The acceleration from 0° to 10° is more marked than that from 10° to 20°, but the ratio of 2 to 1 holds practically true and is 4 to 1 for the rise of 20°.

While this work was in progress there was published by Bigelow, Gore and Howard[1] a description of respiration experiments with apples where there were two lots, one at 0° and the other at 15°. These results were given in percentages of the original weights of apples. From their tables it was found that but four dates could be compared, which are given herewith, together with the percentages of carbon dioxide and the ratio between 0° and 15°.

Date.	0°.	15°.	Ratio 0°: 15°.
Jan. 5.....	0.234 per cent. CO_2	0.794 per cent. CO_2	1:3.3
Jan. 27.....	0.308	0.899	1:2.9
Mar. 2.....	0.436	1.122	1:2.5
Mar. 30.....	0.484	1.375	1:2.8

The average ratio is 1:2.9, which is approximately that of 1:2 for a rise of 10°.

All these results show concordance and prove that apples undergo chemical changes fully twice as fast and in some instances three times as fast with a rise of temperature of 10° between 0° and 20°, or in other words, at summer temperatures apples will undergo respiratory metabolism from 4 to 6 times as rapidly as in modern cold storage. The low temperatures also prove that there must be a limit to the keeping quality even there, since respiration and consequent destruction of cell tissues still goes on.

NEW HAMPSHIRE AGRICULTURAL EXPERIMENT STATION,
DURHAM, N. H.

OBSERVATIONS ON THE STABILITY OF LECITHIN.

BY J. H. LONG.

Received February 19, 1908.

Numerous investigations published in the last four or five years on the subject of the preparation of the lecithin compounds from eggs or from animal and vegetable tissues have discussed more or less vaguely the stability of these products under the influence of light, heat, and atmospheric oxidation. It seems to be assumed that the lecithins in general suffer very ready decomposition, but in the literature I am unable to find much that is definite as to the extent of their decompositions which take place under the influences referred to. In the course of certain experiments in other directions I found the need of this information and felt obliged to carry out some experiments to supply the desired data.

At the outset it may be said that the conception of the term "lecithin"

[1] *U. S. Dept. Agr., Bur. of Chem., Bull. No.* 94, "Studies on Apples."

is still very vague, in spite of the extended studies of Thudichum,[1] Koch,[2] Erlandsen,[3] Stern and Thierfelder,[4] E. Schulze[5] and colleagues, and others, in addition to the well-known investigations of the older literature, and what may be affirmed of egg lecithin, as we know it to-day, does not necessarily apply in full to an analogous product from the vegetable kingdom or from brains. The extended experiments of Thudichum and Erlandsen have contributed greatly to modify our views as to the brain and muscle extracts of a fatty nature containing nitrogen and phosphorus, while the recent paper of Stern and Thierfelder, referred to, shows in clear light the fact that egg lecithin, generally supposed to be comparatively simple and containing probably two main constituents, must in reality be much more complex; but the relatively small yields secured in the fractionations described in these papers are probably not wholly due to imperfect insolubility, but are much more probably due to partial decomposition of some of the individual substances during treatment. That this is the case is shown further by the rather marked acidity of some of the fractions, which is probably due to separated glycero-phosphoric acid, or other phosphoric acid derivative. The modifying influence of the presence of some of these decomposition products on the reactions of "lecithin" is generally overlooked and will be referred to later.

However, it is not my intention to take up the preparation of various lecithin products at the present time, but rather to present data bearing on the stability of some of the best known representatives of the group, as secured through generally recognized methods. Egg lecithin was taken as the first of these products, as representing the simplest and most readily prepared.

Experiments with Egg Lecithin.

Preparation.—The largest quantity of this used was made from yolks without previous drying. The yolks of 72 eggs were treated in lots of 12 eggs each. In each case 300 cc. of ether were added and shaken with the dozen yellows through several days; this was followed by the addition of 500 cc. of alcohol, after which the mixture was well shaken repeatedly and allowed to settle. The alcohol-ether solution was filtered and evaporated to a pasty condition at a low temperature, and finally by aid of vacuum. The residue was taken up in pure ether, the solution filtered, concentrated and precipitated with pure neutral acetone in excess. This operation of dissolving in ether and precipitating by acetone was repeated three times, the last product being carefully dried in a

[1] "*Die chemische Konstitution des Gehirns des Menschen und der Thiere*," 1901.

