From these results, it may be concluded that phthalic acid forms salts readily with the more basic metals. The solubility of these salts in water varies through a wide range and it apparently bears no relation to the atomic weight of the metallic element present. This is shown by the following examples:

The lithium salt is only moderately soluble in water while the berrylium salt is exceedingly soluble.

The cobalt salt is only moderately soluble while the nickel salt is exceedingly soluble.

The lead salt is insoluble while the thorium salt is moderately soluble.

Probably the best method for preparing these salts is the decomposition of the carbonate of the metal by phthalic acid solution. This method does not introduce any foreign acid radicals into the solution and removes the possibility of a mixture of crystals being formed.

BOULDER, COLORADO.

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.]

THE NITROGENOUS HYDROLYSIS PRODUCTS OF HEART LECITHIN.

BY C. G. MACARTHUR, F. G. NORBURY AND W. G. KARR.

Received December 25, 1916.

It has been known for some time[1] that egg lecithin yields on hydrolysis both choline and amino ethyl alcohol. Recently a paper appeared[2] showing the presence of both of these compounds, as well as an unhydrolyzable form of nitrogen in the fatty acid residue, in brain lecithin. The object of the investigation recorded here was to study the hydrolysis products of heart lecithin, to show the presence or absence of each of the above mentioned nitrogenous compounds and to obtain quantitative data on their distribution.

Preparation and Purification.

Beef hearts, each weighing 1800 to 2500 g. in the fresh state, were used as a source of the material for this experiment. After removing the adherent fat, the valves and the connective tissue as completely as possible, the muscle was minced by passage through a grinder. The ground meat was pressed to remove the blood, weighed approximately and dried. Drying was accomplished by one of three methods: (1) dehydration with alcohol, (2) dehydration with acetone, (3) removal of water by current of air in a drier at a temperature of 35–40° C.

Dehydration.—(1) In the dehydration by alcohol the ground material was placed in large bottles and treated with twice its volume of 95%

---

[1] G. Trier, *Z. physiol. Chem.*, 76, 496 (1911–12).

[2] J. E. Darrah and C. G. MacArthur, THIS JOURNAL, 38, 922 (1916).

alcohol, being shaken frequently. The first treatment with alcohol extended 12–16 hours at room temperature. The liquid was then removed by filtration and pressure and fresh alcohol added. The alcohol was allowed to stand on the tissue for two days, the bottle being shaken often during this time. At the end of this period all water was considered as taken up by the alcohol, so the tissue was removed, pressed, dried in a current of air, and either placed in a desiccator or treated immediately for extraction of the phosphatids. After dehydration with alcohol the tissue was of a stringy, fibrous nature and apparently quite porous. The treatment with alcohol removes roughly 77% of the original weight of the material as water. Erlandsen advises against the use of alcohol as a dehydrating medium, on account of what he calls the danger of oxidation of unsaturated acids in the phosphatide molecule. In this work no especial difference was noted in the character of the extracts from heart muscle dehydrated by alcohol and by other methods. The alcohol-water solutions were strongly colored, having dissolved tissue pigments, extractives and some phosphatids. The solubility of the substances in the ether used later is affected by this treatment, for in the case of the alcohol-dried hearts the acetone precipitate (see below) from the ether extract was in every case small in proportion to the weight of material used. However, the amount from both alcohol and ether totalled in proportion to that secured by extraction of tissue dried by other methods.

(2) Water was removed from one lot of tissue by acetone. The procedure was the same as when alcohol was used. By this treatment a loss of water corresponding to 80% of the original weight of the tissue was observed. The tissue after the dehydration was light brown and granular, in excellent shape for extraction. Both acetone-water solutions were colored a yellow-brown, the color in the second being stronger than that of a similar alcohol dehydration of another lot of tissue. On concentration of the liquids and treatment for phosphatids the acetone precipitate was too small to work with. It is probable that the acetone dehydration is without appreciable solvent action on the phosphatids of the heart, only the extractives and pigments being removed with the water.

