ON THE NITROGEN-CONTAINING RADICLE OF LECITHIN AND OTHER PHOSPHATIDES

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PART II

(Received April 13th, 1909)

In a former article¹ it was shown that only about 42 per cent. of the nitrogen of heart muscle lecithin could be accounted for when estimated in the form of the choline-platinum-chloride salt. When a sample of trade lecithin was treated in a similar manner it was found that the yield was considerably higher, being equivalent on an average to about 76 per cent. of the total nitrogen of the substance.

The comparatively low choline content of heart lecithin suggested the probability of part of the nitrogen being represented by some other N-complex differing from choline in its precipitation properties. As the method of preparation of the trade lecithin could not with certainty be ascertained, lecithin was prepared from egg yolk; an analysis of this lecithin agreed in general with that obtained from the heart, but, as will be shown below, the amount of nitrogen that could be accounted for as above described was considerably greater than in heart lecithin, and a good deal lower than in trade lecithin.

This curious result seemed to point to the probability of these different lecithins being really different bodies, at least with regard to the manner of combination of their nitrogen, and in order to test this a long series of experiments was made.

In this paper it is proposed to deal with the results of hydrolysis of egg lecithin, and at the same time describe certain experiments made with a view to ascertaining whether possibly any circumstances were present that might reduce the final output of choline, even assuming that all the nitrogen was present as this base, in accordance with the ordinary formula for lecithin.

PREPARATION OF EGG LECITHIN

Lecithin was prepared from three different portions of eggs. The method adopted was essentially the same as that described in my former papers,² and need not be repeated; in every case (with one exception)

1. This Journal, Vol. IV, p. 38.

2. This Journal, Vol. IV, pp. 47 and 168.

I used the lecithin obtained from the *ethereal* extract of egg yolk, the usual precautions being adopted to exclude, as far as possible, air and light during the process of preparation.

In one set of eggs a curious state of affairs was noticed; these eggs (100) seemed to be perfectly fresh and were all rather large, it being naturally thought that this would ensure a greater yield of lecithin. On extraction, however, it was found that the ether showed quite an abnormal increase of fatty matter, but only a trifling amount of substance precipitated by acetone; after treatment with five consecutive portions of fresh ether, the extract still contained much fat, and the combined yield of lecithin was so small that after purification the total amounted only to a few grammes. On subsequent extraction with alcohol the lecithin yield was also very much below the ordinary average.

In this particular case it would seem as if there was a great increase of fatty matter at the expense of the lecithin; unfortunately, I had not an opportunity of further investigating the nature of this substance, but a comparison of the relationship of fat to lecithin in the egg, and perhaps in other organs, together with an examination into the nature of this fat, would, in the light of the above observation, probably be of some interest.

A curious point with regard to the lecithin here obtained was that it differed greatly in general appearance from lecithin obtained from other eggs; it was from the beginning quite dark brown in appearance and not so plastic as is generally the case, despite the fact that the greatest precautions were taken to prevent oxidation; in general it looked more like a specimen that had been exposed for some time to air than the freshly extracted material, which is usually precipitated as a plastic, more or less whitish mass with a slight brown tinge. The amount of lecithin here obtained was so small that it was not made use of for this investigation.

The first sample purified gave the following figures on analysis; comparison with heart muscle lecithin shows a marked similarity in elementary composition:—

Egg Lecithin			Heart Lecithin
N	(3 experiments) Average	1.876 %	1.87 %
P	2 „ „	3.95 %	3.95 %
C	1 experiment „	64.18 %	66.29 %
H	1 „ „	10.6 %	10.17 %

N : P = 1.05 : 1

HYDROLYSIS OF LECITHIN

(a) *In alcoholic solution of barium hydrate.*—Here about 1 gramme lecithin was boiled for varying periods of from two to six hours with 100 c.c. methyl alcohol containing 5 grammes $\text{Ba}(\text{OH})_2$. After separating certain products of decomposition, as formerly described, the purified alcoholic extract was treated with alcoholic-platinum-chloride solution, and the precipitate washed, dried and weighed as usual.

Two experiments taken at random from a long series gave the following results; percentages are expressed in terms of actual amount obtained, as against theoretical amount calculated on N present:—

1.0268 gm. Lecithin boiled 3 hours	= 0.2766 gm. Choline-platinum-chloride	= 65.4 %
0.8989 " " " 6 " "	= 0.2448 " " "	= 66 %

Here, as in experiments on heart lecithin, it was invariably found that the residue obtained after hydrolysis contained nitrogen, despite the most prolonged and careful washing; experiments described later on suggest that this nitrogen is probably not of choline nature; in the two experiments given above, this insoluble N amounted to 6.08 per cent. and 9.13 per cent. respectively of the total nitrogen contained in the amount of lecithin used. In six experiments in which this residual nitrogen was estimated, the average percentage of the total lecithin nitrogen found in the residue was 6.74 per cent.