[2] *Z. physiol. Chem.*, 36, 134; 37, 181; and elsewhere.

[3] *Ibid.*, 51, 71.

[4] *Ibid.*, 53, 370.

[5] *Ibid.*, 40, 101, where other literature is cited.

vacuum at a low temperature, but not so as to remove *all* the moisture, as may be done by drying over sulphuric acid through a long period. By drying in the latter way a very hard, horny product is secured, which is not easily worked with later. In several lots prepared in my experiments, the average water content left was 6 per cent. In all, about 90 grams of the final product were secured in the form of a light yellow mass, which, on analysis, showed these results for phosphorus and nitrogen:

	Per cent.
P	3.59
N	1.82

These values calculated to the anhydrous condition give:

	Per cent.
P	3.82
N	1.94

which correspond to an atomic ratio of

$$P:N::1:1.12,$$

which suggests a mixture containing some diaminomonophosphatide. With this product the following tests were made, an aqueous emulsion containing in 100 cc. 4.476 grams of the anhydrous substance being employed in most cases.

Effect of Heat.—Two portions of 25 cc. each were evaporated to dryness in a current of carbon dioxide, the temperature being kept at about 60°. The residues found weighed 1.133 and 1.136 grams in place of 1.12 grams, about, as found by long drying over sulphuric acid. This experiment was repeated with three new portions of 25 cc. each. These were evaporated at a temperature of 100° in a vacuum-drying oven, through which a rapid current of carbon dioxide was passed. At the end of two days constant weights were reached as follows:

A.	B.	C.
1.115	1.127	1.122

The dry residues were washed in the small dishes with portions of 25 cc. and then with 10 cc. each of pure dry acetone, the acetone remaining half an hour in contact with the residues. After pouring off the last acetone the dishes were returned to the oven and again dried in carbon dioxide. The new weights found were then:

A.	B.	C.
1.048	1.070	1.063

Nitrogen determinations were made on these residues, which gave the following results:

A. Per cent.	B. Per cent.	C. Per cent.
1.97	1.93	1.95

It is evident that these final residues have about the same composition

as the original, although some loss has occurred in the drying and washing with acetone. This loss is apparently through solubility of the substances as a whole and not through decomposition.

In another experiment 25 cc. of the emulsion were evaporated to dryness in the open air. The product found was very brown and weighed 1.101 grams, that is, less rather than more than the normal. The nitrogen found in it was 0.0214 gram, which corresponds to 1.94 per cent. of the dry weight. In spite of the dark color no important volatile decomposition product had been formed.

Essentially the same result was found by long boiling. Twenty-five cc. of the emulsion were diluted with 50 cc. of water. The mixture was placed in a flask with a stopper furnished with a fine opening and boiled long enough to bring the volume back to 25 cc., which required about an hour and a half. The nitrogen found was 1.94 per cent. again. It is evident that no volatile nitrogen products were formed, and the emulsion remained perfect.

In another experiment 25 cc. of the emulsion were heated in a platinum dish in an autoclave to a temperature of 175°. through two hours. After cooling, the contents of the dish consisted of a clear liquid and a dark fat-like ring on the dish at the surface of the liquid. The total nitrogen in the dish was found to be 0.020 gram, or 1.79 per cent. of the weight of the original dry substance. There was evidently some loss, therefore, of this element.

From these several experiments it is evident that the effect of heat, alone, is not very pronounced, if the conditions for oxidation are absent. It has been noticed in several other cases that even after long heating to 100° in an atmosphere of carbon dioxide, perfect, light-colored emulsions could still be secured, with no change of properties.

Acidity.—The various lecithin preparations which I have examined show a decided acidity to phenolphthalein, and this property in their product is referred to by Stern and Thierfelder.[1] This acidity may be observed directly in the emulsions by titration with 0.1 *N* sodium hydroxide, and more clearly after addition of neutral alcohol. Twenty-five cc. of the above emulsion, containing 1.12 grams of anhydrous lecithin, required directly 1.3 cc. of this weak alkali, and after the addition of alcohol nearly 4 cc. In a second test 1 gram of the lecithin, or 0.94 gram of dry product, was made into an emulsion with 15 cc. of water and 25 cc. of alcohol were added. On titration, I used now 3.6 cc. of the 0.1 normal alkali.

