dissociation and oxidation of whose intermediate hexose-dienols result in the various oxidation products found.

This work was originally undertaken under the mistaken impression that it was a part of certain problems on sugar oxidations granted by Dr. Nef to one of us (Lewis) in 1909. The investigation was well under way before the error, fully acknowledged here, was discovered. The results are now published, however, with the full consent of Dr. Nef, grateful recognition of whose generosity in the matter is herewith freely accorded.

The oxidation products of Fehling's solution on maltose and on lactose are now being studied in this laboratory and, with their completion, the authors will discontinue, as requested by Dr. Nef, all research on the oxidation products of the sugars with inorganic reagents.

BRAIN CEPHALIN: I. DISTRIBUTION OF THE NITROGENEOUS HYDROLYSIS PRODUCTS OF CEPHALIN.

By C. G. MacArthur.

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The constitution of cephalin is uncertain. Though the nature of the glycerophosphoric acid produced on hydrolysis is fairly well established,[1] the nitrogenous substances[2] and the fatty acids[3] present are not definitely known either as to identity or quantity. This series of investigations was started three years ago in an attempt to clear up these two uncertainties in the cephalin molecule.

This paper considers the preparation of cephalin, the methods used in determining quantitatively its various nitrogenous products and the data obtained by these methods.

Preparation and Purification.—Fresh sheep brains were cleaned carefully, ground in a meat grinder with a small amount of thymol, spread in very thin layers on glass plates, and placed in an air drier. By frequent turning the tissue dried in a day. The dry material was scraped off and placed in a vacuum desiccator. In some cases, instead of the above air-drying method, the dehydration was accomplished by adding to the tissue twice its weight of alcohol or acetone and filtering after a day's standing.

After complete desiccation the cholesterol was extracted by continuously shaking the tissue with twice its weight of acetone. Two such treatments of about four hours each removed practically all the cholesterol.

[1] Dimitz, *Biochem. Z.*, **21**, 337.

[2] Thudicum, "Die chemische Konstitution des Gehirns," p. 142; Koch, *Z. physiol. Chem.*, **36**, 134; Neubauer and Frankel, *Biochem. Z.*, **21**, 321.

[3] Cousin, *J. pharm. chim.*, **24**, 101 and **25**, 177; Dimitz and Frankel, *Biochem. Z.*, **21**, 337; Parnas, *Biochem. Z.*, **22**, 411.

The cholesterol-free tissue was then similarly treated with benzene. This removed most of the phosphatids, among them cephalin.

After concentrating the benzene solution the cephalin was precipitated by very slowly adding it to twice the volume of absolute alcohol. The precipitate was evacuated and dried in a calcium chloride desiccator. On dissolving this material in ether, freshly distilled over calcium chloride, an insoluble substance remained. Because it cannot be satisfactorily filtered or centrifuged, it was separated by allowing the ether to stand in tall cylinders in an ice-box. The clear liquid was siphoned off, concentrated in a vacuum and reprecipitated by alcohol, then dried as before. This process of solution in ether and precipitation with alcohol or acetone was repeated until no insoluble material remained. The final product was a yellow-white powder which will be called cephalin (0).

Several different procedures were used to further purify the various preparations of cephalin. One lot was emulsified in water, filtered and precipitated by a slight excess of hydrochloric acid. To separate thoroughly, the liquid was centrifuged and the precipitate washed by the same process. The procedure was repeated and the substance then dried in a vacuum desiccator. On powdering, a brownish yellow, slightly sticky, hydroscopic material resulted. This will be referred to as cephalin (1).

In purifying another preparation, the watery emulsion was salted out by sodium sulfate, the precipitate washed, and the process repeated. Finally the cephalin was dissolved in ether, precipitated with acetone and evacuated. This product had a similar appearance to that of (1). This will be called cephalin (2).

Another method used was to shake the watery emulsion in a separatory funnel with redistilled ether. The ether layer was dehydrated with anhydrous sodium sulfate and precipitated with acetone. After a repetition of this process and a desiccation of the product it was slightly darker than either of the first two preparations; it will be designated as cephalin (3).

