of gall-stones: (1) the constant increase of cholesterin in the blood, and the greatly increased excretion of cholesterin in the bile, first demonstrated by the writer. (2) Pressure on the gall-bladder, with resulting stasis, dependent on the growth of the expanding uterus. These points have, of course, special reference to non-imflammatory gall-stone formation, where the stone arises in an aseptic gall-bladder.

Statistics from Aschoff's laboratory show that over 26 %

of gall-stones pass through an aseptic phase, such stones being either solitary cholesterin stones or the so-called combination stones. The latter begin as pure cholesterin stones, and then from the onset of infection in the gall-bladder, become coated with layers of the usual mixture of cholesterin-pigment-chalk.

LOCAL DEPOSITS of CHOLESTERIN in the TISSUES under PATHOLOGICAL CIRCUMSTANCES.

In the histological study of pathological lesions in the human body, deposits of cholesterin are of fairly frequent occurrence. The substance may be present either in the pure state, or in the form of esters. The pure cholesterin is met with either as the typical rhombic plates, or as bunches of fine needles. Cholesterin-esters are found in two forms, as needle-shaped crystals, and as anisotropic globules. The needles when heated to varying temperatures - 37°C to 70°C - melt to form spherical globules which, on cooling, remain anisotropic for some time. It is possible therefore, that during life the needles of cholesterin-ester do not exist, but only the fluid crystalline stage. All the varieties of cholesterin are readily recognisable in the tissues by their double refraction to light.

The essential conditions under which deposits of cholesterin occur in the tissues are as follows:-

A. Where the cholesterin in the blood is increased.

B. Where necrosis of tissue has occurred.

C. Where lipoid-containing secretions, exudations, or desquamation products have been retained.

D. In tumour growths (Powell White).(54)

A. Dependent on hypercholesterinaemia.

1. The "white spots" on the retina, met with in diabetes-mellitus, chronic nephritis, etc. These two diseases are well known to be associated with increase of cholesterin in the blood, and it was of great interest when it was demonstrated (Lauber & Adamuk,[55] Ginsberg,[56] Chauffard,[57]) that these spots were simply localised cholesterin deposits. V.Noorden[58] has pointed out that these spots may disappear if the cholesterinaemia be got rid of, and may reappear if it returns.
2. The "Arcus Senilis" has now been shown to be a cholesterin-ester infiltration of the cornea (Marie and Laroche)[59]. The association of this condition with arterio-sclerosis, in which the cholesterin content of the blood is always increased, is well known.
3. The "Xanthoma Tuberosum", a peculiar and somewhat rare skin disease. Histologically this condition is found to consist of the heaping up of large foamy cells filled with cholesterin ester. This disease is met with most frequently in diabetes, and in certain liver diseases, both of which are commonly associated with a heightened cholesterin content in the serum.
4. In gall-stones - this has already dealt with in a previous section.

B. Dependent on tissue necrosis.

The deposit of cholesterin crystals is of very common occurrence where the necrosis of tissue has occurred within the body, and where there is no outlet for the necrotic products of the cells. Common examples are tubercular caseation, advanced

atheroma, necrosis in rapidly growing tumours, degeneration cysts of many organs, old pulmonary infarctions etc.

Under such circumstances, the reactions of the neighbouring healthy tissue cells are of much interest, and have recently been studied by Roussy[60] and M.Stewart.[61] If the cholesterin is deposited in the centre of necrotic tissue which is not organised, no cellular reactions occur (tubercular caseation, old atheroma). If, however, the cholesterin is deposited in the midst of living connective tissue, then it acts as an irritant and gives rise to the formation of very characteristic foreign-body giant cells. In paraffin sections, which have,of course, passed through a cholesterin solvent (chloroform or xylol), the appearances histologically are very characteristic. Clefts are left vacant where the cholesterin crystals were, and the clefts are surrounded by closely applied large giant cells. The cholesterin here is in the form of rhombic plates, although the cleft is generally narrow from the way in which the crystal has lain. Occasionally however, a lucky section may show a typical space of rhombic shape. Such cholesterin clefts, associated with giant cell production, occur,as has been stated, where the crystals are deposited contiguous with, or in the midst of, living tissue which can react to them. This condition is fulfilled in many instances, and includes many of the lesions where cholesterin can also be deposited without reaction, (epidermoid cysts, chronic mastitis, chronic pyosalpinx, chronic inflammation in fatty tissues etc.).