(b) *In watery solution of $\text{Ba}(\text{OH})_2$.*—In this case the hydrolysis was carried out in general as above described, certain modifications being adopted in order to obtain the end alcoholic solution of choline as free as possible from impurities. The time during which boiling was continued varied from three to five hours, but in one experiment this was extended to eighteen hours. A reference to the result shows that this prolonged heating had a comparatively trifling effect in destroying choline, and thereby lowering the percentage figures. In general, the results stand in accord with those obtained by the use of alcoholic solution; the following three experiments suffice to show this:—

0.8403 gm. Lecithin boiled 3 hours	= 0.2246 gm. Choline-platinum-chloride	= 64.8 %
0.7821 " " " 5 " "	= 0.2103 " " "	= 65.2 %
0.7411 " " " 18 " "	= 0.1920 " " "	= 63 %

Thus, it is seen that this specimen of lecithin when hydrolysed in a watery or alcoholic solution of $\text{Ba}(\text{OH})_2$ gave practically similar results, varying on an average from 65 to 66 per cent. of the theoretical amount. Here, as with alcohol, the residue obtained after filtration of the hydrolysed solution invariably contained some nitrogen.

In a few experiments a special attempt was made to render this residue free from nitrogen, but without avail. After filtration the substance was thoroughly broken down, returned to the flask, and boiled for fifteen minutes with 50 to 80 c.c. H_2O . Again it was broken down and treated as before, this process being repeated three times; afterwards it was carefully washed on the filter with water almost at boiling temperature. Despite this prolonged washing, examination in every case revealed the presence of a distinct amount of nitrogen in the residue.

(c) *In mineral acid solution.*—In almost all the text-books, both old and modern, there appears the statement that lecithin while easily split up by the action of an alkali, is but very slowly attacked by acids. In testing this, however, it was found that such is not the case, at any rate with regard to the splitting off of choline. After boiling with a weak acid for a comparatively short time, choline seemed to be completely separated off, and as the use of acid in this connection possessed certain advantages some experiments were carried out with it. At first sulphuric acid was used, but the difficulties experienced in getting rid of it after hydrolysis was completed rendered the method somewhat cumbersome, and instead of sulphuric acid use was made of hydrochloric acid. Here it was thought that removal of the acid previous to precipitation by platinum chloride would not be necessary, though after evaporation of the watery part of the solution it was obviously present in fair concentration, and some experiments (described later) showed that this assumption was correct.

In all these hydrolytic processes it is an advantage to avoid if possible the use of barium, owing to the great difficulty of getting rid of it completely afterwards. With an alcoholic solution this is a matter of very great difficulty, and even with water, from which separation is much more easily obtained, it is sometimes found to be present in traces towards the end of the operation of purifying. The danger in the presence of barium results from the fact that this substance forms a double salt with platinum chloride, which may be thrown down with choline-platinum-chloride. It is true that this barium salt is fairly easily soluble in alcohol, but in spite of this fact, great care must be taken to wash the platinum chloride precipitate thoroughly with cold alcohol when there is any chance of barium being present. If this is not attended to somewhat variable results, difficult to account for, may be obtained. In all my experiments in which $\text{Ba}(\text{OH})_2$ was used, particular care was taken to ensure the thorough washing of the platinum chloride precipitate in order to dissolve out any traces of the double barium salt that might be present.

When this precaution is neglected, it is quite possible that traces of barium may sometimes account for the apparent percentage of platinum being somewhat above the theoretical amount when the salt of choline-platinum-chloride is ignited.

With HCl, however, the separation of barium is got rid of, and, so far, a great simplification introduced. Experiment showed that all that is necessary is to boil the lecithin for some time with a weak HCl solution, filter, evaporate to dryness, dissolve in absolute alcohol and precipitate directly with platinum chloride. This method takes up very much less time than when done with $\text{Ba}(\text{OH})_2$, and is to be recommended when estimating the choline of lecithin; here the choline is present from the beginning in the form of the chloride compound, and as such is less likely to undergo decomposition than when present in the free state in alkaline solution.