(3) Dehydration by means of a current of warm air was the method used for the preliminary preparation of the greater part of the tissue. The finely divided fresh material was spread in thin layers on large glass plates and set in the drying apparatus. This consisted of a long wooden chamber containing racks for fifteen plates, fitted with an electric heater at one end and a fan at the other. By adjusting the amount of current in the heating coils and the speed of the fan the temperature within the drier could be regulated at will. A fairly rapid current of air at 35–40°

was used. The tissue was treated in this way for 8 to 12 hours, by which time it had thoroughly dried on top and partly through the layers. It was then turned over on the plates and allowed to remain in the drier 5–12 hours more. At the end of this period the tissue was brown, hard and brittle. It was removed from the plates, ground up somewhat in a mortar and put through a powdering mill. After this treatment it was a fine, light-colored powder, slightly sticky. It gradually became darker on exposure to the air. This procedure removes only the water from the tissue. The average from several lots showed that approximately 77% of the fresh material is water.

Ether Extraction.—The dehydrated tissue was first extracted with ether. Extraction by means of a modified Soxhlet apparatus (designed in this laboratory for use with large quantities of tissue) was carried out as follows: 1200–1400 g. of dried tissue were placed in a cage within the copper extractor used. This tissue was treated by conducting ether vapors from a flask into the condenser above the extractor. The condensed liquid dropped on the tissue was distributed through it by means of cloths, and passed out of the cage carrying with it the soluble substances. When the ether in the extractor reached a height corresponding to that of a siphon tube on the exterior it automatically passed back into the flask. Vaporization and cooling of the solvent before it reached the tissue admitted of the use of distilled products, for the flasks could be changed just before the extract siphoned over, thus keeping it free from contamination by non-volatile bodies in the ether. Also the treatment was carried out in a vapor of ether, thus preventing oxidation of the unstable substances.

This process served, too, as a means of concentrating the ether extract. The ether was boiled off from the solution and used for a second treatment without disconnecting the apparatus. When the extract became quite concentrated the flasks were changed and the concentrated portion worked up as described below. The liquid from the second treatment went through the same process so that the same ether, with some additions, was used several times. It was found that three of these treatments removed all substances obtainable in the primary extracts. The third solution contained only a little phosphatid material.

Following the extraction with cold ether, an extraction with warm ether was made. For this purpose the solvent was placed directly in the copper extractor. On warming, the vapors passed up around the cage containing the tissue to the condenser. The condensed ether then passed through the tissue as in the other treatment. The characteristic feature of this method is that the tissue is completely surrounded and permeated by ether vapor, the extraction going on at 34°.

Alcohol Extraction.—After the primary ether extractions were completed boiling alcohol was used. The method in this treatment was the same as that for warm ether. For 1200–1300 g. of tissue two liters of 95% alcohol were taken. After one extraction by alcohol lasting 13–16 hours, the liquid was siphoned off. The first alcohol extracts were yellowish brown and rather viscous. Another extraction with warm ether for 8–10 hours was now made to see if the hot alcohol had broken up any phosphatid combination within the tissue, releasing more ether-soluble substances. Some phosphatid was found. After the ether fraction had been removed the material was subjected to two more alcohol extractions. The solutions from these were dark brown liquids, very concentrated but not containing much phosphatid.

This completed the extraction of the tissue. The substances in solution were secured by concentration of the extracts when necessary. The liquids were distilled in vapor of CO_2 or ether in order to lessen the chance for oxidation. Since heat aids the oxidation the temperatures were kept low. The extracts with ether were distilled at atmospheric pressure; those with higher boiling solvents, in a partial vacuum. Evaporation was continued until the residue attained a syrupy consistency.