C. Dependent on retention of lipoid containing secretions, exudations, and desquamatory products.

Such circumstances are found in the retained milk of chronic mastitis, the fluids of old empyemata, retained products of old suppuration in many positions, epidermic debris in dermoid cysts, the so-called cholesteatomata etc. (In passing it may be noted that the so-called cholesteatomata, often described as a tumour, is not really a tumour at all. It simply depends on an inclusion of epidermoid tissue which continues to function like epithelium in its abnormal situation, leading to desquamation of keratinous material rich in cholesterin. The condition is most often seen in connection with the cerebral meninges and in the middle ear.)

D. Dependent on tumour growth.

As Powell White (54) pointed out, deposit of cholesterin occurs in various tumours quite apart from necrosis. It occurs among the healthy living cells and consists of cholesterin-ester, seen either in the needle-like crystalline from or as myelin droplets. Both of these forms occur within the cells themselves and are associated with the so-called "foamy" appearance of the cytoplasm as seen in paraffin sections. A similar appearance is of course normally seen in a paraffin section of the suprarenal cortex. The appearances referred to are most commonly seen in carcinomata, and are a striking feature in a specific tumour of the kidney - the hypernephroma.

EXPERIMENTAL WORK with CHOLESTERIN, CHIEFLY in REGARD to the PRODUCTION of CHANGES in the BLOOD VESSELS.

As far as experimental pathology is concerned, the work on cholesterin seems to have originated in Russian Schools, and in the first publications cholesterin was not known to be the active agent in bringing about the effects observed.

Ignatowski (1908) (62) was investigating, in rabbits, the effects of substitution of purely animal food for the normal herbivorous dietary. The diet he used consisted of ox-flesh, eggs, and milk, and the experiments varied in duration between 21 and 198 days. He was astonished to find, especially in the longest experiments, well marked degenerative changes in the walls of the aorta, and other morphological changes in the liver. Starakadomski (63) (1909) repeated these experiments, using egg-yolk as a dietary, and found the same changes in the aortae of his rabbits. He pointed out that the changes affected the intima, amd bore a striking resemblance to those of early atheroma in man.

Stuckey (64) (1910) used meat-juice, egg-white, egg-yolk, and milk, in a series of experiments on rabbits, to see which of these had most, if any, effect. He found that whereas meat-juice, egg-white, and milk were practically without influence, egg-yolk produced a remarkable change in the aortic wall. The intima

became the seat of great proliferation and hypertrophy, followed quickly by infiltration of the thickened area with fat. Pursuing this further, Stuckey tried to find the active constituent of the egg-yolk responsible for these interesting changes. It seemed probable on "a priori" grounds that the albumen fraction could have nothing to do with the result, since other richly albuminous food (egg-white etc.) had been without effect. He was led therefore to consider the other abundant constituents of egg-yolk, namely the fats. Stuckey thereupon proceeded to feed his rabbits on a variety of animal and vegetable fats, but all the experiments were failures, as regards aortic changes, except in the case of a few animals fed on brain tissue, where slight changes resulted.

The clue to the truth was given by the work of Anitschkow and Chalatow (65) (1913), who after feeding rabbits on egg-yolk and brain substance, were struck by certain appearances in the liver. Cells of the liver were found laden with fat, and this fat was recognised by these writers to be doubly refracting, i.e. cholesterin-ester fat. Thus the probability arose that the constituent of egg-yolk responsible for the lesions in the intima was cholesterin, and feeding experiments were at once commenced with cholesterin alone. In these rabbits fed on cholesterin (dissolved in sunflower oil), all the intimal changes were produced with great intensity, and their similarity to those of human atheroma was fully commented upon.

