THESIS for M. D.

by

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"The CHEMISTRY and BIOCHEMISTRY of LECITHIN and other LIPOIDS."

The substances with which this work is concerned form a group which is exceedingly ill-defined and one for which considerable difficulty has been experienced in finding a suitable name. Originally referred to as Phosphatides because of the relation in certain of the more important ones of the elements phosphorus and nitrogen they are now frequently referred to as lipoids or in the terms advocated by Leathes. This writer suggests that compounds of fatty acids containing nitrogen and phosphorus should be called 'Phospholipines', compounds of fatty acids containing nitrogen but no phosphorus 'lipines' and similar compounds containing carbohydrate radicals as well as nitrogen 'galactolipines'. The term "lipoid", originally suggested by Overton for substances resembling in their solubilities the fats, has of late been somewhat extensively employed and while the use of such a term cannot be justified on a purely chemical basis a comprehensive name of the kind is useful since, as is frequently the case, even the approximate chemical constitution of many of the substances concerned is unknown.

Bang includes the following groups under the heading 'Lipoids'

(1) The fats, nitrogen and phosphorus free lipoids of the aliphatic order, containing carbon, hydrogen and oxygen.

(2) The cholesterins, nitrogen and phosphorus free lipoids of the aromatic order containing carbon, hydrogen and oxygen.

(3) The phosphatides, lipoids containing nitrogen, phosphor-us, carbon, hydrogen and oxygen.

(4) The cerebrosides, lipoids containing nitrogen, carbon/

carbon, hydrogen and oxygen, but no phosphorus. This classification, although serving to bring out the chemical relationships of the various groups is unsatisfactory in that it includes the fats since it is from a resemblance to the solubilities of this group that the others have been called lipoids. On the other hand, the inclusion of the cholesterins is questioned by Abderhalden.

In spite of a considerable amount of work on the chemistry of these bodies, our knowledge of their structure and exact relationships is exceedingly scanty. The close similarity of their properties, especially as regards solubility in certain reagents and the ease with which they undergo chemical alteration as well as their complex chemical constitution render them exceedingly difficult to separate in a pure state.

Because of these difficulties much of the work on the chemistry of lipoids is exceedingly unsatisfactory. In many cases crude ether or alcoholic extracts alone have been tested and conclusions have been drawn regarding the action of individual lipoids (usually lecithin and cholesterin) which have not been justified. The most interesting group of the lipoids and the least known is the phosphatids and as this work is more particularly concerned with some members of this group it will be convenient to consider in the first place the general characters of these bodies.

Phosphatides (phospholipines).

With the exception of lecithin, which has been known since 1846, the phosphatides are of comparatively recent discovery. They form a group of substances which have been shown to be of considerable importance in the chemistry of the cell. Although widely distributed in the tissues of animals and plants and in certain organs present/

present in fairly large amounts comparatively little reliable information is to hand as to their real chemical composition. It is still doubtful whether any of the phosphatides which have been described are pure substances and a review of the literature suggests that the same substances or mixtures of substances have been described by different workers under different names. Distinguishing chemical tests for the various substances are practically unknown and separation and identification depend almost solely on the reactions to certain fat solvents and on the determination of the presence, and relative amounts, of nitrogen and phosphorus, and on the character of the fatty acids which are split off on saponification.

It is at present unknown whether the phospholipines occur as such in the animal cell. They are regarded by some as being present in combination with protein from which union they are split off in process of preparation with more or less difficulty depending on the nature of the lipoid concerned; others regard them as primary constituents of protoplasm. Information regarding their distribution in the animal body is deficient. The majority of workers have confined themselves either to the examination of special organs or of individual phospholipines, lecithin being chosen in most cases since it is the most abundant and widely distributed. Unfortunately it has been the practice of a number of writers to regard the total phosphorus content of tissue extracts as giving the amount of lecithin present. The results of such experiments must in the light of present knowledge be regarded as almost useless. It has been shown for example that not only do different organs contain different lipoids but that several phosphatides may occur in the same organ. Since the phosphatides have been shown to differ as regards the proportion

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of phosphorus present in the molecule no figures calculated on the basis of total phosphorus content of an ether or alcoholic extract of tissue can be accepted as giving any indication of the amount of lipoid present in such tissue. Within recent years, however, as the result of more exact methods, our knowledge on the subject of phosphatides has been distinctly extended. The work of Erlandsen has in particular been of service. This author has shown that most of the recognised methods for extraction of lipoids do not give an adequate conception of the nature of the phosphatides present as a number of these are missed in the process of separation. Erlandsen's work will be more fully described later in connection with lecithin.

General characters of Phosphatides.

Speaking generally the phosphatides are non-crystallisable; in the dried state they form wax-like masses which are very hygroscopic and easily altered on standing in air. Comparatively low temperatures cause them to melt with the formation of greasy, viscous fluids. The majority of preparations are yellow in colour from the presence of contaminating pigment. They are for the most part insoluble in water, but readily soluble in the ordinary fat solvents (ether, alcohol, chloroform, benzol, etc) from the majority of which solutions they can be precipitated by the addition of excess of acetone, a property of great value in preparing them.

A prominent character which explains a number of otherwise anomalous occurrences in the process of preparation of phosphatides, is that they markedly influence the solubility of each other. With salts of the heavy metals ($CdCl_2$, $PtCl_4$) additive-like compounds are formed; these have been used by a number of workers for the isolation and purification of phosphatides but,

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as Erlandsen has shown, the precipitation of the lipoid is neither quantitative nor complete and the method therefore cannot be regarded as satisfactory. Dilute acids and alkalis readily effect saponification; partial saponification has also been shown to occur by the action of cadmium and platinum chlorides (Erlandsen).

Chemical Structure.

Chemically considered, the phospholipines are compounds of phosphoric acid, glycerin (or other alcohol), one or more fatty acid radicals and one or more nitrogen containing bases. The cleavage products of lecithin, for example, are glycerophosphoric acid, fatty acids and cholin (hydroxyethyl trimethyl ammonium hydroxide) but considerable doubt exists as to whether these are the only substances formed (MacLean). The fatty acids may belong either to the saturated or to the unsaturated series. Lecithin and cuorin, for example, contain unsaturated acids (Erlandsen) though lecithin is still frequently represented as a fully saturated compound.

Preparation.

In the preparation of phosphatides animal tissues are dried and the material extracted by certain fat solvents especially ether and alcohol. The extract is evaporated, the residue taken up in ether and the phosphatides precipitated by the addition of certain agents which leave neutral fats, fatty acids and cholesterin in solution. In order to effect purification the precipitate may be again dissolved in ether and again precipitated. Individual phosphatides are then obtained from the precipitate, generally by the use of cold or boiling alcohol and the purity of the ultimate product is tested by its reaction to certain solvents and, in particular, by the determination of the nitrogen to phosphorus ratios. In actual practice the separation is usually found to

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be incomplete and, as has been remarked, it is probable that pure preparations are not obtained by any of the methods at present in use. Further it is probable that the tests used do not sufficiently identify individual phosphatides with the result that confusion exists regarding the nature of substances prepared by different methods.

Biological importance.

The study of phosphatides has of late years received a distinct impulse from the discovery of their importance in a number of physiological and biological processes. As a result of experiments on vital staining Overton has suggested that cells possess an external limiting membrane which is composed of a mixture of phosphatide (lecithin) and cholesterin. According to this theory only those dyes which are soluble in a mixture of lecithin and cholesterin can penetrate the cell-wall and produce staining of the cell protoplasm. The experimental evidence in favour of this view has been questioned by a number of writers (Rühland, Höber and others) who have been unable to find the same close relationship between vital staining and lipoid solubility. The most obvious objection to a theory of this kind is that many substances which are easily absorbed by the cell, such as proteid and carbohydrate, are not soluble in lipoids. This difficulty has been explained by reference to the alterations in the solubilities of these substances which result from the presence of phosphatides (proteid and sugars for example become ether-soluble in the presence of phosphatide). Further, it has been found (Overton and Meyer) that the poisonous effects of narcotics stand in direct relationship to their lipoid solubility and it has been suggested that antiseptics and antipyretics may also act on the cell by virtue of the same property.

In the field of haemolysis also the lipoid theory has been applied to explain the action of a member of haemolytic agents of widely different constitution. Thus the organic agents which effect haemolysis (fatty acids, soaps, ether, alcohol etc.,) are either soluble in, or dissolve, lipoids and the escape of haemoglobin is regarded as due to the alterations in the lipoid membrane which result from combination of the lipoid with the haemolytic agent. Similarly, the effect of saponin on corpuscles is related to the disturbance which results from absorption of the saponin by the lipoid (cholesterin) of the corpuscles (Ransom). The well known haemolytic action of cobra venom on the corpuscles of certain species of animals (guinea pig, dog, rabbit, man) is also concerned with lipoids as it has been shown (Kyes) to be due to the formation of a new substance, cobra lecithid, which is actively haemolytic to the corpuscles of even unsusceptible species (ox, sheep, goat,) The marked inhibitory action of cholesterin on most haemolytic processes (saponin, tetanolysin, solanin, cobra venom haemolysis) has been explained by supposing that the cholesterin serves to saturate the affinity of the haemolytic substance for lipoid. It has been shown by Kurt Meyer, Rywosch, and Port that red corpuscles which are rich in cholesterin (sheep, ox) are more resistant towards the haemolytic action of saponin than are those of corpuscles poor in cholesterin (guinea pig, rabbit, dog).

The discovery in 1907 by Wassermann, Neisser and Brück of the Wassermann syphilis test and the relation of lipoids to the reaction has stimulated research on lipoids in quite a new direction. It was demonstrated by these workers that tissue extract in combination with the sera of syphilitic individuals are capable of absorbing or deviating/

deviating 'complement' while the sera of normal individuals tested under precisely similar conditions do not do so. Although no adequate explanation of this reaction has so far been put forward it is certain that lipoids especially lecithin and cholesterin, play an important part in the text (Browning, Cruickshank and Mc'Kenzie). In investigating the phenomena underlying this reaction the part which lipoids play in immunity reactions in general has been examined. This will be discussed more fully later but it may be said here that the action of complement and of immune body have both been attributed to the action of lipoids (Bang, Liebermann, and Fenyvessy).

Until comparatively recently it was generally accepted that the only bodies which are capable of acting as antigens (that is producing specific anti-substances when injected into animals) belong to the proteids. The immunity reactions which have been described as occuring after the injection of nastin (Much) (a fat extracted from leprosy bacilli) and the experiments of Bang and Forssman, Gottlieb and Lefmann who describe the presence of antigenic substances in ether extracts of red blood corpuscles have raised the question as to whether phosphatides and other lipoids can act as Antigens. On the one hand we have experiments to show that specific complement-fixation reactions can be obtained with the sera of animals injected with (1) lipoids from certain animal parasites, tubercle bacilli, echinococcus cysts, round worms, (Kurt Meyer) (2) lecithin (Bergel) (3) ether extracts of red blood corpuscles (Gottlieb and Lefmann, Bang and Forssman) and on the other hand the experiments of Thiele and Embleton, Ritchie and Miller, which demonstrate fairly conclusively that lipoids (with the possible exception of lipoids from certain worms) fail to act as antigens. Attention has also been given to the alterations/

alterations which occur in the fat-splitting power of the sera of animals after injection with lipoids. Bergel states that the sera of animals immunised to foreign red blood cells are about twice as active in splitting foreign fats as the sera of untreated animals. This increased lipolytic activity is however not specific: it is apparently directed against a variety of foreign fats. These results are partially confirmed by Jobling and Bull and the question has been raised (Neuberg and Reicher) as to whether haemolysis is not a kind of lipolysis, or whether the two functions may not be intimately related. The experiments of Jobling and Bull on the fat-splitting power of immune sera, would appear to indicate that the red blood corpuscles of different species possess lipoids peculiar to the species and that these may act as specific antigens.

In a number of pathological conditions an increase in the lipoid content of the blood has been described. Thus Bürger and Beumer describe a high lecithin and cholesterin content in diabetic lipaemia and in cholaemia, raised values in eclampsia and low values in pernicious anaemia, chlorosis, cancer, and atrophy of the pancreas. In pregnancy and eclampsia the lipoid increase runs parallel with an increase in the power to produce haemolysis along with cobra venom (Roemer). An increased lecithin content of the serum has been described as occurring in syphilis (Peritz) but this is denied by Noguchi.

In a large proportion of cases of chronic nephritis (Widal, Weill and Laudet) there is increased lipaemia and cholesterinaemia and the cholesterinaemia cases all have relatively large amounts of albumin in the urine. The fatty infiltration in the retina in cases of albuminuric retinitis is also due to the cholesterinaemia. In this connection it is interesting to note that experimentally

it has been shown by Hueck that the cholesterin content of the blood (as tested by its inhibitory power on saponin haemolysis) is in direct relation to the cholesterin content of the suprarenals. Also cats fed with cholesterin showed increased cholesterin in the suprarenal.

Hess and Fritsch mention the occurrence in the urine of lipaemic diabetics of a lecithin-like phosphorus-containing lipoid which is not present in the urine of normal or non-lipaemic diabetic cases.

Regarding the alterations which occur in the phosphatide content of different organs in pathological conditions practically no information is to hand. Pighini and Carbone in a comparison of the brain of general paralytics with the normal brain, found an increase in cholesterin, a diminution in Kephalin and the presence of an unknown lipoid.

Of considerable importance is the suggestion made by Tallquist that the anaemia in cases of infection with bothriocephalus latus is due to the absorption of haemolytic lipoids from the bodies of the dead worms. The production of fatal anaemia by feeding dogs with dead worms apparently confirms this. (Schaumann & Tallquist). A haemolytic lipoid identical with oleic acid is stated by Faust and Tallquist to be the causative agent.

Since the phosphatides contain phosphorus in organic combination numerous dietetic experiments have been made with the object of determining whether phosphorus in this form is of greater food value than phosphorus in inorganic combination. The results suggest that organically combined phosphorus in the food is probably broken down into simple phosphates before being utilised to form more complex compounds and that the food value of organic phosphorus is in consequence no greater than phosphorus in simple inorganic combination.

Classification of Phosphatides.

Two classifications of phosphatides have been suggested, one by Fränkel depending on the nature of the fatty acids, whether saturated or unsaturated, the other by Thudichum on the relative amounts of nitrogen and phosphorus present. As Thudichum's classification is dependent on the determination of the amounts of more or less stable elements whereas the fatty acids in the case of phosphatides may alter rapidly in the course of preparation the classification of Fränkel has not been generally adopted. Since the methods are in a measure complementary it is probable that a combination of the two will in the future give the best results. According to Thudichum the following classes occur:-

I.	Monamino - monophosphatides	N:P = 1:1	lecithin, kephalin
II.	Monamino - diphosphatides	N:P = 1:2	Cuorin.
III.	Diamino - monophosphatides	N:P = 2:1	sphyngomyelin, amidomyelin.
IV.	Diamino - diphosphatides	N:P = 2:2	
V.	Triamino - monophosphatides	N:P = 3:1	
VI.	Triamino - diphosphatides	N:P = 3:2	

Other phosphatides with even greater ratios have been described, one from bile with N:P = 4:1 (Thudichum) and one from egg yolk with N:P = 8:1 (Fränkel). Compounds such as these must be looked upon with suspicion; in all probability they are impure products containing large amounts of nitrogen-rich phosphorus-free substances (cerebrosides).

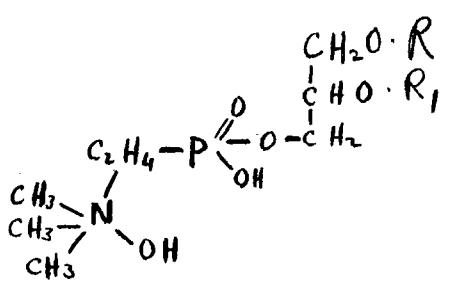
As an explanation of the divergent results which have been obtained with the preparations of different workers I would suggest from the results of my own experiments that sufficient importance has not been paid to the fact that since the amount of nitrogen and of the phosphorus present in the molecule of most phosphatides is very small in/

in proportion to the molecular weight (1.8 per cent in the case of lecithin) it is not unlikely that with the small quantities of substances generally used for the estimation of nitrogen and of phosphorus (0.1-0.2 grms. for example) considerable errors may occur. Further, the practice of a number of writers of accepting as pure only those substances which give an N:P ratio which approximates a round figure, is not wholly free from objection.

Monamino-monophosphatides.

Lecithin.

In 1846 Gobley isolated from egg-yolk and other tissues a body to which he gave the name 'lecithin' and which gave as cleavage products, glycero-phosphoric acid, fatty acid and nitrogen. It is remarkable that the only material advance in our knowledge of lecithin since Gobley's time has been the discovery of the nature of the nitrogen containing products, viz. cholin, the presence of which ~~in lecithin~~ was demonstrated by Hoppe-Seyler and his pupils. Lecithin is tentatively regarded as a compound of glycero-phosphoric acid with cholin on the one hand and two radicals of fatty acid on the other and may be represented by the formula



where R and R_1 represent radicals of fatty acids. The latter which have not yet been identified are most probably some higher members of the unsaturated series (oleic, linoleic, linolenic) (Erlandsen) but it is still common to find lecithin described as a saturated substance containing two radicals of stearic or palmitic acid/

acid. According to Diakonow three lecithins occur, distearyl, dipalmityl and dioleyl, while according to Thudichum oleic acid combinations are most common, oleo-stearyl, oleo-palmityl, and oleo-margaro lecithins being found. Henriques and Hansen, Cousin and Erlandsen believe that the still more unsaturated linoleic and ~~even~~ linolenic acids occur. These ~~different~~ divergent results are at first sight difficult to reconcile but it is probable that the impurity of the products examined and the changes which rapidly occur in unsaturated phosphatides after isolation may in part explain the wide variation. Erlandsen, for example, has shown that in the case of lecithin and cuorin the iodine value (which is an expression of the degree of unsaturation of the fatty acids present) falls rapidly as the preparation is kept. It is not even at present known whether the lecithin out of different tissues is the same. Erlandsen and MacLean in the case of heart lecithin could only identify 42. per cent. of the total nitrogen as present in the form of cholin, while Baskoff found 58 per cent in liver lecithin, and MacLean 66 per cent in the lecithin out of egg yolk. It is possible that the experiments of Von Fürth who has shown that cholin immediately after separation is very rapidly altered, may have a bearing on this subject. The iodine values have also been found to differ for lecithins from different organs. The iodine value of heart lecithin is given as 100.5 (Erlandsen) of liver lecithin as 63 (Baskoff) and of egg yolk as 48.7 (Stern and Thierfelder) Further, Rollett suggests that in the lecithin molecule substances other than fatty acids are present which can absorb iodine. According to MacLean cholin is not the only nitrogen-containing substance present in the cleavage products of lecithin.

In the light of our present knowledge "lecithin" may/

may be defined as a monamino-monophosphatide which can be obtained by extraction of tissues with ether or alcohol and is readily soluble in all fat solvents with the exception of acetone. On saponification glycero-phosphoric acid, cholin and fatty acids, are obtained.

Lecithin has been found in heart and striped muscle (Erlandsen), egg yolk (Stern and Thierfelder) and liver (Baskoff). According to Thudichum it is present in the brain but this is denied by Fränkel. The latter writer has examined several organs (among them the kidney) for lecithin but without success. MacLean has described its occurrence in ether and alcohol extracts of the kidney of the horse.

Attempts to synthesise lecithin have so far failed but the want of success would appear to be attributable rather to the uncertainty regarding the constitution of lecithin itself than to the methods employed. The most recent attempt is that of Grün and Käde. These authors beginning with ethylene glycol have produced by the action of phosphorus pentoxide a distearin glycolester of orthophosphoric acid and by the action on this substance of thionyl chloride and trimethylamine have ~~found~~ formed the trimethyl ammonium salt of distearin-glycol chlorhydrin ester of orthophosphoric acid, a substance which bears a close resemblance to distearyl lecithin as the latter is at present conceived.

Preparation.

Lecithin is prepared by a variety of procedures -

(1) Erlandsen extracts dried tissues repeatedly with ether over a long period (months), evaporates the extracts to dryness in vacuo or under carbondioxide gas and dissolves the accumulated material in ether. The ethereal solution is then precipitated with acetone, the ether-acetone fluid removed and the precipitate shaken with cold absolute alcohol. The alcoholic solution/

solution contains the lecithin.

In Erlandsen's experiments with the phosphatides of ox heart the tissues after thorough extraction with ether were extracted with alcohol. Although this late alcoholic extract contained a much greater amount of phosphatides than the ether extract, Erlandsen was unable to obtain efficient separation of these and could not identify a monamino-monophosphatide with the properties of lecithin. Erlandsen's lecithin therefore is obtained only from ether extracts.

(2) Fränkel's method consists in treating tissues repeatedly with acetone and then extracting with petroleum ether. Lecithin is obtained from the extracted material by means of cold alcohol and further purified by Thudichums method.

(3) Thudichum precipitates alcoholic extracts of tissue with cadmium chloride. The precipitate is treated with benzol which dissolves out the lecithin - cadmium - chloride compound.

(4) In the method of Hoppe Zeyler and Diakonow the dried material is first treated with ether to remove fat and then extracted with alcohol which removes lecithin.

Others recommend treatment with boiling alcohol and subsequent exposure of the extract to 15°C. when precipitation of lecithin results.

Notwithstanding the differences in procedure it is obvious from a comparison of the chemical formulae given below of lecithinsprepared by the different methods that products have been obtained which show a fairly close resemblance -

Lecithin from egg yolk (Diakonow) $C_{44}\ H_{90} NPO_9$

Lecithin from brain (Thudichum) $C_{43}\ H_{84}\ NPO_8$

Lecithin from heart muscle (Erlandsen) $C_{43}\ H_{80}\ NPO_9$

Lecithin from egg yolk (Stern & Thierfelder) $C_{42}\ H_{78}\ NPO_9$

Properties/

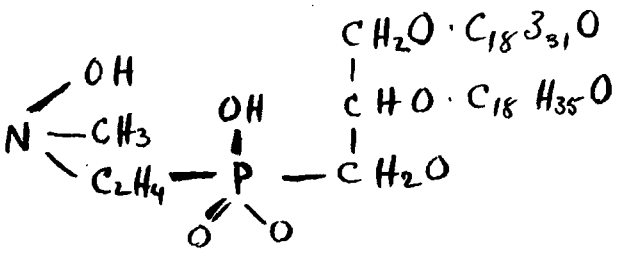
Properties.

In the dry state lecithin is a yellow or yellowish brown somewhat sticky mass which on standing in the air becomes darker in colour and more fluid in consistency. It has a distinct odour which becomes much more marked on warming. Exposed to the action of water lecithin slowly swells to form mucus-like material which on shaking forms an emulsion. In ether, alcohol, chloroform, benzol, petroleum ether and most other fat solvents lecithin is easily soluble. In acetone it is insoluble. By the action of weak alkalies or acids saponification occurs with the separation of fatty acids, cholin and glycero-phosphoric acid. In contact with strong sulphuric acid and sugar lecithin gives Pettenkoffer's reaction on account of the unsaturated acids which it contains. Owing to the unsymmetrical construction of the glycero-phosphoric acid which is present in the molecule lecithin is optically active (Willstater and Lüdecke). In contact with sugar, alkaloids and proteins it forms interesting combinations which, though difficult to break up, are probably not chemical compounds but mixtures. On standing lecithin readily undergoes oxidation with consequent fall in the iodine value. In combination with cadmium chloride it forms a semi-crystalline product which has been extensively examined by a number of workers since it forms a very suitable substance for the estimation of nitrogen and phosphorus. It has however been shown by Erlandsen that partial splitting off of fatty acids occurs in the formation of the compound and by MacLean that the figure representing the amount of cholin in the cadmium chloride compound is much greater than that obtained with lecithin itself. The cadmium chloride compound dissolves in benzol and ether/

ether but is easily rendered insoluble in ether. Compounds of lecithin with platinum chloride, mercuric chloride, sodium chloride and other salts are also described (Bing). Haemolytic compounds of lecithin with bee poison (Morgenroth & Carpi), pancreas juice (Friedemann & Wohlgemuth), ricin (Pascucci) similar to Kyes' cobra lecithid have been described.

Kephalin.

Kephalin was first discovered by Thudichum in the brain and has since been found in egg yolk (Stern and Thierfelder, and MacLean). According to Erlandsen it does not occur in either heart or voluntary muscle. Kephalin is a monamino-monophosphatide which is distinguished from lecithin by its insolubility in cold alcohol. It contains cholin or other base, glycerophosphoric acid and two fatty acid radicals one of which is supposed to be stearic and the other linoleic acid. Like lecithin it readily undergoes oxidation. The constitution is given by Bdng as



It will be noted that the base in this formula is not cholin but hydroxyethyl monomethyl ammonium hydroxide. According to Koch, Fränkel and Neubauer, the base in Kephalin is poorer than cholin in methyl groups. Kephalin is prepared by the same procedures as lecithin but remains behind in the acetone precipitate after treatment of the latter with cold alcohol for removal of lecithin. According to MacLean, kephalin can be separated/

separated from other alcohol insoluble material by treatment with alcohol at 60° centigrade.

Properties. Kephalin is a resinous, easily pulverisable, hygroscopic substance of light yellow colour. It is soluble in ether, chloroform, benzol, petroleum ether, and carbondisulphide. Like lecithin and cuorin it is soluble in hot acetic ether and insoluble on cooling the solution. It is insoluble in alcohol and in acetone. (Thudichum's preparation was almost insoluble in cold but easily soluble in hot alcohol, while that of Koch and Wood was completely insoluble in alcohol). According to Koch a small amount of hydrochloric acid renders it soluble in alcohol. In water it behaves like lecithin.

The cadmium chloride compound is distinguished from that of lecithin by being soluble in ether. Like lecithin it is easily hydrolised by weak acids and bases. On hydrolysis, according to Thudichum, there is found in place of glycero-phosphoric acid a complex kephalo-phosphoric acid (kephalin acid) which is composed of unsaturated fatty acids and glycero-phosphoric acid.

A number of other monamino-monophosphatides have been described - paramyelin, myelin (Thudichum), vesalthin (Pari), and an acetone soluble preparation obtained by Erlandsen out of heart muscle. As the properties of these are not definitely ascertained they will not be discussed further.

Monamino-diphosphatides.

Cuorin.

Cuorin (Erlandsen) occurs along with lecithin in the ether extract of heart muscle and forms the bulk of the phosphatide. It is easily separated from lecithin by its insolubility in cold or hot alcohol, and since, according to Erlandsen, no kephalin is present in heart muscle no difficulty arises in obtaining it as a pure product. Cuorin has been found in the heart, in voluntary/

voluntary muscle (Erlandsen) and recently in horse kidneys (MacLean) but its presence in other organs has not yet been determined.

The formula has been given as $C_{71}\ H_{125}\ NP_2\ O_{21}$ so that the molecule is much bigger than that of lecithin. The cleavage products are also different from those of lecithin consisting of glycero-phosphoric acid, three fatty acids and a base (not cholin). The fatty acids belong to the linoleic, linolenic groups, the iodine values being 130.1 but these have not yet been definitely identified. Like lecithin cuorin is easily altered on standing, especially on exposure to the air, and the oxidation is accompanied by a distinct change in its properties as it loses its solubility in ether and becomes soluble in water.

Freshly prepared cuorin is a yellow brown transparent hygroscopic substance but on standing it becomes a hard, resinous mass which is easily powdered. Cuorin dissolves readily in ether, chloroform, benzol, petroleum ether and in hot acetic ether; from the latter it separates out on cooling. In contact with water it gradually swells and forms a permanent dense emulsion.

According to Bang cuorin does not react with cobra venom.

Liver Phosphatide.

This was prepared by Baskoff from the liver by Erlandsen's method and is in most respects similar to cuorin. MacLean also describes the occurrence of a monamino-di-phosphatide. This was present along with kephalin in the ether extract of egg yolk and was purified by treatment with alcohol at 65° C. which removed the kephalin. No account of the properties of the substance is given; the N:P ratio is the only information available.

Diamino-monophosphatides.

Into/

Into this group fall Thudichum's amidomyelin and sphingomyelin, a phosphatide described by Erlandsen as occurring in ether extract of heart and of voluntary muscle, and a phosphatide prepared by Stern and Thierfelder from the ether extract of egg yolk. As the method adopted by Thudichum for the preparation of amido- and sphingo-myelin is open to a considerable amount of criticism these preparations will not be dealt with in detail. Both are obtained by extracting dried brain (previously treated with alcohol) with absolute alcohol and precipitating the extracts with cadmium chloride. Both substances are difficult to dissolve in cold alcohol.

Of more interest is the diamino-phosphatide of Erlandsen. This was obtained from the secondary alcohol extract and was only examined in the form of the cadmium chloride compound. In general properties this phosphatide resembled lecithin so closely that separation was only possible by taking advantage of the fact that the diamino-phosphatide was not removed by the primary ether extraction.

In the case of heart muscle the great bulk of phosphatide present in the secondary alcohol extract was composed of this material. In its cleavage products also this phosphatide closely resembled lecithin in that glycero-phosphoric acid, fatty acids and a base whose platinum chloride compound contained the same per centage of platinum as that of cholin were formed. Nevertheless the ratio of nitrogen to phosphorus gave a diamino-monophosphatide. As an explanation of the absence of this phosphatide from the primary ether extract it was suggested that the phosphatide in the original tissue was present in firm combination (probably with proteid) which was not broken down by the action of the ether. Recently MacLean has shown in

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an examination of horse kidneys by Erlandsen's method that the great bulk of phosphatide in the secondary alcohol extract consists of lecithin, which, however, is difficult to obtain in a pure state principally owing to the presence of contaminating diamino-mono-phosphatide. From my own observations and MacLean's results I would suggest that Erlandsen's findings with regard to the phosphatide in the secondary alcohol extract, are not correct. The phosphatide in Erlandsen's alcohol extract probably consisted of impure lecithin but as the ratio of nitrogen to phosphorus was almost 2:1 the material was accepted as diamino-monophosphatide and not further purified.

The diamino-phosphatide of Stern and Thierfelder was found in the ether extracts of dried egg yolk and occurred along with lecithin and kephalin in the acetone precipitates. On treatment of the precipitate with ether a small insoluble fraction was found; this was removed by centrifugalising and purified by washing with ether, in which fluid it was only very slightly soluble. Successive acetone precipitates yielded decreasing amounts of the same substance. In its general characters this material was quite different from lecithin or kephalin, being white, semi-crystalline, non-hygroscopic, hardly soluble in ether, soluble in chloroform, insoluble in cold but soluble in hot alcohol, separating out again on cooling. On being heated to 170°C. it melted to a brown oil. From its solution it was precipitated by cadmium chloride and by lead acetate. The iodine value was 34. Out of 100 eggs (887 grms.) only 0.78 grm. of this substance was obtained.

The diamino-phosphatide which MacLean has found in horse kidneys and which is present in both the ether and the alcoholic extracts (in the latter difficult to separate/

separate from the lecithin fraction) is probably the same as that of Stern and Thierfelder.

Triamino-monophosphatides.

A phosphatide of this kind is described by Frankel and Bolaffio as occurring in egg yolk. It is probably Stern and Thierfelder's diamino-phosphatide in an impure state.

Of more interest is the substance called Carnaubon described by Dunham and Jacobson. This was prepared from ox kidneys and differed from Fränkel and Noguiera's phosphatide by being soluble in ether. This substance was a triamino-monophosphatide which contained cholin, three fatty acids and a glycerin-free phosphoric acid. The fatty acids consisted of stearic, palmitic and Carnaubic. Galactose was also present, the preparation in this respect resembling certain plant phosphatides. On the other hand, the presence of this sugar suggests contamination by cerebroside.

Triamino-diphosphatides.

Two preparations of this class have been described, one out of the kidney by Fränkel and Noguiera and the other from the brain by Fränkel. The purity of both products is extremely suspicious.

The Cerebrosides (Galactolipines).

These are compounds of galactose, fatty acids, and certain nitrogen-containing bases. Phosphorus is absent. In animal tissues the separation of these substances from the phosphatides is fairly sharply defined but in plant tissues the compounds of phosphatide and galactose already mentioned serve as an intermediate connecting group with the phosphatides on the one hand and the cerebrosides on the other. Cerebrosides were first prepared by Thudichum and were at first thought to be limited to brain tissue, but have since been found in spleen, pus cells, spermatozoa and in red blood corpuscles. The individual preparations are very similar in properties and it is not impossible that some of them may really be the same substance.

Phrenosin $C_{40}\ H_{80}\ NO_8$.

This was found by Thudichum in the white substance of the brain. The formula is given as $C_{40}\ H_{80}\ NO_8$. It is a white crystalline substance insoluble in cold alcohol, soluble in hot alcohol (separating out again on cooling.) With or without the addition of sugar sulphuric acid produces Pettenkoffer's reaction. On hydrolysis galactose, neuro-stearic acid (an acid isomeric with stearic acid) and a base sphingosin which is supposed to be an amino-fatty acid, are found.

Cerebron $C_{46}\ H_{90}\ NO_9$.

Cerebron was prepared by Thierfelder and Wörner out of Protogan. In its general properties this substance is very like phrenosin. On hydrolysis a fatty acid, galactose, sphingosin and cerebronic acid ($C_{25}\ H_{50}\ O_3$) are found.

Kerasin $C_{36}\ H_{78}\ NO_6$).

This substance was also prepared by Thudichum from the brain/

brain . It was only separable from phrenosin by its slower precipitation from solution in alcohol.

Cerebrin and Homocerebrin.

These were prepared from protagan by Parcus, Kossel, and Freytag and are probably identical with phrenosin and kerasin respectively.

The Cholesterins.

This group includes a number of more or less stable substances which, except for their solubility in fat solvents, bear no relation to the substances already described. A number of them occur naturally, of which Cholesterin is the best known, but a large number have been produced recently by chemical methods. It is not proposed to deal here in detail with these bodies but the general characters of the group may be mentioned. They are for the most part substances which, in their chemical constitution, are related to the polyterpenes especially as regards the proportion of carbon to hydrogen in the molecule, but differ from these in containing alcoholic hydroxl groups. They are also unsaturated and of high molecular weight. With certain reagents (strong sulphuric acid, acetic anhydride, etc.) colour reactions are given which are characteristic for individual members of the group. In ether, benzene and chloroform they are readily soluble, in alcohol, acetone and petroleum ether only with difficulty. From solution in ether and alcohol crystalline forms are produced. The more important members of the group are cholesterin, widely and abundantly distributed in the animal body, coprosterin occurring in human faeces and probably a decomposition product of cholesterin and the phytosterins which are found in plants.

