dialysis, filtration, and precipitation by acetone, gave for the dried substance 15.5% nitrogen (14.0 by aeration).

The corrected (higher) nitrogen contents of these substances (especially those obtained from castor beans) approximate more nearly the analyses of similar substances, described by Osborne and others.[1]

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[CONTRIBUTION FROM THE BIOCHEMISTRY LABORATORIES OF THE UNIVERSITIES OF ILLINOIS AND SOUTHERN CALIFORNIA.]

NITROGENOUS CONSTITUENTS OF BRAIN LECITHIN.

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The object of the work here reported was to study the hydrolysis products of sheep and beef brain lecithin, with the purpose of determining their nitrogenous constituents, both qualitatively and quantitatively.

Preparation of the Lecithin.—Fresh sheep and beef brain, in separate lots, were finely ground in a food-grinder. They were then allowed to stand in acetone for two days. At the end of this time, the material was filtered, thoroughly pressed in a fruit press, and then put into fresh acetone. The acetone was changed in this way at least three times. The second and third extractions removed most of the fat and some extractives, such as cholesterol. The dehydrated material was kept in a good grade of acetone until it was required for use. Portions of this tissue were filtered out, dried on glass plates in a current of air, and placed in double their volume of benzene. The benzene was agitated in contact with the tissue on a shaking machine. Part of the extractions were made, hot, by allowing a stream of water heated to 65° to flow over the bottle containing the mixture, while it was shaking. The extraction period was from two to four hours. The benzene solution was filtered off and the tissue was freed from benzene so far as possible, by the fruit-press. A second benzene extraction was then made. A few trials were made, using ether instead of benzene extraction. This solvent seemed to be much less satisfactory than benzene, and is more likely to cause oxidation of the lecithin.

The benzene extracts were concentrated almost to dryness, by distilling under reduced pressure, in a carbon dioxide atmosphere, over the steam bath. The light brown residue was dissolved in the smallest possible volume of ether, which had been freshly distilled over calcium chloride. This solution was found to contain cephalin, lecithin, and "white substance." The cephalin was precipitated, by addition of a volume of absolute alcohol equal to two and one-half times the volume of the ether solution. As thus precipitated, the cephalin was a very light brown in color, and in waxy condition, easily pressed together.

[1] Cf. Osborne, "The Vegetable Proteins," 1909.

Any suspended cephalin was filtered out, and the alcohol-ether filtrate was evaporated with diminished pressure in a carbon dioxide atmosphere. The temperature was not allowed to rise above 45° during any distillation period. When evaporated almost to dryness, the residue was dissolved in a small portion of redistilled ether. By addition of a double volume of acetone, the lecithin, together with some "white material" was precipitated. The precipitate was yellow or very light brown, and adhered to the walls of the precipitation jar in waxy masses. After filtering off the ether-acetone solution, the precipitate was thoroughly pressed together with fresh acetone, in much the same manner in which butter is worked to remove the buttermilk.

As rapidly as possible this precipitate was freed from acetone and placed in a vacuum desiccator, over sulfuric acid or calcium chloride. The desiccator was wrapped in cloth to exclude light, and allowed to evacuate for several hours. When thoroughly dry, the material was dissolved in freshly distilled ether and placed in tall, stoppered cylinders. It was allowed to stand in the ether overnight. The "white substance" settled out and the clear supernatant solution was syphoned off. This solution was tested for cephalin, by addition of alcohol. Usually cephalin was present, and the alcohol treatment had to be repeated.

After reprecipitation of the lecithin by the above method, the ether treatment was also repeated, to remove any "white substance" still present. The original benzene extract seemed to contain, in addition to lecithin and cephalin, a protagon which was not completely disintegrated until the alcohol-ether treatment had been repeated several times. It was therefore necessary to repeat the test for cephalin at least three times. As a criterion of purity, the following points were observed: (1) absence of cephalin; (2) no deposition of "white substance," when allowed to stand in ether solution.

In the latter part of the work, the lecithin was precipitated by addition of the ether solution of the material to the acetone, instead of the reverse process. The last purification of the lecithin was made by precipitation with acetone, from a petroleum-ether solution. Six samples of lecithin were prepared, designated as follows:

LECITHIN PREPARATIONS.