The work of Anitschkow and Chalatow has been repeated by Wacker & Hueck (66), and by Wilson and the writer, with fully

confirmatory results. Our own experiments (McNee & Wilson) were interrupted by the war, and are not fully complete. A brief account of the main results, which were communicated to the Pathological Society of Great Britain and Ireland in July 1914, is given below.

In all, five experiments have been completed, varying in duration from 31 to 101 days. The technique used was the following. A supply of cholesterin was obtained from gall-stones by the usual method of Soxlet extraction with ether, and the substance so obtained was purified by re-crystallisation from chloroform. A 2.5 % solution of cholesterin was prepared in olive oil, by heating the mixture on a water bath for an hour or more until the cholesterin dissolved completely. The animals used (large rabbits) were fed daily into the stomach through an ordinary soft rubber catheter with 10 c.c. of the oily solution, each animal receiving 0.25 grm of cholesterin daily. Adequate controls, using olive oil only, have been carried out.

In all five animals changes in the aorta were noted, but they varied greatly in degree, according to the duration of the esperiment.

In the first experiment (33 days) the changes were slight, being confined to yellowish pathhes in the neighbourhood of the intercostal vessels.

In the second experiment (36 days) two yellow patches, each 3-4 millimetres in diameter, were present on the ascending part of the aortic arch.

In the third experiment (101 days) very striking lesions were

found, large yellowish patches being present throughout the whole length of the thoracic and abdominal aorta. This case was examined histologically in great detail, and the majority of the illustrations are taken from this experiment.
In the fourth experiment (76 days) abundant yellowish patches were also found, with in addition two very definite small saccular aneurysms situated close above the aortic valves.
In the last experiment (68 days) numerous small patches were found situated on the whole length of the aortic wall.

The experimental lesion in the aorta.

Microscopically, the close similarity of the lesion to that of early atheroma in man is extremely well seen. The intima alone is generally affected, and is raised into a patch. The internal elastic lamina is seen to split at the edge of the patch, and becomes invisible below the central part of the lesion. Frozen sections stained by Sudan III, or Nile Blue, show the patch to be laden with fat, and when examination is made through crossed prisms, the greater part of the fat is found to be doubly refractive to light. The distribution of the fat is important, part being within cells and part between the cells. If a very early small lesion be examined, the fat is seen to be all extra-cellular. It is formed first as small fine droplets, which gradually increase in number, and are doubly refracting from the outset. Soon numbers of large phagocytic cells appear, with rounded nuclei, and much vacuolated protoplasm. These cells absorb the fat and are soon laden with it; they are situated

between the elastic fibrils, which in this way become widely separated from one another. The origin of these "foamy" cells is still in doubt. It is certain that they come into the intima from elsewhere, and at first contain no fat. Anitschkow, who has gone very carefully into the question of their origin, believes them to be either of connective tissue or lymphocytic origin. In the later stages of the lesion, some changes are also found in the innermost layer of the media, the muscle fibres becoming separated and arranged in irregular fashion. In two of the animals fed on cholesterin, patches of calcification of the media were also present, but this change has also been observed in control animals, so that further observations are necessary before any conclusions can be drawn. It is of interest, however, to note that over the site of the area of calcification the intima is raised as if to form a support for the weakened wall. This, of course, is also a phenomenon found in human arterial disease.

In passing, some reference must be made to the changes in other organs of the animals fed on cholesterin. Doubly refracting fat is laid down in abundance in the liver, spleen, bone-marrow, and in the suprarenals, which last increase to double their normal size. In the liver, spleen, and marrow the cholesterin fat is found, on microscopic examination of frozen sections, to be stored in the endothelial cells, and not in the essential parenchymatous cells of these organs.

A series of microphotographs, illustrating the results of the five experiments, is included here.

