XXXVI. THE COMPOSITION OF "LECITHIN," TOGETHER WITH OBSERVATIONS ON THE DISTRIBUTION OF PHOSPHATIDES IN THE TISSUES AND METHODS FOR THEIR EX- TRACTION AND PURIFICATION.

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About the year 1846 Gobley succeeded in obtaining from egg yolk and other organs a substance of a fat-like nature containing nitrogen and phosphorus which he called lecithin [1846]. This substance was soluble in alcohol and in ether, formed an emulsion when rubbed up with water and yielded fatty acids and glycerophosphoric acid on hydrolysis. These observations of Gobley furnished a basis for the structure of the lecithin molecule, which appeared to be completed by the discovery of Liebreich [1865] and Strecker [1868] that the base choline was also obtained as a product of lecithin hydrolysis.

Lecithin was therefore defined as an ester compound of glycerophosphoric acid substituted by two fatty acid radicles with a base, choline, according to the following formula which is generally accepted.

Later it was found that other somewhat similar bodies were present in different organs and tissues; for this general class of substances the name *phosphatides* was suggested by Thudichum [1884].

Lecithin is soluble in all the ordinary organic solvents with the exception of acetone. It differs from a substance known as kephalin in that the latter is insoluble in alcohol. So far, as has already been pointed out, the only phosphatides identified with certainty as distinct substances are lecithin, kephalin, cuorin and sphingomyelin.

A short description of these substances and their probable distribution in the tissues will help to render clear certain problems discussed later.

Both kephalin and lecithin are monamino-monophosphatides ($N : P = 1 : 1$). Cuorin on the other hand is a monamino-diphosphatide ($N : P = 1 : 2$), while sphingomyelin is a diamino-monophosphatide ($N : P = 2 : 1$). The first three of these substances are more or less of a plastic consistency and very hygroscopic, while they are so labile that exposure to air and light for a comparatively short time considerably modifies certain of their properties. They do not crystallise and on account of their liability to oxidation must be preserved in evacuated desiccators. Sphingomyelin on the other hand is a pure white crystalline substance and has physically no resemblance to the other phosphatides mentioned. It is practically non-hygroscopic and does not oxidise on exposure to air.

While these substances have many properties in common, their separation and identification depend chiefly on the following points.

	Lecithin	Kephalin	Cuorin	Sphingomyelin
(1)	Soluble in ether and in alcohol	Sol. in ether Insol. in alcohol	Sol. in ether Insol. in alcohol	Insol. in ether Sol. in hot alcohol Insol. in cold alcohol
(2)	Insol. in acetone	Insol. in acetone	Insol. in acetone	Insol. in acetone
(3)	$N : P = 1 : 1$	$N : P = 1 : 1$	$N : P = 1 : 2$	$N : P = 2 : 1$

The two chief solvents in common use for extracting phosphatides from tissues are ether and alcohol. Ether extracts all four phosphatides to some extent, for although sphingomyelin is insoluble in ether it is fairly soluble in an ether extract of tissue. On the other hand, only a small part of the total tissue phosphatides are capable of being extracted by means of ether. After extracting with ether to a point at which only the merest traces of phosphatides can be obtained, it is found that subsequent extraction with alcohol results in the removal of further large amounts of these substances.

Attempts have been made to explain this phenomenon on the supposition that the ether fails to penetrate the tissues—that the difficulty is simply

a mechanical one. It has been pointed out that when fats or phosphatides are mixed with protein matter and the whole mass dried and then extracted with ether, only a fraction of the original ether-soluble material is removed. While this is true to a degree, it is equally certain that the fraction which can be removed bears a much higher proportion to the total fatty matter present than is the case in extraction of the tissues.

In extracting dried normal kidneys with ether not more than 15 % to 30 % of the total phosphatides can generally be obtained; often the amount is even less than this. On subsequent extraction with alcohol the remainder comes out with ease.

If, however, the kidney is one showing marked fatty degeneration, the greater part of the phosphatides is obtained by ether extraction.

From this it would appear that part of the phosphatide material at any rate is present in some kind of ether-insoluble combination with the proteins; this combination is acted on by alcohol and the phosphatide liberated; it is then easily extracted by ether in so far as physical difficulties permit. In this connexion an interesting observation has just been made by Osborne and Wakeman [1915]. In an investigation on milk they found that the phosphatides were present in the precipitated protein matter; on drying this protein and extracting it with ether no lecithin whatever was obtained, while subsequent treatment with alcohol removed a considerable amount. The explanation advanced by Erlandsen [1907] that the ether-extractable material was "lecithin" while the ether-soluble substance subsequently obtained by means of alcohol was not "lecithin" but a new substance—a diamino-monophosphatide—has been shown to be untenable [MacLean, 1913, 1].

By far the greater part of the tissue phosphatides generally consists of the so-called "lecithin" together with some kephalin. Cuorin appears to be present only in small amount, and from many tissues only a trace of sphingomyelin can be obtained.

ON AN ALCOHOL-SOLUBLE NITROGENOUS IMPURITY ASSOCIATED WITH "LECITHIN."

The observations already made show that alcohol is a more suitable solvent than ether for extracting lecithin from tissues. For the reasons given, alcohol is now generally used, the usual procedure being to dry the tissue as quickly as possible, grind it to a fine powder and extract thoroughly with various changes of the solvent, a shaking machine being used. On evaporating the

alcohol from the combined extracts the residue is taken up with ether, and the phosphatides precipitated by the addition of excess of acetone to the ethereal solution. By repeating the process several times all acetone-soluble bodies such as cholesterol and fatty acids are removed. The final residue is supposed to consist almost entirely of phosphatides and this is divided into "lecithin" and kephalin by fractionation with alcohol. The alcohol-insoluble part is considered as kephalin; the alcohol-soluble as "lecithin."

In every case, however, in which this method is used as a basis for the preparation of phosphatides, the resulting products are far from pure and the "lecithin" fraction often contains as much as 50 % of an extraneous product. This substance which has already been referred to [MacLean, 1912] is soluble in water and on treatment of the "lecithin" fraction by means of acetone and water [MacLean, 1912] it remains in the acetone-water solution. On evaporation of this solution the substance is obtained as a sticky syrupy mass which is insoluble in ether and in *absolute* alcohol but easily soluble in alcohol containing a trace of water. It is exceedingly soluble in water; on standing for some days an aqueous solution gradually deposits small round white crystals. These crystals are insoluble in cold water, but dissolve in boiling water to be reprecipitated on cooling. After recrystallising in this way several times, a substance or mixture of substances is obtained which has a very high nitrogen content and evidently belongs to the purine group.

