

corresponding number of excised host eyes (un-boiled).

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<sup>1</sup> Hadorn, E., and Mitchell, H. K., *Proc. U.S. Nat. Acad. Sci.*, **37**, 650 (1951).

<sup>2</sup> Hadorn, E., *Arch. Julius Klaus Stift.*, **26**, 470 (1951).

<sup>3</sup> Buzzati-Traverso, A. A., *Nature*, **171**, 575 (1953).

<sup>4</sup> Buzzati-Traverso, A. A., *Proc. U.S. Nat. Acad. Sci.*, **39**, 378 (1953).

### Instability of Milk due to a High Content of Calcium Ions

In the nineteen thirties, great difficulty arose in the Netherlands from the frequent occurrence of flocculation of fresh milk on boiling and even during transportation or storing for a short time. This condition was called 'the Utrecht abnormality of milk', for it appeared first in the province of Utrecht. Afterwards this abnormality was found in other parts of the country as well<sup>1</sup>. The abnormality disappeared gradually; at present it is quite rare.

Milk exhibiting the Utrecht abnormality does not show any unusual value for the common numerical data (for example, pH, titration acidity, protein content, total calcium content). The bacterial count is normal. There was evidence, however, that the instability is caused by an abnormally high content of calcium ions<sup>2</sup>. But the evidence was only indirect, for at that time it was not possible to determine the calcium ion content. In recent years a reliable method for the determination of calcium ion content in milk ultrafiltrate has been developed<sup>3,4</sup>.

In recent months we have had the good luck to obtain a few samples of milk showing the Utrecht abnormality.

The method of determination consists in using murexide (the ammonium salt of purpuric acid) as a calcium indicator and measuring with a Unicam S.P.500 spectrophotometer the change of colour due to the amount of calcium ion present. For full details of the method and its theoretical background refs. 3 and 4 should be consulted.

The results for four samples are listed in Table 1.

Table 1. ANALYSIS OF FOUR SAMPLES OF MILK SHOWING THE UTRICHT ABNORMALITY

	1	2	3	4	Normal range
pH	6.7	6.6	6.6	6.4	6.4-6.6
Titration acidity*	7.8	14.5	16.0	19	14-20
Heating test	+	++	+++	++	—
Alcohol test	+	++	+++	++	—
Total Ca (mgm. per cent)	176	144	216	134	120-200
Ultrafiltrable Ca (mgm. per cent)	44	34	45	54	26-46
Ca ions (mgm. per cent)†	14.8	15.2	20	27	8.0-14.4
Citric acid (mgm. per cent)†	161	74	122	208	96-288

\* ml. of 0.1 N sodium hydroxide/100 ml. of milk.

† In the ultrafiltrate.

In complete accordance with Seekles's expectations<sup>1</sup>, we have found the content of calcium ions to be significantly higher than normal.

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<sup>1</sup> Seekles, L., *Proc. XI Congress of Pure and Applied Chemistry*, **3** (London, 1947).

<sup>2</sup> Seekles, L., and Smeets, W. Th. G. M., *Neth. Milk and Dairy J.*, **1**, 1 (1947).

<sup>3</sup> Smeets, W. Th. G. M., and Seekles, L., *Nature*, **169**, 802 (1952).

<sup>4</sup> Smeets, W. Th. G. M., Thesis, University of Utrecht (1952).

### A Toxic Effect of Fluoride

BECAUSE fluoride has been shown to depress bacterial activity, and because the bacteria of the intestinal tract synthesize several vitamins which are utilized by the host, it was considered that the ingestion of large amounts of fluoride might influence the onset of specific vitamin deficiencies in a manner comparable with the effect produced by the insoluble sulphur drugs or the antibiotics.

In one set of experiments large amounts of fluorine ingested orally were found to favour the initiation of a nutritional deficiency (probably a biotin deficiency) in rats under certain conditions. In this experiment 80 parts per million of fluoride, in the form of sodium fluoride, was added to the drinking water of the experimental group. The control group received no fluoride supplements. For three weeks the following semi-synthetic diet was fed: 75 per cent sucrose, 18 per cent vitamin-free casein, 4 per cent lard, 3 per cent salts. To each 100 gm. of ration the following vitamins were added: 1 mgm. thiamine, 1.5 mgm. riboflavin, 0.5 mgm. calcium pantothenate, 0.5 mgm. pyridoxine, 10 mgm. inositol, 10 mgm. *p*-aminobenzoic acid, 10 mgm. niacin, 10 mgm. 2-methyl naphthoquinone, 40 international units vitamin A, 200 mgm. choline chloride. No change in the weights or the appearance of the animals was observed.

The diet was then changed to include 16 per cent raw egg-white, 10 per cent casein, 67 per cent sucrose, 4 per cent lard and 3 per cent salts. The vitamin supplements were the same as listed above. The egg-white was added to aid the onset of a biotin deficiency. At the beginning of the period during which this diet was used, the average weights of the three rats of the control and experimental groups were 204 and 178 gm. respectively. After sixteen days the average weights were 196 and 125 gm. One of the rats of the experimental group died at this stage.

The fur of the experimental animals was bristled and rough, and there was alopecia of the snout, head and legs, mild diffuse dermatitis, and mild 'spectacle eye'. The control animals showed none of these symptoms.

This experiment was repeated and a similar result was obtained.

The diet used had a low vitamin A and pantothenic acid content. Increasing the vitamin A content to 200 international units slightly delayed the onset of the symptoms. Increasing the calcium pantothenate content to 2 mgm. per 100 gm. of ration did not affect the result.

The toxicity of the fluoride alone was not the cause of these symptoms, because other experiments