Fig. I. Photograph of a rabbit's aorta. The animal was fed daily with 2.5 grms of cholesterin in olive oil for 76 days.
Note the raised patches on the aortic wall, and the saccular aneurysms a short distance above the aortic valves.

Fig. 2. Early change in the intima of the aorta of a rabbit fed on cholesterin.
Note that the change is confined to the intima, which is raised into a patch containing "foamy" cells.
Paraffin section. Haemalum & Eosin. X 50.

Fig. 3. Later change in the intima of a rabbit fed on cholesterin.
Paraffin section. Haemalum & Eosin. X 50.

Fig. 4. From the same specimen as Fig.3. Frozen section. Haemalum and Sudan III. Photographed through crossed prisms.
Note the abundant doubly refracting cholesterin fat in the thickened intima. X 50.

Fig. 5. Calcification of the media in a rabbit fed on cholesterin. Note the proliferation of the intima to form a support.
Paraffin section. Haemalum & Eosin. X 20.

Fig. 6. The same as Fig. 5. under higher power. X 50.

Fig. 7. Deposit of doubly refracting cholesterin-ester fat in the liver of a rabbit fed on cholesterin. Photographed through crossed prisms.
Frozen section; Haemalum & Sudan III; X 50.

Fig.8. Deposit of doubly refracting cholesterin-ester fat in the spleen of a rabbit fed on cholesterin. Photographed through crossed prisms.
Frozen section. Haemalum & Sudan III. X 50.

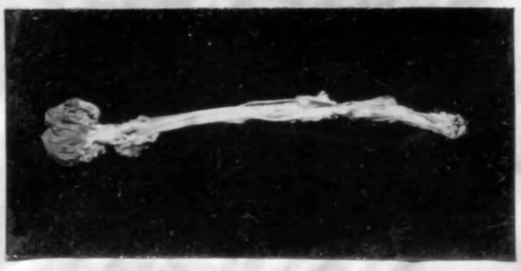

Fig. I

Fig 2.

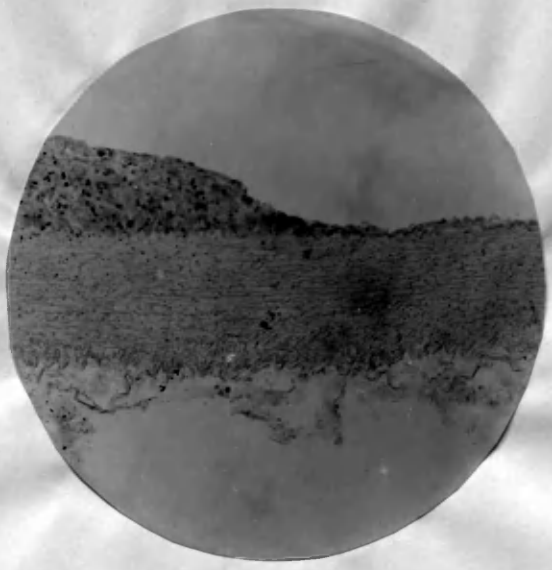

Fig. 3.

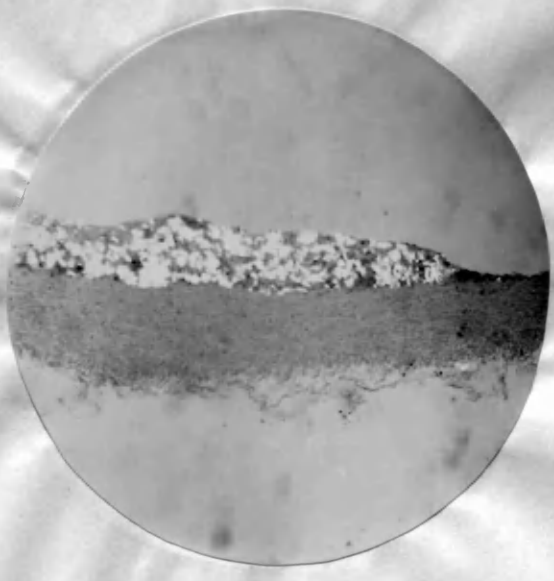

Fig. 4.