Separation and Purification.—These concentrates were taken up in anhydrous ether, prepared by distillation over magnesium bromide. At this point there was always some insoluble material which Erlandsen calls Residue A. It is a grayish white granular substance, insoluble in ether, forming a fine suspension in it. It was separated by allowing the ether solution to stand in the refrigerator when the insoluble portion settled out, or by centrifuging. The former method was the more satisfactory, for evaporation and cooling during centrifuging tended to remove other substances from the solution. A small amount of the compound shaken with an excess of anhydrous ether colored the liquid only slightly. This coloration is probably due to previous incomplete separation of soluble material. However, this liquid gave no precipitate when treated with acetone or alcohol.

MacLean reported the isolation from the kidneys by similar treatment, of a substance probably a diamino-monophosphatid. His compound was present in a very small proportion, 0.55 g. being secured from 2075 g. dry tissue. In this experiment, a little over 2 g. were found in 1310 g. dry tissue. This is the amount present after the inorganic salts have been removed from the insoluble material. Upon analysis of 0.5090 g., 2.43% nitrogen was found. This nitrogen is present partly in choline, and partly in amino ethyl alcohol, and partly as residue nitrogen.

To the clear ether solutions secured from these separations of insoluble material three or four volumes of anhydrous acetone were added to precipitate the phosphatids. In some cases a whitish flocculent precipitate formed at once, and always a yellow to light brown waxy one settled out in a few minutes. The acetone-ether mixture was placed in a desiccator in a vapor of CO_2 and allowed to stand at least ten hours. On standing, a fat-like substance crystallized out and settled on the precipitate and on the walls of the beaker. This is probably not phosphatid. It is difficult to separate it mechanically from the phosphatid precipitate.

In the preparations from tissue dried by alcohol practically all the acetone precipitate from ether extractions was of the flocky, or else of a granular type.

The acetone-ether solutions were filtered off, the precipitates dried and weighed roughly. This acetone precipitate was the only means used of determining extent of extraction. The weights given below on one portion of tissue are of comparative value only since in each case the preparations did not consist solely of lecithin and cuorin.

Dry tissue. Weight in g.	Acetone precipitates from different extracts.				
	1st ether.	2nd ether.	1st alcohol.	3rd ether.	2nd alcohol.
1170	34	10	40	3	6

The acetone precipitates were thoroughly dried, then treated again with anhydrous ether. On this treatment there was in every case much undissolved substance. This seemed to be the same as that found on treating the concentrated ether extracts. Apparently the acetone broke up a combination between the insoluble substances and the phosphatids. The ether solution was precipitated again by acetone and treated with ether. In every case practically all the material dissolved, forming clear or slightly opalescent dark red or brown solutions.

Three or four columns of absolute alcohol were added to each ether solution to secure cuorin. The first alcohol treatments gave two forms of precipitate, a brown waxy substance that settled out immediately and a white flocky portion that separated out after some time. The precipitates were removed for purification of the cuorin. The alcohol-ether solutions containing the lecithin and a small amount of cuorin were evaporated either at atmospheric pressure or in a partial vacuum. The concentrates were taken up in absolute alcohol when some material remained undissolved. The alcohol solution was filtered and evaporated in partial vacuum. The concentrate was taken up in anhydrous ether and finally precipitated with anhydrous acetone. This was used as Sample A. A portion of this was further purified by redissolving in absolute alcohol, evaporation of the clear solution in a partial vacuum, resolution in ether, and precipitation by acetone. This is Sample B.

Hydrolysis and Identification of Products.

For every gram of phosphatid 100 cc. of 4.5% HCl (10 cc. conc. HCl: 90 cc. H_2O) were used. The material and acid were introduced into a large round-bottomed flask and boiled gently for 25 hours, using a return condenser. At one time 21 g. of Sample A and at another time 20 g. of Sample B were thus hydrolyzed.

Fatty Acid Residue.—The visible result of this treatment is a separation of the compound into the insoluble fatty acid residue floating on top of the liquid and into the soluble portions forming a yellow-brown solution with the acid.