In one case small round concentric white crystals were isolated which had all the properties of carnine. After drying at 105°, analysis showed that the substance contained 28.55 % nitrogen. On heating it changed colour about 230° and blackened at 240°. With silver nitrate a white flocculent precipitate was obtained which did not dissolve in ammonia or nitric acid. A precipitate was also given with basic lead acetate and with mercuric chloride, while neither neutral lead acetate nor mercuric nitrate gave any result. In its properties and nitrogen content this substance appears to be identical with or closely related to the base carnine found by Weidel in American meat extract. In another sample a small amount of a substance which appeared to be impure hypoxanthine was isolated; it contained about 40 % nitrogen. The mother liquor from which these substances separate is distinctly acid in reaction, and it is probable that these bodies are set free by a process of decomposition and are not present in the free state in the original liquid. This is suggested by their extreme insolubility in cold water and the difficulty experienced in redissolving them in the

mother liquor after separation has once taken place. The fact that complete separation takes days or even weeks is also suggestive in this connexion.

Owing to the high nitrogen percentage in these bodies it is obvious that a small quantity present in lecithin would materially influence the N : P ratio.

When the separation of these bodies is complete an absolutely clear straw coloured mother liquor is obtained which remains clear for a very long time.

On evaporating, a sticky hard gum-like substance remains. This substance is soluble in alcohol containing a trace of water and appears to dissolve in solutions of lecithin. Further, like phosphatides in general, *it is precipitated by acetone, by cadmium chloride, and also by platinum chloride.* The body appears to be of a very complex nature and so far it has been impossible to determine its chemical constitution. Different specimens contain on an average about 6 % of nitrogen, and there is generally a small amount of phosphorus also present, though the latter may not amount to more than a trace. When tested by Van Slyke's method a considerable part of its nitrogen was found to be present in the amino form. On hydrolysis some specimens yielded a very small amount of fatty acids, but this was probably due to contamination with lecithin, for a specimen has been obtained recently which, on hydrolysis with HCl, gave no trace of fatty acids. It decolourised Fehling's solution on heating but no precipitate occurred: on prolonging the boiling however a dense precipitate was formed.

Attempts to fractionate the water-soluble substance.

Some of the substance was rubbed up thoroughly with ether to remove any traces of fatty acids or phosphatides that might be present. The ether was decanted off and the residue dissolved in about 20 cc. water to which 30 cc. alcohol and 50 cc. ether were added. On the addition of the ether, part of the substance was precipitated as an oily liquid. This was dried and analysed. It contained 5.7 % nitrogen and 0.9 % phosphorus.

The part that remained soluble in the water-alcohol-ether mixture was obtained by evaporating off the solvent. It was of a brown colour and thick syrupy consistency. This was dissolved in water and excess of mercuric chloride added which produced an immediate precipitate.

After standing for two hours this precipitate was filtered off = *Precipitate A*. The filtrate was allowed to stand, and gradually deposited some more substance

which was filtered off after 24 hours = *Precipitate B*. *Precipitate A* was decomposed in water by means of sulphuretted hydrogen, the insoluble sulphide separated and the filtrate evaporated to dryness *in vacuo*. A crop of crystals was obtained; the nitrogen estimation gave the following figures:

0.0164 g. required 4 cc. N/10 H_2SO_4 = 34.1 % N.

In certain of its properties it agreed with creatinine, though on treatment with sodium nitroprusside and alkali, or picric acid and alkali, no red colour was produced. Sufficient of the substance was not obtained to identify it with certainty; it was probably not a pure substance. *Precipitate B* was treated in the same way as above, and a small amount of crystalline residue was obtained. Unlike the corresponding substance obtained from *precipitate A*, this substance gave marked creatinine reactions and presumably contained creatinine which might have been formed from creatine during the various manipulations employed.

Thus far there is evidence that the original water-soluble impurity contained carnine or somewhat similar bases, and a trace of creatinine, the latter perhaps being an artificial product. The filtrate *M* from the mercuric chloride precipitate was decomposed by sulphuretted hydrogen and the sulphide separated by filtration. The filtrate was evaporated to dryness on the water bath and the residue dissolved in alcohol. After filtration the alcohol was removed *in vacuo* and the residue dried and analysed for N and P. It was a brownish hard hygroscopic material.

0.0708 g. required 3.09 cc. N/10 H_2SO_4 = 6.11 % N.

0.2234 g. gave no trace of phosphorus.

These results sufficiently indicate the complex nature of this substance; the fact that it is so exceedingly difficult to separate from the phosphatides by the ordinary methods of purification is undoubtedly responsible for the appearance in the literature of many supposed new phosphatides.

In very many cases it is quite obvious from the methods employed by several investigators that the phosphatides obtained and supposed to be pure substances must have been contaminated with this impurity.

The phosphatides can be entirely separated from this impurity by emulsification with water and precipitation with acetone as already described in a former paper [MacLean, 1912].

SOME ANOMALOUS RESULTS OBTAINED IN THE EXTRACTION
AND PREPARATION OF PHOSPHATIDES.

The simplest method for the extraction of the chief bulk of the phosphatides from the tissues is by means of alcohol, the dried tissue being thoroughly exhausted with this solvent. If the cuorin is also wanted it is necessary to use ether, as this substance is apparently quite insoluble in alcohol or in alcoholic solutions of phosphatides. When the alcohol filtrates are concentrated, the residue dissolved in ether and the phosphatides precipitated by excess of acetone, it is generally found that a good deal of the acetone precipitate is insoluble in absolute alcohol. This insoluble part on further purification is found to have a N : P ratio of 1 : 1 and constitutes the substance known as kephalin; the alcohol-soluble "lecithin" generally predominates in amount. If, however, the same kind of tissue is obtained in as fresh a condition as possible and carefully and quickly dried, either by immersing the finely divided substance for a few minutes in excess of alcohol or by exposing it in thin films to a current of air at 30°, it is often found that the alcoholic extract, when treated in the usual way with acetone, gives a precipitate which is entirely soluble in alcohol. In this case the whole of the extracted phosphatide, since it is alcohol-soluble, would be held to be "lecithin." Now these divergent results can be obtained from the same tissue, so that according to the treatment of the material one observer may find nothing but "lecithin" while another may find abundance of both "lecithin" and kephalin.

Again, it very often happens that "lecithin," which dissolves easily in alcohol immediately after its separation from the tissues, becomes partly alcohol-insoluble even when preserved in a carefully evacuated brown glass desiccator. The alcohol-insoluble substance formed has the properties and general composition of kephalin. This change in solubility is much more in evidence if the organic solvents used are not quite pure, and in general the greater care taken to purify the various organic reagents—ether, acetone, etc.—the more "lecithin" is obtained. Particularly does this appear to be the case with ether.

In one experiment bearing on this point heart tissue was extracted with alcohol in the ordinary way: the residue was taken up in ordinary "good" ether as supplied by the dealers. Another portion was treated in exactly the same way, ether from the same source being used, but only after careful purification. Using ordinary ether a considerable amount of the phosphatide

ultimately obtained was insoluble in alcohol and part of the "lecithin" fraction became alcohol-insoluble after a week; on the other hand the specimen in which purified ether was used gave nearly all "lecithin" which, up to the present time (six weeks after preparation), remains quite soluble in alcohol.