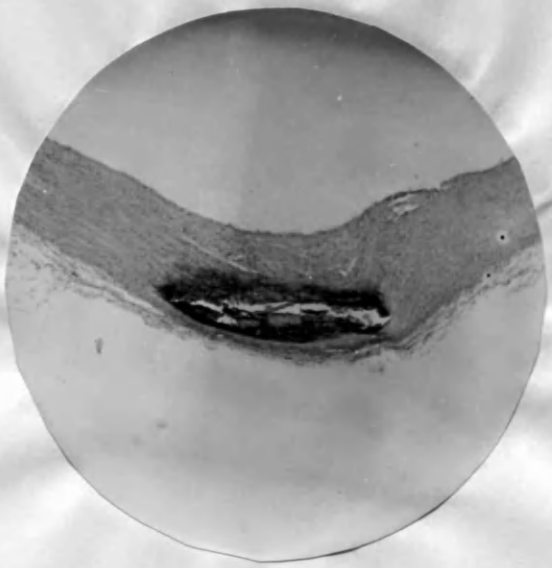

Fig. 5.

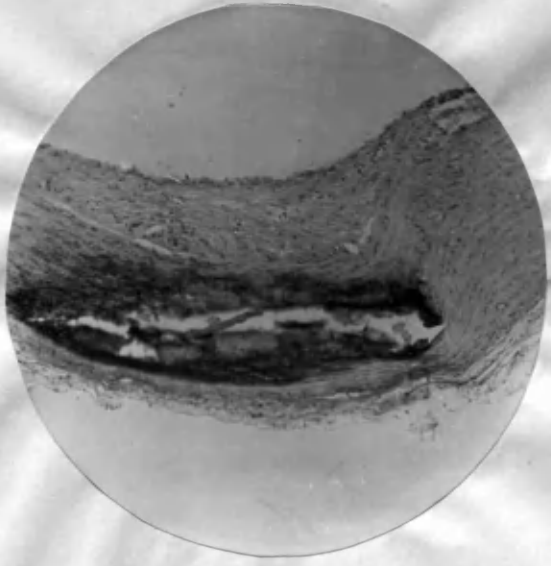

Fig. 6.

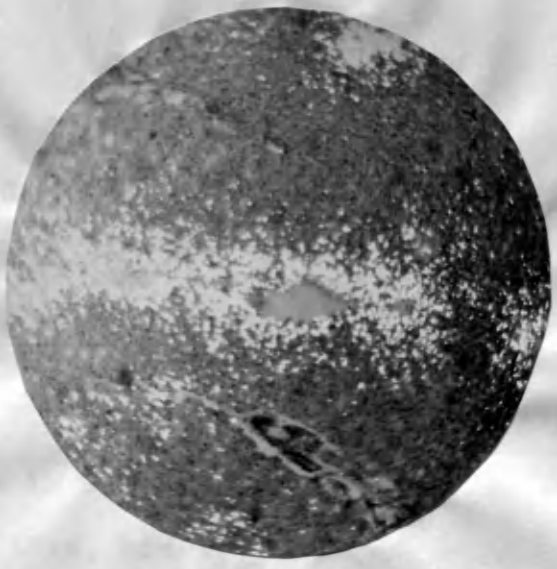

Fig. 7.

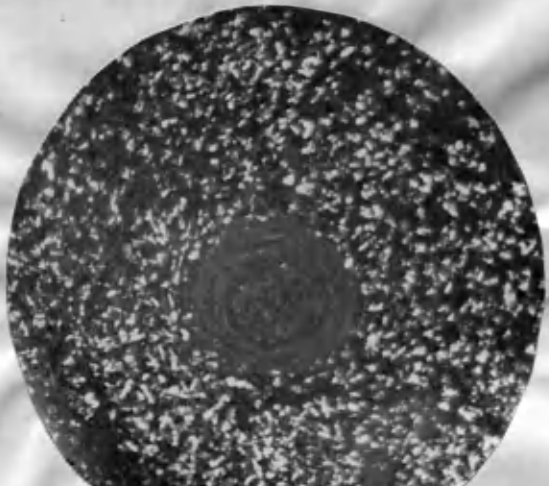

Fig. 8.