The majority possess, at least in some degree, the property of inhibiting the action of most haemolytic substances but the function which they serve in the animal body is quite unknown.

The chemical and bio-chemical characters of cholesterin and its derivatives will be fully described later.

EXPERIMENTAL PART

During the last four years I have investigated the chemical and bio-chemical characters of lecithin and cholesterin and certain other lipoids. The work was in the first place suggested by the discovery of the important part which 'lipoids' in the form of alcoholic extracts, played in certain biological tests, the Wassermann syphilis reaction in particular. It had been demonstrated by a number of observers that the sera of syphilitics in combination with emulsions of alcoholic tissue extracts absorbed or deviated complement whereas the sera of normal individuals did not do so. The nature of the bodies in the alcoholic extracts which participated in the reaction had been investigated by several workers and a number of chemical agents of lipoid nature had been tested for their power to act as antigens. The lipoids examined included lecithin (Porges and Meier, Landsteiner, Müller and Pötzl), cholesterin (Fleischmann), protagon and certain salts of the fatty acids. About this time I was engaged in examining the alterations which occur in organs undergoing autolysis and, as an increase in the lipoid content of tissues in this condition had been described, it seemed of interest to compare the antigenic properties of an extract of an organ undergoing autolysis with the extract of the same organ in the fresh state. An alcoholic extract of a guinea pig's liver which had undergone aseptic autolysis for several days was made, and was tested along with the extract of a fresh guinea pig's liver. It was found that the amount of complement deviated in the presence of syphilitic serum by an emulsion of the extract from the autolised tissue was much greater than that absorbed by a similar amount of emulsion prepared from the extract of the normal organ tested under/

under similar conditions. The extract from the autolised organ had apparently a greater antigenic value than the extract from the normal tissue. It was soon discovered, however, that the extract of autolised tissue was by itself much more haemolytic for ox's red blood corpuscles than the extract of fresh tissue and that it was, in consequence, unsuitable as a test reagent for syphilitic sera. It was further noted that this haemolytic action was inhibited to very different degrees by different syphilitic sera. An attempt was made to get an extract free from this objectionable feature. After a number of unsuccessful methods had been tried it was found that by evaporating the extracts to dryness, dissolving the residue in ether and adding acetone in excess a precipitate was obtained which had comparatively slight haemolytic action. The haemolytic substances being soluble in acetone were retained by the acetone-ether fluid. The precipitated material was found to be only partially soluble in cold alcohol. The alcohol soluble or 'lecithin' fraction was found to possess the property of deviating complement in the presence of syphilitic serum though the amount of complement absorbed was much less than that deviated by the corresponding amount of crude extract similarly tested. Further, it was found that the antigenic value of the lecithin fraction of the acetone precipitate in the case of an extract of autolised tissue was not greater than that of the corresponding fraction prepared from an extract of fresh tissue. The acetone-soluble fractions from both fresh and autolised tissue extracts in addition to being markedly haemolytic were also very anticomplementary - that is to say, these fractions alone, without the addition/

addition of syphilitic serum were capable of absorbing considerable amounts of complement. The phenomena seemed sufficiently interesting to warrant further examination and it was therefore decided to prepare a large quantity of extract and to purify and test the lecithin fractions more thoroughly. As Erlandsen had shown by chemical methods that ethyl acetate was a valuable agent for the purification of lecithin and other phosphatides, this substance was also employed as a preliminary to the treatment with acetone.

Fresh ox liver was minced and added to 95 per cent ethyl-alcohol in the proportion of 1 part of liver to 4 of alcohol. Extraction was allowed to proceed for fourteen days at room temperature, the mixture being stirred up daily. The fluid was then pressed out of the insoluble residue by hand, and filtered through paper. In the course of several days a small amount of sediment separated out from the clear, yellow-tinted fluid: this was rejected. The solution now constituted the crude extract, which, on evaporation at 60°C. to constant weight was found to contain 1.9 per cent. of solid matter. Five hundred c.c. of crude extract were evaporated at 60°C. till a brown viscid mass resulted. The residue was rapidly dissolved in 170 c.c. of ethyl-acetate at 60° C. and filtered at this temperature through paper, leaving a residue which was quite insoluble in hot ethyl-acetate and which was not further investigated. The hot ethyl-acetate solution was put in the ice chest and left over night. A bulky precipitate separated out. The clear fluid was decanted and kept in the ice chest for several days, but no further sediment appeared. Two components were thus obtained one of which was soluble only in hot ethyl-acetate, the other also in cold ethyl-acetate.

(a)/

(a) The portion insoluble in ethyl-acetate in the cold was highly soluble in water-free ether at room temperature. To the clear ethereal solution 5 volumes of acetone were added, which caused a yellowish-white precipitate to separate out immediately (crude lecithin). No further precipitation occurred from the acetone-ether mixture after several days in the ice chest, nor did the addition of acetone to the mixture cause precipitation. On evaporating it, however, and dissolving again in ether, the addition of acetone caused a further precipitate, thus showing that all the acetone-insoluble constituents were not removed by the first treatment with acetone. The crude lecithin was redissolved in ether and reprecipitated with acetone; these processes were repeated twice further. The final precipitate was dissolved in absolute ethyl-alcohol at room temperature, a 1.7 per cent. solution being prepared. A small amount of the acetone-insoluble substance was insoluble in cold alcohol. About 2 grms. "pure lecithin" were thus obtained from 500 c.c. of crude extract, a fifth of the total solid matter. This represented somewhat less than the total amount of lecithin present. The alcoholic solution of purified lecithin had a faint yellow tint. It gave an abundant precipitate with alcoholic $CdCl_2$ solution. A fairly permanent emulsion was got by adding 1 part of the alcoholic solution to 7 parts of 0.85 per cent NaCl solution; the reaction was neutral to litmus paper.

(b) The portion soluble in ethyl-acetate in the cold was freed from the solvent by distillation under diminished pressure at 50°C. The dried product weighed 0.4 grms. It dissolved readily in absolute alcohol, a 2 per cent solution being prepared which had a deep yellow tint. With alcoholic Cd Cl_2 solution no precipitate occurred/

occurred. One part of the 2 per cent alcoholic solution with 7 parts of 0.85 per cent. NaCl solution produced a very turbid emulsion from which flocculi rapidly separated out; the reaction was slightly acid to litmus paper. Further experiments showed that the portion soluble in ethyl-acetate in the cold was almost entirely soluble in water-free ether, and that the addition of acetone caused practically no precipitate from the ethereal solution even after several days in the ice chest. This product was therefore apparently free from lecithin.

The fractions were examined for -

(1) Haemolytic action.

(2) Effect on complement.

(3) Power of causing the combination of a large amount of complement in the presence of syphilitic serum.

(4) Action along with cobra venom.

The method of procedure in the tests involving the use of complement was as follows:-

Three series of tubes were prepared -
(A) contained 0.6 c.c. of "lipoid" emulsion (organ extract, etc.) along with 0.05 c.c. of syphilitic or normal serum, which had been heated previously for half to three-quarters of an hour at 57°C.
(B) contained 0.6 c.c. emulsion alone.
(C) contained 0.05 c.c. serum in 0.6 c.c. salt solution.
Increasing amounts of complement were added to the tubes, and at the same time the dose of complement (fresh guinea-pig's serum at least twenty-four hours after withdrawal of the blood) was estimated by placing suitable amounts in 0.6 c.c. salt solution. All the tubes were incubated for one and a half hours at 37°C., then to each 1 c.c. of 5 per cent. washed ox-blood suspension, sensitised previously by the addition of five minimum haemolytic doses of immune serum from the rabbit, was added. After further incubation for an hour and a quarter the tubes were set at room temperature; on the following day the final reading was taken. An emulsion of the crude extract was generally used for comparison. This was prepared by diluting the extract with salt solution in the proportion of 1 to 5. The emulsions of lipoid were in all cases made of maximum turbidity by floating the alcoholic solution on to the surface of the salt solution in a test tube and slowly rotating the tube.

Lecithin Component.

Lytic Properties.- The pure lecithin was only slightly lytic for ox's red blood corpuscles; 2 c.c. of a 1 in 5 dilution of the 1.7 per cent. alcoholic solution of lecithin caused not quite complete lysis of 1 c.c. of 5 per cent suspension of washed ox blood after three hours at 37°C. (Some specimens of ox blood were more sensitive to the lytic action than others.)

Action on complement.- A 1.7 per cent. solution in alcohol was adopted as most satisfactory; 0.6 c.c. of the emulsion made by diluting 1 part of the alcoholic solution with 7 parts of saline usually caused scarcely any lysis of 1 c.c. of the test corpuscles in twenty-four hours. This quantity of emulsion had only a very slight inhibitory effect on complement by itself, lysis usually being complete with two to three doses. In general this amount of lecithin emulsion had less inhibitory action on complement than had the standard amount of crude extract emulsion. The inhibitory effect of the lecithin emulsion also was more uniform with different specimens of complement than that of the crude extract. This is well seen in Table I.

Wassermann reaction.- Along with syphilitic serum the lecithin emulsion in the amount mentioned above caused increased absorption of complement; but this was much less marked than in the case of a crude extract (vide Table II,) even although the amount of lecithin in the latter represented only a fraction of that present in the standard amount of pure lecithin emulsion. With sera which gave only a slight positive reaction with crude extract there was practically no increase in complement absorption with the pure lecithin. It was obvious, therefore, that lecithin was not the sole component to which the crude extract owed its activity. At the same time the comparatively/

comparatively slight lytic effect of the crude extract emulsion by itself excluded the presence of oleic acid salts to any great extent, unless there was also some other body which acted powerfully in preventing their haemolytic action.

Cobra-venom activation. Complete lysis of 1 c.c. ox blood suspension was caused by 0.0017 c.c. of a 1 per cent emulsion of lecithin in the presence of 0.1 c.c. of 1:1000 cobra venom. The ratio of the lytic dose with venom to that without venom was 1:400.

The Component Soluble in Cold Ethyl-Acetate.

Lytic Properties.- This component was distinctly more lytic for ox corpuscles than was the pure lecithin,- 0.025 c.c. of a 1 per cent solution causing just complete lysis of 1 c.c. of ox blood suspension; but the lytic action was weak as compared with oleic or linoleic acid (vide Table 4).

Action on complement.- The emulsion had a very powerful anti-complement effect which was neutralised to a great extent by lecithin, when the alcoholic solutions were mixed and the emulsion was then made; on the other hand, when the emulsions were made separately and were then mixed the anti-complement effect remained practically unaltered. These results are shown in Table III.

In series A, 0.9 c.c. of the 1.7 per cent. alcoholic lecithin solution were mixed with 0.1 c.c. of 2 per cent. alcoholic solution of the ethyl-acetate soluble portion and 0.1 c.c. of absolute alcohol, and the mixture was emulsionised in the usual way. In series B, the same quantity of alcoholic lecithin solution was emulsified in 3.5 c.c. salt solution, and the ethyl-acetate soluble component along with the absolute alcohol were emulsified in another 3.5 c.c. of salt solution; the two emulsions were then mixed. In both cases the mixtures were neutral to litmus paper and were of approximately equal turbidity. To 0.6 c.c. of emulsions increasing amounts of guinea-pig's complement were added, and after one and a half hour's incubation at 37°C.1 c.c. of the test suspension of sensitised ox corpuscles was added and the mixture was again incubated as usual. The result was that in series A 0.025 c.c. of complement caused complete lysis, whereas in series B more than 0.1 c.c. of complement required/

required to be added before complete lysis occurred.

Similar phenomena in other colloid reactions would suggest as an explanation that, where the alcoholic solutions are mixed before the emulsion is made, lecithin comes into more intimate contact with the other components, and thus protects the complement from the latter, whereas when the substances are mixed in the form of emulsions they persist for a considerable period in the form of isolated globules, so that absorption processes must be delayed.

Wassermann effect.- The presence of syphilitic or normal serum inhibited slightly the anti-complement effect in the amounts employed, and no Wassermann reaction occurred. Table IV shows that the test amounts of a syphilitic serum and a normal serum had practically no influence on the complement inhibition.

When the lecithin and the ethyl acetate components were mixed in alcoholic solution and then emulsified it was found that the Wassermann reaction was increased as compared with that given with lecithin alone in the presence of syphilitic serum (vide Table V). An effect equal to that of the crude extract was not obtained, however.

Cobra-venom activation.- The emulsion of this component activated cobra-venom haemolysin; but only to a slight degree, as the ratio of the lytic dose with venom to the lytic dose by itself was only 1:6. Table VI shows the result of estimations of the cobra venom activating dose and the lytic dose of the pure lecithin and of the ethyl-acetate soluble component as well as the lytic doses of oleic acid and linoleic acid. The tests were all made at the same time and with the same specimen of ox blood, and serve as a representative example of repeated observations. To ensure uniformity, the

amounts/

amounts of alcohol in the different series were kept as far as possible equal; thus with the exception of the estimation of the lytic power of lecithin, stock dilutions were made up just before use containing 25 per cent. of alcohol. The amount of alcohol present in the doses employed was therefore by itself practically negligible. For the estimation of the lytic dose of lecithin by itself a solution containing 20 per cent. alcohol was employed. The amounts of the various solutions were added to 1 c.c. of 5 per cent. washed ox blood suspension, and after incubation at 37° for two and a half hours with repeated shaking the results were read. Table VII shows the respective doses in grammes of the various substances. Smaller amounts of the ethyl-acetate soluble component distinctly inhibited lecithin activation. This held practically equally whether the alcoholic solutions of lecithin and of the component soluble in ethyl-acetate were mixed and then emulsionised or were emulsionised separately and the emulsions then mixed.

As the ethyl acetate component was a complex mixture of bodies (fatty acids, neutral fats, cholesterin etc.) it was not clear whether the effect of this fraction was due to an individual substance or to the interaction of a number of bodies. At this stage in my experiments it was discovered that the action of the component soluble in cold ethyl acetate could be regarded as due in some respects to the presence of cholesterin. It was found by Dr Browning that the addition of cholesterin to the alcoholic lecithin solution greatly increased the antigenic effect of the lecithin in the presence of syphilitic serum while at the same time the anticomplementary effect was not appreciably/

appreciably altered. The lecithin-cholesterin mixture was in fact comparable to the crude extract in regard to its action in the Wassermann test. The results of the examination of a weak and of a powerful syphilitic serum with emulsions of lecithin, lecithin-cholesterin, and crude extract are given in tables 8 and 9. As this discovery was thought to be of some importance with regard to the production of a reagent which could be standardised for use in the Wassermann reaction the attention of Dr. Browning and myself was given for some time to the examination of a number of syphilitic and normal sera. The results of this examination were published in a joint paper along with Dr. Mackenzie, who supplied a number of the syphilitic sera. The lecithin-cholesterin method, as it has been called, has since been used by a number of workers (Gilmour, Muirhead, Watson) and has been found to be of considerable service especially in the diagnosis of weak or doubtful syphilitic sera.

In the course of this work, however, it was found that different specimens of lecithins from the same organs gave on occasion slightly different results and as it was thought that information regarding the nature of lecithin might be obtained from more extended observations it was decided to prepare lecithins from a number of different sources and to examine these for their action in the various biological tests. In the preliminary part of this investigation I was associated with Drs Browning and Gilmour. Work which I have myself done in this connection is as follows:-

Action of Lecithins from Different Sources.

In order that comparisons of the different lecithins could be made it was necessary that a uniform method of preparation should be employed throughout. As a result of the experience gained during the course of the experiments already described the method of preparing lecithin was slightly altered as follows:

The crude extract obtained by macerating the minced tissues with alcohol (1 part of tissue to 4 parts of 95 per cent. alcohol) for 7 or 10 days at room temperature, was evaporated on the water bath at 60°C, this operation lasting about 5 hours. The residue was then rubbed up with quartz sand (previously washed with water and dried) and extracted with ethyl acetate at 60°C for 10 or 15 minutes. The solution in ethyl acetate was placed in the ice chest and the precipitate which formed was removed and redissolved in ethyl acetate. This was again allowed to precipitate in the ice-chest. These processes were repeated till the supernatant fluid was colourless (usually three times was sufficient). The ultimate precipitate was dissolved in water-free ether and the solution precipitated with excess of acetone, the treatment with ether and acetone being repeated three times. Finally the acetone precipitate was pressed in a mortar in order to get rid of fluid, rapidly rubbed up with quartz sand and the mass treated with absolute alcohol at room temperature. The portion soluble in alcohol constituted the lecithin. The strength of the solution was determined by evaporating a measured quantity and weighing the residue. A 0.75 per cent solution was usually prepared.

All the lecithins were tested for 1) effect as antigen in the Wassermann syphilis reaction, 2) haemolytic action along with cobra venom, 3) degree of saturation as tested by the iodine-value.

The lecithin was employed in the form of an emulsion with salt solution (1 part of alcoholic lecithin to 7 parts of 0.85 per cent. NaCl-solution) this being made as turbid as possible by floating the alcoholic solution on to the surface of the salt solution in a test tube and mixing by slow rotation of the tube. The importance of the turbidity of lipoidal emulsions in determining the amount of complement absorbed had been shown by Sachs and Rondoni for the crude extract, and

by/

by Browning and Cruickshank in the case of lecithin-cholesterin mixtures. A rapidly made emulsion deviates less complement than a slowly made, more turbid one, In all the experiments, therefore, with a view to comparing the action of different lecithins, only the most turbid emulsions of each were employed.

Preliminary experiments were carried out to test the effect of varying the amounts of lecithin and of alcohol in the emulsions. As regards varying the quantity of lecithin, the alcohol being kept constant, it was found that only a very small amount of lecithin was necessary to produce a positive Wassermann reaction in the presence of syphilitic serum. In general, increased amounts of lecithin produced increased absorption of complement with positive sera (v. table 10). Occasionally, a slight zone effect was found (v. table XI), that is, an increase in the amount of lecithin beyond a certain optimum quantity, caused the absorption of less complement; but the zone effect was practically negligible. The addition of a fixed amount of cholesterin to the lecithin solutions of varying strength, while causing increased absorption of complement in the presence of syphilitic serum did not cause any notable differences in the various series (v. table XII). The effect of varying the amount of alcohol is shown in table XIII. Increase of alcohol (short of an amount sufficient to destroy complement) caused increased deviation of complement in the presence of syphilitic serum, while not increasing the inhibitory effect of the emulsion itself on complement.

The results showed that where the amounts of syphilitic serum and of alcohol were kept constant very considerable variation in the amount of a given preparation of lecithin, within certain limits, produced only

a/

a comparatively small difference in the amount of complement absorbed. The strength of the lecithin solution usually employed to bring out differences in individual preparations was 0.75 per cent. To obtain an idea of the average efficiency of the various lecithins it was necessary to test them as far as possible at the same time and with the same complement and serum. By using also a standard ox-liver lecithin throughout the experiments, it was possible to compare the results obtained on different occasions. All the lecithins were tested with more than one syphilitic serum. This was important in view of the fact that the ratio of the antigenic value of two given lipoid emulsions may vary to some extent with different sera. The inhibitory effects of the lecithin emulsions by themselves on complement and the lytic effects for ox's red blood corpuscles were also estimated.

Lecithins from the following sources were prepared, ox's heart, liver and kidney, sheep's liver and egg yolk. A number of commercial preparations of lecithin (Merck, Kahlbaum, Riedel Nos. I amd II, Poulenc Frères) were also tested. (In every case a clear alcoholic solution was prepared in the first instance, any insoluble material being removed by centrifugalising).

Bio-chemical Actions.

Wassermann Syphilis Reaction.

The results of a large number of experiments showed that considerable differences existed in the amounts of complement absorbed by the various lecithins in the presence of syphilitic serum. In general, the greatest amounts of complement were absorbed by the heart lecithins, the least by the yolk lecithins, while the liver lecithins were intermediate. Ox-liver

lecithin was slightly superior to that from the liver of the sheep (v. table XIV); but specimens of lecithin prepared from different ox-livers showed some variation in this respect.

It was noted that the emulsions of the heart lecithins were constantly more turbid and the yolk lecithins less turbid than the emulsions of liver lecithin so that there was apparently a direct correspondence between the density of the emulsion and its deviating property in the presence of syphilitic serum. The inhibitory effect of the lecithins by themselves on complement was in all cases very slight. All the emulsions reacted neutral to litmus. The commercial ovo-lecithins had a comparatively weak action as syphilitic antigen.

The addition of cholesterin to the lecithin solutions produced in every case an increase in the amount of complement absorbed in the presence of syphilitic serum, as compared with the amount absorbed by the corresponding lecithins themselves (vide tables XV. XVI). The absolute increase was greatest in the case of the heart and least with yolk lecithins. Ox-liver lecithin was generally inferior to ox-heart lecithin in this respect. The inhibitory effects of the various lecithin-cholesterin emulsions by themselves on complement were practically identical with those of the lecithins.

An attempt was made to effect further purification of some of the lecithin preparations by keeping the ethereal solutions at a low temperature by means of ice and salt. An ether-insoluble body separated out. The portion remaining in solution was precipitated with acetone, redissolved in ether, and again cooled. This process was repeated a number of times over a period of days, a considerable amount of ether insoluble matter being thus removed, until at last practically no further precipitate/

precipitate separated out on cooling. The acetone precipitate was finally dissolved in alcohol. The lecithin so obtained was compared with the original preparation. Practically no difference was found.

The action as syphilitic antigen of preparations of lecithin from different specimens of the same tissue was thus fairly constant. The difference between the lecithins from two different tissues, e.g. heart and egg-yolk, could not be explained as due to the presence of any gross and easily separable impurity such as cholesterin. This was obvious from the fact that the addition of even a large amount of cholesterin to egg-yolk lecithin did not produce as great deviation of complement in the presence of syphilitic serum as did "pure" ox-heart lecithin, which could only contain at the most a mere trace of cholesterin as impurity.

Haemolysis with Cobra Venom.

The most actively haemolytic lecithins were the preparations from egg-yolk, those from ox's heart were the least active (v. table XVII). The other lecithins occupied an intermediate position. In these comparative experiments the turbid emulsions were employed. In the case of ox-liver lecithin I found that with the rapidly made, clear emulsions haemolysis occurred at first more rapidly than with the corresponding turbid emulsions, but that the end results (24 hours) were practically identical in both cases. In each experiment the haemolytic action of the emulsions by themselves for ox's corpuscles was also tested; in the amounts used they were always non-haemolytic. Slight differences were observed in the amounts necessary to produce complete lysis of the test corpuscles in the absence of venom, the heart lecithins being usually least/

least lytic and the yolk lecithins most lytic (v. table XVIII).

Iodine Values.

The method used for these estimations was that of v. Hubl. This depends on the fact that when the alcoholic solution of a fat which contains unsaturated acids is allowed to stand in contact with an alcoholic solution of iodine to which mercuric chloride has been added, a certain amount of iodine is absorbed. By titrating the mixture subsequently with sodium thiosulphate or other suitable reagent the amount of free iodine is determined and from this the amount of iodine which has been absorbed can be calculated. The weight, expressed in grammes, of iodine absorbed by 100 grammes of fat is the "iodine-value".

Considerable variation was found in the amount of iodine absorbed by the different lecithins. Table IX shows an actual experiment; all the preparations noted were, of course, tested simultaneously and 24 hours was allowed for the absorption of iodine by the lecithin solutions. It will be seen that not only were there distinct differences between the lecithins from different tissues, but also between lecithins obtained from different specimens of the same organ. Thus ox-liver lecithin No. 1 was apparently highly unsaturated while ox-liver lecithin No. 2 was only slightly unsaturated. In comparing the iodine values of the lecithins with their effects in the Wassermann reaction it will be observed that no correspondence has been found to exist between these functions. For example, ox-liver lecithin No. 1 resembles the lecithin from a fatty human liver in being highly unsaturated, but the amounts of complement deviated in the Wassermann reaction by these two lecithins differ very markedly, the/

the human liver lecithin having a much higher "antigenic" value (table XX). Ox-liver lecithin No. 2 and yolk lecithin No. 1 have approximately the same iodine value, but the liver lecithin was greatly superior to the yolk lecithin in deviating power. Again, the more unsaturated ox-liver lecithin (No. 1) was a less efficient syphilitic "antigen" than specimen No. 2; but the difference between the two preparations in this respect was much less than the variation in their iodine-values. I have not found any alteration to occur in the iodine-value of lecithins, prepared as described above, which had been allowed to stand in alcoholic solution for some months.

These results differ from those of Noguchi and Bronfenbrenner who, in examining the acetone-insoluble portions of alcoholic extracts from pathological human organs found that a high iodine-value corresponded with a high antigenoc-value in the case of the lipoids from liver and heart.

Table I.

	Haemolytic Dose of Guinea-pig's Complement for 1 c.c. of 5 per cent. Ox Blood Suspension + 5 Doses of Immune Body.	Guinea-pig's Complement + Emulsion, 0.6 c.c.			
		Lecithin		Crude Extract of Ox Liver.	
		Incomplete Lysis	Complete Lysis	Incomplete Lysis	Complete Lysis
1	0.0075 c.c.	0.025 c.c.	0.035c.c.	0.035 c,c.	...
2	0.005 c.c.	...	0.01 c.c.	0.04 c.c.	0.045 c.c.
3	0.005 c.c.	...	0.015 c.c.	0.03 c.c.	0.04 c.c.
4	0.005 c.c.	...	0.01 c.c.	0.015 c.c.	0.02 c.c.
5	0.005 c.c.	...	0.01 c.c.	...	0.01 c.c.
6	0.005 c.c.	0.01 c.c.	.015 c.c.	0.02 c.c.	0.035 c.c.

Table 2.

yphilitic Serum (½ hour at 7°C.) 0.05 c.c. + Emulsion, .6 c.c.	Amounts of Guinea-pig's Complement			
	0.075 c.c.	0.1 c.c.	0.15 c.c.	0.2 c.c.
Lecithin	Just complete lysis	Complete lysis	Complete lysis	Complete lysis
Crude extract	0	Faint trace of lysis	Distinct lysis	Complete lysis

CONTROLS.

Lecithin emulsion, 0.6 c.c. + 0.01 c.c. complement = just complete lysis.

Crude extract emulsion 0.6 c.c. + 0.01 c.c. complement = just complete lysis.

Syphilitic serum, 0.05 c.c. + 0.6 c.c. NaCl solution + 0.01 c.c. complement = complete lysis.

Dose of complement = 0.005 c.c.

Table 3.

ecithin + thyl-Acetate oluble Com-onent.	Amounts of Guinea-pig's Complement.						
	0.02 c.c.	0.025c.c.	0.035c.c.	0.05c.c.	0.075c.c.	0.1c.c.	0.15 c.c.
a) Alcoholic mixture emul-sionised	Very marked lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis	Com-plete lysis
b) Emulsions made separ-ately, then mixed	0	0	0	Faint trace of lysis	Distinct lysis	Marked lysis	Com-plete lysis

Dose of complement = 0.0075 c.c.

Table 4.

per cent. Alcoholic Solution of the Ethyl-Acetate Soluble Component, 0.05 c.c. Absolute Alcohol, 0.45 c.c. Emulsionised in 7 c.c. Salt Solution: 0.6 c.c. of the Emulsion.	Amounts of Guinea-pig's Complement.			
	0.08 c.c.	0.12 c.c.	0.17 c.c.	0.25 c.c.
0	Trace of lysis	Marked lysis	Very marked Lysis	Just complete lysis
normal serum (57°for ½ hour). 0.05 c.c.	Faint trace of lysis	Trace of lysis	Marked lysis	Very marked lysis
syphilitic serum (57° for ½ hour). 0.05 c.c.	Trace of lysis	Marked lysis	Very marked lysis	Just complete lysis

Table 5.

Syphilitic Serum (57°C. for 30 minutes), 0.05 c.c. + 0.6 c.c. Emulsion.	Amounts of Guinea-pig's Complement			
	0.05 c.c.	0.075 c.c.	0.1 c.c.	0.15 c.c.
Lecithin, 1.7 per cent, 0.4 c.c. + absolute alcohol 0.6 c.c. in 7 c.c. salt solution	Faint trace of lysis	Marked lysis	Just complete lysis	Complete lysis
Lecithin, 1.7 per cent. 0.4 c.c. + 2 per cent ethyl-acetate soluble component, 0.165 c.c. + absolute alcohol, 0.435 c.c. in 7 c.c. salt solution	0	0	Trace of lysis	Almost complete lysis

CONTROLS.

Emulsions alone, 0.6 c.c. + 0.02 c.c. complement = complete lysis.

Serum, 0.05 c.c. + NaCl solution, 0.6 c.c. + 0.01 c.c. complement = complete lysis.

Dose of complement = 0.0065 c.c.

Table 6.

Substance.	Concentration in Solution in 25 per cent. Alcohol .	Dose activating 0.1 c.c. of 0.1 per cent. Cobra Venom Solution + 1 c.c. of 5 per cent. Ox Blood	Dose lytic by itself for 1 c.c. of 5 per cent. Ox Blood
Lecithin . . .	0.085 per cent	0.017 c.c.	(0.34 per cent. solution in 20 per cent alcohol) 2 c.c.
Ethyl-acetate soluble component	0.1 "	0.04 c.c.	0.25 c.c.
Oleic acid .	0.05 "	...	0.07 c.c.
Linoleic acid	0.05 "	...	0.07 c.c.

Table 7.

Substance	Absolute Amounts.		Ratio
	Activating Dose for 0.1 c.c. of 0.1 per cent. Cobra Venom + 1 c.c. of 5 per cent. Ox Blood.	Lytic Dose for 1 c.c. of 5 per cent. Ox Blood	
Lecithin	0.000017 grms.	0.0068 grms.	1:400
Ethyl-acetate soluble component	0.00004 "	0.00025 "	1:6
Oleic acid	...	0.000035 "	...
Linoleic acid	...	0.000035 "	...

0.6 c.c. emulsions (a) and (b) + 0.01 c.c. complement = complete lysis.
0.6 c.c. emulsion (c) + 0.02 c.c. complement = almost complete lysis.
Syphilitic serum, 0.05 c.c. + 0.8 c.c. NaCl. solution + 0.01 c.c. complement = complete lysis.

Dose of complement = 0.005 c.c.

Table 8.

yphilitic Serum owerful, ½ hour t 57°C.) 0.05 c.c. 0.6 c.c. Emulsion.	Amounts of Guinea-pig's Complement.					
	0.075 c.c.	0.1 c.c.	0.135c.c.	0.18c.c.	0.22c.c.	0.26 c.c.
a) Lecithin	Trace of lysis	Complete lysis	Complete lysis	Complete lysis	Complete Lysis	Complete lysis
b) Lecithin-cholesterin saturated	0	0	0	0	Faint trace of lysis	Distinct lysis
c) Crude extract	0	Faint trace of lysis	Faint trace of lysis	Faint trace of lysis	Trace of lysis	...

CONTROLS.

Emulsions alone - (a) and (b) + 0.015 c.c. complement = complete lysis.
(c) + 0.04 c.c. complement = just complete lysis
Syphilitic serum, 0.05 c.c. + 0.6 c.c. NaCl solution + 0.01 c.c. complement = complete lysis.

Dose of complement = 0.005 c.c.

Table 9.

yphilitic Serum eak, ½ hour at 7°C.) 0.05 c.c. Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.				
	0.015 c.c.	0.025 c.c.	0.04 c.c.	0.065 c.c.	0.1 c.c.
a) Lecithin	Very marked lysis	Complete lysis	Complete lysis	Complete lysis	Complete lysis
b) Lecithin-cholesterin saturated	0	0	Faint trace of lysis	Trace of lysis	Complete lysis
c) Crude extract	0	0	Faint trace of lysis	Trace of lysis	...

CONTROLS.

0.6 c.c. emulsions (a) and (b) + 0.01 c.c. complement = complete lysis.
0.6 c.c. emulsion (c) + 0.02 c.c. complement = almost complete lysis.
Syphilitic serum, 0.05 c.c. + 0.6 c.c. NaCl. solution + 0.01 c.c. complement = complete lysis.

Dose of complement = 0.005 c.c.

Table 10.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion of Ox-liver Lecithin 0.6 c.c.		Amounts of Guinea-pig's Complement.				
		0.09 c.c.	0.12 c.c.	0.15 c.c.	0.18 c.c.	0.24 c.c.
Weak Positive Serum	Lecithin 0.6. per cent	Very faint trace	Marked	Very Marked	Complete	Complete
	Lecithin 0.2 per cent	Trace	Very Marked	Complete	Complete	Complete
	Lecithin 0.06 per cent	Distinct	Almost complete	Complete	Complete	Complete
Strong Positive Serum	Lecithin 0.6 per cent	0	0	0	Faint trace	Almost Complete
	Lecithin 0.2 per cent	0	Very Faint Trace	Distinct	Marked	Complete
	Lecithin 0.06 per cent	Trace	Very Marked	Complete	Complete	Complete

CONTROLS: All emulsions 0.6 c.c. + Compl. 0.04 c.c. = Just complete Lysis.

Sera 0.05 c.c. + Salt Solution 0.6 c.c. + Complement 0.038 c.c. = Complete Lysis.

Dose of Complement = 0.01 c.c.

Table 11.

Syphilitic Serum .05 c.c. + Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.				
	0.08 c.c.	0.12 c.c.	0.17 c.c.	0.24 c.c.	0.36 c.c.
x-liver Lecithin 0.75 per cent	0	0	Very faint Trace	Almost complete	Complete
x-liver Lecithin 0.25 per cent	0	0	0	Marked	Almost Complete
liver lecithin 0.083 per cent	0	0	0	Very Marked	Complete
x-liver lecithin 0.027 per cent	Faint trace	Trace	Almost Complete	Complete	Complete

CONTROLS: Emulsions alone 0.6 c.c. + Complement 0.03 c.c. = Complete.

Serum 0.05 c.c. + Salt Solution 0.6 c.c. + Complement 0.04 c.c. = Complete.

Dose of Complement = 0.015 c.c.

Table 12.