Source.	Weight of product. G.
I. Beef brain (old material repurified)	5.0000
II. Beef brain	17.4149
III. Sheep brain	4.0620
IV. Sheep brain	18.6333
V. Beef brain	9.7450
VI. Sheep brain	11.5000

Hydrolysis.—The lecithin was hydrolyzed with 3% HCl, or with

1.6–5% KOH in aqueous solution. The hydrolyzing mixture was refluxed for from 15 to 20 hours in an atmosphere of carbon dioxide. After acid hydrolysis, the free fatty acids floated on the surface. In case hydrolysis by KOH had been used, the acids were liberated by acidifying with dilute HCl. The acids were filtered out on a hardened filter, and were washed on the filter with water containing a trace of acid.

Qualitative Study of Nitrogenous Products.

Natural Amino Ethyl Alcohol by Method I.—Lecithin Samples I and II were hydrolyzed with 3% HCl for 15 hours. The filtrates from the fatty acids were combined. The nitrogenous bases remained in the filtrate, together with the glycero-phosphoric acid. This solution was evaporated to dryness, using a low temperature. The residue was taken up in the smallest amount of water necessary, and barium chloride solution was added in excess. Ninety-five per cent. alcohol was then added to precipitate the barium glycerophosphate. This precipitate was filtered off, and the filtrate was evaporated to dryness. The residue was taken up in alcohol and evaporated repeatedly, to free it from alcohol-insoluble material. Finally, the residue was taken up once in absolute alcohol and evaporated. It was then dissolved in distilled water. To a volume measuring 120 cc. were added 18 cc. of concentrated HCl, and a solution of 15 g. of phosphotungstic acid dissolved in 30 cc. of water. The solution was made up to 200 cc.; it was warmed slightly, then allowed to cool in the refrigerator.

The choline-phosphotungstate, thus precipitated, was filtered out and washed on the filter with 200 cc. of a solution containing 5 g. of phosphotungstic acid and 6 cc. of concentrated HCl. To the filtrate, phenolphthalein was added, and 50% sodium hydroxide was run in, until alkalinity was reached. The solution was then diluted to 800 cc., and a 20% solution of crystalline barium chloride was added in excess. This removed the excess of phosphotungstic acid. The precipitate was filtered out and washed free from chlorides. Washings and filtrate combined were acidified with HCl, and evaporated to dryness on the steam bath. The residue was extracted with absolute alcohol.

The alcohol solution, thus obtained, was filtered and a warm saturated alcoholic solution of picrolonic acid (1,*p*-nitro phenyl-3-methyl-4-nitro-5 pyrazolon) was added. The picrolonate of amino ethyl alcohol precipitated slowly, after standing in a desiccator, over sulfuric acid or calcium chloride. The crystals had the characteristic appearance, *i. e.*, tufts of yellow needles. After several recrystallizations from alcohol, this derivative was found to soften at 221°, and melted with decomposition at 225°. The salt prepared from synthetic amino ethyl alcohol showed exactly the same crystalline form and melting point. On mixing

the synthetic with the natural derivative, no change in melting point was observed.

Melting point of natural and synthetic, 221–225°.	Wt. sample.	Wt. N_2.	% N.	Theoretical % N_2.
Natural derivative.....	0.1028	0.0218	21.20	21.51
Synthetic derivative.....	0.0743	0.0158	21.26[1]	...

Synthetic Amino Ethyl Alcohol.—It may be well to outline the procedure followed in the synthesis of the base. The available starting point in the synthesis seemed to be ethylene bromide. A mixture of 175 g. of potassium acetate and 175 g. ethylene bromide with 350 g. of 85% ethyl alcohol were refluxed together for two days on the water bath. The resulting mixture was distilled. The fraction boiling from 130° to 190° was redistilled. Most of it distilled at 182°. This glycol-acetate was saturated with dry HCl gas. The chloracetic ester separated as a brown oil. This oil was placed in a separatory funnel and dropped slowly into a 50% solution of KOH in water. The alkali was warmed slightly at the same time. The resulting vapors were passed through a condenser, cooled by means of ice-water drawn through by the suction of a water pump. The cooled vapors of ethylene oxide were thus conducted into a collecting flask containing a saturated solution of ammonia in ethyl alcohol, and surrounded by a freezing mixture. The flask was provided with a small outlet tube, to release pressure. The alcohol solution, containing the ethylene oxide and ammonia, was allowed to stand tightly stoppered, for two days.