This acidity does not appear to be due to acid liberated on formation of the emulsion with water, as it is also observed on titration of a solution in strong neutral alcohol. A solution made by dissolving 1.157

[1] *Loc. cit.*

grams of lecithin (anhydrous) in neutral alcohol, to which a little neutral ether was added, required 4.2 cc. of the 0.1 normal alkali with phenolphthalein. The acid substance is therefore present in the original lecithin as separated by the process outlined, and the fact that in absence of alcohol a weak acidity is shown, while after addition of the latter a much stronger acidity is developed, is evidence of the presence of two kinds of acid substances. One of them is soluble in water and is indicated in the first part of the titration, while solution in alcohol is necessary to bring out the second substance. It is likely that the glycero-phosphoric acid complex is responsible for the first reaction, while separated fatty acids, on solution in alcohol, bring out the second. Pure glycero-phosphoric acid behaves as a dibasic acid with phenolphthalein and is monobasic with methyl orange, while the acid complex, as separated from lecithin, would doubtless act as monobasic with the first indicator and neutral with the second. The lecithin emulsions I have made are not acid to methyl orange. From the above it appears possible, if not probable, that the acidity observed in the lecithin is due, in part, at least, to small amounts of dissociation or hydrolysis products, rather than to the substance itself. If we may assume that the free acid hydrogen of the phosphoric group is fully combined in the titration in aqueous solution, each cc. of the 0.1 normal alkali used would measure 8.07 mg. of lecithin decomposed, or in the above case, would correspond to about 10 per cent. of the whole. But this assumption cannot be correct, as a part of the phosphoric acid is apparently already combined with calcium. The ash of the lecithin contains this metal, as shown by Thudichum for the brain lecithin, and by Stern and Thierfelder for the egg product. Further light on the question of acidity will be given in some experiments to be referred to below.

Electrical Conductivity.—Since a determination of electrical conductivity seems to furnish very interesting evidence as to the progress of hydrolysis or other change with liberation of acid in the lecithin emulsion, I have made a large number of tests on this and other samples of lecithin from various sources. At best, the conductivity is low, and its range is indicated in the following table. The measurements were made by the usual Kohlrausch telephone method, and always at a temperature of 20°, accurately maintained. The lecithin was made into an emulsion with water of high purity, the conductivity of which may always be neglected for these tests. The emulsion employed contained, like the one referred to above, 4.476 grams of anhydrous lecithin in 100 cc. The variations in the conductivity with the dilution are shown below.

The emulsion once formed, is comparatively stable, as shown by this experiment. Twenty-five cc. of the original were mixed with 75 cc. of water and heated on an actively boiling water-bath two hours, in a flask. After

cooling, the remaining liquid was made up to 100 cc., accurately, and the conductivity found. It was $\kappa_{20} = 0.000300$, that is essentially the same as in the second dilution below.

Conc. in 100 cc.	κ_{20}.
4.476	0.000798
2.238	0.000508
1.119	0.000299
0.559	0.000172
0.279	0.000096
0.140	0.000054

To what is this conductivity due? In the usually accepted formula for lecithins there is a free hydrogen in the phosphoric group, but as intimated above, the acid value of this must be low. In the preparation of these lecithins, alcohol, ether and acetone of a high degree of purity were used throughout. These liquids were tested for conductivity and residues from evaporation of 50 cc. of each one, taken up with water, were also tested. In no case was a conductivity found which was at all appreciable. The triple solution in ether and precipitation by acetone insured the freedom from electrolytes originally present. As a corresponding degree of conductivity has been found in many other samples of lecithin from different sources it would seem to be inherent in the molecule, but that this is probably not the case the next experiment will show. Another emulsion of the same strength as the last was made up and examined in the same cell, which contained the usual platinum black-covered electrodes, with capacity, $C = 0.306$. The conductivity was found to be $\kappa_{20} = 0.000718$. Thinking the nature of the electrodes might have some effect, a new test was made in a cell with bright electrodes and $C = 0.383$. I found now $\kappa_{20} = 0.000715$, and the value was not changed after washing the electrodes with ether and alcohol.