Syphilitic r. 0.05 c.c. + ulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.					
	0.08 c.c.	0.12 c.c.	0.18 c.c.	0.24 c.c.	0.32 c.c.	0.4 c.c.
-liver lecithin .75 per cent	Distinct	Just Complete	Complete	Complete	Complete	Complete
-liver lecithin .75 per cent + holesterin 1.3 er cent	0	0	Very Faint Trace	Trace	Very Marked	Complete
-liver lecithin .2 per cent	Trace	Almost Complete	Complete	Complete	Complete	Complete
-liver lecithin .2 per cent + holesterin 1.3 er cent	0	0	0	Very Faint Trace	Very Marked	Complete
-liver lecithin .075 per cent	Trace	Very Marked	Complete	Complete	Complete	Complete
-liver lecithin 0.075 per cent Cholesterin 1.3 per cent	0	0	0	Very Faint Trace	Very Marked	Complete

CONTROLS: Emulsions alone 0.6 c.c. + Complement 0.035 c.c. = Complete.

Serum 0.05 c.c. + Salt Solution 0.6 c.c. + Complement 0.025 c.c. = Complete.

Dose of Complement = 0.015 c.c.

Table 13.

2.37 per cent. solution of ox-heart lecithin in alcohol; 1 part emulsionised with 7 parts of saline, so as to produce the maximum turbidity = stock emulsion.

A. 2.5 c.c. of stock emulsion + 5.5 c.c. of NaCl-solution

B. 2.5 c.c. of stock emulsion + 5.2625 c.c. of NaCl-solution + 0.2375 c.c. absolute alcohol.

C. 2.5 c.c. of stock emulsion + 4.8125 c.c. of NaCl-solution + 0.6875 c.c. absolute alcohol.

Syphilitic r. 0.05 c.c. Emulsion 0.6 c.	Amounts of Guinea-pig's Complement..					
	0.06 c.c.	0.09 c.c.	0.13 c.c.	0.17 c.c.	0.22 c.c.	0.3 c.c.
ulsion A	First trace	Trace	Distinct	Very Marked	Complete	Complete
ulsion B	0	Very Faint Trace	Faint Trace	Trace	Very Marked	Complete
ulsion C	0	0	Very Faint Trace	Faint Trace	Trace	Very Marked

CONTROLS: Emulsions 0.6 c.c. + Complement 0.02 c.c. = Complete

Serum 0.05 c.c. + Salt Solution 0.6 c.c. + Complement 0.02 c.c. = Complete

Dose of Complement = 0.01 c.c.

Table 14.

Syphilitic Serum .05 c.c. + Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.				
	0.04 c.c.	0.07 c.c.	0.1 c.c.	0.12 c.c.	0.2 c.c.
Ox-liver lecithin	0	0	Faint Trace	Trace	Almost Complete
Ox-kidney lecithin	0	0	Faint Trace	Faint Trace	Faint Trace
Ox-heart lecithin	0	0	0	0	Faint Trace
Sheep-liver lecithin	0	0	Faint trace	Just Complete	Complete
Egg-yolk lecithin	0	Very Faint Trace	Very Marked	Complete	Complete

CONTROLS: All emulsions alone 0.6 c.c. + Complement 0.035 c.c. = complete.
Serum 0.05 c.c. + Salt Solution 0.6 c.c. + Complement 0.015 c.c. = Complete.

Dose of Complement = 0.005 c.c.

Table 15.

yphilitic Serum .05 c.c. + Turbid mulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.					
	0.02 c.c.	0.04 c.c.	0.07 c.c.	0.1 c.c.	0.14 c.c.	
x-liver lecithin	0	Very Faint Trace	Trace	Marked	Complete	
x-heart lecithin	0	0	0	0	Very Faint Trace	
	0.04 c.c.	0.08 c.c.	0.13 c.c.	0.18c.c.	0.26 c.c.	0.34 c.c
x-liver lecithin + Cholesterin 1 per cent	0	0	Very Faint Trace	Marked	Complete	
x-heart lecithin + Cholesterin 1 per cent	0	0	0	Very Faint Trace	Marked	Complete
Crude Extract (Ox-liver)	0	Trace	Marked	Just Complete		

CONTROLS: All emulsions alone 0.6 c.c. + Complement 0.025 c.c. = Complete.

Serum alone 0.05 c.c. + Salt Solution 0.6 c.c. + Complement 0.025 c.c. = Complete.

Dose of Complement = 0.008 c.c.

Table 16.

Emulsions in every case of maximum turbidity.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement			
	0.1 c.c.	0.14 c.c.	0.2 c.c.	0.32 c.c.
Ox-heart lecithin	0	0	Faint trace	
Ox-heart lecithin + Cholesterin 1 per cent	0	0	0	0
Ox-liver lecithin	Faint trace	Trace	Almost Complete	Complete
Ox-liver lecithin + Cholesterin 1 per cent	0	0	Faint Trace	Trace
Egg-yolk lecithin	Very Marked	Complete	Complete	Complete
Egg-yolk lecithin + Cholesterin 1 per cent	0	Distinct	Complete	Complete

CONTROLS: All emulsions 0.6 c.c. + 0.03 c.c. Complement = Complete.

Serum alone 0.05 c.c. + Salt Solution 0.6 c.c. + 0.015 c.c. Complement = Complete.

Dose of Complement = 0.005 c.c.

Table 17.

Turbid mulsions of .75 per cent ecithin 1 rt + NaCl-olution 7 arts	1 c.c. 5 per cent Ox-blood Suspension + Cobra Venom 1: 10,000 + Emulsion.					
	0.005 c.c.	0.01 c.c.	0.015 c.c.	0.02 c.c.	0.025 c.c.	0.03 c.c.
x-heart	0	0	Faint Trace	Trace	Distinct	Complete
x-liver	Faint Trace	Distinct	Marked	Complete	Complete	Complete
gg-yolk	0	Distinct	Marked	Complete	Complete	Complete

Table 18.

Turbid Emulsions of .75 per cent. Lecithin part + NaCl-solution 7 parts	0.25 c.c. 5 per cent. Ox-blood Suspension + Emulsion.		
	0.5. c.c.	0.75 c.c.	1.0 c.c.
Ox-heart	0	0	Faint Trace
Ox-liver	0	Faint Trace	Very Marked
Egg-yolk	Trace	Distinct	Complete

Table 19.

0.05 g Lecithin + 10.0 c.c. v. Hübl solution (mixture allowed to stand for 24 hours).

	Amount of $Na_2S_2O_3$ solution equivalent to unabsorbed Iodine	Amount of $Na_2S_2O_3$ solution equivalent to absorbed Iodine.
[O]x-liver No. 1	5.45 c.c.	17.15 c.c.
" " No. 2	18.2 "	4.4 "
[F]atty human liver	3.7 "	18.9 "
[E]g-yolk No. 1	18.1 "	4.5 "
" " No. 2	19.0 "	3.6 "
" " (No. 2 redissolved)	19.45 "	3.15 "
[O]x-heart	16.5 "	6.1 "

CONTROLS: 10.0 c.c. v. Hübl solution = 22.6 c.c. $Na_2S_2O_3$ solution.

20.0 c.c. standard $K_2Cr_2O_7$ solution = 16.5 c.c. $Na_2S_2O_3$ solution

Table 20.

Turbid Emulsions .6 c.c. + Syphilitic erum (55° C ½ hour) 0.05 c.c.	Amounts of Guinea-pig's Complement.				
	0.02 c.c.	0.03 c.c.	0.05 c.c.	0.08 c.c.	0.12 c.c.
x-liver lecithin No. 1	Distinct	Marked	Very Marked	Just Complete	Complete
" " No. 2	Trace	Distinct	Marked	Very Marked	Complete
atty human liver lecithin	0	0	0	0	Trace
gg-yolk lecithin No. 1	Complete	Complete	Complete	Complete	Complete
" " " No. 2	Just Complete	Complete	Complete	Complete	Complete
" " " (No. 2 redissolved)	Distinct	Marked	Almost Complete	Just Complete	Complete

CONTROLS: Emulsions alone 0.6 c.c. + 0.02 c.c. Complement = Complete.

Syphilitic Serum 0.05 c.c. + Salt solution 0.6 c.c. + 0.02 c.c.
Complement = Complete.

Dose of Complement = 0.004 c.c.

As the results of these experiments pointed to the fact that lecithin as prepared by a uniform method from different tissues possessed very different properties and as these differences might be due either to impurity in the lecithin as obtained by the method employed or to actual differences in the constitution of the products I decided to extend the range of observation and at the same time give attention to the character of the other substances which were present along with lecithin in the acetic ether and acetone precipitates. For the latter purpose it was found necessary to dispense with the use of sand for, although this agent facilitated extraction of material soluble in hot acetic ether and in alcohol, it obscured the nature of the substances which were not soluble in these reagents.

The procedure was, therefore, as follows:-

The evaporated extract was treated with small amounts of hot acetic ether until no further solution was obtained. The accumulated acetic ether extract was cooled in the ice chest and the precipitate which resulted removed by centrifugalising. The precipitate was treated with hot acetic ether. A fraction generally remained insoluble; this was removed by filtration and kept for further examination. The hot acetic ether solution was cooled and the precipitate again treated with hot acetic ether, any insoluble material being removed and kept. This treatment was repeated until the cold acetic ether solution was quite colourless and the precipitated material was completely soluble in hot acetic ether. The precipitate from this final solution was treated with ether. As a rule solution was not complete, a variable amount of white granular material being insoluble. This was removed by centrifugalising and the ether-soluble fraction precipitated by the addition of several volumes of acetone. This precipitate was allowed to settle and the supernatant fluid was decanted. In most cases the precipitate was found to be only partially soluble. The treatment with ether and acetone was repeated until the whole of the precipitate was soluble in ether when it was again treated with acetone. Finally the precipitate was rapidly washed with a small amount of absolute alcohol (to remove traces of acetone) and allowed to stand in contact with absolute alcohol for some hours. The material soluble in cold alcohol was removed by centrifugalising. This constituted the lecithin solution. The greater portion of the precipitate was found to be insoluble in cold alcohol. This fraction will be dealt with later.

In view also of Erlandsen's findings with ox heart

lecithin/

lecithin it became of importance to test the products obtained by extraction of tissues with ether, For such extraction the tissue must be dried and powdered as ether does not mix with water.

The drying of tissues for fat extraction has always been a matter of great difficulty. The methods which have been employed fall under these heads:-

(1) Drying by means of warm air.
(2) " " " " Alcohol.
(3) " " " " Anhydrous salts, e.g. sodium sulphate, calcium sulphate.

(1) This method, which is at once the most easy and the most rapid permits degenerative and putrefactive changes to occur unless the temperature to which the tissues are exposed is fairly high when it is most likely that the unsaturated acids undergo oxidation. In Erlandsen's experiments the heart muscle after being freed from fat and fibrous tissues was finely minced and exposed in front of an electric fan driving a current of slightly warmed air. The time taken for drying was said to be about 12 hours. It is difficult to see how complete drying of the material was effected by such means.

(2) The tissue is finely minced and treated with a large volume of alcohol. This is removed after some hours and fresh alcohol added. Four or five such extractions may be necessary before the bulk of the water is removed. As a large amount of phospholipine is removed by this method it is necessary to collect the alcoholic extracts, evaporate to dryness, and add the residue to the material obtained later by extraction with ether. The phospholipine ultimately obtained is thus extracted from a mixture of alcohol and ether extracts.

(3) Drying by means of anhydrous salts is in my experience exceedingly unsatisfactory and inconvenient. When large amounts of tissue are being dealt with the mass of material is most unwieldy. Complete dehydration generally does not result or it is difficult to secure proper penetration of the tissue by the salt.

In consideration of these difficulties I decided to try the effect of preliminary treatment of the tissue with formalin. Thin slices of fresh ox liver were placed in 10 per cent formalin, layers of cotton wool being used to separate the slices from each other in order to secure rapid and thorough fixation. After 48 hours the tissue was washed in water, dried between towels and minced. A moist, coarsely granular material was obtained which dried very rapidly on exposure in an oven to a temperature of 50°-60°C. A reduction in weight equivalent to 80 per cent of the original tissue was obtained in a few hours. The final drying was facilitated by again passing the tissue through a Universal mincer, a very fine dust-like powder being thus obtained.

Before proceeding to extract lecithin by means of ether from such dried tissue it was necessary in the first place to determine whether the formalin fixation and drying had altered the characters of the lecithin as obtained by extraction with alcohol. Fresh liver tissue and a sample of the same material after fixation in formalin and drying to constant weight were extracted with absolute alcohol for several days. The extracts were filtered off, evaporated to dryness, and lecithin prepared as described above. In process of preparation of the lecithins certain differences in the characters of the various extracts were noted. Thus the alcohol extract of dried tissue was much more readily evaporated than that from the fresh tissue (owing to the absence of water) and the residue obtained dissolved readily and almost completely in hot acetic ether whereas that from the fresh tissue extract was largely composed of insoluble inorganic salts and protein material from which residue the fats and phospholipines were difficult to remove by means of acetic ether.

The results obtained in the Wassermann reaction and in the test along with cobra venom showed (v. table 21) that there was practically no difference between the two lecithins. A number of other experiments of a similar nature were carried out with the same result.

A large amount of dried liver tissue was then prepared. This was divided up into several portions of equal weight. These were extracted for several days with equal amounts of

(1) Absolute Alcohol.
(2) Methylated Spirit.
(3) 75 per cent alcohol.
(4) 50 " " "
(5) Ether.

The extracts were then removed and fresh reagents added. This was repeated after an interval of two days. The accumulated extracts from each portion were then evaporated down and lecithin prepared. The characters of the products obtained from each extract are given in the following table:-

	Absolute Alcohol	Spirit	75 per cent alcohol	50 per cent alcohol	Ether
Colour of Extract	Dark Amber	Dark amber	Amber	Pale Straw	Yellowish brown
Result of treatment with hot acetic ether	Completely soluble	Almost completely soluble	Considerable residue insoluble(this residue insoluble in alcohol soluble in water	Practically insoluble residue insoluble in alcohol but soluble in water	Almost completely soluble
Acetic ether precipitate	Abundant, Yellow flocculent	Abundant, Yellow viscid	Abundant, Yellow, viscid	Mere trace	Small, white and granular
Treatment with 1st Ether	Mere trace insoluble	Mere trace, insoluble	Abundant granular residue insoluble; this residue soluble in water	---	Very considerable residue insoluble.
Solubility of 1st Acetone precipitate in ether	Complete	Partial, white, granular residue	Partial, white, granular residue	---	Partial, considerable residue insoluble
Solubility of 2nd Acetone precipitate	Partial, small insoluble residue	Partial, considerable insoluble residue	Partial, considerable insoluble residue	---	Partial, distinct insoluble residue
Solubility of 3rd Acetone precipitate	Complete	Complete	Complete	---	Complete
Colour of Lecithin	Yellow	Dark Yellow	Dark Yellow	---	Clear, colourless

It will be seen that the products from the extracts containing water have somewhat different characters from the extract with absolute alcohol. The differences are, however, only due to the differences in amounts of certain 'impurities'. It will be noted that the yield of acetone precipitate from the 50 per cent alcohol extract was so small that it was not proceeded with further. In the case of/

of the ether extract the lecithin in addition to being quite colourless was exceedingly small in amount in spite of the fact that the original extract was very highly coloured and contained a comparatively large amount of extracted material. This experiment has been repeated a number of times with liver and other tissues, the result being practically the same. In every case the yield of lecithin from the ether extracts was very small as compared with that from the alcohol and spirit extracts.

It was found in general that a greater amount of lecithin was obtained from the spirit extracts and from alcoholic extracts containing about 15 per cent of water than from the absolute alcohol extracts but some variation was found according to the nature of the tissue under examination but in some cases the difference was not marked. The following experiment will serve as a typical example of these examinations. An amount of dried finely powdered ox heart weighing 350 grms. was divided into 7 portions of 50 grms. each and to these respectively were added 400 c.c's of

(1) Absolute Alcohol

(2) 80 per cent alcohol

(3) 60 per cent "

(4) Mineralised Spirit

(5) Industrial "

(6) Ether

(7) Boiling absolute alcohol (This mixture kept boiling for 12 hours under a reflux condenser).

Extraction was allowed to proceed for 5 days. The Extracts were then filtered, the amount of each extract was measured and the strength of the solution estimated by evaporating a known volume.

Extracts/

Extracts in order of Depth of Colour.	Amounts obtained	Percentage of solid material	Total solids.
(1) Boiling alcohol	240 c.c's	1.48	3.55 grms.
(2) Mineralised Spirit	255 c.c's	0.86	2.23 grms.
(3) Industrial Spirit	250 c.c's	0.85	2.04 grms.
(4) 80 per cent alcohol	250 c.c's	0.86	1.97 grms.
(5) Absolute alcohol	250 c.c's	1.11	2.66 grms.
(6) 60 per cent alcohol	235 c.c's	0.68	1.6 grms.
(7) Ether	225 c.c's	1.66	3.73 grms.

	Amounts of Lecithin obtained	Colour of Lecithin.
(1) Absolute alcohol	0.33 grms.	Pale amber
(2) 80 per cent alcohol	0.35 "	Amber
(3) 60 per cent "	Negligible	-
(4) Boiling "	0.37 grms.	Amber
(5) Industrial Spirit	0.57 "	Amber
(6) Mineral Spirit	0.68 "	Amber
(7) Ether	0.04 "	Colour-less.

Of the above preparations the only one which corresponds to Erlandsen's lecithin is that from the extract with ether. It is interesting that a substance which is much more soluble in ether than in alcohol should be removed in only comparatively small amounts by extraction with ether. Further, tissues thoroughly extracted with ether yield considerable amounts of lecithin on further extraction with alcohol.

In the Wassermann reaction very marked differences have been elicited between 'ether extracted' and 'alcohol extracted' lecithins. Thus the ether extracted lecithins had generally a distinct anti-complementary effect which was so much increased by the addition of cholesterin that these lecithins were quite unsuitable reagents for the diagnosis of syphilitic sera (v. table 22 a&b). In addition

a/

a positive Wassermann reaction was usually given with sera, which, tested at the same time with 'alcohol extracted' lecithins, were quite negative.

As haemolysins along with cobra venom the ether lecithins were very inefficient, differing markedly in this respect from the preparations obtained from alcohol extracts (v. table 22c,23a,24). This result is of great interest as considerable controversy has taken place regarding the nature of the product which acts along with cobra venom in the production of haemolysis. The haemolytic effect of 'ether extracted' lecithins for unsensitised corpuscles was practically the same as that of 'alcohol extracted' lecithins (v. table 23b).

A number of human tissues in a condition of fatty degeneration (human liver and kidney from a case of peritonitis, liver and kidney from a case of pernicious anaemia) have also been examined. The amounts of lecithin obtained by means of alcohol were much smaller than those from normal human tissues but in the various biological tests no differences were found. In the case of ether extracts, however, the lecithin solutions were much less anticomplementary than the ether extracts of normal tissues and were more efficient along with cobra venom; the iodine values were also higher than the values of the corresponding 'ether extracted' lecithins of normal tissues (v. table 24,25,26).

It is probable that the further examination of tissues in various pathological conditions will yield interesting results. Owing to the unsatisfactory state of our present knowledge regarding lecithin itself I have not further pursued this line of investigation as it seemed of more importance to confine attention to the problems connected with the chemistry of lecithin from normal tissues.

The lecithins extracted from dried tissue by methylated spirit, mineralised spirit, 10 per cent, 15 per cent/

cent and 20 per cent alcohol have not differed materially from that obtained with absolute alcohol. A comparison of the action of some of these in the Wassermann reaction and in the test with cobra venom is given in tables 21 to 24. In a few cases the lecithins from watery alcohol mixtures have been slightly more haemolytic by themselves for ox's corpuscles than the corresponding lecithin extracted by means of absolute alcohol. As the preparation of pure lecithin involves the use of large amounts of costly reagents (acetic ether, ether, acetone) in addition to the large volumes of absolute alcohol generally used for the extraction, I have given particular attention to the lecithins extracted by means of methylated spirit since the use of this reagent for purposes of extraction in place of alcohol would very considerably diminish the cost of production. I have convinced myself that absolute alcohol can for all practical purposes be replaced by methylated spirit, either industrial or mineralised. In the experiments which follow it will be understood, unless where it is stated to be otherwise, that the extracting agent used is industrial methylated spirit.

Amounts of Lecithin in Different Tissues.

Attention has already been drawn to the imperfect nature of our knowledge regarding the distribution of lecithin in different organs. I have therefore made an examination of a variety of tissues for the presence of lecithin and have compared the action of these in the various biological tests. An attempt has also been made to measure the amount of lecithin present. For the latter purpose it was found necessary to make repeated extractions over fairly long periods as a fraction only of the extractable lecithin was removed by one or two treatments with spirit. In the majority of cases ordinary minced (or 'wet') tissue has been used and the amount of lecithin in each extract has been measured. In a number of cases from eight to ten extractions have been necessary in order to remove the last traces.

The following tissues have been examined, the extraction of each proceeding simultaneously, ox's heart, liver, kidney, pancreas, thyroid, thymus, submaxillary glaud, spleen, testicles, lungs and red blood corpuscles, sheep's liver, pancreas, blood corpuscles and serum, human brain, kidney, liver and heart. Certain of these tissues have also been dried after fixation in formalin and comparisons made of the properties of the lecithins extracted by ether and spirit respectively.

Considerable differences were found in the Lecithin content of the different tissues, as shown by the following table.

	Amount of Lecithin per 100 grms. of 'wet' tissue.
Ox Heart	0.36 grms.
" "	0.35 grms.
Sheep's Liver	1.6 "
Ox Kidney	0.48 "
Spleen	0.14 "
Lungs	0.40 "
Testicles	0.62 "
Thyroid	0.3 "
Pancreas	0.68 "
Submaxillary	0.3 "
Human Brain	0.5 "

The details of this experiment were:-

Ox Heart 1015 grms (wet tissue)

Extract No.	1.	1.07 grms
" "	2.	1.02 "
" "	3.	1.06 "
" "	4.	0.28 "
" "	5.	0.19 "
" "	6.	0.095 "
Total		3.715 grms.

Human Brain (dried) 214 grms.

Extract No.	1.	2.38 grms.
" "	2.	0.1 "
" "	3.	0.53 "
" "	4.	0.137 "
" "	5.	0.354 "
" "	6.	0.013 "
Total		3.514 grms.

Ox Heart 1350 grms (wet tissue)

Extract No.	1.	0.204 grms.
" "	2.	2.14 "
" "	3.	1.325 "
" "	4.	0.712 "
" "	5.	0.186 "
" "	6.	0.163 "
" "	7.	0.063 "
Total		4.793 grms.

Brain Fresh 1230 grms.

Extract No.	1.	-
" "	2.	0.48 grms.
" "	3.	0.56 "
" "	4.	5.1 "
" "	5.	0.15 "
Total		6.29 Grms.

Liver (Sheep) 460 grms (wet tissue).

Extract No.	1.	3.480 grms.
" No.	2.	1.890 "
" No.	3.	1.240 "
" No.	4.	0.408 "
" No.	5.	0.100 "
" No.	6.	0.035 "
" No.	7.	0.045 "
" No.	8.	0.002 "
Total		7.400 grms.

Ox Kidney (wet) 460 grms.

Extract No.	1.	0.0 grms.
" "	2.	1.07 "
" "	3.	0.0 "
" "	4.	0.845 "
" "	5.	0.304 "
Total		2.219 grms.

Dried Ox Liver 105 grms = 500 grms. (wet tissue)

Extract No.	1.	3.44 grms.
" No.	2.	0.4 "
" No.	3.	0.1 "
" No.	4.	0.014 "
" No.	5.	0.067 "
" No.	6.	0.01 "
Total		4.031 grms.

Ox Spleen, 800 grms.

Extract No.	1.	0.28 grms.
" "	2.	0.265 "
" "	3.	0.216 "
" "	4.	0.3654 "
" "	5.	0.06 "
Total		1.1864 grms.

Lungs 900 grms (wet tissue)

Extract No.	1.	0.62 grms.
" "	2.	1.37 "
" "	3.	1.0 "
" "	4.	0.54 "
" "	5.	0.134 "
Total		3.664 grms.

Testicles 755 grms. (wet tissue)

Extract No.	1.	0.25 grms.
" "	2.	1.0 "
" "	3.	2.57 "
" "	4.	0.87 "
Total		4.69 "

Thyroid 315 grms. (wet tissue)

Extract No.	1.	0.17 grms.
" "	2.	0.59 "
" "	3.	0.19 "
" "	4.	0.006 "
Total		0.956 "

Submaxillary, 95 grms.

Extract No.	1.	- grms.
" "	2.	0.25 "
" "	3.	0.035 "
" "	4.	0.01 "
" "	5.	-
Total		0.295 grms.

It will be seen that of these tissues the organ which contained most lecithin was the liver. This has been generally found. The only tissue which contains more lecithin than liver is egg yolk. The pancreas and testicles of the ox have a comparatively high lecithin content. Lecithin in considerable amount was also found in brain tissue. I have examined a number of brains and have in each instance found lecithin to be present. This agrees with the observations of Thudichum but is opposed to those of Fränkel. I have also found lecithin in ether extracts of dried brain. From one human brain weighing 1230 grammes, 6.3 grms. of lecithin were obtained by extraction with spirit. In the case of a brain which had been kept in formalin for about 15 years and which after drying weighed 214 grms. the amount of lecithin removed by extraction with spirit was over 3.5 grms. This lecithin possessed unusual properties which will be referred to later.

Owing to the importance which has of late years been given to the presence of lipoid material in the blood/

blood I have made several examinations of red blood corpuscles and of serum. In one experiment 330 c.c's. of the red blood corpuscles of the sheep, freed from serum by centrifugalising, were treated with 500 c.c's. of spirit for 3 days. The extract was yellow in colour and had a trace of smoky appearance but no bands were present on examination with the spectroscope. The residue obtained on evaporation was small and a fraction only was soluble in hot acetic ether. The precipitate which resulted on cooling the hot solution was not completely soluble in a fresh portion of hot acetic ether. The insoluble fraction was removed and the precipitate obtained on cooling the soluble fraction was treated with ether. It was found that complete solution did not result, there being a greyish-white insoluble residue. The solution was treated with acetone. A small amount of flocculent precipitate formed which was almost insoluble in cold ether. Solution was effected by gentle warming and acetone again added. From the precipitate thus obtained 0.32 grms. of lecithin was obtained. Several further extractions of the original tissue yielded only 0.08 grms. more of lecithin - that is to say, the total amount of lecithin in 330 c.c!s of red blood corpuscles was 0.4 grms. As 1 c.c. of corpuscles weighs about 1 gramme this corresponds to 0.12 grm. of lecithin per 100 grms. of corpuscles, ~~this~~ an amount ~~being~~ less than that from any of the above tissues. In another experiment 660 grms. of corpuscles free of serum were treated with 10 per cent formalin. Haemolysis occurred and the mixture set to a firm jelly. This was minced and thoroughly dried, a somewhat coarse, black powder weighing 160 grms. being obtained. The powdered material was divided into two equal portions of which the one was treated with 500 c.c's of spirit,

the/

the other with 500 c.c's of ether. From the spirit extract only 0.05 grms. of lecithin was separated, from the ether extract only a trace of acetone precipitate was obtained which was not proceeded with further. The amount of lecithin from the 'wet' corpuscles was thus about 8 times that obtained from the corresponding amount of corpuscles dried. Similar differences in the amounts of lecithin from 'wet' and dried tissues have occurred with brain and kidney. These differences are apparently due to want of penetration of the dried tissue by the spirit. In the case of wet tissue the amount of water which is present probably secured much more efficient penetration and extraction. It was noted that the dried material from the blood corpuscles and the brain was very hard and difficult to reduce to fine powder. For the thorough extraction of dry tissue it is most important that the material should be very finely divided. The amount of lecithin extracted from dried serum was also very small. Four hundred and fifty grammes of sheep's serum were dried to constant weight, a brownish powder weighing 40 grms. resulting. This was treated with 200 c.c's of spirit for two days when the extract was so well coloured that it was withdrawn and fresh spirit added. The extract contained 1.6 grms. of solid matter but no lecithin was found to be present. The amount of colouring matter in the serum was considerable; repeated extractions of the dried serum with spirit did not cause appreciable diminution in the depth of colour of the various extracts. The pigment was soluble in the cold acetic ether fraction.

The amounts of lecithin obtained from each extract of a series of extractions of the same sample of tissue varies. If the tissue is dry the 1st extract generally contains/

contains the bulk of the lecithin. With wet tissue the 1st extract frequently contains no material insoluble in cold acetic ether; in other cases small amounts of lecithin are obtained. The amount of lecithin removed is apparently dependent on the relative amounts of spirit and water present. If a small volume of spirit is added to a large amount of 'wet' tissue no lecithin will be obtained and a second extract will probably contain only a small amount. It is possible in this way by using small volumes of extract to remove a fair amount of water without extracting much fatty material. As already noted this procedure has been used for the drying of tissues, preliminary to extracting with ether. Where relatively large amounts (4-6 times the amount by volume of the tissue) are added from the first, the bulk of the lecithin will be found in the first two extracts. This is shown in the above tables in the case of the liver of the sheep where 3.68 grms. of the total 7.4 grms. were found in the first extract and 1.89 grms. in the second. With the ox heart (1015 grms.) on the other hand, the first three extracts each contained about the same amount of lecithin whereas with the ox heart weighing 1550 grms. the first extract contained very little owing to the bulk of fluid being relatively small. In the case of the fresh human brain which was treated with small amounts of spirit the first three extractions removed only 1 grm. of lecithin whereas the fourth extract contained 5.1 grms.

Numerous extractions were necessary in order to remove the last traces of lecithin. In Erlandsen's experiments several months were occupied in obtaining the last traces of lecithin by means of ether from ox heart. Prolonged shaking would probably shorten

this/

this period. In my experience only the first two or three ether extracts contain an appreciable amount of phospholipine. This is generally the case with dried tissues. It is advisable to remove the earlier extracts at short intervals (from three to four days) and to give longer periods for the later extracts. In the experiment detailed above the more bulky tissues were extracted over a period of nearly three months. As a result of repeated extraction with alcohol the tissues lose markedly in weight. Thus 460 grms. of liver after eight extractions weighed 79 grms. and 1015 grms. of ox heart became 150 grms. after 6 extractions. In estimating the amount of lecithin in any large amount of tissue it is not advisable to accumulate a number of extracts before proceeding to the further preparation of lecithin. The manipulation of large quantities of acetic ether and acetone precipitates is comparatively easy but it is exceedingly difficult to extract lecithin from a bulky acetone precipitate unless the method of triturating with sand or other inert material be adopted. On the addition of alcohol the particles of precipitate tend to cohere and shaking produces rounded masses which adhere to the glass and to each other. From these masses lecithin is not removed even after prolonged contact with cold alcohol. Gentle warming is frequently of service especially where the particles are not firmly glued to each other. When sand is used the final precipitation of acetone must be performed in a mortar or in a flat dish so that access may be had to the precipitate. If an acetone precipitate is boiled with alcohol, solution of a much larger amount of precipitate occurs. On cooling the hot alcoholic solution a considerable amount of material separates out. (This fraction will be dealt with later, also the residue insoluble in boiling alcohol).

Action of the Lecithins from the same and from different Tissues.

Anticomplementary Effects. The lecithins have all been tested in the form of slowly made emulsions. In the majority of cases the effect of the addition of 1 per cent cholesterin has also been investigated. Considerable differences have been found. Thus in table 28 the lecithins obtained from the first and second extracts of ox heart give just complete lysis with 0.025 c.c. of complement, but the preparations from the third and fourth extract of the same tissue only gave complete lysis with 0.075 c.c. complement. In the presence of cholesterin the lecithins from the first, second, and fourth extracts show complete lysis with 0.1 c.c. complement while the lecithin from the third extract is very anticomplementary there being only a trace of lysis with 0.1 c.c. of complement. Again, the lecithin from sheep's red corpuscles shows by itself little anticomplementary effect whereas in the presence of cholesterin only a trace of lysis occurs with 0.1 c.c. of complement; a similar result was got with the lecithin out of dried corpuscles. The lecithin from dried brain, on the other hand, is not altered as regards this action by the addition of cholesterin. The lecithin which was obtained from the acetone precipitate of the second extract by boiling this precipitate with alcohol and cooling the solution is also seen to be very anticomplementary in the presence of cholesterin, differing in this respect from the lecithin obtained by the usual method. In table 29 further results of this sort are shown. Attention is particularly drawn to the fact that, although the anticomplementary effects of the lecithins from the third and fourth extract/

extract of ox heart are the same, the presence of cholesterin brings out very considerable differences while in the case of the lecithin from the sixth extract the addition of cholesterin produces a very anticomplementary mixture. Further, the lecithin from the first extract of ox spleen both alone and in the presence of cholesterin does not deviate much complement but the corresponding lecithin from the second extract shows very marked anticomplementary properties on the addition of cholesterin. These results have been repeatedly obtained. Considerable variation however may occur with individual specimens of complement and it is not possible to compare the results of two experiments at different times using different complements. For example the action of the lecithin from the fourth extract of 460 grms. of ox liver is shown in both the tables just referred to. With the one complement this lecithin, with cholesterin added, is very anticomplementary whereas with the other complement no such effect is shown. In order therefore to bring out differences between a number of specimens of lecithin it is necessary to test them all at the same time with the same specimen of complement. I have therefore endeavoured to test as many preparations as possible at the same time. It is obvious from these experiments that any specimen of lecithin chosen at random is not necessarily a suitable reagent for use in the diagnosis of syphilitic serum. Only those preparations can be used which have been tested with a number of different complements and found to have uniformly very little anticomplementary effect especially after the addition of cholesterin. It is a somewhat striking fact that the lecithins prepared from successive extracts of the same specimen

of/

of tissue do not give the same results. In this connection it is perhaps important to remember that the results given are these obtained with lecithins from "wet" tissue and that the different degrees of dehydration of the tissue resulting from repeated extraction with spirit may affect the nature of the products removed by each extract.

Reactions with Cobra Venom.

