

T H E S I S

on

CHOLESTEROSIS OF THE GALL-BLADDER;

A Clinical and Experimental Study.

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Presented for the Degree of

M. D.

by

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VOLUME I.

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## I N T R O D U C T I O N .

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Attention has been directed during recent years to a curious condition of the gall-bladder characterised by the deposit in its wall of large collections of lipoid substances, of which the most important is cholesterol.

Since this condition was first described in 1909 numerous case reports, and papers dealing particularly with the surgical aspects of the disease, have accumulated, but on the other hand the pathological problem presented by it has received singularly little attention, and such reasons as have been advanced as to its origin have been largely hypothetical. It has therefore been felt that a fuller consideration of the disease and of several related pathological and physiological problems would be of use.

The writer has been fortunate in meeting with 35 examples of Cholesterosis of the Gall-bladder, the majority of them occurring in patients under the care of Professor D.P.D. Wilkie, and this collection forms/

forms the basis for the present thesis. In many of the cases it has been possible to carry out complete investigations of the patients, both before and after operation, to obtain full histories and to correlate the impressions thus gained with the actual findings at operation and with careful naked-eye and microscopic examination of the gall-bladder after its removal. It is proposed to present the clinical and pathological observations made upon this series of cases, along with certain other relevant features of clinical interest, as Part I. of this thesis.

The occurrence of cholesterol deposits in the wall of the gall-bladder has appeared to the writer to be of more than passing interest, for the following reasons:-

(1) Although cholesterol is of widespread distribution and may be laid down in many sites throughout the body in large collections, yet little is known either of its real function in regard to the general body economy or of the conditions which control its deposition. Such deposits consequently attract interest in inverse proportion to our knowledge of their causation.

(2) The biliary tract is known to bear very particular relationship to cholesterol, for, with the exception of the milk during lactation, the bile forms by/

by far the most important vehicle for its excretion, and it therefore becomes a very attractive hypothesis that the gall-bladder, as a specialised part of the biliary tract, bears some close relationship to this excretory process and that this relationship renders it particularly subject to the deposition of cholesterol.

Part II. of this thesis is devoted to a consideration of these and other aspects of the disease, and to a description of experimental work which has been carried out in relation to them. Firstly, Cholesterosis of the Gall-bladder will be considered in its relation to similar deposits of cholesterol in other organs. Secondly, an excursion will be made into the realms of Comparative Pathology, to describe a similar change which it has been the writer's good fortune to observe in the gall-bladder of a cat. Thirdly, the possible causes of Cholesterosis in the human being will be considered, and the experimental production of the disease in animals under controlled conditions will be described. And lastly, experimental work will be recorded which goes to indicate the relation of Cholesterosis to the function of the gall-bladder and biliary tract.



Whatever may be the pathogenesis of Cholesterosis of the Gall-bladder, a feature of very practical interest is its relation to the formation of gall-stones. It is well recognised that Cholesterol forms the chief constituent of the great majority of gall-stones, and it will be shown later that those stones which consist almost entirely of Cholesterol seem particularly apt to be associated with Cholesterosis of the Gall-bladder. The relationship between these two conditions therefore invites examination.

In Part III. the present-day views as to the formation of gall-stones are considered, and in this connection several cases from the series here reported are described, which indicate the relationship existing between the origin of stones and cholesterol deposition in the gall-bladder wall.

ACKNOWLEDGMENT

The clinical work reported here was carried out in Professor Wilkie's wards in the Royal Infirmary of Edinburgh; the experimental and laboratory investigations in the Department of Surgery of the University. Except where otherwise stated, the cases reported were from the hospital and private practice of Professor Wilkie.

I would express my deep indebtedness to Professor Wilkie for the facilities which he has unstintingly afforded me and for his personal guidance and encouragement; to Professor Lorrain Smith and Dr. T. Rettie for much helpful advice and kindly criticism; and lastly, to the technical staff of the Department of Surgery for willing and skilled assistance.

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PART I.

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P A R T I.  

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CLINICAL AND PATHOLOGICAL FEATURES.  

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I. DEFINITION.

The term Cholesterosis of the Gall-bladder was proposed by Mentzer<sup>(33)</sup> to include two conditions, previously recognised, which are characterised by the deposit in the mucous membrane of the gall-bladder of lipid substances and especially of cholesterol. The former of these two conditions is that known variously as the "strawberry" gall-bladder, "fish-scale" gall-bladder, lipid gall-bladder, etc; the latter condition is that described as Cholesterol Polyposis or Lipoid Papillomatosis.

These two conditions differ essentially only in one particular, the distribution of the lipid. In the former, "strawberry" change, lipid deposits are scattered more or less diffusely over the mucous surface of the gall-bladder and are seen as innumerable tiny yellow specks, which, in a somewhat congested mucosa, give an appearance sometimes closely/

closely similar to that of a ripe strawberry. In the condition of Polyposis, on the other hand, the lipoid deposits are aggregated into one, two or more sites, giving rise, as the accumulations increase, to a gradual projection and pedunculation of the mucous membrane surrounding them.

Note as to Terminology. It is unfortunate that such a well-defined pathological entity as Cholesterosis of the Gall-bladder should have received such an abundance of names, and it is felt that, whilst certain of these in virtue of their descriptive character and of common usage should be retained, others are unnecessary and even actually misleading.

For the purpose of this thesis "Cholesterosis" will be used to signify the condition as a whole (although the term "Lipoidosis", which has also been suggested, would probably be more accurately descriptive). "Strawberry" change deserves retention as a convenient and well-recognised term for the diffuse form of Cholesterosis, and the localised type of the disease is most accurately described as Lipoid Polyposis.

The term Papillomatosis seems particularly misleading in connection with such polypi. It appears to have been originally based upon the view that/

that the epithelial hyperplasia sometimes associated with Cholesterosis was of a neoplastic nature, but this must now be quite definitely recognised as incorrect. The polypi simply result from the mechanical effect of a large accumulation of lipoids and have no other significance, and any associated epithelial hyperplasia is purely inflammatory in origin and course.

## II. HISTORICAL

The first description of "Cholesterosis" of the Gall-bladder is usually attributed to MOYNIHAN<sup>(36)</sup> in 1909, but while it is true that Moynihan deserves the credit for bringing this condition to the notice of the clinical and especially of the surgical world, it must be admitted that Pathologists had recognised its existence and had indeed conducted much patient research work in connection with it many years previously.

Thus, VIRCHOW<sup>(47)</sup> in 1857 noted that fat-like granules were frequently found in the epithelial cells of the wall of the gall-bladder, and ASCHOFF<sup>(3)</sup> in 1906, recognising the importance of this finding in relation to the question of the excretion of cholesterol via the bile, performed numerous experiments/

experiments to ascertain its significance.

It is MOYNIHAN, however, who first recognised the disease clinically. In a characteristically lucid paper he gave a very accurate description of the naked-eye manifestations in three cases, but, although he noted the importance to the surgeon of being able to recognise the disease, he interpreted its significance incorrectly as an intramural formation of gall-stones. The true understanding of Cholesterosis, as a pathological accumulation of lipoids, was not appreciated by clinicians till several years later.

In the following year, 1910, MacCARTY<sup>(28)</sup> in an elaborate classification of the inflammations of the gall-bladder, again drew attention to the naked-eye appearance of Cholesterosis, which he regarded as merely a form of chronic catarrhal Cholecystitis, the yellow colouration of the "specks" being attributed to an inflammatory desquamation of the epithelium resulting in subepithelial fibrosis and secondary bile staining.

In the same paper MacCARTY also described a single case of a cholesterol polypus in the gall-bladder, which he classified under the title "catarrhal papillomatous cholecystitis".

Though/

Though he failed to advance our knowledge of the essential pathological processes underlying Cholesterosis, MacCARTY did much, however, to attract attention to this condition, both by his happy bestowal of the name "strawberry" and by his insistence in several subsequent papers <sup>(29)</sup> upon the frequency of the lesion.

Much of our knowledge of the pathological aspects of Cholesterosis we owe to BOYD <sup>(6)</sup>, whose masterly paper in 1923 placed our knowledge of this condition on a sure footing. In particular BOYD gave a lucid description of the naked-eye and microscopic appearance of the disease and gave convincing proof of the nature of the lipid chiefly involved.

In France much attention has recently been devoted to Cholesterosis from both clinical and pathological aspects by CHIRAY and PAVEL <sup>(12)</sup>, whose views upon the aetiology will be referred to frequently in the course of this work, by LECÈNE and MOULONGUET <sup>(26)</sup> and by GOSSET <sup>(20)</sup> and his associates, who have dealt particularly with the clinical and surgical aspects of the lesion and its relation to stone formation.



III. DESCRIPTION OF SPECIMENS.

For the sake of lucidity it is proposed to give here a general description of the Morbid Anatomy, the Histology, and the various other aspects of Cholesterosis rather than to depict in oft recurring details the individual specimens which have been examined. The descriptions given here are, however, based upon examination of the actual specimens of 35 cases of Cholesterosis which form the present series and individual variations from the general type will be referred to as they are encountered.

(1) Macroscopic Appearance

(a) "Strawberry" Type. Figs. 1, 2, 3, and 4 illustrate the naked-eye appearance of some of the best examples of "strawberry" change which have occurred in this series. It will be seen that the appearance is striking in the extreme.

The mucous membrane of the whole gall-bladder has in most cases been distinctly congested and sometimes of dark red colour, so that in the fresh specimens the tiny nodular yellow "specks" of lipoid stand out in great contrast, giving an appearance which in many cases closely resembles that of a ripe strawberry.

The appearance is even more striking, as BOYD has pointed out, if the gall-bladder is examined under a binocular dissecting microscope. (The close-view photograph in Fig.2 gives a somewhat similar impression/

impression.) In the healthy organ the surface of the mucosa is raised up into numerous tall, thin, gossamer-like ridges, surrounding deep oval or polygonal hollows. (It is these ridges which, owing to their appearance in cross section, are usually described as villi. The gall-bladder has no true villi, but common usage necessitates the retention of this term.). In the "strawberry" gall-bladder these ridges, instead of being thin and tenuous, are stout and swollen, and within them the lipid is seen as dense or streaky yellow masses, which, in the words of BOYD, load the villi "much as the delicate birch tree might be weighed down by a load of snow".

In all the cases of this series the lipid has been confined (on naked-eye examination) to the summits of the ridges; other cases have been reported, however, where even the deeper recesses of the wall have also been invaded. In the well-marked case illustrated in Figs. 1 and 2 the lipid had infiltrated extensively, affecting practically the whole extent of the summits of the ridges, and as a result the deposit appears in this specimen as yellow linear streaks, following the general line of the ridges and running longitudinally along the gall-bladder.

The/

The strict localisation of the lipoid to the gall-bladder is a very interesting phenomenon, for, however extensively the gall-bladder is infiltrated, the lipoid appears invariably to stop short of the cystic duct, which is entirely free from the deposit. In the gall-bladder of Figs. 1 and 2 this delimitation was especially striking, though unfortunately it is not well reproduced in the photograph. The infiltrated ridges, which for the most part were arranged longitudinally, merged quite suddenly together into a more or less transverse yellow line, and beyond that line the cystic duct showed no trace of lipoid. The actual line of delimitation seems to vary in position in different cases. That in Fig.1 was situated well down into the cystic duct itself; in some of the other cases on the other hand it was in the region of the neck of the gall-bladder, some distance from the duct.

The lipoid infiltration is not always so extensive as seen in Figs. 1 and 2. Instead of occurring in linear masses it may be found as small scattered pin-head nodules, and the distribution, instead of being generalised, may be patchy, the patches varying in size down to the most insignificant.

Of the 31 cases of "strawberry" change in this series only 11 showed an extensive generalised distribution/

distribution, the remainder being affected in a patchy fashion. It is a point of interest that in these "patchy" cases there is a very definite tendency for the infiltration to be localised to the proximal end of the gall-bladder, and the fundus, on the other hand, is only rarely affected. Thus, in the 20 cases of this series, the patch of infiltration was at the fundus in only a single instance.

(b) Cholesterol Polypi. In these cases the lipoid, instead of being scattered diffusely over the gall-bladder, <sup>is</sup> aggregated to one, two or more sites. In this series 9 cases have occurred of this type, 5 of which were also associated with varying degrees of "strawberry" change. Fig. 14 shows a well-marked specimen of this type, and Fig. 5 shows one which occurred in an otherwise healthy gall-bladder. These two are particularly large polypi — the latter one measured more than half a centimetre in its largest diameter, but in the remainder of the cases the polypi have been considerably smaller. Thus, Fig. 36 illustrates a case in which a large number of polypi were scattered in the region of the neck, and in this case all the polypi were approximately the size of grains of rice. Usually they are seen to be markedly pedunculated masses which are attached to the underlying wall by the finest of filamentous stalks, which seem barely sufficient to maintain an adequate blood supply/

supply to the masses. Less commonly the polypi may be almost sessile.

The smaller polypi have been yellow in colour, the large ones greenish or brownish, probably owing to pigmentation from the bile. The polypi are not all composed entirely of cholesterol; in some of the smaller ones especially it has been noted that, in addition to the yellow lipoid, part of the mass has been occupied by red, fleshy, submucous tissue.

(2) Microscopic Appearance.

(a) General Histological Characters. As will be stated more fully in a later part of this paper, Cholesterosis of the Gall-bladder occurs most commonly in association with other pathological lesions, and especially with mild or moderate degrees of Cholecystitis. Consequently, the histological appearance is usually complicated by varying degrees of inflammatory change. There may be an extensive fibrosis of the wall of the gall-bladder, or a less chronic lesion with engorgement of the vessels and some round-celled infiltration, or, on the other hand, the inflammatory change may be extremely slight.

However marked this associated change, however, certain characteristic features will be noted. The inflammatory changes, however old-standing, have in all the cases of this series been confined mainly to the outer coats of the wall, and although some hyperplasia/

hyperplasia of the epithelium has frequently been present, the mucous membrane has in no case shown such marked changes as the outer coats. Extensive scarring or atrophy of the mucosa has never been noted. The villi have usually been enlarged, elongated, and almost pedunculated. The epithelial layer, which has been found complete unless eroded by pressure or stones, may be thrown into numerous folds which, in sections, sometimes give a false impression of glandular acini. The stroma of the mucous membrane, by some incorrectly called the submucous coat, also shares in the hyperplasia. The connective tissue cells are proliferated, and in addition there is often a mild degree of infiltration with leucocytes. Giant cells of the "foreign body" type, which occur around many cholesterol deposits, have never been seen in any of the cases of this series. A characteristic feature in the stroma is the vascular dilatation. The main vessels of the stroma lie immediately superficial to the muscular coat, and these and the ramifications which ascend to the villi may be greatly congested.

(b) Situation of the Lipoid. The main mass of the lipoid is deposited in the mucous coat, though occasionally in advanced cases traces may also be visible in the fibromuscular layer. In the mucosa the deposits may be either in the epithelium or in the stroma. In some/

some specimens the whole of the lipid has been in the one situation, in others in both; sometimes in the same gall-bladder one portion of the section showed the epithelial deposit, another that in the stroma (Fig.6). Moreover, different portions of the epithelial cells or of the stroma may be affected in different cases, giving rise to variations of appearance which are not readily explicable.

(1) The Lipoid in the Epithelial Cells may be found either superficial to or deep to the cell nucleus. Of these, the latter has been found to be the more characteristic. In this basal position the lipid occurs in the form of large globules, one globule filling the whole of the proximal portion of the cell, so that in a stained section the adjacent globules overlap. This deposit is invariably more marked towards the tips of the villi, but in well-marked cases it may also be found in the epithelium of the intervening depressions, giving the appearance, in sections stained with Scharlach R. or Sudan 4, of a bright scarlet streak running as a margin along the base of the epithelial layer (Fig.6).

Less commonly the lipid is placed superficial to the cell nucleus (Fig.7), and in this position it differs from the more deeply placed deposit both in appearance and in chemical character. Here it/

it is in the form of minute droplets which may be scanty or very numerous. The droplets are always localised to that part of the cell immediately superficial to the nucleus, and never approach completely to its free surface, a clear zone being invariably present there.

When the chemical characters of the lipoids are considered, it will be shown that in the two situations within the epithelial cells we have to deal with substances of entirely different characters, that in the basal region resembling the lipoid of the stroma in responding to the tests for cholesterol esters, whilst the supranuclear lipoid is more probably of the nature of an unsaturated fatty acid.

(2) The Lipoid in the Stroma is found in several types of cell, and in rare cases, though not in this series, has also been observed as an intercellular deposit. This latter situation is, however, exceptional, and probably represents merely a late phase consequent upon disintegration of the lipid-bearing cells.

The cells of the stroma which contain lipid in greatest abundance are large mononuclears which in paraffin sections have a striking appearance (Figs. 8, 9, 10, 11.). They are of large size (25-30 m.), round, oval, or polygonal in shape, with a small, highly/



highly pyknotic nucleus which may be central or displaced to the periphery. The cell protoplasm, from which the lipoid has been dissolved, contains numerous minute vacuoles, and its fine, delicate reticular appearance has been accurately described as "foamy".

These foamy cells are packed together, with little or no intercellular substance, in rounded clumps which are especially prominent close to the tips of the villi, but may also extend down as a leash of cells through the base of the villi towards the muscular coat. When present in considerable numbers they tend by their size to expand the villi into the broad, almost globular form already described, the appearance then being very like that of tiny air-balloons attached to the subjacent wall by delicate threads of tissue (Fig.8). In frozen sections the appearance of the foamy cells is equally striking, the vast amount of lipoid which they contain showing up brilliantly when stained (Figs.12, 13, 14).

Such foamy cells are by no means peculiar to Cholesterosis of the Gall-bladder, but may be associated with an excess of certain lipoids in many situations throughout the body, e.g. in arterial atheroma, in the subcutaneous tissue in Xanthoma, and in the spleen in certain conditions of lipoidosis. Their origin is not clearly understood. By some they are believed to be/

be fixed cells of endothelial origin, by others, wandering phagocytes, and it seems likely that they may arise from either of these sources. Their nature is best studied in early cases of Cholesterosis, where the sheer number of the cells does not hide their origin. They first tend to appear towards the tips of the villi, where they may be arranged as rounded rosettes (Fig.15), or disposed in linear fashion along the length of the villi (Fig.6b). In these early cases they are never related closely to the blood vessels, but, on the other hand, frequently lie directly in the line of the lymphatics.

In addition to the foamy cells, lipid in the stroma may occur in the following situations:-

(1) In small spindle-shaped or polygonal cells, in the form of minute droplets. These have occurred in several cases of this series.

(2) In the endothelium of blood vessels. Such a situation, which has been noted by BOYD and others, has only been found in one case of the present series. The lipid here, as in the small spindle-shaped cells, differs chemically from that in the foamy cells.

(3) In the lumen of blood vessels. BOYD noted one example in which he believed this to be the case, and in which the lipid appeared to be lying in  
a/

a small vessel of the stroma. Owing to the fact that the section had been subjected for a short time to a solvent, the appearance might have been due to displacement of the lipid from the tissues, and complete proof was therefore lacking. More definite is an observation of BERGERET and DUMONT (5), who have described and illustrated a case in which cells of typical foamy appearance, containing lipid, were found in the lumen of a small venule in the mucosa. These authors believed that the cells had reached this situation by a process of diapedesis.

To recapitulate, it may be stated that the lipoids of Cholesterosis occur in a variety of situations in the epithelium or in the stroma of the mucous membrane. In the present series the most frequent occurrence has been in two situations, namely, in the basal portions of the epithelium and in foamy cells in the stroma. Either of these positions may be affected alone or in combination, and it has been not uncommon to find different sites of involvement in different parts of the same specimen.

(c) The Microscopic Appearance of Lipoid Polypi differs from that of "strawberry" change only in degree. The polypi (Figs. 17, 18, 19) are composed almost entirely of foamy cells with a scanty covering of epithelial cells which are frequently lacking in places.