The method least open to objection is a reprecipitation of the ether or benzene or petroleum ether solution a large number of times by alcohol or acetone. The solvent is changed each time, as is also the precipitating agent. A better and more rapid purification is obtained by adding the solution slowly with continuous stirring to the alcohol or acetone. This cephalin is a light, yellowish white, hydroscopic powder (4).

By choosing an amount of alcohol (95%) or acetone not quite sufficient to produce a complete precipitation, a partial separation of the more soluble cephalin occurs. One lot of cephalin (4) which had been precipitated three times was put through this partial separation. The first four fractions remaining dissolved were united and carefully evaporated to a light brownish, waxy, non-powderable material, to be called cephalin

(5). The next four fractions were treated similarly, but gave a yellow, hard, powderable preparation (6). The residue which had been precipitated twelve times was brittle, light brown, and not sticky. This should be very pure cephalin. It will be referred to as preparation (7).

These seven products differed but little in their nitrogen and phosphorus content. Many nitrogen analyses gave an average of 1.60%. The phosphorus was never far from 3.65%.

Quantitative Methods and Data.—After trying out the various acids and alkalies used for hydrolysis, it was found that the best results were obtained with 1% hydrochloric acid. Three grams of cephalin were hydrolyzed 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 ammonium chloroplatinate, and so the nitrogen in it, when added to the alcohol-insoluble nitrogen, would be *ammonia nitrogen.* To distinguish between these two determinations, the nitrogen in the platinum precipitate will be labeled *platinum nitrogen.* After removing the excess of platinum by hydrogen sulphide the above filtrate was evaporated to dryness and taken up in water. It was made slightly alkaline with sodium carbonate and mercuric acetate was added as long as a precipitate formed while keeping the solution alkaline with the sodium carbonate.[1] An equal volume of alcohol was added and the solution filtered. The nitrogen in the precipitate was labeled *mercury precipitate nitrogen,* that in the filtrate *mercury filtrate nitrogen.* The precipitate has been found to be amino acid and amino alcohol, the filtrate mostly amino alcohol,[2] the latter being but partly precipitated as the mercury compound. Two of many analyses by this method follow:

	Impure cephalin (0).		Cephalin (1).	
Residue nitrogen	0.53		0.25	
Alcohol-insoluble nitrogen	0.05	0.20	0.11	0.25 ammonia nitrogen
Platinum nitrogen	0.15		0.14	
Mercury precipitate nitrogen	0.66		0.89	
Mercury filtrate nitrogen	0.15		0.22	
Total nitrogen	1.54		1.51	

Many attempts were made to reduce the amount of nitrogen in the residue. Seventy-five hours' hydrolysis in dilute acid or alkali did not

[1] Neuberg and Kerb, *Biochem. Z.*, 40, 498.

[2] A later publication of this series describes the finding of these two compounds. See also Bauman, *Biochem. Z.*, **54**, 30 for the finding of amino alcohol.

lower it, nor did twenty hours in 10% sulfuric acid or 10% potassium hydroxide. It was not reduced by alcoholic hydrochloric acid. It seems to be a constant quantity of any preparation of cephalin. During purification the amount is lowered to a definite limit, beyond which further treatment does not reduce it. An unpurified cephalin (0) gave 0.53% residue nitrogen, but after purification, as cephalin (2), this became 0.28%. Results of a similar nature have been obtained for brain lecithin, heart cuorin, and heart lecithin.[1] All give a part of the nitrogen in the residue.