Twenty-five cubic centimeters of the last emulsion were measured out and precipitated with 45 cc. of acetone in a small separatory funnel, and the precipitate washed with 10 cc. and finally with 5 cc. more of acetone. The residue was dried in a current of washed carbon dioxide and emulsified with water to again make 25 cc. In the same cell, with the bright electrodes, I found now a resistance over 10 times as great, or $\kappa_{20} = 0.000066$. After standing 18 hours, the result was unchanged. The acetone was evaporated and the residue made up to 25 cc. with water. During the evaporation a small amount of insoluble matter separated, which appeared to consist of some of the dissolved lecithin, but which was very light in color, while the lecithin residues proper are usually quite dark. The aqueous solution contained a soluble substance, or substances, since a conductivity, $\kappa_{20} = 0.001576$, was found and on titration 0.8 cc. of 0.1 normal sodium hydroxide was required with phenolphthalein.

The last emulsion was treated anew with acetone, when the surprising observation was made that a very large quantity of the latter must be added. About 100 cc. were required to do now what was accomplished with 45 cc. in the first case, and more was needed to complete and to wash the precipitate. The latter was made up to a 25 cc. emulsion with water, as before, and tested for conductivity, giving $\kappa_{20} = 0.000041$. This very considerable decrease may be here due in part to the loss of the portion soluble in the excess of acetone, but the change in the first case, after precipitation, cannot be so explained. Several experiments have shown that the recovered lecithin, after the first acetone precipitation and washing, is about 88 to 90 per cent. of the original weight. The loss after the second precipitation is apparently much greater, while in an attempt to precipitate a third time, nearly the whole of the substance went into solution with the acetone. The emulsions found after precipitation and taking up with water are practically neutral to phenolphthalein.

It is evident from the above that the observed conductivity of the first emulsion is due to something not true lecithin, and that when this is separated precipitation by acetone is very difficult. To test this point, some of the supernatant acetone from a first precipitation was evaporated and the residue taken up with water. A few drops of this solution added to the second emulsion caused it to precipitate with acetone immediately. The same result was secured by adding a few drops of a very dilute glycero-phosphoric acid solution; in this case a sharp result followed at once, which suggests that this acid may be the fraction split off from the original lecithin and is responsible for the observed behavior. In precipitating lecithin from ether solution by acetone, in the process of preparation, glycero-phosphoric acid and other possible decomposition products seem to be carried down and remain with the finished mass, but in precipitating from an aqueous emulsion this is not the case apparently, and in this way a purer final product seems to be secured. On this point, however, futher work is necessary, as experiments carried out show, in some cases, a slightly lower nitrogen and higher phosphorus content in the so-purified lecithin than in the other, which suggests that the acetone, in presence of water, may have a splitting or hydrolyzing action and leave a residue poorer in the fatty acid groups, and relatively richer in the phosphoric acid group. It is possible, also, that a part of the nitrogen may be split off from the latter as the following figures suggest: Two emulsions were made, having 5 and 6 grams to 100 cc. These were precipitated with acetone, and the residues washed and dried in carbon dioxide without loss. On weighing, it was found that 88.9 per cent. of the original anhydrous lecithin was recovered. The two recovered products were made up into emulsions again and portions taken

for phosphorus and nitrogen determinations, with the following results, considering the recovered lecithin as anhydrous:

	A. Per cent.	B. Per cent.
P	4.27	3.98
N	1.65	1.68

It will be recalled that in the original dry material the nitrogen and phosphorus were 1.94 per cent. and 3.82 per cent., respectively. It is evident, therefore, that some splitting has followed, but the nature of the reaction is not clear, especially in view of the facts of lower conductivity and lower acidity in the last emulsions.

Salt Precipitation.—It is stated above that an emulsion which will not precipitate by addition of acetone, or at best imperfectly, may be caused to yield a good precipitate by the addition of a trace of glycero-phosphoric acid. It was found that weak salt solutions have the same action, and this, with the original weak emulsions, as well as those made up after acetone treatment. In this respect experiments have been made with solution of sodium chloride, barium chloride, silver nitrate and aluminum sulphate in various dilutions and with many other salts in certain molecular proportions. The precipitates are markedly colloidal and do not settle quickly. These findings do not seem to agree with the results of Koch[1] for brain lecithin. According to this author, dilute emulsions of brain lecithin yield precipitates with dilute solutions of divalent metals, but not with mono- or trivalent metals. My findings are quite sharp and conclusive, and, as will be shown below, hold for brain lecithin also.