IV. NATURE OF THE LIPOIDS DEPOSITED IN THE GALL-BLADDER WALL.

For a proper review of the nature of the lipoids present in Cholesterosis of the Gall-bladder it will be advisable to consider first of all the types of fatty substances which are met with elsewhere throughout the body and to outline briefly the methods which are in use at the present day for their recognition.

Classification of the Lipoids.

The term lipid, proposed by KLETZINSKY (25) in 1858, is applied to a large number of substances of widely different chemical composition which have, however, certain common properties. Thus, they are all soluble, to a varying extent, in the fat solvents - ether, chloroform, benzine, alcohol, and the like, and similarly they all respond to certain of the fat stains, such as Sudan III, Sudan IV. and Scharlach R.

The lipoids which commonly occur in the human body may be grouped into four different categories:-

(1) The phosphatids are lipoids characterised by the presence of nitrogen and phosphorus. They include the monophosphatids or lecithins, Cephalin and Sphingomyelin. The members of this group are found chiefly in the brain, but also to a great extent in the cortex of the adrenal glands, and/

and they form an important constituent of the fatty substances found in the corpus luteum, ovary, thyroid and elsewhere.

(2) The second group comprises substances containing nitrogen but no phosphorus - the so-called Cerebrosides.

(3) The third group includes the fatty pigments or lipochromes. They occur in many situations throughout the body, but little is known of their chemical nature or the rôle they play in pathological processes.

(4) The fourth group comprises a somewhat heterogeneous group of substances which contain neither nitrogen nor phosphorus; it includes Cholesterol, the fatty acids (oleic, palmitic, stearic), cholesterol esters (combinations of the fatty acids with cholesterol), and the neutral fats or glycerine esters.

It is with the lipoids of this fourth group that we are particularly concerned, and especially with two of those mentioned, namely cholesterol and its ester.

Cholesterol,  $C_{27}H_{46}O$ , is a cyclic compound and a secondary alcohol. It was first isolated (from gall-stones) in 1775 by CONRADI<sup>(13)</sup> and was named cholesterin/

cholesterin by CHEVREUL<sup>(11)</sup> in 1815. In virtue of its character as an alcohol it is now generally known as Cholesterol, though the older nomenclature is still often retained.

Cholesterol is a constant constituent, in minute quantities, of most or all of the cells of the body, and is present (sometimes in large amount) in all animal fats and oils, and in the bile, blood, milk and egg yolk. Physiologically it occurs in greatest amount in three situations in the body, in the cells of the adrenal cortex, in the corpus luteum, and in medullated nerve sheaths. In pathological conditions its occurrence is widespread; thus it is present in the internal layer of the aorta in atheroma, in the subcutaneous tissues in xanthoma, in necrotic, caseating and suppurative foci, in the spleen in certain splenomegalies, and in numerous other sites.

In many of these situations cholesterol may occur in the pure state, and in some, for instance in the bile, this is its common condition, but on the other hand it is present within the tissues far more commonly in association with other lipoids, such as lecithin or the fatty acids, or in the form of loose fatty acid combinations or esters.

When/

When isolated in the pure state cholesterol is found to be a white, crystalline substance, which is chemically fairly stable, with a high melting point of 148-150°. The crystals occur in two forms: in the first, anhydrous form, the crystals are of fine acicular shape and frequently arranged in characteristic bristle-brush bundles; in the second form, in which crystallisation has occurred with one molecule of water, flat rhombic plates are seen, which frequently have one re-entering angle.

The ester of cholesterol may also occur in the form of crystals of fine acicular shape, which closely resemble those of pure cholesterol. The two can, however, be easily distinguished, for whereas cholesterol melts at a comparatively high temperature, namely 148-150° C., the crystals of its ester melt at a considerably lower point, the oleate at 37-42°C., the palmitate at 77-78°C., and the stearate at 82°C.<sup>(15)</sup>

In addition to its crystalline forms, cholesterol ester, especially when it is associated with traces of other lipoids, has the property, possessed also by many other lipoids, of assuming the so-called myelin figures.

In the tissues the ester of cholesterol most frequently occurs in a non-crystalline form, as minute droplets of homogeneous appearance, dispersed, sometimes in large numbers, within the cells. At other/

other times, and especially when the deposit is associated with a degenerative process, the fine acicular crystals may be visible, and these are usually extracellular.

Both Cholesterol and its ester have the property of anisotropism. That is to say, when they are viewed by polarised light through crossed Nicol's prisms, they stand out as masses of a brilliant white luminous appearance, and when they are present in the tissues their appearance contrasts markedly with the darkness in which the surrounding non-refractile tissues are cloaked. This property of anisotropism is valuable as a method of distinguishing cholesterol and its ester from other non-refractile lipoids such as the fatty acids and neutral fats, and with the aid of the polarising microscope it is an easy matter to make this distinction absolute.

If Cholesterol ester, such as the oleate, is viewed under the polarising microscope, and the section heated by means of a hot stage, it melts, as has been stated, in the neighbourhood of  $37-42^{\circ}$  C, If the heating is applied slowly, at this point the ester passes into what is known as the "fluid crystalline state". In this condition it has the physical properties of fluid, but remains anisotropic, and when/



when viewed by polarised light it gives the appearance of brilliant white Maltese crosses on a black background. This appearance may be maintained until the temperature of about 49° C. is reached, and at this point the ester passes into a completely fluid, non-refractile state, when it becomes completely invisible under the polariscope.

#### Staining Reactions.

Cholesterol and its esters, like other lipoids, can be stained by many of the so-called fatty stains. Some of these stains affect equally the cholesterol products and other types of lipoids; other stains, on the contrary, give peculiar colour reactions which are of assistance in the recognition of the particular lipoid involved. Thus, Sudan III, which stains fats with a yellowish brown colour, and Sudan IV, and Scharlach R., which give a scarlet reaction, affect fatty acids, neutral fats, cholesterol and its ester in the same way, though the colour with the last two lipoids is not usually quite so brilliant as in the case of the first. Other stains, which give a differential colour reaction, will be considered separately.

Osmic Acid,  $\text{OsO}_4$ , is reduced to  $\text{OsO}_2$  by neutral fats and fatty acids, and gives rise to a dense black staining. With Cholesterol and its ester, on/

on the other hand, little or no reduction occurs, and the lipoid remains unstained or at best but a faint grey.

Nile blue Sulphate has certain colour reactions which render it valuable in the recognition of the nature of lipoids. With neutral fats it forms a light rose-pink colour, and in contradistinction to this, fatty acids are stained blue, and cholesterol and its esters violet.

For the detection of cholesterol and its esters in the tissues BOYD has introduced a micro-chemical test based upon the well-known chemical method of SALKOWSKI. It consists in applying to a frozen section a solution of 5 parts of conc.  $H_2SO_4$  and 1 part of water, and it gives to cholesterol a light red colour, to its ester a terra-cotta or henna brown.

From the foregoing review it will be gathered that we have at our disposal three methods of investigation by which we may recognise the individual lipoid substance with which we have to deal:-

(1) Direct microscopical examination of frozen sections for the crystals of Cholesterol or its ester, recognisable by their shape and melting point;

(2)/

(2) Examination by polarised light for anisotropism and for the fluid crystalline phase;

(3) Examination of staining reactions.

The Lipoids in Cholesterosis.

Thanks especially to the work of BOYD, it is now clearly recognised that the most important constituent of the lipoids in the "strawberry" gall-bladder is Cholesterol, which BOYD believes to be present in the form of an ester. It is not generally recognised, however, that Cholesterol is by no means the only lipid present, and, moreover, that whereas Cholesterol is invariably found in certain situations of a "strawberry" gall-bladder, e.g. in the foamy cells, in other situations the lipid is of a different nature.

In this series frozen sections of the gall-bladder of 20 of the cases have been examined by some or all of the methods described above, and the following conclusions have been arrived at:-

(a) The lipoids in two situations, the basal portions of the epithelial cells, and the "foamy" cells of the stroma, give the following reactions:-

By polarised light anisotropism of a brilliant white colour is present. The melting point, varying in different specimens, is from 37° to

42° C., and at this point, if the heat is gradually applied, small and large "Maltese" crosses appear, later to be lost as heating proceeds and the lipid passes into the completely non-refractile phase.

The staining reactions with Sudan and Scharlach stains do not differ from those of other lipoids. Osmic acid, however, gives but a faint grey stain. Nile blue sulphate stains the lipid a bluish violet colour; BOYD'S Sulphuric acid test gives a henna brown reaction.

It is from these reactions that BOYD has concluded that the cholesterol is in the form of an ester, but while this conclusion is sufficiently correct for ordinary purposes, it should perhaps be remarked that acquaintance with the behaviour of cholesterol in other situations suggests that probably simple mixtures or loose combinations of cholesterol and fatty acids are more common than true esters.

(b) The greater part of the lipid in the foamy cells was deposited in the form of fine droplets, which were frequently in large numbers in the cells. In the epithelium, on the other hand, medium-sized droplets, and at the bases of the epithelial cells solitary large globules, were present.

(c)/

(c) Crystals visible under the microscope have been observed in several cases in one situation only, namely, inside the foamy cells. The crystals were invariably of fine acicular shape and arranged in characteristic bristle-brush fashion, and in all cases they were situated inside foamy cells towards the centre of a large cellular collection - a position which suggests that crystallisation (if indeed it was an intra vitam phenomenon) was perhaps associated with a commencing degenerative change in the part furthest removed from the blood supply. Extracellular crystallisation was not observed in any of the cases examined - a fact which may account for the entire absence of giant cells of the foreign body type.

(d) The lipid in the supranuclear portions of the epithelium, in small cells in the stroma, and in the walls of blood vessels (of one case) had an entirely different character. It was not doubly refractile, stained intense black with Osmic acid, and blue with Nile blue, thus corresponding in reaction with the unsaturated fatty acids.

V. CHARACTER OF THE BILE IN CHOLESTEROSIS.

The characters of the bile are of particular importance in regard to the hypothesis of the origin of Cholesterosis which will be advanced in the latter part of this paper. They have therefore been the subject of special study in the cases under review.

In his original paper in 1909 MOYNIHAN noted that the bile in the gall-bladders which he described was dark, tarry, and extremely concentrated, and this finding has been large confirmed in the present series. The appearance of the bile in 24 cases is recorded in Table I. (see Page 33).

Seven of the 24 specimens of bile examined were from gall-bladders which presented a well-marked "strawberry" change. In every case the bile was dark and "tarry". In six of these cases dilution with water showed that, apart from its extreme concentration, the bile was perfectly clear, with no trace of turbidity. In the seventh case no turbidity was present, such as might be caused by purulent debris or "biliary mud", but the bile contained innumerable minute yellow flakes. Microscopic examination of one of these flakes showed it to be composed of an amorphous/

TABLE I.

Character of the Bile in Cholesterosis.

<u>Cholesterosis</u>	<u>No. of Cases</u>	<u>Clear Bile</u>			<u>Turbid Bile</u>
		<u>Dilute</u>	<u>Tarry</u>	<u>Tarry with Cholesterol flakes</u>	
Generalised "Strawberry"	7	0	6	1	0
Patchy "Strawberry"	15	6	7	0	2
Polypi	2	0	2	0	0

amorphous, somewhat granular mass with no crystals visible, but after repeated heating and cooling the crystalline rhombs characteristic of Cholesterol appeared. Fifteen of the specimens were from gall-bladders which showed only patches of "strawberry" change, and here the appearance of the bile was less characteristic. In thirteen the bile was clear, and of these, seven were concentrated and six dilute (pale yellowish colour). In the remaining two turbid bile was present.

Of the two cases in which polypi alone were present, the bile of each was extremely dark and tarry.

#### Discussion in regard to the Naked-Eye Appearance of the Bile.

Two outstanding features of the bile have been noted in the gall-bladders which show Cholesterosis, namely (1) that the bile is in almost all cases free from turbidity, and (2) that it is highly concentrated in the majority of cases and especially so where the degree of Cholesterosis is marked.

The absence of turbidity from the great majority of specimens is a feature of importance in regard to the Pathogenesis of Cholesterosis, indicating as it does that no gross infection of the bile and no marked degeneration or desquamation of epithelial cells is present.

The/



The high concentration of the bile is also of importance. It is true that the condition of the bile in a patient prepared for operation does not accurately represent that usually present; when bile reaches the healthy gall-bladder a rapid concentration of it takes place by the absorption of water through the mucous membrane, and in the condition of modified fasting which usually precedes operation this concentration is frequently unduly exaggerated; but comparing the series here recorded with the bile of other patients operated upon under similar conditions, there is no doubt that undue concentration of the gall-bladder bile is the rule in Cholesterosis.

#### Cholesterol Content of the Bile in the Gall-bladder.

This estimation<sup>\*</sup> has been carried out in six cases of the present series, and in addition in three control cases in which mild Cholecystitis, but no Cholesterosis, was present. The findings are recorded in Table II. (see Page 37). It will be noted that a very marked deviation from the normal was found. Whereas the cholesterol content in the three control cases averaged 329.3 mgms. per cent, in examples of mild Cholesterosis the average finding was 503.6 and in the three cases of well-marked Cholesterosis/

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\* The method used for estimation of the cholesterol content of the bile is described in the Appendix. (Page 146).

Cholesterosis it reached the high figure of 969.6. It is true that the cholesterol content of the gall-bladder, like its degree of concentration, varies very much in health, after varying periods of fasting. (50) FOX has collected estimates of the normal cholesterol content of the bile, as estimated by different workers, which range from 160 to 910 mgms. per cent., and although estimations by different methods are not at all comparable, there seems no doubt that a wide range of readings is to be expected in health.

In the series reported here, however, it is believed that these variations have been reduced to the minimum. In all cases the bile was removed at operation (in some of FOX's it had been obtained by duodenal tube), and in all cases the duration and extent of pre-operative preparation of the patients was uniform. Moreover, those cases were chosen for estimation in which a corresponding degree of cholecystitis was present, for it was obvious that the bile from a grossly diseased gall-bladder in which concentration was inadequately carried on, would give faulty readings.

The raised bile cholesterol content will be considered at length in Parts II. and III., when it will be shown that it is of importance from two points of view, namely, firstly, as a factor predisposing to and mainly responsible for the onset of Cholesterosis, and, secondly, as a factor leading to the formation of pure cholesterol stones.

TABLE II.

Cholesterol Content of Gall-bladder Bile.

<u>Gall-bladder</u>	<u>Cases</u>	<u>Cholesterol Content (mgms. %)</u>	<u>Averages</u>
controls (No Cholesterosis)	(1)	156	} 329.3
	(2)	290	
	(3)	542	
Patchy "Strawberry"	(1)	466	} 503.6
	(2)	445	
	(3)	600	
Generalised "Strawberry"	(1)	636	} 969.6
	(2)	1033	
	(3)	1240	

VI. DISEASES ASSOCIATED WITH CHOLESTEROSIS.(a) Cholecystitis.

In nearly all the case reports of Cholesterosis emphasis has been laid upon its association with inflammatory lesions of the gall-bladder - cholecystitis - and so frequent is this association that the Cholesterosis is regarded by many as merely one manifestation of Cholecystitis.

It is the writer's opinion, however, that this view is incorrect, for although it is true that in the vast number of recorded cases cholecystitis has coexisted, cases are on record in which no trace of an inflammatory lesion was evident. When, moreover, it is recalled that the great majority of the reported cases of Cholesterosis have been those observed in gall-bladders removed surgically, it becomes evident that the predominance of coexisting inflammatory lesions may be more apparent than real.

GOSSET, LOEWI and MAGROU<sup>(20)</sup> have reported a case in which on histological examination no evidence of cholecystitis was forthcoming, and MENTZER<sup>(34)</sup>, in a large series seen at autopsy, has come to the conclusion that these are not infrequent.

In the present series of cases only one case has occurred in which histological examination yielded no evidence of an inflammatory change. The history and/

and other clinical features of this case will be described later (Case No.4. Mrs.M.). The gall-bladder, viewed at operation, appeared to be thin-walled, of healthy blue-green colour, with no excess of subserous fat and no trace of pallor. Indeed, so healthy did it appear that it was only in view of a very clearly defined clinical history pointing to the biliary tract that cholecystectomy was performed. Sections of the gall-bladder, both frozen and paraffined, were prepared (see Figs. 20 and 21), which show that, although the villi of the mucous membrane are distended with immense masses of cells containing lipoid material, there is no trace of an inflammatory reaction, either around the lipoid-containing cells or in the deeper layers of the tissue. The bacteriological examination of this specimen also gave no evidence of an infection, cultures in glucose-broth from the gall-bladder wall, the bile, and the cystic lymphatic gland proving sterile.

In all the remaining cases of this series, however, some degree of cholecystitis coexisted. In some cases the inflammatory lesion was well marked and of long duration, but in the majority was of mild degree.

For the purpose of this paper the 35 cases of the series have been arranged approximately according /

according to the degree or the duration of cholecystitis present. Several classifications of cholecystitis have been suggested at various times, the most complete one being that of MacCARTY<sup>(28)</sup>, but none of them appears to be entirely satisfactory. It has been considered that for the present purpose the most serviceable classification should be as simple as possible and based upon readily recognisable changes in the gall-bladder wall (see Table III, Page 41).

It will be seen from the Table that in the great majority of the cases of this series some degree of chronic cholecystitis has been found. Only two cases occurred in which an acute exacerbation of a chronic inflammation had occurred, and in each of the two cases both the naked-eye and the microscopic appearance strongly suggested that the cholesterol deposition was of old standing and had long preceded the acute exacerbation.

Of the cases which showed a chronic inflammation the change was of mild degree in the majority. Thus, of the total number of 32 cases with minor change, four showed no definite deviation from the normal until submitted to microscopic examination, and/

TABLE III.

Relation of Cholesterosis to Cholecystitis.

<u>Type of Cholesterosis</u>	<u>No. of Cases</u>	C H O L E C Y S T I T I S					
		<u>None</u>	<u>Chronic Cholecystitis</u>				<u>Acute</u>
			<u>Grade (1)</u>	<u>Grade (2)</u>	<u>Grade (3)</u>	<u>Grade (4)</u>	
* Generalised "Strawberry"	11	1	1	3	4	1	1
† Patchy "Strawberry"	20	0	1	9	3	6	1
Polypi alone	4	0	2	2	0	0	0
Total Cases	35	1	4	14	7	7	2
Total Cases with Stones	17	0	0	5	4	6	2
Total Cases without Stones.	18	1	4	9	3	1	0

\* One of these also contained polypi.

† Four of these also contained polypi.

\* Grade (1) - Microscopic disease only.  
 " (2) - Gall-bladder slightly opaque.  
 " (3) - Gall-bladder wall moderately thickened.  
 " (4) - Gall-bladder wall grossly thickened.

and fourteen were of the group in which a slight opacity of the wall, as seen at operation, was the only macroscopic evidence of inflammation. Moreover, the cases which showed moderate or marked degrees of inflammatory change were mainly those in which calculi had formed, and if these cases be excluded, the predominance of minor degrees of cholecystitis becomes still more marked, for moderate or gross infection was present in only four of the eighteen cases without calculi.

Experience of these cases has led one to the belief that Cholesterosis is most commonly found in gall-bladders which at operation have the following appearance. At first sight, when the abdomen is opened and the viscera are inspected, the projecting fundus of the gall-bladder frequently shows little abnormality. It is thin, moderately distended, and of healthy blue-green colour. Further examination of the whole organ reveals, however, that mild inflammatory changes are present, and these are frequently most marked in the region of the neck of the gall-bladder. The subserous coat is often infiltrated with fat, not in marked excess, but a delicate leash of fat of a deep yellow colour. The gall-bladder has, moreover, in this region lost its bluish colour; it is opaque or pale, and to the touch some slight/



slight thickening of its wall is evident. Not infrequently also a marked degree of fibrosis of the liver adjacent to the gall-bladder has been present, a further evidence of mild cholecystitis. In other cases, however, more marked degrees of cholecystitis have been present, and in 7 cases the degree has been gross, the gall-bladder thickened by old-standing fibrous tissue which in one case was half a centimetre in depth (see Fig.34). It is remarkable, though, that in all of these cases the inflammatory change has been almost entirely confined to the outer coats of the wall. The mucous membrane in many cases has been found to show some hyperplasia, and in several a marked degree of round-celled infiltration, but gross chronic changes, such as scarring of the mucosa and disappearance of the epithelium, have never been encountered.