As pointed out above, however, the action of $\text{Ba}(\text{OH})_2$ on choline in saturated watery or alcoholic solution is not of much importance, the loss in a sample boiled for eighteen hours with 5 per cent. $\text{Ba}(\text{OH})_2$ in H_2O amounting only to about 2 per cent. of the total choline present. In my experiments the HCl hydrolysis was performed as follows:—

About 1 gramme substance was taken and boiled for varying periods with 100 c.c. of a 10 per cent. watery solution of hydrochloric acid (10 c.c. HCl sp. gr. 1.81 + 90 c.c. H_2O), boiling being continued with the aid of a reflux condenser for from two to five hours. The fatty material separated out as an oily mixture, and could not be conveniently filtered off while the solution was hot, but on cooling formed a solid mass, which was easily separated from the liquid. This fatty residue was returned to the boiling flask, and again boiled with 100 c.c. H_2O for ten to fifteen minutes; the mixture was then allowed to cool and filtered as before, the process being in many cases repeated three times. The total filtrates mixed together were now evaporated to dryness on the water bath, and the residue extracted with absolute alcohol, filtered, evaporated to small bulk and precipitated directly with platinum chloride. It was then left to stand till next day, and the precipitate then filtered, washed, dried and weighed as usual.

The obvious object of the above prolonged washing of the fatty residue was, if possible, to render it nitrogen free. This, however, could not be accomplished, and it was found quite unnecessary to return the residue to the flask more than once, as further washing did not lessen the nitrogen content.

It is noteworthy that in whatever manner lecithin is split up the residue always contains a certain amount of nitrogen, and since this residue is composed practically of fatty matter, it suggests the probability of this nitrogen being in close relationship with the fatty acid radicle of the lecithin body.

The following is an example of the figures obtained with HCl:—

0.7601 gm. Lecithin	boiled 1½ hours	= 0.2012 gm. Choline-platinum-chloride	= 64.2 %
0.8274 „ „ „	3 „	= 0.2222 „ „ „	= 65.1 %
0.9124 „ „ „	5 „	= 0.2425 „ „ „	= 64.5 %

The results are practically identical with those obtained when barium hydrate was used, and other samples of egg lecithin gave almost similar results. One sample, however, differed somewhat from the above in its nitrogen percentage,¹ and in this case a slightly larger yield of choline was obtained when calculated on the total nitrogen present, though when calculated on the weight of substance it was practically in agreement with the above sample.

A trade lecithin, having a nitrogen percentage of 1.77 and a N : P ratio of almost exactly 1 : 1, was now taken, and samples treated alongside of the egg lecithin as follows:—About 1 gramme of this lecithin and a similar amount of egg lecithin were hydrolysed under exactly similar conditions as parallel experiments; they were then treated as nearly as possible in the same manner, the same amount of fluid being used to wash the residue, &c., and the choline precipitated in the usual way.

One experiment was done using alcoholic Ba(OH)₂, another with watery Ba(OH)₂, and a third with HCl. The following results were obtained, and clearly prove that the two lecithins, though having a fairly similar elementary composition, are certainly not identical substances with regard to their nitrogen complex:—

Fluid used for hydrolysis	Percentage of choline of egg lecithin found	Percentage of choline of trade lecithin found	Difference in percentage
Alcoholic Ba(OH) ₂	65.8	80	14.2
Watery Ba(OH) ₂	64.6	79.6	15
„ HCl	65.3	80	14.7

Again, heart lecithin differs from these results in a greater degree than they differ from each other; a comparison of the three substances gives the following figures with regard to choline recovered as the double platinum salt:—

Heart lecithin	= 42 %
Egg lecithin	= 65 %
Trade lecithin	= 80 %

1. This sample gave an average of 1.77 % N.

From these results it is obvious that so-called lecithins are really bodies of different composition, despite their general agreement when viewed from the results of elementary analyses.

RELATION OF N OF END FILTRATE TO CHOLINE-PLATINUM-CHLORIDE
ACTUALLY OBTAINED

As in the case of heart muscle lecithin, some experiments were done in order to test directly what proportion of the nitrogen in the end filtrate—i.e., the end alcoholic solution obtained after hydrolysis, and purified as much as possible from other decomposition products and ready for precipitation with platinum chloride—could be obtained as the double platinum salt. In some respects this direct estimation gives more definite results than the ordinary lecithin estimations described above, for it is known exactly what amount of the nitrogen appearing in the end solution is actually precipitated as choline. To determine this, hydrolysis was performed as before, and the alcoholic solution ultimately obtained divided into two equal parts. One part was used for N estimation, and the other precipitated directly with platinum chloride. In three experiments it was found that 23 per cent., 24 per cent. and 26 per cent., respectively, of the N actually present was not recoverable as choline-platinum-chloride.