In this connection another interesting observation was made. It was found very difficult to extract the lecithin from aqueous emulsions by means of ether; in fact traces only seem to go into solution, and this has been observed not only for the simple emulsions used in these experiments, but also in some of the commercial lecithin emulsions on the market. The addition of sodium chloride brings about an immediate solution, which is first shown by the color of the upper layer when the emulsion is shaken with ether in a tube, and which can be proven by decanting the ether layer and evaporating. This behavior seems to depend on the power of precipitating colloidal substances, possessed by many electrolytes, and was shown by several other salts as well as by sodium chloride, but not by urea and sugar, which are crystalline but not electrolytes. Further work is in progress on this interesting reaction.

Digestion Experiments.—The behavior of the fat-splitting ferment of the pancreas was first pointed out, apparently, by Bokay,[2] but his experiments were not extensive enough to show the rapidity of the action. I undertook some investigations in this direction but did not carry them

[1] *Z. physiol. Chem.*, **37**, 181.

[2] *Ibid.*, 1, 157.

far, as meanwhile the work of Schumoff-Simanowski and Sieber[1] came to my notice. In their extended experiments the rate of digestion by several ferments in addition to that by the steapsin is satisfactorily demonstrated. My method of work was in principle very different and was intended to show the rapidity of acid liberation rather than the amount liberated.

It is shown above that the pure lecithin in the form of emulsion has little or no conducting power, while that mixed with small amounts of decomposition products shows electrical conductivity in rather marked degree. It was further shown that this was not increased by standing or by warming on the water-bath. I found, in preliminary experiments, that the emulsions, after being mixed with pancreas extracts prepared in the laboratory, and incubated at 40° through a number of hours, showed a greatly increased conductivity and increased acidity. But in such cases part of the increased acidity is often due to the acids formed by changes in the ferment mixture itself by enzymes or bacteria, and in testing several laboratory extracts and commercial pancreas preparations in incubated solutions, I have observed this increased acidity and increased conductivity. In working with the lecithin emulsions it was necessary to guard against this source of error as far as possible by the use of toluene or thymol in making up the ferment solutions, and even the emulsions themselves.

In carrying out the tests the following solutions were used: First, an egg lecithin emulsion of approximately 1 per cent. strength in thymolyzed water. This was found to have a conductivity, $10^4\kappa_{20} = 2.66$. At the same time a moderated active pancreas extract in thymolyzed water was made and this had a conductivity, $10^4\kappa_{20} = 5.27$. A mixture of equal volumes of the two liquids gave $10^4\kappa_{20} = 4.03$. This mixture was kept in the thermostat at 40°, and portions were withdrawn for tests from time to time with the following results:

Time.	$10^4\kappa_{20}$.
0 hours	4.03
3 "	4.12
21 "	6.77
45 "	10.16
69 "	12.50
96 "	13.02

In the 96 hours through which the experiment was carried the conductivity of the pancreas alone increased from $10^4\kappa_{20} = 5.27$ to 6.34, or 20.3 per cent., while the increase in the mixture was 223 per cent.

At the same time there was a very marked increase in the total acidity of the mixture determined by titration with 0.1 normal sodium hydroxide after the addition of alcohol. At the beginning 25 cc. of the mixture re-

[1] *Z. physiol. Chem.*, **49**, 50.

quired 1.6 cc. of the alkali, while at the end of the experiments over 6 cc. were required. The increased acidity was about 4.5 cc. of 0.1 *N* alkali which measures a marked degree of acid liberation, including, apparently, part of the phosphoric acid. From the results given above for the increased conductivity of the pancreas solution alone, it is probable that a part of the developed acidity must be due to the ferment itself. This is a point which is usually overlooked in fat-splitting experiments, and it appears to have been overlooked in one of the results of Schumoff-Simanowski and Sieber,[1] in which the acid formed is evidently more than could have been liberated, under the conditions of the experiment, from the lecithin molecule.

Additional Experiments with Egg Lecithin.—Many of the tests made above were repeated with egg lecithin obtained by somewhat different processes, but it will not be necessary to go into the details of the results. Some data from two cases only need be referred to.