As regards the lecithin from ox heart, ox liver and egg yolk the results of the previous examination have in the main been substantiated, that is to say, lecithins from ox heart were slightly less active with venom than were ox liver lecithins while the lecithins from egg yolk were still more active. In table 30 a comparison of the results obtained with the lecithins from the first and second extracts of ox heart and ox liver respectively are given. Both the liver lecithins were a little more active than the corresponding heart extracts. In the same table the action of a lecithin from a sample of the same liver after drying is also shown. It is distinctly less active than the corresponding lecithins from "wet" liver although by itself it is more lytic. This result has been obtained not infrequently. Along with cobra venom the lecithins obtained from ox liver would appear to be much more constant in action than those from ox heart. This is shown in table 30 where the action of the lecithins from the first three extracts of a specimen of ox liver is given. In each case haemolysis is complete in the corresponding tubes. In table 31 the result with lecithins from corresponding extracts of ox heart is given. The lecithins from the first extract is very much less active than the others. This difference in the actions of these lecithins/

lecithins (prepared from the same sample of ox heart) has been shown to persist over a period of some months. Further, there is some evidence that the lecithins from later extracts are more active that those from earlier extracts and that the later heart lecithins may ever be more lytic along with venom than an active liver lecithin. A very interesting result is that shown with the lecithins obtained from the red corpuscles of the sheep,(v.tables 31 & 32). These were always found to be very inactive along with venom. The same result was obtained from lecithin from ox's corpuscles,(v table 33). These results do not support the theory that haemolysis of corpuscles in the presence of venom is due to the combination of the venom with the lecithin of the corpuscles. The fact that lecithin is not the only lipoid which is capable of forming a haemolysin along with venom, as I have been able to show,is important in this connection. While in the great majority of cases the ratio of the haemolytic activity in the presence of venom to the haemolytic power in the absence of venom was from about 150:1 to 200:1, yet in a considerable number of cases the ratio was much smaller. This was for the most part due to the lecithin being more actively lytic than was usual in the absence of venom. In table 34, for example, the ratio in the case of the lecithin obtained by boiling the acetone precipitate with alcohol and cooling the solution is only about 50:1 whereas with most of the other lecithins the ratio is about 200;1. In some cases ratios of 300;1 and 400:1 have been obtained. The lecithin already referred to which was extracted from a dried human brain which had been kept in formalin for some years was so actively lytic in the absence of venom that the addition of venom only increased the haemolytic power/

(v. table 32)

power by about three times. This also occurred with the lecithins obtained from succeeding extracts. A somewhat similar result occurred with a specimen of lecithin from dried blood corpuscles. The results with these lecithins are shown in table 35 .

Lytic Effects.

Except for the lecithin just mentioned none of the preparations have shown by themselves marked haemolytic action. Variation does occur within certain limits. For example in table 31. the lecithin from the first extract of ox heart shows very marked lysis with 0.5 c.c. of emulsion while the third extract shows no lysis in the corresponding tube. Again, the liver lecithin (460 grms. fourth extract) is more lytic than usual since haemolysis is almost complete with 0.5 c.c.. Such a lecithin would not be suitable for use in the diagnosis of syphilitic sera.

Iodine Values.

These have been tested for the most part by a modified von Hübl method in which sodium thiosulphate was replaced by antipyrine. In the coarse of the inquiry into the iodine values of heart, liver and egg yolk by means of the ordinary von Hübl test difficulties were encountered which by this method seemed insurmountable. In the first place the use of chloroform as a solvent for the lecithins necessitated the evaporation of the alcoholic solutions of lecithins to dryness, a procedure which I thought should be avoided as far as possible as the iodine value was probably altered in the process. Difficulties also occurred with certain lecithins from the formation of precipitates or dense emulsions during the process of titration. In addition, the titre of the thiosulphate solution varied considerably from time to time and constant controls with/

with potassium bichromate were necessary. Under the circumstances I decided to try the method of Borde. This depends on the fact that free iodine in the presence of mercuric chloride solution can be accurately titrated by means of antipyrine solutions. The advantage of this method lies in the fact that the determination can be carried out in alcoholic solutions. The formation of layers as occurs with sodium thiosulphate is thus avoided.

The procedure was as follows:-

A 5 per cent solution of Iodine in 95 per cent alcohol was carefully prepared, also a 6 per cent solution of mercuric chloride in 80 per cent alcohol. 10 c.cs of each of these were carefully measured out and mixed in a clean glass bottle. To this mixture 0.1 grms of the lecithin was added. The mixture was shaken and allowed to stand in the dark for about 18 hours when a solution of antipyrine (18.8 grms. in 1000 c.c.s of 50 per cent alcohol) was added from a burette till the mixture was just colourless. A control test without the addition of lecithin was examined in the same way. The difference between the amounts of antipyrine added in each case gave the amount of antipyrine equivalent to the amount of iodine which had been absorbed by 0.1 grms. of lecithin. A number of careful tests were made to determine the amount of iodine equivalent to the antipyrine solution: comparisons were also made of the results obtained with a number of lecithins using the antipyrine and ordinary von Hübl methods. It will be seen in table 36 that the results were practically the same. It was found that the titre of the antipyrine solution did not vary to any appreciable extent and that the five per cent iodine solution altered only very slightly

on/

on standing. As a control experiment was made in every case this alteration was of little importance. By the use of antipyrine the process of titration was much simplified and it was possible to test the lecithins in the form of their solutions in alcohol without the production of precipitates or densely turbid mixtures which in the case of titration with sodium thiosulphate interfered with the exact determination of the end point.

Inspection of tables 37 - 41 will show that the iodine values of the different lecithins have differed very markedly. In no case, however, has a saturated product been found. In a number of instances very high iodine values were obtained; this is shown in table 38 (ox heart sixth extract). In other cases the values were low (ox pancreas and ox spleen). In a large number of the earlier estimations of iodine values no special attention was paid to the age of the lecithins under examination. It was, however, found that considerable differences existed even in the iodine values of lecithins extracted from the same sample of tissue. This result was somewhat unexpected. In order to make the results more comparable each lecithin was tested within a few hours of isolation from the precipitate with acetone. The iodine values of about 20 different lecithins were tested in this way. (v. table 36) The values still differed. In the case of lecithins from the same tissue it was found that in general the iodine value increased with the number of the extract, that is to say, the iodine values from the earlier extracts were lower than those of the lecithins obtained from the later extracts. This is shown in tables 37 & 38 . In order to test this point more fully a special experiment was arranged. "Wet" liver/

liver tissue was treated with a known volume of spirit for a period of two days. The fluid was then removed, fresh spirit added and this mixture also allowed to stand for two days. Other two extracts were made in similar fashion. The earlier extracts were kept in the dark until the last extract had been obtained when they were all separately evaporated, a sample of each being retained for examination. Lecithin was prepared from each extract, close attention being paid throughout to the uniformity of the method. The initial extracts and the lecithins were tested for (1) iodine values, (2) action along with venom and (3) degree of turbidity when mixed with salt solution. The results are given below.

Solutions 0.75 per cent: emulsions made 1 part in 8 parts of 0.85 per cent NaCl solution.	0.5 c.c. Ox Blood Suspension + Cobra Venom + Emulsion.					0.5 c.c. Ox Blood Suspension + Emulsion.			
	0.005 c.c.	0.01 c.c.	0.015 c.c.	0.025 c.c.	0.05 c.c.	0.025 c.c.	0.075 c.c.	0.1c.c.	0.2 c.c.
Lecithin from old dried brain	0	Trace	Complete	Complete	Complete	Distinct	Complete	Complete	Complete
Lecithin from dried Blood Corpuscles	0	0	Distinct	Very Marked	Complete	0	Distinct	Marked	Complete

The iodine values of the extracts and of the lecithins were as follows:-

	Iodine Values.
1st Extract	20
2nd do.	80
3rd do.	96
4th do.	137
1st Lecithin	122
2nd do.	118
3rd do.	139
4th do.	175

The successive extracts were, therefore, increasingly unsaturated. The lecithins showed the same phenomenon except that the iodine values of the lecithins from the first and second extracts were for practical purposes the same. As haemolysins along with cobra venom the earlier extracts were more active than the later ones while the lecithins were practically equal in activity (v. table)

	0.5 c.c.	Ox Blood Corpuscles + Cobra Venom + Emulsion			Corpuscles + Emulsion
Extract	0.3 c.c.	0.6 c.c.	0.9 c.c.	1.5 c.c.	2.0 c.c.
No. 1	0	Marked	Almost Complete	Complete	0
" 2.	0	Marked	Just Complete	Complete	0
" 3.	0	0	0	0	0
" 4.	0	0	0	0	0

In the form of slowly made emulsions the extracts showed increasing turbidity with the rise in iodine value. When these emulsions were allowed to stand over night other differences were found. These are shown in the table.-

	Turbidity of Emulsion.	After standing over night
Extract No. 1.	Trace	Clear fluid, separation of flocculi
Extract No. 2.	Distinct	Do. Do.
Extract No. 3.	Marked	Turbid fluid, very slight separation of flocculi
Extract No. 4	Very Marked	Turbid fluid, no separation

As it was possible that the increased iodine value of the lecithins from the late extracts was only apparent and in reality due to a fall in the value of the lecithins from the early extracts during the interval of/

of waiting this point was investigated. The original tissue was again treated with spirit and the fluid removed after an interval of two days and allowed to stand in the dark for two weeks. The iodine values of the extract and of the lecithin were still high. The results with other lecithins obtained by successive extraction of the same tissue were also against this view. The iodine values of the lecithins from the early extracts tested immediately after preparation were almost always lower than those from later extracts.

A possible explanation may be that the amount of water in the tissue under extraction affects the nature of the products removed by the alcohol. It is not unlikely that the more unsaturated acids, which do not mix with water, are not removed until the tissue is almost dehydrated and that the presence of these in the extracts causes the increased, and greater permanency of, turbidity which results on mixing the extracts with salt solution. The differences in the iodine values of the extracts may of course be due to differences in the amounts and not to differences in character of the fatty material extracted. This, however, does not apply to the lecithins if these are pure substances. The results of an experiment in which dried ox heart tissue was extracted with various agents, may be mentioned in this connection.

50 grms. of Dried Ox Heart extracted with	Iodine value of extract.
(1) Absolute alcohol	54
(2) Boiling "	54
(3) 80 per cent "	54
(4) 60 per cent "	33
(5) Spirit	48
Lecithins from above extracts	**Iodine values**
(1) Absolute alcohol	133
(2) Boiling "	152
(3) 80 per cent"	162
(4) ---	---
(5) Spirit	126

While the spirit and 60 per cent alcohol extracts have lower iodine values than the other extracts the 80 per cent is the same as those free from water and the lecithin from the 80 per cent alcohol extract is higher than any of the others. These results do not give much support to the view that the amount of water in the extract is the principal factor in determining the nature of the unsaturated products removed but the conditions of the two experiments are not strictly comparable.

Attention must be drawn to the relatively very high iodine values which were obtained with the lecithins in the above experiment with wet tissue and in a number of other cases. In much of the earlier work it was not considered necessary to examine the iodine values of the lecithins on the day of preparation. In order to make better comparisons it was the practice to prepare as large a number of preparations as possible about the one time in order that these should be tested all together. In view of my later findings such an arrangement did not give a proper conception of the real iodine values. The latter must be tested as soon as is possible after the preparation of the lecithin since a fall in the value would appear to occur very rapidly in many cases. This is shown below where the iodine values of the lecithins on the day of preparation, five days later and four weeks later are given.

	Iodine Value on the day of preparation.	Iodine value five days later	Iodine value four weeks later.
Liver lecithin No. 1.	122	86	78
" " " 2.	118	83	83
" " " 3.	139	112	79
" " " 4.	175	152	83

These results along with observations made in the course of other iodine value estimations would suggest that where the iodine value is high, immediately after preparation, a fall very rapidly occurs to a value which remains constant for a considerable period (several months). In the case of lecithins tested some time after preparation the iodine value most frequently found lies, in the case of liver lecithins, between 70 and 80, in the case of yolk lecithins, between 50 and 65 (v. tables 40a and 40b).

It is interesting to compare the iodine values which I have obtained with those of the lecithins of other workers. Erlandsen's heart muscle lecithin had a value of 100.5, Baskoff's liver lecithin 63 and Thierfelder and Stern's egg lecithin 48.7. It is probable that these iodine values do no more than express the degree of unsaturation of the lecithin at the time of testing and after the products had been dried. It must be remembered too that in my experiments the lecithins have been prepared from extracts which have been evaporated down in open vessels at moderate temperatures, a procedure which is not regarded by Leathes as likely to yield lecithins with high iodine values.

The iodine values of commercial lecithins were found to be very low (table 40 a). Commercially prepared lecithins would appear to be very far from pure. From the preparations which I have examined it seems to be the practice in some cases to regard the whole acetone precipitate from extracts of egg yolk as lecithin.

Degree of Turbidity along with salt solution.

The lecithins have also differed as regards the degree of turbidity which was produced on emulsifying them with salt solution and also as regards the physical state of the emulsions which resulted from the introduction of cholesterin into the lecithin solutions and slow mixture with salt solution.

Care was taken to make the emulsions as uniform as possible. In the case of the lecithin-cholesterin solutions used the amount of cholesterin added to the lecithin was 1 per cent in all cases.

Comparisons of the results with a series of lecithins are given in tables 42 - 43 .

It will be seen that the turbidities produced by the lecithins from the same sample and from different samples of tissue differ very markedly and that the amount of separation of cholesterin from the lecithin-cholesterin emulsions bears no relationship either to the turbidity of the lecithin emulsions or to the iodine values of the lecithins.

As a result of the differences in different preparations of lecithin which the foregoing experiments had elicited, it seemed of interest to attempt to alter the characters of a lecithin; a few experiments in this connection have recently been made.

Effect of Heating Alcoholic solutions of Lecithin.

An alcoholic solution of lecithin (from dried liver of the sheep) was placed in a flask fitted with a reflux condenser and kept at or near the boiling point for 24 hours. The only visible change was a marked deepening of the colour. The iodine values of a sample of the same lecithin unheated and of the heated lecithin ~~untested~~ were:-

Iodine value (unheated sample) 40.
" " (heated sample) 50.

This result was somewhat unexpected as a fall in value was thought to be the most probable result of such treatment. A rise in the iodine value is difficult to explain unless it is supposed that slight hydrolysis of the lecithin occurred.

In another experiment an ox liver lecithin solution, 9.46 per cent, was heated on a warm plate for some hours and slowly evaporated to dryness. It was found to be still soluble in alcohol. The alcoholic solution which was very much deeper in colour than the original solution was precipitated by the addition of acetone. The supernatant fluid was evaporated to dryness, the residue taken up in ether and acetone added. A further precipitate occurred. This was redissolved in ether and precipitated again by acetone. Two precipitates were thus obtained:-

(1) Precipitate by addition of acetone to solution in alcohol.

(2) Precipitate by addition of acetone to the ether solution/

solution of the fraction not precipitated by addition of acetone to solution in alcohol.

1. This first precipitate was found to be not completely soluble in ether, a small amount of white material being insoluble. The latter was removed and the highly coloured ether solution precipitated by the addition of alcohol. A trace of precipitate was obtained; this was removed and the alcohol ether mixture evaporated down, the residue was taken up in ether and precipitated by the addition of acetone. The precipitate was shaken with cold alcohol; even on standing for a prolonged period complete solution did not result. The alcohol soluble part was removed (Product A).
2. This precipitate was treated with cold alcohol and found to dissolve readily and completely (Product B). The iodine value of product A was 88, of product B 112. Thus from a lecithin originally completely soluble in ether and alcohol several products were obtained with apparently different properties. It was found, however, that a sample of the same lecithin not heated gave somewhat similar products. The iodine values of the two alcohol soluble products corresponding to those tested above were 88 and 103 respectively. The only alteration therefore which the heating had produced was a rise in the iodine value of one of the components.

The experiment is, however, very suggestive and further experiments along the same lines are indicated. It appears probable that the precipitation of alcoholic solutions with acetone may afford a means of further purifying lecithin.

Effect of oxidising and Reducing Agents.

Only one experiment of this kind has so far been tried.

Three/

Three equal volumes of the same specimen of lecithin solution were taken. To one zinc dust was added, to another platinum black while the third was kept as a control to the other two. The three lecithins were allowed to stand in the light for about a week, being well shaken at intervals. No alteration was visible. The lecithins were then removed, retitrated, and the iodine values tested.

Control Lecithin	66
Zinc Dust "	76
Platinum Black "	66

No other differences in the preparations were found.

Effect of Water on Lecithin.

The third acetone precipitate from an ox liver extracted with spirit was divided up into a number of portions in a series of flasks. To these were added respectively -

(1) Absolute alcohol
(2) 80 per cent "
(3) 65 " " "
(4) 50 " " "
(5) Water.

Each flask was heated to boiling point, when complete solution occurred in the case of mixtures 1 to 3 and emulsions in the case of 4 and 5. The flasks were allowed to cool. Precipitates resulted in the flasks containing absolute 80 per cent and 65 per cent alcohol; in the other two flasks, although slight precipitation took place, permanent emulsions were formed. The supernatant fluids were removed and tested after careful titration. The results were as follows:-

	Iodine Values.
(1) Supernatant Fluid	71
(2) " "	53
(3) " "	50
(4) Emulsion	81
(5) "	24

Supernatant or other Fluid 1 part to 7 parts of salt solution.	0.5 c.c. Ox Blood Corpuscles + Venom					
	0.005 c.c.	0.1 c.c.	0.15 c.c.	0.25 c.c.	0.04c.c.	0.075 c.c.
(1) Fluid from absolute alcohol	0	Almost Complete	Complete			
(2) Fluid from 80 per cent alcohol	0	Almost Complete	Complete			
(3) Fluid from 65 per cent alcohol	0	0	0	0	Trace	Trace
(4) Emulsion with 50 per cent alcohol	0	Almost Complete	Complete			
(5) Emulsion with water	0	0	0	Trace	Almost Complete	Complete

The material which had fallen out of the hot solutions from flasks 2 and 3 was washed well with cold alcohol; boiling alcohol was then added and complete solution occurred. The hot fluids were cooled. Precipitates settled out in each case. The fractions remaining in solution in the cold alcohol were tested.

	Iodine values.
Fraction soluble in cold alcohol from precipitate with 80 per cent alcohol	68
" " " "alcohol from precipitate with 65 per cent	94

These fractions were further tested along with cobra venom and for their haemolytic effects and found to give the same results as a control specimen of lecithin. It seemed probable therefore that the precipitates from the 80 per cent and 65 per cent alcohol mixtures contained considerable amounts of lecithin. The low iodine value of the supernatant fluid from the flask containing 65 per cent alcohol along with the inefficiency to act with venom suggests that in this case very little lecithin had remained in the watery alcohol. It is somewhat more difficult to explain the results with the 80 per cent alcohol. The supernatant/

-natant fluid apparently contains lecithin with a lower iodine value (53) than the lecithin in the fraction obtained from the secondary treatment of the precipitate with absolute alcohol. The high iodine value of the fluid obtained from the precipitate with 65 per cent alcohol (94) as compared with the value of the primary supernatant fluid with absolute alcohol (71) is also striking. These experiments have been repeated with other specimens of acetone precipitate and somewhat similar results have been obtained (see tables 40a & 41).

Treatment of the acetone precipitate with water along somewhat similar lines has recently been suggested by MacLean as a method of obtaining pure lecithin. This worker recommends that the acetone precipitate should be rubbed with successive small amounts of water containing a little salt. A water soluble substance is in this way removed from the acetone precipitate which otherwise remains in the lecithin fraction and prevents a N:P ratio of 1:1 being obtained.

Lipoids other than lecithin which occur in the Acetone Precipitates.

It has been already noted that lecithin is only one constituent of the acetone precipitate, viz., the constituent soluble in cold alcohol, and that by treatment of this precipitate with boiling alcohol another substance soluble in boiling alcohol, insoluble on cooling, could be shown to be present. A further examination of the acetone precipitates from a number of organs has been made. It has been found that three fractions are always present.-

(1) A fraction soluble in cold alcohol (lecithin)

(2) A fraction soluble in boiling alcohol, insoluble on cooling: this fraction is also soluble in water.

(3) A fraction insoluble in cold or boiling alcohol, soluble in water.

These fractions have been found in the acetone precipitates from all the tissues which have been extracted with alcohol and are also present in the precipitates from ether extracts.

Fraction initially soluble in boiling alcohol.

On cooling the hot alcoholic solution this fraction separated out as a yellowish-white flocculent precipitate which adhered in part to the walls of the vessel. In order to effect the removal of "lecithin," the procedure of repeated solution in hot alcohol and precipitation by cooling was resorted to. But on carrying out this method a difficulty was encountered in obtaining the purified product owing to the behaviour of the precipitate. Thus it was found that a certain proportion of the precipitate which formed on cooling adhered firmly to the glass, and the amount of precipitate adhering in this fashion increased when the solution was allowed to stand for a considerable time. This portion of the precipitate adhering to the glass was no longer completely/

-pletely soluble in boiling alcohol, though the flocculent moiety dissolved readily. On each occasion on which the material was allowed to separate out on cooling from solution in hot alcohol a certain quantity of precipitate became resistant to solution, and further treatment with hot alcohol caused it to become viscous and to assume a darker brown colour. In this way, by repeating the treatment with hot alcohol sufficiently often, the whole of the material could be rendered insoluble in boiling alcohol. Both the original material (product A) and that which had become insoluble in boiling alcohol (product B) dissolved in ether from which solutions they were incompletely precipitated by acetone, as an emulsion was formed from which separation very slowly occurred. In water both substances readily formed moderately turbid "solutions." The iodine value of the material soluble in boiling alcohol was always higher than that of the material which had been rendered insoluble in hot alcohol.

Fraction of the acetone precipitate which remains undissolved after treatment with boiling alcohol.

This material, which was of a dark colour, dissolved in water to form a clear yellow or slightly turbid solution. On evaporation of the watery solution to dryness two bodies were obtained, the one soluble, the other insoluble, in ether. The ether-soluble portion (product C), which constituted the greater part, was readily precipitated out of ethereal solution by the addition of acetone or cold alcohol, and the precipitate, like the original material, dissolved in water to form a clear yellow or slightly turbid solution, which gave a precipitate with alcohol and with watery cadmium chloride. Unlike "lecithin," it was not readily removed/

removed from watery solution by shaking with ether. The ether-insoluble portion (product D) was also soluble in water, forming a transparent yellow solution which did not give a precipitate on the addition of alcohol, but was precipitated by alcoholic or watery cadmium chloride. Owing to the small amounts available for examination, it was not possible to make a satisfactory estimation of the iodine values or of the N:P ratios. Phosphorus was shown to be present in both fractions. The amounts of the various bodies present in the acetone precipitates of different extracts have differed for different organs and for different specimens of the same organ, but in no case have more than very small amounts been obtained even from large quantities of tissues. (1700 grms. of fresh ox liver yielded only a small fraction of a gramme of each of products A, B and C, and only traces of product D.)

All the substances described above yielded haemolysins along with cobra venom. Watery solutions were employed, and the ratio of the haemolytic power for ox's red blood corpuscles with venom to that without venom was determined. With the exception of product A, the addition of cobra venom increased the haemolytic activity of all these bodies from seventy to two hundred times (vide Tables 25, 30, 32, 33, 34, 44, 45.). In the case of the substance soluble in boiling alcohol (product A) marked haemolysis of the corpuscles occasionally occurred without venom, but even in these cases the activity was increased from ten to twenty times by the addition of venom.

In the case of all the substances mentioned it was found to be of importance to test the haemolytic activity as soon as possible after preparation.

When the lecithins obtained from red blood corpuscles were under discussion it was pointed out that

these were strikingly inefficient along with cobra venom. It is interesting to note that a component active for venom was present in the residue of the acetone precipitate. The fraction of the precipitate which was insoluble in boiling alcohol when dissolved in water gave an active haemolysin in the presence of venom (v. table 32)

The relation of the above fractions to known lipoids has not yet been determined. The product soluble in boiling alcohol, insoluble on cooling, agrees in most respects with the characters of certain kephalins According to Erlandsen, however, kephalin is not present in ox heart. In my experiments this substance (Product A) was found in ox heart as in other tissues. The nature of product B is still more obscure. In a number of the tables in which the action of product A is described reference is made to it as 'the Kephalin-like material.' The fraction of the acetone precipitate which is insoluble in boiling alcohol bears a certain resemblance to courin but is distinguished from it by the fact that it generally yields an active haemolysin along with cobra venom, whereas courin, prepared by Erlandsen's method, is quite inactive in my experience (v. table 46). In addition freshly prepared courin is not readily soluble in water: the residue insoluble in boiling alcohol, on the other hand, dissolves readily. In a number of the tables which are appended it is referred to as 'the cuorin-like product'

A considerable number of other products have been isolated in course of the work. It has been already noted that the first acetone precipitate as a rule does not dissolve completely in ether. The insoluble fraction consists of white, granular material which is obtained by centrifugalising the mixture. This material is insoluble in cold or warm ether, soluble in boiling alcohol/

alcohol, insoluble on cooling. After repeated solution in hot alcohol and subsequent cooling a white, crystalline material is obtained which can be dried without the characters altering. In the dried state it is a pure white powder which has a somewhat greasy feeling to the finger and melts readily (in the case of egg yolk the material melted sharply at 49° C.) This substance was quite inactive along with cobra venom; when dissolved in alcoholic lecithin the mixture gave the same reactions as a mixture of lecithin and cholesterin both as haemolysin with venom and as antigen in the Wassermann test (v. table 47). The melting point does not correspond with any of the known cholesterin products, and the material does not give any of the colour reactions of cholesterin or its derivatives. This product although insoluble in ether is difficult to separate completely from the ether solutions of the acetone precipitates. For its removal it is important that the solution of the acetone precipitate in ether should be centrifuged very thoroughly as soon as possible as traces of the material are obtained from apparently clear ~~fluids~~ ether solutions. Further, although successive acetone precipitates are treated in this way a trace of the material may be found later to separate out from the alcoholic lecithin solution.

In its reactions to fat solvents it bears a close resemblance to the "diamino fraction" obtained from egg yolk by Stern and Thierfelder and to the somewhat similar diamino-monophosphatide described by MacLean as occurring in extracts of horse kidneys. I have, however, failed to demonstrate the presence of phosphorus in the material after it was purified by repeated solution in hot alcohol and precipitation

on/

on cooling. Nitrogen has been found present in small amount (about 1 per cent). It is possible that the substance belongs to the class of cerebrosides. This white material, although present in very small amount in all the extracts, is much more abundant in lung, kidney, spleen and brain extracts than in other tissue extracts. In ether extracts it forms a considerable fraction of the early precipitates with acetone especially if the tissue has been first extracted with spirit. Tissues in a condition of fatty degeneration would appear to yield greater amounts than normal tissues. The amount obtained from an ether extract of 287 grms. of dried ox heart was 0.25 grms.

In the case of certain extracts (brain, egg yolk, liver) it was found that the acetic ether precipitate contained considerable amounts of yellowish white material insoluble in ether. This material was for some time regarded as the same as the white product just described. Further examination, however, showed that after this material had been thoroughly washed with ether it could be split by the use of alcohol into three fractions.-

(1) Soluble in cold alcohol.

(2) Insoluble in cold alcohol, soluble in boiling alcohol, insoluble on cooling.

(3) Insoluble in cold or boiling alcohol.

Fraction (1). In all respects this fraction resembled lecithin. The solution was yellow in colour, gave turbidity with water, a precipitate with cadmium chloride, formed an active haemolysin with cobra venom and acted like lecithin in the Wassermann reaction (v. tables 21-26). Phosphorus and nitrogen were both present.

Fraction (2). This possessed all the characters of the "white product" with which it was probably identical/

identical.

Fraction (3). This was small in amount and appeared to consist largely of protein material.

MacLean has also described the presence, in extracts of horse kidneys, of ether insoluble material which could be divided into three fractions reacting to alcohol in similar fashion.

I have recently found that the ether insoluble material from acetic ether precipitates of brain may dissolve almost completely if left in contact with ether at room temperature for some weeks. The solution so obtained yielded a precipitate with acetone which, on further treatment with ether, gave a soluble and an insoluble fraction. The insoluble fraction consisted of "white product". The ether soluble portion treated with a further amount of acetone yielded a precipitate which had the properties of the ordinary acetone precipitate.

During the examination of the lipoids obtained from an ether extract of ox heart by a method somewhat different from that generally employed, traces of other lipoids were found which gave haemolysins along with venom. (v. table 48). The amounts obtained were too small to permit of critical examination of their properties.

Separate tables showing the action of a number of these different lipoids have not been prepared as it was not considered advisable to disassociate them from the other preparations tested on the same day. They will, therefore, be found throughout the tables. Table 49 shews the result obtained in the Wassermann test with the kephalin-like products from an ox liver and a fatty human liver respectively. It will be seen that whereas the product from the ox liver gives

a/

a negative reaction in the presence of the normal serum the similar product from the fatty liver gives a positive reaction. In the presence of syphilitic serum both products give a positive reaction. Examination of the anticomplementary effects of the emulsions by themselves shows, however, that these results are due in great part to the fact that both substances lack the power to remove the normal anticomplementary effect of cholesterin. It will also be noted that the anticomplementary effect of the cholesterin in the presence of the ox liver kephalin is greatly reduced by the presence of the normal serum. This effect of serum has been frequently observed.

Optical Activity of Lecithin.

The fact that lecithin is optically active is fairly well known but the exact amount of rotation does not appear to have been measured.

Owing to the marked absorption of light due to the colour of most lecithin solutions and the necessity to use a strong solution in order to obtain a measurable rotation, it is somewhat difficult to obtain a good reading with the ordinary sodium flame. I have obtained the following result with a lecithin out of egg yolk. A 3.52 per cent solution in alcohol was used with a tube of 200 m.m. The reading was 2.5.

$$[\alpha]^{D} = \frac{2.5 \times 100}{2.0 \times 3.52} = 35.5^{\circ}$$

In the above experiment the absorption of light seemed to be out of proportion to the depth of colour and suggested that the solution of lecithin in alcohol was of the nature of a colloidal solution. Provided that lecithins could be obtained free from pigment the measurement of the optical activity might be used as a test for the purity of the preparations.

The colouring matter of Lecithin.

It has been already noted that the great majority of preparations of lecithin are contaminated by the presence of yellow pigment. I have been unable to find in the literature any reference to the nature of this colouring matter. A number of facts regarding its occurrence and properties have been accumulated during this work. In the first place it is almost constantly present in lecithin from alcoholic extracts and is more abundant in the lecithin from watery alcohol extracts. Lecithins from ether extracts are practically colourless although the ether extract itself is very highly coloured. It is therefore probable that the pigment is more soluble in alcohol, especially watery alcohol, than in ether. Repeated treatment with acetic ether does not completely remove it from the precipitate although as a rule, the first two or three cold acetic ether soluble fractions are well coloured. It is soluble in ether, insoluble in acetone. Alcoholic solutions examined by means of the spectroscope show no bands; but a marked absorption of the violet end of the spectrum occurs. An alcoholic lecithin solution heated for some hours becomes much darker in colour; if the heating be prolonged a very dark brown solution results.

These properties are somewhat similar to those of urochrome. This pigment which is the colouring matter of normal urine and of serum has the following characters (Garrod). It is easily soluble in water and rectified spirits, less soluble in absolute alcohol and ether, sparingly soluble in acetic ether, amyl alcohol, acetone, almost insoluble in chloroform and benzol. Watery solutions show no characteristic spectrum only diffuse absorption of the violet end of the spectrum. The golden yellow watery solution becomes dark brown on heating/

heating or on standing.

Probably more pigments than one are present in the original extracts. The cold acetic-ether-soluble component is, for example, always well coloured. In the case of ether extracts all the colouring matter goes over into this fraction. If treatment with acetic ether is omitted and the ether solution precipitated directly with acetone the colouring matter remains in the acetone-ether fluid.

Repeated extraction of the same sample of tissue with alcohol does not produce much diminution in the intensity of the colour of the extracts. Each extract has a golden yellow appearance. In the case of ether extracts the colour rapidly diminishes until a colourless extract is obtained. Tissue thoroughly extracted with ether gives a yellow extract on further treatment with alcohol. Alcoholic extracts of dried serum contained a comparatively large amount of pigment; ether extracts of the same were quite colourless. All the tissues examined yielded coloured alcohol extracts. It is possible that the pigment is derived from the amount of blood present. Ox blood corpuscles, however, freed from serum by repeated washing with salt solution also gave a coloured extract. Further observations on these matters are in progress.

Examination of Lecithins for N : P ratio.

A number of preparations of lecithin have been tested by Kjeldahl's method for the amount of nitrogen and by Neumann's method for phosphorus. The results were extremely disappointing. The Kjeldahl examinations were carried through with great care but constant results could not be obtained. A number of samples of the same lecithin tested at the same time by the same method did not give the same result. Control tests with alanine and urea performed at the same time showed that the technique (at least for these substances) was not faulty. Various catalytic agents copper sulphate, copper sulphate with potassium sulphate, red oxide of mercury and metallic mercury have all been tried but the results have not been encouraging. I do not, therefore, propose to submit any of my results in this connection.

An examination of the results of other workers in this field does not suggest that the examination of the ratio of the nitrogen to phosphorus yields much information regarding the nature of the substances concerned. The same view has been expressed by Leathes. In many cases the purity of particular lipoids has been judged solely by the ratio of nitrogen to phosphorus and in many such cases the ratio has only approximated 1:1 (or 2:1, as the case may have been). Substances which have been accepted as pure because the N : P ratio approximated whole numbers have later been shown to be impure. For example, in MacLean's experiments with extracts of horse kidneys, a substance with a ratio N:P = 3:1 and, in consequence, accepted as pure was investigated later and found to be an impure mononamino-mono-phosphatide. A ratio accurately 1:1 could not, however, be obtained. In Erlandsen's experiments with ox heart the bulk of the lipoid in the alcoholic extract

was/

was regarded as being a diamino-monophosphatide as the N:P ratio approximated 2:1. In the case of horse kidneys MacLean, using Erlandsen's methods, found that the bulk of the phosphatide in the alcoholic extracts consisted of lecithin. This could only be obtained tolerably pure by repeated solution in ether and precipitation with acetone and by rapidly centrifugalising the ether solutions each time in order to remove material which was insoluble in ether but which dissolved readily in an etheral solution of lecithin. From these results and my own experiments with ox heart I think it is probable that the diamino-monophosphatide of Erlandsen's alcoholic extracts was in reality impure lecithin.

In my opinion figures of much greater interest and importance would result from a comparison of the absolute amounts of nitrogen and of phosphorus present in a series of 'pure' lecithins prepared by a uniform method from the same and from different tissues.

Where the ratio only of nitrogen to phosphorus for any particular substance is estimated it is possible to obtain a satisfactory ratio from figures which do not express the real proportion of nitrogen and phosphorus.

I have already criticised the technique of nitrogen estimation (as applied to these substances) especially as regards the small amount of substance which is generally used. In my experience it is often a matter of considerable difficulty to obtain clearing of the fluid under combustion with sulphuric acid and in some cases it has been found necessary to continue heating overnight, a procedure which is regarded by competent critics as likely to prejudice the results.