All these results prove beyond any doubt that the nitrogen of the end filtrate is not present as choline, and suggests strongly that in lecithin the generally accepted formula is insufficient for the facts obtained.

In the light of the above results, two considerations present themselves—

(1) That the nitrogen of the lecithin is really not all present in the form of choline or similar basic compound; or

(2) That all the nitrogen is really present as choline, but that some interaction with the chemicals employed during hydrolysis, or with some of the substances of hydrolytic decomposition, gives rise to some unknown nitrogenous complex which is not precipitated by platinum chloride.

In order, therefore, to strengthen the probability that lecithin N is not all present in the form of choline, it was necessary to thoroughly investigate this point; for this purpose pure choline chloride was used, and the following experiments performed:—

EXPERIMENTS WITH CHOLINE CHLORIDE

Samples of the choline-platinum-chloride salt obtained in the above experiments were mixed together and dissolved in water, filtered and re-crystallised. The typical crystals obtained were again dissolved in water, and the process of re-crystallisation twice repeated. On ignition practically the theoretical amount of platinum was obtained. A strong watery solution of the crystals was then treated with H_2S to remove the platinum, the fluid being heated during the passage of the gas in order to ensure complete separation. The mixture was then freed from platinum sulphide by filtration, and the filtrate, which was water clear, evaporated to dryness on the water bath. Residue was then dissolved in absolute alcohol, and this solution used in the experiments.

The object of these experiments was to find out what percentage of the choline chloride actually present in the alcoholic solution could be recovered as the double platinum salt after the solution had been treated with $\text{Ba}(\text{OH})_2$ in alcoholic, and in watery solution, and with HCl , exactly as in the hydrolysis of lecithin.

For each experiment an equal amount of the above solution was taken, and the following tests performed:—

A. For Nitrogen

5 c.c. choline solution was directly run in to a Kjeldahl flask and the N-content estimated with the following results:—

- (1) Gave 9.24 mgr. N equivalent to 0.2031 grm. Choline-platinum-chloride
- (2) „ 9.24 „ „ „ 0.2031 „ „ „

Here in both experiments the results were absolutely identical.

B. For Choline-platinum-chloride by Direct Precipitation

- (1) 5 c.c. directly precipitated by 10 % alcoholic platinum chloride solution, allowed to stand 18 hours, filtered, dried and weighed:—

Result = 0.1961 grm. Choline-platinum-chloride

- (2) Same as above, but 5 c.c. evaporated to about 2 c.c. before precipitated with platinum chloride:—

Result = 0.1968 gm. Choline-platinum-chloride

From the above it is seen that the amount of choline actually present in the solution was, on the nitrogen calculation, equivalent to 0.2031 gramme of the double platinum salt, and calculated on direct precipitation about 0.1964 gramme, taking the average of the two experiments. This slight difference may be accounted for by the difficulty of absolute exactness in allowing for the necessary reduction of nitrogen formed from

the chemicals used in the Kjeldahl estimation, coupled with the ordinary experimental errors and perhaps a slight loss due to traces of the choline remaining in solution. It would seem that evaporation of the choline containing fluid to very small bulk is not necessary, as the above results with volumes of 5 c.c. and 2 c.c., respectively, are practically the same. As a result of all these figures, it is clear that 5 c.c. of this solution ought to yield at least 0.1960 gramme of the double salt when precipitated by platinum chloride.

C. Choline Solution Boiled with Different Reagents to Imitate Hydrolysis of Lecithin

(1) *Watery solution of Ba(OH)₂*.—To 100 c.c. of a 5 per cent. solution of Ba(OH)₂ in water, 5 c.c. of choline solution was added and the mixture boiled for three hours, a reflux condenser being used. After cooling the solution was filtered, the filter paper thoroughly washed, and the combined filtrates treated with CO₂ to precipitate the barium. Mixture was now filtered and the barium carbonate residue thoroughly washed with hot water. Filtrates were united, a few drops HCl added, and evaporated to dryness. Residue was then dissolved in alcohol, evaporated to small bulk and precipitated as before.

Gave 0.1963 gm. Choline-platinum-chloride

„ 0.1959 „ „ „

(2) *Methylic alcohol solution of Ba(OH)₂*.—This was carried out as above, with a few modifications mentioned in a former article, to ensure the removal of the barium. Owing to an accident, only one experiment was completed; it gave—

0.1957 gm. Choline-platinum-chloride

(3) *Hydrochloric acid*.—(a) To 100 c.c. of a 10 per cent. watery solution of HCl, 5 c.c. choline chloride was added and the mixture boiled for two hours; it was then filtered and the filter paper thoroughly washed. Filtrate was then evaporated to dryness over the water bath, residue dissolved in absolute alcohol, filtered, evaporated to a few c.c.'s and precipitated as usual.