From these anomalous results it is easy to explain the divergence of opinion with regard to the occurrence of kephalin in the tissues.

Thus, Erlandsen in heart muscle could obtain no trace of kephalin [1907], while Koch and Woods [1905] found kephalin in the heart tissue in excess of the lecithin in the proportion of 100 to 61. In many other cases these observers found more kephalin than lecithin as the following figures show.

Substance analysed			Total phosphatide per cent.	Lecithin per cent.	Kephalin per cent.
Egg yolk	...	...	9.9	3	6.9
Pancreas	...	...	1.89	0.59	1.3
Milk (cow)	...	...	0.086	0.049	0.037
Liver	...	...	2.3	0.8	1.5
Kidney	...	...	2.6	1.2	1.4

How are we to explain these results? The explanation appears to be that kephalin, when rapidly extracted from fresh tissues, is soluble in an alcoholic solution of "lecithin." Its insolubility is probably brought about by oxidation, so that when impure solvents are used or precautions to prevent chemical changes are not taken, some of the kephalin becomes insoluble. If these oxidation changes are very marked some of the lecithin may also become insoluble so that the "kephalin" represents in this case a mixture of true lecithin and kephalin; it would appear however that true lecithin is much less liable than kephalin to become alcohol-insoluble.

A good deal of controversy exists with regard to the question whether kephalin is absolutely or relatively insoluble in alcohol. It is obvious, however, that kephalin is not entirely alcohol-insoluble, more especially in the presence of other phosphatides. If, therefore, a tissue is treated with alcohol, two monamino-monophosphatides are extracted—lecithin and kephalin—but it depends on circumstances whether the kephalin is insoluble in the alcohol or remains dissolved in the alcoholic solution of lecithin. In the latter case the phosphatide would have all the general properties of "lecithin" and little or no kephalin would be obtained. The small amount of sphingo-myelin present in the tissues would also be present in the alcohol extract.

From this it appears that the substance known as "lecithin" must in many, if not in all cases, be represented by a mixture of two substances—

kephalin which tends to become insoluble in alcohol, and a substance which remains alcohol-soluble—true lecithin. The lecithin of the text books is really a lecithin-kephalin mixture. While it is possible that the two substances may be present in some kind of feeble combination there is obviously no combination in the ordinary chemical sense; this is indicated by results to be described later. For the further discussion of the subject it is necessary to point out the chief differences which have been established between kephalin and true lecithin.

Kephalin. Kephalin owing to its insolubility in alcohol and the conditions just described can be obtained in the pure state. The pure substance seems to have the same general structure as true lecithin, but in the case of kephalin the base present is not choline but β -amino-ethyl alcohol. This base is difficult to isolate in that its combination with platinum chloride is soluble in alcohol, especially in the presence of glycerol or other products of phosphatide hydrolysis. The salt is precipitated from its alcoholic solution by ether. It may, however, be obtained as the platinum chloride or gold chloride double salt by careful concentration. It yields all its nitrogen when treated with nitrous acid and can be estimated quantitatively by Van Slyke's method. Its compounds are so soluble that a gravimetric method of estimation has not yet been found.

Kephalin is precipitated by cadmium chloride in alcohol solution, the *resulting combination being soluble in ether*.

True lecithin. Unlike kephalin, true lecithin has theoretically all its nitrogen in the form of choline. Hitherto it is probable that no one using the ordinary methods has succeeded in obtaining a lecithin in which the choline accounted for the whole of the nitrogen. That the "theoretical" compound does exist will be shown later. Like kephalin, lecithin is precipitated by cadmium chloride, but *under certain circumstances the precipitate is insoluble in ether*.

THE COMPOSITION OF ORDINARY "LECITHIN."

The above observation, that ordinary lecithin is a mixture, is strengthened by the results of many investigators who found that the amount of choline obtainable from lecithin, and generally separated as the platinum compound, was not nearly sufficient to account for the whole of the nitrogen present. The figures given indicate sufficiently the variations found.

Investigator	Source of "lecithin"	Percentage of nitrogen of "lecithin" obtained as choline platinum chloride
Heffter [1891]	Liver	25
Erlandsen [1907]	Heart	42
Moruzzi [1908]	Egg yolk	77
MacLean	Heart	42 to 75
"	Egg yolk	65
"	Other specimen egg yolk	77
"	Trade lecithin (Riedel)	80
"	Trade lecithin (Kahlbaum)	90
Osborne and Wakeman [1915]	Milk	39.5

It is exceedingly suggestive that "lecithins" from the same source but prepared in different ways did not always contain the same percentage of choline, while control experiments with the same sample gave almost identical results. As a result of similar experiments the writer many years ago came to the conclusion that "the generally accepted lecithin formula in which the whole of the nitrogen was represented by a base—choline—is incorrect and can no longer be accepted" [1909, 1]. The correctness of this view was questioned by Malengreau and Prigent [1912] who, working with Kahlbaum's lecithin, obtained nearly the whole of the nitrogen as choline. They made use of the cadmium chloride salt of lecithin and hydrolysed for long periods by means of N/10 H_2SO_4 . That certain trade lecithins do contain a large percentage of their nitrogen as choline had been already emphasised by me [1908], but in cases where I employed acid hydrolysis the acid used was much stronger than the above. I therefore thought it well to repeat the experiment of Malengreau and Prigent with "lecithins" prepared from heart and from eggs.

Method of separation of choline from lecithin.

The lecithin was boiled for several hours (14 to 72) with excess of N/10 H_2SO_4 ; the mixture was then allowed to cool, when the fatty acids separated out as a solid cake on the surface. This was separated by filtration. The fatty acids were then returned to the flask, some distilled water added and the whole boiled for several minutes. On cooling the acids which again separated as a solid cake were as before removed by filtration. This process was carried out from four to six times.

An aliquot part of the total liquor obtained was taken and neutralised with $\text{Ba}(\text{OH})_2$ solution; the precipitate formed was separated by filtration and carefully washed many times with boiling water. The filtrate, acidified with hydrochloric acid, was evaporated to dryness on the water bath, the residue extracted with alcohol, the alcoholic solution filtered, made up

to a definite volume and divided into two equal parts. One part was used for estimation of the nitrogen, and the other for choline platinum chloride. The results given below were very similar to those obtained in my earlier experiments with stronger acids.