In the first of them a product was obtained from boiled eggs by extracting with ether only. The eggs were boiled until thoroughly hardened, and then the yellows were separated and ground up with clear quartz sand without any preliminary drying. The mass so obtained was thoroughly extracted in the Soxhlet apparatus. The crude ether extract was concentrated and the residue dried at a low temperature. It was taken up with dry ether and precipitated with acetone in the usual way. The precipitate was washed with acetone and dried in carbon dioxide. A nitrogen determination gave 2.08 per cent. The electrical conductivity of an emulsion made up as before was found to be much lower than with the former product, pointing, possibly, to the presence of smaller amounts of dissociation products. The purified lecithin obtained by treatment of the emulsion with acetone, when made up into a new emulsion, gave, likewise, a very low conductivity.

In the preparation of this last lecithin from hard-boiled eggs, the mass left in the Soxhlet apparatus after ether extraction, was extracted through several days with redistilled alcohol. The alcoholic solution was evaporated at a low temperature, leaving a considerable residue, in fact nearly as large as that from the ether extraction. Most of this residue was found to be soluble in absolute ether, which was somewhat remarkable, in view of the preliminary treatment. On concentration of the ether solution and precipitation with acetone a light mass was secured closely resembling that from the ether extraction. After repeated washings with acetone it was dried and used as in the other case. A determination of nitrogen gave a high result, *viz.*, 2.34 per cent. This would suggest the presence of a considerable amount of diaminophosphatide, and is not at variance with the results of some of the experiments of Stern and

[1] *Loc. cit*

Thierfelder.[1] A phosphorus determination was not made because of lack of material.

An emulsion was made with the portion not used in the nitrogen test, and this had a concentration of 3.1 per cent. It was characterized by a relatively high conductivity, and for equivalent concentrations almost four times as great as for the portion extracted with ether alone. The emulsions in both cases precipitate metallic solutions readily and both have an acid reaction when tested directly. That the two extracts are markedly different is shown not only by the different nitrogen contents but by the great variation in conducting power. It is apparent that the alcohol has brought a larger quantity of decomposition products into solution than was the case with the ether.

Experiments with Brain Lecithin.

It is well known that the product termed lecithin, as obtained from the brain, is usually a mixture of considerable complexity, and that some, at least, of the constituents of this mixture are very unstable compounds. This is well illustrated by a few commercial substances which are now sold under the name "lecithin," and obtained from brain extracts. In beginning some experiments with brain lecithin, I attempted to use a crude product made by a local manufacturing firm, and which was obtainable in quantity. This material dissolved readily in ether, and the solution gave a good precipitate with acetone, but on attempting to redissolve and purify it, it became dark. Even after repeated solution in ether and precipitation by acetone the products secured remained dark and failed to yield a characteristic emulsion with water. The crude extract had been prepared by extraction with the light hydrocarbon sold as "hexane," and in some stage of the work had probably been exposed to a high temperature, which brought about a marked decomposition. The products of decomposition were evidently carried down with the acetone precipitate in the first and following attempts at purification. I mention these facts because they throw light on some of the commercial "lecithin" preparations on the market, which in recent years have been highly advertised as curative agents.

Not being able to utilize the commercial product, I dried down about a kilogram of minced calves' brains in a current of warm air, as recommended by Erlandsen,[2] and extracted with ether thoroughly. This ether extract was concentrated and the solid portion finally dried at a low temperature in a rapid current of carbon dioxide. The pasty mass was taken up with absolute ether, which left a residue amounting to about 60 per cent. of the crude solid extract, and this new ether extract was concen-

[1] *Loc. cit.*

[2] *Z. physiol. Chem.*, 51, 71.

trated to a small bulk. This was precipitated with acetone, which furnished a nearly white mass. The latter was dried and after expelling the acetone completely was redissolved in absolute ether and again precipitated with acetone in a large flask. The greater part of the supernatant liquid was easily removed by pouring and the remainder was drawn out by a current of carbon dioxide passed rapidly through the flask, which meanwhile was immersed in a vessel of warm water. In this way a good yield of a *light yellow* mass was recovered, with which the following experiments were made. On analysis the phosphorus content was found to be 3.76 per cent. and the nitrogen content 2.13 per cent., both calculated on the anhydrous basis. This gives an atomic ratio,

$$P : N :: 1 : 1.27.$$

The product is naturally a mixture of different phosphatides, and for the purposes of the present examination is sufficient. The experience of Stern and Thierfelder[1] shows the extreme difficulty of securing products of constant composition, in quantity, from the analogous egg lecithin, as well as the great losses which accompany re-solution and precipitation.