(b) Here a 5 per cent. HCl solution was used, and boiling was continued for one and a half hours; otherwise it was identical with above.

(a) Gave 0.1970 g.m. Choline-platinum-chloride

(b) „ 0.1951 „ „ „

It is thus obvious that HCl when used as above does not interfere to any extent with choline chloride. The only point in the manipulation at which some action might be expected is during evaporation to dryness of the dilute acid solution, for it is obvious that towards the end the concentration of HCl is very great; however, this apparently exerts no destructive action.

The following gives an indication in tabular form of the results obtained:—

CHOLINE-PLATINUM-CHLORIDE IN GRAMMES

Calculated from N found	Found by direct precipitation	Found, using HCl	Found, using alcoholic Ba(OH) ₂	Found, using watery Ba(OH) ₂
0.2031	0.1961	0.1970	0.1957	0.1963
0.2031	0.1968	0.1951	—	0.1959

The above experiments were carried out with every possible precaution in order to ensure parallel results, and a comparison of the figures shows that the greatest difference between any two amounts only to 0.008 gramme, the average difference being very slight indeed. When it is considered that a rather long process of manipulation, including several filtrations and the necessity of changing from vessel to vessel (evaporation, &c.), was involved the results agree remarkably well, and show conclusively that the loss of choline-platinum-chloride in the experiments on lecithin is not due to any destructive action on the part of the hydrolysing agents; further, they indicate that the necessary manipulations can be conducted with little or no loss, though in doing this the greatest care is necessary.

In view of the above results there remained only the possibility, already mentioned, that some inter-action between choline and some other product of lecithin decomposition might ensue, and so yield a nitrogenous complex of obscure nature which was not precipitated by platinum chloride. In order to test this a similar solution to the above was used, but to the Ba(OH)₂ or acid mixture some of the known products of lecithin decomposition were added before boiling. From the results obtained it is obvious that no such inter-action takes place. The experiments were carried out as follows:—

EXPERIMENTS WITH LECITHIN DECOMPOSITION PRODUCTS

For these experiments a solution of choline chloride similar to above was used, but of somewhat different strength; 5 c.c. gave on direct precipitation 0·2200 gramme of the double platinum salt.

(a) 5 c.c. of this solution was added to 100 c.c. water containing 2 c.c. glycerophosphoric acid. This mixture was boiled for half an hour and 5 grammes $\text{Ba}(\text{OH})_2$ added; boiling was continued for two hours, and after cooling a whitish residue was obtained on filtration. This residue was thoroughly washed three times with 100 c.c. boiling water, being each time returned to the flask and boiled for ten minutes; finally it was washed with hot water on the filter. Filtrates were then treated with CO_2 , and the usual manipulations performed.

(b) This experiment was conducted exactly as above, only that $\text{Ba}(\text{OH})_2$ was present at the beginning of boiling. Results were as follows:—

- (a) Gave 0·2173 gm. Choline-platinum-chloride
 (b) „ 0·2161 „ „ „

A number of experiments were now made with different amounts of other known decomposition products, such as glycerine, phosphoric acid, oleic acid, &c. The average results obtained showed that only a trifling loss was accounted for by the presence of these materials, and as their introduction gave rise to marked difficulties in the way of filtration and washing of residues, this small loss cannot be held to account for the great loss in lecithin experiments; in any case this loss did not amount to more than 6 per cent. of the total, and since much greater quantities of above products than would ever be obtained as the result of the hydrolytic decomposition of lecithin containing a similar amount of choline (equivalent to 0·2200 gramme platinum salt) were used, much more difficulty was experienced in filtering than is the case with lecithin.

The following experiment is sufficient to show that, even when excessive quantities of substances representing lecithin decomposition products are used, the final yield of lecithin is not materially decreased.

5 c.c. choline solution was mixed with the following substances:—

Glycerine	...	...	...	...	...	1 c.c.
Glycerophosphoric Acid	...	...	...	...	...	1 c.c.
Phosphoric Acid	...	...	...	...	...	0·5 gm.
Stearic Acid	...	...	...	...	...	0·5 gm.
Oleic Acid	...	...	...	...	...	0·5 c.c.

and the mixture boiled in a 5 per cent. watery solution of $\text{Ba}(\text{OH})_2$ for four hours; it was then treated in the ordinary way to remove impurities. In this case the difficulties in filtration were very great, the experiment taking a very long time owing to the fatty nature of the residues obtained. The final yield, however, gave 0.2110 gramme choline-platinum-chloride, or about 95.5 per cent. of the total obtained on direct precipitation, showing definitely that when choline is really present as such at the beginning, such treatment does not materially lessen the final amount obtained.