Source of lecithin				Time of hydrolysis in hours	Percentage of nitrogen of final filtrate obtained as choline platinum chloride
Kahlbaum's trade lecithin	...	...	...	14	91.4
"	"	...	...	35½	92
Heart (old specimen)	...	...	...	16	89
" (by ether extraction)	...	...	...	32	63.8
" (by subsequent alcohol extraction)	...	...	...	32	69
" (direct alcohol extraction)	...	...	...	35½	72
" (ether extraction)	...	...	...	35½	61.4
" " " (control)	...	...	...	35½	62
" (ether extraction, very fresh specimen)	...	...	...	30	52

The difference in choline content of "lecithins" from the same source is well brought out and is important in considering whether the "lecithin" is a mixture of true lecithin and kephalin or a combination. These results suggest that it is merely a mixture and not a combination, for in the latter case it is difficult to see how this variation in nitrogen content could arise.

Further evidence in support of this contention is furnished by an observation made several years ago [MacLean, 1909, 2] that when "lecithin" is precipitated by cadmium chloride, the cadmium compound obtained contains considerably more of its nitrogen as choline (in one case 10 % more) than the lecithin from which it was obtained. This observation has been confirmed by Eppler [1913] and also by Trier.

In my experiments, the cadmium chloride precipitate was washed with ether and it is possible that this, rather than the alcohol, was the cause of the difference. This, however, does not in any way influence the deduction to be drawn—that we are dealing with a mixture and not a compound.

There still remained the theoretical possibility that none of the methods used to estimate choline was satisfactory, and that all the nitrogen might still be present as choline.

These objections were answered by the discovery of Trier [1911], who found β -amino-ethyl alcohol $\text{CH}_2(\text{NH}_2) \cdot \text{CH}_2 \cdot \text{OH}$ among the products of hydrolysis of the phosphatides of bean meal (*Phaseolus vulgaris*). Later the same observer recovered amino-ethyl alcohol from egg lecithin, identifying it by means of the gold chloride combination [1912, 1, 1913]. Trier has shown that amino-ethyl alcohol when methylated with methyl iodide, methyl alcohol and potassium hydroxide is converted into choline [1912, 2].

Amino-ethyl alcohol was also discovered independently in egg lecithin by Eppler [1913] working in Thierfelder's Laboratory, and has been found several times by the writer in heart "lecithin" [MacLean, 1913, 2]. It has also been found by Trier in various other "lecithins" particularly of plant origin. The next point to determine was whether the whole of the nitrogen of "lecithin" can be accounted for by the choline and amino nitrogen present. The amino nitrogen may be assumed to be present as amino-ethyl alcohol, since there is abundant evidence that this base is always present in "lecithin"; this view is supported by the fact that no other base containing amino nitrogen can be obtained from lecithin. There are also many chemical considerations such as the extreme solubility of the platinum and gold chloride combinations of this substance which are suggestive. In hydrolysed lecithin, platinum chloride precipitates only choline while another combination with a base remains in solution in the filtrate. On carefully evaporating this filtrate, the only base obtained is the amino-ethyl alcohol salt which appears to be invariably present. Before giving details of the experiments performed to determine the distribution of nitrogen in "lecithin" the method by which the "lecithin" was prepared will be described, as this differs considerably from the ordinary methods and has been somewhat modified of late. The "lecithin" used in all the experiments to be described later, together with several "lecithins" already mentioned, was prepared in this way. The following are the chief steps, which may be somewhat varied according to circumstances.

Preparation of "Lecithin."

(1) The tissue used (heart, kidneys or egg yolk) was in the case of solid organs ground to a fine paste by a mincing machine, the paste spread out in a thin layer on a glass plate and dried at 30° by means of an air current generated by a fan. Sometimes the original paste was put into excess of alcohol for a few minutes, the alcohol separated by filtering through a cloth and the solid material finally pressed to a hard mass by a laboratory hand press. In either case, the dried tissue was passed through a coffee mill and a fine powder obtained.

(2) The dried tissue was thoroughly extracted several times (generally four to six) with excess of absolute alcohol, the various extracts mixed and concentrated to small bulk under reduced pressure at 40°.

(3) The residue was taken up with a small volume of ether, in which much of it remained insoluble. To the mixture, without any attempt at

filtration, acetone was added in excess and the precipitate obtained pounded together by a pestle and separated. The precipitate was again mixed with ether, precipitated by acetone and treated as before. By this process, which was repeated three times, all but traces of acetone-soluble bodies were removed.

(4) The precipitate from above was rubbed up in a mortar with a large excess of water and a good emulsion made; to the emulsion from one-quarter to one-third of its volume of acetone was added. On the addition of the acetone a large amount of substance separated in the form of large white flakes and floated on the surface of the liquid. This was removed partly by means of a glass spatula, and partly by filtration. It was again emulsified and precipitated three times more. The filtrate obtained from the first emulsification always contained much soluble matter—the nitrogenous impurity previously referred to—but after the first treatment only traces of this impurity were found in the liquid.

The solid substance which separated was now dried by treating it several times with fresh additions of acetone. Finally, as much acetone as possible was pressed out of the mass by means of a pestle and the whole taken up in ether, in which it was still partly insoluble, forming an opalescent mixture.

(5) The ether mixture was centrifuged when a clear supernatant fluid and a white precipitate were obtained. This white precipitate was found to be impure sphingomyelin. The ethereal solution was decanted off and treated with excess of acetone. The resulting precipitate was again taken up with ether when an almost clear solution was obtained. Centrifuging was repeated as before and the clear supernatant ethereal solution again precipitated with acetone. Generally it was only necessary to centrifuge twice, but if the phosphatide was not quite soluble in ether after a second treatment the process was repeated until a precipitate was obtained which was quite soluble in ether.

(6) The substance was now dissolved in alcohol, and if the solution was not quite clear it was allowed to stand for some time until the insoluble substance (crude kephalin) settled on the bottom of the flask. The amount of alcohol-soluble substance obtained depended on circumstances already explained. After separation of the alcohol-insoluble part by decantation the solution was filtered.

(7) The solution was now evaporated under reduced pressure at 40°, the residue taken up with ether, and the phosphatide separated from the

etheral solution by acetone. The precipitate obtained gave a perfectly clear solution with ether and with alcohol. It was treated with acetone several times and dried in the desiccator *in vacuo* over sulphuric acid. This substance had all the properties of "lecithin." The samples obtained contained about 1.85 % of nitrogen and 4 % of phosphorus and had a N : P ratio of approximately 1 : 1.

Distribution of nitrogen in "lecithin."

At first, attempts were made to estimate the amount of nitrogen obtained by Van Slyke's method [1912], using the unhydrolysed lecithin. These experiments were rendered difficult, partly on account of the frothing which resulted, and partly owing to the difficulty of finding a suitable solvent for the lecithin. The solvent used was strong acetic acid, but it was found that after the preliminary shaking of the sodium nitrite and acetic acid according to Van Slyke's instructions, the addition of acetic acid (instead of the usual aqueous solvent or very weak acid or alkali) acted in such a way as to give a good deal of gas which was not taken up by the permanganate absorbing mixture. This introduced an error which had to be allowed for by controls, but even then the results were not satisfactory. In spite of these disadvantages, however, the numbers obtained are very suggestive. On hydrolysing the lecithin and then using the liquid containing the soluble products of hydrolysis no difficulty whatever was experienced.