(b) Malignant Disease of the Gall-Bladder.

BOYD has described and illustrated a case in which Cholesterosis has been noted in a case of carcinoma of the gall-bladder. No case of this lesion has, however, occurred in the present series.

(c)/

(c) Gall-Stones.

Gall-stones have been present in 17 of the 35 cases of the series, and in no fewer than 11 of these the stones were of the "pure" cholesterol varieties. The whole question of the relationship between such stones and Cholesterosis will be discussed at length in Part III.

(d) Disease in Organs other than the Gall-bladder.

In considering the association of Cholesterosis with other diseases it must be remembered that statistics based upon surgical material do not give an accurate impression of their true incidence. Bearing this in mind, it would appear that in this series no undue predominance of any one lesion was present. Duodenal ulcers coexisted in two cases, hydronephrosis once, and pronounced visceroptosis once. Gross chronic appendicitis was present in two cases.

VII. BACTERIOLOGY OF CHOLESTEROSIS.

Wherever possible in the cases of this series a bacteriological examination has been carried out of the tissues removed at operation. The method of procedure has been as follows:- The tissue, gall-bladder or cystic gland has been received directly/

directly from the surgeon into a sterile glass dish and portions have been immediately taken for culture. In all cases the culture medium used has been 0.2% Glucose Broth, with the addition, in earlier cases, of fragments of sterile brain. Table IV. (P.46) shows the results of this investigation

It will be seen that only a small proportion of the tissues examined showed any growth on culture, and that in these cases no characteristic flora occurred.

#### VIII. CLINICAL FEATURES.

##### (1) Case Reports.

It will be convenient at this point to study the case reports of selected cases of the series. It has already been remarked that in this series Cholesterosis of the Gall-bladder has in most cases been associated with other lesions, Cholecystitis, Gall-stones, etc., and it will be evident that these coexisting diseases must considerably complicate the clinical features of the cases affected. It has therefore appeared to the writer that a detailed description of the clinical features of all these cases would complicate the issue unnecessarily and perhaps actually mislead.

Accordingly/

TABLE IV.

Bacteriology of Cholesterosis.

<u>Tissue</u>	<u>No. of Cases</u>	<u>Sterile</u>	<u>Infected</u>	<u>Strepto- cocci</u>	<u>B.coli</u>	<u>Other organisms</u>
Gall-bladder Wall	22	16	6	3	2	1
Bile	23	19	4	2	1	1
Cystic Gland	21	19	2	1	0	1
Stone	3	2	1	1	0	0

Accordingly, eight cases have been selected for detailed description, namely those cases of which a full history was available and which presented no complicating coexisting lesion other than the inevitable minor grades of Cholecystitis.

CASE/1.

CASE 1.

Mrs. T. aet.34. Housewife. Multiparous.

Complaint: Pain below the right ribs.

Duration: Two years.

History:

During the past two years patient has been much troubled with a pain in the right side. At first it came on in attacks of great severity which doubled her up and were the worst pains she has ever experienced. It is much worse than labour pains. It is felt just below the rib margin on the right side and passes to the back to a point just below the angle of the right scapula. She often has pain also over the right shoulder which makes her right arm feel heavy.

Most of the attacks of pain come on during the day, and they may last for a few hours or for two or three days. When the pain is present it comes on in spasms like colic. Ever since these attacks began she has had a constant soreness in the right side, so that she sometimes cannot bear to wear tight clothing of any sort. She says that she is sometimes a little yellow in colour and she often has a rigor.

Patient has no indigestion and she never complains of flatulence and can eat any kind of food. She never obtains any relief from her discomfort after a good meal.

Patient vomits when the pain is severe, and every morning when she rises she feels sick and nauseated. She often vomits in the morning when she feels like this, and if she has had supper the night before, she recognises her supper in the vomited material.

Previous History:

Slight inflammation in the uterus nearly three years ago.

Family History:

Husband alive and well. Three of a family. None dead.

Menstrual History:

Considerable leucorrhoea, otherwise no abnormality.

On Examination:

Patient appears to be in good health.  
There is no definite jaundice.

Teeth: Only a few lower ones remaining,  
and these show some pyorrhoea.  
No upper teeth.

Tongue: Clean.

Abdomen: Normal in appearance, moves freely  
on both sides with respiration.

There is a slight degree of cutaneous hyper-  
aesthesia above the umbilicus on the right side  
but not extending round the chest. There is  
some definite rigidity and some tenderness over  
the gall-bladder point on the right rectus.  
Gall-bladder is not palpable and nothing ab-  
normal is felt in the abdomen. There is no  
tenderness elsewhere.

Circulatory System: Heart - not enlarged;  
both sounds closed.

Respiratory System: Lungs - no cough; exami-  
nation of chest shows  
no abnormality.

Urinary System: Urine - contains some  
pus cells. No fre-  
quency of micturition  
or dysuria.

Blood Cholesterol: 294.6 mgms. per cent.

Cholecystogram: 30.1.28.

Control film: No foreign shadow in gall-  
bladder area.

Film after S.T.I.P.P.: Gall-bladder well  
outlined.

Film after fat meal: Gall-bladder almost  
completely empty.

Stomach: Radiologically normal. Duodenal  
cap shows the effects of pressure from  
the gall-bladder on its right margin.

Conclusion: Radiologically normal gall-  
bladder.

Operation: 3.2.28. Professor Wilkie.

Stomach, duodenum and other viscera appeared  
healthy. The gall-bladder seemed a little  
pale and opaque towards the fundus, and there was  
some definite perihepatitis in its neighbourhood.  
In view of this and of the definite history the  
gall-bladder was removed. It was found to con-  
tain thick tarry bile, no stones.

The mucosa had the typical appearance of a "strawberry" gall-bladder with deposition of cholesterol in linear streaks over a red congested mucous membrane, stopping short at a point proximal to the cystic duct.

Microscopically there was evidence of a mild chronic cholecystitis.

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CASE 2.

Mrs. T. aet. 45. Housewife. Multiparous.

Complaint: Severe pain under right costal margin.

History:

Two years ago, whilst apparently in good health, patient had a sudden attack of severe pain under the right costal margin which lasted for three hours and was followed by a soreness there. She had nausea but no vomiting during this attack. One week later she had a second attack - not so severe. Since that time she has had 3 or 4 similar bad attacks, the last being 14 days ago, and this lasted several hours and was followed by soreness under the right costal margin which has not quite gone away yet. During these attacks patient has never been jaundiced definitely and has never vomited but always has nausea. Between the attacks she seems to be quite well - good appetite, not troubled with flatulence. Periodically she has a feeling of fullness after food with sickly sensation. This has been much worse during the last three months.

Bowels are usually very constipated; occasionally diarrhoea for a few days. If anything, she is gaining weight.

Patient has 8 children. During latter half of first pregnancy, 26 years ago, she was jaundiced and had much vomiting. The child only lived 9 days and was also jaundiced.

Menstrual/



Menstrual History:

Menstrual periods regular.

Urinary System:

Nothing to note.

Respiratory System:

~~renal~~ No cough; no "night sweats".

Circulatory System:

Shortness of breath on exertion recently;  
no swelling of ankles.

Heart: Sounds closed.

Pulse: Regular in time and force; good  
volume; wall not thickened.

On Examination:

Patient has a sallow appearance but shows  
no sign of jaundice; well nourished.

Remaining teeth good, gums healthy.

Tongue moist but slightly pale and coated.

Abdomen: Wall very fat and atonic. Moves  
freely on respiration; no ab-  
normal swelling visible. No superficial hyper-  
aesthesia. There is an area below the right  
costal margin which is slightly tender; no  
rigidity. Gall-bladder and liver not palpable  
below the right costal margin. Spleen and  
kidneys not palpable. No splashing in stomach  
3 hours after meal.

Operation, 16.6.25. Professor Wilkie.

Gall-bladder small, slightly thick-walled,  
with a considerable excess of subserous fat.  
No other pathological lesion within the abdominal  
cavity.

The gall-bladder contained clear, dilute  
bile, with no stones. The mucosa presented a  
small patch of "strawberry" change in the region  
of the neck, and there was a solitary cholesterol  
polypus of moderate size at the fundus.

Progress:

Reported three and a half years after  
operation. Symptom free and perfectly well.



CASE 3.

Mrs. B. aet. 45. Housewife.

Complaint: Attacks of pain in stomach for two years - jaundice three times.

History:

Two years ago, whilst working in the house, patient was seized without warning with severe abdominal pain. The pain was felt most intensely under the right costal margin, radiating round the side to the right scapula and across the abdomen to the left hypochondrium. It came on suddenly, was colicky in character and passed away gradually in about 24 hours. The pain made her roll about and perspire profusely. It was accompanied by nausea, but there was no actual vomiting. There was no further attack for a year, and during that interval she felt well.

One year ago she had a similar attack, in which the pain was more constant in character and not so severe. The pain passed off gradually in about 48 hours. She cannot state as to the presence or absence of tenderness. The attack was accompanied by nausea but no vomiting. For a week after that she states she was "yellow". In each of the attacks she was given morphia by her doctor.

Another similar attack occurred 6 months ago and was also accompanied by jaundice. Since the second attack, one year ago, she has not been in good health. For a few days at a time she has a dull pain under the right costal margin which has no relation to food and which disappears for 7-14 days at a time, to return again without obvious cause. There has also been a feeling of discomfort and distension in the epigastrium after food, worse by evening, when she has to loosen her clothing to get ease. She states that there is no excessive flatulence. She very seldom eats fats, and there is no special difficulty with any other kind of food. She has had no shivering attacks. The bowels are not constipated. There have been no urinary symptoms.

Menstrual History:

Regular 7/28 day type; considerable loss.

Previous/

Previous History:

Curettage twice in Ward 35 for menorrhagia.

Family History:

Husband - alive and well.

5 children - 2 of whom were still-born;

1 died aged 7 days. Youngest 14 years.

On Examination:

There is no obesity and patient is not jaundiced.

Alimentary System:

Teeth: Upper artificial; lower much decayed.

Abdomen: Well covered; no localised swelling. On palpation, there is slight rigidity of the upper half of the right rectus muscle and definite tenderness over a point  $1\frac{1}{2}$ " below the right costal margin at the lateral edge of the right rectus, also over a point 1" to the right of and below the umbilicus. There is also less marked, diffuse tenderness over the right hypochondrium. The gall-bladder is not palpable. The liver and spleen are not enlarged.

Circulatory System: Heart not enlarged; sounds closed in all areas, regular.

Respiratory System: No symptoms; no abnormal signs at the right base or elsewhere.

Urine: S.G. 1010. Acid. No abnormal constituents.

Cholecystogram: Dense shadow of gall-bladder present, which appeared in every respect normal.

Operation: 25.5.26. Professor Wilkie.

Stomach, duodenum, appendix and other viscera appeared normal. Gall-bladder showed little pathological to the naked eye. It contained clear bile, and the common duct, which was carefully examined, seemed healthy. The gall-bladder contained no stones. The mucosa appeared/

appeared somewhat engorged and presented a single cholesterol polyp, the size of a millet seed, situated close to the fundus. Microscopically, marked hyperplasia of the mucous membrane was visible, and a round-celled infiltration of the deeper coats was present. The polypus consisted almost entirely of large "foamy" cells, mainly unprotected by epithelium (Fig.17).

Progress:

Reported 2½ years after operation. Has had no recurrence of severe pain or jaundice, but has an occasional ache in the region of the wound. Appetite good, but is unable to eat eggs or most fatty foods.

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CASE 4.

Mrs. M. aet. 54. Housewife.

Complaint: Flatulent distension with eructations. Periodic vomiting. Jaundice on one occasion. Dull ache situated just below the right costal margin. Loss of appetite. Slight pain in right loin.

History:

In 1919, after prolonged medical treatment, perforation of a duodenal ulcer occurred; laparotomy was performed and the perforation closed. Thereafter, for two years, the patient enjoyed good health.

Early in 1921 she began to suffer from more or less continuous indigestion. After meals, and especially after tea, she had the sensation "as if she had eaten too much", and would feel distended and have to loosen her clothes. Frequently relief would be obtained by bringing up wind.

In October 1921 she had a sudden attack of agonising pain across the epigastrium, shooting up to the left shoulder. The pain lasted several hours and necessitated an injection of morphia. Three/

Three days later she was definitely jaundiced and remained so for about a week.

Since that attack the patient had had several short spasms of similar, very acute pain in the right hypochondrium, occurring at intervals of 2 to 8 months. The pain was usually followed by the vomiting of deeply bile-stained fluid.

Patient stated that for years previous to admission (in March 1928) she was rarely free from a dull ache in the right hypochondrium immediately below the costal margin. She was rarely able to take a good meal, and starchy or fatty foods disagreed with her.

On Examination:

Temperature subnormal. Pulse 80. Respirations 20.

Patient is of the pasty, fat, soft type. No sign of anaemia. No jaundice present.

Muscular development fair but muscles are of poor tone.

Eyes: sight good - pupils react to light and accommodation.

Teeth: edentulous.

Tongue: moist, shows a patchy grey fur.

No deformities. Cervical, inguinal and axillary glands show no enlargement.

Abdomen: Striae present. Right paramedian incision scar below the umbilicus. Moves freely with respiration. Superficial and deep hyperaesthesia present in right half of abdomen, except in lower iliac region. Rigidity and definite tenderness throughout right half of abdomen above the level of the umbilicus. Maximum point of tenderness not very definite. No tumour mass palpable. No abnormal dulness. Liver and spleen not enlarged. No splashing elicited.

Urinary System: There is tenderness over middle portion of right kidney. The renal organ can be palpated but does not appear to be unduly mobile nor enlarged.

Urine: S.G. 1020. Acid. No abnormal findings.

Cardio- /

Cardio-vascular System:

Pulse: 80, regular in time and force; systolic pressure is raised; vessel wall not appreciably thickened.

Heart: not enlarged; all sounds pure; no accentuations.

Respiratory System:

Lungs: both diaphragms move equally posteriorly; no abnormal dulness; breathing vesicular, no accompaniments; vocal resonance unaltered.

N.B. No crepitations heard at right base.

Blood Cholesterol: 209.6 mgms. per cent.

Cholecystogram:

Control film: No foreign shadow in gall-bladder area.

After S.T.I.P.P. Gall-bladder well outlined. No negative shadows.

After fatty meal: Gall-bladder shadow greatly reduced in size.

Barium Meal: shows a posterior gastro-enterostomy. Food was leaving rapidly through the stoma. No food was leaving by pylorus but there was marked tenderness of the cap area.

After 6 hours: small residue distal to stoma. Head of meal in descending colon.

After 24 hours: meal almost entirely evacuated. Residue in caecum.

Operation: 6.4.28. Professor Wilkie.

The small scar of the previous duodenal ulcer was found, and there were widespread adhesions throughout the peritoneal cavity, the result of the previous perforation. The gall-bladder presented little abnormality to the naked eye, but in view of the clear history it was decided to remove it. The common duct, which was closely inspected, appeared normal.

The gall-bladder contained clear concentrated bile. No stones were present, but floating in the bile were numerous minute buttery masses (the size of pin-heads) of cholesterol. The mucosa presented a typical "strawberry" appearance.

Microscopic/

Microscopic sections showed complete absence of signs of inflammation (Fig.20).

Bacteriological examination:

Cultures from wall, bile and cystic gland proved sterile.

Progress:

Five months after operation the patient reported, complaining of pain in the left lumbar region, and pyelography showed a mild degree of hydronephrosis on this side. The patient affirmed that since the operation she had had complete cessation of the right-sided symptoms.

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CASE 5.

Robert M. aet. 28. Labourer.

Complaint: Heartburn and waterbrash.  
Pain in umbilical region.

History:

In the summer of 1921 the patient had his first attack of indigestion, which began with a burning sensation in the region of the navel, accompanied by heartburn and waterbrash. These symptoms continued for the best part of a week. There was no vomiting, and jaundice did not follow. He was able to carry on work throughout this first attack.

Since 1921 the patient has had similar attacks of indigestion, lasting two to three months and occurring once or twice a year. During these periods of indigestion heartburn is a constant trouble to him, and a beating sensation in the epigastrium continues much of the day and most of the night. Regularly one hour after food a dull pain starts in the umbilical region and usually continues until food is taken, remaining, however, in the same spot. Vomiting is not associated with these periods of indigestion, but he brings up repeatedly mouthfuls of hot, acid fluid.

Since/

31 Since January 1926 the symptoms of heart-burn, waterbrash and abdominal pain after food have been continuous instead of periodic as previously.

In February 1928 the patient was admitted to Kirkcaldy Hospital and had a barium series of X-rays taken. A diagnosis of chronic appendicitis was made and operation carried out. He was discharged "cured", with the wound dry, at the end of 11 days. For six weeks afterwards he was free of indigestion. Following the operation he was jaundiced for seven days.

For the past 18 months the patient has been leading a life somewhat as follows:- Wakes with umbilical pain at 7.30 a.m. This continues until breakfast is taken at 8.30 a.m. Pain then passes off, to return about 10.30 a.m., and again continues till dinner time, unless soda or prescribed powders containing alkalies, including bismuth, are taken. Dinner at 12.30 - free of pain till 2 p.m. Pain then remains till 5 p.m., unless food is taken. From 8 p.m. till 7.30 a.m. patient is conscious of umbilical pain or a throbbing sensation in the upper abdomen. For many months he has been kept awake by the abdominal pain.

Patient's appetite is very poor. There is a marked tendency to constipation, requiring daily medicine. Habits temperate. During the war he smoked 30-40 cigarettes a day. Recently he has almost stopped smoking entirely.

Previous History:

Very healthy subject.

In 1917, accident at sea whilst in a mine layer - nearly drowned, in water for many hours; unconscious for a short period. A red rash came out in the skin and remained for three months. Was treated by arsenic by the mouth for several weeks.

Troubled with teeth during the war - two extracted.

February 1928 - appendicectomy.

On Examination:

Temperature 97.2°, Pulse 88, Respirations 20.  
Patient is only fairly well nourished.  
Muscular development is good. No deformities, no morbid appearances. Skin loose and lax.  
Subcutaneous/



Subcutaneous fat sparse. Mucous membranes of good colour. Small areas of psoriasis are present over both shins and extensor aspects of both elbows.

Pupils: equal and react to light and accommodation.

Teeth: several carious in upper jaw with some slight pyorrhoea around them. Lower teeth and gums healthy but several are absent.

Tongue: moist but slightly furred.

Abdomen: Movements free and symmetrical. Superficial hyperaesthesia well demonstrated over bands on either side corresponding to the distribution of the 8th intercostal nerves. No rigidity. Tenderness definitely elicited over a strictly localised area  $\frac{3}{4}$ " cranial and medial to the umbilicus. Liver and spleen not enlarged. No tumour mass felt. No sign of free fluid.

Respiratory System: Lungs resonant throughout; vesicular respiration in all areas; no accompaniments; vocal resonance and vocal fremitus unchanged.

Cardio-vascular System:

Pulse regular in time and force, expansion good; vessel not thickened nor tortuous.

Heart not enlarged; sounds closed; no accentuations.

Urinary System: No costovertebral nor suprapubic tenderness.

Urine: free of albumin and sugar.

Blood Cholesterol: 160 mgms. per cent.