In addition, most 'pure' lecithins contain colouring matter which is probably urochrome or an allied substance/

substance. As the amount of nitrogen present in urochrome is about 10 per cent some degree of error in the nitrogen estimation must result from the presence of even a small amount of such substance.

A very large number of lecithins and other products have now been accumulated and it is intended to pursue this side of the subject more thoroughly. Until it has been found that constant values are obtainable with individual preparations I do not regard it as profitable to compare the various preparations which have been isolated.

Some discussion regarding the relation of these results as a whole to the work of others may perhaps be in place here. In the first place no attempt has been made by any other workers to determine differences in lecithins by means of biological tests. The purity of a particular lecithin has always been estimated by the solubility reactions and, in some cases also, by the N:P ratio. In my experiments the method of preparation which has been used is superior in most respects to the methods generally employed. The repeated treatment with acetic ether and acetone undoubtedly removes all the impurities (neutral fats, fatty acids, cholesterin) which can be removed by such methods of treatment. I have tested the efficiency of the method in the following way. Various mixtures of lecithin with cholesterin and fatty acids were made. These were evaporated to dryness and the residues treated with acetic ether and acetone as in the preparation of lecithin from the tissue extracts. It was found that the lecithin finally obtained was in all respects the same as the lecithin which had been used at the beginning/

-ning of the experiment. As a result of the experience gained in the work I am of opinion that the biological tests form a valuable addition to the list of measures used for ascertaining the purity of lecithin. By means of the biological reactions differences can be brought out which by the ordinary chemical tests cannot be elicited. Further, any additions to the list of tests for lecithin are of the greater importance since it must be obvious to any worker in the subject that the criteria by which the purity of lecithin is judged are quite insufficient. This is a matter to which much attention does not seem to have been paid. At the present time it is, I believe, even doubtful if a single substance with the properties generally ascribed to lecithin exists. However, owing to the fact that lecithin contains two fatty acid groups and that the number of possible combinations of these is very large it is possible that a number of different lecithins exists. On the other hand it is even more likely that the differences in different preparations are due to the presence of other substances which at present cannot be separated from lecithin. It is difficult to say what the properties of a pure lecithin may be. I have frequently attempted further purification of lecithin. Some of my experiments have been already mentioned in the paragraphs dealing with the chemical alteration of lecithin as I have considered it advisable, in the present state of our knowledge, to regard certain of the products obtained as "altered" rather than as "purified" lecithins.

Certain other experiments may be shortly mentioned. If alcoholic solutions of "pure" lecithin are well cooled in mixtures of ice and salt a fairly abundant yellowish white precipitate is formed. This precipitate/

-tate is very difficult to remove as it redissolves very rapidly on the removal of the lecithin from the ice mixture. By alternate cooling and rapid centrifuging it is possible to remove the majority of the material. The results of such an experiment are given in tables 34 and 41. It will be seen that the cold alcohol soluble or "lecithin" fraction has a higher iodine value than the original substance while the material insoluble in the cold (which was repeatedly washed with ice cold alcohol) has a very low iodine value. It was found that the insoluble fraction dissolved very readily in alcohol at room temperature and that the solution gave an active haemolysin along with cobra venom. An attempt has also been made quite recently to analyse lecithin by an examination of the products obtained when lecithin is treated with cobra venom using the method of Kyes for the preparation of cobra-lecithid. This substance is described as containing only one fatty acid radicle and it appeared possible that some information regarding the nature of the other group might result from an examination of the bye-products. An investigation of this nature had already been made by Lüdecke but the lecithins used were commercial preparations which are by no means pure. Several cobra lecithids have already been made by Dr. Browning from ox heart, ox liver and egg yolk lecithins, which I have prepared. These lecithins I have tested for their iodine values (v. table 39). They were found to be practically saturated. The bye-products from these cobra lecithids I am at present testing but no results can be given. The fact that these specimens of "pure" lecithin have yielded "cobra lecithids" which were actively haemolytic contradicts the statement of Bang that cobra-lecithid is only formed from impure commercial/

commercial lecithins. It is perhaps important in this connection to remember that 'ether extracted' lecithins in my experiments were greatly generally very inactive along with cobra venom and it is possible that the pure lecithin used by Bang may have been in the first place extracted with ether. Noguchi has also found that ether extracts of corpuscles insusceptible to the haemolytic action of cobra venom do not serve to activate cobra venom haemolysis. It has been asserted by Bang and Noguchi that the activating effect of lecithin on cobra venom haemolysis is due to fatty acids. The lecithins used in my experiments, in addition to having been prepared by a method likely to give a lecithin free from fatty acids, were, with a very few exceptions non-lytic for unsensitised corpuscles. If an appreciable amount of free fatty acid had been present the preparations would have been actively haemolytic. Further, Moore has shown that in the case of fatty acids the haemolytic action is in direct proportion to the degree of unsaturation. It has been already observed that in the case of lecithins no such relation has been found. The fact that lecithin is not the only substance which forms a haemolysin along with venom would suggest that the activating effect is dependent on the presence in these substances of certain common groups and that the substances as a whole do not participate in the reaction. This view receives some support from the fact observed in the preparation of cobra-lecithid that only a very small amount of lecithid substance results from the use of a very large amount of lecithin. Further information may result from attempts to prepare 'lecithids' by the action of venom on some of the substances, other than lecithin, which give haemolytic combinations with cobra venom.

Table 21.

Syphilitic Serum ($\frac{1}{2}$ hour at 57°C) 0.025 c.c. + Emulsion 0.3c.c.	Guinea-pig's Complement. 0.012 c.c.	0.024 c.c.	0.038 c.c.	0.55 c.c.	0.078 c.c.	Emulsions alone. 0.24 c.c.	0.038cc
Lecithin (human liver) wet method. case of pernicious anaemia	Marked	Complete				Just Complete	Complete
+ Cholesterin	-	Trace	Very Marked	Complete		Complete	
Lecithin extracted with watery alcohol (liver from case of pernicious anaemia)	Complete					Just Complete	Complete
+ Cholesterin	-	Trace	Just Complete	Complete		Complete	
Lecithin extracted with spirit (liver from case of pernicious anaemia)	Just Complete	Complete				Complete	
+ Cholesterin	-	0	Trace	Just Complete	Complete	Just Complete	Complete
Lecithin from ox heart with ether cuorin removed first	Very Marked	Just Complete	Complete			Very Marked	Complete
+ Cholesterin	-	0	0	Trace	Complete	0	0
Ether Insoluble, alcohol soluble of Acetic Ether precipitate (P.A. Liver + watery alcohol)	Complete					Complete	
+ Cholesterin	-	0	Distinct	Complete		Just Complete	Complete
Ether Insoluble alcohol soluble of Acetic Ether precipitate (P.A. Liver + alcohol	Complete					Complete	
+ Cholesterin	-	Complete				Just Complete	Complete

	Lecithin Emulsions + Cobra Venom					Lecithin-Cholesterin Emulsions + Cobra Venom.					
	0.005 c.c.	0.012 c.c.	0.025 c.c.	0.045 c.c.	0.085 c.c.	0.0 25 c.c.	0.045 c.c.	0.085 c.c.	0.13 c.c.	0.2 c.c.	0.4 c.c.
1.	0	Complete +				Very Marked	Complete				
2.	0	Distinct	Complete			Complete					
3.	0	Marked	Complete			Trace	Marked	Complete			
4.	0	0	Distinct	Almost Complt.	Complete	0	0	0	0	0	0
5.	0	Marked	Complete			Distinct	Very Marked	Complete			
6.	0	Faint trace	Complete			0	0	0	0	Distinct	Marked

Table 22a

Serum ($\frac{1}{2}$ hour at 57°C) 0.025 c.c. + Emulsion 0.3 c.c.		Amounts of Guinea pig's Complement						Emulsions alone Amounts of Complement		
		0.015 c.c.	0.025c.c.	0.04 c.c.	0.06 c.c.	0.09 c.c.	0.13 c.c.	0.01 c.c.	0.02 c.c.	0.035 c.c
Syphilitic Serum	1.Lecithin extracted by absolute alcohol	Almost Complete	Complete					Very Marked	Complete	
	. . . . . .+ Cholesterin	-	0	0	0	Trace	Very Marked	Distinct	Just Complete	Complete
	2. Lecithin extracted by spirit '	Almost Complete	Complete					Just Complete	Complete	
	. . . . . .+ Cholesterin	-	0	0	0	Distinct	Almost Complete	-	Almost Complete	Complete
	3. Lecithin extracted by .75 per cent Alcohol	Just Complete	Complete					Complete		
	. . . . . .+ Cholesterin	-	0	0	0	Trace	Almost Complete	Distinct	Complete	
	4. Lecithin extracted by Ether	Complete						Marked	Very Marked	Just Complete
	. . . . . .+ Cholesterin	-	0	0	0	0	0	0	Faint trace	Trace
Normal Serum.	1.Lecithin extracted by absolute alcohol	Very Marked	Almost Complete	Complete						
	. . . . . .+ Cholesterin	-	Distinct	Almost Complete	Complete					
	2.Lecithin extracted by spirit	Very Marked	Almost Complete	Complete						
	. . . . . .+ Cholesterin	-	Distinct	Very Marked	Complete					
	3.Lecithin extracted by 75 per cent Alcohol	Very Marked	Almost Complete	Complete						
	. . . . . .+ Cholesterin	-	0	Distinct	Complete					
	4.Lecithin extracted by Ether	Distinct	Very Marked	Complete						
	. . . . . .+ Cholesterin	- -	0	0	Very Marked	Almost Complete	Complete			

Sera alone 0.025 c.c. + Complement 0.02 c.c. = Just Complete Lysis. Dose of Complement = 0.01 c.c.

Syphilitic Serum (½ hour at 55°C) 0.025 c.c. + Emulsion, 0.3 c.c.	Amounts of Guinea pig's Complement						Emulsions alone		
	0.01c.c.	0.022c.c.	0.04c.c.	0.06c.c.	0.09c.c.	0.13c.c.	0.01c.c.	0.022c.c.	0.04 c.c.
1.Lecithin (human liver) extracted with ether	0	0	Distinct	Almost Complete	Complete	Complete	Distinct	Just Complete	Complete
. . . . . + Cholesterin	-	0	0	0	0	Distinct	0	Faint trace	Trace
2.Lecithin (same tissue) extracted with spirit	Very faint trace	Very Faint Trace	Very Faint Trace	Almost Complete	Complete	Complete	Very Marked	Just Complete	Complete
. . . . . + Cholesterin	-	0	0	0	Just Complete	Complete	0	0	Almost Complete
3.'Lecithin' from precipitate obtained by treating acetone precipitate with water (egg yolk)	0	Distinct	Almost Complete	Complete	Complete	Complete	Marked	Almost Complete	Complete
. . . . . + Cholesterin	-	0	0	Trace	Almost Complete	Complete	Trace	Very marked	Complete
4.Lecithin (egg yolk) extracted with spirit	Trace	Marked	Complete	Complete	Complete	Complete	Marked	Almost Complete	Complete
. . . . . + Cholesterin	-	0	0	Distinct	Complete	Complete	Distinct	Very marked	Complete
5.Ether insoluble fraction 1st Acetone precipitate (mixture of tissues) alcohol soluble portion	Trace	Distinct	Just Complete	Complete	Complete	Complete	Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Very faint Trace	Trace	Almost Complete	Complete	Marked	Just Complete	Complete
6.Kephalin-like material from mixed tissues	Distinct	Marked	Complete	Complete	Complete	Complete	Complete	Complete	Complete
. . . . . + Cholesterin	-	0	0	Distinct	Complete	Complete	0	Just Complete	Complete
7.'Lecithin' from precipitate obtained by boiling acetone precipitate with 80 per cent alcohol and cooling solution	Distinct	Very Marked	Just Complete	Complete	Complete	Complete	Marked	Almost Complete	Complete
. . . . . + Cholesterin	-	0	Very Marked	Just Complete	Complete	Complete	Trace	Very Marked	Just Complete

(Contd.)

Table 22b contd.

Syphilitic Serum (½ hour at 55°C) 0.025 c.c. + Emulsion, 0.3 c.c.	Amounts of Guinea pig's Complement						Emulsions alone.		
	0.01c.c.	0.022c.c.	0.04c.c.	0.06c.c.	0.09c.c.	0.13c.c.	0.01c.c.	0.022c.c.	0.04c.c.
8.'Lecithin' by similar procedure by using 50 per cent alcohol	Marked	Almost Complete	Complete	Complete	Complete	Complete	Very Marked	Almost Complete	Complete
. . . . . .+ Cholesterin	-	0	Very Marked	Complete	Complete	Complete	Marked	Almost Complete	Complete
9.Lecithin from mixture of tissues (treatment of acetone precipitate with watery alcohol	Trace	Very Marked	Almost Complete	Complete	Complete	Complete	Marked	Almost Complete	Complete
. . . . . .+ Cholesterin	-	0	0	Distinct	Complete	Complete	Distinct	Just Complete	Complete
10.Lecithin from mixture of tissues	Distinct	Marked	Just Complete	Complete	Complete	Complete	Distinct	Almost Complete	Complete
. . . . . .+ Cholesterin	-	Faint trace	Faint Trace	Trace	Complete	Complete	Marked	Complete	Complete
11.Kephalin-like substance from egg yolk	Faint Trace	Distinct	Complete	Complete	Complete	Complete	0	0	Distinct
. . . . . .+ Cholesterin	-	0	Trace	Distinct	Just Complete	Complete	0	0	0
12.Control lecithin	0	0	Faint trace	Distinct			Distinct	Very Marked	Almost Complete
" " + Cholesterin	-	0	0	0	0	Distinct	Trace	Distinct	Just complete

Serum alone 0.025 c.c. + Complement 0.015 c.c. = Just Complete.
Complement dose = 0.015 c.c.

Table 22c

Emulsions same as in previous experiment.

Turbid Emulsions of 0.4 per cent 1 part + NaCl solution 7 parts	1 c.c. 5 per cent Ox-blood Suspension + Cobra Venom 1: 10,000 + Emulsion.					
	0.01 c.c.	0.02 c.c.	0.035 c.c.	0.06 c.c.	0.09 c.c.	0.15 c.c.
1.	0	0	Distinct Trace	Distinct	Just Complete	Complete
2.	0	Trace	Distinct	Very Marked	Complete	Complete
3.	0	Very Marked	Almost Complete	Complete	Complete	Complete
4.	0	Distinct	Just Complete	Complete	Complete	Complete
5.	0	0	Trace	Distinct Trace	Just Complete	Complete
6.	0	0	0	Trace	Distinct	Almost Complete
7.	0	Trace	Distinct	Marked	Just Complete	Complete
8.	Faint Trace	Trace	Distinct	Marked	Complete	Complete
9.	0	0	0	0	0	Very faint trace
10.	0	0	0	0	0	Distinct trace
11.	0	Very Faint Trace	Trace	Distinct	Just Complete	Complete
12.	Very Faint Trace	Faint Trace	Very Marked	Complete	Complete	Complete

Table 23a

Alcoholic Solutions 0.75 per cent 1 part + 0,85 per cent NaCl 7 parts.	1.0 c.c. Ox Blood Suspension + Cobra Venom 1:10,000 + Emulsion.					
	0.005 c.c.	0.01 c.c.	0.015 c.c.	0.03 c.c.	0.05 c.c.	0.08 c.c.
1. Lecithin (absolute alcohol)	0	0	Very faint trace	Complete	Complete	Complete
2. Lecithin (spirit)	0	0	Trace	Almost Complete	Complete	Complete
3. " (75 per cent alcohol)	0	0	Faint Trace	Complete	Complete	Complete
4. " (ether)	0	0	0	0	0	0

Table 23b

	1.0 c.c. Ox Blood Suspension + Emulsion.			
	0.1 c.c.	0.2 c.c.	1.0 c.c.	2.0 c.c.
I. Lecithin (absolute alcohol)	0	0	Marked	Almost Complete
II. " (Spirit)	0	0	"	" "
III. " 75 per cent alcohol	0	Trace	Very Marked	Just Complete
IV. " (Ether)	0	0	Marked	Almost complete

Table 24.

Lecithin 0.75 per cent 1 part + parts 0.85 per cent NaCl.	0.5 c.c. 5 per cent Ox Blood Suspension + Cobra Venom + Emulsion					Turbidity of Emulsions
	0.005 c.c.	0.01 c.c.	0.025 c.c.	0.5c.c.	0.075 c.c.	
1.Ox Liver treated with Spirit	0	Faint Trace	Marked	Complete	Complete	Very Faint Trace
2. " " treated with 75 per cent Alcohol	0	0	Trace	Complete	"	Practically Clear
3. " " treated with Absolute Alcohol	0	0	Trace	Almost Complete	"	Faint Trace
4. " " treated with Ether	0	0	0	0	0	Distinct
5.Fatty Liver treated with Ether	0	0	Complete	Complete	Complete	Trace
6. " " treated with Alcohol	0	0	Complete	Complete	"	Very faint Trace

Table 25.

Lecithins 0.12 per cent in alcohol 1 part + 7 parts 0.85 per cent NaCl: slowly made emulsions	1.0 c.c. Ox Blood Corpuscles + cobra Venom + Emulsion.								1.0 c.c. Ox Corpuscles + Emulsion.		
	0.025 c.c.	0.05c.c.	0.1 c.c.	0.2c.c.	0.325c.c.	0.4 c.c.	0.6c.c.	1.0c.c.	0.4 c.c.	0,6 c.c.	1.0 c.c.
1. Liver (human) spirit extract	0	0	Complete						0	Faint Trace	Marked
2. Liver (human) (Spirit + 150 c.c. H_2O)	0	Faint trace	"						0	Very Faint Trace	Trace
3. Liver (human) (Spirit + 300 c.c. H_2O)	0	Distinct	"						0	Trace	Marked
4. Kidney (human) Alcohol extract	0	0	0	0	0	0	Trace	Marked	0	0	0
5. Fatty Liver (human) Ether extract	0	0	0	Complete					0	0	0
6. Fatty Kidney (human) Alcohol extract	0	0	0	Faint trace	Complete				0	0	0
7. Ox Heart, spirit extract	0	0	Trace	Complete					0	0	Very ft. trace
8. Control Lecithin	0	Complete							0	Distinct	Marked
9. Kephalin-like material from acetone precipitate (liver with spirit)	0	0	Faint Trace	Complete					0	0	Distinct
10. Kephalin-like material from acetone precipitate (Fatty Liver with ether)	0	0	0	0	0	0	0	0	0	0	0

Syphilitic Serum (½ hour at 57°C) 0.025 c.c. + Emulsion 0.3 c.c.	Amounts of Guinea pig's Complement						Emulsions alone.		
	0.01 c.c.	0.02 c.c.	0.04 c.c.	0.06 c.c.	0.085 c.c.	0.12 c.c.	Guinea pig's Complement 0.01 c.c.	0.02 c.c.	0.04 c.c.
1. Lecithin from Fatty Liver (alcohol)	0	Very faint trace	Very Marked	Just Complete	Complete	Complete	Almost Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Faint trace	Trace	Very Marked	Almost Complete	Almost Complete	Complete	Complete
2. Lecithin from Fatty Liver (spirit)	Very faint trace	Distinct	Just Complete	Complete	Complete	Complete	Just Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Very faint trace	Distinct	Marked	Very Marked	Marked	Very Marked	Complete
3. Lecithin from Fatty Liver (ether)	0	Very Ft. Trace	Distinct	Almost Complete	Complete	Complete	Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Faint Trace	Trace	Distinct	Complete	Trace	Distinct	Complete
4. Lecithin Fatty Kidney (alcohol)	Trace	Marked	Just Complete	Complete	Complete	Complete	Just Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Trace	Marked	Just Complete	Complete	Almost Complete	Complete	Complete
5. Ether Insoluble alcohol soluble fraction from acetic ether precipitate (fatty liver)	0	Distinct	Almost Complete	Complete	Complete	Complete	Just Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Faint Trace	Trace	Just Complete	Complete	Just Complete	Complete	Complete
6. Ether Insoluble alcohol soluble fraction from acetic ether precipitate (alcohol after ether)	Trace	Marked	Just Complete	Complete	Complete	Complete	Just Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Very faint trace	Trace	Just Complete	Complete	Very Marked	Complete	Complete

(Contd)

Table 26 contd.

(Contd).

Syphilitic Serum (½ hour at 57°C) 0.025 c.c. + Emulsion 0.3 c.c.	Amounts of Guinea pig's Complement						Emulsions alone.		
	0.01 c.c.	0.02 c.c.	0.04 c.c.	0.06 c.c.	0.085 c.c.	0.12 c.c.	Guinea pig's Complement 0.01 c.c.	0.02 c.c.	0.04 c.c.
7. Ether Insoluble alcohol soluble fraction from Acetone precipitate (Fatty liver spirit)	0	0	Trace	Very Marked	Complete	Complete	Just Complete	Complete	Complete
. . . . . + Cholesterin	-	0	Faint trace	Distinct	Just Complete	Complete	Complete	Complete	Complete
8. Control Lecithin	0	Very faint Trace	Distinct	Just Complete	Complete	Complete	Almost Complete	Complete	Complete
" " + Cholesterin	-	0	0	Trace	Very Marked	Complete	Just Complete	Complete	Complete

Lecithin solutions 0.5 per cent: 1 part + 7 parts 0.85 per cent Na Cl: emulsion of maximum turbidity 0.3 c.c.	Amounts of Guinea pig's complement.				
	0.015 c.c.	0.025 c.c.	0.04 c.c.	0.075 c.c.	0.1 c.c.
I. Ox Heart (1350 gms), 1st Extract	Very Marked	Just Complete	Complete	Complete	Complete
" " " " + Cholesterin	Faint Trace	Faint Trace	Distinct	Marked	Just Complete
II. " " " 2nd Extract	Very Marked	Just Complete	Complete	Complete	Complete
" " " " + Cholesterin	Faint Trace	Trace	Distinct	Marked	Just Complete
III. " " " 3rd Extract	Marked	Very Marked	Almost Complete	Just Complete	Complete
" " " " + Cholesterin	Faint Trace	Faint Trace	Faint Trace	Faint Trace	Faint Trace
IV. " " " 4th Extract	Marked	Very Marked	Almost Cpl.	Just Complete	Complete
" " " " + Cholesterin	Trace	Trace	Distinct	Almost complete	Just Complete
V. " " " 2nd Extract, by boiling	Very Faint Trace	Faint Trace	Distinct	Very Marked	Just Complete
" " " " by boiling + Cholesterin	0	0	0	0	Faint Trace
VI. Ox Liver (460 gms) 4th Extract	Faint Trace	Trace	Distinct	Marked	Complete
" " " " + Cholesterin	0	0	0	0	Trace
VII. Fresh Brain 2nd Extract	Marked	Very Marked	Distinct	Complete	Complete
" " " + Cholesterin	0	0	0	0	0
VIII. Sheep's red corpuscles, 1st Extract	Distinct	Just complete	Complete	Complete	Complete
" " " " + Cholesterin	Trace	Trace	Trace	Trace	Trace
IX. Dried Brain, 2nd Extract	Marked	Distinct	Marked	Just Complete	Complete
" " " + Cholesterin	Distinct	Marked	Very Marked	Almost Complete	Complete
X. " " alcohol soluble fraction of ether insoluble substance Dried Brain + Cholesterin	Just Complete Distinct	Complete Marked	Complete Distinct	Complete Trace	Complete Distinct
XI. Dried Sheep's Blood Corpuscles, 1st Ext:	Distinct	Marked	Very Marked	Complete	Complete
" " " " + Cholesterin	Trace	Trace	Trace	Trace	Trace

Table 29.

Lecithin 0.75 per cent 1 part + 0.85 per cent Na Cl 7 parts: emulsions slowly made	Lecithin emulsions				Lecithin cholesterin emulsions				
	Amounts of Guinea pig's complement				Amounts of Guinea pig's complement.				
	0.015c.c.	0.025c.c.	0.045c.c.	0.075 c.c.	0.005 c.c.	0.01c.c.	0.015 c.c.	0.025 c.c.	0.05 c.c.
Ox Heart (1015 gms), 3rd Extract	Very Marked	Almost Complete	Complete	Complete	Marked	Almost Complete	Complete	Complete	Complete
" " " , 4th "	Marked	"	"	"	0	0	0	Distinct	"
" " " , 5th "	"	"	"	"	0	0	0	"	"
" " (1350 gms), 5th "	"	Just Complete	"	"	0	Trace	Almost Complete	Just Complete	"
" " " , 6th "	0	Distinct	Just Complete	"	0	0	0	0	Very Faint trace
Ox Lungs, 1st Extract	Distinct	Just Complete	Complete	"	Marked	Almost Complete	Just Complete	Complete	Complete
" Testicles 1st "	Complete	Complete	"	"	"	"	Complete	"	"
" Kidney, 2nd "	Very Marked	Just Complete	"	"	Very Marked	Just Complete	Complete	"	"
" " 4th "	Just Complete	Complete	"	"	Almost Complete	Complete	"	"	"
Dried Ox Kidney 2nd "	Complete	"	"	"	Distinct	Very marked	"	"	"
Ox Spleen 1st "	"	"	"	"	Almost Complete	Complete	"	"	"
" " 2nd "	Just Complete	"	"	"	0	0	0	0	0
" Thyroid 1st "	Complete	"	"	"	Almost Complete	Complete	Complete	Complete	Complete
" Pancreas 1st "	"	"	"	"	"	"	"	"	"
Dried Brain 3rd "	"	"	"	"	Distinct	Marked	Almost Complete	"	"
Fresh Brain 3rd "	"	"	"	"	"	"	"	"	"
Liver (460 gms) 4th "	Marked	Complete	"	"	"	"	"	"	"

Table 30.

Lecithin 0.75 per cent 1 part + 0.85 per cent NaCl 7 parts: rapidly made emulsions	0.5 c.c. Ox Blood Suspension + Cobra Venom + Substance.					0.5 c.c. Ox Blood Suspension + Substance.		
	0.005 c.c.	C.01 c.c.	0.015 c.c.	0.025 c.c.	0.05 c.c.	0.3c.c.	0.6 c.c.	1.0 c.c.
Ox Liver (460 gms) 1st Extract	Distinct	Complete	Complete	Complete	Complete	0	Trace	Marked
Ox Liver (460 gms) 2nd Extract	Distinct	Complete	"	"	"	0	Trace	Marked
Ox Liver (460 gms) 3rd Extract	Distinct	Complete	"	"	"	0	Faint trace	Distinct
Ox Heart (1015 gms) 1st Extract	Trace	Almost complete	Complete	"	"	0	Trace	Distinct
Ox Heart (1015 gms) 2nd Extract	0	Almost complete	Complete	"	"	0	Trace	Distinct
Dried Liver, 2nd Extract	0	Trace	Very marked	Almost Complete	Complete	0	Distinct	Almost complete
Product B (ox liver)	Complete	Complete	Complete	Complete	Complete	0	0	0
Product D (ox liver) 1st extract	0	0	Trace	Distinct	Complete	0	0	0
Product D (ox liver) 2nd Extract	0	0	0	0	0	0	0	0
Product C (ox liver)	Complete	Complete	Complete	Complete	Complete	0	0	0

Product B is the substance initially soluble in boiling alcohol rendered insoluble by treatment.

" D is the ether-insoluble fraction of the cuorin-like material resistant to boiling alcohol.

" C is the ether-soluble fraction of the same material.

Table 31.

Reading at end of 4 hours.

Lecithin solutions 0.75 per cent 1 part + 7 parts 0.85 per cent NaCl solution, rapidly made emulsions.	0.5 c.c. Ox Blood Suspension + Cobra Venom + Emulsion						0.5 c.c. Ox Blood Suspension + Emulsion.				
	0.01 c.c.	0.025c.c.	0.045c.c.	0.07c.c.	0.1c.c.	0.2 c.c.	0.1c.c.	0.2c.c.	0.5 c.c.	1.0c.c.	1.25c.
1.Ox Heart, 1350 gms, 1st Extract	0	0	0	0	0	Distinct	0	0	Very Marked	Complete	Complete
2." " 1350 gms 2nd Extract	0	Almost Complete	Complete	Complete	Complete	Complete	0	0	Trace	"	"
3." " 1350 gms 2nd Extract by boiling	0	Very Marked	Complete	"	"	"	0	0	0	"	"
4." " 1350 gms, 3rd Extract	Distinct	Just Complete	Complete	"	"	"	0	0	0	Almost Complete	"
5." " 1350 gms 4th Extract	"	Just Complete	"	"	"	"	0	0	0	Just Complete	"
6.Ox Liver, 460 gms, 4th Extract	Trace	Very Marked	"	"	"	"	0	Trace	Almost complete	Complete	"
7.Fresh Brain, 2nd Extract	0	Very Marked	"	"	"	"	0	0	Trace	"	"
8.Sheep's Blood Corpuscles 1st Extract	0	0	0	0	0	Marked	0	0	0	Marked	Almost Complete
9.Sheep's Blood Corpuscles 2nd Extract	0	0	0	0	0	0	0	0	Distinct	Complete	Complete

Table 32.

Reading after 15 hours.

Lecithin 0.75 per cent 1 part + 7 parts 0.85 per cent NaCl, emulsions rapidly made.	0.5 c.c. Ox Blood Suspension + Cobra Venom + Emulsion						0.5 c.c. Ox Blood Suspension + Emulsion.			
	0.05 c.c.	0.1 c.c.	0.3 c.c.	0.5 c.c.	0.75 c.c.	1.0 c.c.	0.2 c.c.	0.5 c.c.	0.6 c.c.	2.0 c.c
Sheep's Red Corpuscles	0	0	0	0	Faint trace	Trace	0	0	0	0
Sheep's Red Corpuscles	Trace	Distinct	Marked	Very marked	-	-	0	0	0	0
	0.01 c.c	0.025c.c.	0.05c.c.	0.075c.c	0.125c.c.	0.3 c.c.	0.05c.c.	0.1 c.c.	0.2 c.c.	0.5 c.c
Dried Brain	Trace	Complete	Complete	Complete	Complete	Complete	Distinct	Marked	Just Complete	Complete
Dried Brain, alcohol soluble fraction of ether insoluble material	0	Distinct	Marked	Very Marked	Complete	Complete	0	Very faint trace	Almost Complete	Complete
	0.005c.c.	0.01 c.c.	0.015c.c.	0.025c.c.			0.3 c.c.	0.3 c.c.	2.0 c.c.	4.0c.c.
Cuorin-like substance from Sheep's Blood Corpuscles	Marked	Very marked	Complete	Complete			0	0	0	0

Table 33.

Solutions 0.25 per cent emulsions or dilutions 1 part to 7 parts of 0.85 per cent NaCl.	0.5 c.c. Ox Blood Suspension + Cobra Venom + Solution.						0.5 c.c. Ox Blood Suspension + Solution.				
	0.015c.c.	0.03c.c.	0.045c.c.	0.075c.c.	0.13c.c.	0.25c.c.	0.1c.c.	0.25c.c.	0.5c.c.	1.0c.c.	1.6 c.c.
1. Lecithin from Ox Blood (Spirit)	0	0	0	0	Trace	Just Complete	0	0	0	0	0
2. " " Fresh Brain No. I	0	0	0	Marked	Complete		0	0	0	0	0
3. " " Fresh Brain No. II	0	Marked	Complete				0	0	0	0	0
4. " " Ox Heart (ether)	0	0	Marked	Almost Complete	Complete		0	0	Trace	Very Marked	Almost complete
5. Kephalin-like product from Fresh Brain	Trace	Distinct	Marked	Complete			0	0	0	0	0
6. Cuorin-like product from same	Just Complete						0	0	0	0	0
7. Residue after lecithin removed (ox heart + 80 per cent alcohol)	Complete						Very Marked	Almost Complete	Complete		
8. Residue after lecithin removed (ox heart + spirit)	Trace	Trace	Distinct	Marked	Complete		Very Marked	Just Complete	Complete		

TABLE. 34.

Solutions 0.75 per cent emulsified 1 in 8 with 0.85 per cent Na Cl solution	0.5 c.c. 5 per cent Ox Blood Suspension + Cobra Venom + Emulsion.				0.5 c.c. 5 per cent Ox Blood Suspension + Emulsion.				
	0.005 c.c.	0.01 c.c.	0.015 c.c.	0.025 c.c.	0.75 c.c.	0.4 c.c.	0.2 c.c.	0.1 c.c.	0.05 c.c.
1. Lecithin from 1st Extract of Ox Liver	Just Complete	Complete	Complete	Complete	Marked	Distinct	Trace	0	0
2. Lecithin from 2nd Extract of Ox Liver	Just Complete	Complete	Complete	Complete	Marked	Distinct	0	0	0
3. Lecithin from 3rd Extract of Ox Liver	Almost Complete	Complete	Complete	Complete	Trace	0	0	0	0
4. Lecithin after treatment at 0° C	Just Complete	Complete	Complete	Complete	Trace	0	0	0	0
5. Material insoluble at 0° C	0	Distinct Trace	Complete	Complete	Very Marked	Trace	"	"	"
6. Lecithin obtained by emulsifying acetone precipitate with 50 per cent alcohol	Just Complete	Complete	Complete	Complete	0	0	0	0	0
7. Lecithin obtained by emulsifying acetone precipitate with Water	Complete	Complete	Complete	Complete	Marked	Distinct	Trace	0	0
8. Kephalin-like substance (Product A)	Very Marked	Almost Complete	Just Complete	Complete	Very Marked	Marked	Distinct	0	0
9. Ether Insoluble fraction of material insoluble in boiling alcohol (Product D)	Trace	Trace	Distinct	Complete	Trace	0	0	0	0
10. Ether Insoluble fraction of material insoluble in boiling alcohol (Product D.)	Trace	Complete	Complete	Complete	Trace	0	0	0	0
11. Ether Soluble fraction of material insoluble in boiling alcohol (Product C.)	Trace	Complete	Complete	Complete	Complete	Marked	Trace	0	0
12. Lecithin from 1st Extract of Ox Liver obtained by boiling acetone precipitate with alcohol	Trace	Complete	Complete	Complete	Complete	Almost complete	Trace	0	0

Table 35.