THE RELATIONS of the AORTIC CHANGES PRODUCED EXPERIMENTALLY in RABBITS to those of HUMAN ATHEROMA.

The question of whether the experimental results obtained in rabbits throw any light on the obscure problem of the causation of atheroma in man, requires very careful consideration. Before proceeding to this, however, it is necessary to review briefly the theories of causation of human atheroma, most of which are due to German pathologists. A careful watch must be kept on the terminology of such writers, or great confusion may arise from the study of the theories set forth. For many German writers the term "arterio-sklerose" includes all forms of arterial disease, both those primarily of the intima and those of the media. British pathologists, on the other hand, as a rule carefully separate atheroma (a primary disease of the intima) from arterio-sclerosis (a primary hypertrophy of the media, followed by degenerative sclerosis of fibrosis). It must be admitted, however, that the two conditions were thoroughly recognised by Marchand, who distinguished our "atheroma" as "atherosklerose" - a disease of later life, in contrast to "arterio-sklerose" where the medial hypertrophy occurs in middle life.

The theory of etiology of atheroma (atherosklerose) which has probably received the widest acceptance, is the mechanical theory of overstrain or overstretching, associated with the name

of Thoma,[67] and developed by him in a long series of papers in Virchow's Archives. This view was actively supported by Marchand,[68] Romberg, Jores, etc. This theory takes note of the fact that the changes occur in the degenerative period of life, long after the vessels have attained their full size. The development of the lesion is attributed primarily to physical and mechanical overstrain of the vessel walls, and the actual localisation of the patches to ~~secondary~~ damage of the elastic tissue, brought about by bacterial or alimentary poisons in the broad sense. This view has in recent times been attacked by Lubarsch,[69] Benda etc., and again stoutly upheld by Aschoff,[70] Torhorst, and others. Aschoff points out as a histological fact that the growth of blood vessels is always characterised by gradual hyperplasia of the elastic tissue (well seen during the establishment of a collateral circulation after arterial ligature), whereas during the degenerative period of life there occurs a definite mixing of fibrous tissue with the elastic tissue.

How does Thoma's theory, with its more modern developments, fit in with, and explain, the peculiar degenerative changes with deposit of cholesterin crystals and lime salts, so characteristic of human atheroma? Jores states that the fatty change is the result of fatty degeneration of the elastic fibres, but Aschoff and Torhorst have shown that the site of fatty degeneration is the inter-fibrillar cement substance, and not the elastic fibrils themselves. These authors describe a process of swelling and loosening of the cement substance, which they attribute to absorption of plasma through the intima. They believe that such

a process of absorption through the intima can explain all the degenerative changes characteristic of atheroma. In their view cholesterin-ester is drawn in with the plasma, and deposited in the inter-cellular cement substance between the elastic fibres. At first this fat is rapidly taken up by cells (just as in the experimental lesion in the rabbit), but as more and more fat accumulates, the cells finally die. The cholesterin-ester, set free again in this way, is soon broken up to liberate crystals of cholesterin, while the free fatty acids combine with calcium to form soaps, and this finally results in the characteristic deposit of lime. This view, it will be seen, is simply superadded to the theory of Thoma, in order to explain the peculiar degenerative changes in atheroma.

