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 highest interest, the behavior of their solutions or suspensions toward salt solutions has attracted recently much attention. Hofmeister[1] was among the first to call specific attention to the precipitating action of many solutions on certain proteins, and these suggestions were followed up by extended investigations of Pauli,[2] Spiro,[3] Hardy,[4] and others. The work of Pauli was especially valuable in showing the order of the precipitating power followed by the different cathions, and the modifying influences of the anions, while in the later article of Spiro, data are presented to bring these phenomena in comparison with others and so lead to a theory of the processes.

Considering the lecithins as colloids, Koch[5] has attempted to show the relations between valence of cathions and the precipitation of weak emulsions of this substance by solutions of numerous salts. According to this author the behavior of lecithin emulsions is in many respects analogous to that of the true colloids just referred to, but the precipitating power of the salt solutions on the emulsions seems to be confined to certain groups only. Mono- and trivalent metals are said to be without action, while the solutions of the common bivalent metals, Mg, Ca, Sr, Ba, Co, Ni, Fe, Zn, Cd, Cu, etc., are active precipitants. Acid solutions (H ions) were also found to act as precipitants, while a number of anions, investigated in their combinations with metals of the first group, were found to be without specific action. In this paper Koch notes further the behavior of mixtures of salts in the precipitation of lecithin and finds that certain amounts of the mono- and trivalent metals neutralize the precipitating action of the bivalent metals. This is especially interesting in the case of ferric chloride, which was found to prevent precipitation by calcium nitrate.

While experimenting on the extraction of lecithin from certain solutions and emulsions on the market as remedies, or "tissue builders," we made the observation that the ease or completeness of extraction is very much influenced by the character of mineral matters or salts present at the same time. Emulsions in water or glycerol which gave up no lecithin directly to ether or chloroform were found to extract perfectly by a shaking-out process after the addition of various salts. In following up the question it was found that these salts all produced a more or less perfect precipitation in water emulsions, and that this separation by precipitation, or salting out, must evidently precede the actual ether extraction. As some of this experience seems to be the reverse of that

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[1] *Archiv. exper. Path. u. Pharm.*, 25, 1; 27, 295; 28, 210.

[2] *Beitr. chem. Phys. u. Path.*, 2, 1; 3, 225; 5, 27; 6, 233.

[3] *Ibid.*, 4, 300.

[4] *Z. physik. Chem.*, 33, 385.

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

reported by Koch, we were led to give it a fuller study with a larger number of substances. Before the results were completely worked out, however, an interesting paper by Hoeber[1] came to hand in which he reports observations leading to the same conclusions which we had reached, and which fail to confirm the findings of Koch. As our experiments cover a somewhat wider range than those of Hoeber on this particular point we give them in full, although as far as the simple question of the precipitation of lecithin emulsions by monovalent metals is concerned it might not be considered necessary.

In a previous paper by one of us,[2] on certain properties of lecithin, attention was called to the fact that acetone precipitates from *emulsions* of this substance a product which is different from the original and in some respects purer; at any rate it is free from the acid reaction usually found in the lecithin from other processes. In our experiments, given below, we have used both kinds of lecithin, with practically the same results, and have tried emulsions of various strengths from 0.005 *N* to 0.05 *N*. For all the reported tests, however, we have used the weakest emulsion, which contaned 4 grams to the liter, as the molecular (here normal) weight of the egg lecithin employed is about 800. The preparation of this lecithin is described in the last paper referred to. It may be added that essentially the same results were secured with some brain lecithin, described in the same paper. The weakest emulsion used is so dilute that it may be filtered, yielding an opalescent filtrate in which a precipitate is readily visible. In the first series of tests we used in each trial 5 cc. of the 0.005 lecithin and 1 and 5 cc. of the salt solutions in normal strength, where the solubility permitted. With salts of low solubility saturated solutions were employed. In the table below "t" indicates increased turbidity, "p" actual precipitation, while by "op" a slight increase in opalescence is indicated. The action of a few acids is included in the table given below.

