THE CORRELATION BETWEEN THE NUMBERS OF LIVING BACTERIA AND THE MONTHS ELAPSED SINCE MANUFACTURE IN POWDERED MILK I.

STUDIES ON SPRAY-DRIED SKIM MILK POWDER

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(Received for publication, Feb. 13, 1958)

At the beginning of March, 1955, an explosive outbreak involving 1,936 cases of food poisoning resulting from the consumption of skim milk powder manufactured in Japan and supplied for school lunches, occurred among primary school children in Tokyo. The investigation to determine the cause of this food poisoning and to ascertain the degree of possible contamination in the skim milk powder on the market showed that some of the samples contained more than 50 thousand living bacteria. At that time, not only skim milk powder of home manufacture but also imported powder had been used for lunches in primary school. The former had usually been supplied immediately after manufacture but in some of the latter many months had already elapsed since processing and they also contained a lot of bacteria. Therefore, in order to ascertain any possible correlation between the numbers of living bacteria and the months elapsed since manufacture, a research project was designed making use of some of spray-dried skim milk powder of home manufacture.

A part of the results obtained were reported at the 5th Meeting of the Japanese Society of Veterinary Public Health held in Sept., 1956.

SAMPLES AND METHODS OF EXAMINATION

Twenty-six bags of skim milk powder were used for this research. The bags were of poly-ethylene, 375 g net weight. They had been processed between Aug., 1954 and Mar., 1955, so the periods elapsed since manufacture varied from 1 month to 7 months at the first inspection. Concerning their initial bacterial counts, the smallest one was 400 and the largest 81,000 (Table 1).

These samples were kept in room temperature (5~25°C) for 18 months, and, during this period they were respectively sampled for calculation of their bacterial counts every 3 months, 7 times in all.

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TABLE 1. *Changes in Numbers of Living Bacteria in 26 Samples of Spray-Dried Skim Milk Powder Preserved in Room Temperature*

SAMPLE NO.	MONTHS ELAPSED SINCE MANUFACTURE AT THE FIRST INSPECTION	NUMBERS OF BACTERIA COUNTED EVERY 3 MONTHS						
		1	2	3	4	5	6	7
500	1	750	370	360	430	460	430	490
501	"	850	900	480	330	550	370	470
499	"	900	540	540	470	380	480	520
68	"	1,300	1,500	1,100	800	470	520	420
502	"	8,000	8,700	8,000	5,100	5,300	5,300	4,300
509	2	42,000	19,000	13,000	14,000	16,000	7,200	4,200
507	"	53,000	58,000	19,000	20,000	15,000	12,000	5,300
494	3	5,000	4,900	2,200	1,300	1,300	900	830
510	"	16,000	10,000	8,700	7,200	6,800	3,000	1,500
508	"	61,000	16,000	14,000	8,100	6,300	6,100	2,300
492	4	850	620	680	470	460	360	280
518	"	4,800	4,900	5,000	3,600	2,900	3,500	3,100
504	"	4,900	4,900	4,100	2,100	1,300	1,100	980
515	"	14,000	6,600	3,600	3,000	4,600	4,400	2,000
498	5	400	180	120	200	190	200	330
487	"	450	290	410	170	170	350	590
484	"	1,000	580	330	400	310	280	260
513	"	8,100	3,000	2,100	2,000	1,800	1,200	730
512	"	81,000	30,000	17,000	19,000	13,000	9,500	6,900
390	6	2,000	1,400	1,300	2,000	930	620	480
511	"	7,800	6,000	4,200	3,600	4,500	3,200	2,900
516	"	14,000	12,000	3,100	5,600	5,300	4,100	2,500
398	7	1,300	2,300	1,800	630	490	530	540
517	"	2,800	900	850	670	820	590	460
418	"	4,400	5,000	3,700	1,400	1,000	1,800	1,100
453	"	15,000	8,800	7,700	9,200	9,300	5,600	4,600

Bacterial counts were estimated following the standard agar plate method described in the "Manual of Food Hygienic Inspection" compiled by the Ministry of Health.