This mechanical theory of Thoma held almost undisputed sway until lately, when numerous workers - Josué (France)[37] Ignatowski[62] (Russia), and Saltykow[71] (Germany), began to advance the alimentary theory to the front. The French and Russian work has already been referred to, while Saltykow's original experiments described the effects of injection of cultures of staphylococci into rabbits, and the aortic lesions which resulted. A critical examination of Saltykow's work, however, showed that his rabbits were fed on milk and eggs, i.e. substances containing abundant cholesterin. Thus Saltykow, in his latest communication admits that his findings were quite probably due to the feeding, and may not have been direct effects of the bacterial toxin introduced.

This brings us on to discuss the place of cholesterin in the pathology of human arterial diseases such as atheroma.

It is certain that the mere deposit in the intima of small spots of cholesterin-ester does not constitute atheroma. Such spots are quite common in young children dying after infective fevers, at a time, in fact, when the cholesterin content of the serum is known to be reduced. It is evident, too, that mere hypercholesterinaemia is not sufficient to bring about human atheroma. Otherwise, atheroma would be a common disease in women who have borne many children. In diabetes, however, a disease commonly associated with hypercholesterinaemia, large yellow patches of fatty deposit are common in the aortic intima. In one young soldier (aged 21) who died of acute diabetes, the writer found the aorta almost covered with large patches of intimal thickening, laden with cholesterin fat. Histological examination showed, however, that no degeneration and no deposit of lime salts or crystals of free cholesterin had occurred, so it may well be doubted if these thickenings were truly atheroma at all. v.Noorden has pointed out that the white spots in the retina, which occur in diabetes, may disappear if the hypercholesterinaemia passes off, and it seems quite probable that the aortic changes might do likewise. Other local deposits of cholesterin-ester in the tissues are known to occur in man where hypercholesterinaemia exists. An example of this is seen in the development of xanthoma, in cases of jaundice, and more rarely in diabetes. There must, however, be some additional factor at work in the production of this condition,

since xanthoma is far from being a common complication of such diseases.

With regard to the experiments on rabbits, certain points must be borne in mind.

1. Rabbits are herbivora, and the aortic changes have been produced only by giving them a quite abnormal animal diet. All experiments on dogs and cats have so far been failures, although more require to be done.

2. In rabbits, the amount of cholesterin the the serum can be kept up by feeding to fourteen times (McNee) the normal, and it is only in long continued experiments that the changes are found. Such a hypercholesterinaemia never occurs in man. In rabbits, experiments have shown that this tremendous hypercholesterinaemia can result because very little cholesterin passes through the liver even normally (rabbit's bile contains mere traces of cholesterin), whereas in man, except in complete biliary obstruction, cholesterin readily filters out by the liver.

On grounds such as are referred to above, the writer has arrived at the following conclusions.

1. In man, the presence of hypercholesterinaemia is not a sufficient cause for the occurrence of atheroma, although it may account, in part at least, fro the occurrence of fatty patches in the aortic intima.

2. The presence of cholesterin-ester in a true atheromatous patch is simply to be regarded as an outward visible sign of a degeneration in the intima, brought about in a way which is (up to the present) uncertain. In other words, the damaged (overstrained-

-Thoma) area of the intima is simply made clearly visible and demarkated by the peculiar secondary degenerative changes - deposit of cholesterin and lime salts - found in atheroma.

3. The occurrence of cholesterin-ester fat in the degenerative change of atheroma is probably accounted for by a direct absorption from the plasma passing over the intima.

4. Until our knowledge extends, the mechanical theory of overstrain, originally developed by Thoma and elaborated by others, must be left in possession of the field.

GENERAL SUMMARY.

It is hoped that, from the survey of the subject presented here, some idea may be gained of the present state of our knowledge of cholesterin metabolism. In some parts, our knowledge is well advanced; in others, our information is merely fragmentary. The importance of the investigation of cholesterin is sufficiently obvious from the questions raised with regard to both physiological and pathological states.

It may be indicated again that the study of cholesterin merely touches the fringe of the larger question of the metabolism of the lipoidal substances in general. Until, however, the other compounds are more accurately identified, and suitable chemical means for their isolation and investigation are evolved, advances in our knowledge of their functions and metabolic activities in the body are likely to be slow.

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