Here again it is seen that the residue obtained on boiling choline with $\text{Ba}(\text{OH})_2$ and other substances such as the above products gives a residue which *can* be rendered N-free. From this it must be assumed that the nitrogen remaining in the first and other residues in the hydrolysis of lecithin does really not represent nitrogen of choline, but is present in the form of another nitrogen complex.

The result of all the above experiments seems to show definitely that if choline were really present in lecithin to the amount represented by the nitrogen content, a much greater yield would necessarily be obtained on hydrolysis and precipitation with platinum chloride.

Since little or no evidence of the breaking down of choline can be obtained experimentally under the conditions present in the hydrolysis of lecithin, it is but fair to assume that a similar state of matters holds good for lecithin itself, and that the results actually obtained gave a very fair indication (allowance being made for slight losses due to possible defects of the method) of the amount of choline actually present as such in the original substance. A consideration of these facts shows the absolute futility of endeavours made at one time to estimate the amount of lecithin present in an organ in terms of the yield of choline obtained. It is obvious that this method would give widely divergent results when applied to, say, heart lecithin and egg lecithin respectively.

Thus the accepted formula for lecithin does not account for the facts, and in view of the differences existing between apparently similar lecithins but derived from different sources, can hardly be taken as a representation of any lecithin as obtained by the best methods at our disposal at present.

It was next thought that an investigation of some of the salts of lecithin such as the cadmium chloride compound might yield some information; the results obtained strengthen the above view.

CADMIUM-CHLORIDE-LECITHIN

32.53 grammes lecithin were dissolved in absolute alcohol, and to this an alcoholic solution of cadmium chloride was added till no more precipitation occurred. After standing for eighteen hours precipitate was filtered off and thoroughly washed with cold alcohol, filtrate and wash alcohol being preserved. It was then dried and weighed, and yielded 36.41 grammes lecithin-cadmium-chloride.

Filtrate and wash alcohol together was now carefully evaporated to dryness and gave a total residue of 2.64 grammes, part of this obviously consisting of cadmium chloride.

This residue (which may be termed Residue A) was now treated with water in order to dissolve out the cadmium chloride, and was extracted till the solution gave no evidence of the presence of cadmium on the passage of H_2S .

On evaporating down this wash water it was tested and found to contain a good deal of nitrogen. A mere trace of phosphorus could be detected, the relation of N : P standing as 14.1 : 1.

Here it was obvious that some nitrogen must have been split off from the lecithin; on boiling this watery solution with HCl and treating in the usual way with platinum chloride, no precipitate could be obtained, indicating that this nitrogen was not present in the form of choline.

After this extraction Residue A weighed only 1.16 grammes, so that 1.48 grammes must have gone into solution, the greater part of this being cadmium chloride. Thus, the total amount of substance obtained from 32.53 grammes lecithin after removal of excess of cadmium chloride was 36.41 grammes + 1.16 grammes = 37.57 grammes. The remaining portion (1.16 grammes) was now thoroughly dried and analysed, with the following results:—

Nitrogen	$\left\{ \begin{array}{l} 1.73 \% \\ 1.77 \% \end{array} \right\}$	average 1.75 %
Phosphorus	2.18 %	

On treating in the usual way for choline, only about 20 per cent. of the theoretical yield was obtained, but the small amount of substance rendered it difficult to get quite an accurate result.

Since it is well known that lecithin is not entirely precipitated out of alcoholic solution by cadmium chloride, this residue might be expected to be composed of lecithin-cadmium-chloride; the analysis shows plainly,

however, that something more containing a higher percentage of nitrogen and a lower percentage of phosphorous than this compound must have been present as well. The choline-platinum-chloride found probably represents the choline of the double lecithin salt, while the remaining nitrogen was present in some other form. All these points go to strengthen the conclusion hitherto suggested, that the nitrogen of lecithin is not all represented by choline or such basic body.

ANALYSIS OF LECITHIN-CADMIUM-CHLORIDE

In the light of the above results it was of interest to ascertain what percentage of the nitrogen in the double cadmium salt could be recovered in the form of choline-platinum-chloride. Since, in the manipulation described, nitrogen was found which did not seem to represent choline nitrogen, it was naturally concluded that the percentage of nitrogen actually representing choline in the double cadmium salt ought to be somewhat higher than in lecithin. This was found to be the case.