Test Meal: (3.8.28)

Free Hcl.	Nil
Total acidity	14
Benzidine	Negative.

Barium Series. (6.8.28)

Radiologically normal stomach. Duodenal cap regular in all positions. No tenderness on palpation.

After/

After 6 hours: Minute gastric residue.  
Head of meal in transverse colon.

After 24 hours: Head of meal in sigmoid.

Conclusion: No lesion found.

Operation. 14.8.28. Professor Wilkie.

In spite of definite duodenal ulcer symptoms, pre-operative diagnosis was in favour of pylorospasm rather than ulcer. X-rays tended to confirm this.

Right paramesial incision, splitting right rectus in its medial third. No duodenal nor gastric ulcer (appendix previously removed). Slight perihepatitis over right lobe. Gall-bladder somewhat oedematous and thickened. Cystic gland definitely enlarged. In view of symptoms, gall-bladder appearances and enlarged cystic gland, it was determined to do a cholecystectomy. This was carried out in the usual way. An abnormal antero-inferior relation of the cystic artery was very clearly seen, also a very large, clearly defined right hepatic duct. The cystic gland was abnormal in position, being much lower down near the junction of the cystic and common ducts; this gland was considerably enlarged and was removed for culture.

After ligation of cystic artery and duct the gall-bladder was removed from its neck towards the fundus. One fine catgut suture was inserted in the liver to enfold the gall-bladder bed. Usual cigarette drain and closure in layers.

The gall-bladder contained clear, concentrated bile and no stones. The mucosa presented a slight degree of "strawberry" change, most marked over the neck of the gall-bladder.

Microscopically there was evidence of mild cholecystitis. In addition there were numerous large masses of foamy cells containing anisotropic lipoids. These cells were situated in the stroma of the mucous membrane and especially in the projecting villi. There was no lipid deposition in the epithelium.

Progress: Patient reported two months after operation, symptom free and perfectly well.

CASE 6.

Mrs. F. aet. 44. Housewife.

Complaint: Flatulent distension with eructations.  
Slight abdominal pains.

History:

Since before the war patient had been troubled with "wind". Any time during night or day a feeling of acute distension would come over her and she would feel tight around the abdomen. After some time she would usually be able to eructate, this giving some relief; rarely did she pass flatus per rectum. This periodic distension had no relation to the taking of food, and might come on during a meal, through the night or after food.

Patient has never had any acute abdominal pain, but since May 1928 she has been subject to a rheumatic-like aching in the right shoulder blade periodically. During the past month she has been conscious of a gnawing sensation two or three times a day in the right hypochondrium, and in May 1928 for three weeks this area was tender to touch.

There has been no vomiting, jaundice nor marked constipation, although she has had to take medicine now and again. Her appetite has always been quite good and she does not find that any particular food other than cheese aggravates her symptoms.

Previous History:

Nothing to note.

Family History:

One boy aged 3 years.  
One girl aged 7 years.  
One miscarriage (3 months) in 1920.

On Examination:

Pulse 68. Temperature 97.4. Respiration 20.  
Patient is pale, soft, rather flabby, but moderately nourished. Very slightly cyanosed. Mucous membranes all pale.  
Pupils/

Pupils: equal and react to light and accommodation. Can only read with very strong glasses; sees movements with both eyes without glasses but cannot count fingers.

Teeth: upper artificial; 6 teeth in lower jaw, 2 carious with pyorrhoea around them.

Tongue: moist and clean; protruded symmetrically.

Abdomen: rather protuberant; no hyperaesthesia nor rigidity. An indefinite, small, slightly tender mass can be felt protruding from below the right costal margin, opposite the 9th costal cartilage. This is dull on percussion. Liver and spleen not enlarged. No splashing. No signs of free fluid.

Cardio-vascular System:

Heart: not enlarged; sounds faint but closed; no accentuations.

Pulse: regular in time and force; poor expansion; vessels not thickened nor tortuous.

Respiratory System: No abnormality.

Urinary System: No costovertebral nor suprapubic tenderness.

Urine: free of albumin and sugar.

Blood Cholesterol: 160 mgms. per cent.

Cholecystogram: (26.7.28)

Gall-bladder outlined.

After barium. (Screen) No barium could be palpated beyond the distal end of the pyloric canal, suggesting pylorospasm. By the time a film was taken, however, barium had entered the cap, which on the single film looks irregular. The "dye"-filled gall-bladder is seen and not related to the stomach.

After fatty meal. This film was taken about 2½ hours after barium drink. The gall-bladder is still outlined and there is a large amount of barium still in the stomach.

Conclusions: (1) The gall-bladder has not emptied after fatty meal. (2) There is a suggestion of stasis in the stomach.

Operation/

Operation: (2.8.28) Professor Wilkie.

Kocher incision. Liver healthy. Gall-bladder slightly opaque, fat laden and thickened. Common duct normal; pancreas normal. No evidence of ulcer in stomach or duodenum.

Appendix slightly thickened in its distal half; it was removed. Pelvic organs normal.

The gall-bladder was removed after dividing cystic duct and vessels. Cystic gland kept for culture. Cigarette drain. Wound closed in layers.

The gall-bladder was fat-laden and somewhat opaque. It contained clear concentrated bile and no stones. To the naked eye a mild degree of generalised "strawberry" change was apparent, most marked in the region of the neck of the gall-bladder.

Microscopically, there was a moderate degree of chronic cholecystitis, with fibrous replacement of the muscle and some increase in the thickness of the subserous coat. The lipid was situated in foamy cells in the stroma and also to a less extent in the bases of the epithelial cells.

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CASE 7.

Mrs. C. aet. 41. Housewife.

Complaint: Flatulent distension and a feeling of nausea after food.

History:

For at least 18 months the patient has noticed that after meals her abdomen became very swollen. This symptom would bother her perhaps 3 times daily for 7-10 days and then not recur for 5 weeks. During these periods of indigestion, after/

after the taking of a meal, she may feel intensely sick and perhaps vomit. If she has been very distended for an hour or two an indefinite ache may set in under the right costal margin, but this never amounts to true pain. Owing to these attacks she has had to stop wearing corsets on account of the tight feeling produced by them. Usually she gets relief from the flatulence by massage of the abdomen and repeated eructations follow. Jaundice has never been noticed. She does not suffer from heartburn or waterbrash.

Previous History:

No accidents; no disorder of micturition.

1912. One year after marriage, owing to dysmenorrhoea following a miscarriage (3 months), dilatation and curettage.

1917. Right oophorectomy.

1922. Acute rheumatism, in bed for 3 weeks.

Menstrual History:

Menarche 12; type 2-3/28; regular until marriage; after marriage the intermenstrual period varied from 6 weeks to 2 months. Before 1917 considerable dysmenorrhoea for first day; since operation she has had no pain. Loss has never been marked; no intermenstrual discharge. Last menstrual period - 3 months ago. The menstruation previous to that occurred 24 months ago.

On Examination:

Temperature 98. Pulse 78. Respiration 24.

Rather stout, well developed female. No morbid appearances. Marked anterior convex deformity of both tibia. Mucous membranes rather pale. No jaundice.

Eyes: pupils equal and react to light and accommodation.

Teeth: all false.

Tongue: moist and clean.

Abdomen: midline suprapubic incision scar. Movements free and symmetrical; no hyperaesthesia nor rigidity; tenderness present over the medial half of the upper third of the right rectus. No tumour mass felt. No splashing two hours after a meal. Liver and spleen not enlarged.

Cardio- /

Cardio-vascular System:

Pulse: regular in time and force; expansion only fair; vessel not thickened nor tortuous.

Heart: no enlargement. Loud systolic murmur replaces the whole of the first sound in the mitral area and is well conducted into the axilla; second pulmonary slightly accentuated; remaining sounds closed.

Lungs: no abnormal findings.

Urinary System: no costo-vertebral nor suprapubic tenderness.

Urine - free of albumin and sugar.

Blood Cholesterol: 330 mgms. per cent.

Cholecystogram: (23.8.28)

- (1) Control Film: no foreign shadow in gall-bladder area.
- (2) Film after S.T.I.P.P: gall-bladder densely outlined.
- (3) After Barium: Stomach - no radiological abnormality; cap small and doubtful but not tender. Gall-bladder visualised.
- (4) After fat meal: Opacity of gall-bladder much diminished.

Conclusion: Radiologically normal gall-bladder.

Operation: (27.8.28) Professor Wilkie.

Kocher incision. No perihepatitis. Gall-bladder rather opaque and definite thickening could be felt at the neck. The gall-bladder emptied satisfactorily. Cystic gland just palpable; no stones present. Ducts patent. No duodenal or gastric ulcer. Right ovary absent; uterus and left appendages normal.

The appendix, which showed no definite signs of disease, was removed and the stump invaginated.

It was decided to do a cholecystectomy, which was carried out in the usual way from neck to fundus after ligation of the cystic duct and artery. The cystic gland was removed for bacteriological examination. Abdomen closed in layers.

The/

The gall-bladder was opaque and thick-walled. It contained clear, very concentrated bile, no stones. The mucosa presented a well-marked degree of "strawberry" change, with innumerable tiny seedlets of lipoid in a rather congested wall. The cholesterol infiltration was absent from the cystic duct and from an area one inch in diameter at the fundus. In this area the mucosa was more inflamed than elsewhere, and was somewhat scarred. In addition to the diffuse cholesterosis, a single large pedunculated cholesterol polypus was present. (see Fig.4)

Progress:

Patient reported two months after operation, symptom-free and perfectly well.

CASE 8.

Mrs. H. aet. 31. Housewife.

History:

During the last two months of the last three pregnancies she had had pain in the left hip, with stiffness of the hip-joint and shooting pains down the leg. During the last pregnancy, which terminated 7 weeks before admission, the lameness was much worse. It continued after delivery, though with slight improvement during the three weeks before admission. The patient had also had occasional heartburn during her last pregnancy, associated with some flatulence.

The hip-joint condition was considered to be the result of a toxæmia from some focal infection, and the patient was examined and treated from this point of view.

Two years before, for a period of 3 weeks. the patient had an attack of "biliousness", with loss of appetite and occasional vomiting, but apart from this, careful questioning could elicit no symptoms referable to the biliary tract.

On Examination:

Gross inflammatory enlargement of the tonsils was found, and there was a considerable degree of pyorrhoea.

The/



The abdomen showed no hyperaesthesia, but there was definite tenderness on pressure over the gall-bladder, with a considerable degree of guarding of the muscles over this region.

Blood Cholesterol: 196.2 mgms. per cent.

Cholecystogram:

Dense shadow of the gall-bladder obtained which appeared in all respects "normal".

The tonsils and teeth were first dealt with, and at a later date the abdomen was explored.

Operation:

The gall-bladder was thin-walled and of normal blue-green colour. Shining through it, against the background of the bile, could be seen the faint streaky lines of cholesterol infiltration. No other intra-abdominal lesion could be demonstrated.

The gall-bladder contained clear, very concentrated bile. No stones. The mucosa showed a well-marked degree of cholesterosis - a typical "strawberry" gall-bladder - the lipid extending over the whole organ with the exception of the cystic duct.

Microscopically, definite evidence of an inflammatory lesion of the gall-bladder was evident. The vessels of the stroma were markedly congested, there was a mild infiltration of the stroma and muscularis with round cells, and a slight degree of fibroblastic reaction.

Progress:

Patient reported seven months after operation, when she felt perfectly well, with no abdominal symptoms, good appetite, and no indigestion. No pain or stiffness in the hip.

(2) Frequency of Cholesterosis.

Twenty-one of the gall-bladders of this series have been received in the course of twelve months, during which period the whole number of gall-bladders examined totalled exactly one hundred. It would appear, therefore, that in surgical practice Cholesterosis of the Gall-bladder is a lesion of distinctly frequent occurrence. The fact that such a frequency is not generally recognised may be attributed to two factors: (1) Unless the gall-bladder is examined in the fresh state within a short time after removal, the erosive action of the bile may discolour the wall and mask a minor degree of Cholesterosis;

(2) Cholesterosis is most frequently associated with mild degrees of Cholecystitis and is not commonly found in gall-bladders which show gross thickening and fibrosis. Its occurrence tends therefore to be greatest in the practice of those surgeons who particularly favour the operation of cholecystectomy for mild cholecystitis.

It is interesting to note that other observers on the look-out for Cholesterosis report a similar or greater frequency in their cases. Thus LECÈNE and MONLONGUET <sup>(26)</sup> found typical Cholesterosis in 15 of 84 cases, and MENTZER <sup>(33)</sup>, in an autopsy series of 633 cases, noted it in 34.7 per cent.

(3) Incidence.

(a) Sex. The great majority of the cases occurred in women, but when the actual numbers are taken in conjunction with the total number of the two series operated upon, the disparity between the incidence is negligible.

Thus, during twelve months, 4 (or 20%) of 20 gall-bladders from men showed Cholesterosis, as compared with 17 (or 21.5%) of 80 from women. It is rightly arguable that these figures, based upon surgical cases alone, may not represent the true incidence, and it is therefore of interest to know that a similar equality in regard to the sexes was noted by MENTZER (33) in an autopsy series.

(b) Age. In this series the youngest patient was 28, the eldest 60, and the great majority exceeded 45 years.

(c) Social State. Remarkable similarity has been noted in the incidence of the disease in hospital and in private cases. Cholesterosis occurred in 12 (or 21%) of 57 consecutive cholecystectomies in hospital, as compared with 9 (or 20.9%) of 43 private cases.

(4) Symptomatology.

It has been one's object in considering the clinical features of Cholesterosis of the Gall-bladder to endeavour to discriminate between those symptoms and clinical signs which may be due to the Cholesterosis per se and those attributable to accompanying lesions of the gall-bladder or of other organs, and, although this discrimination has often proved difficult or impossible, in favourable cases it has been possible to obtain relatively reliable data.

From these, it appears that in regard to clinical features the cases may be divided into three distinct groups, (1) those with severe symptoms, (2) those with mild symptoms, (3) symptomless cases.

(1) Cases with severe symptoms. Four such cases have occurred in this series. All of them have been already described in full (Cases 1 to 4). It will be observed that all those patients complained of attacks of pain, which appear to have been intense, so that one patient volunteered that it was worse than childbirth, and another stated that it made her roll about and perspire freely. The pain in all these cases closely resembled that of biliary colic, striking the patient in the right hypochondriac region and radiating to the scapula or to one or other shoulder and necessitating the administration of morphia.

In two cases an attack of pain was followed by definite jaundice of several days' duration.

In the intervals between attacks these patients complained of symptoms like those of chronic cholecystitis, aching pain in the hypochondrium, soreness of the skin in this region, flatulence or abdominal distension.

In spite of these clearly defined histories of attacks of severe pain, the gall-bladder in these four cases showed extremely little change except for the gross deposits of cholesterol. No stones were present, and the degree of cholecystitis was very limited. In one case there was absolutely no histological trace of an inflammatory lesion; in a second such evidence was only obtained on microscopical examination, and in the remaining two the cholecystitis was of very mild degree. Yet in all these cases the absence of any other gross intra-abdominal lesion and the curative effect of cholecystectomy indicated that the symptoms had originated in the biliary condition.

(2) Cases with mild symptoms. Three of the cases of this type have been described in full (Cases 5, 6 and 7). Their symptoms, briefly, were those of "indigestion". In the first case (No.5) the symptoms/

symptoms closely simulated those of duodenal ulcer, with "hunger pain" relieved by food and by alkalies, and, occurring as they did in a man aged 28 and of rather less than average weight, they in no way suggest a biliary lesion, and only a "normal" radiographic examination prevented a confident diagnosis of peptic ulceration.

The second and third cases of this group (Nos. 6 and 7) are typical of a large number of the cases of the series. The indigestion complained of was of the type generally associated with chronic cholecystitis, and as there was a fair degree of this condition present in each case it seems likely that this, and not the Cholesterosis, was responsible for the symptoms.

(3) Only one case (No.8) of the fourth group ("Symptomless" Cases) occurred, as one would expect in a series culled from a surgical theatre, but it is of particular interest in two respects:

(a) It is probably representative of a large class, unrecognisable surgically and only found at autopsy if specially sought for;

(b) In spite of the lack of symptoms, the gall-bladder, when examined microscopically, showed unmistakable evidence of recent mild inflammatory change in addition to widespread cholesterol infiltrations.

Discussion of Symptomatology.

The symptoms presented by these patients possess several points of clinical and pathological interest.

In regard to Group (1), the main difficulty is to reconcile a history of severe colicky pains, in two cases followed by jaundice, with an apparently innocuous deposit in the gall-bladder wall. Biliary colic accompanied by jaundice is usually regarded as indicating an obstructive or gross inflammatory lesion in the ducts or at the neck of the gall-bladder, yet in the cases under review, although careful inspection of the bile ducts was carried out, no evidence of such a lesion was obtained, and, moreover, the apparent cures after cholecystectomy indicate that the causative lesion was centred in the gall-bladder. Three possible reasons for the severe pains in these cases present themselves: (a) Transient attacks of cholecystitis, which had completely subsided by the time of operation. In view, however, of present-day conceptions that cholecystitis invariably leaves behind it some greater or less degree of change, this view seems inadmissible. (b) Transient attacks of Catarrhal Hepatitis or Cholangitis. The absence of fever, the severity of the pain, and the relatively infrequent occurrence of jaundice prevent the acceptance of this view. (c) Passage of cholesterol crystals/

crystals. In the writer's opinion this seems the least <sup>a</sup> unlikely hypothesis. The passage of crystalline substances down the relatively wide and well-protected ureter is well recognised to cause attacks of agonising pain, and it may quite well be that a similar condition exists in the biliary tract, though it must be recognised that cholesterol crystals are frequently found in the bile in symptomless cases too.

Cases of Group (1) are probably infrequent; when the symptoms in Cholesterosis can be clearly discriminated from those of gall-stones, they most often resemble the features of chronic cholecystitis. Symptomless cases are probably the most frequent of all.

#### (5) Clinical Signs.

As the case reports indicate, the clinical signs give no help in the diagnosis of Cholesterosis. The general appearance of the patients has been in no way dissimilar from that usually associated with gall-bladder disease, and the patients have been mostly, though not all, well nourished or stout. Local signs have been merely those of chronic Cholecystitis.



(6) Special Diagnostic Measures adopted.

In an endeavour to discover some clinical feature characteristic of Cholesterosis and of value as a diagnostic measure, two methods of examination have been investigated in a number of cases, namely, Cholecystography and the estimation of the cholesterol content of the blood, these being two of the measures adopted as a routine in cases to be submitted to operations on the upper abdomen.

(a) Chole<sup>cy</sup>stography. It would be superfluous here to detail the full history of the discovery of the method now known as "Cholecystography", and it will be enough to summarise briefly the value of the method and the information which it may be expected to furnish. Essentially, Cholecystography consists in the administration of a substance which is excreted by the biliary passages and which, when sufficiently concentrated by absorption of water from the bile in the gall-bladder, is rendered sufficiently opaque to X-rays to give a visible shadow of the gall-bladder. A "normal" gall-bladder shadow therefore postulates three processes: (a) excretion of the substance in sufficient amount from the liver; (b) access to the gall-bladder via the cystic duct, and (c) adequate concentration of the substance by the absorption of water from the bile.

(21)

E.A. GRAHAM, who introduced the method of Cholecystography, has shown that no fewer than 13 substances fulfil these desiderata, but various disadvantages, such as undue toxicity, reduce the number of any value to 4, and only two are now in general use, namely, sodium tetra-iodo-phenolphthalein and its isomer, sodium phenol-tetra-iodophthalein.

Two general methods of administration are in common use, the oral and the intravenous. While various disadvantages are attributed to either, it is generally admitted that the intravenous route is more certain of giving accurate results.