BACTERIAL COUNTS

As shown in table 1, in all samples generally the bacterial counts tended to decrease with the lapse of months of preservation, although the counts increased slightly in some cases. When the results are observed in detail more, it is found that the samples containing a higher bacterial count than 10,000 per g at the first inspection decreased in count rapidly,

while the ones with less than 10,000 bacteria decreased in number of bacteria slowly; when their bacterial counts decreased under 1,000, the count seemed to continue almost constant or invariable. On the other hand, when the sorts of bacteria in these samples were inspected, cocci were generally found in the samples which showed decreasing bacterial counts, but spore-forming bacilli exhibited the most part of the bacteria in samples maintaining almost invariable counts.

It was proved experimentally that the living bacterial counts in skim milk powder tend to decrease in parallel with the passage of months after manufacture, and then this fact was analysed statistically.

TABLE 2. *Classification of Samples Preserved*

MONTHS ELAPSED SINCE MANUFACTURE	CLASSIFICATIONS							TOTAL	BACTERIAL COUNTS	
	1	2	3	4	5	6	7		Maximum	Minimum
1	5	·	·	·	·	·	·	5	8,000	750
2	·	2	·	·	·	·	·	2	53,000	42,000
3	·	·	3	·	·	·	·	3	61,000	5,000
4	5	·		4	·	·	·	9	14,000	370
5	·	2	·	·	5	·	·	7	81,000	400
6	·	·	3	·	·	3	·	6	16,000	2,000
7	5	·	·	4	·	·	4	13	15,000	360
8	·	2	·	·	5	·	·	7	30,000	180
9	·	·	3	·	·	3	·	6	14,000	1,400
10	5	·	·	4	·	·	4	13	8,800	330
11	·	2	·	·	5	·	·	7	20,000	120
12	·	·	3	·	·	3	·	6	8,100	1,300
13	5	·	·	4	·	·	4	13	7,700	380
14	·	2	·	·	5	·	·	7	19,000	170
15	·	·	3	·	·	3	·	6	6,800	1,300
16	5	·	·	4	·	·	4	13	9,200	370
17	·	2	·	·	5	·	·	7	13,000	170
18	·	·	3	·	·	3	·	6	6,100	900
19	5	·	·	4	·	·	4	13	9,300	360
20	·	2	·	·	5	·	·	7	9,500	200
21	·	·	3	·	·	3	·	6	4,100	620
22	·	·	·	4	·	·	4	8	5,600	280
23	·	·	·	·	5	·	·	5	6,900	260
24	·	·	·	·	·	3	·	3	2,900	480
25	·	·	·	·	·	·	4	4	4,600	460

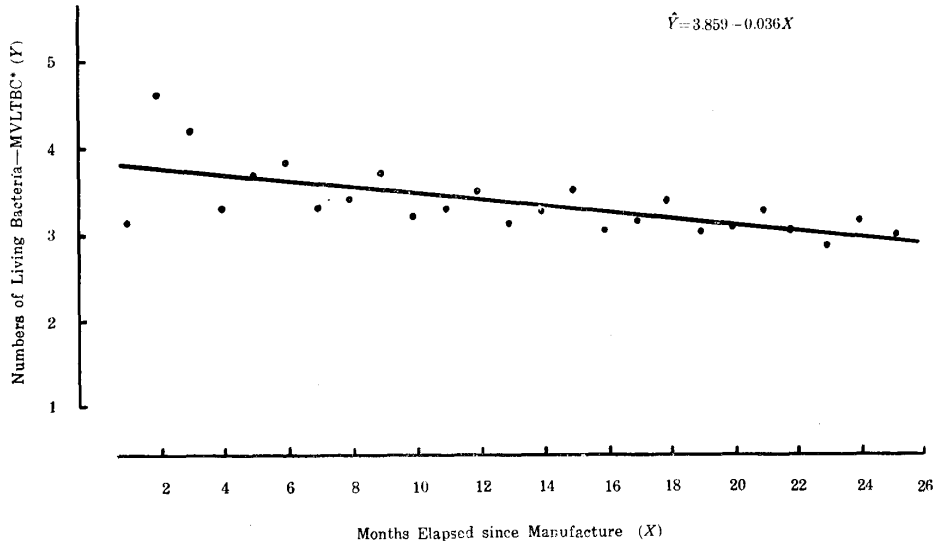
Statistical Analysis

1. Correlation

The bacterial count of each sample at the first inspection was distributed in the range between maximum 81,000 per g and minimum 400. Also the samples differed in age since manufacture and the size of sample was not so large. Therefore, without testing the region of rejection of correlation for each sample but taking each sample as a random sample derived from a possible population, the writers classified the samples into 25 groups according to months elapsed since manufacture as shown in table 2.

