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SIMPLE QUANTITATIVE DETERMINATION OF THE NON-PROTEIN
PORTION OF MILK, BASED ON GREENWALD'S MODIFICATION OF
THE DETERMINATION OF THE NON-PROTEIN NITROGEN IN BLOOD.

A Thesis Submitted to the Faculty of the
College of Arts and Sciences
of the
UNIVERSITY OF LOUISVILLE
Through the Chemical Department,
in Partial Fulfillment for the Degree
"Master of Science,"
by
Sarah Huntoon Vance.

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SIMPLE QUANTITATIVE DETERMINATION OF THE NON-PROTEIN
PORTION OF MILK, BASED ON GREENWALD'S MODIFICATION
OF THE DETERMINATION OF THE NON-PROTEIN NITROGEN IN
BLOOD.

As far as I have been able to determine from the scant literature at hand, no attempt has been made as yet to determine the percentage of non-protein nitrogen in milk by a procedure simple enough for use as a clinical method. This problem was suggested to me by Dr. Charles J. Robinson, with the request that I should base my method on some of the methods of determination of the non-protein nitrogen portion of other physiological fluids.

The preparation of protein for milk has been made at various times, both from whole milk and milk powders. Mitchell and Nelson describe a method of preparing a protein for milk using trichloroacetic acid as the precipitating agent for the proteins. They made use of milk powder and fifty percent solution of trichloroacetic acid. Funk has prepared the pure non-protein nitrogen compounds of milk and other substances by using the alcoholic extract of the dried whole milk. These methods give an idea of the character of the work which has been done on the non protein nitrogen portion of milk as a whole. Other investigations have been carried out on the determination of individuals constituents of the non-protein nitrogen portion.

Method.

The method employed was adopted from Greenwalds trichloroacetic acid method for the determination of the non protein nitrogen in blood. A Solution of 25% trichloroacetic acid was found more satisfactory than one of 2.5% which Greenwalds suggests for use in determining the non protein nitrogen in blood. Whole cows milk was used throughout the investigation.

50c.c. whole milk well mixed were diluted in a volumetric flask to 100 c.c. with 25% solution of trichloroacetic acid.

The flask was shaken well and allowed to stand from fifteen minutes to a half hour or until the precipitate had settled somewhat. The supernatant liquid was decanted through a dry filter. One c.c. of the filtrate was then treated in a large pena test tube (70cc) with 2 C.C. Sulphite acid; 1 gram of potassium sulphate, powdered; and three drops of a 5% solution of copper sulphate. The mixture was heated carefully over a burner until clear and then allowed to cool but not solidify. 5-10 c.c. of water were added carefully, and then the acid solution was made strongly alkaline with about 10 c.c. of a 33% sodium hydroxide solution, the ammonia liberated was aspirated into 2 c.c. $\frac{N}{10}$ sulphuric acid, diluted to 10 c.c. The excess acid was then titrated with $\frac{N}{100}$ or $\frac{N}{50}$ sodium hydroxide. The non protein nitrogen portion was calculated as number of milligrams of nitrogen per 100 c.c. milk.

Sample No. 1.	of whole milk average	42	Mg. per 100 c.c.
" " 2.	" " " "	36.4	" " " "
" " 3.	" " " "	56	" " " "

Sample No. 5. Evaporated milk average 35 " " " "

Conclusion.

The non protein nitrogen of whole milk was found to vary from 36 - 56 milligrams of nitrogen per 100 c.c. The non-protein nitrogen of one sample of evaporated milk undiluted was found to be 70 mg. of nitrogen per 100 c.c. The label on the evaporated milk can suggested diluting with an equal quantity of water to make the product approximately whole cows milk in composition. In that case, the non protein nitrogen would be 35 milligrams per 100 c.c. The significance of the non protein nitrogen containing constituents of milk has not been determined but various authors have pointed out the presence of substances in milk which come under that class. Funk estimated the vitamine fraction of milk as C H O N and found that there were .1 - .25 milligrams of vitamine nitrogen per 100 c.c. of milk. If the residue of non protein nitrogen be considered as allantoin, there would be 2-5 mg. of allantoin nitrogen per 100 c.c. of milk. Urea in fresh milk has been determined as 18 mg. of urea nitrogen per 100 c.c. The amount of creatine and creatime, and the purine bases, ademine and guanine have been found not to exceed a few milligrams of the non protein nitrogen per 100 c.c. The ammonia in fresh milk has been estimated as 0.3 - 0.4 milligrams per 100 c.c. of milk.

The sum total of these figures 21 - 25 milligrams of nitrogen per 100 c.c. does not total the number of milligrams of non protein as determined by the above method. However the lecithin nitrogen as well as the amino acid nitrogen has not been determined.

It is interesting to note that the number of milligrams of non protein nitrogen per 100 c.c. of milk determined by the above method very closely approximated the amount of non protein nitrogen per 100 c.c. of blood determined by Greenwald's method.

- (1) Mitchell and Nelson, Uni. Ill. Biol. Chem
23. 459-67 (1915)
- (2) Funk
- (3) Greenwalds J. Biol. Chem. 21, 61, 1915.
- (4) Funk, London Biol. Chem. J. 7, 211, 3.
- (5) Jour. Biol Chem. 17 -475.81.
- (6) Spittgerlar - Rilfart Zeit. Natur. Genus. 27, 59, 67.
- (7) "Our Pet" Brand Evaporated milk.
Helvatia Milk Condensing Co.,
Highland, Ill. U.S.A.