The method of examination which has been employed in the cases described here is as follows:-

Before administration of the dye, a preliminary radiogram is taken, for comparison and to discriminate between shadows previously present and those due to the dye. The dye (sodium tetra-iodophenolphthalein) has been administered intravenously in the evening, after preliminary preparation directed to the bowels. On the next morning, 12 to 16 hours after the injection, an X-ray plate has been taken. At this period the healthy gall-bladder should be clearly outlined by the dye and should be of moderately large size, owing to the previous fasting condition/

condition of the patient. Immediately after this X-ray, in many cases a Barium Meal examination has been carried out, to demonstrate the precise relationship of the stomach and duodenum with the gall-bladder.

Then follows what is frequently the most important part of the examination. A meal is administered composed largely of fat and including the whole of an egg-yolk, and, two or three hours later, a last X-ray plate is taken. BOYDEN and WHITAKER<sup>(7)</sup> have shown that such a meal causes the gall-bladder to empty itself of bile, so that in the course of a few hours the shadow of the dye, previously large and lax, becomes greatly reduced in size. This emptying has been shown to be due to an intrinsic muscular contraction on the part of the gall-bladder, and the "emptying test" is therefore a criterion of this property.

Findings in Cholesterosis. 17 cases of Cholesterosis have been examined by the method detailed above. In 11 cases the picture was complicated by the presence of gall-stones or of gross cholecystitis, and in these, as one would expect, the cholecystograph showed corresponding deviations from the normal. Interest centres on the remaining 6 cases in which no such complicating factor coexisted. Here the findings were clearly defined. Although several/

several of the cases showed a considerable degree of cholesterol infiltration, yet the cholecystograph in all cases appeared perfectly "normal". The shadow of the gall-bladder was dense, uniform throughout, of average size and shape, and with well-defined margins. Moreover, after the administration of a fat meal, in all cases except one the normal response, by contraction of the gall-bladder and partial emptying of its contents, was obtained. There is therefore adequate support for the following definite conclusions in regards to cholecystography:- (a) As, in uncomplicated cases, an apparently "normal" shadow of the gall-bladder may be obtained, cholecystography is only of value in the diagnosis of cholesterosis as a means of excluding grosser disease of the gall-bladder, such as marked cholecystitis or stones. (b) The cholecystographic findings are of value in a consideration of the pathogenesis of Cholesterosis, for they indicate very clearly two features, firstly, that the concentrating function of the gall-bladder may be unimpaired, and, secondly, that the emptying mechanism of the gall-bladder is not affected, i.e. there is no biliary stasis present.

(b) Estimation of Cholesterol Content of the Blood. It is a curious circumstance that, although most of the existing hypotheses as to the pathogenesis of Cholesterosis of the gall-bladder are based/

based upon the assumption that in these cases the cholesterol content of the blood is raised, yet the whole literature contains only two instances in which this estimation has been carried out in cases of Cholesterosis. Moreover, in these two cases the findings were entirely dissimilar, for in the case recorded by CHIRAY and PAVEL the blood cholesterol index was raised to 210 mgms. per cent, whereas in the other case, reported by GOSSET, LOEWI and MAGROU it was of a rather low normal value (150 mgms. per cent.).

In this series the estimation has been carried out in 9 patients subsequently proved to have Cholesterosis of the gall-bladder. The blood has been taken during a fasting period, usually on the morning of operation. For controls, other cases have been taken in which operation upon the gall-bladder was proposed and in which cholecystitis or gall-stones but no Cholesterosis was subsequently demonstrated. Table V. (Page 80) gives the actual readings obtained. In Table VI (Page 81) the variation from the normal readings in cases of Cholesterosis are compared with those of the controls.

Discussion/

TABLE V.

Blood Cholesterol Estimations.

<u>Appearance of Gall-bladder</u>	<u>No. of Cases</u>		<u>Stones present</u>	<u>Blood Cholesterol</u>
Generalised "Strawberry" Cholesterosis	6	(1)	+	150
		(2)	-	160
		(3)	+	196
		(4)	-	209
		(5)	-	294
		(6)	-	330
Patchy "Strawberry" Cholesterosis	3	(1)	-	160
		(2)	+	190
		(3)	+	190
Controls Cholecystitis without Cholesterosis	14	(1)	-	110
		(2)	-	135
		(3)	-	140
		(4)	+	147
		(5)	+	160
		(6)	-	170
		(7)	-	180
		(8)	+	180
		(9)	-	190
		(10)	+	190
		(11)	-	210
		(12)	+	235
		(13)	+	270
		(14)	-	280

TABLE VI.

Blood Cholesterol Estimations.

<u>Gall-bladder</u>	<u>No. of Cases</u>	<u>Blood Cholesterol</u>			
		<u>Low</u> ( <u>Below</u> <u>150</u> )	<u>Low normal</u> (150-175)	<u>High normal</u> (175-200)	<u>Raised Index</u> (Over 200)
Generalised "Strawberry"	6	0	2	1	3
Patchy "Strawberry"	3	0	1	2	0
Controls: Cholecystitis no Choles- terosis.	14	4	2	4	4

Discussion of Findings in regard to Blood Cholesterol Index.

The cholesterol content of the blood is subject to such a wide range of "normal" variations, and is abnormally raised in so many physiological and pathological states, e.g. Pregnancy, Diabetes, Parenchymatous Nephritis, Jaundice, Xanthoma, etc., that in any particular case the interpretation to be placed upon a raised Blood Cholesterol Index must be considered with care. Moreover, even in the above-mentioned conditions, such as Xanthoma, a raised index is by no means an invariable finding, and it therefore <sup>not</sup> could/be expected that any such regular deviation from the normal would occur in Cholesterosis. Nor has this been the case. Reference to the table shows that, of the 6 cases with marked Cholesterosis, distinctly raised readings were obtained in 3; one was within the "high normal" range, and the remaining two were "low normal". Of the group showing patchy "strawberry" change, two gave "high normal" and the remaining one a "low normal" reading.

The cases showing Cholesterosis were thus equally distributed between the three groups, "low normal", "high normal", and "raised". Comparison with the control series of cases, in which no Cholesterosis was present, indicates that little or no difference exists between the two groups.

Conclusions/



Conclusions in regard to Blood Cholesterol Estimation.

These may be summarised as follows:-

(a) In the 9 cases of Cholesterosis of the Gall-bladder which have been examined, the blood cholesterol content has been distinctly raised in 3 cases, high normal in 3, and low normal in 3.

(b) The range of figures in these cases only differs from those obtained in the controls in that no abnormally low reading was obtained, whereas, of the controls, 5 of 14 cases gave distinctly low figures.

(c) Estimations of the blood cholesterol content cannot be regarded as of value in the diagnosis of Cholesterosis of the Gall-bladder.

(d) In regard to Pathogenesis, this series affords no support for the view that a hypercholesterolaemia is an essential causative factor.

(7) Diagnosis.

It will be obvious from the varied symptomatology that the diagnosis of Cholesterosis must present great difficulties. On the one hand, severe pain simulating biliary colic and followed by transient jaundice, will lead to the suspicion that a stone/

stone is present in the gall-bladder, and, on the other, the symptoms may be so trifling as to be missed.

In the majority of cases, however, the symptoms and signs point clearly to some lesion of the biliary tract, and the diagnosis of Cholesterosis must depend either upon the exclusion of grosser lesions or upon special methods of examination.

Of these, the one which seems to offer most assistance in the diagnosis is Cholecystography.

It is now generally recognised that Cholecystography, though by no means an infallible diagnostic measure, does give an indication either of gross chronic cholecystitis or of the presence of all stones except the smallest, and a "normal" cholecystographic shadow excludes these conditions. In a patient with a clear biliary history and a "normal" shadow the diagnosis is therefore narrowed down to one of the following lesions, namely, mild chronic cholecystitis, Cholesterosis, or small calculi.

It must be admitted, however, that in the present state of our knowledge the diagnosis of this condition can only be tentative.

(8). Treatment.

As Cholesterosis is rarely recognised until an extirpated gall-bladder has been opened, consideration of the treatment is of academic rather than practical interest. In two circumstances, however, Cholesterosis may be recognised at an earlier stage.

The yellow deposits in the mucous membrane may be discovered when the gall-bladder is opened for the purpose of cholecystostomy, or occasionally they may be recognised in the course of an exploratory laparotomy, when they may be visible through the thin translucent gall-bladder wall.

In the former circumstance the indication for treatment seems clear. The gall-bladder having already been opened, only two courses are available, either drainage or extirpation. Drainage can have no therapeutic effect upon a disease localised to the wall of the gall-bladder, and is more likely to result in increased traumatisation, infection, and scarring of the mucosa. Cholecystectomy is therefore clearly desirable.

The indication in the second circumstance, where Cholesterosis is recognised in an untouched gall-bladder, is not so clear, and must be based upon our knowledge of the aetiology of Cholesterosis and upon its symptoms and the possibility of spontaneous cure. In regard to this possibility, little is at present known, and it is conceivable that in the/

the absence of inflammatory lesions cholesterol deposits may eventually undergo absorption, with complete restitution to the normal. The observations of BOYD and others, however, indicate that the disappearance of cholesterol is usually associated with an extension of the inflammatory process already present, and the treatment should therefore be directed towards the prevention of this. Medical measures are not known to have any influence upon lesions of the gall-bladder wall, and if symptoms are present which point to the biliary tract, in the absence of contra-indications the operation of cholecystectomy is called for. This treatment has been carried out in all the cases of this series, and, though the period since operation is in most cases too short to justify a final opinion, the results so far seem eminently satisfactory. Of the six cases of uncomplicated Cholesterosis which can be traced, the period since operation has varied from six months up to four years. These six cases include the four patients who gave histories of severe symptoms, and all six state that since the operation no recurrence of these symptoms has taken place.

PART II.

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P A R T II.

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PATHOGENESIS OF CHOLESTEROSIS.

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I. GENERAL CONSIDERATION OF PATHOGENESIS OF CHOLESTEROSIS.

Cholesterol deposits, like those of neutral fats and other lipoids, have been classified into two groups, according as they originate by degeneration of the tissue in which they lie (i.e. an unmasking of cholesterol previously present in masked form), or by infiltration of the tissue with cholesterol derived from other sources; and in spite of the artificial nature of this classification it is a point of primary importance to determine to which of these two groups Cholesterosis belongs.

The whole appearance of sections of "strawberry" gall-bladders strongly favours the view that their cholesterol is derived from other sources by a process of infiltration. The amount of lipid material in the large collections of foamy cells seems altogether too great to have been previously present in a relatively acellular and scanty stroma, and, moreover, in most cases neither the cells of the epithelium nor those of the subjacent layers show any tendency to degenerative changes.

The question has also been quite definitely proved by BOYD, on the basis of chemical estimations of the cholesterol content of gall-bladders, for, whereas this content in the healthy gall-bladder amounted to from 0.5 to 1.7 per cent of the dry/

dry weight, and in an inflamed but non-cholesterol specimen to 0.36 per cent, the corresponding estimations in a series of "strawberry" gall-bladders reached the immense figures of 34.6 to 60.5.

Two principal views have been held in regard to the aetiology of Cholesterosis since its morbid histology was clearly recognised. The one view, most widely held, is that the "strawberry" change is merely one result of an inflammatory process in the gall-bladder. As only a small proportion of cases of Cholecystitis present any cholesterol deposits, it is usually presumed that Cholesterosis is a manifestation only of mild Cholecystitis, and that as the inflammation grows more severe the cholesterol deposit tends to vanish. Some workers, e.g. CHIRAY and PAVEL, have gone further and have believed that Cholesterosis depends upon a very special type of inflammation of the gall-bladder wall, or possibly upon an infection with certain specific organisms. In their view, the essential cause of the lesion is an obstruction of the lymphatics which drain the gall-bladder.

Other workers, on the contrary, consider that Cholesterosis is in no way associated with inflammatory change in the gall-bladder, but rather that it is a result of some upset of the general cholesterol/

cholesterol metabolism. GOSSET and his collaborators regard it as merely one stage in the formation of gallstones; MENTZER has correlated it with alterations of the general lipoid metabolism, and STEWART<sup>(46)</sup> believes that it is associated with an increase in the cholesterol content of the blood. Clinical records of the blood cholesterol content in cases of Cholesterosis are unfortunately scanty, and in addition to those reported here, only two cases have been found in the literature. Few though the records are, however, it seems clear that though a proportion of "strawberry" gall-bladders will be found to be associated with a hypercholesterolaemia, in some cases this index is within the limits of the normal.

In addition to the question of a general increase in the cholesterol content of the blood, the possibility must also be considered of a local increase of this substance - an excess in the bile - and in this relationship must also be considered the special function of the gall-bladder <sup>in regard</sup> to bile cholesterol. It is well known that, excepting the milk during lactation, the bile forms the only important excretory channel for cholesterol, and the amount which the bile contains, depending to some extent upon that in the blood, may sometimes be considerable. When the/



the bile reaches the gall-bladder the diminution of volume effected there by absorption of water causes a further increase in its cholesterol concentration, and this may be still further augmented by any factor which tends to promote biliary stasis. MOYNIHAN, in his original paper on the "strawberry" gall-bladder, drew attention to the thick, dark tenacious bile in the cases he reported, and this association of extreme concentration of the bile, and of a high bile-cholesterol content, with Cholesterosis has been confirmed in many of the cases here reported

(Tables I. and II.). In one well-marked case, this had further progressed to the deposition of cholesterol in numerous minute masses of a buttery consistency, and in many others deposition in the form of stones of more or less pure cholesterol had occurred.

The function of the gall-bladder in relation to cholesterol has been the subject of diverse views. NAUNYN (31) has always maintained that the gall-bladder serves to excrete cholesterol into the bile, a process which he believes to be effected mainly or entirely by disintegration of the epithelial cells of the mucosa. Most other workers, on the contrary, believe that the reverse process takes place, and that, when the bile from the hepatic ducts reaches the gall-bladder/

bladder, some of its contained cholesterol undergoes reabsorption through the wall. This latter view is supported to some extent by our knowledge of the other properties of the gall-bladder, which is known to be capable of absorbing water<sup>(41)</sup>, crystalloids such as sodium iodide<sup>(14)</sup>, potassium sulphocyanide<sup>(23)</sup>, potassium ferrocyanide<sup>(6)</sup>, and even particulate substances such as carmine or Indian ink<sup>(33)</sup>, and further evidence of it is offered by work to be described later. The question is of great importance in regard to the aetiology of Cholesterosis, for, if it can be shown that the normal gall-bladder has the power to absorb cholesterol from the bile, it only remains to determine what pathological circumstance perverts this process to a piling up of such excessive deposits in the mucous membrane.

It will be seen, therefore, that in a study of Cholesterosis of the gall-bladder, several well defined problems require consideration, some of them concerned particularly with the functions of the gall-bladder, others with more widespread processes.

These relevant problems will be considered in the following order:-

(1) As Cholesterosis of the gall-bladder has been shown to result from an infiltration with cholesterol/

cholesterol, a brief reference will be given to similar infiltrations in other parts of the body, and especially to two which appear to have several features in common with the gall-bladder lesion.

(2) Cholesterosis of the gall-bladder, as a disease of spontaneous occurrence in animals, is unknown, and the literature contains no reference to cholesterol deposits in other organs in animals. In the course of this research work, however, the writer has met with a condition of the gall-bladder of a cat which, though not identical, is closely similar to Cholesterosis, and from a comparative standpoint it is felt that a description of it will be of value.

(3) The aetiological importance of Hypercholesterolaemia, of Cholecystitis, and of Biliary Stasis will be considered.

(4) A method will be described by which it has been found possible to reproduce experimentally in animals the condition of Cholesterosis of the gall-bladder.

(5) Since there is much evidence that the origin of Cholesterosis is intimately related with some alterations of the function of the biliary passages in regard to cholesterol, these functions will be considered and relevant experimental research described.

(6) Lastly, as the basis of the foregoing, an hypothesis of the causation of Cholesterosis will be advanced.

## II. CHOLESTEROL INFILTRATIONS IN OTHER SITUATIONS.

Pathological infiltrations with cholesterol occur in widespread distribution throughout the body, and as results of very varied types of pathological change. M.J. STEWART<sup>(45)</sup> has classified them as follows:-

### (1) Infiltrations due to local pathological changes.

(a) Around caseating and suppurative foci, in subacute and chronic salpingitis, subacute inflammations of adipose tissue, and cerebral softenings; in association with certain specific infections such as Actinomycosis and Mycosis fungoides; and in the lesion known as "Myelin Kidney" \*.

(b) Due to retention of fluids rich in cholesterol, such as retention cysts of the breast, dermoid cysts, etc.

(c) In certain tumours, as in the myelomata of tendon sheaths.

(2)/

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\* Experimental work reported later indicates that this condition should be classified under Group (2).

(2) Infiltrations resulting from an increase of the cholesterol content of the blood, such as Cutaneous Xanthoma, Cholesteatoma of the Choroid, and possibly Arcus Senilis and certain forms of Atheroma. Certain splenomegalies may also be included in this group.

In these infiltrations cholesterol occurs in a variety of forms. Frequently, as for instance in arterial atheroma, large rhombic crystals are present. In other cases, however, (which are of great interest in relation to Cholesterosis of the gall-bladder) the cholesterol is situated inside cells of "foamy" type, and here, moreover, it is usually in the form of an ester or fatty acid mixture of a constitution very similar to that occurring in the gall-bladder. Or again, rarely, cholesterol occurs in an entirely different form in which it is entirely unrecognisable by the ordinary microscopical methods, being combined, probably with proteins, in a masked form.

Of the infiltrations classified above, two deserve especial description in relation to the study of Cholesterosis. These are (a) Cutaneous Xanthoma and (b) Xanthosis of the Fallopian Tubes.

(a) Cutaneous Xanthoma or Xanthosis is a lesion characterised by the presence in subcutaneous tissues/

tissues, especially in certain regions such as the eyelids or the extensor aspects of the elbows, of yellow coloured deposits. These deposits contain fibrous tissue and often small round cells and multinuclear giant cells, but their most interesting and important feature is the presence of large, "foamy", lipid-containing cells, which have an appearance and lipid content closely similar to those occurring in the gall-bladder.

The Pathogenesis of Xanthoma is generally believed to be closely related to alterations of the metabolism of cholesterol and other lipoids. Nearly all observers have found an increase in the blood-cholesterol content, and alteration in the total fat content of the blood has also been noted in several cases. On the other hand, there are a few well-authenticated cases in which the cholesterol values have been within normal limits<sup>(10, 39, 40)</sup>, and there are, of course, innumerable instances in which hypercholesterolaemia is not followed by the somewhat rare Xanthosis.

It seems evident, therefore, that Xanthoma is more than a mere overflow-infiltration of cholesterol and other lipoids into the tissues. On the one hand, some local factor must be presumed in addition to the general factor of hypercholesterolaemia, and/

and this is usually regarded as of an irritative nature, though such is only rarely demonstrable (as, for instance, in a case in which Xanthoma followed at the site of a mosquito bite<sup>(31)</sup>). On the other hand, an explanation must be forthcoming for these cases in which no increase in the blood cholesterol is found, and these are usually ascribed to the persistence of Xanthoma lesions after a causative hypercholesterolaemia has disappeared. ANITSCHKOW<sup>(1)</sup> has produced experimental evidence in support of these views. He found that, whereas in normal dogs the subcutaneous injection of infective or aseptic irritants caused the appearance of macrophages of the ordinary type, which contained particles of non-refractile fats, in animals in which the blood cholesterol content had been raised by appropriate feeding the macrophages contained esters of cholesterol. The lesions produced in these animals bore a close resemblance, it is stated, to subcutaneous Xanthoma.

(b) Xanthosis of the Fallopian Tubes.

