(4) It will be noted that the bluish-green color developed in each test is transient. Changes occurring after the lapse of several minutes are to be disregarded.

(5) As a further corroboration of the presence of palm oil, the refractive index of the fatty acids extracted by the alkali should be determined. The refractive index of the fatty acids extracted in the above manner from an oleomargarine made up of oleo oil, neutral, cotton-seed oil, and a small amount of palm oil, will not exceed 1.4615 at 25° C. On the other hand, if corn, mustard, or, in fact, almost any of the other vegetable oil be used in the manufacture of the oleomargarine, the refractive index of the fatty acids extracted will be much higher, depending on the quantity of the oils used.

Bleached palm oil does not give the color reactions described. They are, therefore, dependent, either upon the coloring-matter of the palm oil itself, or upon some constituent which is destroyed by the process of bleaching.

ON A GLOBULIN OCCURRING IN THE CHESTNUT.

BY WILLIAM EDWARD BARLOW.

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THIS paper presents the first results of an investigation which the writer hopes shortly to continue. It is offered as a slight addition to the sum of our knowledge of the vegetable proteins —a knowledge for which we are indebted chiefly to the well-known researches of Osborne, Harris, Campbell and others.

The globulin studied by the writer was obtained as follows: Spanish chestnuts, of the edible variety (*Castanea vesca*), were peeled, bruised in a mortar, and treated with ether in an extraction-apparatus until most of the oil was removed. The resulting mass was then finely powdered and again extracted with ether until a fat-free meal was obtained. This meal was treated with a 10 per cent. solution of sodium chloride for several hours at 50° C. The resulting solution, strained and filtered until almost clear, gave a flocculent precipitate on saturation with ammonium sulphate. This precipitate was filtered out, drained free from the liquor, washed, and dissolved in 10 per cent. sodium chloride solution. The solution was filtered repeatedly until perfectly clear and bright and then dialyzed for four days against running

hydrant-water, and, finally, for several hours against frequent changes of distilled water. The precipitate which formed inside the dialyzer was removed, washed, and dried in a vacuum over sulphuric acid.

The substance so isolated was a grayish powder. Under the microscope it was seen to be apparently quite homogeneous, consisting entirely of small spheroids, all of approximately the same size and shape. The first portion to separate inside the dialyzer consisted of minute plates bound by three curved edges, resembling a form which the writer has seen in impure excelsin, but showing no inner nucleus.

The proteid was entirely insoluble in distilled water, even on warming to 45° C. In a 10 per cent. solution of sodium chloride it dissolved almost completely. Separate portions of this cloudy solution, filtered clear, gave the following reactions:

(1) Saturation with sodium chloride produced only a slight precipitate.

(2) Saturation with magnesium sulphate gave a heavier precipitate, but, on testing the filtrate from this, much of the proteid was found to be still in solution.

(3) Saturation with ammonium sulphate precipitated the substance completely.

(4) An approximately 5 per cent. solution of the proteid in 10 per cent. sodium chloride solution gave, on dilution with an equal volume of water, a comparatively slight precipitate which became more considerable on addition of more water.

(5) Mercuric chloride produced a slight precipitate; and both tannic acid and picric acid gave a heavy precipitate.

(6) The substance responded to all the general proteid reactions, such as the Biuret test, the Xanthoproteic reaction, Millon's reaction, etc.

(7) Extremely dilute hydrochloric acid, or acetic acid, precipitated the globulin from its saline solutions in a form not easily soluble in excess of the precipitant.

The dry proteid dissolved easily and completely in a solution of sodium carbonate of a concentration of about 0.2 per cent., and on careful neutralization with very dilute hydrochloric acid it was completely reprecipitated. On adding crystals of sodium chloride until the liquid contained 10 per cent. of this salt only a part of the precipitated proteid dissolved. The filtrate from

the cloudy solution contained some—and apparently the greater part—of the proteid in unaltered form.

The proteid dissolved completely in 0.02 per cent hydrochloric acid, and was reprecipitated from this solution by the addition of a drop of saturated ammonium sulphate solution, or by the addition of a small crystal of salt. This precipitate was almost entirely insoluble in stronger sodium chloride solution.

The solid substance mixed with a little concentrated sulphuric acid and twice the volume of glacial acetic acid gave, at once, a pale violet coloration.

Dissolved in cold concentrated hydrochloric acid, and allowed to stand for some time without warming, the proteid developed an amethyst coloration.

Attempts to determine the coagulation-temperature of saline solutions of the globulin led to the following results: Opalescence began at 74° to 75° C. Flocks were produced in small quantity by heating for some time at 96° to 97° C. On addition of a trace of hydrochloric acid at this temperature a complete precipitation of the proteid in flocks took place at once, indicating that the substance is precipitable only slowly and incompletely by heat alone. By actually boiling its solution the proteid coagulated in flakes somewhat slowly, but the addition of a trace of acid instantly completed the precipitation.

The precipitation limits with ammonium sulphate were determined by the method given by Osborne and Harris[1]. The lower limit was 3.0 cc. The bulk of the proteid was precipitated between 3.0 and 4.1. To remove the last traces required 4.2 cc.

The amount of the substance obtained in this first investigation was so small that it was impossible to determine its percentage composition or its specific rotation. The facts brought out so far show, however, that the body is a true plant globulin, and that in most of its reactions it resembles the globulin of the filbert (corylin) more closely than it does other members of the group, although it differs from corylin in coagulation-temperature and in precipitation limits. A further investigation is to be undertaken in this laboratory with the object of determining whether the chestnut globulin is or is not identical with corylin or any other of the vegetable proteids hitherto isolated. If it is not—as appears probable—the writer would propose for it, as indicative of the source, the name castanin

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[1] This Journal, **25**, 837-842 (1903).