The table shows the wide range of salts which possess the power of precipitating the lecithin emulsions, and the most marked difference which may be noted is in the time required to cause actual subsidence of the several precipitates. We have tried to distinguish between opalescence and the appearance of turbidity, but the distinction in many cases is far from sharp. It will be noticed that the action with acetic acid is weak while with boric acid no effect whatever was observed. The precipitates formed by calcium, strontium and barium salts are at the outset heavier, apparently, than those formed by salts of the alkali metals, but after 24 hours the differences disappear and all the precipitates settle out. In most cases these precipitates do not appear to be true

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[1] *Beitr. chem. Phys. u. Path.*, 11, 35 (Dec., 1907).

[2] Long, THIS JOURNAL, preceding article.

chemical unions, as the lecithin may be separated by an extraction process with ether, as referred to below, and as is shown also by the following behavior. After subsidence of the precipitates the greater part of the supernatant liquid may be poured off. This liquid carries, of course, the larger part of the added salt. If the same volume of distilled water is then poured over the precipitate and the mixture shaken a new emulsion is formed which, however, is denser than the original emulsion, and, besides, is not stable. A separation soon follows, due apparently to the presence of small amounts of salt not removed in the decantation. On repeating these operations once or twice, and thus removing all the salt, stable emulsions like the original are secured.

TABLE I.—PRECIPITATION OF 0.005 *N* LECITHIN.

	At once.		In 2 hours.		In 24 hours.	
Salts used.	1 cc.	5 cc.	1 cc.	5 cc.	1 cc.	5 cc.
NaCl	op	op	t	p	p	p
KCl	op	op	t	p	p	p
NH_4Cl	op	op	t	p	p	p
$NaNO_3$	op	op	t	p	p	p
KNO_3	op	op	t	p	p	p
NH_4NO_3	op	op	op	t	p	p
Na_2SO_4	op	op	p	p	p	p
K_2SO_4	op	op	p	p	p	p
$(NH_4)_2SO_4$	op	op	p	p	p	p
$CaCl_2$	t	t	p	p	p	p
$SrCl_2$	t	t	p	p	p	p
$BaCl_2$	t	t	p	p	p	p
$Ca(NO_3)_2$	t	t	p	p	p	p
$Sr(NO_3)_2$	t	t	p	p	p	p
$Ba(NO_3)_2$	t	t	p	p	p	p
$Fe(NH_4)_2(SO_4)_2$	t	t	p	p	p	p
$FeCl_3$	t	t	p	p	p	p
$Fe'''(NH_4)(SO_4)_2$	t	t	p	p	p	p
Tl_2SO_4	op	op	p	p	p	p
$Pb(NO_3)_2$	op	op	p	p	p	p
$CdCl_2$	op	op	p	p	p	p
$CuSO_4$	t	t	p	p	p	p
HCl	p	p	p	p	p	p
HNO_3	p	p	p	p	p	p
H_2SO_4	p	p	p	p	p	p
$H_2C_4H_4O_6$	p	p	p	p	p	p
$HC_2H_3O_2$	t	t	t	t	p	p
H_3BO_3	o	o	o	o	o	o

The behavior with ether is indicative of the same thing, that is, the physical rather than the chemical nature of the precipitations. To show this a 0.025 *N* emulsion from the same lecithin as before was used, and to it were added certain volumes of ether and salt solutions. After shaking and allowing the mixtures to stand the ether layer which gradually separated showed always some color, from the lecithin, even with the weakest dilutions of the salts, as shown in the table below for sodium chloride solutions. Essentially the same results have been found for other salts, and need not be repeated. The color of the ether layer measures roughly the amount of lecithin separated, within certain limits, but it is evident that the amount of salt required to secure the maximum color is soon reached in the successive trials. This amount of salt is small. As the ether becomes colored the water layer below clears up perfectly.

TABLE II.—EFFECT OF MIXING EQUAL VOLUMES OF LECITHIN EMULSION SALT SOLUTION AND ETHER.