The alcohol solution was fractionated. At first, ammonia vapor and alcohol passed over. Fractions from 85° to 130° were redistilled. Fractions boiling at 120–180° were also redistilled. Finally, combining the higher boiling fractions, all distilled over at 120–140°. This fraction was a light brown oil,[2] which rapidly grew dark in color. It had a strong basic odor and attacked cork readily. Allowing for small amounts of water and alcohol probably present, the yield was approximately 2 g. The picrolonate derivative of the synthetic base proved to have the same properties as the derivative of the natural base, as previously described. The melting point was 225°. The crystals formed characteristic tufts of yellow needles, with twinning.

Natural Amino Ethyl Alcohol by Method II.—A somewhat different method was used in lecithin Samples III–VI than in Samples I and II, for isolation of the amino ethyl alcohol. Sample III was hydrolyzed with 3% HCl, Samples IV and V with 5% KOH, and Sample VI with 1.6% KOH. Hydrolysis products of Samples III, IV, and V, were com-

[1] This agrees well with Knorr's value, *i. e.*, 21.31% N_2. M. p. 221–225°. *Ber.*, 30, 909 (1897).

[2] Knorr, *Ber.*, 30, 909 (1897); Wurtz, *Ann.*, 114, 51 (1890); 121, 228 (1862); Atkinson, *Ibid.*, 109, 232 (1859); Simpson, *Ibid.*, 113, 115 (1860).

bined and examined together for amino ethyl alcohol, while Sample VI was treated separately. The solution of chlorides of the base was evaporated to dryness and extracted with absolute alcohol, then a solution of platinum chloride was added to precipitate the choline. The precipitate came down in fine yellow flocks. By recrystallization from dilute alcohol, and ignition of a sample in a porcelain crucible, 31.8% platinum was obtained. The calculated value is 31.64% platinum.[1]

The excess platinum was removed from the alcoholic solution of the bases, by saturation with H_2S. The glycero-phosphoric acid was not removed as was done in the first preparations, but potassium hydroxide in sticks was added to the strongly concentrated alcoholic solution in a distilling bulb until the solution reacted alkaline. Amino ethyl alcohol was then distilled off, leaving a residue of potassium glycerophosphate. The largest fraction distilled over at 160–165°. The purest fraction distilled at 177–185°. We obtained the same substance (amino ethyl alcohol) as was obtained by the previous method; and with much less trouble. The picrolonate and the chloroaurate were prepared.

The chloroaurate was made by adding concentrated HCl to the base, and then a molecular equivalent of gold chloride in a 3% aqueous solution. After standing a week in a good vacuum desiccator over sulfuric acid, the salt crystallized in needles over a centimer long, of a golden yellow color. This salt was so hydroscopic that it was difficult to obtain a melting point. After heating in a glass tube immersed in sulfuric acid maintained at 120–150° for two hours, the salt became free from water and melted at 186°.[2] An attempt to crystallize the salt from water solution failed. It was necessary to recrystallize it from concentrated HCl.[3] Metallic gold separated as the salt crystallized, therefore it was necessary to determine the percentage of gold present as metallic gold, and to deduct this value from the total percentage of gold in the salt. This was done by use of an alundum filter cone.

After treatment with concentrated HCl, washing with water, then with alcohol and ether, the cone was dried in a steam oven. It was then ignited to constant weight. Then a sample of the chloroaurate was weighed out in the cone, treated with concentrated HCl, washed with water, then with alcohol and ether, then dried and ignited as before. The free metallic gold was found to be 2.13%. The total gold was found, by ignition of the chloroaurate, to be 51.31% and 51.52%, in two checks. By subtraction of the metallic gold, 49.18% and 49.39% gold content

[1] Gulewitsch obtained 31.80% platinum. *Z. physiol. Chem.*, **24**, 517 (1898).

[2] Knorr gives 190°.

[3] This was also the experience of Trier, *Z. physiol. Chem.*, **86**, 153 (1913).

were obtained.[1] The salt obtained by crystallizing the filtrate, from metallic gold in the alundum cone, showed a gold content, after ignition, of 49.40%. Calculated for C_2H_7NO-HCl-$AuCl_3$: gold equals 49.17%.