Lecithins 0.75 per cent; emulsions 1 in 7 of 0.85 per cent NaCl solution	0.5 c.c. Ox Blood Suspension + Cobra Venom + Emulsion.					0.5 c.c. Ox Blood Suspension + Emulsion	
	0.005 c.c.	0.01c.c.	0.025 c.c.	0.05c.c.	0.1 c.c.	0.2 c.c.	0.35 c.c.
I.Dried Blood, with spirit	0	0	Very Marked	Complete		Complete	Complete
II.'Wet' Blood, 3rd Extract with spirit	0	0	Distinct	Marked	Just Complete	0	0

Table 36.

Iodine Values.		
	Antipyrine Method	Von Hübl.
Lecithin A	112	112
- B	88	85
- C	89	90

Table 37.

0.1 gm. Lecithin + 10 c.c. Iodine solution + 10 c.c. $HgCl_2$ solution.	Amounts of Antipyrin equivalent to unabsorbed Iodine.	Antipyrin Equivalent to absorbed Iodine	Iodine Values
Ox Liver (460 gms) 1st Extract	13.3 c.c.	3.2 c.c.	81
" " 2nd "	13.4 c.c.	3.1 c.c.	79
" " 3rd "	12.1 c.c.	4.4 c.c.	112
Heart (1015 gms.) 1st Extract	13.65c.c.	2.85c.c.	72
" " 2nd "	12.8 c.c.	3.7 c.c.	94
Dried Liver, 1st Extract.	14.95c.c.	1.55c.c.	40
Dried Liver, after 24 hrs. on hot plate.	14.55c.c.	1.95c.c.	50
Ox Heart Lecithin, 2 months old.	14.8 c.c.	1.7 c.c.	43
Ox Liver, 4th Extract, 7 months old.	13.0 c.c.	3.5 c.c.	90
Ox " , " " (lecithin removed from acetone precipitate by hot alcohol)	13.8 c.c.	2.7 c.c.	68
Control (no lecithin)	**16.5 c.c.**		

Table 38.

0.05 grms. of substance + 5 c.c. Iodine Solution + 5 c.c. $HgCl_2$ solution.		Amount of Antipyrine equivalent to un-absorbed iodine	Amount of Antipyrine equivalent to absorbed iodine	Iodine Values
0x Heart (1015 grms)	3rd Extract	7.2	1.1	63
" " " "	4th "	6.75	1.55	90
" " " "	5th "	6.0	2.3	132
" " (1350 grms)	5th "	6.3	2.0	115
" " " "	6th "	5.1	3.2	179
" Lungs,	1st Extract	6.05	0.9	52
" Testicles,	1st "	6.25	2.05	117
" Kidney	2nd "	7.15	1.15	65
" "	4th "	6.65	1.65	95
" Dried Kidney,	2nd "	6.95	1.35	77
" Spleen,	1st "	7.65	0.65	37
" "	2nd "	7.05	1.25	71
" Thyroid,	1st "	7.1	1.2	69
" Pancreas,	1st "	7.65	0.65	37
" Dried Brain,	3rd "	7.4	0.9	52
" Fresh "	3rd "	6.75	1.55	90
0x Liver (460 grms)	4th "	6.05	2.25	129
0x Heart (1350 grms)	4th "	6.65	1.65	95
Control		8.3		

Table 39.

0.1 gm. Lecithin + 10 c.c.s Iodine Solution + 10 c.c. $HgCl_2$ solution	Amounts of Anti-pyrine equiva-lent to unabsorb--ed Iodine	Amounts of Anti-pyrine equiva-lent to Absorbed Iodine	Iodine Values
Ox Heart (1350 gms) 1st Extract 3 week's old	9.25 c.c.	6.55 c.c.	166
" " " 2nd " 2 days' old	12.1 c.c.	3.7 c.c.	94
" " " 2nd " by boiling acetone precipitate	12.6 c.c.	3.2 c.c.	81
" " " 3rd Extract, 1 day old	10.65 c.c.	5.15 c.c.	131
" " " 4th " 1 day old	12.4 c.c.	3.4 c.c.	86
Ox Liver (460 gms) 4th Extract, 5 days' old	10.0 c.c.	5.8 c.c.	147
" " " 5th " 1 day old	12.5 c.c.	3.3. c.c.	84
Cobra Lecithid prepared from ox liver	15.7 c.c.	0.1	2
Cobra Lecithid " " egg yolk	15.7 c.c.	0.1	2

Control 10 c.c. Iodine + 10 c.c. $HgCl_2$ solution = 15.8 c.c.

Table 40a.

0.1 grms. of Substance + 10 c.c. Iodine solution + 10 c.c. $HgCl_2$ solution.	Amount of antipyrine equivalent to unabsorbed iodine.	Amount of antipyrine equivalent to absorbed Iodine	Iodine Values
1. Ether insoluble fraction of 1st Acetone precipitate of dried egg yolk, portion soluble in cold alcohol.	12.5 c.c.	4.4 c.c.	112
2. Ether insoluble fraction of 1st Acetone precipitate from mixture of dried tissues extracted with spirit, portion soluble in cold alcohol.	15.9 c.c.	1.0 c.c.	25
3. Ether insoluble fraction of 1st Acetone precipitate from mixture of dried tissues extracted with spirit, portion soluble in boiling alcohol, tested in watery solution.	16.1 c.c.	0.8 c.c.	20
4. Ether insoluble fraction of Acetic Ether precipitate (egg yolk)	14.0 c.c.	2.9 c.c.	73
5. Ether insoluble fraction of Acetone precipitate of Acetic Ether soluble portion (egg yolk extracted with spirit)	14.6 c.c.	2.3 c.c.	58
6. Lecithin egg yolk	14.7 c.c.	2.2 c.c.	56
7. Lecithin (egg yolk)	14.8 c.c.	2.1 c.c.	54
8. Lecithin (egg yolk) obtained by treating acetone precipitate with salt solution.	14.7 c.c.	2.2 c.c.	56
9. 'Lecithin' from precipitate obtained by treating 3rd Acetone precipitate with 80 per cent alcohol (egg yolk).	14.2 c.c.	2.7 c.c.	69
10. 'Lecithin' by same procedure using 50 per cent alcohol (egg yolk)	14.2 c.c.	2.7 c.c.	69
11. Lecithin from mixture of dried tissue obtained by treatment of acetone precipitate with watery alcohol.	15.8 c.c.	1.1 c.c.	27
12. Lecithin from mixture of dried tissue obtained by treatment of acetone precipitate with absolute alcohol.	15.8 c.c.	1.1 c.c.	27
13. Lecithin human liver, case of nephritis.	13.3 c.c.	3.6 c.c.	91
14. Riedel I	15.0 c.c.	1.9 c.c.	48
15. Riedel II	14.8 c.c.	2.1 c.c.	53
16. Poulenc	15.2 c.c.	1.7 c.c.	43
17. Merck	15.4 c.c.	1.5 c.c.	38

Table 40.b.

	Iodine Values.
1. Egg Yolk lecithin extracted by spirit after preliminary treatment of tissue with ether . . .	51
2. Lecithin from Malt Glidine	51
3. Egg Lecithin	61
4. Lecithin from 1st Extract of Ox Liver	76
5. Lecithin from 2nd Extract of same Liver	76
6. Liver Lecithin	86
7. Ox Liver Lecithin	81
8. Human " "	76
9. Egg Lecithin (old)	63
0. Egg Lecithin (new)	72
1. Ether Insoluble, alcohol soluble fraction of acetic ether precipitate of egg yolk	63

Table 41.

0.1 gm. Substance + 10 c.c. Iodine solution + 10 c.c. $HgCl_2$ solution	Amounts of Antipyrin equivalent to unabsorbed Iodine	Difference from Control	Iodine Values.
1. Lecithin 3rd Extraction of ox liver with spirit	4.7 c.c.	2.7 c.c.	70
2. Same lecithin after removal of material insoluble at 0°C.	3.4 c.c.	4.0 c.c.	101
3. Material Insoluble at 0°C.	6.9 c.c.	0.5 c.c.	13
4. Lecithin obtained after emulsifying acetone precipitate with 50 per cent alcohol	2.9 c.c.	4.5 c.c.	114
5. Lecithin obtained after emulsifying acetone precipitate with water.	3.4 c.c.	4.0 c.c.	101
6. Kephalin-like substance (watery solution)	3.7 c.c.	3.7 c.c.	94
7. Kephalin-like substance (dissolved in hot alcohol)	3.3 c.c.	4.1 c.c.	104
8. Materially initially soluble in hot alcohol but rendered insoluble by treatment.	4.9 c.c.	2.5 c.c.	63

Control, 10 c.c. alcohol + 10 c.c. Iodine solution + 10 c.c. mercuric chloride sol. = 7.4 c.c. antipyrin.

Table 42.

	Turbidity of the Lecithin Emulsions	State of the Lecithin-cholesterin emulsions after 12-18 hours
x Heart(1350gms)1st Extract	Very Marked +	Very Turbid Fluid, distinct sediment
" " 2nd "	Distinct	Turbid Fluid, marked sediment
" " 3rd "	Distinct	Milky Opalescent Fluid, no sediment
" " 4th "	Very Faint trace	Milky Opalescent Fluid, no sediment
" " 2nd " (by boiling)	Marked	Turbid Fluid, marked sediment
x Liver(460 gms)4th Extract	Very Faint Trace	Very Turbid Fluid, slight sediment
Fresh Brain, 2nd Extract	Distinct	Almost Clear Fluid, very marked sediment
Sheep's Red Corpuscles, 1st Extract	Very Distinct	Very Turbid Fluid, distinct separation
Dried Brain, 2nd Extract	Very Faint Trace	Almost Clear Fluid, very marked sediment
Dried Brain, alcohol soluble fraction of ether insoluble substance	Very Faint Trace	Distinctly Turbid Fluid, marked sediment
Dried Sheep's Corpuscles 1st Extract	Faint Trace	Very Turbid Fluid, slight separation

Table 43.

Preparation of lecithin	Turbidity of lecithin emulsions	Turbidity of lecithin-cholesterin emulsions	Iodine Values
Heart(1015gms)3rd Extract	Marked	Very Marked; distinct separation	56
" " 4th "	Very Marked	" " + trace of separation	80
" " 5th "	Very Marked	" " distinct separation	117
" (1350gms) 5th "	Very Marked	" " + trace of separation	101
" " 6th "	Very Marked +	" " distinct separation	162
Lungs, 1st Extract	Distinct	" " + distinct separation	46
Testicles, 1st Extract	Marked	" " distinct separation	104
Kidney, 2nd Extract	Distinct	" " distinct separation	58
" 4th "	Distinct	" " + trace of separation	84
Dried Kidney, 2nd Extract	Trace	Marked very distinct separation	68
Spleen, 1st Extract	Distinct	Very Marked, distinct separation	33
" 2nd "	Marked	" " distinct separation	63
Thyroid, 1st "	Marked	" " distinct separation	61
Pancreas, 1st "	Faint Trace	Trace Very marked separation	33
Dried Brain, 3rd Extract	Very faint Trace	Very Marked + Trace of separation	46
Fresh " 3rd "	Very Marked	" " Distinct separation	80
Liver(460gms)4th "	Very Marked	" " + Faint Trace of separation	114
Heart(1350) 4th "	Distinct	" " + Faint Trace of separation	84

Table 44.

Solutions 0.09 per cent in 0.85 per cent NaCl.	0.5 c.c. Ox Blood Suspension + Cobra Venom + Solution.			0.5 c.c. Ox Blood Suspension + Solution.		
	0.01 c.c.	0.025 c.c.	0.05 c.c.	0.4 c.c.	0.8 c.c.	2.8 c.c.
Product A, ox heart	Just Complete	Complete	Complete	Trace	Trace	Complete
" C, "	Complete	"	"	0	Faint trace	"
" A, ox liver	"	"	"	Trace	Marked	Almost Complete
" C, "	Very marked	"	"	"	"	"
" B "	Just complete	"	"	0	Faint trace	Marked

[illegible] indicate the results after 1 hour and after [illegible] hours in each case.

Table 45.

Solutions 0.75 per cent in 0.85 per cent NaCl diluted to 1 in 8 with 0.85 per cent NaCl.	0.5 c.c. Ox Blood Suspension + Cobra Venom + Solution						0.5 c.c. Ox Blood Suspension + Solution		
	0.01 C.C.	0.025 c.c.	0.05 c.c.	0.1c.c.	0.2c.c.	0.4c.c.	0.4c.c.	1.0c.c.	2.8c.c
1.Kephalin-like substance from Ox Heart 2nd extraction	0 Just Complete	Marked Complete	Very Marked Complete	Complete Complete	Just Complete Complete	Marked Complete	0 Trace	0 Trace	0 Complete
2.Kephalin-like substance from Ox Liver (460 gms) 3rd Extraction	0 Complete	Marked Complete	Very Marked Complete	Marked Complete	Distinct Complete	0 Complete	0 Trace	0 Marked	0 Almost Complete
3.Cuorin-like material from Ox Heart 2nd Extraction	0 Complete	Marked Complete	Very Marked Complete	Marked Complete	Marked Complete	0 Complete	0 0	0 Faint Trace	Marked Complete
4.Cuorin-like material from Ox Liver (460 gms) 3rd Extraction	0 Just Complete	Marked Complete	Very Marked Complete	Marked Complete	Marked Complete	0 Complete	0 0	0 Faint Trace	0 Marked

The readings indictate the results after 1 hour and after 15 hours in each case.

Table 46.

Cuorin, prepared by Erlandsen's method : emulsion in salt solution 3.5 per cent.

0.5 c.c. 5 per cent Ox Blood Suspension.

	0.01 c.c.	0.03 c.c.	0.06 c.c.	0.09 c.c.	0.3 c.c.
+ Cobra Venom	0	0	0	0	0
	0.3 c.c.	0.5 c.c.	0.75 c.c.	1.0 c.c.	1.5 c.c.
No cobra venom	0	0	0	0	0

Table 47.

Serum ($\frac{1}{2}$ hour at 57°C) 0.025 c.c. + Emulsion 0.3 c.c.		Amounts of Guinea pig's Complement.				
		0.01 c.c.	0.03 c.c.	0.06 c.c.	0.09 c.c.	0.15cc
Syphilitic Serum	Liver Lecithin	0	Trace	Distinct	Just Complete	Complete
	" " + 1 per cent Cholesterin	0	Very Faint Trace	Faint Trace	Trace	Very Marked
	Liver Lecithin + 1 per cent White Product	0	Trace	Trace	Distinct	Complete
		0.01 c.c.	0.025c.c.	0.04 c.c.	0.06 c.c.	0.09cc
Normal Serum	Liver Lecithin	Distinct	Almost Complete	Just Complete	Complete	Complete
	" " + 1 per cent Cholesterin	Very faint trace	Distinct	Just Complete	Complete	Complete
	Liver Lecithin + 1 per cent White Product	Marked	Just Complete	Complete	Complete	Complete

	Emulsions alone.			
	0.01 c.c.	0.02 c.c.	0.03 c.c.	0.6c.c
Liver Lecithin	Marked	Almost Complete	Complete	Complete
" " + Cholesterin	Trace	Almost Complete	Complete	Complete
" " + White product	Very Faint trace	Faint Trace	Marked	Very Marked

	1.0 c.c. Ox Blood Suspension + Cobra Venom + Emulsion.						
	0.005 c.c.	0.01c.c.	0.015c.c.	0.025c.c.	0.05c.c.	0.075c.c.	0.1 c.c.
Lecithin	Distinct	Almost Complete	Complete	Complete	Complete	Complete	Complete
Lecithin + White product	0	0	0	0	0	0	0
Lecithin + Cholesterin	0	0	0	0	0	0	0

Table 48.

Solutions 0.1 per cent in 0.85 per cent NaCl.	1 c.c. Ox Blood Suspension + Venom + Product.			1 c.c. Ox Blood Suspension + Solution		
	0.005 c.c.	0.01 c.c.	0.025 c.c.	0.5 c.c.	1.0 c.c.	2.0 c.c.
Fraction of acetone precipitate insoluble in cold or hot alcohol (ox liver)	Distinct	Very marked	Complete	0	0	0
Fraction of first acetone precipitate insoluble in ether and alcohol (ox liver)	Complete	Complete	"	0	0	0
Fraction of first acetone precipitate from ether extract of ox heart, insoluble in cold alcohol	Just Complete	"	"	0	0	Trace

b) Kerry Liver Kephalin. Faint Trace | Trace

" " + Cholesterin 0 | 0

Horse serum 0.025 c.c. + 0.018 c.c. Complement = Inc

Cytolytic serum 0.025 c.c. + 0.025 c.c. " = "

Dose of Complement = 0.0075 c.c.

Table 49.

Kephalin-like substances 0.75 per cent 1 part + 0.85 per cent Na Cl 7 parts, emulsions slowly made 0.3 c.c. + Serum (½ hr. at 57°C) 0.025 c.c.		Amounts of Guinea Pig's Complement.			
		0.02 c.c.	0.04 c.c.	0.06 c.c.	0.09 c.c.
Normal Serum	a) Ox Liver Kephalin	Just Complete	Complete	Complete	Complete
	" " " + Cholesterin	Very Marked	Just Complete	Complete	"
	b) Fatty human liver Kephalin	Just Complete	Complete	"	"
	Fatty human liver Kephalin + Cholesterin	0	Marked	Very Marked	Complete
Syphilitic Serum	a) Ox Liver Kephalin	Just Complete	Complete	Complete	Complete
	" " " + Cholesterin	0	Very faint trace	Marked	Complete
	b) Fatty human liver Kephalin	Very Marked	Complete	Complete	Complete
	Fatty human liver Kephalin + Cholesterin	0	0	0	Trace

Emulsions alone.	0.015 c.c.	0.03 c.c.	0.05 c.c.
a) Ox Liver Kephalin	Distinct	Marked	Complete
" " " + Cholesterin	0	0	0
b) Fatty Liver Kephalin	Faint Trace	Trace	Complete
" " " + Cholesterin	0	0	0

Normal serum 0.025 c.c. + 0.015 c.c. Complement = Just Complete

Syphilitic serum 0.025 c.c. + 0.015 c.c. " = " "

Dose of Complement = 0.0075 c.c.

The effect of introducing lecithin into serum.

It has been known for some time that the haemolytic action of cobra venom may be affected by the presence of serum. Thus, the fresh serum of the guinea pig markedly increases the haemolytic effect of cobra venom. Certain human sera, after heating at 58°C. produce haemolysis of horse's corpuscles in the presence of venom and this property has been used for diagnostic purposes in cases of tuberculosis and carcinoma. In a number of other pathological conditions, to some of which reference has already been made, increased haemolytic activity of the serum along with cobra venom has been regarded as indicating an increased lecithin content of the blood. In the case of fresh guinea pig's serum, however, the action would appear to be somewhat different. It has been shown by Kyes and Sachs that the haemolytic effect of fresh serum in the presence of venom can be removed by a variety of procedures which do not affect lecithin-venom-haemolysis and that the fresh serum in sub-lytic doses does not aid but inhibits the haemolytic effect of lecithin. Further, the fluid which results from the haemolysis of corpuscles susceptible to the action of venom causes haemolysis along with venom of blood corpuscles which are insusceptible to cobra venom. This has been attributed to the liberation of lecithin from the stromata of susceptible corpuscles. Kyes is, therefore, of opinion that the activating effect of fresh serum in the presence of venom results from a preliminary lysis of corpuscles by the action of the serum itself and that the lecithin so set free ('endocomplement') further increases the haemolysis by acting along with cobra venom. It has been shown (Morgenroth and Kaya) that a solution of cobra venom in normal salt solution when heated/

heated at 70°C. loses the property of causing haemolysis along with fresh serum but retains the power of causing haemolysis along with lecithin. By the use of such heated venom Browning and Mackie have shown recently that the haemolytic effect of laked blood (endocomplement) is due to lecithin. If Kyes' conclusions regarding the indirect nature of the activating effect of fresh serum be accepted as correct it is somewhat difficult to understand why such heated venom does not act with fresh serum since in this case also the haemolysis is due to endocomplement set free by the lytic effect of the serum itself. While I was considering this question it occurred to me to try the effect of the introduction of lecithin into serum. The results were highly interesting.

To 1 c.c. of ordinary human serum 1 c.c. of a 9.22 per cent yolk lecithin in alcohol was added. A fairly abundant granular precipitate was formed. This was removed by centrifuging when the supernatant fluid was found to be densely turbid. The haemolytic activity of this fluid for ox red blood corpuscles was the same as that of the original serum. In the presence of cobra venom, however, the turbid lecithin serum mixture was found to be about 100 times more active than was the serum itself similarly tested. (v. table I) A considerable amount of lecithin active for venom was therefore present. In addition, it was found that the amount of precipitate which was produced by a given amount of lecithin depended on the mode of mixing the lecithin with the serum. Rapid mixture, as by ejecting the lecithin from a pipette, caused more lecithin to remain in solution than slow admixture, as by floating the lecithin on to the surface and slowly rotating the tube.

A control experiment using alcohol in place of lecithin/

lecithin solution was carried out. It was found that the addition of this amount of alcohol caused a precipitate to form (albumen). It appeared probable therefore that the precipitate produced by the addition of the lecithin did not consist wholly of lecithin. On reducing the amount of lecithin it was found that a considerable amount could be introduced without a precipitate resulting. The haemolytic activity in the presence of cobra venom was in direct proportion to the amount of lecithin added.

Fresh guinea pig's serum gave similar results. Somewhat unexpectedly it was found that the complement dose of the serum remained almost unaltered even after the addition of an amount of lecithin sufficient to cause an abundant precipitate. Removal of this precipitate by centrifugalising did not alter the complement effect.

As it had been shown by a number of workers that the complement activity of a serum was removed or markedly diminished by procedures which removed portion of the globulin (dialysis-Ferrata, Brand; the addition of HCl - Sachs and Altmann; passage of carbonic acid gas - Liefmann) it seemed of interest to examine the lecithin-serum more closely. 1 c.c. of fresh guinea pig's serum was diluted with 9 c.cs. of ice-cold distilled water and 0.15 c.cs. of the egg lecithin rapidly added by ejecting from a pipette. A turbid mixture resulted of which the haemolytic activity for sensitised corpuscles was the same as that of the fresh serum similarly diluted. The lecithin serum was then treated with carbon dioxide gas which precipitated portion of the globulin. Two fractions were thus obtained, a globulin fraction and a lecithin-albumen fraction. A sample of the same serum without lecithin was similarly treated. It was found that/

that the albumen fraction of the serum treated with lecithin was as active in haemolysis as the unsplit complement whereas, in confirmation of the results of others, the albumen fraction of the untreated serum was relatively without action. The experiment was repeated with another specimen of guinea pig's serum and the same result was obtained. An examination of a larger number of sera was then proceeded with. In this I was associated with Dr Mackie. The exact method of procedure was as follows.-

A steady stream of carbon di-oxide gas was allowed to pass through the serum mixture (kept ice-cold) for a period of ten minutes. A flocculent precipitate resulted. The mixture was allowed to stand for about 5 minutes at 0°C. and the precipitate was then removed rapidly and thoroughly by centrifugalising. Occasionally the supernatant fluid was again treated with carbon di-oxide; the fact that in no case was a further precipitate obtained showed that all the globulin precipitable by this method had been removed in the first instance. 10 per cent NaCl solution was added to bring the concentration of the fluid up to 0.85 per cent NaCl and the fluid was then exposed in a flat dish in the ice-chest for about an hour to remove the carbon di-oxide gas. The resulting fluid, which retained the turbidity of the original lecithin-serum dilution, constituted the lecithin-albumen fraction or "lecithin-end-piece". The globulin precipitate was washed once with ice-cold distilled water and was then rubbed up with a few drops of 0.85 per cent salt solution to effect solution. Finally 0.85 per cent salt solution was added to make up an amount equivalent to two volumes of original undiluted serum. A control sample of the same serum without lecithin was always treated in the same way and

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at the same time, the fractions so obtained serving as controls to those from the lecithin-serum. It was found that whereas the globulin precipitate in the case of the lecithin-serum was always easily and completely soluble (giving a turbid solution) the globulin from the native serum was incompletely soluble, a flocculent residue generally remaining in suspension.

Ledingham and Dean have stated recently that complete solution of the globulin can always be obtained if care is taken to keep the precipitate at a temperature of 0°C. I have not found this to occur with the guinea-pig sera which I have examined. These authors further state that particles of "foreign material" were generally present in the solutions of globulin. In my experience particles are constantly present in the solutions of guinea-pig's globulin and are not "foreign matter" but natural constituents of the globulin precipitate. No such particles occur in the solutions of the globulin from rabbit, ox and horse sera prepared under precisely similar conditions. In the case of guinea-pig sera the presence or absence of such particles is immaterial to the activity of the globulin when this is tested along with ordinary end-piece.

In these experiments the test corpuscles consisted of a 5 per cent suspension of washed ox-blood sensitised with five doses of immune body from the rabbit. The readings were taken after 1½ hours incubation at 37°C. and also after the tubes had stood at room temperature for about 18 hours further.

Results with Guinea-pig Serum.

In correspondence with the results of others 9 have found that in general the albumen and the globulin fractions prepared from ordinary complement serum have by themselves little haemolytic effect as compared with

the/

the whole serum, while a mixture of the two in suitable proportions is as active as the original complement. In the case of sera treated with lecithin the results were however very different. The lecithin-albumen fractions were always found to be as actively haemolytic as the whole complement. The results of the examination of 25 sera are shown in table 2. The globulin fractions from the lecithin sera were by themselves inactive but possessed in equally as full a degree as the ordinary globulins the power of restoring the complement effect to the albumen fractions of normal complement sera, (v. table 3). Careful repeated comparisons of the globulin fractions of the lecithin sera with those of the corresponding sera without lecithin did not reveal any differences in haemolytic activity when these fractions were tested in combination with the albumen fractions of untreated sera. The lecithin-globulin fractions were generally by themselves slightly more haemolytic than the corresponding ordinary globulin fractions. It was evident therefore, that the addition of lecithin to complement serum did not merely have the effect of preventing the "splitting" of the complement. On the other hand, the increased haemolytic activity of the lecithin-albumen fraction could not be explained merely by the presence of the lecithin, as the addition of lecithin to ordinary end-piece after separation did not result in any increase in the activity of the latter. It was also observed that the full effect of the lecithin was only obtained by adding the lecithin to the whole serum... Partial or complete separation of globulin before the addition of lecithin resulted in partially or completely inactive albumen fractions (v. table 4). The complementing action of the lecithin-albumen fractions was in fact directly proportional to the amount of whole serum present when the lecithin was added. The colloidal

state/

state of the lecithin in the serum was also of importance. Slow admixture of the lecithin solution with the serum, while giving a more turbid mixture, yielded lecithin end-pieces which were as inactive by themselves as ordinary albumen fractions (v. table5). To obtain active albumen fractions it was necessary to effect rapid mixture of the lecithin with the serum, e.g. by ejecting the lecithin solution rapidly from a pipette into the serum water. It was found to be immaterial whether the lecithin was first mixed with distilled water and the serum added or the serum first diluted and the lecithin then added. The whole procedure was carried through as rapidly as possible and the mixture treated with carbon di-oxide gas immediately. This was found to be of importance especially in the case of certain sera which on dilution with ice-cold water rapidly yielded globulin precipitates without further treatment.

This effect was not produced by the presence of alcohol as control experiments using alcohol in place of lecithin solution showed.

The lytic effects of the lecithin-sera and their fractions for unsensitised ox's corpuscles were also tested. Even in comparatively large amounts there was absolutely no haemolysis (table 15). The age of the complement serum was found to be an important factor in some cases. As a rule the sera were employed within a few hours after withdrawal from the animal. In the case of older sera (24 - 36 hours) the lecithin end-piece was generally somewhat less active than the whole serum. Sera which had stood for some days without much loss of complement activity gave lecithin-albumen fractions which were by themselves almost as inactive as ordinary end-piece (v. table 6). As a rule the haemolysis with lecithin end-piece proceeded more slowly than that in the corresponding/

corresponding tubes containing either whole complement or complement reconstituted by mixing ordinary albumen and globulin fractions. The end point was, however, usually the same in each case.

The fact that the lecithin albumen fractions possessed a haemolytic activity equal to that of whole complement suggested that this fraction should be tested in other complement reactions and by the usual complement-absorbing agents. Table 7 shows the results obtained in the Wassermann reaction with lecithin end-piece and whole complement respectively. It will be seen that lecithin end-piece behaved like the whole complement in being deviated by the syphilitic serum in the presence of lipoid emulsions but not by the normal serum. A similar result was obtained on other occasions with different syphilitic and normal sera. As Michaelis and Skwirsky have shown that in the Wassermann reaction it is the globulin fraction or mid-piece of normal complement which is absorbed or deviated it was conjectured that lecithin end-piece might also possess middle-piece properties. In order to test this point the procedure suggested by Sachs & Bolkowska was resorted to.

This consists in treating complement for one hour at 0°C. with ox's corpuscles which have been previously sensitised with 40 doses of the corresponding immune body from the rabbit, centrifugalising the mixture and testing the sedimented corpuscles by adding ordinary end piece. Lysis occurs owing to the corpuscles having become per-sensitised by the absorption of mid-piece from the complement.

Lecithin-end-piece, ordinary end piece and whole complement were tested in this manner. It was found that the corpuscles in the lecithin end piece series were per-sensitised like those treated with the original complement-containing serum whereas those subjected to treatment with ordinary/

ordinary end piece were unaffected. The results of actual experiments are given in table 8.

Lecithin end piece therefore contained middle piece properties.

Action of Complement absorbing Agents.

It is well known that the complement action of fresh serum can be abolished or markedly diminished by treatment of the serum with certain agents, e.g. sensitised red corpuscles or sensitised stromata. It seemed of interest to examine the action of such agents on the lecithin-albumen fraction. It was found (v. table 9) that the complement action of lecithin-end-piece was markedly diminished by such treatment although not to the same degree as the whole complement treated similarly at the same time. In the case of such 'absorbed' lecithin end-piece the addition of a small amount of mid-piece completely restored the complement activity whereas, the complement itself after absorption was not reactivated by this means.

The addition of lecithin to complement after absorption did not restore its haemolytic value. Also, lecithin-complement was absorbed quantitatively to the same extent as ordinary complement when treated with these absorbing agents. In the course of these experiments the albumen and globulin fractions obtained by splitting a serum after treatment with sensitised ox's corpuscles or sensitised stromata were examined. It was found that the albumen fractions did not produce haemolysis along with ordinary mid-piece, whereas, the globulin fractions were quite active when tested along with ordinary end-piece, that is to say, the albumen fractions only were deficient in activating power while the same 'absorbed' complement was apparently deficient in mid piece.

Experiments/

Experiments with Rabbit and other Sera.

In the case of rabbit's serum most workers have found that the complement action is generally not restored by mixing the albumen and globulin fractions in the proportions present in the original serum. This has been shown to be due to some defect of the albumen fraction. For example the globulin of rabbit's serum activates the albumen fraction of guinea-pig's serum when used in guinea-pig doses, but the albumen fraction of rabbit's serum does not act along with guinea-pig globulin in any doses. In my experience restoration of the complement action by mixtures of the albumen and globulin fractions of the rabbit occurred not uncommonly.

The addition of lecithin to rabbit's serum, as in the case of the guinea-pig, alters the action of the complement components, that is to say, the lecithin albumen fraction is as active as the whole serum (v. tables 10&11). It was noted, however, that the addition of lecithin to rabbit's serum frequently caused a marked increase in its complement activity and, in addition, that the lecithin-albumen fraction from a lecithin-serum was also more active (3 or 4 times) than the ordinary serum. The complement activity of rabbit's serum to that of guinea-pig serum was generally in the ratio of 1-20. The albumen fractions of lecithin rabbit sera, nevertheless, when used in doses corresponding to those of guinea-pig's end-piece were found in some instances to be capable of producing haemolysis along with guinea-pig globulin in guinea-pig doses. In other words the complement value of the lecithin rabbit end-piece was quantitatively very greatly increased as compared with ordinary rabbit end-piece (vide table 10).

Two/

Two specimens of horse complement were tested. In both cases the serum was several days old; the lecithin end-piece was found to be not more active than the ordinary horse end-piece. On two occasions however, the addition of lecithin caused a distinct increase in the complementing power of the whole serum(vide table 12).

When the constancy of the phenomena described above in the case of guinea-pig and rabbit sera had been established, it became necessary to enquire further into the nature of the alteration which had been brought about and especially to consider whether the increase in haemolytic power depended really on 'bodies' of the nature of complement. It was found in the first place that procedures which destroyed complement also destroyed the action of the lecithin serum and of its components. Heating at 55°C. for half an hour, for example, completely inactivated lecithin-serum, lecithin-end-piece and lecithin-mid-piece, while the addition of lecithin to serum previously inactivated by heating did not cause any restoration of the activity of the serum or of its components. The action of the lecithin therefore was dependent on the presence of 'complement' in the serum. Further, the addition to ordinary end-piece of lecithin-end-piece, obtained from a serum previously inactivated, did not enhance the haemolytic action of the former. Similarly a mixture of lecithin with mid-piece previously inactivated by heat did not increase the effect of ordinary end-piece. Lecithin-albumen and lecithin-globulin prepared from fresh egg-white also failed to produce haemolytic action along with the albumen and globulin fractions of fresh guinea-pig's serum.

The amount of lecithin which was necessary for the production of the reaction was tested (vide table 13). It is seen that diminution of the amount of lecithin is accompanied by decreasing activity of the lecithin-albumen fractions. Amounts of lecithin greater than that generally used while giving a much greater turbidity did not yield more active albumen fractions.

All specimens of lecithin were not suitable for the production of the phenomena. The results of the examination of a number of preparations are given in tables 4 & 7. It will be seen that a considerable number is quite inefficient. This group includes all the commercial preparations except one. Practically all the specimens gave a turbidity when added to serum but in quite a number the lecithin was precipitated along with the globulin fraction the albumen fraction thus being clear or almost clear. The clear albumen fractions without exception, were inefficient. (Some idea of the amount of lecithin which remains in the end-piece can be obtained by testing with cobra venom (v table 15). On the other hand, certain specimens which gave turbid albumen fractions also failed to give the reaction. The egg yolk lecithins were almost all active, as also the alcohol soluble portion of an ether insoluble fraction obtained in course of preparing lecithin from egg yolk. One freshly prepared egg yolk lecithin which failed to give the reaction was tested several weeks later and found to be quite efficient. The lecithin with which the reaction was first discovered still retains the property after an interval of a year. No correspondence between this function and any other property of any of the lecithins has yet been found.

