With less alkali, distillation was incomplete, although no acid was present originally in the solution. With Mixture B, the distillation was incomplete with 75 cc. alkali, sp. gr. 1.444 or 1.470, sulfuric acid and potassium sulfate having been added to the solution in amounts corresponding to digestion mixtures. With Mixture D the presence of different quantities of sulfuric acid originally, made only a comparatively small difference in the error of the aeration procedure.

## Conclusion.

The aeration procedure in the ordinary Kjeldahl method for nitrogen very often gives inaccurate and therefore unreliable results, and should not be used.

NEW YORK, N. Y.

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[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK.]

# STUDIES ON ENZYME ACTION. CORRECTIONS.

BY K. GEORGE FALK AND KANEMATSU SUGIURA.

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In the course of the study of lipolytic actions, some of the results of which were communicated in previous papers, a number of solid preparations were described and analyzed. The nitrogen contents were determined by the Kjeldahl method with the aeration procedure recommended by Kober. The unreliability of this procedure was pointed out in the preceding paper[1] and it is the object of this paper to correct the nitrogen results given in former papers on the basis of the customary heat distillation procedure in the Kjeldahl method.

The esterase preparations from castor beans,[2] water extract of castor beans, dialyzed, filtered clear, precipitated with acetone, designated E II gave 16.2% nitrogen for the dried material whether dialyzed 5 or 20 hours, while the ash-free material gave 17.0% nitrogen (as against 15 7 and 16.3% by the aeration procedure). The lipase preparations[3] L I, saturated NaCl extraction of castor beans residues after water extractions, dialysis, filtration and washing with acetone, gave 16.8% nitrogen for the dried material, and 17.9% for moisture and ash-free material (17.0 by aeration). L II, obtained similarly with 1.5 *N* NaCl solution, gave 17.1% for moisture-free or 17.9% for ash- and moisture-free material (16.7 by aeration). L II reprecipitated from NaCl solution, gave 18.2% for dried or 18.4% for dried and ash-free substance (17.6% by aeration). The lipase preparation from soy beans,[4] preparation by water extraction

[1] Cf. also Dillingham, THIS JOURNAL, 36, 1310 (1914).

[2] XII paper, THIS JOURNAL, 37, 223 (1915).

[3] *Ibid.*, p. 227.

[4] XIII paper, THIS JOURNAL, 37, 651 (1915).

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.

BY J. E. DARRAH AND C. G. MACARTHUR.

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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.