The mean values in logarithmic transformation of bacterial counts (this term is afterwards abbreviated as MVLTBC) were obtained for each group of samples. When the relation between the months after manufacture and the MVLTBC was graphed, it was found that there may be a correlation between the two (Figure 1).

FIGURE 1. *Correlation between the Numbers of Living Bacteria and the Months Elapsed since Manufacture in Spray-Dried Skim Milk Powder*



* MVLTBC: Mean Values in Logarithmic Transformation of Bacterial Counts

Then, taking X as the months elapsed since manufacture and Y as the MVLTBC, the correlation coefficient (r) between the two was calculated, and $r=-0.657$ was obtained. The negative correlation between X and Y was found significant at the 1% level of significance.

2. Regression Equation

It follows from the above results that a regression equation: $\hat{Y}=3.859-0.036\,X$ is found by the method of least squares as shown in figure 1, where $\hat{Y}$ is the regression of Y: the MVLTBC of each group on X: the months elapsed since manufacture. The second

power of this equation is not significant at the 5% level of significance.

In other words, there is a linear regression between the MVLTBC and the months elapsed since manufacture of samples, as shown in figure 1.

3. Statistical Test of the Stability of the Regression Coefficient

The samples were classified into 7 groups from 1 month to 7 months old, respectively according to the months elapsed since manufacture at the first inspection as shown in table 1. A regression equation for each group was obtained as follows:

Months Elapsed since Manufacture at the First Inspection	Regression Equation	Sample Size
1	$\hat{Y} = 3.155 - 0.049\,X$	5
2	$\hat{Y} = 4.793 - 0.146\,X$	2
3	$\hat{Y} = 4.334 - 0.163\,X$	3
4	$\hat{Y} = 3.675 - 0.038\,X$	4
5	$\hat{Y} = 3.304 - 0.075\,X$	5
6	$\hat{Y} = 3.829 - 0.088\,X$	3
7	$\hat{Y} = 3.667 - 0.096\,X$	4

These regression equations can not be stable for their regression coefficients at the 1% level of significance, and the regression coefficients have no relation with the months elapsed since manufacture. But it is necessary to consider that these facts seem to have resulted from the varieties of bacterial species, bacterial counts and so on in samples.

Summary

In order to know how the numbers of living bacteria in spray-dried skim milk powder change as the months after manufacture go on, the bacterial counts were calculated in 26 samples of home manufacture (Table 1), which differed in months elapsed since manufacture at the first inspection and were kept in room temperature for 18 months being respectively sampled at random for calculation of the numbers of bacteria every 3 months, 7 times in all, and the changes in bacterial counts were analysed statistically (Table 2 and Figure 1).

The results show that there is a negative correlation between the mean values in logarithmic transformation of bacterial counts and the months elapsed since manufacture of the groups of samples, and the regression equation between the two mentioned above is $\hat{Y} = 3.859 - 0.036\,X$ (Figure 1).

It can be seen that the regression coefficient in each equation is not stable and that there is no relation between the regression coefficients and the months elapsed since manufacture.

As all these results are derived from the field materials, it will be necessary to undertake a desk-work designed statistically to supply the deficiency in this field-work.

NOTE

Spray-dried skim milk powder of home manufacture has recently been so improved in quality that it is now scarcely possible to obtain such as contain more than 10,000 living bacteria per g.

The writers' thanks are offered to Dr. K. TAKAHASHI, Department of Physical Therapy and Internal Medicine, Faculty of Medicine, Tokyo University, for his kind advice on statistical estimation. Indebtedness is also acknowledged to Dr. T. TASAKA and Dr. H. GOTO, members of our Department, for their assistance with this work.