This curious condition, which was noted by PICK<sup>(38)</sup> in 1910, is of particular interest on account of the marked similarity of appearance, both to the naked eye and under the microscope, which it bears to Cholesterosis of the gall-bladder. DANIEL and BABÈS<sup>(17)</sup> have/

have described three cases in great detail. In one, a case of chronic salpingitis, the mucosa of the tube was swollen and yellow coloured, raised in prominent branching villi, which microscopically were seen to contain "foamy" cells with much cholesterol. In a second case, a pyosalpinx, in addition to a similar diffuse yellow infiltration there was a single pedunculated mass, yellow coloured and of the size of a haricot bean, consisting almost entirely of a mass of "foamy" cells, and apparently closely resembling a cholesterol polypus. In the third case, the tube was almost obliterated, and projecting from the outer surface of it was a row of polypi, varying in size up to that of a pea, and extending the whole length of the tube.

Discussion. The two types in infiltration described, Cutaneous Xanthoma and Xanthosis of the Fallopian Tubes, serve to indicate the widespread distribution and character of these lesions and the great similarity in appearance which they may bear to Cholesterosis of the gall-bladder.

In the case of Xanthoma, the aetiological factors seem to be well defined, and the relation to hypercholesterolaemia is undeniable. In Xanthosis of the tubes, on the other hand, although no case is on record in which the estimation has been carried out, there/



there would appear to be no reason to suspect that the blood cholesterol index would be raised, and in these cases the presence of a local suppurative focus renders it probable that a local rather than a general increase of cholesterol is the essential causative factor.

From these observations the question naturally arises:- Can Cholesterosis of the gall-bladder be classed with Xanthoma as a lesion depending on an increase in the cholesterol content of the blood, or is it rather comparable to the tubal lesion as a result of a local excess of cholesterol? In later pages these possibilities will be considered.

### III. SIMILAR LESIONS IN ANIMALS.

In laboratory animals pathological accumulations of cholesterol appear to be extremely rare, except after the experimental administration of cholesterol (which will be considered later).

By a curious chance, during the course of his experimental researches upon Cholesterosis, the writer has encountered an example of a disease of the gall-bladder which occurred in a cat spontaneously (i.e. not as a result of experimental conditions), and which presented much resemblance to Cholesterosis in man. This case was met with, moreover, in circumstances/

circumstances which rendered an immediate and complete examination of all the organs possible. In view both of its intrinsic interest and its importance in relation to the pathogenesis of Cholesterosis in man, this case is described here somewhat fully.

In the cat, wide variations occur in the anatomy of the gall-bladder — it is not infrequently absent, and varying degrees of duplication and other abnormalities are common — but, in contrast with this, pathological changes in the biliary tract are almost unknown. Text-books of Veterinary Medicine dismiss the subject with the brief comment that gall-stones are rare, and an extensive search of the literature has failed to reveal any record of Cholesterosis. In 200 cats examined by the writer, no instance similar to the present one has been seen, and as the gall-bladder of the cat is thin-walled, and any change in the colour or opacity therefore readily noticeable, it is safe to assert that only a rare disease could have escaped detection.

The cat was a male, of moderate size and well nourished though not obese. (Orchidectomy had not been performed). It had been obtained from a dealer two days before examination, and no details of its past history are available. While in the laboratory it received the usual fare of fish and milk.

For the purpose of an experiment upon the gall-bladder, laparotomy was performed with full aseptic technique, but the gall-bladder appeared so/

so abnormal that the experiment was abandoned and instead an autopsy was performed.

### The Gall-bladder.

The gall-bladder was large, of almost double the normal capacity. The wall, in contrast to the usual translucent green, appeared a pale, almost creamy colour, and on palpation a thickening, with much oedema, became evident. Traced down towards the cystic duct, these changes were found to be localised entirely to the gall-bladder itself, the cystic and common ducts being of healthy appearance and normal calibre. There were no omental or other adhesions to the gall-bladder, and the adjacent liver appeared healthy, but, when the gall-bladder was removed it was found to be densely adherent on its deep surface to the liver, and, unlike the normal organ, could only be stripped off with difficulty.

The gall-bladder contained turbid bile of a greenish brown colour, thin and with no trace of mucus. No stones were present.

The most interesting feature was the mucous membrane, which over the greater part of the organ had a pale yellow colour. The wall was thickened and had a peculiar velvety feel. Seen from a distance it resembled a soft chamois leather in appearance. Careful examination showed that the yellow colouration was most evident at the tips of the "villi" or ridges of the mucosa, which were prominent and closely packed together. At the fundus, where the ridges were separated, the intervening surface had a somewhat congested appearance. Towards the other end of the gall-bladder the mucosa appeared relatively healthy. Cystic and common ducts seemed normal.

Sections of the gall-bladder wall increased the interest of the case. In the normal cat this wall is a thin, rather flattened membrane, from which the "villi" only project a short distance into the lumen, but here, on the contrary, the gall-bladder wall was thickened to almost four times the normal, and the mucous membrane was raised into innumerable closely ranged ridges, of extreme height and great delicacy, consisting mainly of a columnar epithelium with only a scanty/

scanty supporting stroma of connective tissue, the whole appearance so closely resembling the intestinal wall that a second section was required to exclude any possibility of mistake (Fig. 22).

In addition to this remarkable change in the mucous membrane the wall of the gall-bladder presented evidence of a mild chronic inflammation. The vessels, especially those of the subserous coat, were greatly dilated, there was some slight fibrosis in addition to considerable oedema, and in a few situations collections of small round cells were seen.

In a section stained with Osmic acid (Fig. 23) it was recognised that the yellow colour of the naked-eye specimen was due to the deposit of lipid substances in the mucous membrane. This lipid, which reduced Osmic acid to a dense black, was entirely confined to the tips of the villi, and was situated in the epithelial cells.

The epithelium of the bases of the villi and of the intervening depressions was lipid-free, as was the connective tissue stroma and the muscular coat. In the epithelial cells the lipid was situated both superficial to and deep to the nuclei, but mainly in the latter position.

#### Nature of the Lipoid.

In view of the similarity to the condition of "Strawberry" gall-bladder, it was confidently expected that the nature of the lipid present would also be the same, namely an ester or a loose combination of cholesterol and fatty acids, but this did not prove to be the case, for the characteristic feature of anisotropism was lacking, and even when a chloroform extract of a portion of the wall was examined by the Lieberman-Burchard reaction, only the faintest trace of cholesterol was found.

Further recognition of the lipid involved was obtained by its colour reactions in frozen sections. Scharlach R. and Sudan 4 gave the usual scarlet colouration, but with Nile Blue sulphate a vivid blue was obtained, contrasting markedly with the rose-pink neutral fat in the subserous coat, and indicating that the lipid was of the nature of an unsaturated fatty acid.

#### Examination/

### Examination of other Organs.

A complete autopsy was carried out immediately after the condition of the gall-bladder was recognised, and, as it was at first regarded as a case of Cholesterosis, particular attention was paid to those situations in which an excess of cholesterol might be evident.

On both naked-eye and microscopic examination, however, no pathological lesion could be recognised outside the biliary tract, nor were there any other unusual deposits of lipoids. The Adrenal Glands were not enlarged and contained no excess of cholesterol; the Aorta, so frequently attacked in experimental hypercholesterolaemia, was not atheromatous; Liver, Spleen and Kidney appeared healthy, and there were no deposits of cholesterol in the Choroid Plexus, the Iris, or other abnormal situations.

Discussion. Apart from its rarity, the case described above is of interest for several reasons. As an illustration of a pathological lesion rare in animals, it points the moral that in experimental as in clinical research, carefully controlled conditions are essential; a spontaneous disease such as this might render worthless the experimental series in which it was included, and since the occurrence of this case it has therefore been one's invariable custom to exclude similar ones by an exploratory operation preliminary to any experiment.

This case also tends to impress one with the importance of the factor of a chronic inflammation in the causation of Cholesterosis. In the cat, both Cholecystitis/

Cholecystitis and Lipoidosis are each so rare as to have escaped previous mention, and their association in this one case tends to emphasize the relationship.

The strict localisation of the deposits to the gall-bladder and the entire freedom from participation by the bile ducts serve to emphasize the similar observations made clinically, which very strongly suggest some intimate connection between the condition present and the particular function of the gall-bladder as compared to the ducts. The absence of evidence of lipoid deposits elsewhere in the body supports this view.

The one main distinction between this disease of the cat and the "Strawberry" gall-bladder in man is the character of the lipoid present. However, the essential similarity of the two conditions, it is believed, can be reconciled easily to this difference. In the human being, cholesterol occurs in considerable quantity in the diet as well as in the tissues, and it is present in large amount in the bile; in the cat, on the other hand, both the blood cholesterol and the bile cholesterol contents are low, and any lipoid infiltration consequently tends to involve other lipoids rather than cholesterol.

IV. RELATION OF HYPERCHOLESTEROLAEMIA AND CHOLESTEROSIS OF THE GALL-BLADDER.

(1) Clinical Evidence.

It has for many years been well recognised that in certain diseases characterised by an increase in the cholesterol content of the blood there is a definite tendency to the deposition of cholesterol in the tissues, a most obvious example of this sequence being given by Subcutaneous Xanthoma. M.J.STEWART<sup>(46)</sup> and others have attributed to Cholesterosis of the gall-bladder a similar origin, but the evidence for this view is somewhat incomplete. Proof of such a hypothesis should rely mainly upon the demonstration of a constant, or fairly constant, occurrence of hypercholesterolaemia in cases of "Strawberry" gall-bladder, and such proof is entirely lacking. The literature contains only two cases in which the cholesterol estimation has been carried out — one case showed a raised index; in the other it was normal — and the results obtained in the present research and shown in Table V. (see Page 80) emphasize still further the inconstancy of the relation. It is true that the finding of a normal cholesterol index in Cholesterosis, as in the rare cases of Xanthoma, might be passed over on the presumption of a former hypercholesterolaemia, but not in the entire absence of other,

other evidence in favour of the hypothesis.

MENTZER has regarded hypercholesterolaemia as an aetiological factor of primary importance, and in support of this view he has shown that, in an autopsy series, Cholesterosis of the gall-bladder occurred most commonly in individuals in whom an aortic atheroma or cholesterol infiltration in other situations indicated a present or previous alteration of lipid metabolism. These findings, in a large series of cases, must be accorded due weight, but it must be recognised, nevertheless, that there is much contrary evidence.

Whatever the importance of Hypercholesterol-  
aemia, there is much clinical proof that it alone cannot cause Cholesterosis of the gall-bladder. Many of the control cases recorded in Table V. had a considerable excess of cholesterol in the blood, yet neither gross nor microscopic deposits were found in the gall-bladders. Diabetes, obstructive jaundice, and parenchymatous nephritis are frequently associated with hypercholesterolaemia, yet the "strawberry" gall-bladder does not appear to be common in these diseases, and occasional cases are on record in which the blood cholesterol content has risen to tremendous heights (as in a case of DYKES<sup>(19)</sup>), in which it reached/



reached 1250 mgms. per cent.) with no deposits in the gall-bladder.

Clinically, therefore, there is no evidence that Cholesterosis depends upon an increased cholesterol content of the blood.

(2) Experimental Evidence.

(a) Effects of Experimental Hypercholesterolaemia. Experimental research upon the effects of hypercholesterolaemia originated with the discovery by certain Russian workers that characteristic changes in different organs followed the administration, for a prolonged period, of foods rich in cholesterol.

IGNATOWSKI<sup>(24)</sup> in 1908 observed changes in the aorta, liver, adrenals and kidneys of dogs fed for six months upon meat, eggs and milk. STAROKADOMSKI<sup>(45)</sup> and STUCKEY<sup>(44)</sup> confirmed these findings, and ANITSCHKOW and CHALATOW<sup>(2)</sup> showed that they were due to the deposit of cholesterol esters, that they depended solely upon the cholesterol content of the foods given, and that they could be produced by the administration of pure cholesterol. Since that time many workers have followed up these experiments and have investigated the remarkable results of cholesterol feeding, which may be briefly summarised here.

It/

It is found that, of laboratory animals, the ones which respond most readily to cholesterol feeding are rabbits, and this finding is generally attributed to the fact that normally the rabbit needs very little cholesterol and is correspondingly less able to deal with large amounts. In cats, dogs, and guinea-pigs, however, a similar, though less marked, response to feeding is obtained.

The administration of cholesterol, either as it occurs in egg yolks and in brain, or as purified crystals, rapidly and constantly causes an increase in the cholesterol content of the blood, and if the administration is maintained for a sufficient length of time, characteristic changes in certain organs and tissues are found.

The Aorta is one of the earliest tissues to show changes. These consist in a cholesterol infiltration of the intima with large collections of "foamy" cells, which may be so numerous as to present a naked-eye appearance closely similar to Atheroma. The lipoid subendothelial collections appear as prominent yellow masses projecting into the lumen of the vessel. They occur earliest in the ascending aorta, later in the descending portion, and, as in Atheroma, they tend first to involve the openings into branch vessels, the coronary, subclavian, intercostal and lumbar arteries.

Smaller/

Smaller Vessels also undergo the same change. In the writer's experience, the vessels of the lung have appeared particularly prone to it. In some cases the mass of "foamy" cells is so great as completely to fill the lumen of even large vessels, giving a remarkable appearance such as is seen in Figs. 24 and 25.

The Adrenal Glands constantly respond to cholesterol feeding by an increase in their size and lipid content, so that they may be three, four or more times their natural size and contain innumerable deposits of anisotropic fats.

In the Spleen "foamy" lipid-containing cells appear, especially in the sinusoids close under the capsule (Fig. 26).

The Liver responds first by the accumulation of cholesterol in numerous enlarged K<sup>u</sup>pfer cells. Later, prolonged feeding may lead to cirrhosis.

Occasionally gross changes occur in the Kidney, giving an appearance closely similar to the so-called "myelin" kidney occasionally found in man.

Figs. 27 and 28 illustrate the naked-eye and microscopic appearance of the kidney of a rabbit, fed for 12 weeks upon, in addition to its ordinary diet, 0.2 grams of cholesterol daily. At the end of this period the blood cholesterol index had risen from its normal value of 105 up to 1136 mgms. per cent. In the kidney large masses/

masses of yellow lipid are seen, situated chiefly in the intermediate zone at the junction of the cortex and medulla, but also extending through the medulla almost to the pyramids. The lipoids had all the physical and chemical properties characteristic of cholesterol-fatty acid mixtures. The microscopic section shows an extraordinary collection of large "foamy" cells, situated in the interstitial tissues between the tubules, which have undergone varying degrees of compression, obliteration or obstruction.

The cholesterol content of the bile is now generally recognised to vary to some extent, though by no means exactly, with that of the blood<sup>(30)</sup>, and the administration of cholesterol for a sufficient period of time leads to its excretion by the biliary tract. In certain cases this excretion is so great as to lead to the formation of small concretions of cholesterol in the bile<sup>(22)</sup>.

In a rabbit, which had been fed with 0.2 grams of pure cholesterol daily for fifteen weeks, the precipitation of cholesterol from the bile had continued to a remarkable degree. The gall-bladder, when viewed as it lay in the abdomen, was thought at first to have undergone a "strawberry" change, for visible through the thin wall against the dark green background of bile were innumerable tiny yellow spots. When the gall-bladder was opened, these spots proved to be flakes of a buttery consistency floating in the bile, and gave the chemical reactions of cholesterol. Microscopically, they presented an amorphous appearance and no crystals were visible.

(b) Effects of Experimental Hypercholesterolaemia on the Gall-bladder Wall. Many workers have

sought to show that cholesterol deposition in the gall-bladder may be produced by a simple increase in the cholesterol content of the blood, hoping thus to prove that this hypercholesterolaemia is the sole aetiological factor in Cholesterosis.

CHALATOW<sup>(16)</sup>, DEWEY<sup>(18)</sup>, and BLAISDELL and CHANDLER<sup>(8)</sup> have demonstrated that in rabbits, after a prolonged course of feeding, cholesterol may appear in the cells of the gall-bladder wall, and this finding has been confirmed in the course of the present work. It is felt, however, that this finding cannot be accurately compared with Cholesterosis in man. Cholesterosis of the gall-bladder may occur as a unique manifestation of pathological lipoid infiltration in an individual who is in other respects healthy. In an autopsy recently conducted by the writer, in which the gall-bladder presented a very marked "strawberry" appearance, naked-eye and microscopic examination revealed no trace of pathological cholesterol deposition in any other situation. In animals, on the other hand, if feeding with cholesterol is maintained for a sufficient period of time to ensure its infiltration in the gall-bladder wall, excessive deposition also occurs in the liver, kidneys, spleen, adrenal glands, and in the aorta and other vessels —

lesions which quite overshadow the rather scanty microscopical deposit in the gall-bladder.

#### Experiment.

Cholesterol was administered to four healthy rabbits over periods of 11, 15, 14, and 12 weeks respectively, each rabbit receiving daily, in addition to its usual portion of bran and green-stuff, 0.2 grams of pure cholesterol, which was administered, for convenience, mixed with butter.

The blood cholesterol contents were raised from 85, 70, 98 and 105 mgms. per cent, up to 342, 363, 469 and 1136 mgms. per cent. respectively.

In all the animals, at autopsy, gross deposition of cholesterol was found in the aorta, adrenal glands, and liver; in one, marked excretion of cholesterol in the bile had led to the formation of numerous small biliary concretions, and in another (referred to above) extremely extensive deposits were found in the kidney, spleen and other organs; yet in only two were traces of cholesterol visible in the gall-bladder wall, and in these two the scanty deposit, visible only under the microscope, contrasted very markedly with the large deposits elsewhere.

#### Conclusions in regard to the Importance of Hypercholesterolaemia.

It has been shown that, clinically, there is no support for the view of those who regard Cholesterosis as a mere result of a hypercholesterolaemia, and experimentally also all the evidence is against this view.. The fact that microscopical deposits appear in the gall-bladder wall after very prolonged feeding with cholesterol can be disregarded on account of the very excessive deposits which are also found elsewhere. Indeed, considering how massive some of these/

these latter deposits are, it would be very surprising if the gall-bladder wall did not share in the process.

On the other hand, it cannot be maintained that the amount of cholesterol in the circulating blood is entirely without importance in the pathogenesis of Cholesterosis. Clinically, it was noted that the only outstanding feature of the blood cholesterol content was that in Cholesterosis it is never below normal, whereas, in the control series, a low reading occurred frequently. It may well be, therefore, that a slight tendency to hypercholesterolaemia predisposes to Cholesterosis.

#### V. RELATION OF CHOLECYSTITIS TO CHOLESTEROSIS.

Diametrically opposed views have been held upon the importance of the infective or inflammatory factor in Cholesterosis, and, especially in French literature, great controversy has raged upon this question.

GOSSET and his associates have repeatedly affirmed that Cholesterosis is an aseptic, non-irritative lesion (in their opinion it is merely one stage in the formation of gall-stones), and they have described cases in which the gall-bladder presented no trace of inflammatory change. CHIRAY and/

and PAVEL, LECENE and MOULONGUET, and others equally insist upon an inflammatory basis, accounting for the occasional absence of histological evidence by the assumption that a former Cholecystitis has undergone complete resolution.

In the New World, also, there are adherents to either view. MacCARTY and BOYD have regarded Cholesterosis as merely one manifestation of Cholecystitis. MENTZER, on the other hand, from autopsy studies inclines to the opposing view.

Whilst it is generally conceded that in the great majority of cases a greater or less degree of Cholecystitis is present, occasional cases undoubtedly occur in which no evidence of this can be found; and in view of the fact that most investigations of Cholesterosis are based upon surgical cases, which are usually brought to operation by the accompanying inflammation, these exceptional non-inflammatory cases are of particular importance.

In the series reported in Part I. several cases occurred in which, to the naked eye, the gall-bladder presented little sign of inflammation, but in all of these except one the microscopic examination gave undoubted evidence of mild cholecystitis (Table III., see Page 41). In one case, however, although a very gross degree of "strawberry" Cholesterosis was present/



present, neither histological nor bacteriological examination yielded any trace of evidence of an inflammatory lesion (see Case 4 and Figs. 20 and 21).