After hydrolysis was considered completed, the solution was filtered off and the flask and residue washed with 5% HCl, which was added to the filtrate. The filtrate was evaporated on the steam bath. More fatty acids separated out on concentration and were added to the residue.

The nitrogen-containing compound of this residue has not been very completely investigated. It is probably similar to the corresponding compound in brain lecithin. It seems to be a saturated complex because its solubilities do not alter on bromination. One analysis on the fairly pure product gave 1.2% nitrogen.

Considerable evidence favors the view that this substance connects equal quantities of choline lecithin and amino alcohol lecithin and is therefore a part of the molecule. On purification of the lecithin a point is reached where no more "white substance" separates and the solution in ether or petroleum ether is perfectly clear. Further treatment with water and acetone or with absolute alcohol produces no further separation of this white compound.[1] Even this lecithin contains 0.2% nitrogen in this fatty acid form.

Alcohol Insoluble Fraction.—The filtrate from the fatty acids was further concentrated until it reached a syrupy consistency, when it was treated several times with small amounts of absolute alcohol. Solubility in alcohol seems to depend somewhat on the extent of the previous concentration. If evaporation is prolonged there is a rather large amount of insoluble material and some of the nitrogen-containing substance may be held in it.

The residue was dried and treated in several ways. One portion of the solid was tested for NH_3 with $Ca(OH)_2$ and H_2O. The result was weakly positive. The solution of another portion tested with Nessler's reagent gave slight positive results. When the solution was treated with $H_2(Pt)Cl_6$ a yellow amorphous precipitate was secured. On recrystallization this was found to consist of regular octahedra. This is the characteristic crystal form of the NH_4Cl compound with platinum. Undoubtedly there is some ammonia in the hydrolysis products but very likely considering the small and somewhat variable amounts found, this is a contamination.

Choline Fraction.—In Sample A the alcohol extract of the bases was pre-

---

[1] H. MacLean, *Biochem. J.*, **6**, 355 (1911–12).

cipitated with a saturated alcohol solution of mercuric chloride. Upon recrystallization of this compound from water, crystals similar to those of choline mercuric chloride were obtained.

The alcohol extracts from Sample B were treated with alcohol platinum chloride as long as a precipitate formed. This precipitate was recrystallized twice from a mixture of alcohol and water. The small yellow octahedra had the characteristic form of choline platinum chloride. Upon ignition 0.0779 g. of this compound gave 0.0238 g. of platinum. This is 30.55%. The theoretical platinum content of choline platinum chloride is 31.64%.

Amino Alcohol Fraction.—The filtrate from the choline was diluted with water, warmed and treated with hydrogen sulfide to remove the platinum. After filtration the solution was concentrated and placed in a small distilling flask, then made alkaline with solid potassium hydroxide. Upon slow (because of foaming) distillation a liquid was obtained that had the characteristic basic odor of amino ethyl alcohol. The fractions around 180° were the nearly pure compound. The distillation was discontinued at 200° C.

The fractions distilling near 180° were united and dissolved in a small amount of concentrated hydrochloric acid, to which gold chloride had been added. Upon standing, long, slender, yellow needles appeared. After washing with strong hydrochloric acid and drying 0.0494 g. of this gold derivative gave upon ignition 0.0240 of gold. This is 49.19% gold, as compared with the theoretical amount of 49.17%. The melting point of the gold derivative was 190°.

The remainder of the above distillates were united and picrolonic acid added. After concentration the characteristic yellow twinned prisms of amino ethyl alcohol picrolonate were obtained.

Quantitative Methods and Data.

Method I.—1.0414 g. of lecithin from Sample A were hydrolyzed in $4^1/_2$% HCl by boiling for twenty hours, cooled and filtered. The nitrogen in the fatty acid residue was determined by the usual combustion method and called *residue nitrogen.* The filtrate, after careful evaporation to dryness, was repeatedly extracted with absolute alcohol. The insoluble material contained nitrogen as ammonium chloride. This was determined and labeled *alcohol-insoluble nitrogen.* The alcohol solution was precipitated by alcoholic chloroplatinic acid. This precipitate was found to be choline chloroplatinate, and so the nitrogen in it will be labeled *choline nitrogen.* The nitrogen in the filtrate will be referred to as *amino alcohol nitrogen.* An analysis by this method follows: In Column A is given the percentage of nitrogen in the lecithin; in Column B the percentage of total nitrogen.