Effect of Heat.—Tests were made here as with the egg product, and the general results were essentially the same. However, this difference was noted: in all the evaporations, whether of the aqueous emulsion or of the ether solution, the brain product remained much lighter in color than was the case with the other. This suggests a greater degree of stability, although from the apparently greater complexity the reverse might be assumed. After evaporating the emulsions to dryness and making up to the original volume with water, no perceptible change in color followed, and no essential change in conductivity. From my various experiments in this direction I must conclude that the lecithin compound, such as is secured in the method of extraction outlined, is much more stable than would be inferred from many statements in the literature. In the process of preparation, that is, while the lecithin is mixed with other substances, it evidently changes readily, but when isolated is apparently much more stable. In view of many observations I have made, I must consider the statement of Bang[2] on this point as too strong.

Acidity.—The brain lecithin shows a greater acidity toward phenolphthalein than was noted with the egg product. As before, this is best observed in the aqueous emulsion. To test the point quantitatively, an emulsion was made containing in 100 cc. 4.52 grams. When directly tested, 25 cc. of this, containing 1.13 grams, required 2.1 cc. of 0.1 normal alkali for neutralization. For a second 25 cc. mixed with an excess of neutral alcohol, 7.4 cc. of the dilute alkali were required. This is a strong degree of acidity.

[1] *Loc. cit.*

[2] "*Ergebnisse der Physiologie*," VI Jahrgang, p. 162.

A second 50 cc. of this emulsion was precipitated and washed with pure neutral acetone, using 120 cc. in all. This acetone solution was divided into equal portions, one of which was titrated for acid and then used for a phosphorus test, while the second portion was tested for nitrogen. In the titration, 6.5 cc. of 0.1 normal alkali were required, and this for a volume corresponding to 25 cc. of the original emulsion. A good test for phosphoric acid was also secured. The portion reserved for nitrogen was concentrated and decomposed in the usual manner for the Kjeldahl determination. The ammonia obtained was 12.6 mg., corresponding to nitrogen equivalent to 0.92 per cent. of the original lecithin in the volume taken.

The purified lecithin residue left after the acetone treatment was dried in carbon dioxide, then freed from this gas by a current of air with gentle warming, and made up with water to a volume of 50 cc. A portion titrated was found to be *perfectly neutral* with phenolphthalein and alkali, even after addition of alcohol.

Twenty cc. of the new emulsion furnished 0.0106 gram nitrogen, which amounts to 1.17 per cent. of the anhydrous lecithin originally present in the equivalent volume.

Ten cc. of the emulsion gave 0.0105 gram phosphorus, corresponding to 2.34 of the lecithin originally present in the equivalent volume.

It is evident that the treatment of the emulsion with excess of acetone has resulted in precipitating lecithin, apparently, with considerable loss, and also in changing the ratio of the nitrogen to the phosphorus. In the original substance we had 3.76 per cent. P and 2.15 per cent. N, with an atomic ratio of P:N::1:1.27. Here, in the "purified" emulsion, we have 2.34 per cent. of phosphorus and 1.17 per cent. of nitrogen for the same recovered weight, or a ratio of P:N::1:1.17. In other words, there is a relatively greater loss of nitrogen than of phosphorus, as was found to be the case with the egg product, and the acetone treatment may have then the effect suggested for the egg lecithin.

No quantitative determination of the weight lost on treating the brain lecithin emulsion with acetone was made, but superficial observation showed it was much higher than with the egg lecithin, and besides this the supernatant liquid was not perfectly clear as in the other case. We have then a rather marked degree of solubility in the acetone, and this seems to be accompanied by the formation of some decomposition products which are likewise soluble. It must be recalled, however, that the total acidity of the acetone solution is not greater than the original, but, in fact, a little less, which complicates any attempt at explanation of what actually takes place in the treatment.