In such a case as this it seems clear either that a previous Cholecystitis may completely resolve and leave behind it no visible sign, or that Cholesterosis requires no inflammatory factor. In their staunch advocacy of an infective origin of Cholesterosis, CHIRAY and PAVEL have accepted the former view, but with our present knowledge of diseases of the gall-bladder this seems unsupported. All clinical evidence goes to show that a gall-bladder, once affected by inflammatory disease, can never return completely to its normal state, and any exceptions to this must be rare. Judging by these criteria, therefore, it must be admitted that Cholecystitis is not an essential factor, though probably usually taking an important part in the production of Cholesterosis.

#### VI. RELATION OF BILIARY STASIS TO CHOLESTEROSIS.

The dark, tarry nature of the bile has been regarded as an evidence of undue stasis in the gall-bladder, and to this stasis has been attributed a major rôle in the origin of Cholesterosis. Clinical evidence, however/

however, is against this view. Whilst an extreme concentration of the bile is one of the effects of stagnation in the gall-bladder, it may also result from two other causes, namely, excretion of an unduly concentrated bile by the liver, and an increased absorption of water through the gall-bladder wall.

The entire absence of any degree of stasis in Cholesterosis may be most readily demonstrated by Cholecystography. It has been shown by BOYDEN and WHITAKER<sup>(7)</sup> that the gall-bladder responds to the administration of a fat meal by emptying its contents into the duodenum, the emptying process resulting from a slow tonic contraction of its muscular coat. In many of the cases of Cholesterosis reported in Part I., in which no gross degree of Cholecystitis coexisted, this emptying mechanism remained intact, and after the administration of a fatty meal a rapid and sometimes almost complete emptying of the gall-bladder occurred. There is therefore clear evidence that in these cases no stasis of bile existed.

VII. EXPERIMENTAL PRODUCTION OF CHOLESTEROSIS.

It was considered that the experimental production of Cholesterosis in animals would afford the readiest means of assessing the importance of different factors in its causation, and this was accordingly attempted in a long series of experiments.

In spite of the fact that either of the two factors, Hypercholesterolaemia and Cholecystitis, may be lacking clinically, it was felt that their use gave the best prospect of a successful production of Cholesterosis in animals, and as the former factor, acting alone, fails to give rise to more than microscopic deposits of cholesterol, it was decided to employ the two factors together. This method proved successful.

The method employed to produce a chronic cholecystitis was the inoculation, directly into the wall of the gall-bladder, of avirulent organisms. It is well recognised that in a considerable proportion of chronically inflamed gall-bladders cultures taken from the wall will yield a growth of streptococci, which are non-haemolytic, slow-growing and avirulent, and A.L. WILKIE has shown that if these organisms are injected between the layers of the gall-bladder wall, no acute inflammation occurs but a slowly progressive chronic cholecystitis appears, at first localised to the site of inoculation, later spreading over the whole gall-bladder. The whole process resembles that seen clinically in cases of mild or moderate chronic cholecystitis, in which inflammatory changes are chiefly localised to the outer coats of the wall, the mucosa remaining relatively intact.

The procedure adopted was as in the following experiment:-

Experiment.

A healthy male chinchilla rabbit was fed with 0.2 grams of pure cholesterol in butter daily, along with its ordinary ration of bran and greenstuff, for a period of 13 weeks.

One week after the commencement of the feeding laparotomy was performed. The gall-bladder appeared healthy. With a syringe and fine needle an intramural injection of a saline suspension of streptococci was then made into the inferior wall of the gall-bladder at its mid-point. The streptococcus was one which had been isolated several months previously from a case of Cholecystitis, and belonged to the viridans group. For the inoculation the growth from a 24-hour agar slope culture was suspended in 10 ccms. of saline solution and about one minim was injected.

Thirteen weeks after the commencement of the experiment the rabbit was killed. At this time it was extremely well nourished and appeared healthy. The abdomen was free from adhesions, the scar of the operation wound being barely discernible. The gall-bladder was of average size. Its inferior surface had an opaque, pearly-white appearance and on palpation seemed thickened. Its deep wall, when separated from the liver, appeared relatively normal.

The other viscera showed relatively little change to the naked eye, though microscopically deposits of lipoid were visible in the liver and adrenal glands.

The gall-bladder contained a large, semi-solid concretion and a few drops of turbid bile. The amount present was insufficient for an accurate estimation, but rough tests showed the presence of cholesterol in excess.

The inner aspect of the gall-bladder presented a striking appearance (Fig. 29). Through its whole extent the mucosa was raised up into linear ridges of a bright yellowish white colour, the whole appearance being very like that of a clinical "strawberry" gall-bladder, and this similarity was further enhanced by the fact that, close up to the commencement of the cystic duct, the infiltration stopped completely, the cystic and common ducts being to all appearances free from lipoid.

In/

In frozen sections from the gall-bladder (Fig. 30) the similarity to clinical Cholesterosis was completely demonstrated, for the lipid was found in the two regions where it would be expected, namely in the stroma and the epithelial cells of the mucous membrane, and much of the lipid conformed in physical and chemical characteristics with an ester of cholesterol, i.e. it was anisotropic and melted, with the production of Maltese crosses, at from 37° to 42° C.

Paraffin sections confirmed the similarity, for, in addition to numerous small cells of inflammatory origin, the stroma of the mucosa contained a number of typical "foamy" cells, which were most marked in the rather prominent villi, but also extended into the deeper part of the stroma (Fig. 31). In sections stained with Osmic acid these foamy cells were seen to contain lipid granules stained a faint grey.

In another rabbit, similarly treated except that the streptococcal inoculation was made only 4 weeks before the animal was killed, the appearance of the gall-bladder was even more striking. The gall-bladder, which was small and somewhat thickened, contained a few drops of fluid bile and a large soft yellow mass, which was taken at first to be thick pus adherent to the mucosa. More careful examination showed, however, that this was incorrect, and in microscopic sections it was seen that the whole of this mass was composed of large "foamy"-celled areas situated within the mucosa, the condition being that of a very much exaggerated "strawberry" change.

It will be seen, then that Cholesterosis of the gall-bladder can be readily produced experimentally by inducing an inflammation of the gall-bladder in a hypercholesterolaemic animal. It remains to be shown how this experimental finding can be correlated with the clinical observation that either Cholecystitis or Hypercholesterolaemia may be lacking. It is believed that this correlation can best be effected by a study of the function of the gall-bladder in regard to

VIII. RELATION OF FUNCTION OF GALL-BLADDER TO CHOLESTEROSIS.

The strict localisation of cholesterol deposits to the gall-bladder itself, and their invariable absence from the cystic duct, points clearly to some relationship between the function of the gall-bladder as opposed to that of the ducts. It is proposed first to consider their known functions and properties, and later to consider how they bear upon the problem of Cholesterosis.

The functions of the gall-bladder, so far as they are known at present, may be summarised thus:-

(1) As a blind diverticulum of the biliary tract, it serves as a reservoir in which bile is stored during the intervals of digestion.

(2) To compensate for its inadequate storage capacity, the gall-bladder absorbs water from the bile and thus concentrates it to a fraction of its former bulk.

(3) It acts as a pressure regulator to the biliary system.

(4) In response to certain stimuli during digestion it pours the contained bile into the duodenum.

(5) It has the property of absorbing certain substances from the bile. These have already been enumerated on Page 91.

The bile ducts are usually regarded as mere passive channels for the conduction of bile, their only other property being that of secreting a thin watery fluid, which becomes evident after an obstruction of the common duct and is then known as "white bile". These therefore do not share any of the functions of the gall-bladder, and for this reason presumably are not subject to Cholesterosis.

The bile forms a most important vehicle for the excretion of Cholesterol, which is present in large quantity, being held in colloidal suspension by the bile salts (probably chiefly desoxycholic acid). Two opposing views are held as to the method of this excretion. NAUNYN believes that the cholesterol in the bile is derived mainly or entirely from the desquamation of lipid-containing epithelial cells in the gall-bladder; practically all other authorities, however, regard it as a result of actual excretion from the liver.

From the point of view of Cholesterosis, it is important to determine the relation of the gall-bladder to cholesterol in the bile. Does the gall-bladder excrete cholesterol, re-absorb it, or take neither action? It is believed that the localisation of Cholesterosis in itself is sufficient to exclude the third possibility. What we know of the absorptive property of the gall-bladder towards several other substances suggests that cholesterol too is absorbed, and this view is further supported by the close similarity in distribution (i.e. towards the tips of the villi) which the lipoids of Cholesterosis bear to those of the intestinal wall during fat absorption.

In/

In the experimental field efforts have often been made to demonstrate the absorption of cholesterol from the bile, but these efforts have had little success. Other lipoids, on the contrary, can readily be shown to be absorbed into the gall-bladder mucosa.

(1) Absorption of other Lipoids.

The demonstration of lipid absorption by the gall-bladder wall was first given by ASCHOFF and later by MENTZER. The former introduced olive oil, butter and sterile milk into the gall-bladders of dogs, the cystic ducts being ligated to prevent expulsion of the inoculum. In each case it was found that after a period of a few days lipid deposits were present in the epithelial cells, whereas in control animals in which simple ligature of the duct was performed, no such appearance was seen.

MENTZER noted similar findings. Even after as short a period as half an hour the lipid could be seen in the epithelial cells, and after longer periods it could be followed to the stroma and even to the vascular endothelium. No particles of fat could be observed in the lymph node draining the gall-bladder, and MENTZER therefore concluded that the absorption took place directly into the blood vessels.

The/



The following experiments are typical of many that have been carried out, using a variety of fatty and lipoid substances.

Experiment.

In a cat the gall-bladder was exposed by laparotomy, and the bile removed by a needle inserted at the fundus. In place of the bile about 1 ccm. of oleic acid (insufficient to distend the gall-bladder completely) was injected, the needle puncture being then closed by a fine silk ligature. To prevent expulsion of the lipoid, the common duct was ligated close above its entrance into the duodenum. (In other experiments the cystic duct instead was ligated, with similar results. A disadvantage of ligating the cystic duct is that a certain amount of damage to the surrounding lymphatic vessels draining the gall-bladder is inevitable; on the other hand, ligation of the common duct introduces the complication of a complete biliary obstruction, with consequent raising of the pressure within the gall-bladder. Control experiments, using both sites for ligation, were therefore always carried out.)

Two days later the animal was killed. The mucosa of the gall-bladder appeared undamaged, but throughout the entire organ it showed numerous milky-white, tiny deposits, scattered throughout the mucosa along the summits of the ridges - an appearance closely similar to that of a typical clinical "strawberry" gall-bladder.

Microscopically this similarity was confirmed (except, of course, as regards the nature of the lipoid). In sections stained by Osmic acid or by Sudan 4 innumerable fatty deposits were visible in the epithelial cells of the mucosa, in exactly the situation most characteristic of Cholesterosis, namely at the bases of the cells just below their nuclei, and especially in those cells situated towards the apices of the "villi". In other sections the lipoid was sometimes found also in the more superficial/

superficial parts of the cells, and in some the epithelium was completely loaded with fatty particles (Figs. 32 and 33).

The whole appearance of such sections so strongly resembles that of early cases of Cholesterolosis that it is difficult to believe that in the latter condition the cholesterol can have reached its site of deposition except by absorption from the lumen into the epithelium of the mucosa. The only respect in which the clinical condition differs from that induced experimentally is in the nature of the lipoid, which in the experiment here cited had the staining reactions of a fatty acid and failed to show anisotropism.

In similar experiments the absorption of butter, olive oil and lecithin has been demonstrated.

## (2) Absorption of Cholesterol.

The demonstration of the absorption of cholesterol through the gall-bladder wall has proved difficult. ASCHOFF in 1906 showed that if cholesterol in olive oil is placed in the gall-bladder, though lipoid appears in the epithelial cells, it does not have the reactions characteristic of cholesterol.

MENTZER performed similar experiments, using cholesterol ester, with equally negative results. In addition, cholesterol dissolved in oleic acid was used, and in this case it is stated that absorption took place, though not so marked as in the case of other lipoids. Apart from the simple/

simple statement, however, MENTZER advances no proof that the absorbed lipid really was cholesterol, and, unless polaroscope examinations are carried out a fallacy may here crop up, as in the following experiment.

#### Experiment.

In a cat the bile in the gall-bladder was replaced by 1 ccm. of oleic acid, in which was dissolved 0.2 gm. of pure cholesterol. The common duct was ligated.

Two days later the mucosa of the gall-bladder showed evident absorption, and microscopically there was lipid in many of the epithelial cells. Polaroscope examination showed, however, that this lipid was not anisotropic, and even chemical tests of ethereal extracts of the gall-bladder wall showed but faint traces of cholesterol.

Working from another aspect TORINOUMI has shown how difficult it is to prove that the gall-bladder can absorb cholesterol. TORINOUMI, working with dogs, estimated the total cholesterol content of the bile in the gall-bladder before and at the end of a period during which the cystic duct was closed by ligature, and endeavoured to determine whether any reduction of this total took place. Several difficulties attended the performance of these experiments (especially the accidental product of inflammation in the gall-bladder), and the findings were therefore very variable. In those experiments, however, where at the end of the period the gall-bladders appeared healthy, there was a distinct diminution in their cholesterol content, so they do give some evidence of an absorptive function.

It is evident that the proof of the absorption of cholesterol, if it does occur, must be difficult to obtain for the following reasons:-

(1) The experiments of TORINOUMI indicate that absorption is very limited in extent, possibly depending partly upon a relatively high concentration of cholesterol in the bile as compared to the blood, and slowing down as these concentrations equalise.

(2) The absorption may not be demonstrable by histological methods. Little is known about the normal method of transport of cholesterol through the body, but it seems probable that the cholesterol is usually bound up with other lipoids in such a manner as to render it unrecognisable by its staining or physical properties. This masking of cholesterol is very evident in absorption through the intestinal mucosa. Thus in a rabbit, which for several weeks had been fed with cholesterol, microscopic sections from several regions of the intestinal tract showed no trace of doubly refractile material, although at the time of death some absorption of cholesterol must undoubtedly have been taking place. It seems possible that the passage of cholesterol through the gall-bladder mucosa may be similarly obscured.

(3) In attempts to demonstrate experimentally absorption by the gall-bladder, the physical and chemical state and the environment of the cholesterol used/

used must be of great importance and should correspond as nearly as possible to that present in the normal bile and tissues.

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In the course of this investigation numerous experiments have been carried out with the object of giving histological demonstration of cholesterol absorption, but this end has not been achieved. The experiments have been performed on lines similar to that described on Page 122, cholesterol in a great variety of mixtures being injected into the gall-bladders of cats and rabbits.

At first, pure cholesterol was used, suspended in vaseline, agar agar, or butter, or dissolved in oleic acid or olive oil, but it was soon realised that in the crystalline state absorption would be unlikely, and other mixtures were then adopted; by means of lecithin, complex watery emulsions were prepared, after the method of CASHIN and MORAVEK,<sup>(51)</sup> in which much of the cholesterol was in the form of amorphous or granular masses, or as "myelin bodies"; in other cases, cholesterol in complex mixture with other lipoids was obtained by ethereal extraction of the adrenal glands of hypercholesterolaemic animals. All these experiments, however, yielded the same results, namely, that although/

although absorbed lipoid could usually be demonstrated in the mucosa of the gall-bladder, it failed to give the reaction for cholesterol.

Although histological proof has been found lacking, it has been possible, however, to demonstrate very clearly the absorption of cholesterol by other methods.

#### Experiment.

A supply of cholesterol, mixed with other lipoids as nearly as possible in the state in which it normally exists in the body, was obtained as follows:- The adrenal glands were removed from a number of rabbits which for three months had been on a diet rich in cholesterol. Frozen sections of these glands showed a large excess of cholesterol, chiefly in the form of esters. The glands were extracted in the cold with chloroform-ether mixture, and a sticky extract was obtained which also showed a high content of cholesterol esters. This extract was emulsified by means of lecithin in a small quantity of cat's bile.

Laparotomy was then performed on two cats, and the same procedure carried out in each. The cystic duct was carefully exposed and freed from the cystic artery, and cut across. With a fine Record needle inserted along the cystic duct the bile in the gall-bladder was withdrawn, and in place of it 1 ccm. of the prepared bile was injected. The cystic duct was then doubly ligatured and the wound closed.

Five days after operation both cats were killed. The gall-bladders, which appeared healthy, free from adhesions, with unimpaired blood supply and lymph drainage, and with no evidence of leakage, were removed and their total contents carefully collected.

Cholesterol/

Cholesterol estimation\* was then performed upon these contents, and at the same time upon 1 cc. of the original injected fluid, all the steps of the three estimations being carried out together to minimise any error.

The results of this experiment are seen in Table VII (Page 128a), which shows that during the space of five days more than half of the cholesterol injected has gone from the gall-bladder. This amount far exceeds any possible error of estimation, and as every care was taken to prevent loss during the operative procedure or at autopsy, it seems justifiable to attribute it to actual absorption through the gall-bladder wall.

It is concluded, therefore, from these experiments that cholesterol, when in excess and in certain physical mixtures, can be absorbed by the gall-bladder wall, probably in masked form, which prevents its histological demonstration.

It remains to be shown what bearing this absorptive function has upon the origin of Cholesterolosis. Is any proof forthcoming that in Cholesterolosis the cholesterol deposited in the mucosa reaches that situation after absorption from the lumen of the gall-bladder?

Experimentally, indirect evidence of this mode of origin is given by the following series of experiments. In these the method previously adopted successfully for the experimental production of Cholesterolosis (viz. the production of a mild chronic Cholecystitis in a hypercholesterolaemic animal) was again followed/

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\* For method of cholesterol estimation used in this and other experiments see Appendix (Page 146).

TABLE VII.

Absorption of Cholesterol from the Gall-bladder.

	<u>Cat 89</u>	<u>Cat 98</u>
Cholesterol injected into gall-bladder	34.425 mgms.	34.425 mgms.
Cholesterol present after five days	15.67 mgms.	13.29 mgms.
Amount absorbed	18.755 mgms.	21.135 mgms.



followed, with the important exception that access of bile to the gall-bladder was prevented by ligature of the cystic duct. Any resulting cholesterol deposit in the mucosa could, therefore, only have reached that situation by way of the blood stream. In these experiments, however, no Cholesterosis resulted, and they therefore give indirect support for the absorption theory.

#### Experiment.

Three rabbits were fed with pure cholesterol suspended in butter, over a period of 12 weeks, 0.3 grams of cholesterol being administered daily to each. During this period the blood cholesterol index in each case was very markedly raised (Table VIII., Page 129a). Three weeks after the commencement of the experiment laparotomy was performed. By delicate blunt dissection the cystic duct was exposed, separated from the artery, ligated in two places, and cut, great care being taken to avoid damage to the vessels. With the object of producing a mild progressive chronic cholecystitis, a suspension of avirulent streptococci was then introduced by means of needle and syringe, between the layers of the inferior wall of the gall-bladder. The wound was then closed.

All three animals progressed well, gaining considerably in weight. They were killed at the end of twelve weeks.

Autopsy Findings. All the animals were well nourished to the point of obesity. Cholesterol was present in excess in the adrenal glands, and to a lesser extent in the liver and spleen. In two of the animals there was a considerable degree of aortic atheroma. The gall-bladders in all cases showed a mild cholecystitis, with opacity of the wall and some thickening on palpation. The blood supply appeared intact and omental adhesions were scanty. Microscopic examination revealed an entire absence of visible cholesterol from the mucosa.

TABLE VIII.

Effect of Cholesterol Feeding  
upon the Blood Cholesterol Content.

Rabbits fed with pure Cholesterol (0.3 grams daily)  
in butter.

Rabbit (1) Female; weight at onset, 5 lb. 5½ oz.  
Rabbit (2) Male ; " " " 4 lb.  
Rabbit (3) Male ; " " " 5 lb. 1 oz.