These experiments are of interest as regards their bearing on the nature of complement action. By the use of certain lecithins the character of the components of complement are markedly altered and the complement activity in the case of certain rabbit and horse sera may even be increased. The results cannot be explained by supposing that incomplete splitting of the complement occurs as the globulin fractions from the lecithin sera have/

have been shewn to retain in apparently undiminished degree the capacity of acting along with ordinary albumen fractions. Noguchi and Bronfenbrenner have recently suggested that 'splitting' of the complement does not occur in treating sera with globulin precipitating agents but that the whole complement is retained by the albumen fraction, the complement action of this fraction being however inhibited by the presence of certain acids or alkalis which are developed in the process of precipitating the globulin. In support of this theory these workers have shown that ordinary albumen fraction is reactivated by the addition of certain neutral substances such as alanine. It does not appear that lecithin acts in this way. Otherwise the addition of lecithin to ordinary albumen fraction after splitting of the serum would render this fraction active. It seems probable that the lecithin acts by rendering active a component of complement which is normally present in an inactive or latent state. In the case of guinea-pig's serum this component resembles in its properties ordinary globulin fraction but differs in not being precipitated by the passage of carbon di-oxide gas. Some modification of or addition to this theory is, however, necessary in order to explain the increase of complement activity which has been shown to result with certain rabbit and horse sera merely on the addition of lecithin. In the case of rabbit's sera, as already noted, it has been shown that the globulin fraction is present in apparent excess of the amount necessary to complement the albumen fraction. The lecithin in this instance would appear to increase the complement activity by acting like albumen fraction. As an active albumen fraction is, however, obtained on splitting lecithin rabbit serum, the globulin fraction/

fraction properties must also have been altered. In considering these matters it is necessary to remember that in the opinion of a number of workers no rigid line of separation can be drawn between the properties of the two complement components since in certain reactions they are apparently capable of replacing each other when used in considerable amount.

Certain workers (Liebermann and Fenyvessy, Bang) are of opinion that complement is of lipoid nature. The experimental evidence in support of this depends mainly on the fact that sodium oleate in the presence of rabbit's serum is more haemolytic towards corpuscles sensitised with the corresponding immune body than towards the same corpuscles unsensitised. In the experiments described above the production of an actively haemolytic albumen fraction by means of lecithin was so inseparably associated with the presence of 'complement' in the serum that the results cannot be said to give any support to such a theory.

As regards the differences which have been elicited in the various lecithins by means of this test no explanation can be given. It has not yet been determined whether the property belongs to lecithin itself or to some associated impurity.

Table 1.

1 c.c. of 9.22 per cent egg yolk lecithin added to 1 c.c. of human serum : resulting precipitate removed	1.0 c.c. ox blood suspension + Cobra Venom 0.1 c.c. of 1:1000 + Serum.			
	0.000125 c.c.	0.0025 c.c.	0.00125 c.c.	0.0025 c.c.
Lecithin Serum	0	0	Complete	Complete

	0.01 c.c.	0.03 c.c.	0.06 c.c.	0.1 c.c.	0.14 c.c.
Serum	0	Very faint trace	Distinct	Just Complete	Complete

14.	0.005 c.c.	0.005
15.	0.0075 c.c.	0.0075
16.	0.0075 c.c.	0.02 c
17.	0.005 c.c.	0.005
18.	0.005 c.c.	0.0075
19.	0.005 c.c.	0.0075
20.	0.01 c.c.	0.015

Table 2.

Minimum Haemolytic Dose, for 0.5 c.c. 5 per cent suspension of ox blood + 5 doses of immune body.

Experiment.	Untreated guinea-pigs' serum.	Albumen fractions of same sera treated with lecithin. (doses corresponding to amounts of undiluted serum).
1.	0.005 c.c.	0.005 c.c.
2.	0.015 c.c.	0.015 c.c.
3.	0.01 c.c.	0.01 c.c.
4.	0.01 c.c.	0.01 c.c.
5.	0.01 c.c.	0.0075 c.c.
6.	0.015 c.c.	0.018 c.c.
7.	0.02 c.c.	0.015 c.c.
8.	0.005 c.c.	0.01 c.c.
9.	0.0075 c.c.	0.02 c.c.
10.	0.0075 c.c.	0.005 c.c.
11.	0.005 c.c.	0.0075 c.c.
12.	0.01 c.c.	0.01 c.c.
13.	0.0075 c.c.	0.0075 c.c.
14.	0.005 c.c.	0.005 c.c.
15.	0.0075 c.c.	0.0075 c.c.
16.	0.0075 c.c.	0.02 c.c.
17.	0.005 c.c.	0.005 c.c.
18.	0.005 c.c.	0.0075 c.c.
19.	0.005 c.c.	0.0075 c.c.
20.	0.01 c.c.	0.015 c.c.

Table 3.

Lysis of 0.5 c.c. 5 per cent suspension ox blood + 5 doses of immune body.

	0.05 c.c.	0.075 c.c.	0.1 c.c.	0.125 c.c.	0.15 c.c.
Complement (diluted 1 in 10)	Very marked	Almost Complete	Just Complete	Complete	Complete
"Lecithin - Complement" (diluted 1 in 10)	Marked	Just Complete	Complete	Complete	Complete
"Lecithin-end-piece" (diluted 1 in 10)	Very Marked	Just Complete	Complete	Complete	Complete
	0.05 c.c.	0.1 c.c.	0.2 c.c.	0.4 c.c.	0. 6 c.c.
Ordinary end-piece (diluted 1 in 10)	0	0	0	0	0
	0.01 c.c.	0.02 c.c.	0.04 c.c.	0.08 c.c.	0.12 c.c.
"Lecithin-middle-piece" (diluted 1 in 2).	0	0	0	Trace	Very marked
Ordinary middle piece (diluted 1 in 2)	0	0	0	0	0
"Lecithin middle-piece" dil. 1 in 2 + Ordinary end piece (dil. 1 in 10)	0.01 c.c. + 0.05 c.c. Distinct	0.02 c.c. + 0.1 c.c. Very Marked	0.04 c.c. + 0.2 c.c. Complete	0.08 c.c. + 0.4 c.c. Complete	0.12 c.c. + 0.6 c.c. Complete
Ordinary middle-piece dil.1 in 2 + Ordinary end-piece (dil. 1 in 10)	0.01 c.c. + 0.05 c.c. Distinct	0.02 c.c. + 0.1 c.c. Marked	0.04 c.c. + 0.2 c.c. Almost Complete	0.08 c.c. + 0.4 c.c. Complete	0.12 c.c. + 0.6 c.c. Complete
	0.05 c.c.	0.1 c.c.	0.2 c.c.	0.4 c.c.	0.6 c.c.
Ordinary end-piece (dil. 1 in 10) + Lecithin	0	0	0	0	0

Table 4.

Lysis of 0.5 c.c. 5 per cent suspension of ox blood + doses of immune body.

	0.05c.c.	0.075 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.	
"Lecithin-end-piece" diluted 1 in 10.	Almost Complete	Just Complete	Complete			
"Lecithin-end-piece" diluted 1 in 10 Globulin separated partially before lecithin added to serum.	Faint Trace	Trace	Distinct	Almost Complete	Just Complete	
Ordinary End-piece diluted 1 in 10.	0	0	0	0	0	

Dose of Complement 0.0075 c.c.

Table 5.

Lysis of 0.5 c.c. 5 per cent suspension of ox blood + 5 doses of immune body.

	0.05 c.c.	0.075c.c.	0.1c.c.	0.2 c.c.	0.3 c.c.
End-piece of complement treated with lecithin in form of slowly made emulsion. (dilution 1 in 10)	0	0	0	0	0
Lecithin-end-piece prepared in usual way. (dilution 1 in 10)	Almost Complete	Complete			

Complement dose 0.005 c.c.

Table 6.

Lysis of 0.5 c.c. 5 per cent suspension of ox blood + 5 doses of immune body.

	0.05 c.c.	0.075 c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.
"Lecithin-end-piece" (diluted 1 in 10). Serum a few hours old.	Almost Complete	Just Complete	Complete		
"Lecithin-end-piece" (diluted 1 in 10). Serum one week old.	0	0	0	0	0

Dose of Complement, serum a few hours old = 0.01 c.c.
" " " " " one week old = 0.01 c.c.

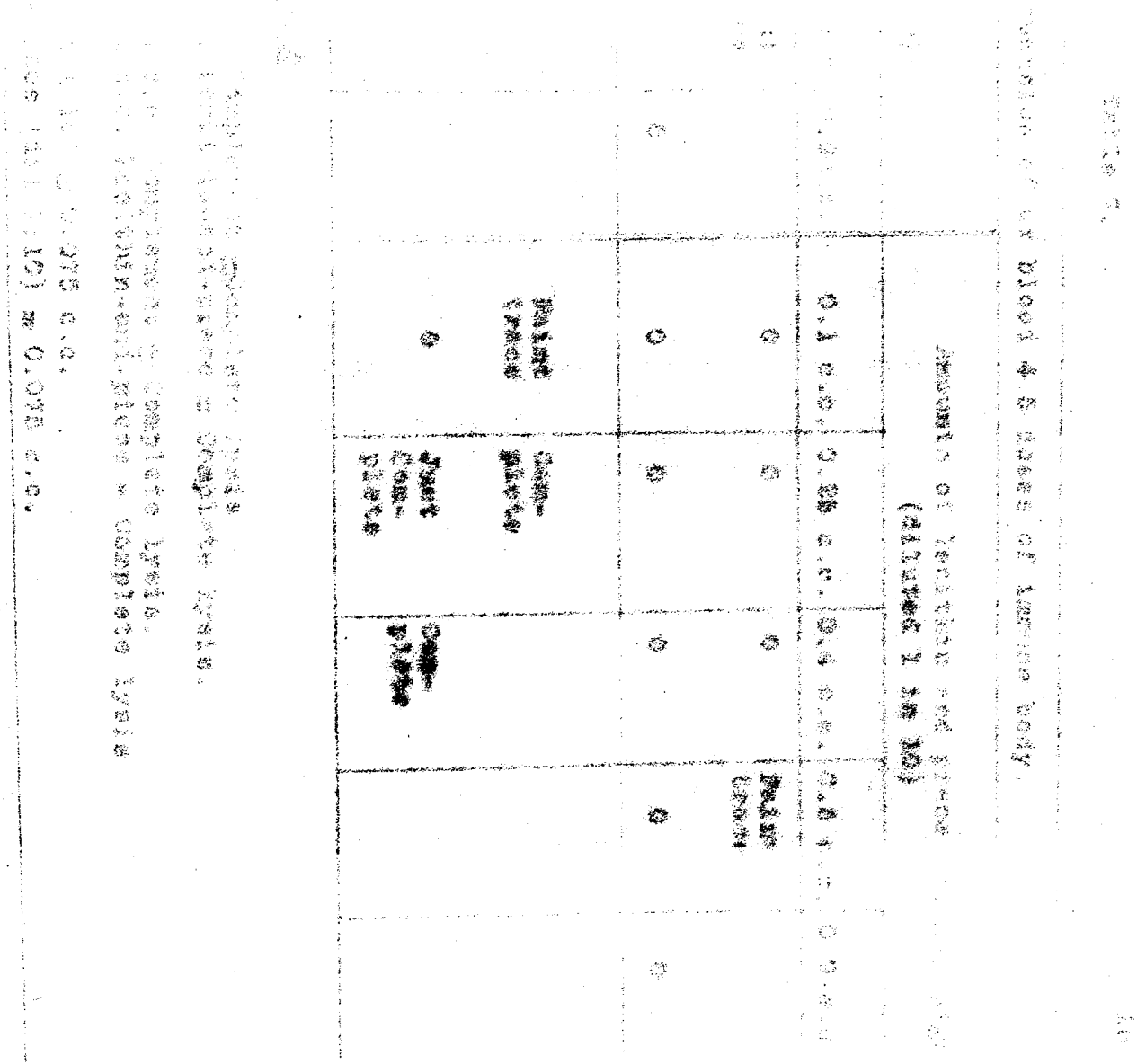


Table 7.

Lysis of 0.5 c.c. 5 per cent suspension of ox blood + 5 doses of immune body.

Human Sera. (55°C for ½ hour)	Emulsion	Amounts of guinea-pigs' complement (diluted 1 in 10)					Amounts of lecithin end piece (guinea-pigs') (diluted 1 in 10)				
0.025 c.c.	0.3 c.c.	0.1c.c.	0.25c.c.	0.4c.c.	0.6c.c.	0.9c.c.	0.1 c.c.	0.25 c.c.	0.4 c.c.	0.6 c.c.	0.9 c.c.
Syphilitic	Lecithin	0	0	0	Faint trace		0	0	0	Faint trace	
	Lecithin-Cholesterin	0	0	0	0	0	0	0	0	0	0
Normal	Lecithin	Faint trace	Just Complete	Complete			Faint trace	Complete			
	Lecithin-Cholesterin	0	Very Marked	Complete			0	Just Complete	Complete		

CONTROLS

Sera alone) + 0.1 c.c. Complement = Complete lysis.
0.025 c.c.) + 0.1 c.c. Lecithin-end-piece = Complete lysis.

Emulsions alone) + 0.1 c.c. Complement = Complete lysis.
0.3 c.c.) + 0.1 c.c. Lecithin-end-piece = Complete lysis

Dose of Complement (dil 1:10) = 0.075 c.c.
Dose of Lecithin-end-piece (dil 1:10) = 0.075 c.c.

Table 8.

In each tube, amounts of lecithin end-piece, diluted complement, and ordinary end-piece, equivalent to 0.05 of original complement, treated for 1 hour at 0°C. with 1 c.c.5 per cent ox blood suspension + 40 doses of immune body from the rabbit: corpuscles separated by centrifugalization and tested with varying amounts of ordinary end piece.

1 c.c. ox blood suspension + 40 doses I B. after treatment at 0°C with	Ordinary-end-piece diluted 1 in 10					No End-piece
	0.05 c.c.	0.1c.c.	0.2c.c.	0.3c.c.		
1. Lecithin end-piece	Marked lysis	Very marked lysis	Almost Complete lysis			No lysis
2. Complement	No lysis	Trace of lysis	Distinct lysis	Almost complete lysis		No lysis
3. Ordinary end piece	No lysis	No lysis	No Lysis	No lysis		No lysis

Dose of Complement for 1 c.c. ox suspension + 5 doses of immune body = 0.01 c.c.

Dose of lecithin-end-piece " = 0.1 c.c.
dilution 1 in 10

Table 9.

Guinea pig's complement (diluted 1 in 10) and lecithin end-piece (dil. 1 in 10) treated for 1½ hrs. at 37°C with excess of sensitised Ox red corpuscle stromata, and tested with 0.5 c.c. 5 per cent suspension of ox blood + 5 doses of immune body.

	0.05 c.c.	0.075 c.c.	0.1 c.c.
Complement (dil, 1 in 10)	Very marked lysis	Almost Complete lysis	Complete lysis
Lecithin end-piece (dil. 1 in 10)	Distinct lysis	Almost Complete lysis	Complete lysis
	0.3 c.c.	0.5 c.c.	1.4 c.c.
Absorbed Complement (dil. 1 in 10)	No lysis	Distinct trace lysis	Very marked lysis
	0.1 c.c.	0.3 c.c.	0.6 c.c.
Absorbed lecithin end-piece (dil. 1 in 10)	No lysis	Distinct trace lysis	Very marked lysis
Absorbed complement (dil, 1 in 10) + Middle piece (dil. 1 in 2)	0.1c.c. + 0.02 c.c. No lysis	0.5 c.c. + 0.1 c.c. distinct lysis	
Absorbed lecithin end-piece (dil. 1 in 10) + Middle piece (dil, 1 in 2)	0.1c.c. + 0.02 c.c. Complete lysis		
Middle piece (dil. 1 in 2)	0.06 c.c.	0.1 c.c.	0.24 c.c.
	No lysis	No lysis	No lysis

Table 10.

Lysis of 0.5 c.c. 5 per cent ox blood suspension + 5 doses of immune body.

Experiment	Untreated Serum	Serum treated with lecithin (lecithin serum)	End-piece from "lecithin-serum" (lecithin-end-piece)	Lecithin-end-piece + (Rabbit Ordinary end-piece (Guinea pig)
1	0.12 c.c. = complete		0.045 c.c. = Complete	
2	0.25 c.c. =complete	0.06 c.c. = just complete	0.06 c.c. = Complete	of each 0.01 c.c. = complete
3	0.07 c.c. =complete	0.075 c.c. = Complete	0.075 c.c. = Complete	of each 0.02 c.c. = complete
4	0.02 c.c. Just =Complete		0.05 c.c. = Complete	of each 0.04 c.c. = complete
5	0.2 c.c. =Almost Complete	0.15 c.c. = Almost complete	0.05 c.c. = complete	of each 0.2 c.c. = complete

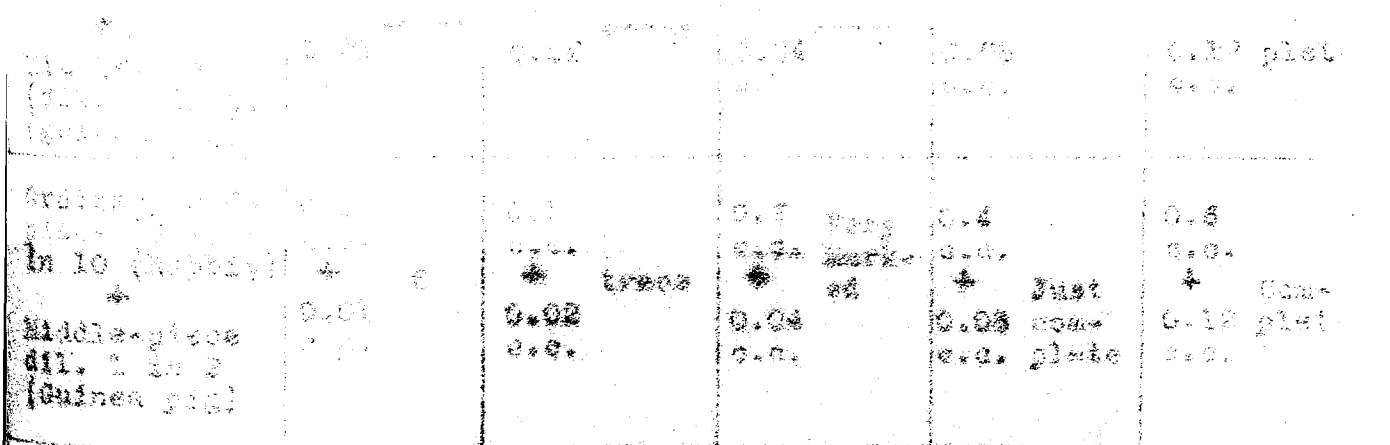


Table 11.

Rabbit's Serum.

Lysis of 0.5 c.c. 5 per cent suspension of ox blood + 5 doses of immune body.

	0.6 c.c.	1.0 c.c.	1.4 c.c.	1.7 c.c.	2.5 c.c.
Complement (dil, 1 in 10)	Trace	Distinct	Marked	Very Marked	Complete
Lecithin-complement (dil.1 in 10)	Just Complete	Complete			
Ordinary end-piece (dil. 1 in 10)	0	0	0	0	0
"Lecithin end-piece" (dil. 1 in 10)	Just Complete	Complete			
	0.12 c.c.	0.2 c.c.	0.28 c.c.	0.34 c.c.	0.5 c.c.
Ordinary middle-piece (dil.1 in 2)	0	0	0	0	0
"Lecithin middle-piece" (dil. 1 in 2)	0	0	0	0	Trace
"Lecithin-end piece" (dil. 1 in 10(Rabbit) + Middle-piece (dil. 1 in 2) (guinea-pig)	0.05 c.c. + 0.01 c.c. Distinct	0.1 c.c. + 0.02 complete	0.2 c.c. + 0.04 c.c. Complete	0.4 c.c. + 0.08 c.c. Complete	0.6 c.c. + 0.12 c.c. Complete
Ordinary end-piece (dil. 1 in 10 (Rabbit) + Middle-piece dil. 1 in 2 (Guinea pig)	0.05 c.c. + 0.01 c.c. 0	0.1 c.c. + 0.02 c.c. trace	0.2 c.c. + 0.04 c.c. Very Marked	0.4 c.c. + 0.08 c.c. Just complete	0.6 c.c. + 0.12 c.c. Complete

Table 12.

Lysis of 0.25 c.c. 5 per cent Suspension of ox blood + 5 doses of immune body.

Experiment	Untreated Horse Serum	Horse Serum treated with lecithin	End-piece from untreated serum (dil. 1 in 10)	End-piece from lecithin serum
1	0.6 c.c. = faint trace	0.1 c.c. = trace 0.25 c.c. = Complete	2.5 c.c. = No lysis	2.5 c.c. = No lysis
2	0.1 c.c. = 0 0.15 c.c. = trace 0.3 c.c. = marked	0.1 c.c. = Almost Complete 0.15 = Complete		

Lysis of 0.5 c.c. 5 per cent ox blood suspension + 5 doses of immune body.

Amount of lecithin solution (per c.c. of guinea pig's serum) and in preparation of lecithin end-piece	Lecithin end-piece diluted 1 in 10					
	0.03c.c.	0.05c.c.	0.075c.c.	0.1 c.c.	0.2 c.c.	0.3 c.c.
0.15c.c. of 9 per cent lecithin solution	Distinct	Marked	Almost Complete	Just Complete	Complete	
0.15c.c. of 4.5 per cent lecithin solution	0	0	0	Marked	Just Complete	Complete
0.15c.c. of 2.25 per cent lecithin solution	0	0	0	Faint trace	Trace	Very marked
0.15c.c. of 1.125 per cent lecithin solution	0	0	0	0	0	Distinct
0.15c.c. of .5625 per cent lecithin solution	0	0	0	0	0	Faint trace
	0.2 c.c.	0.5 c.c.	0.8 c.c.			
End-piece from untreated complement (dil. 1 in 10)	0	Very faint trace	Faint trace			

Complement dose (Serum diluted 1 in 10) = 0.05 c.c.

Table 14.

Lysis of 0.5 c.c. 5 per cent ox blood suspension + 5 doses of immune body.

End-piece of guinea-pig's serum (dil. 1 in 10) treated with	0.05c.c.	0.075 c.c.	0.1c.c.	0.2c.c.	0.3c.c.	0.4c.c.	Turbidity of end-piece
Lecithin 1 (Egg yolk)	Just complete	Complete					Marked
Lecithin 2 (fresh egg yolk)	Faint trace	Trace	Trace	distinct	Marked		Marked
Lecithin 3 (Hypernephroma)	0	0	0	0	0		No turbidity
Lecithin 4 (egg yolk)	0	0	0	Marked	Complete		Marked
Lecithin 5 (egg yolk)	0	0	0	Distinct	Marked	Complete	Distinct
Lecithin 6 (egg yolk)	Just Complete						Marked
Lecithin 7 (egg yolk)	Distinct	Distinct	Marked	Marked	Just Complete	Complete	No turbidity
Lecithin 8 (ox liver)	Trace	Trace	Distinct	Distinct	Distinct	Distinct	No turbidity
Lecithin 9 (Liver)	Just complete						Marked
Lecithin 10 (egg yolk)	Complete						Marked
Lecithin (Poulenc)	0	Very faint trace	Very faint trace	Distinct	Distinct	Distinct	No turbidity
Lecithin Riedel (a) (egg yolk)	0	0	0	0	0		No turbidity
Lecithin Riedel (b) (egg yolk)	Almost complete	Complete					Distinct
Lecithin Merck (egg yolk)	0	0	0	trace	Distinct	Almost Complete	Marked
Lecithin 2 8 weeks after preparation	Complete						Marked

Lysis of 0.5 c.c. 5 per cent ox blood suspension + 5 doses of I.B.

End Piece (diluted 1 in 10) prepared from	0.05 c.c.	0.01 c.c.	.015 c.c.	0.25c.c.	0.5 c.c.	Turbidity of End piece
1.Heart Lecithin 1350 gms.	0	0	0	0	0	Very faint trace
2. " " 1015 "	0	0	0	0	0	Clear
3.Malt Glidine Lecithin	0	0	0	0	0	Clear
4.Liver Lecithin 4th Extract	0	0	0	0	0	Clear
5.Liver Lecithin 3rd Extract	0	0	0	0	0	Clear
6.Egg Yolk Lecithin No.I	Trace	Marked	Just complete	Complete	Complete	Very Marked
7.Egg Yolk No.II	Just Complete					Marked
8.Ether Insoluble, alcohol soluble of egg yolk	Marked	Very Marked	Just complete	Complete	Complete	Marked

	0.5 c.c. Ox Blood + Cobra Venom + End Piece.				0.5 c.c. unsensitised Corpuscles
	0.01 c.c.	0.028c.c.	0.05 c.c.	0.1c.c.	1.0 c.c.
1. Heart Lecithin 1350 gms	0	Distinct	Complete	Complete	0
2. " " 1015 "	0	Just Complete	Complete	Complete	0
3.Malt Glidine Lecithin	0	0	0	0	0
4.Liver Lecithin 4th Extract	0	0	0	0	0
5. " " 3rd Extract	0	0	0	Almost complete	0
6.Egg Yolk Lecithin No.I	Just Complete	Complete	Complete	Complete	0
7.Egg Yolk No.II	Just Complete	"	"	"	0
8. Ether Insoluble, alcohol soluble of egg yolk	Just Complete	"	"	"	0

Table 16.

Lysis of 0.5 c.c. 5 per cent Ox Blood Suspension + I.B.

Lecithin end-piece prepared by use of	0.005c.c.	0.01c.c.	0.02c.c.	0.04c.c.	0.075c.c.	
1.Ox Heart Lecithin	0	0	0	0	0	Clear
2.Sheep's Liver	0	0	0	0	0	Clear
3.Dried Human Brain	Trace	Just Complete				Clear
4.Ox Liver Lecithin	0	0	0	0	Distinct	Almost clear
5.Ether Insoluble alcohol soluble fraction of acetic ether precipitate (egg yolk)	0	0	0	0	Distinct	Almost clear
6.Yolk Lecithin	Trace	Marked	Complete			Turbid

The dried brain lecithin end-piece in the above table was found to be actively lytic for ox's corpuscles unsensitised in an amount of 0.005 c.c. The other lecithin end-pieces were non lytic.

Table 17.

Effect of using inactive lecithins in solutions of much greater concentration than that generally used.

	0.005c.c.	0.01 c.c.	0.02c.c.	0.035c.c.	0.05c.c.	Turbidity
Fresh Kidney Lecithin	0	0	0	0	0	Very Marked
Ox Liver Lecithin	0	0	0	0	0	Marked
Fresh Human Brain Lecithin	0	0	0	0	0	Very Marked
Ox Heart Lecithin	0	0	0	0	0	Distinct
Control Lecithin (active)	Distinct	Complete	Complete	Complete	Complete	Very Marked

The Biological Action of Cholesterin and Its Derivatives.

The inhibitory action which cholesterin exerts on most haemolytic processes has been already shortly referred to, also the effect which it produces when combined with lecithin in the syphilis reaction and in the test along with cobra venom. In the course of the investigation into the action of cholesterin in the Wassermann reaction it was thought that the investigation of a number of the derivatives of cholesterin might yield results of interest especially as it was possible that some relationship between the biological action and the chemical constitution of cholesterin might be elicited. This subject had already been investigated by Hausmann as regards saponin haemolysis and by Abderhalden and Le Count as regards lecithin-venom lysin, tetanolysin, saponin and solanin. Hausmann had found that the compounds in which substitution of the hydroxyl group had occurred (cholesteryl chloride, acetate, benzoate, etc) were without inhibitory action but that the action was still present, although weakened, in compounds in which the double bond was saturated while Walbum had pointed out that the physical state of the cholesterin had a marked effect on its action in the biological tests.

Along with Dr. Browning I made an examination of a number of cholesterin derivatives principally as regards their action in the Wassermann test and along with cobra venom.

The following compounds I have myself tested:-

1. Cholesterin.
2. Cholesteryl chloride
3. " acetate
4. " Benzoate } Esters
5. " oleate
6. Cholesterin dibromide
7. Dibromcholesteryl acetate
8. Dihydrocholesterin (B-cholestanol)
9. Cyclocholesterin (a-cholestanol)
10/

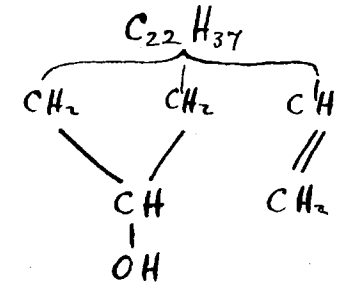
10. Nitrodehydrocholesteryl nitrate.
11. " " acetate.
12. Cholestenone.
13. Dehydrocholestanonol.
14. Dehydrocholestendion (oxycholestenone).
15. Ethyl ether of dehyrocholestendion
16. Dehydrocholestendionol (oxycholestendiol).
17. Dehydrocholestandion.

The esters, dibromide, dibromcholesteryl acetate and nitrodehydrocholesteryl nitrate and acetate were prepared by myself, the other compounds were supplied by Messrs Windaus and Mauthner.

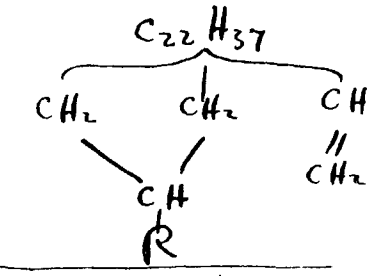
The more important chemical features of these compounds are as follows:-

Cholesterin.- The following groups have been determined:-

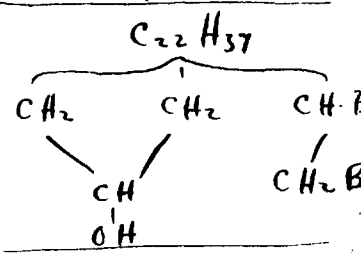
1. A secondary alcoholic hydroxyl group $C\begin{matrix}H\\OH\end{matrix}$ standing between two methylene groups (CH_2);
2. A vinyl group($CH{:}CH_2$); there is thus an unsaturated carbon double bond. The vinyl group is terminal, i.e. it stands at the end of an open chain



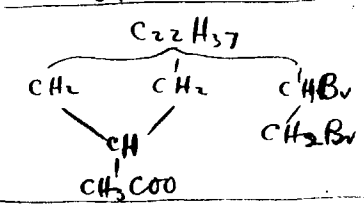
Cholesterin esters.- The alcoholic hydroxyl is replaced by an acid radicle, chloride (Cl-), acetate ($CH_3.COO$-), benzoate ($C_6H_5.COO$-), oleate $C_{17}H_{33}.COO$-)



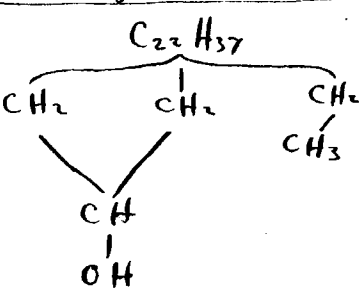
Cholesterin dibromide.- The addition of one molecule of bromine saturates the carbon double bond. The esters also add Br_2, thus cholesteryl acetate yields



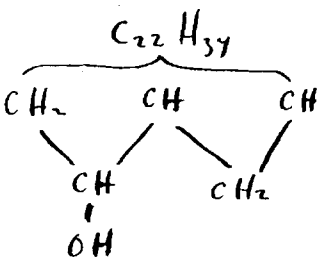
Dibromcholesteryl acetate . . . . .



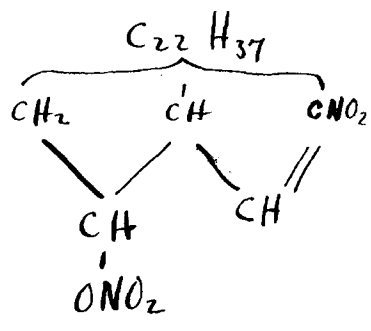
Dihydrocholesterin (B-cholestanol) is the normal reduction product of cholesterin: it differs from cholesterin only by the vinyl group being converted into ethyl ($-CH_2.CH_3$); the secondary alcoholic hydroxyl is intact



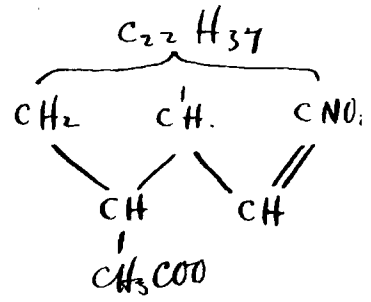
a-Cholestanol (cyclocholesterin).- It has not been determined whether this is a reduction product of cholesterin or a product of molecular rearrangement



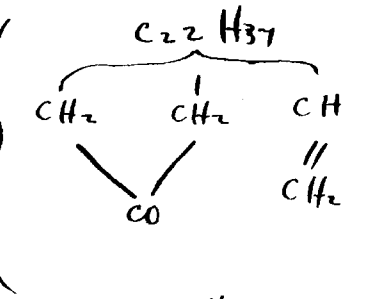
Nitrodehydrocholesteryl nitrate is the product obtained by nitrating cholesterin



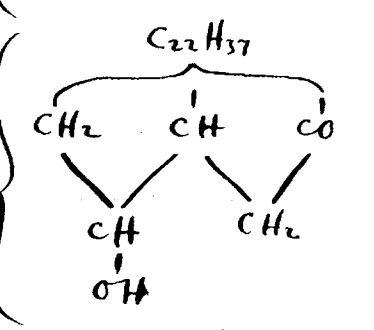
Nitrodehydrocholesteryl acetate is obtained by nitrating cholesteryl acetate



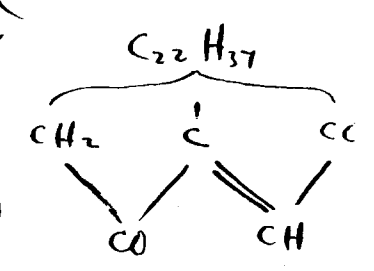
Cholestenone is the ketone corresponding to cholesterin, i.e. the alcoholic hydroxyl has been converted into carbonyl (-CO)



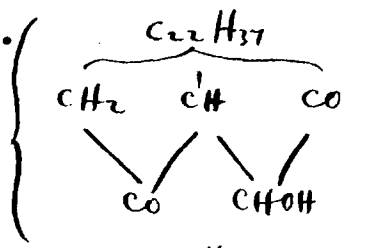
Dehydrocholestanonol . . . . . .