The following figures for N and P were obtained.

Nitrogen	$\left\{ \begin{array}{l} 1.414 \% \\ 1.416 \% \end{array} \right\}$	average 1.415 %
Phosphorus	$\left\{ \begin{array}{l} 3.09 \% \\ 3.10 \% \end{array} \right\}$	average 3.095 %
N : P = 1 : 1		

From these figures it is, of course, easy to deduce the theoretical yield of cadmium-chloride-lecithin that should be obtained from a given quantity of lecithin. In the above sample the average nitrogen content of the lecithin used was 1.876 per cent., and of the cadmium salt 1.45 per cent. If all the nitrogen of the lecithin were contained in the cadmium salt a simple calculation shows that 1 gramme lecithin contains sufficient nitrogen to yield 1.294 grammes of the cadmium salt, and, therefore, the quantity used (32.53 grammes lecithin) ought to furnish 42.093 grammes lecithin-cadmium-chloride. The amount actually obtained after removal of excess cadmium chloride was, as mentioned, only 37.57 grammes—another proof that all the nitrogen present in the lecithin did not go to form the double cadmium salt.

Although, as shown, a certain amount of the lecithin-cadmium-chloride remained in solution, this was much too small to account for the loss of about 4.5 grammes calculated on the theoretical amount, using nitrogen as a basis.

HYDROLYSIS OF LECITHIN-CADMIUM-CHLORIDE

The salt was treated with watery solution of $\text{Ba}(\text{OH})_2$ in the usual way, and the choline calculated as choline-platinum-chloride with the following results:—

0.6348 gm. Salt	= 0.1470 gm. Choline-platinum-chloride	= 74.5 %
0.5587 „ „	= 0.1777 „ „ „	= 75 %

These figures show that the cadmium salt of lecithin yields about 10 per cent more choline-platinum-chloride than lecithin itself does, results being in both cases calculated on the original N-percentage of the substance. This again shows that some nitrogen, which was not present as choline, must have been thrown off from the lecithin, otherwise the results cannot be explained. On the other hand, it is obvious that this lecithin salt must, like lecithin itself, contain a good deal of nitrogen which is not present as choline.

It is intended to again recover this lecithin from the salt, and to estimate its choline content; then to repeat the process of precipitation and analysis in order to ascertain whether this splitting off of nitrogen would be in evidence on a second precipitation.

It is quite possible that this particular part of the nitrogen may be present as an impurity in the form of some other complex not really belonging to the lecithin molecule; if so, it must have the same general properties as the lecithin itself, both in regard to solubility in ether and precipitation by acetone, and is likely present in many so-called lecithins. Of course it represents only a comparatively small part of the total lecithin nitrogen.

In the hydrolysis experiments carried out with lecithin-cadmium-chloride mentioned above, it was found that the residue obtained gave, as usual, a distinct indication of the presence of nitrogen; as before it was found quite impossible to get rid of this however prolonged washing was attempted; as in lecithin, it seems certain that this residual insoluble nitrogen has nothing to do with choline.

Since it has been shown that on the hydrolysis of lecithin a considerable percentage of nitrogen actually present in the end alcoholic solution is not precipitated as choline-platinum chloride, this nitrogen ought to be present in the filtrate after the separation of the platinum chloride precipitate. That the substance obtained was really the double platinum salt of choline is indicated by the results of ignition experiments, which generally gave a residue of platinum but slightly wide of the theoretical amount; it is, of course, quite likely that in some cases,

at any rate, the salt was not of *absolute* purity, but any admixture of other material must have been so slight as to be of little or no importance in judging of the general results. To substantiate this some experiments were made in order to ascertain if the amount of nitrogen used up actually corresponded to the amount necessary for the quantity of choline-platinum-chloride isolated. This was found to be so, the remainder of the nitrogen being present in the filtrate.

A number of these final filtrates were now united, some water added, and the excess of platinum separated by means of H_2S , the solution being heated during the passage of the gas. It was then filtered from platinum sulphide and the clear filtrate evaporated to dryness; residue was then dissolved in a small quantity of absolute alcohol and again treated with platinum chloride; in each case a slight precipitate was obtained, but it invariably proved to consist of a mixture of a small amount of choline-platinum-chloride with some of the barium salt of platinum chloride. This was in turn filtered off and filtrate again treated with H_2S , as above. The final solution obtained was then treated with various general precipitating reagents, but no definite substance could be isolated in this way. Experiments in this direction are at present being carried out, but it seems certain that the excess nitrogen of lecithin is not present as an ordinary basic compound; on the other hand there is some evidence that part of it, at any rate, is present in the form of an amino-acid, but this will be entered into in a later communication. The purpose of the present investigation was to ascertain the reason why in the hydrolysis of lecithin the actual amount of choline isolated should invariably fall so far short of the theoretical amount, and also to obtain some definite evidence with regard to the correctness or otherwise of the generally accepted formula with regard to its nitrogen distribution.