Strength of salt solution.	Color of ether layer.
0.00500 *N*	very slight color.
0.00625 *N*	slight, but increased.
0.00830 *N*	more distinctly colored.
0.01250 *N*	decidedly yellowish brown.
0.02500 *N*	marked brown color.
0.05000 *N*	" " "
0.07500 *N*	" " "
0.10000 *N*	" " "
0.12500 *N*	" " "

No increase of color seemed to follow after using the 0.025 *N* salt solution, and this amount appears to be sufficient to separate the whole of the lecithin, as was found in some experiments in which larger volumes were taken for the trials. In the above table the amount taken was 5 cc. of each liquid, but with the larger volumes it was possible to recover most of the lecithin in the ether layers. It was found also that the lecithin was separated as such, and not as a salt or combination. This was shown clearly in two experiments in which sodium chloride and barium chloride were employed in relative excess. The supernatant ethereal layers containing the lecithin were removed, evaporated, and the residues ignited. Only a very minute trace of chlorine was found in either case, and no barium in the second case by the sulphate test. These two tests represent typical cases, as the precipitation by the sodium salt is relatively slow, while that by the barium salt is rapid.

The behavior of salts of cadmium and lead in this respect is interesting. Thudichum[1] has shown that these and several other salts give true chemical precipitates with lecithin in alcoholic solution, and it is important to note their action with emulsions. In making the actual tests it was soon recognized that as far as the simple precipitation is concerned, lead and cadmium salts do not differ essentially from the others of the bivalent group, as Koch[2] points out. In making the precipitates in pres-

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

[2] *Loc. cit.*

ence of ether, and after a time decanting the layer of this substance which separated, we found that not more than the minutest traces of the heavy metals had gone into solution. These metals, as all the others, remain in the aqueous layer while the lecithin dissolves in the ether. In making a test for lead in the residue from the evaporated ether, nothing more than a faint coloration with hydrogen sulphide was secured at any time, and this might well come from the slight solvent action of the water held by the ether. The results with some other metals were the same, indicating that the lecithin must exist *wholly* in the colloidal form in the water solution, in which condition it does yield a true metallic combination.

A few experiments have been made with some salts in which one or both ions are relatively weak. In this list are included mercuric chloride, cyanide and acetate, aluminum acetate, chromium acetate, and the sulphates of both metals. With the three mercuric salts the precipitation reactions were extremely faint, if at all present, and on adding ether practically no coloration appeared. This behavior is especially plain in the case of mercuric cyanide, from which it may be inferred that the degree of dissociation of the salts may have some bearing on the problem. With the acetates of chromium and aluminum, very weak reactions were observed. These salts were made by double decomposition between the pure sulphates and lead acetate, and held a trace of lead in the water solutions. Solutions obtained by dissolving the washed hydroxides in acetic acid were even more inert with the lecithin, but these solutions were weaker. With the two sulphates good reactions were noticed.

In carrying out the last experiments it was observed that the age of the lecithin emulsions has some influence on the results. Old emulsions appear to be much less reactive than fresh ones, as we noticed in a number of the later tests. With an emulsion which had been prepared several days, no reactions were obtained with the acetates of aluminum and chromium, and rather weak tests with the sulphates. The results reported above were obtained with fresh emulsions in general, and we have carried out no tests to explain this exceptional behavior.

In this connection the action of non-electrolytes is interesting. It was stated above that ether fails to extract lecithin from glycerol emulsions, and in following up this point glycerol, glucose, saccharose, urea and egg albumen were added in molecular proportions to the same kind of emulsion used in the other tests. With the glycerol no increase of turbidity was observed, while with the others it is possible that there was a little increase in the opalescence. According to Pauli, as quoted above, the sugars, urea and other non-electrolytes have no action on the true colloids. With some specimens of urea a slight precipitate may be obtained, but this is due, doubtless, to the traces of sulphates present in much of the product as obtained from chemical dealers. With pure urea the reaction is extremely weak. On adding ether to the mixtures, and shaking, as before, no color was found in this when it came to the surface on standing. These compounds cannot have, therefore, the same action on the emulsion which was noted with the salts. When, however, to each one of the mixtures a few drops of salt solution were added, and the tubes shaken, an immediate coloration of the ether layer followed as with the regular electrolyte solutions, described above. The extraction with ether is again seen to depend on preliminary salt action, and the presence of the non-electrolytes does not appear to inhibit this. It is possible that some of these substances form loose compounds with the lecithin which are not soluble in the ether, but which are readily decomposed by the addition of salts. A number of such compounds have been described,[1] but in most cases tangible proofs of their existence are lacking. From various physiological reactions the existence of combinations between lecithins and certain toxins has been much better established.