<u>Weeks after Onset of Experiment</u>	<u>Blood Cholesterol Content (mgms. %)</u>		
	<u>Rabbit (1)</u>	<u>Rabbit (2)</u>	<u>Rabbit (3)</u>
0	87	109	70
1	100	369	95
2	110	467	118
3	217	683	294
4	339	657	305
6	410	617	388
8	393	576	315
10	563	521	395
12	532	500	666

IX. CONCLUSIONS IN REGARD TO PATHOGENESIS.

It is believed that there are now sufficient data available to justify a tentative opinion as to the aetiology of Cholesterosis.

The fact that similar infiltrations with cholesterol in various tissues of the body are frequently or usually associated with two demonstrable lesions, namely, an increase in the cholesterol content of the blood and an inflammatory change, necessitates a careful consideration of these two factors in particular. .

It cannot be doubted, for the reasons already cited, that neither of these factors alone can give rise to Cholesterosis, but it is an attractive hypothesis, and one frequently maintained, that they are jointly responsible. Unfortunately this does not completely agree with the available facts, for either Hypercholesterolaemia or Cholecystitis may be lacking, the former indeed commonly.

In addition, the frequent cases in which Hypercholesterolaemia and mild Cholecystitis exist, yet fail to give rise to Cholesterosis, indicate clearly that other causative factors must be sought for. It seems probably that an indication of the nature of these/

these may best be obtained by a careful consideration of the normal functions of the gall-bladder in relation to cholesterol.

From the experiments described above it seems clear that the gall-bladder can absorb cholesterol from the bile. This does not necessarily mean that such an action goes on to any appreciable extent in the normal gall-bladder (the experiments of TORINOUMI indicate that such action must at least be very limited in degree), and it is likely that an essential feature in determining the absorption of cholesterol is the concentration of this substance in the bile, absorption taking place only when it is in excess. Moreover, it is clear that the cholesterol, when absorbed into the healthy mucosa, yet remains indistinguishable under the microscope, its characteristic optical properties being masked in some way not fully understood.

This being the case, it becomes evident that deposit of visible cholesterol in the gall-bladder (Cholesterosis) postulates essentially two processes:-

(1) Absorption of cholesterol into the mucosa, depending probably upon an increase in the cholesterol content of the bile, and

(2) Some change which unmasks this absorbed but invisible cholesterol, and which furthermore,

furthermore prevents or delays its transport and leads to its accumulation in the mucous membrane.

At this point we may see how it is possible to link up this hypothesis with clinical observations.

(1) An increased cholesterol content of the bile was found clinically in all the cases in which the estimation was carried out. In 17 of the cases, moreover, it had progressed to the formation of stones. This cholesterol increase in the bile is regarded as a primary factor of importance; the blood cholesterol may or may not be increased. In the experimental production of Cholesterosis (as in Experiment, page 117) an increase of blood cholesterol is a necessary intermediate step in raising the bile cholesterol, but there is much evidence that in man the latter alone may be increased.

(2) In the second essential process we can see how the factor of Cholecystitis may be linked up with the pathogenesis of Cholesterosis, for it is believed that the part played by the inflammation consists simply in interfering with this absorptive process, so that the absorbed cholesterol is rendered visible and at the same time is accumulated in large quantity in the gall-bladder wall.

The/

The exact rôle of the inflammation is open to speculation. CHIRAY and PAVEL have suggested that it acts by actual blockage of the lymphatic drainage of the gall-bladder, but against the acceptance of this hypothesis are two facts, namely (1) there is no proof that absorption usually occurs by the lymphatic path, and (2) there is no histological evidence of lymphatic obstruction and no oedema or fibrosis of the gall-bladder wall.

A more feasible hypothesis would appear to be that the inflammatory process occurs in virtue of some chemical action directly upon the absorbed cholesterol which would at the same time render the cholesterol visible, interfere with its normal transport, and lead to the characteristic endothelial response. Such a hypothesis has the additional advantage that it is also applicable to those cases in which no inflammatory element is recognisable. In these cases one may presume some non-inflammatory change in the nature of the absorbed cholesterol, which prevents its transport away from the gall-bladder.

PART III.

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P A R T    I I I .

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RELATION OF CHOLESTEROSIS  
TO THE FORMATION OF GALL-STONES.

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Mentzer has noted that Cholesterosis of the gall-bladder is frequently associated with the presence of certain types of gall-stones, namely those which are composed mainly of cholesterol; and this observation has been confirmed in the series of cases recorded here.

There are two common types of cholesterol-rich gall-stones,—the single stone or "solitaire" and the multiple stones of "mulberry" appearance,—and both these types occur especially frequently in gall-bladders which are affected by Cholesterosis.

(P.134a)  
Table IX. <sup>A</sup> shows the incidence of stones in 35 cases of Cholesterosis, and in a control series, exactly double in number, of gall-bladders in which no cholesterosis was present. It will be seen that 17 of the 35 gall-bladders of the former series contained stones, and in no fewer than 11 cases the stones were of "pure cholesterol" varieties ("solitaires" in four cases, multiple/



TABLE IX.

Incidence of Gall-Stones with Cholesterosis

<u>Type of Stone</u>	<u>Number of Cases</u>	
	<u>With Choles- terosis</u>	<u>Without Cholesterosis (Controls)</u>
Pure Cholesterol Solitaire	4	5
Multiple Pure Cholesterol ("Mulberry")	7	2
Other Types of Stone	6	30
Stones present in	17	37
No Stones present in	18	33
Total:	35	70

multiple "mulberry" stones in seven). The undue proportion of these stones is clearly seen by comparing the control series, in which they only occurred seven times out of a total of 37 calculous cases. Thus most "pure cholesterol" stones are found in gall-bladders affected by Cholesterosis. Moreover, it is a fact which has been frequently noted, that where such a stone is unaccompanied by Cholesterosis, a considerable degree of inflammatory thickening of the wall of the gall-bladder usually exists, such as might be presumed to have obscured a pre-existing Cholesterosis. A thin-walled gall-bladder which contains a pure cholesterol stone almost always, in the writer's experience, presents some degree of Cholesterosis.

A clear relationship between the two conditions, Cholesterosis and the formation of these particular types of stones, may therefore be taken as established, and its nature must now be considered. It will be profitable at this stage to describe the stones in question and to review the theories which have been put forward in regard to their origin.

#### Characters of "pure cholesterol" stones.

A "solitaire" stone is light in weight, round or oval in shape, and of pale yellow colour. It consists, to the extent of 95 per cent to 98 per cent/

cent, of cholesterol, which is mainly in the form of coarse crystals. In a cross section of a stone, the crystals are seen to be built up in radiating coarse columns, extending from the centre of the stone to its periphery, and intertwined and twisted like close-growing tree trunks. A characteristic feature, which differentiates pure cholesterol stones from others, is the entire absence of lamination. At the centre of the stone is often seen a small pigmented area, dark green or brown in colour, which has been regarded by some as a nucleus of bile pigment.

Stones of Mulberry Type occur as multiple formations, two, three, or as many as thirty being found together. Usually, though not invariably, all the "mulberry" stones in a gall-bladder are of the same size, and they may vary from the size of grains of rice up to that of Barcelona nuts. Individually, they closely resemble "solitaires" in outward appearance. They are of light weight, rounded, and with a nodular surface. In colour they are often a very brilliant yellow when removed at operation, but after a short time they fade to a dull fawn. Less commonly, "mulberry" stones may be almost pure white, when they may closely resemble paraffin wax both in appearance and consistency. On cross section, "mulberry" stones/

stones have usually a somewhat amorphous, waxy appearance, without either gross crystallisation or lamination. In other cases, though less commonly, intertwined coarse crystalline columns, exactly like those of the "solitaire", may be seen. It is of great interest to note that, just as with the "solitaire", in many of the "mulberry" stones of this series a "nucleus" of black bile pigment has been found.

Theories as to formation of "pure cholesterol" stones.

Modern investigation of the formation of gall-stones may be said to have originated with NAUNYN<sup>(37)</sup>, who elaborated the earlier theory of MECKEL v. HEMSBAUGH<sup>(32)</sup>, that gall-stones of all types result from two predisposing factors, namely, stasis of the bile, and an infection, or "stone forming catarrh", of the gall-bladder wall.

ASCHOFF and BACMEISTER<sup>(4)</sup>, while accepting this hypothesis in regard to the majority of stones, differentiated another fundamentally different process for certain types, and especially for stones comprised mainly of cholesterol. These stones they regarded as arising from a non-inflammatory process, an alteration of metabolism, which resulted in the deposition of cholesterol out of its solution in the bile. Such a process ASCHOFF believes to be attributable/

attributable to a transitory increase in the excretion of cholesterol in the bile, which might account for the well-known association of cholesterol stones with pregnancy (a state in which hypercholesterolaemia is frequently observed), or it might rather be due to a diminution of those bile constituents which normally maintain cholesterol in solution.

It is now generally believed that cholesterol is normally held in colloidal solution in the bile in virtue especially of the bile salts, and LICHTWITZ<sup>(27)</sup> has suggested that the factor which leads to its deposition out of the bile is rather an alteration in the acid-base reaction of the bile than any quantitative change.

In the actual building up of the stone a primary importance has been attributed to the presence of a nucleus, around which cholesterol is laid down. Reference has already been made to the frequent finding of a small central pigmented area both in solitary stones and in those of "mulberry" type. NAUNYN regards these as minute concretions of bilirubin calcium which have arisen as a consequence of cholangitis and have been later washed down into the gall-bladder; in ROVSING's view they are "bile thrombi" formed in the smaller intrahepatic radicles of the bile ducts as a result of some alteration in pigment/

pigment metabolism. ASCHOFF, on the other hand, emphatically controverts this view that cholesterol stones result from precipitation around pre-existing nuclei. In his opinion, the pigmented centre of cholesterol stones is a secondary manifestation, a result of secondary absorption of bile pigment to the centre of a primary cholesterol formation, which has occurred independently, from a slow crystallisation out of the bile.

While this biliary origin of cholesterol stones is widely accepted, more recently CHAUFFARD, GOSSET, and his collaborators, and BOYD, have supported another hypothesis, namely, that the stones arise, not in the bile, but in the gall-bladder wall, as collections of cholesterol which later become freed into the lumen. These workers have noted the close similarity of appearance which exists between cholesterol "polypi" of the gall-bladder wall and small cholesterol stones, and they have pointed out how easily such polypi, suspended on narrow delicate stalks, might be set free into the bile.

It will be seen, then, that the relationship between Cholesterosis of the gall-bladder and the formation of cholesterol stones may be that of cause and effect, the stones resulting from broken-off portions/

portions of the intramural deposit, or it may consist merely in dependence upon a common origin, namely a hypercholesterinosis or dyscholesterinosis of the bile. In the series of cases reported here, several have occurred which are of particular interest from the point of view of these hypotheses, and they will therefore be described more fully.

Case (a). This gall-bladder, which is seen in Fig. 34, merely illustrates the very frequent association between Cholesterosis and pure cholesterol stones. In the gall-bladder wall, the outer coats of which are considerably thickened by old-standing disease, is a very marked degree of generalised "strawberry" change. The stones are typical of the "mulberry" variety.

The next two cases are of interest in that they tend to support the intramural origin of stones by the setting free of "cholesterol polypi".

Case (b). This case is illustrated in Fig. 36. The gall-bladder, which was thin-walled, contained about twenty stones in addition to a number of polypi. The stones were all small, the largest being the size of a split pea and the majority the size of grains of rice. All were practically pure white/

white in colour, shaped like a mulberry, and of an almost waxy appearance and consistency. No coloured nuclei were present, and the stones were composed almost entirely of cholesterol in amorphous form.

The polypi were about ten in number. They were situated in the region of the neck of the gall-bladder, and attached to the mucous membrane by fine threads of tissue. In size, colour, and appearance they bore a very close resemblance to the stones, so much so, in fact, that one stone, which was lodged in a small crypt at the cystic duct, was for a long time regarded as a polypus.

In the illustration (Fig. 36) both polypi and stones are visible; it will be noted that they are almost indistinguishable. In such a case as this it is difficult to avoid the conclusion that the polypi represent an earlier stage of the process of stone formation.

Case (c). This gall-bladder\* is seen in Fig. 37. At one part of the mucous membrane, close to the fundus, is a small patch of "strawberry" change; at another part two "cholesterol polypi" are seen, and in the lumen are two "mulberry" stones, one small and the other the size of a large pea. The natural inference/

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\* Mr. J.J.M. Shaw's case.



inference is that these different lesions represent merely stages in the process of stone formation.

Case (d). This case, on the other hand, indicates a totally different method of stone formation. In a gall-bladder which presented a very minor degree of Cholesterosis, and no polypi, were thirty stones. All but one of these are illustrated in Fig. 38, and a closer view of several is given in Fig. 39. In this series all stages of one method of stone formation can be traced.

The smallest stone is simply a minute black particle, which appears to consist entirely of bile pigment with no visible trace of cholesterol. As the series of somewhat larger calculi is followed, all are seen to consist mainly of pigment, but in each a varying amount of cholesterol is also added. The cholesterol is deposited, that is to say, on a pigment nucleus. At first, in the smaller stones, the deposition is excentric, and the cholesterol forms one, two, or three rounded lobules attached to one side of the pigment particle. In somewhat larger stones the deposit is seen to have spread almost completely round the nucleus, which is now only visible at one point of its circumference. Last of all, in the completely formed mulberry stones at the right of the Figure, the pigment is completely hidden. Its presence in the centre of one of them was demonstrated by cross section.

DISCUSSION.

Of the four cases described, the first represents the condition most commonly met with, namely, fully formed pure-cholesterol stones associated with a "strawberry" gall-bladder. In such cases it is usually impossible to do more than guess the mode of origin of the stones, and it is the earlier stages which may be expected to give more information.

Cases (b) and (c) illustrate clearly all that has ever been advanced in favour of the intramural origin of gall-stones. The remarkable similarity between polypi and some gall-stones is clearly evident in Case (b). Fig. 5, moreover, illustrates how easily a polypus might break off and become free in the bile, and Case (c) demonstrates how all the stages of this method of origin may be seen in a single gall-bladder.

On the other hand, there are certain considerations which militate against a full acceptance of this theory except as a possible explanation of a small minority of stones. Pure cholesterol stones, when multiple, are usually all of equal size (cases such as (c) and (d) are rare), and it seems clearly evident that they originate simultaneously, like the much more common faceted gall-stones. Moreover, pure/

pure cholesterol stones are usually of moderate size, and are composed mainly of crystalline pure cholesterol, whereas polypi rarely exceed 0.5 cm. in diameter and consist chiefly of esters of cholesterol. It is possible that small polypi, set free into the bile, might increase in size by the accretion of fresh cholesterol around them, and it is conceivable that their lipid content might be de-esterized to pure cholesterol, but that several polypi are simultaneously set free in the bile to undergo these changes at exactly the same rate is a hypothesis which may be allowed to account for a rare case, but cannot be accepted in explanation of the relatively frequent "mulberry" gall-stones.

In the writer's view, it is much more plausible to regard both Cholesterosis and "pure cholesterol" stones as arising from a common cause, a hypercholesterinosis or dyscholesterinosis of the bile. Such a cause has been shown to be present in all the clinical cases of Cholesterosis in which the bile cholesterol content has been estimated, and there seems no reason to doubt that it may always be present in this condition.

In regard to the actual building of calculi, there seems much evidence that nuclei of bile pigment do/

do, in at least a proportion of cases, play an important part. The appearance in Case (d) can be interpreted in no other way. On the other hand, pure cholesterol stones do occur in which no nucleus is evident, and their formation is no doubt attributable to a primary crystallisation out from the bile. The two views in regard to the occurrence of nuclei are by no means incompatible; a crystallisation out of the bile will no doubt occur more readily around a pre-existing nucleus and will give rise to multiple formations if more than one nucleus is present; but it will also occur, though more slowly and as a solitary formation, if a nucleus is lacking.

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APPENDIX

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A P P E N D I X.

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METHODS USED FOR THE ESTIMATION OF THE  
CHOLESTEROL CONTENT OF THE BLOOD AND BILE

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Blood.

For blood, the method adopted has been as follows:-

- (1) 5 ccs. of oxalated or citrated venous blood, carefully measured by pipette, is added, drop by drop with continual shaking, to a 100 ccs. measuring flask containing about 75 ccs. of a mixture of 3 parts of 95% Alcohol to 1 part of Ether.
- (2) The resulting mixture is raised to boiling point for a few seconds by placing the flask in a bowl of boiling water. It is then thoroughly shaken and allowed to cool overnight to room temperature.
- (3) /

(3) The mixture is made up to 100 ccs. volume by the addition of Alcohol-Ether mixture.

(4) After a further shaking, the fluid is filtered through No.1 filter paper.

The remaining steps are carried out in triplicate, to minimise error.

(5) Three portions, each of 20 ccs. of the filtrate are added to three Erlenmeyer flasks, and to each is added a few drops of concentrated NaOH. The mixture is then boiled on a boiling water bath until the volume is reduced to approximately one-third. At this point sulphuric acid is added, drop by drop, sufficient to render the mixture just on the alkaline side of neutrality, and boiling is then continued to the point of dryness. (Note: It is important that neutralisation should be carried out sufficiently early in this stage, to prevent discoloration of the fluid by over-concentration of the alkali.)

(6) To the dried extract, after cooling, is added 10 ccs. of pure Chloroform. The mixture, after being thoroughly shaken and allowed to stand for half an hour, is filtered into a second small flask/

flask. The washings, each of 5 ccs. Chloroform, from the first flask, are then added to the filtrate. The total Chloroform extract is then reduced, by boiling over a water bath, to volume of about 4 ccs., which, after cooling, is transferred to a 10 cc. graduated tube for colorimetric estimation. Chloroform washings from the second flask are added to make a total volume of approximately 8 ccs.

- (7) For the colorimetric estimation, six 10 cc. graduated tubes are required. Three have already been filled, as described, each with 8 ccs. of chloroform extract derived from the blood to be tested. Into the remaining three tubes standard solutions of cholesterol in chloroform, each 8 ccs., containing respectively 1 mg., 1.5 mg., and 2 mg. cholesterol, are placed for comparison.
- (8) The colour reaction is obtained by the Liebermann-Burchard method. To each of the six tubes is added 1 cc. of Acetic Anhydride and 0.1 cc. of concentrated  $H_2SO_4$ ; the mixtures are stirred and made up to exactly 10 ccs., and allowed to stand in the dark for half an hour until/



until the colour develops. Comparative readings are then obtained in a micro-colorimetre from each of the test solutions against the most suitably coloured standard solution (i.e. the standard most nearly approaching a similar strength.

- (9) Based upon the quantities stated above, the cholesterol content of the blood (in mgms. per 100 ccs.) is estimated as follows:-

$$\text{Cholesterol Content}_{\text{mgms. \%}} = \frac{\text{Colorimetric Reading of Standard}}{\text{Colorimetric Reading of Unknown}} \times 100 \times \text{Cholesterol Content of Standard in mgms.}$$

The estimation is carried out independently for each of the three test solutions, and an average taken.

#### Bile.

The above method, simple and satisfactory for blood, was found to be completely unsuitable, without modification, for bile, owing to the fact that bile pigments, carried through the alcohol and ether and chloroform extracts, completely obscured the colour reaction.

After much experiment, however, it was found that this discoloration could be very easily prevented by a preliminary alkalisation of the bile. 2 ccs. of/

of bile was mixed with 2 ccs. of normal Sodium Hydroxide, and the mixture added to alcohol-ether as in the case of blood. The method used for blood was then followed, except that complete neutralisation by sulphuric acid was not carried out, a distinctly alkaline reaction being preserved. By this method it was found possible to rely upon obtaining a reaction unimpaired by adventitious pigments. Owing to the rather higher cholesterol content of bile, as compared with blood, it was customary to use a smaller quantity (2ccs.) for the estimation, the calculation being suitably modified.

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