	A.	B.
Residue nitrogen	0.231	11.91
Alcohol-insoluble (ammonia) nitrogen	0.103	5.31
Choline nitrogen	0.808	41.65
Amino alcohol nitrogen	0.798	41.11
	1.940	99.98

Probably the ammonia, as reported in this analysis, is somewhat too high because of other forms of nitrogen held by the salts present. It is difficult to be certain that all the alcohol-soluble forms of nitrogen but inappreciable amounts of ammonium chloride are dissolved. It is not definitely shown from this analysis that all the amino alcohol nitrogen is in amino ethyl alcohol. However, excepting the small amount of amino acid nitrogen, it probably is in this compound.

Method II.—The lecithin Sample B (about 2.5 g. in each experiment) hydrolyzed with 4% HCl (10 cc. HCl to 100 cc. H_2O) for 30 hours. The fatty acids were filtered off and washed three times with dilute hydrochloric acid. A nitrogen determination was run on the fatty acids. This nitrogen is *residue nitrogen.* The filtrate was slowly evaporated to dryness on a water bath to remove the excess of hydrochloric acid and a small amount of fatty acids. The residue was treated with several small portions of water, which were filtered into a 50 cc. measuring flask. The solution was made up to exactly 50 cc. The nitrogen determination on 10 cc. of the filtrate gave *total filtrate nitrogen.* Another 10 cc. portion was used for the *amino nitrogen* by means of amino nitrogen apparatus. This indicated the amount of amino ethyl alcohol. 10 cc. were carefully evaporated to dryness, treated with absolute alcohol in several small amounts, filtered, and chloroplatinic acid added in slight excess. The choline platinum chloride was filtered off and a nitrogen determination run on the precipitate. This represented *choline nitrogen.* Ammonia *nitrogen* was determined on 10 cc. by making it alkaline with 1 g. of potassium carbonate and drawing over the ammonia into standard acid in the usual way. An estimation was made of the *amino acid nitrogen* on the remaining 10 cc. by the copper method.

The following quantitative data were thus obtained. The figures indicate the percentage of nitrogen in the lecithin:

Residue nitrogen	0.25		0.22	
Ammonia nitrogen	0.04		0.03	
Amino acid nitrogen		0.07		0.03
Amino alcohol nitrogen (diff.)		0.72		0.80
Total amino nitrogen	0.79	——	0.83	——
Choline nitrogen	0.80		0.84	
Total filtrate nitrogen		1.52		1.63
Total nitrogen	1.88		1.92	

It is rather unexpected that approximately the same relative amounts of fatty acid nitrogen, choline nitrogen, and amino alcohol nitrogen should be found in heart lecithin as in brain lecithin.[1] This may mean that the two lecithins are identical. If the nitrogen of the residue was present as an impurity one would not expect two lecithins from such different sources, prepared by rather dissimilar methods, to have about the same amounts of it.

It is possible that the amino alcohol reported in these analyses is not an integral part of the lecithin molecule. But if it is not why do we find so much of it in about the same amounts as choline? Why is it so difficult to remove? Why should three different investigators[2] find approximately 42% choline in heart lecithin if there should be 100%? The data lend themselves best to the following hypothesis: Heart lecithin may have several choline-containing portions fastened to several amino acid-containing portions by means of a saturated nitrogen-containing complex. Very probably the first two of these complexes, and maybe all three, have structures similar to that usually assigned to a theoretical lecithin.

When purifying lecithin white matter separates after many of the procedures, indicating strongly a decomposition of some labile complex. Probably the lecithin of this investigation is such a complex, but of a more stable nature than the original tissue complex.

