day. After the infection was well established, following intrabronchial insufflation of relatively large doses (0.1 cc of 10^{-4} and 10^{-5} of 18-hour bouillon cultures) of pneumococci, serum was more efficacious than sulfapyridine in preserving life, but a combination of the 2 did not show a significant reduction in mortality even with the smaller infecting dose.

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Factors Affecting the Vitamin B_1 Content of Evaporated Milk.

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The work of several investigators who have studied the vitamin B_1 content of evaporated milk has been recently reviewed by Daniels.[1] She reported two trends in results: certain workers had found about 50% destruction of vitamin B_1 when evaporated milk was compared with raw milk, whereas others had observed only 0-20% destruction. Using a modification of the 10-day rat growth technic of Schlutz and Knott,[2] Daniels assayed raw Guernsey milk and commercial evaporated milk. Her results indicated 60% less vitamin B_1 in the evaporated milk than was present in the raw milk. Since certain studies in our laboratory differed from the results presented by Daniels, and because she had used repeated assays on the same animals but had not employed an improvement we had reported for our basic ration,[3] we have thought it advisable to investigate further the effect of the process of evaporation upon vitamin B_1.

Fresh raw milk was immediately iced and delivered by special messenger together with evaporated milk prepared from the same lot. By careful refrigeration the raw milk was kept sweet for the duration of the test. The evaporated milk was stored at room temperature.

Sixty-gram rats were fed the following ration: 15.0% vitamin-free casein, 15.0% dried autoclaved liver,* 45.5% sucrose, 17.0% Crisco, 3.0% cod liver oil and 4.5% modified Wesson's salts. After

1 Daniels, A. L., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 212.

2 Schlutz, F. W., and Knott, E. M., *J. Nutr.*, 1936, **12**, 583.

3 Schlutz, F. W., and Knott, E. M., *J. Biol. Chem.*, 1937, **119**, lviii.

* Fresh hog liver was ground and autoclaved for 5 hrs at 120°.

22-25 days when a rat had been depleted of its vitamin B_1 reserve and was losing weight slowly but steadily, it was given a daily supplement of the material to be assayed. The dose was continued for 10 days, the amount being changed from day to day, if necessary, to maintain a gain of about 2 g per day. After administration of the dose was completed a rat was continued on the unsupplemented basic ration until it was again losing weight. The total dose which had been administered was then divided by the total grams of gain to obtain a growth unit for calculating the vitamin B_1 content. Crystalline thiamin was used as a standard control of the assay technic. For the sake of comparison with Daniels' results, certain groups of rats were given autoclaved whey in the basic ration instead of the autoclaved liver, and some rats were used for second and third assays when they had again become depleted after the first assay. As a further control, the curative technic as described by Supplee and Bender[4] was used to assay one lot of evaporated milk.

Results are reported in Table I. The raw milk was found to contain from 92 to 117 International Standard Units of vitamin B_1 per quart. The evaporated milks prepared from these raw milks contained 61 to 93 units per reconstituted quart, indicating losses of 34, 24, 21 and 20% respectively. There was no difference observed in the vitamin B_1 content of the irradiated and non-irradiated milks which had been prepared from the same lot of raw milk.

Remarkably comparable results were obtained when one lot of evaporated milk (M4591) was assayed by both the growth and curative technics. In the positive controls for these 2 technics, an average of 1.45 γ thiamin gave one gram of growth, while an average of 0.56 γ was needed to maintain a "cure" of one day. With the milk assays, averages of 3.83 and 1.50 cc were required respectively for the 2 technics, therefore 2.64 cc of milk were equivalent to one gamma of thiamin when growth was the criterion while 2.68 cc of milk were equivalent to one gamma of thiamin when the curative technic was employed.

When pure thiamin was administered as a supplement to the basic ration containing autoclaved whey, 2.38 γ were needed for a gram of growth in contrast to the 1.45 γ required with the liver ration. This poorer growth on whey was apparently due to a deficiency of the ration for some factor of the vitamin B-complex other than thiamin, since it was impossible to maintain growth with thiamin supplements to whey during second assay periods

[4] Supplee, G. C., and Bender, R. C., *Ind. and Eng. Chem.*, 1938, **10**, 636.

TABLE I.

Raw Milk				Evaporated Milk						
Date	No. of animals	cc milk per γ B_1	I.S.U. per qt	No.	Months since evaporation	No. of animals	cc milk per γ B_1	I.S.U.	% destroyed	Remarks
9-'38	9	3.01	105	M4591	0	8	1.99	80	24	Assayed with regular liver ration.
				M4591	2	6	2.50	63	40	Assayed with whey ration—first depletion.
				M4591	2	6	2.32	68	35	Assayed with whey ration—second depletion.
				M4591	2	6	2.29	69	34	Assayed with whey ration—third depletion.
				M4591	4	8	2.64	60	43	Assayed by regular growth technic.
				M4591	4	6	2.68	59	44	Assayed by curative technic.
6-'38	10	3.45	92	N6685	0	9	2.60	61	34	
10-'37	11	2.70	117	N4101	0	11	1.72	92	21	Irradiated
				N4101	8	7	3.24	49	58	"
10-'37	11	2.70	117	409N1	0	10	1.68	93	20	Non-irradiated
				409N1	8	6	3.62	44	62	"
				M6711	22	9	3.75	42		Assayed by regular technic.
				M6711	22	4	3.80	42		Assayed with a thiamin supplement added to the milk.

with the same animals. The whey ration therefore cannot be used indiscriminately for vitamin B_1 assays.

When milk M4591 was assayed by means of the whey ration, second and third 10-day growth assays showed 68 and 69 units of vitamin B_1 per quart as compared to only 63 units which were obtained with the first assay. Evidently evaporated milk contained the factor which the whey failed to supply, and thus made possible the uniformity in results for these repeated assays. Since the time interval between first and second assays was but a few days (as compared to the 3 weeks for initial depletion) the first assay was able to restore the rat's reserve of this factor sufficiently to make the second and third assays reliable.

Examination of the data for milk M4591 shows that changes had occurred in its vitamin B_1 content. When this milk was first brought to the laboratory and assayed it contained 80 International Standard Units of vitamin B_1. After 2 months the assay showed 68 units per quart, while still later assays gave only 59 units. The amount of vitamin B_1 destroyed had therefore increased from 24% for the original assay to 35% and 44% for the 2 later assays. This milk had been stored at room temperature. When milks N4101 and 409N1 were assayed after 8 months only 49 and 44 units were found, showing that decreases of 58% and 62% had occurred in the vitamin B_1 content of these 2 milks. A sample of milk (M6711) which had been stored in the laboratory for 22 months was assayed and found to contain 42 units of vitamin B_1.

The increased destruction of vitamin B_1 as evaporated milks are stored would appear to explain the differences in assay results which have been reported in the literature. Daniels who found 60% destruction studied milk purchased on the open market. Samuels and Koch[5] who found 16-20% destruction studied milk obtained directly after evaporation and stored at 5°C for several months. Dutcher, Francis and Combs[6] who reported only slight or negligible destruction prepared their own evaporated milk at intervals of only 3 weeks, and preserved it by refrigeration.

Further work is now in progress to determine just what change has taken place in the thiamin content of the milk, and to determine also to what extent pH, temperature, and time affect this change.

[5] Samuels, L. T., and Koch, F. C., *J. Nutr.*, 1932, **5**, 307.

[6] Dutcher, R. A., Francis, E., and Combs, W. B., *J. Dairy Sci.*, 1926, **9**, 379.