in several different forms, namely: (1) as occlusion water, (2) capillary water, (3) osmotic water, (4) colloidal water, bound by physical forces, and (5) chemically bound water. The water which freezes at $-12.5°$ or below is probably either occlusion, capillary or osmotic water. It would not be expected that the osmotic pressure of the water in the white would be sufficient to prevent freezing at $-12.5°$. The ash of the white is about 0.60%. It is also probable that this bound water comes almost entirely under the class of colloidally bound water.

Summary

Using a freezing method for differentiating between bound and free water in a hydrophilic biocolloid, it is shown that a temperature of $-12.5°$ is sufficient to freeze all of the freezable (free) water and that the remaining water (bound water) is not frozen at temperatures ranging between -12.5 and $-35°$. The average amount of bound water in the thick portion of egg white is found to be about 26% as determined by this method. An improved formula for the calculation of free water is presented.

PULLMAN, WASHINGTON

[CONTRIBUTION FROM THE DIVISION OF LABORATORIES AND RESEARCH, NEW YORK STATE DEPARTMENT OF HEALTH]

A NOTE ON THE PREPARATION OF CEPHALIN

BY FRANK MALTANER

RECEIVED MAY 13, 1931 PUBLISHED NOVEMBER 5, 1931

The preparation of cephalin, of uniform composition and free from associated phosphatids, is of considerable importance not only for the study of the chemical and biological activity of this material but also for the study of its structure. While the results obtained by Levene and West,[1] with reduced cephalin, lend strong support to the generally accepted formula, $C_{41}H_{78}NPO_8$, the analytical figures found with the unreduced material, correspond more closely to the formula $C_{41}H_{78}NPO_{13}$.[2]

In a recent publication[3] a method was described for the preparation of cephalin, free from lecithin, and approximating in composition the latter formula. Different samples prepared by this method were of constant composition and contained all of their nitrogen in the amino form. The yield, however, was small and variable and the product was yellow in color, when dried, evidently due to slight decomposition. A better and more uniform yield (about one gram per pound of fresh brain) of a pure white prod-

[1] P. A. Levene and C. J. West, *J. Biol. Chem.*, **35**, 285 (1918).

[2] Hugh Maclean, "Lecithin and Allied Substances, the Lipins," Longmans, Green & Co., London, 1918, p. 45.

[3] Augustus Wadsworth, Frank Maltaner and Elizabeth Maltaner, *Am. J. Physiol.*, **97**, 74 (1931).

uct of similar chemical composition and biological activity was obtained by the following modification of this method.

Ten- instead of 50-pound lots of fresh beef brain were dried with acetone and pulverized. This dried tissue was first exhaustively extracted with acetone, from which it was subsequently separated by filtration and final drying *in vacuo*, then extracted with about 8 liters of U. S. P. ether containing 3% of added water. The ether extract, chilled below 0°, filtered to remove white matter and concentrated to a small bulk (500–600 cc.) *in vacuo*, was precipitated in 10 volumes of cold absolute ethyl alcohol, redissolved in ether and cooled below 0° overnight. This process was repeated until no further precipitation occurred in the cooled ether extract. It was then poured into 5 volumes of boiling hot absolute methyl alcohol. The precipitate, removed by filtration, was dissolved in ether and the ether solution poured once more into 5 volumes of boiling methyl alcohol, this second precipitate being discarded. The total methyl alcohol solution from the two precipitations was treated with an amount of saturated solution of sodium chloride to give a final concentration of 2% of water in the mixture. The precipitated cephalin was separated by centrifugalization, dissolved in ether and recentrifugalized to remove the sodium chloride. The ether solution was cooled to below 0° overnight to remove any cerebrosides which might have been carried over. The final clear ether solution was poured into ten volumes of absolute ethyl alcohol and the white precipitate, cephalin, separated by centrifugalization. It was preserved under absolute alcohol to prevent oxidation, as suggested by Page and Bülow.[4]

MICRO-ANALYSIS OF CEPHALIN

Source	Nitrogen, %	Phosphorus, %	Ratio Amino nitrogen: total nitrogen	Ratio Nitrogen: phosphorus
Beef brain No. 1.....	1.51	3.64	98:100	1.00:1.09
Beef brain No. 2.....	1.58	3.55	101:100	1.00:1.01

ALBANY, NEW YORK

[4] I. H. Page and Margarete Bülow, *Z. physiol. Chem.*, **194**, 166 (1931).