The question involved is undoubtedly the same as the much-discussed protagon one. Just as there is a rather unstable complex called phosphocerebroside, which is a weak combination of phosphatids and cerebrosides, so there is a lecithin one as above described which by suitable means can be decomposed and separated into its constituents.[3]

It is profitable to study the more complex, less stable compounds because it is these, and not the more stable, simpler, so-called pure compounds, that are more largely concerned in biological phenomena. But of course these more complex ones cannot be understood thoroughly until their simpler constituents are investigated.

Summary.

1. Heart lecithin as prepared for this investigation has about 12% of its nitrogen in a form insoluble in water on hydrolysis.

2. About one-half of the soluble nitrogen is in the form of choline.

3. The other half of the soluble nitrogen is present in amino ethyl alcohol.

4. There are small amounts of ammonia in the hydrolyzed solution; but this is probably a contamination and not a part of the lecithin molecule.

---

[1] J. E. Darrah and C. G. MacArthur, THIS JOURNAL, **38**, 922 (1916).

[2] H. MacLean, *Biochem. J.*, **4**, 38 and 240 (1909); A. Erlandsen, *Z. Physiol. Chem.*, **51**, 71 (1907).

[3] H. MacLean, *Biochem. J.*, **9**, 351 (1915).

5. The small amount of amino acid found may be due to a small quantity of an amino acid containing phosphatid as impurity.

6. Heart lecithin has about the same amounts of each of the three main constituents as brain lecithin. The two lecithins may be the same compound. It is not to be expected that lecithin from two such different tissues prepared by rather different methods should yield by hydrolysis nitrogen products qualitatively identical and quantitatively so similar, unless each of these constituents was represented in the molecule by about the quantities found.

7. Acetone dehydration left the tissue in the most satisfactory condition for further work on the phosphatids.

BERKELEY, CAL.

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE ARKANSAS AGRICULTURAL EXPERIMENT STATION AND JOHNS HOPKINS UNIVERSITY.]

THE INOSITE PHOSPHORIC ACIDS OF COTTONSEED MEAL.[1]

BY J. B. RATHER.

Received January 3, 1917.

In a previous paper on the phosphorus compounds of cottonseed meal,[2] the writer showed that the inorganic phosphorus of that material was only 5% of the total phosphorus, and that cottonseed meal contained an organic phosphoric acid which gave all the reactions relied on by Hardin[3] and by Crawford[4] to prove the presence of meta- and pyrophosphoric acids in that material. The writer concluded that there is no evidence that cottonseed meal contains these acids. Anderson[5] about a year later came to the same conclusion, and further stated that cottonseed meal contains an organic phosphoric acid very similar to phytic acid, $C_2H_8P_2O_9$. In continuation of the study the present writer[6] showed that cottonseed meal contains an inosite phosphoric acid richer in carbon and poorer in phosphorus than phytic acid, and ascribed the empirical formula $C_{12}H_{41}O_{42}P_9$ to this acid on the basis of analyses of silver salts which had been freed from bases and inorganic phosphoric acid. Anderson[7] later repeated his work and concluded that the acid was not the one claimed to be present by the writer, but that it was inosite hexaphosphoric acid,

---

[1] Read in part at the Seattle Meeting of the American Chemical Society and the 1916 Meeting of the Association of Official Agricultural Chemists. Full details of this work will be published as a bulletin of the Arkansas Agricultural Experiment Station.

[2] Texas Agr. Expt. Sta., *Bull.* 146.

[3] South Carolina Agr. Expt. Sta., *Bull*, **8**, n. s.

[4] *J. Pharm. Expt. Therapeutics*, 1, 51 (1910).

[5] *J. Biol. Chem.*, 13, 311 (1912).

[6] THIS JOURNAL, **35**, 890 (1913).

[7] *J. Biol. Chem.*, 17, 41 (1914).