It seems evident from the boiling point of the free base and analyses of the chloroaurate and picrolonate derivatives, together with a comparison of the same with the synthetic preparations, that one of the nitrogen-containing bases of beef and sheep brain lecithin is amino ethyl alcohol. That choline is also present was confirmed by analysis of the platinum salt, as above described. Sheep and beef brain lecithin, therefore, contain the same nitrogenous constituents as egg lecithin, but in different proportions.

Quantitative Study of Nitrogenous Products.

For some of the quantitative determinations the lecithin used in the qualitative study just described was further purified. It was emulsified in distilled water, enough dilute hydrochloric acid added drop by drop to give a flocculent precipitate. This was rapidly filtered, washed till it began to dissolve, then redissolved in a large volume of water and reprecipitated with hydrochloric acid, washed with water then with acetone. The acetone was removed in a vacuum desiccator. This preparation will be designated *HCl Lecithin*.

The lecithin sample (1, 2 or 3 g., usually 3 g.) was hydrolyzed with 200 cc. of a dilute hydrochloric acid solution (20 cc. HCl + 180 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.[2] This indicates the amount of amino ethyl alcohol.

Ten 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*.

[1] Knorr's value, % gold equals 49.15% and 49.02%; Trier's value, % gold equals 49.27%.

[2] Van Slyke, *J. Biol. Chem.*, 12, 275 (1912).

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.[1]

An estimation was made of the *Amino Acid Nitrogen* on the remaining 10 cc. by the copper method.[2]

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

	Sheep HCl lecithin (new).	Sheep HCl lecithin (new).	Sheep HCl lecithin (5 mo. old).	Sheep lecithin (5 mo. old)	Beef lecithin (new).
Residue nitrogen.....	0.24	0.26	0.31	0.36	0.35
Ammonia nitrogen.....	0.03	0.02	0.05	0.04	0.05
Amino acid nitrogen.....	0.04	0.04	0.05	0.05	0.04
Choline nitrogen.....	0.72	0.75	0.72	0.70	0.53
Amino (alcohol) nitrogen...	0.74	0.72	0.58	0.60	0.75
Total filtrate nitrogen.....	1.55	1.54	1.50	1.38	1.42
Total nitrogen.....	1.79	1.80	1.81	1.74	1.77

Discussion of Quantitative Data.

The residue nitrogen is slightly larger in beef brain lecithin than in sheep brain lecithin. This is undoubtedly due to the fact that the beef brain lecithin available for this work had not been treated with hydrochloric acid and water as had most of the sheep brain samples. Where the latter had not been so treated the amount of residue nitrogen is about the same as in beef brain lecithin. It is not known just what this residue nitrogen represents. However, the study of this fatty acid residue through a separation of the fatty acids by the usual lead acetate method and then a bromination of the unsaturated acids showed that the nitrogen containing part of this residue was with the oleic bromide and could be separated from it by its insolubility in acetone, the oleic bromide being soluble in this reagent. The amount of nitrogen in this acetone-insoluble material was 1.3%. It looks then as though the residue nitrogen represented a substance that has about the same solubilities as the rest of the lecithin that is attached to it either by chemical combination or adsorption so firmly that no known method of purifying the lecithin removes it. That it becomes constant at about 0.25% and that no amount of purification lowers this value lead one to believe it is an integral part of the molecule. Yet it is rather difficult to reconcile this view with the fact that this residue nitrogen substance has the same solubilities it had in the original lecithin even after alkali hydrolysis, the lead acetate separation and bromination. This makes one look upon it as a saturated impurity with properties so similar to lecithin that no known method removes it. Some preliminary experiments on brominating the lecithin

[1] Denis, *J. Biol. Chem.*, 8, 427 (1910).
Kober, THIS JOURNAL, 35, 1546 (1913).

indicate that through the bromo derivative the lecithin can be purified.

The presence of a small amount, almost negligible in most cases, of ammonia is possibly due to a small amount of an ammonium lecithinate, or more probably to a contamination of ammonia from the laboratory during the preparation and hydrolysis, though the usual precautions against contamination were taken. The fact that the amount was so small and so variable supports the latter view.