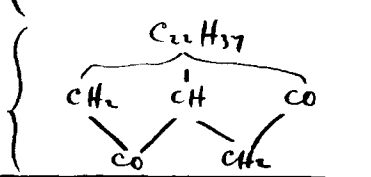
Dehydrocholestendion (oxycholestenone).- One of the carbonyl groups can react in the "Enol" form, since on treatment with HCl and alcohol an ethyl-ether is formed



Dehydrocholestandionol (oxycholestendiol) . .



Dehydrocholestandion . . . . . .



In investigating the actions of these substances on lecithin attention was particularly directed to the effects of -

(1)Alterations in the physical conditions as they affected the action of the same compound;

(2)Alterations in the chemical constitution, the physical state being the same.

The Effect of the Physical State.

In general the physical state depended on the manner in which the mixture with water was effected. Thus emulsions made by slowly diluting the alcoholic solution of lecithin plus cholesterin derivative with salt solution were in the form of turbid suspensions from which crystals separated out more or less rapidly. On the other hand, rapid mixture led to colloidal solutions of varying stability. The stability of colloidal solutions was influenced by the amount of cholesterin derivative present along with a fixed quantity of lecithin. A mixture of 0.5 per cent. cholesterin dibromide with 0.75 per cent. ox liver lecithin in alcohol, when mixed rapidly with 7 parts of normal salt solution, yielded a colloidal solution which was kept for days at room temperature without the occurrence of any definite precipitate; if the cholesterin dibromide was present in a quantity of 1 per cent. the solution was at first colloidal, but within a few minutes precipitation began, and was practically complete in twenty-four hours; when the dibromide was increased to 1.3 per cent. the precipitate separated out at once, apparently without the intervention of an appreciable colloidal phase. The effect of the physical state of the watery mixture in modifying the biochemical action was well illustrated by the action of cholesterin dibromide on lecithin/

lecithin.

The alcoholic solution of 1 per cent. cholesterin dibromide and 0.75 per cent ox liver lecithin (1 volume) was mixed with salt solution (7 volumes) (a) rapidly, (b) slowly. The mixtures were left for twenty-four hours at room temperature, by which time an abundant precipitate had separated out from both, leaving the supernatant fluid of (a) perfectly clear and colourless, of (b) slightly opalescent. The lytic effect of the two separated fluids along with ox corpuscles and cobra venom was then tested (Table 1).

The result showed that it was only where the mixture of the alcoholic solution with water had been effected rapidly, so that the emulsion initially passed through the colloidal state (Series A), that the lecithin was removed from the solution. The same effect followed when 1.3 per cent cholesterin dibromide was employed, even though the rapid mixture formed a precipitate practically instantaneously. This supplied an experimental explanation of the view that the inhibitory action of cholesterin on lecithin-venom haemolysis was due to the cholesterin fixing (absorbing) the lecithin.

Sachs and Rondoni first showed, in the case of a crude alcoholic organ extract, that the turbid emulsion caused the absorption of more complement in the presence of syphilitic serum than did the clear emulsion. This held for mixtures of lecithin with cholesterin and also with cholesterin acetate. Emulsions of maximum turbidity were therefore employed for testing the syphilis reaction. The inhibitory effect of cholesterin on lecithin-venom haemolysis is, on the other hand, most marked when the cholesterin is in the colloidal state (Walbum). The effect of all the cholesterin derivatives in both the suspended and the colloidal states/

states was tested. In the case of cholesterin and cholesterin dibromide, the lytic effect of the colloidal mixture was much less than that of the turbid emulsion (Table 2). Dehydrocholestanonol, however, had a much more marked antilytic effect when the emulsion was turbid than when it was colloidal; in addition, the mixtures of this compound with lecithin showed a marked zone effect, there being an optimum amount of the mixture which caused more rapid lysis of the blood corpuscles in the presence of venom than did greater or smaller amounts (Tables 3 & 4). Most of the cholesterin derivatives had little effect on lecithin-venom haemolysis in either state.

The Effect of Differences in Chemical Constitution.

In the case of cholesterin dibromide the inhibitory effect on lecithin-venom haemolysis was due to the removal of lecithin from the solution. When the dibromide precipitated out from the colloidal solution the lecithin was also removed. Where the mixture was permanently colloidal, e.g. lecithin-cholesterin, it was probable that the condition was similar, and was of the nature of adsorption. Such adsorption was closely dependent on chemical constitution. Thus the cholesteryl esters (chloride, acetate, benzoate) and many other cholesterin derivatives formed very stable colloidal solutions with lecithin, and yet the inhibitory effect of these bodies on lecithin-venom haemolysis was minimal (Tables 5 and 16). Dibromcholesterin was deprived of its inhibitory action by esterification; thus dibromcholesteryl acetate was practically without effect on lecithin-venom haemolysis (Table 5). In the syphilis reaction there was also a marked relationship between constitution and biochemical action.

The/

The action of these cholesterin derivatives along with lecithin in (1) the syphilis reaction, and (2) cobra-venom haemolysis is given in Table 6 .

The compounds were dissolved in 0.75 per cent. lecithin (ox liver) in absolute alcohol, and the amounts employed were equimolecular usually with 1 per cent. cholesterin. Table 7 shows the physical characters of the rapidly formed emulsions. In no case was the emulsion of lecithin and cholesterin derivative more lytic by itself than lecithin emulsion alone; that is to say, in the amounts employed the emulsions had by themselves practically no effect on the red corpuscles.

The results of the experiments showed that in the syphilis reaction any alteration in the cholesterin molecule diminished the effect; that neither the hydroxyl group nor the double bond appeared to be essential, since (1) cholestenone and the esters and (2) dibromcholesterin and dihydrocholesterin gave the reaction. In regard to more marked alterations in the molecule, the nature and position of the side chains and the unsaturated bond probably influenced the action, but a comparison of the structural formulae with the effects of the various compounds showed that no general conclusions could be drawn.

The results with regard to lecithin-venom haemolysis were in accord with Walbum so far as cholesterin esters and cholesterin dibromide were concerned. Thus the esters, which retain the double bond but lack the alcoholic hydroxyl, were almost without antilytic effect, whereas the dibromide, which possessed the hydroxyl but not the unsaturated bond, was fairly active. The replacement of hydroxyl by acetyl in the dibromide (dibromcholesteryl acetate) abolished the antilytic power/

power. The presence of the alcoholic hydroxyl, however, was not necessarily accompanied by antilytic properties, since cyclocholesterin was practically without effect on lecithin-venom haemolysis. Cholesterin dibromide in which the double bond is saturated by Br_2 was more antilytic than dihydrocholesterin in which the double bond is saturated by H_2 (table 20).

There was no parallelism between the effect with lecithin in the syphilis reaction and on lecithin-venom haemolysis (Table 21).

Table 1.

	1 c.c. Ox Blood Suspension + 1 : 10,000 Cobra Venom + Fluid.				
	0.06 c.c.	0.09 c.c.	0.13 c.c.	0.18 c.c.	0.24 c.c.
1. Fluid from rapid emulsion of lecithin + cholesterin dibromide	0	0	0	0	0
2. Fluid from slow emulsion of lecithin + cholesterin dibromide	0	complete	Complete	Complete	Complete

Table 2.

Lecithin +	1 c.c. Ox Blood Suspension + 1: 10,000 Cobra Venom + Emulsion.					
	0.05 c.c.	0.08 c.c.	0.12 c.c.	0.17 c.c.	0.23 c.c.	0.3 c.c.
holesterin 1 per cent. rapid emulsion (colloidal)	0	0	0	0	0	0
holesterin 1 per cent. slow emulsion	0	0	Faint trace	Very marked	Complete	Complete
holesterin dibromide 1 per cent. rapid emulsion (colloidal)	0	0	0	Trace	Complete	Complete
holesterin dibromide 1 per cent. slow emulsion	0	Very marked	Complete	Complete	Complete	Complete

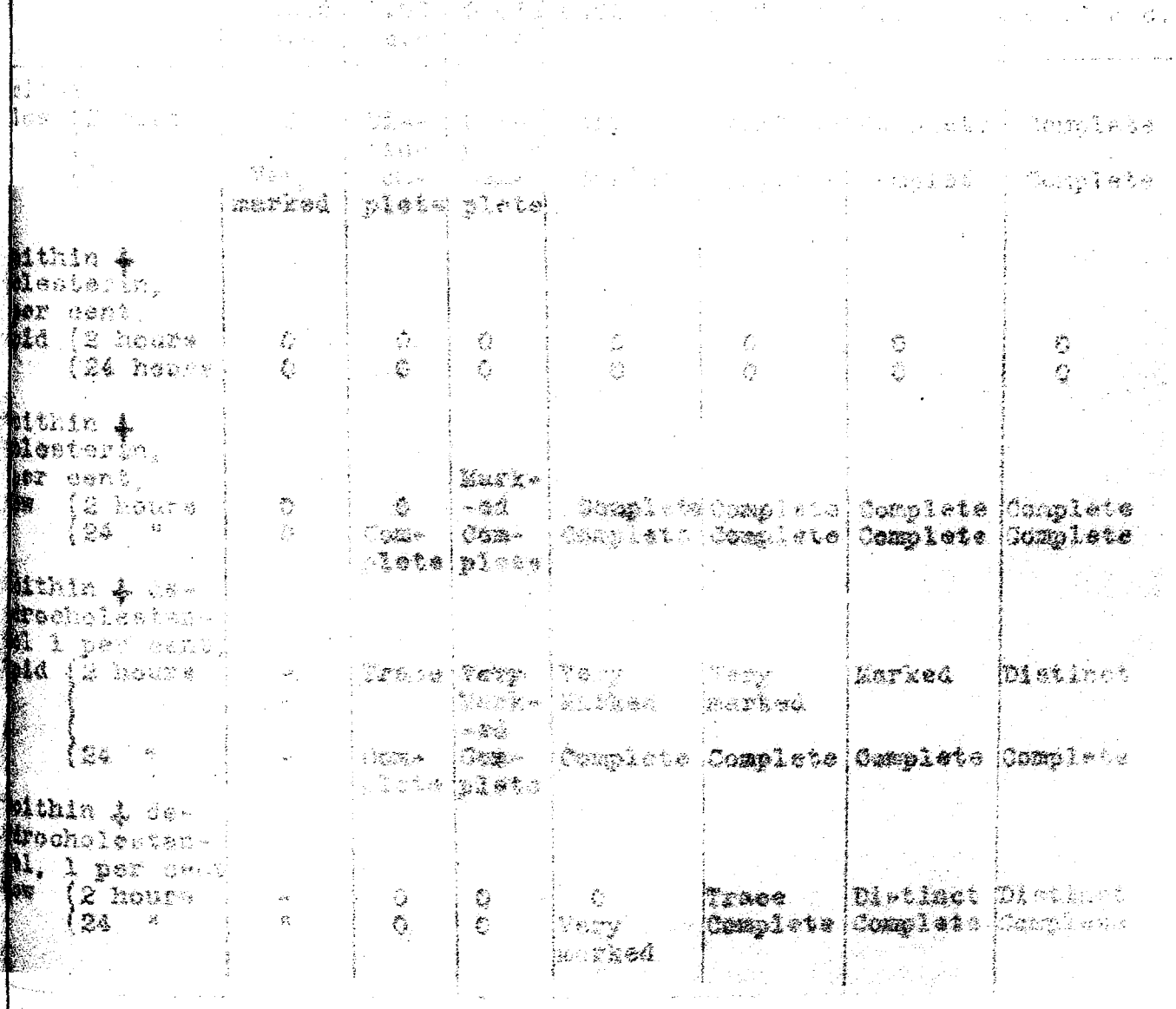


Table 3.

ulsion of cithin + hydrocholes-anonol, 1 er cent.	1 c.c. Ox Blood Suspension + 1: 10,000 Cobra Venom + Emulsion					
	0.02 c.c.	0.03 c.c.	0.045 c.c.	0.06 c.c.	0.09 c.c.	0.13 c.c.
pid	Com-plete ←	Com-plete ←	Com-plete →	Com-plete →	Almost complete →	Very marked
ow	0	0	0	0	0	Trace

The results show the amount of lysis in two hours at 37° C.
The arrows indicate the directions in which lysis proceeded.

Table 4.

mulsion.	1 c.c. Ox Blood Suspension + 1: 10,000 Cobra Venom + Emulsion.						
	0.015 c.c.	0.02 c.c.	0.035 c.c.	0.05c.c.	0.08c.c.	0.12 c.c.	0.17 c.c.
cithin, low (2 hours	0	Dis-tinct	Com-plete	Complete	Complete	Complete	Complete
(24 "	Very marked	Com-plete	Com-plete	Complete	Complete	Complete	Complete
cithin + olesterin, per cent pid (2 hours	0	0	0	0	0	0	0
(24 hours	0	0	0	0	0	0	0
cithin + olesterin, per cent, low (2 hours	0	0	Mark-ed	Complete	Complete	Complete	Complete
(24 "	0	Com-plete	Com-plete	Complete	Complete	Complete	Complete
cithin + de-drocholestan-ol 1 per cent, pid (2 hours	-	Trace	Very Mark-ed	Very Marked	Very marked	Marked	Distinct
(24 "	-	Com-plete	Com-plete	Complete	Complete	Complete	Complete
cithin + de-drocholestan-ol, 1 per cent low (2 hours	-	0	0	0	Trace	Distinct	Distinct
(24 "	"	0	0	Very marked	Complete	Complete	Complete

Table 5.

Rapid Emulsions.	1 c.c. Ox Blood + Cobra Venom 1: 10,000 + Emulsion.					
	0.025 c.c.	0.05 c.c.	0.08 c.c.	0.15 c.c.	0.23 c.c.	0.3 c.c.
Lecithin	Almost complete	Complete	Complete	Complete	Complete	Complete
cithin + cholesterin, 1 per cent	0	0	0	0	0	0
cithin + cholesteryl hloride	0	Complete	Complete	Complete	Complete	Complete
cithin + cholesteryl cetate	0	Trace	Complete	Complete	Complete	Complete
cithin + cholesterin ibromide	0	0	0	Faint trace	Complete	Complete
cithin + dibromchol- steryl acetate	0	Very marked	Complete	Complete	Complete	Complete

Table 6.

Substance.	Effect in syphilis Reaction	Inhibitory Effect on Lecithin-Venom Haemolysis.
Cholesterin	Very Marked	Very marked
Cholesterin esters	Marked	Practically none
Cholesterin dibromide	Marked	Marked
Dibromcholesteryl acetate	Distinct to marked	Practically none
Dihydrocholesterin	Distinct	Distinct
Cyclocholesterin	Slight	Practically none
Nitrodehydrocholesteryl nitrate	Distinct to marked	Practically none
Nitrodehydrocholesteryl acetate	Distinct	(not tested)
Cholestenone	Distinct to marked	Practically none
Dehydrocholestanonol	Practically none	Marked
Dehydrocholestendion	Marked	Practically none
Dehydrocholestendion ethyl-ether	Marked	Practically none
Dehydrocholestandionol	Distinct	Practically none
Dehydrocholestandion	Distinct	Practically none

The successive degrees of effect are: very marked, marked, distinct, slight.

Table 7.

Substance, Per Cent		Nature of Rapid Mixture
Cholesterin . . .	1.0	Permanent colloidal, almost clearsolution
Cholesteryl chloride .	1.0	" " opalescent solution
" acetate .	1.1	Colloidal opalescent solution, precipitate in 24 hours
" benzoate .	0.6	" solution, permanent
Cholesterin dibromide .	1.3	Immediate precipitate
" " .	1.0	Colloidal, precipitate in a few minutes
" " .	0.5	Permanent, colloidal, almost clear solution
Dibromcholesteryl acetate		Colloidal solution, less stable than with corresponding concentration of cholesterin dibromide
Dihydrocholesterin .	1.0	Permanent jelly-like emulsion
Cyclocholesterin . .	1.0	" colloidal solution, opalescent
Nitrodehydrocholesteryl nitrate	1.27	" " "
Cholestenone . . .	1.0	Colloidal permanent solution, opalescent
Dehydrocholestanonol .	1.0	Permanent colloidal solution, clear
Dehydrocholestendion .	1.0	Colloidal opalescent, precipitate visible in 2 hours
" ethyl ether	1.0	Permanent colloidal solution, opalescent
Dehydrocholestandionol	1.1	Colloidal opalescent, precipitate in 24 hours
Dehydrocholestandion .	1.0	Colloidal opalescent, visible precipitation in 2 hours

Table 8.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement		
	0.14 c.c.	0.2 c.c.	0.28 c.c.
Lecithin	Marked	Almost complete	Complete
" + cholesterin, 1 per cent	Trace	Distinct trace	Complete
" + cholesteryl chloride, 1 per cent	Trace	Distinct	Complete

CONTROLS: Emulsions alone 0.6 c.c. + complement 0.05 c.c. = almost complete.

Dose of complement = 0.03 c.c.

Table 9.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.			
	0.15 c.c.	0.2. c.c.	0.27 c.c.	0.36 c.c.
Lecithin	Marked	Complete	Complete	Complete
" + cholesteryl benzoate, 0.55 per cent	0	0	Trace	Just complete
" + cholesteryl oleate, 0.57 per cent.	0	0	Trace	Just complete

CONTROLS: Emulsions alone 0.6 c.c. + complement 0.02 c.c. = complete.

Dose of complement = 0.008 c.c.

Table 10.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.			
	0.14 c.c.	0.2. c.c.	0.28 c.c.	0.36 c.c.
Lecithin	Very marked	Almost complete	Complete	Complete
" + cholesterin, 1 per cent	0	0	Very marked	Complete
" + cholesteryl acetate 1.1 per cent	Faint trace	Distinct	Almost complete	Complete
" + nitrodehydrocholesteryl nitrate, 1.26 per cent	Faint trace	Very marked	Complete	Complete
" + dehydrocholestendion, 1 per cent	0	Distinct trace	Almost complete	Complete

CONTROLS: Emulsions alone 0.6 c.c. + complement 0.05 c.c. = complete.

Dose of complement = 0.03 c.c.

Table 11.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's complement.				
	0.2 c.c.	0.24 c.c.	0.28 c.c.	0.34 c.c.	0.4 c.c.
Lecithin , ...	Trace	-	-	-	-
" + cholesterin, 1 per cent	0	0	0	0	Faint trace
" + cholesteryl acetate 1.1 per cent	0	Faint trace	Distinct	Complete	Complete
" + cholesterin dibromide, 1.3 per cent	0	0	Very faint trace	Marked	Complete
" + dibromcholesteryl acetate, 1.4 per cent	Very faint trace	Distinct	Marked	Complete	Complete

CONTROLS: Emulsions alone 0.6 c.c. + complement 0.02 c.c. = complete

Dose of complement = 0.02 c.c.

Table 12.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement						
	0.07 c.c.	0.09 c.c.	0.11 c.c.	0.14 c.c.	0.18 c.c.	0 24 c.c.	0.33 c.c.
Lecithin	Almost Complete	Complete	Complete	Complete	Complete	Complete	Complete
Lecithin + cholesterin 1 per cent	0	0	0	0	0	Faint trace	Complete
" + cholestenone	0	0	0	Faint trace	Marked	Complete	Complete
" + dehydrocholestendion	0	0	0	Faint trace	Marked	Complete	Complete
" + dehydrocholestandionol	0	0	Very faint trace	Trace	Just Complete	Complete	Complete
" + cyclocholesterin	0	0	Trace	Marked	Complete	Complete	Complete
" + dihydrocholesterin	0	Trace	Trace	Trace	Almost Complete	Complete	Complete
" + dehydrocholestandion	0	0	Trace	Marked	Complete	Complete	Complete
" + dehydrocholestanonol	Just Complete	Just Complete	Complete	Complete	Complete	Complete	Complete

Emulsions alone 0.6 c.c. + complement 0.04 c.c. = complete.

Serum 0.05 c.c. + salt solution 0.6 c.c. + complement 0.02 c.c. = complete.

Dose of complement = 0.008 c.c.

Cholesterin derivatives in amounts equimolecular with cholesterin 1 per cent.

Table 13.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.			
	0.07 c.c.	0.1 c.c.	0.14 c.c.	0.2 c.c.
Lecithin	Trace	Complete	Complete	Complete
" + cholesterin, 1 per cent	0	0	Faint trace	Complete
" + nitrodehydrocholesteryl acetate, 1 per cent	0	Faint trace	Trace	Complete

CONTROLS: Emulsions alone 0.6 c.c. + complement 0.05 c.c. = complete

Dose of complement = 0.015 c.c.

Table 14.

Syphilitic Serum 0.05 c.c. + Turbid Emulsion 0.6 c.c.	Amounts of Guinea-pig's Complement.			
	0.04 c.c.	0.08 c.c.	0.13 c.c.	0.2 c.c.
Lecithin	Complete	Complete	Complete	Complete
" + cholesterin, 1 per cent	0	0	Distinct	Complete
" + dehydrocholestendion, 1 per cent	0	Faint trace	Complete	Complete
" + dehydrocholestandion, 1 per cent	Faint trace	Distinct	Complete	Complete
" + cholestenone, 1 per cent	Trace	Marked	Complete	Complete
" + cyclocholesterin, 1 per cent	Distinct	Complete	Complete	Complete

CONTROLS: Emulsions alone 0.6 c.c. + complement 0.04 c.c. = complete.

Dose of complement = 0.008 c.c.

Emulsion	1 c.c. Ox Blood + Cobra Venom 1: 10,000 + Emulsion.				
	0.01 c.c.	0.02 c.c.	0.05 c.c.	0.07 c.c.	0.4 c.c.
Lecithin, rapid	Very faint trace	Complete	Complete	Complete	Complete
" slow	Very faint trace	Almost complete	Complete	Complete	Complete
Cholesterin, rapid	0	0	0	0	0
" slow	0	0	Marked	Complete	Complete
Dehydrocholestendion, rapid	Very faint trace	Complete	Complete	Complete	Complete
Dehydrocholestendion, slow	Very faint trace	Complete	Complete	Complete	Complete
Dehydrocholestendion ethyl ether, rapid	0	Complete	Complete	Complete	Complete
" slow	0	Complete	Complete	Complete	Complete
Dehyrocholestandionol, rapid	0	Complete	Complete	Complete	Complete
" slow	0	Almost complete	Complete	Complete	Complete

Table 15.

Emulsion	1 c.c. Ox Blood Suspension + Cobra Venom 1: 10,000 + Emulsion.			
	0.02 c.c.	0.035 c.c.	0.055 c.c.	0.5 c.c.
Lecithin, slow . .	Distinct	Complete	Complete	Complete
" + cholesterin, 1 per cent, rapid	0	0	0	Trace
" + cholesterin, 1 per cent, slow	0	Distinct	Complete	Complete
" + dihydrocholesterin, 1 per cent, rapid	0	0	Just complete	Complete
" + dihydrocholesterin, 1 per cent, slow	0	0	Very marked	Complete
" + cyclocholesterin, 1 per cent, rapid	Very faint trace	Almost complete	Complete	Complete
" + cyclocholesterin, 1 per cent, slow	Faint trace	Complete	Complete	Complete
" + cholestenone, 1 per cent, rapid	0	Very marked	Just Complete	Complete
" + cholestenone, 1 per cent, slow	0	Marked	Just Complete	Complete

Table 16.

Rapid Emulsion	1 c.c. Ox Blood Suspension + Cobra Venom 1: 10,000 + Emulsion.				
	0.01 c.c.	0.02 c.c.	0.035 cc.	0.055 cc.	0.085 c.c.
Lecithin	0	Complete	Complete	Complete	Complete
" + cholesterin, 0.5 per cent	0	0	0	0	Trace
" + cholesteryl benzoate, 0.55 per cent	0	Complete	Complete	Complete	Complete
" + cholesteryl benzoate, 0.225 per cent	0	Complete	Complete	Complete	Complete

Table 17.

Emulsion.	1 c.c. Ox Blood Suspension + Cobra Venom 1: 10,000 + Emulsion.			
	0.01 c.c.	0.02 c.c.	0.035 c.c.	0.5 c.c.
Lecithin, slow	0	Complete	Complete	Complete
" + cholesterin, 1 per cent, rapid	0	0	0	Very faint trace
" + cholesterin, 1 per cent, slow	0	0	Complete	Complete
" + nitrodehydrocholesteryl nitrate, 1.27 per cent, rapid	0	Complete	Complete	Complete
" + nitrodehydrocholesteryl nitrate, 1.27 per cent, slow	0	Complete	Complete	Complete

Table 18.

Syphilitic Serum (55°C.), 0.05 c.c. + Turbid Emulsions 0.6 c.c.	Amounts of Guinea-pig's Complement 0.25 c.c.	0.32 c.c.	0.4 c.c.	0.5 c.c.
Lecithin	Very faint trace	Complete	Complete	Complete
" + cholesterin, 1 per cent	0	0	Veryfaint trace	Complete
" + dehydrocholestendion, 1 per cent	0	Very faint trace	Complete	Complete
" + dehydrocholestendion ethyl ether, 1 per cent	0	Very faint trace	Complete	Complete

Dose of complement = 0.015 c.c.

Table 19.

Emulsions.	1 c.c. Ox blood + Cobra Venom 1:10,000 + Emulsion. 0.01 c.c.	0.02 c.c.	0.5 c.c.
Lecithin, turbid	Trace	Complete	Complete
" + cholesterin, 1 per cent	0	0	Faint trace
" + dehydrocholestandion, 1 per cent, clear	Trace	Complete	Complete
" + dehydrocholestandion, 1 per cent, turbid	Trace	Complete	Complete

Emulsions.	1 c.c. Ox blood Suspension + 1:10,000 Cobra Venom + Emulsion.			
	0.035 c.c.	0.055 c.c.	0.085 c.c.	0.125 c.c.
ecithin + cholesterin dibromide 1.3 per cent, rapid	Very faint trace	Marked	Almost complete	Complete
" + cholesterin dibromide, 1.3 per cent, slow	Marked	Complete	Complete	Complete
" + cholesterin dibromide 0.75 per cent, rapid	Very faint Trace	Marked	Complete	Complete
" + cholesterin dibromide 0.75 per cent, slow	Marked	Complete	Complete	Complete
" + cholesterin dibromide 0.375 per cent, rapid	0	0	Marked	Complete
" + cholesterin dibromide, 0.375 per cent, slow	Distinct	Almost complete	Complete	Complete
" + dihydrocholesterin, 1.0 per cent, rapid	Complete	Complete	Complete	Complete
" + dihydrocholesterin, 1.0 per cent, slow	Very marked	Complete	Complete	Complete
" + dihydrocholesterin, 0.5 per cent, rapid	Almost complete	Complete	Complete	Complete
" + dihydrocholesterin, 0.5 per cent, slow	Complete	Complete	Complete	Complete
" + dihydrocholesterin, 0.25 per cent, rapid	Complete	Complete	Complete	Complete
" + dihydrocholesterin, 0.25 per cent, slow	Complete	Complete	Complete	Complete

Lytic dose of lecithin emulsion (slow) with venom = 0.02 c.c.

Table 21.

Substance.	Effect in Syphilis Reaction.	Inhibition of Lecithin-Venom Haemolysis
Cholesteryl esters	Marked	Practically none
Cholesterin dibromide	"	Marked
Dehydrocholestendion	"	Practically none
Dehydrocholestanonol	Practically none	Marked
Cholesterin	Very marked	Very marked

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SUMMARY and CONCLUSIONS.

(1) The properties of alcoholic extracts of normal and of autolised liver have been compared as regards -

(a) Haemolytic action on ox's red blood corpuscles.

(b) Effect on complement.

(c) Effect when used as 'antigen' in the Wassermann syphilis test.

(d) Action along with cobra venom.

(2) Extracts of autolised tissue as compared with those of normal tissues were found to be much more haemolytic and, as a result were unsuitable antigens in the Wassermann test.

(3) The haemolytic action of autolised tissue extracts was inhibited in different degree by different syphilitic sera.

(4) The crude alcoholic extracts of normal and of autolised tissues have been divided into a number of components by the use of acetic ether and acetone.

(5) The component soluble in cold acetic ether from both normal and autolised tissues was always markedly haemolytic, distinctly anticomplementary and unsuitable as syphilitic antigen.

(6) The lecithin component was always relatively non-haemolytic, had practically no anticomplementary action and yielded an active haemolysin along with cobra venom.

(7) In the presence of syphilitic serum the lecithin fraction had a lower antigenic value than the corresponding crude extract; addition of the component soluble in cold acetic ether to the lecithin fraction markedly increased the antigenic effect.

(8) The combination of cholesterin and lecithin formed a very suitable antigen for the detection of syphilitic sera.

(1) Lecithins have been prepared from a variety of tissues by a special method which has been found to give, as compared with other methods, a very pure product.

(2) The action of different lecithins in the following biological tests has been examined (a) haemolytic effect on ox's red blood corpuscles, (b) effect on complement, (c) power of causing the combination of a large amount of complement in the presence of syphilitic serum, (Wassermann syphilis test) and the effect of the addition of cholesterin, (d) power to form haemolysins along with cobra venom.

(3) In the presence of syphilitic serum the addition of cholesterin caused in all cases a marked increase in the amount of complement absorbed; this increase was greatest in the case of heart lecithins and least with yolk lecithins.

(4) The most suitable lecithins for use in the Wassermann reaction, because the most uniform in action, were the liver lecithins.

(5) Lecithins from heart muscle were found to be uniformly more anticomplementary, less lytic and less active along with cobra venom than were the lecithins from the liver and the egg yolk, while the yolk lecithins were generally the most actively lytic in the presence of venom.

(6) Considerable differences were found in the degree of unsaturation of the lecithins as tested by the Iodine Values; egg yolk lecithins gave generally lower iodine values than liver or heart lecithins; differences were also found in the iodine values of lecithins obtained from different samples of the same tissue.

(7) No parallelism between the iodine values of the lecithins and their action as syphilitic antigens or as haemolysins with cobra venom could be found.

(1) The effect of preliminary formalin fixation as an aid to the drying of tissues has been tested.

(2) Lecithins from formalin-fixed and dried tissues do not differ materially from lecithins obtained from the same tissues extracted in the 'wet' condition.

(3) Evidence has been produced to show that in the case of both 'wet' and dried tissues the amount of lecithin obtained from any particular extract depends on the degree of dehydration of the tissue at the time of extraction, i.e. the relative proportions of water and alcohol present.

(4) Only a small proportion of the amount of lecithin actually present can be obtained by the extraction of tissues with ether.

(5) Lecithins obtained from ether extracts of tissue differ in their biological reactions from the lecithins obtained from the same tissue by means of alcohol.

(6) 'Ether-extracted' lecithins are generally quite unsuitable for use in the Wassermann reaction and are relatively inactive along with cobra venom.

(7) Methylated spirit can be used in place of absolute alcohol for the extraction of lecithin, the cost of production being thus much reduced.

(8) The lecithin content of a large number of different tissues has been examined and observations have been made regarding the effect of repeated extraction of wet tissues with alcohol.

(9) All the lecithins obtained (numbering from 400 to 500) have/

have been tested for their biological reactions and for their iodine values and considerable differences have been elicited.

(10) Lecithins obtained from successive alcohol extracts of the same sample of tissue may also differ.

(11) Results have been obtained which suggest that the iodine values of lecithins may in part depend on the amount of water present in the mixture of tissue and alcohol used for extraction.

(12) All the lecithins examined have been unsaturated: no fully saturated lecithin has been met with.

(13) The iodine values of lecithins tested immediately after preparation were high and a fall generally occurred to values which were fairly constant for a considerable period.

(14) The iodine values obtained in many cases have been much higher than the values hitherto recorded for lecithin by other observers.

(15) Commercial lecithins were generally impure.

(16) A few experiments with the object of altering the characters of lecithin have been made but the results have been inconclusive.

(17) A number of facts regarding the occurrence and properties of certain pigments have been made in the course of the work.

(18) Numerous attempts to determine the purity of lecithins by an examination of the N:P ratio have been made but the results have in all cases been unsatisfactory as constant results could not be obtained.

(1) A number of substances having properties differing from those of known lipoids have been separated from the residues of the acetone precipitates.

(2) These substances, with one exception, were soluble in ether and were precipitated out of ethereal solutions by the addition of acetone or alcohol.

(3) They were all soluble in water, giving with some clear, and with others, turbid solutions.

(4) These substances all yielded haemolysins along with cobra venom.

(5) The property of giving rise to a haemolysin along with cobra venom is not, therefore, a characteristic of lecithins as has hitherto been supposed.

(6) A number of other lipoids isolated as bye-products from the acetic ether precipitates in the course of preparing lecithins have also been examined; certain of these have also given haemolysins along with cobra venom.

(1) The introduction of lecithin into complement-containing serum of the guinea-pig does not materially alter the complement dose; in the case of rabbit's serum the complement activity is frequently increased.

(2) The albumen fraction from a serum treated with certain lecithins is as actively haemolytic for sensitised corpuscles as the original complement while the globulin fraction retains the property of effectively acting along with ordinary albumen fraction.

(3) The addition of lecithin to ordinary albumen fraction after separation does not enhance the complement activity of the fraction.

(4) The lecithin must be mixed rapidly with the serum or with the water used for dilution in order to produce the effect described; slow admixture does not yield an active lecithin albumen fraction.

(5) The albumen fraction of a serum treated with lecithin is 'absorbed' by complement-absorbing agents; it can also replace complement in the Wassermann reaction.

(6) The activity of the lecithin albumen fraction is dependent on the presence of complement in the original serum.

(7) Lecithins differ markedly with regard to their power to produce the alterations described; a large number of preparations are quite inefficient.

(8) These results have an important bearing on the nature of complement action.

(1) The biological actions of a number of cholesterin derivatives have been tested and observations made regarding the effect of the physical state and of alterations in the cholesterin molecule.

(2) In the syphilis test turbid emulsions were more efficient in causing complement deviation than clear (colloidal) solutions.

(3) The inhibitory effect of cholesterin dibromide on lecithin-venom-haemolysis was greater when these substances were in the colloidal state; the reverse was the case with dehydrocholestanonol.

(4) Alterations in the cholesterin molecule produced differences in action which were independent of variations in the physical state of the mixtures.

(5) Cholesterin was more efficient in the syphilis test and in inhibiting lecithin-venom-haemolysis than were any of its derivatives.

(6) No parallelism existed between the effect with lecithin in the syphilis reaction and the effect on lecithin-venom-haemolysis.

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