XXI. AN ATTEMPT TO ESTIMATE THE VITAMINE-FRACTION IN MILK.

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(Received February 17th, 1913.)

The isolation of the beri-beri vitamine from milk [Funk, 1912, 1], together with the important fact observed by Andrews [1912], that infantile beri-beri occurs when the children are fed by mothers suffering from beri-beri, suggest a new line of investigation into the etiology of infantile scurvy and rickets. The problems which present themselves in connection with these investigations have been already set forth [Funk, 1912, 2] and will be fully treated in an account in the *Ergebnisse der Physiologie*, Vol. 13. These problems are briefly the following. (1) What is the normal amount of vitamins in milk of different species, including the human? (2) Is there a definite relationship between the amount of vitamins secreted in the milk and that ingested in the food? This point is of special interest because of the periodical appearance of rickets in winter and disappearance in summer, a period when the cows return to pasture. (3) What effect have boiling and pasteurisation on the vitamine content of milk? All these points await their solution until a method for determining such small quantities is available. The present inquiry shows that the ordinary chemical methods for estimating vitamins can hardly suffice and attention at present must therefore be directed to colorimetric methods.

Nevertheless the results described below give an idea of the amount of vitamins possibly present in milk. The whole vitamine fraction (nitrogen precipitated by phosphotungstic acid in the alcoholic extract of the dried milk) from 1 litre of milk amounts to 1-2.5 mgr. nitrogen. This would correspond to 1-3 cg. vitamine ($C_{17}H_{20}O_7N_2$). After the elimination of the vitamine-fraction the residual nitrogen amounts to 20-50 mgr. per litre milk. This residual nitrogen represents in all probability allantoin; assuming this to be correct, one litre of milk contains 0.06-0.15 gm. allantoin, a figure in good agreement with that obtained by Ackroyd [1911] by means of a direct method, namely 0.199 gm.

The results further indicate that for a chemical investigation of the vitamins of milk it will be necessary to start on a large amount of milk. At the same time they show that the artificial protein-free milk, such as was used by Osborne and Mendel [1912] in their experiments on growth, differs from true protein-free milk in lacking these nitrogenous substances. It is therefore quite conceivable, as the experiments of Hopkins and Neville [1913] point out, that these substances play an important part in the process of growth.

As the figures below show, the milk after removal of fat by centrifuging has lost about 50 % of vitamins and allantoin.

The milks used in these experiments were from London dairies. For each experiment a litre was evaporated in vacuo at 30°, using a distilling flask constructed of two parts from which therefore the residue could be quantitatively removed. The process of drying the residue was hastened in the last stages by an addition of alcohol. The residue was powdered in a mortar and dried in a vacuum desiccator to constant weight. The fine powder was then shaken with a definite amount of alcohol (400 c.c.) in a shaking machine for two hours. The extract was filtered and of the filtrate an aliquot part was evaporated in vacuo. The residue was extracted with water and the extract transferred to a measuring flask. The watery extract was filtered and the filtrate found in every case to be entirely free from proteins. A measured quantity was acidified slightly with sulphuric acid and precipitated with 10 % phosphotungstic acid. After standing for 48 hours the precipitate was filtered and washed with dilute sulphuric acid. In accordance with my previous results this phosphotungstic acid precipitate can be regarded as the vitamin-fraction. Both in the precipitate and filtrate the nitrogen was estimated by Kjeldahl, N/20 normal solutions being used for titration.

		Weight of residue	Nitrogen of the vitamin fraction in mgr. (phosphotungstic acid precipitate)	Residual nitrogen in mgr. (phosphotungstic acid filtrate)
1.	{ Centrifuged milk	100	2.54	50.9
	{ Control	99.5	2.46	49.6
2.	{ Uncentrif.	133	1.7	30.7
	{ Centrif.	98	1.53	16.8
3.	{ Uncentrif.	127	2.5	22.7
	{ Centrif.	90	1.4	12.1
4.	{ Uncentrif.	129	1.96	29.6
	{ Centrif.	92	1.2	15.3
5.	{ Uncentrif.	132	2.4	49.2
	{ Centrif.	96	1.6	27.3
6.	{ Centrif.	89	1.06	22.4
7.	{ Centrif.	105	1.83	44.9

In this connection the behaviour of allantoin when boiled with Ruhemann's reagent (triketohydrindene hydrate) should be noted. According to Abderhalden and Schmidt [1911] allantoin when boiled for one minute with Ruhemann's reagent does not give any colouration. But although this is so, if the boiling be continued for 2-3 minutes a violet colouration appears and this reaction might be used as a test for allantoin. Since the crude product obtained by oxidation of uric acid by permanganate gave the reaction at once, probably due to admixture with allanturic acid, and allantoin is known to be destroyed by boiling, it was thought that a decomposition product of allantoin, probably allanturic acid, was the cause of this reaction. A sample of pure allantoin was therefore boiled with PbO_2 and another with water. Both samples gave a strong allanturic acid reaction after a certain time of boiling, the sample with PbO_2 giving however the stronger reaction. The reaction seemed to disappear after prolonged boiling. The reaction may also be obtained from hydantoin.

It is interesting to note, that we have in allantoin an example of a substance, which like vitamine is destroyed by boiling.

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