There is but little amino acid nitrogen in lecithin. This differentiates lecithin from cephalin. The latter has a considerable part of its nitrogen in an amino acid.[1]

Choline contains about one-half of the soluble nitrogen of both sheep and beef lecithin. In cephalin, on the other hand, there is no choline.

Most of the other half of the soluble nitrogen is in the amino ethyl alcohol. The amount present is not largely different in sheep and beef brain lecithin, though as a rule there is appreciably more amino alcohol nitrogen and slightly less choline nitrogen in beef brain lecithin. It will be noticed in the analysis that the older samples of sheep lecithin give lower values for amino nitrogen. This would indicate that on oxidation the amino nitrogen slowly changes to some other form.

From this study it can be concluded that sheep and beef brain lecithin contain an unhydrolyzable portion of nitrogen and a hydrolyzable portion, the latter comprizing about 85% of the total. Of this soluble portion about one-half of the nitrogen is in choline and the other half in amino ethyl alcohol.

It seems probable that it is merely a coincidence that in brain lecithin the amount of amino ethyl alcohol should approximately equal that of choline. Lecithins from other sources have decidedly different ratios between these two substances. In fact these lecithins are undoubtedly to be distinguished from one another by the varying amounts they contain of these nitrogenous bases. It is even likely that beef and sheep brain lecithin can be differentiated by the higher choline content of the latter.

Conclusion.

(1) The presence of amino ethyl alcohol was demonstrated by analyses of the gold double salt and of the picrolonate derivative. In every respect these analyses were in agreement with the analyses of the corresponding derivatives of synthetic amino ethyl alcohol.

(2) By analysis of the platinum salt, the presence of choline in brain lecithin was confirmed.

(3) These bases (choline and amino ethyl alcohol) were present in both beef and sheep brain lecithin prepared by the above method.

[1] MacArthur, THIS JOURNAL, 36, 2397 (1915).

(4) In both sheep and beef brain lecithin the amount of choline nitrogen is about equal to the amino alcohol nitrogen, the two comprising 85% of the total nitrogen in the lecithin.

(5) The other 15% is in the form of an unhydrolyzable residue.

(6) If lecithin is a single substance, and it probably is not, or at least a much more complex one than is usually believed, it probably contains one molecule of each of the nitrogenous bases, choline and amino ethyl alcohol. This associated lecithin molecule is rather firmly combined with a saturated nitrogenous substance which it has been impossible, as yet, to remove.

LOS ANGELES, CAL.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGY OF THE UNIVERSITY OF CHICAGO, THE MEMORIAL INSTITUTE OF INFECTIOUS DISEASES, AND RUSH MEDICAL COLLEGE, CHICAGO.]

THE EFFECTS OF COMMERCIAL GLUCOSE WHEN FED TO WHITE RATS.

BY A. J. CARLSON, L. HEKTOEN AND E. R. LECOUNT.

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In order to study the general effects of commercial glucose when consumed regularly in considerable quantities for a relatively long period of time, young white rats were fed over a period of six months with bread containing a certain amount of glucose. The white rat was selected because it is an easily handled, omnivorous animal whose average span of life covers about three years. For purposes of control another set of rats was fed on bread containing a certain amount of granulated cane sugar and a third set on bread without sugar.

The bread, which was unleavened, consisted of flour, 1000 parts; lard, 25 parts; baking soda, 15 parts; hydrochloric acid, enough to neutralize the soda; water as required for flour, about 655 parts; commercial glucose, 34 parts, in the case of "Glucose Bread," and granulated cane sugar, 26.5 parts, in the case of "Cane Sugar Bread." Fresh lots of the three kinds of bread were baked each Wednesday and Saturday.

In the laboratory in which the experiment took place, white rats are fed on white bread (baker's) about seven days old, small quantities of carrots, and occasionally a little meat. Fresh water is supplied each day. Careful special observations showed that 25 rats of the average weight of 75 g. would eat 275 g. of bread and 170 g. of carrots a day. On the basis of these results and of the general experience, the feeding experiment was conducted with young rats, 25 in each cage, and a daily ration for each cage, to begin with, of 300 g. of bread, 12 g. per rat, 150 g. of carrots or other vegetables, or of meat occasionally, and plenty of