The choline was estimated by hydrolysis with weak acid followed by the steps already described.

Experiments with whole "lecithin."

(1) *Amino nitrogen.* After dissolving the "lecithin" in strong acetic acid the solution was allowed to stand for 24 hours; unless this was done concordant results could not be obtained, for a fresh solution of "lecithin" in acetic acid invariably gave too high results. The estimation was done exactly as described by Van Slyke, secondary octyl alcohol being used to prevent frothing as much as possible.

Experiment. 2.35 g. "lecithin" dissolved in 25 cc. strong acetic acid; different amounts of this were taken and amino nitrogen estimated.

(1) 5 cc. (equal to 0.47 g. "lecithin") = 4.9 cc. nitrogen at 20°, 769.

(2) 5 cc. (equal to 0.47 g. "lecithin") = 4.95 cc. nitrogen at 20°, 769.

(3) 4 cc. (equal to 0.376 g. "lecithin") = 3.9 cc. nitrogen at 20°, 769.

Therefore 1 g. "lecithin" contains 6.0097 mg. N as amino nitrogen = 31.4 %.

(2) *Choline nitrogen.* 0.9714 g. "lecithin" boiled for several hours with N/10 H_2SO_4 , filtered, treated with $\text{Ba}(\text{OH})_2$, filtered, residue taken up with alcohol and made up to 25 cc.

10 cc. gave 0.1040 g. choline Pt. chloride = 66 % of total nitrogen.

The nitrogen in this sample was distributed as follows.

N in fatty acids	=	4 %
N as choline	=	66 %
N in amino form	=	31.4 %
Total	=	101.4 %

Other results obtained in this way are shown in the table.

No.	Amino nitrogen found in percentage of total nitrogen	Choline nitrogen found in percentage of total nitrogen	Nitrogen retained by fatty acids in percentage of total nitrogen	Percentage of total nitrogen accounted for
1	30	68.6	3	101.6
2	38.2	63	4.4	105.6
3	28	64	6	98
4	30	68	6	104

Although these results are not altogether satisfactory, it must be remembered that there are great difficulties in getting exact quantitative results when using platinum chloride as a precipitant for choline; also the very small amount of nitrogen retained in the fatty acids is naturally difficult to estimate accurately. When to these are added the difficulties experienced in dealing with whole "lecithin" in Van Slyke's apparatus the results are as accurate as could be reasonably expected. They suggest that all the nitrogen of "lecithin" is present, partly as choline, and partly in the amino form—as amino-ethyl alcohol. This is better brought out in the next set of experiments, where hydrolysed lecithin was used.

Experiments with hydrolysed "lecithin."

In these experiments hydrolysis was carried out with dilute hydrochloric or sulphuric acid. Since the fatty acids always retain a small amount of nitrogen, and the nature of this small fraction of the total is difficult to ascertain, the investigation was limited to the determination and distribution of the filtrate nitrogen. On account of technical considerations it was found most convenient in some cases to estimate the choline in one portion of the lecithin, a separate portion of the same sample being used for the amino-ethyl alcohol determination. This was done in two experiments. In other cases aliquot parts of the same filtrate were taken.

(1) *Amino nitrogen.* For the determination of this fraction hydrochloric acid was used. About 1 g. of the "lecithin" was boiled with excess of the acid for several hours. After separation of the fatty acids they were thoroughly washed several times with hot water, separated by filtration, and the combined extracts evaporated to a small bulk on the water bath. The hydrochloric acid was then nearly neutralised with sodium hydroxide, the liquid filtered, the residue extracted with water and the total filtrates made up to a definite volume (10-20 cc.). Of this 2 cc. were taken and the amino nitrogen estimated by Van Slyke's micro-apparatus.

(2) *Choline nitrogen.* For the quantitative determination of choline N/10 H_2SO_4 was used, the process being carried out in the manner already described. The results of two experiments are given below.

No.	Source of Lecithin	Percentage of filtrate nitrogen recovered as choline	Percentage of filtrate nitrogen recovered as amino-ethyl alcohol	Percentage of nitrogen accounted for
1	Egg yolk	70	31	101
2	"	66.4	35.8	102.2

Experiments with "lecithin," using aliquot parts of the same filtrate.

1.0935 g. lecithin were boiled with 100 cc. N/5 H_2SO_4 for 48 hours. After separation of the fatty acids the usual manipulations were performed and a final alcoholic filtrate of 25 cc. obtained. Separate portions of this were taken for the determination of (1) choline, (2) amino-ethyl alcohol, (3) total nitrogen.

(1) *Determination of choline.* To 10 cc. of filtrate an alcoholic solution of platinum chloride was added. The precipitate obtained after drying to constant weight at 105° weighed 0.115 g. This figure was obtained after deducting the weight of the double salt of amino-ethyl alcohol platinum chloride which was present to the extent represented by 0.07 mg. amino-nitrogen.

(2) *Determination of amino-nitrogen by Van Slyke's method.* 10 cc. alcoholic filtrate were acidified with hydrochloric acid and carefully evaporated to dryness. The residue was dissolved in 2.5 cc. distilled water, 2 cc. of this being used for the determination.

2 cc. aqueous solution gave 2.55 cc. N at 20° and 755,
i.e. 10 cc. contained 1.802 mg. amino-nitrogen.

(3) *Determination of total nitrogen.* 3.5 cc. alcoholic filtrate required 1.75 cc. N/10 H_2SO_4 = 2.45 mg. N, i.e. 10 cc. = 7 mg. N.

From these figures:

$$\begin{aligned}\text{Total nitrogen in filtrate} &= 17.5 \text{ mg.} \\ \text{Nitrogen present as choline} &= 13.07 \text{ mg.} \\ \text{Nitrogen present as amino-ethyl alcohol} &= 4.505 \text{ mg.} \\ \text{Total} &= \underline{17.575 \text{ mg.}}\end{aligned}$$

In this case the nitrogen of the filtrate was all accounted for, 74.6 % being present as choline and 25.7 % as amino-ethyl alcohol.

Somewhat similar results were obtained in other experiments as shown by the table.

No.	Source of Lecithin	Total nitrogen of filtrate in mg.	Choline Pt. chloride obtained in g. (calculated from amount obtained from aliquot part)	Mg. nitrogen present in choline chloride obtained	Mg. nitrogen present as amino-ethyl alcohol	Percentage nitrogen as choline	Percentage nitrogen as amino-ethyl alcohol	Percentage of total filtrate nitrogen accounted for
1	Egg yolk	16.5	0.2860	13	3.9	78.8	23.6	102.4
2	Ox heart	20.2	0.3298	15	4.95	74.2	24.5	98.7
3	Horse kidney	23	0.3364	15.3	8	66.5	34.8	101.3

From these results there can be no doubt that the whole of the water-soluble nitrogen of lecithin is present in the form of choline and amino-ethyl alcohol. It is probable that the nitrogen retained in the fatty acids is also in the amino form though some of this may be due to traces of impurity in the lecithin; it is worth noting that in many cases the nitrogen content of the lecithin is often slightly higher than is required to give a N : P ratio of 1 : 1. At any rate the part retained by the fatty acids is small.