Another method of studying the nitrogen distribution was worked out which was found more satisfactory than the above. After the hydrolysis of the cephalin in 1% hydrochloric acid for twenty hours the fatty acids were filtered off and the filtrate very slowly and carefully evaporated to dryness. The residue was taken up in water made slightly alkaline with potassium carbonate or hydroxide, and air passed several hours to drive off the ammonia, which was collected in standard acid.[2] The solution was then made slightly acid with hydrochloric acid and carefully evaporated to dryness, taken up in water and made to 25 cc. in a measuring flask. In 10 cc. the total amino nitrogen was estimated by the amino apparatus.[3] A 5 cc. portion was used for the determination of the amino acid nitrogen by the copper method.[4] The nitrogen in the other 10 cc. was estimated by combustion. This gives the total nitrogen in the filtrate from the fatty acids and is a control on the total amino nitrogen. Several typical analyses by this method are given below:

	Cephalin (4).	Cephalin (7).	
	Per cent.	Per cent.	Per cent.
Residue nitrogen	0.21	0.25	0.27
Ammonia nitrogen	0.21	0.22	0.21
Amino acid nitrogen	0.39	0.70	0.73
Amino alcohol nitrogen (by diff.)	0.79	0.40	0.40
Total amino nitrogen	1.18	1.10	1.13
Total filtrate nitrogen	1.20	(1.18)	1.18
Total nitrogen	1.60	1.57	1.61

In this table it will be noticed that the residue nitrogen, the ammonia nitrogen, and the total amino nitrogen are nearly constant in the different preparations, but that the amounts of amino acid and amino alcohol nitrogen vary largely. To find out whether this might not be due to cephalin being a mixture, an attempt was made to partially separate the two fractions. This was done as described above for the preparations

[1] See a later article for data. See MacLean, *Biochem. J.*, 4, 38 and 240.

[2] Denis, *J. Biol. Chem.*, 8, 427.

[3] Van Slyke, *Ibid.*, 12, 275.

[4] Kober, THIS JOURNAL, 35, 1546.

of cephalins (5), (6), (7). It will be seen from the accompanying table that the amino alcohol content is larger in the more soluble portions, while the amino acid increases in the more insoluble part.

	Cephalin (0, 4). Per cent.	Cephalin (5). Per cent.	Cephalin (6). Per cent.	Cephalin (7). Per cent.
Amino alcohol nitrogen.....	0.82	0.82	0.87	0.38
Amino acid nitrogen.....	0.32	0.25	0.22	0.74

Cephalin (0, 4) is a sample partially purified according to the cephalin (4) method. It was probably similar to the preparation from which cephalins (5), (6) and (7) were derived.

With the purification of cephalin (7) the per cent. of amino acid constantly increased. After the first treatment there was 0.46% nitrogen in this form. Later in the process this became 0.64% and finally 0.73%. This last value is not a final one. It simply indicates the extent of the separation.

Conclusions.

It may be concluded from the above data that:

1. There is neither choline nor neurine in cephalin.

2. Cephalin has its nitrogen in four forms: These are residual nitrogen, about 0.20%; ammonia nitrogen, about 0.20%; amino alcohol nitrogen, 0.80%; and amino acid nitrogen, 0.40%. These are values for a typical pure product.

3. Ordinary cephalin is probably made up of at least two cephalins, one containing a large percentage or all its nitrogen as amino alcohol, the other having the larger amount of its nitrogen as amino acid.

I wish to express my indebtedness to the late Waldemar Koch for his suggestion of this line of work and for his valuable advice during the first months of the investigation. I am also indebted to Professor A. P. Matthews for suggestions during the progress of this work.

LABORATORIES OF BIO-CHEMISTRY OF THE
UNIVERSITIES OF CHICAGO AND ILLINOIS.

[CHEMICAL SECTION, IOWA AGRICULTURAL EXPERIMENT STATION.]

CHEMICAL CHANGES DURING SILAGE FORMATION.

BY RAY E. NEIDIG.

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Introductory.

Studies on the volatile aliphatic acids and the lactic acid in corn silage have been reported in previous publications.[1] It was shown that both nonvolatile and volatile acids are present in considerable amount, and occur in the ratio of about four parts of the former to three of the latter. The principle volatile acids were acetic and propionic, these being pres-

[1] Dox and Neidig, *Iowa Agr. Exp. Sta. Research Bulletins* 7 and 10.