In the light of the above experiments it seems to me that the reason for the discrepancies mentioned depend on the fact that different lecithins contain varying amounts of choline; also that the ordinary formula cannot be accepted. Again, it is likely that many of the specimens formerly examined contained mixtures of other phosphatides besides lecithin, and so gave even lower results than recorded above.

With regard to other phosphatides examined mention may be made of two—the mon-amino-diphosphatide isolated from the heart muscle by Erlandsen, and from eggs by the writer. As mentioned in a former paper these two substances differ somewhat in their general analyses, but in each case no definite evidence of choline or any other substance of basic nature could be obtained. On hydrolysis in the usual way, platinum

chloride may give a slight often ill-defined precipitate, but the amount is so exceedingly small even when a large quantity of the substance is used, as to make it impossible to say what it really consists of. It would seem to be more of the nature of an impurity, and is in no way equivalent to the amount of nitrogen present. With the comparatively small amounts of substance at my disposal it is difficult to make any definite statement, but so far, in the case of the egg phosphatide, I have failed to obtain any substance of a basic nature in the ordinary sense though many experiments in this direction have been made. At present a large supply of heart 'cuorin' has been prepared, and it is hoped that some future experiments with large amounts of substance may settle the point.

The general results of the above investigation and the chief conclusions inferred in the light of these results may be shortly summed up as follows:—

SUMMARY

From different lecithins (heart, egg, etc.) prepared with the greatest care, and having a ratio of N : P as almost 1 : 1, different amounts of choline are obtained under similar conditions. On the other hand the amount obtained from any given specimen is practically constant, the result being the same whether watery or alcoholic solution of $\text{Ba}(\text{OH})_2$, or watery solution of hydrochloric acid be employed. From this it is obvious that these different lecithins, though showing somewhat similar figures as the result of elementary analyses, are not identical in composition, particularly with regard to their nitrogen distribution.

A long series of experiments with choline chloride has shown that if choline is really present at the beginning of hydrolysis, a very large percentage of it can be ultimately recovered as the double platinum salt, and the presence of substances corresponding to the known decomposition products of lecithin do not, by means of any more or less obscure reaction with the choline present, prevent this base being ultimately recovered as the double platinum salt; in one experiment containing excessive amounts of all the known products of hydrolytic decomposition of lecithin, there was a loss of only 5 per cent. of the choline, obviously due in great part to the difficulty experienced in thoroughly washing the bulky residues present.

Since these insignificant losses entirely fail to account for the small yield of choline obtained from lecithin, it is necessary to assume that the whole of the nitrogen of lecithin is not represented by choline.

In some lecithins, however, more of the nitrogen present is actually

represented by choline than is the case in other lecithins, a comparison of the amount of choline-platinum-chloride salt obtained from heart and egg lecithin, respectively being roughly 42 per cent. and 65 per cent, while from a trade lecithin 80 per cent. was obtained.

Results substantiating the above were obtained on precipitating egg lecithin with cadmium chloride and examining the filtrate and salt; in the sample examined some nitrogen was split off by CdCl_2 in a form soluble in H_2O ; this nitrogen did not represent choline. The cadmium-lecithin-chloride salt contained 10 per cent. more choline (based on its N-content) than the lecithin from which it was obtained. This practically proves that some of the nitrogen originally present in the lecithin was not present as choline.

Other evidence with regard to direct estimation of nitrogen and choline in different filtrates all points in the same direction, and lends additional weight to the inferences derived from a consideration of the above experiments. Whether lecithin is really a chemical unit, or a mixture of two or more substances having the same properties does not affect these facts.

CONCLUSIONS

1. In the light of the results obtained, it is the opinion of the writer that the generally accepted lecithin formula, i.e.—

in which the whole of the nitrogen present is represented by a base—choline—is *incorrect, and can no longer be accepted*; probably part of this nitrogen at any rate is present in the form of amino-acid.

2. Lecithins obtained from different sources differ, often to a great extent, with regard to the percentage of nitrogen actually represented by choline, and though often giving somewhat similar results on elementary analysis, cannot be regarded as identical chemical substances.

3. These results fully explain the general failure of former investigators to obtain anything corresponding to the theoretical yield of choline from lecithin.