When an alcoholic solution of choline containing amino-ethyl alcohol is precipitated by platinum chloride it frequently happens, if the solutions are concentrated, that some amino-ethyl alcohol platinum chloride is carried down with the choline double salt. This was found to be the case to a very slight extent in the above experiments. Here the amino nitrogen actually present in the platinum chloride precipitate was estimated and due allowance made. In this connexion, it is best not to have the alcoholic choline solution too concentrated; again the precipitate should be very thoroughly washed with absolute alcohol before drying. It must be dried at 105° to remove water of crystallisation. In pure alcoholic solution amino-ethyl alcohol gives a distinct precipitate with platinum chloride, especially if the latter is added in excess, but in the presence of glycerol and other impurities the double salt tends to remain in solution¹.

¹ In this connexion Osborne and Wakeman [1915] record the fact that the final alcoholic extract of some hydrolysed lecithin yielded on the addition of platinum chloride only a certain

Experiments with phosphatides differing in solubility in alcohol.

When lecithin is prepared from a tissue and kept for some time, a good deal of it tends gradually to become insoluble in alcohol while some remains easily soluble as already described. Between these two extremes, however, fractions of varying solubility in alcohol can be obtained.

Thus, in one experiment about 20 g. of some old heart lecithin were rubbed up three times in a mortar with about 80 cc. alcohol. The alcohol was separated by filtration and contained lecithin readily soluble in alcohol which was separated and dried in the usual way: Fraction I. The solid residue from above was now extracted thoroughly with excess of alcohol, and a lecithin fraction rather less soluble in alcohol obtained: Fraction II.

The alcohol-insoluble part remaining was dissolved in ether and great excess of alcohol added. A great deal was precipitated but some remained in solution: Fraction III.

The precipitate was dissolved in ether and precipitated by acetone. This was practically alcohol-insoluble kephalin: Fraction IV.

From the results already obtained it was to be expected that the amount of choline present should decrease and the amount of amino-ethyl alcohol increase in proportion to the relative insolubility in alcohol of the different fractions.

This proved to be the case; the results given were obtained by the methods already described.

Phosphatide	Percentage of nitrogen as choline	Percentage of nitrogen as amino-ethyl alcohol
Fraction I	67.7	33
" II	—	38.2
" III	43	55.3
" IV	—	81

These figures substantiate the statement previously made that "lecithin" is a variable *mixture* and not a *compound*. Osborne and Wakeman in the communication already referred to state that the ether-soluble milk phosphatide obtained by them may be a mixture or combination of two substances, one yielding choline and the other a base containing amino-nitrogen. They intend to fractionate the substance to settle this question. In the light of the above observations it is probable that this phosphatide will be found

fraction of the total choline; after evaporating the filtrate, taking the residue up in alcohol and adding more platinum chloride another precipitate was obtained. This was repeated four times. It is probable, however, that some of these latter precipitates at any rate contained amino-ethyl alcohol platinum chloride.

to fall into line with other phosphatides in that it consists of a mixture of true lecithin and kephalin.

Attempts were now made to separate "lecithin" into its component parts. After many unsuccessful experiments I succeeded in doing this, and obtained a true lecithin with all its nitrogen present as choline.

SEPARATION OF "LECITHIN" INTO ITS COMPONENTS.

The lecithin used was derived from ox hearts and from eggs, the extraction and purification being carried through as quickly as possible.

The first experiments described were conducted with ox heart lecithin which was at first completely soluble in alcohol. On analysis for nitrogen, phosphorus and distribution of nitrogen, it gave the following results.

Nitrogen (Kjeldahl). 0.8007 g. required 10.85 cc. N/10 H_2SO_4 = 1.89 % N.

Phosphorus (Neumann). 0.5990 g. required 43.7 cc. N/2 NaOH = 4.04 % P.

$$\text{N} : \text{P} = 1.03 : 1.$$

Distribution of nitrogen.

1.3202 g. lecithin boiled with 120 cc. N/10 H_2SO_4 for 48 hours gave, after the usual treatment and addition of platinum chloride to the final alcoholic solution, 0.3673 g. choline platinum chloride (platinum content = 31.63 %).

The fatty acids contained 0.448 mg. nitrogen.

From above figures :

Total nitrogen of 1.3202 g. "lecithin" = 24.95 mg.

Nitrogen found in fatty acids = 0.448 mg.

Nitrogen of filtrate (by difference) = 24.502 mg.

Now 24.502 mg. nitrogen = 0.5387 mg. choline platinum chloride and choline platinum chloride obtained = 0.3673 mg., i.e. 68 % of filtrate nitrogen is derived from choline.

By Van Slyke's method the filtrate from choline platinum chloride was found to contain 28 % of its nitrogen in amino form.

After standing for four days in an evacuated desiccator this "lecithin" formed a slightly opalescent alcoholic solution which on standing for some time deposited a very small amount of a sticky brown substance. The

solution was filtered and treated with excess of an alcoholic solution of cadmium chloride.

The resulting precipitate of cadmium chloride compound was thoroughly washed with alcohol, the latter being finally drained off by means of the suction pump. The precipitate was then dried *in vacuo*.

The dried powder was now ground up in a mortar with excess of pure dry ether. By this means it was hoped to extract the kephalin fraction, since kephalin cadmium chloride is said to be soluble in ether, while, on the other hand, the corresponding lecithin salt is supposed to be insoluble.

Here, however, the whole of the cadmium chloride precipitate dissolved in ether to form an almost clear solution. The ether was partly evaporated under reduced pressure; the concentrated solution obtained being spread out in a thin layer under a fan and freed from ether. The residue was taken up with fresh ordinary ether which had not been specially purified; a good deal remained in suspension as a brownish white powder. This was separated by the centrifuge and dried; it was then thoroughly ground in a mortar, extracted with ether several times and finally obtained as a white powder.

The ether-soluble part was freed from ether and the residue finely powdered. On treating a fraction of this with ether it was found to be entirely ether-soluble.

The cadmium chloride compound was now divided into two parts:

- A. An ether-insoluble part forming a white powder.
- B. An ether-soluble part forming a brownish white powder.

Treatment of ether-insoluble fraction A.

This fraction was further purified by twice recrystallising it from a mixture consisting of ethyl acetate, 2 parts, 80 % alcohol, 1 part.