An important combination, however, has apparently been completely overlooked, and that is, the complex formed by the union of lecithin and bile salts. Lecithin in the form of emulsion is dissolved perfectly in an aqueous solution of bile salts, and in considerable proportion. The combination formed seems to be remarkably stable, and not readily decomposed in such a manner as to give up the lecithin to the usual solvents. We are at present engaged in a fuller study of this complex, which is interesting from several standpoints, especially in the separation of lecithin from bile.

Results.—It has been shown in these investigations that weak emulsions of lecithin are precipitated by a large number of solutions of salts and acids and that the completeness of precipitation does not appear to bear any relation to the valence of the cathions concerned. Within each group, the alkali group for example, we have not been able to distinguish any characteristic differences. It seems to be true, however, that the precipitation is in some way related to the degree of dissociation of the various compounds. While, for example, the ordinary acids and tartaric acids are very active, acetic acid is weak and boric acid quite inert. The mercuric salts tested were all weak, and the activity decreased from the chloride through the acetate to the cyanide, which is practically inert.

It has been found also that ether and similar solvents have a very slight extracting power for the pure aqueous emulsions, which in most cases is scarcely appreciable, and that after the addition of salts to the

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[1] See convenient literature references in paper by Ivar Bang in *Ergebnisse der Physiologie*, 6, 163.

emulsions the lecithin is immediately taken up by the solvents. This action, which is best shown with ether, is related to the precipitating power of the salts, and it appears necessary to break up the emulsions before extraction is possible. Mercuric cyanide and boric acid do not precipitate the emulsions and after treatment with ether no lecithin is dissolved by the latter.

Certain non-electrolytes tested do not appreciably precipitate the emulsions, and their presence does not aid the solution by ether, but the addition of traces of salt solutions to the mixtures brings about an immediate solution of the lecithin, even when great excesses of the non-electrolytes are used. This behavior has certain practical applications.

Finally, attention is called to the peculiar behavior of bile salts.

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ON THE OCCURRENCE OF COPPER IN OYSTERS.

BY J. T. WILLARD.

Received March 19, 1908.

Last spring the attention of Dr. S. J. Crumbine, secretary of the Kansas State Board of Health, was called to some cases of illness following the use of fresh oysters in which these were suspected to be the cause. Two samples were sent to the writer for examination. They had a distinctly greenish blue color, and qualitative tests showed the presence of copper. The oysters also possessed a noticeable coppery taste. Quantitative determinations of the amount of copper were made. One of the samples was found to contain 0.0437 per cent. of copper, or 0.302 per cent. calculated on the dry substance; the other contained 0.0324 per cent., or 0.211 per cent. in the dry substance. As copper has been recognized as a constituent of many species of mollusks it seemed desirable to test other samples. Two other samples of fresh oysters and six samples of canned oysters, sold under the name of Cove Oysters, were examined and in every case copper was found to be present. As the oyster season was practically at an end at that time, further investigation was postponed until October, when analyses were made of a considerable number of samples secured chiefly with reference to determination of water content. In all 34 distinct samples were analyzed. The results are shown in the following table.

With the exception of those marked as bulk samples these oysters were placed in glass jars as they were taken from the shells. In most cases the amount of liquor present was too small to determine the copper, but in other cases where the liquor was examined copper was found present, and in no instance was copper absent from the oysters although in sample No. 12,117 the amount was very small. The uniformity of the