Electrical Conductivity.—For these tests some of the same emulsion,

with 4.52 grams to 100 cc., was employed, and the observations were made as before at 20°. The following results were obtained:

Conc. in 100 cc.	κ_{20}
4.52	0.000721
2.26	0.000529
1.13	0.000337
0.565	0.000195
0.283	0.000111

In general, the values found are not greatly different from those for the egg emulsions. It appears in this case also that precipitation with acetone furnishes a product with much lower conductivity, as was shown by precipitating 25 cc. with acetone, in a bottle, pouring off the acetone and washing several times with fresh portions. The residue was dried in an atmosphere of carbon dioxide and after expulsion of the gas was emulsified with water and made up to 25 cc. again. The conductivity was now found to be $\kappa_{20} = 0.000127$, or about one-sixth of what it was originally. The new emulsion was neutral in reaction. The great change must be due to the loss of decomposition product, rather than to the loss of lecithin itself through solubility.

Some of this last emulsion was allowed to stand about two weeks and examined again to detect a possible increase in conductivity by hydrolysis through long contact with water, but no such increase was found, and this again speaks for the comparative stability of the substance.

Precipitation by Salts.—It was found that emulsions of the egg lecithin are readily precipitated by solutions of several salts and in a manner quite distinct from that described by Koch.[1] Similar experiments were made with the brain lecithin emulsions, and with the same general result, which will not be given in detail here, as the observed relations are made the subject of fuller investigations. Since the completion of the experimental part of this paper, an article by Hoeber[2] has come to hand, in which the author shows that carefully purified egg lecithin made up into emulsion yields precipitates with many neutral salts without regard to valence of the metallic ions. This is in full accord with the results of my experiments.

Action of Light.—In various methods of preparation of lecithin given in the recent journal literature, much is said about keeping the product, as far as possible, in the dark. In some of my experiments I have done this, while in others no such precaution was taken. To test the behavior of light, I have made emulsions of both egg and brain lecithin and allowed them to stand in stoppered flasks through periods of two weeks or more in a well-lighted room with south and west exposure, and part of the

[1] *Loc. cit.*

[2] *Beitrage zur. chem. Physiol. und Path.*, 11, 35.

time in direct sunlight. I have not observed in any of the flasks a change of color, change in acidity, change in conductivity, or change in behavior toward weak salt solutions, from which I am forced to conclude that the light effect, if present at all, is very slight.

Summary of Results.

In this work it has been shown that:

1. Emulsions of egg and brain lecithin are comparatively stable with respect to temperature. Increase of temperature, or long-continued heating of the emulsions does not appear to increase the dissociation as measured by acidity or conducting power. The action of light on the emulsions appears to be very slight.

2. Lecithin emulsions have an acid reaction which is marked. On precipitating the emulsions with an excess of pure acetone the residues left, on being again brought into emulsion form with water, are neutral. Precipitation of lecithin from ether solution by means of acetone seems to furnish a product which becomes acid when treated with water. The acetone precipitation from water effects also some decomposition, shown by change in the P:N ratio.

3. The electrical conductivity found in the emulsions suggests the presence of acid or basic groups, but after purification by acetone the conductivity is so much reduced as to indicate that this phenomenon as observed is not due to the lecithin itself, but to decomposition products. It is likely that many of the reactions assumed to be characteristic of lecithin are due to hydrolysis or other products.

4. Emulsions of both brain and egg lecithin are readily precipitated by weak salt solutions. No relation between the precipitating power and the valence of the metallic or acid ions of the salts is apparent. The extraction of lecithin from emulsions is aided by the addition of salts.

My thanks are due to my assistant, Mr. Frank Gephart, who has made the above lecithin preparations.

NORTHWESTERN UNIVERSITY MEDICAL SCHOOL,
CHICAGO, February, 1908.

ON THE BEHAVIOR OF EMULSIONS OF LECITHIN WITH METALLIC SALTS AND CERTAIN NON-ELECTROLYTES.

BY J. H. LONG AND FRANK GEPHART.

Received March 13, 1908.

Although lecithin may be obtained, like many other fats, in a crystalline condition, its behavior is ordinarily colloidal, and when mixed with a large quantity of water its relation, physically at least, to the colloids is very marked.

Among the properties of the colloids which must be regarded as of the