The compound was dissolved by heat in this mixture and on cooling the greater part separated as a white precipitate, which under the microscope showed beautiful white clusters of well-formed needles arranged in stars. These crystals were washed with ethyl acetate and with ether and dried.

Analysis of crystals.

Nitrogen (Kjeldahl). 0.2998 g. required 3.1 cc. N/10 H_2SO_4 = 1.45 % N.

Phosphorus (Neumann). 0.1310 g. required 7.45 cc. N/2 NaOH = 3.15 % P.

N : P = 1.02 : 1.

Distribution of nitrogen in crystals.

0.5896 g. was boiled with excess of N/10 H_2SO_4 for 42 hours, the final alcoholic solution containing the soluble nitrogen being obtained in the usual way. Filtrate gave 0.1644 g. platinum chloride salt (Pt. content 31.55 %). Fatty acids contained 0.95 mg. nitrogen.

From above figures:

Total nitrogen of 0.5896 g. compound = 8.54 mg.

Nitrogen found in fatty acids = 0.95 mg.

Filtrate nitrogen (by difference) = 7.59 mg.

Now 7.59 mg. nitrogen = 0.1669 g. choline platinum chloride and choline platinum chloride obtained = 0.1644 g. i.e. 98.5 % of filtrate N is derived from choline.

The filtrate from the platinum chloride precipitate gave no amino nitrogen by Van Slyke's method. The whole of the filtrate nitrogen was therefore removed by platinum chloride.

With the exception of the variable amount of nitrogen always retained by the fatty acids this powder contained all its nitrogen as choline, while the "lecithin" from which it was derived contained only 68 % of its soluble nitrogen as choline.

This separation proves that "lecithin" is a mixture from which its choline-lecithin component can be separated by making use of the insolubility of its cadmium chloride salt in ether.

Investigation of the ether-soluble part of the cadmium chloride precipitate (Fraction B above) showed that it still contained some choline. Since the whole of the cadmium chloride precipitate originally obtained from lecithin dissolved in ether on the first attempt at separation, this result was to be expected. Choline to the extent of about 30 % of the total nitrogen was found. Attempts to reduce the amount of choline were frustrated by the extreme solubility of the salt in ether, and it is probable that although true lecithin cadmium chloride is insoluble in ether, it dissolves to a certain extent in an ethereal solution of kephalin cadmium chloride.

A fresh sample of lecithin was now obtained from hearts, and the cadmium chloride compound prepared as before. Here the fractionation of the salt gave rise to great difficulties, for when treated with ether an opalescent solution was formed which it was impossible to filter or centrifuge. Evaporating the ether and again taking up the dried substance with ether

—a procedure which was very effective in the former separation described—proved quite useless here. It was found, however, that if the dried material was made into a thick paste with alcohol, and ether containing 2 % alcohol was used, an opalescent solution was obtained which filtered very well. By this means the compound was divided into two parts as before.

A. Fraction insoluble in ether = true lecithin.

B. Fraction soluble in ether = kephalin contaminated with lecithin.

Now Thudichum [1884] makes the statement that lecithin cadmium chloride is soluble in cold benzene, and that the part which remains insoluble in the cold but dissolves on heating, to fall out again on cooling, is not lecithin but a substance which he calls paramyelin. An attempt at fractionating the white powder *A* by means of benzene was therefore made. This, however, was unsatisfactory and in my case the process did not appear to be of much value. When cold benzene was added to the powder very little indeed appeared to dissolve, but on heating the mixture nearly all the solid disappeared. On leaving this solution to stand in the ice chest for some hours an opalescent layer formed at the bottom with a clear liquid above. On filtration an absolutely clear benzene solution was obtained, while a fair amount of a semitransparent gelatinous substance was left on the filter. When this was treated with hot benzene part of it dissolved but did not form a clear solution. On again leaving it to stand at low temperature a marked deposit formed. This deposit constituted fully one half of the total substance.

When treated with hot benzene some of this apparently goes into solution, and the process of separation of lecithin cadmium chloride into benzene-soluble and benzene-insoluble fractions appears to be unsatisfactory. It seems that practically the whole can be got into solution if sufficiently large volumes of benzene are used and any differences of solubility that may exist are probably due to slight oxidation or other changes; the “insoluble” and “soluble” fractions are probably the same substance.

The ether-insoluble fraction was now divided into

(1) Fraction remaining soluble in cold benzene (original solution being accomplished by means of heat),

(2) Fraction insoluble in cold benzene (soluble more or less in hot benzene).

The cold benzene solution was treated with twice its volume of alcohol and the precipitate obtained separated by the centrifuge.

The cold benzene-insoluble fraction (2) was spread out in a thin film under a fan and dried at room temperature. Both substances were then dissolved in the ethyl acetate-alcohol mixture as before and white crystalline precipitates obtained. The following scheme represents the fractionation of the original "lecithin."

The distribution of the nitrogen in these compounds is given in the table. Hydrolysis¹ was carried out in the usual way and aliquot parts of the filtrate used for choline and for total nitrogen. The amino nitrogen was estimated in the filtrate after the separation of choline as the platinum salt. No account is taken of the nitrogen retained by the fatty acids or other precipitate.

The numbers refer to the total nitrogen of the filtrate.

No.	Substance	Percentage nitrogen as choline	Percentage nitrogen in amino form	Percentage of total filtrate nitrogen accounted for
1.	Lecithin	62	37.2	99.2
2.	Cadmium chloride lecithin, before ether	65	33	98
3.	Ether-insoluble fraction (benzene-soluble)	94	3	97
4.	Ether-insoluble fraction (benzene-insoluble)	99	None	99
5.	Ether-soluble fraction	45	54	99

Here the two fractions (3 and 4) contained all their nitrogen (with the exception of a trace in No. 3) as choline. A cadmium chloride compound of a true choline-lecithin has therefore been obtained from an original lecithin which contained only 62 % of its nitrogen as choline.

It is impossible to get the kephalin part quite free from true lecithin by this process, but kephalin can be obtained in the pure state by treatment with alcohol and suitable purification, and as such has been isolated and

¹ Considerable difficulty was sometimes experienced in hydrolysing cadmium chloride compounds of phosphatides with N/10 H₂SO₄. Often a great deal of the nitrogen was retained in the precipitates obtained during the preparation of the final alcoholic extract for precipitation by platinum chloride. Sometimes the whole of the nitrogen (with the exception of the small amount retained in the fatty acids) was found in the filtrate. The reason of these anomalous results I have been unable to ascertain. When lecithin itself was used no difficulty of this kind was encountered.

analysed by many investigators. It contains the whole of its nitrogen as amino-ethyl alcohol.

So far, only the cadmium chloride salt of true lecithin had been isolated, but on separation of the free phosphatide by means of ammonium carbonate, free lecithin was obtained which had all its nitrogen in the form of choline. This particular sample of lecithin was derived from egg yolk and as its method of preparation differed considerably in certain respects from the methods already given, this will now be described.

PREPARATION OF A SAMPLE OF LECITHIN CONTAINING ALL
ITS NITROGEN AS CHOLINE.

Dried egg yolk was powdered and extracted with alcohol: to the alcoholic extract was added excess of alcoholic solution of cadmium chloride.

The resulting precipitate was washed with alcohol and then rubbed up with about fifteen times its volume of ether containing a trace of alcohol. A dense opalescent mixture was obtained which on centrifuging separated into a brownish deposit and clear supernatant liquid. The deposit was thoroughly washed with ether, dried and decomposed by boiling in alcohol with ammonium carbonate according to the method recommended by Bergell [1900].

The alcoholic solution was concentrated, the residue taken up with ether and excess of acetone added. The precipitate obtained was emulsified with water and treated by the usual process for the purification of lecithin [MacLean, 1912]. The lecithin was dissolved in alcohol and again precipitated by cadmium chloride. The double compound obtained was recrystallised from the ethyl acetate mixture already described. Beautiful white feathery crystals of pure lecithin cadmium chloride were thus obtained.

Analysis of pure lecithin cadmium chloride (egg).

Nitrogen (Kjeldahl). 0.5604 g. required 5.85 cc. N/10 H_2SO_4 = 1.46 % N.

Phosphorus (Neumann). 0.2600 g. required 15.8 cc. N/2 NaOH = 3.37 % P.

N : P = 1 : 1.04.

Test for amino nitrogen.

1.8615 g. were boiled for 10 hours with dilute hydrochloric acid (96 cc. H_2O + 4 cc. conctd. HCl); after cooling the fatty acids were separated by filtration, the filtrate evaporated to a small bulk on the water bath and nearly

neutralised with alkali. The whole was then made up to 20 cc. Of this 2 cc. were used for each experiment.

The following results were obtained by Van Slyke's method.

Controls with reagents $\left\{ \begin{array}{l} (1) \text{ 0.12 cc. nitrogen} \\ (2) \text{ 0.13 cc. ,,} \end{array} \right\}$ at 752 and 20°.

Experiments $\left\{ \begin{array}{l} (1) \text{ 0.14 cc. nitrogen} \\ (2) \text{ 0.15 cc. ,,} \\ (3) \text{ 0.15 cc. ,,} \end{array} \right\}$ at 752 and 20°.

Average 0.146 cc. nitrogen.

2 cc. therefore contained 0.021 cc. nitrogen and from this 1.8615 g. (20 cc. solution) gives 0.210 cc. nitrogen = 0.117 mg. nitrogen in amino form.

Total nitrogen in 1.8615 g. compound = 27.17 mg.

Total nitrogen in filtrate = 26.80 mg.

Retained by fatty acids, etc. = 0.37 mg.

From above figures:

Percentage of total nitrogen in amino form = 0.430

Percentage of filtrate nitrogen in amino form = 0.437

Separation of pure lecithin from its cadmium chloride compound.

This was done as already described by ammonium carbonate. The substance obtained was thoroughly purified by means of acetone and water and finally dried *in vacuo* over H_2SO_4 .

Analysis.

Nitrogen (Kjeldahl). 0.3747 g. required 5 cc. N/10 H_2SO_4 = 1.87 % N.

Phosphorus (Neumann). 0.2710 g. required 20.3 cc. N/2 NaOH = 4.15 % P.

N : P = 1 : 1.

Estimation of choline.

1.8426 g. were boiled with 150 cc. N/10 H_2SO_4 and after the necessary manipulations 25 cc. of a final alcoholic solution obtained.

10 cc. of this solution gave 0.2993 g. platinum salt (platinum content 31.7), therefore total filtrate = 0.7483 g. platinum salt.

Now total nitrogen of 1.8426 g. substance = 34.46 mg. = 0.7581 g. choline platinum chloride; obtained 0.7483 g. choline platinum chloride, i.e. 98.7 % of total nitrogen is derived from choline.

Since, however, a small amount of nitrogen is always retained in the fatty acids the whole of the soluble nitrogen of this specimen may be regarded as choline. The platinum chloride removed every trace of the filtrate nitrogen.

SOME CONSIDERATIONS ON THE CHEMICAL CONSTITUTION OF "LECITHIN."

Fatty acids. The fact that lecithin has been shown to consist of two components throws some light on the difficulties experienced by many investigators when endeavouring to ascertain the nature of the fatty acids present. Theoretically only two acid radicles exist in the molecule, and separation and identification ought to be easy, but, generally, mixtures are obtained which appear to consist of more than two acids. It is hoped, however, that examination of the true lecithin obtained by the processes described will give better results.

Glycerophosphoric acid. From the chemical standpoint this acid can exist in two modifications—the α - and β -forms.

The α -form contains an asymmetric carbon atom and is therefore optically active, while the β -variety is inactive.

After the discovery by Willstätter and Lüdecke [1904] that the glycerophosphoric acid of egg lecithin was optically active, rotating the plane of polarisation of polarised light to the left, it was generally assumed that the acid present in lecithin was α -glycerophosphoric acid, though Tutin and Hann [1906] disputed this and held that lecithin glycerophosphoric acid consisted of a mixture of the α - and β -forms. Recently Grimbirt and Bailly [1915] claim to have definitely shown that egg lecithin is a mixture of at least two isomers containing the symmetrical and unsymmetrical varieties of glycerophosphoric acid.

It is possible that the true lecithin part of "lecithin," containing all its nitrogen as choline, may have one form of this acid, while the kephalin part may have the other form. Some experiments bearing on this point were begun some time ago but have had to be stopped for the present. I hope, however, to take up this point in the future and to settle some of these problems in a later communication.

SUMMARY.

(1) When phosphatides are extracted from tissues by means of alcohol they invariably contain large amounts of a nitrogenous impurity. This is very difficult to remove by any of the ordinary methods for preparing "lecithin" and many bodies described as new substances are simply "lecithin" contaminated with this body.

(2) This nitrogenous impurity is very complex chemically and contains bodies of a purine nature.

(3) The whole of the nitrogen of "lecithin" is accounted for by the choline and amino nitrogen present.

(4) "Lecithin" is a mixture in variable proportions of two somewhat similar substances, one having the whole of its nitrogen in the form of choline—true lecithin—while the other contains no choline but has all its nitrogen represented by amino-ethyl alcohol—kephalin.

(5) Reasons for the divergent statements as to the occurrence of kephalin in different tissues are given.

(6) By fractionation of the cadmium chloride salt of "lecithin" with ether, "lecithin" has been divided into its two components—true lecithin and kephalin. True lecithin in which every trace of the nitrogen was present as choline was obtained. The kephalin fraction contained a certain amount of choline, but had the greater part of its nitrogen as amino-ethyl alcohol.

(7) Various points on the mode of occurrence, distribution and purification of the phosphatides are discussed.

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