THESIS

A BACTERIOLOGICAL STUDY OF THE HOMOGENIZING PROCESS IN MAKING ICE CREAM

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Miller - Burroughs

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By

Frederick W. Fabian.

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INTRODUCTION.

In a previous paper (1) a bacteriological study was made of the influence of various manufacturing operations upon the bacterial content of ice cream. One of the operations studied was homogenizing. In this operation, which takes but a short time to complete, an increase in bacterial count was noted in the majority of the samples studied. The increase noted in the mix was presumed to be due to two causes; first, bacterial contamination of the mix from the homogenizer, and second a breaking up of the clumps of bacteria as the mix passed through the homogenizer. It was to determine whether there is an actual breaking up of the bacterial clumps as the ice cream mix passes through the homogenizer that the present work was undertaken.

PREVIOUS WORK.

Hammer and Sanders (2) made a bacteriological study of the influence of homogenizing the ice cream mix upon the bacterial count both with and without pressure. When the mix was run through the homogenizer without pressure, they found an increase in the numbers of bacteria in all cases. However, all the samples analyzed were from the first material passing through. This

material would serve to partially free the machine from bacterial contamination so that subsequent material passing through should not be contaminated from this source to nearly as great an extent. When the mix was passed through the homogenizer under pressure, an increase in the number of bacteria was shown in all cases except one. The increase in the number of bacteria when the mix was homogenized under pressure was not as great in most cases as without pressure. They state that, "At least two factors are operating to change the bacterial content when the pressure is thrown on; first, the machine has been in operation longer and the contamination from it is becoming less; second, the agitation in the machine tends to break up any clumps that may be present and thus apparently increase the count."

Peterson and Tracy (3) in a study to determine the relative importance of each step in the manufacture of the mix say that, "The increase after homogenizing and freezing is probably due, for the most part, to the breaking up of the bacterial clusters, which results in a higher count by the plate method."

PRESENT WORK.

The present work was designed to determine whether there was a breaking up of the bacterial clumps during the process of homogenizing. The materials going into the ice cream mix were placed in a starter can and heated to 145°F for thirty minutes. The mix was thoroughly stirred by mechanical paddles during the entire pasteurizing process which usually took from forty-five minutes to an hour to complete. After the mix had been pasteurized

(To be inserted before 'Present Work' on page 3)

* Hoy and Olson (10) in their study of the homogenizing process state that, "In general, however, the increases were larger than the decreases, and the averages of all the counts showed an increase of 33 percent after homogenizing. Such an increase in bacterial count is, no doubt, more apparent than real, being due chiefly to the breaking up of clusters of organisms each individual of which may give rise to a separate colony on an agar plate." From their study of this process they conclude that, "Homogenization of the mix usually causes an increase in the bacterial count as determined by the agar-plate method. Such an increase is probably due to the breaking up of clumps of bacteria."

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* This paper appeared after thesis had been written.

it was then passed through the homogenizer at 2000 pounds pressure. Samples were taken as the mix passed into the homogenizer and as it came out on the other side. The samples were always collected after about half the mix had passed through the homogenizer or toward the end but never at the beginning since it was desirable to reduce the bacterial contamination from the homogenizer to a minimum.

METHOD.

The samples were analyzed by two methods; the plate method and the direct microscopic method. The numbers of bacteria by the plate method were determined as follows: 1 gram of the mix was weighed into an Erlenmeyer flask and 99 cc. of sterile physiological saline solution added to it. Suitable dilutions were made from this and plated on milk-powder agar (4). The dilutions used were 1:100; 1:1000 and 1:10,000. The plates were incubated at 37°C and counted at the end of 48 hours. All plates were made in duplicate and the counts represent an average of the two best plates unless otherwise noted. All counts are therefore per gram and not per cubic centimeter.

Breed's direct microscopic method (5), (6), (7), (8) with slight modifications was used to determine the numbers of individual and of groups of bacteria present. The method used was as follows: 0.01 gram of the mix was weighed on each end of a clean slide, and spread evenly over an area of one square centimeter. The slides were made in duplicate so that for each sample of mix four one square centimeter areas were made. After

the slides had been dried they were immersed in xylol for five minutes to remove the fat. The surplus xylol was removed and the slides dried again. They were then fixed in 95 percent alcohol for twenty minutes and dried. After this they were stained for thirty seconds in Loeffler's alkaline methylene blue and decolorized to a light blue in 95 percent alcohol. The bacteria stained a deeper blue than the rest of the material. Because of the larger amount of fat present in ice cream the fields are more pitted than in the case of milk. However, satisfactory preparations for comparison and other detailed information may be obtained. Twenty-five or more fields from each area were counted and averaged. This made a total of 100 fields for each sample. The counting was done under a 1.9 millimeter oil-immersion lens. The factor used was 500,000 and was derived as follows: Diameter of the field was .16 mm. By substituting in the following equation $\frac{x}{\pi R^2} \times 100 = y$ where x equals the area of the smear in square millimeters, R the radius of the field in millimeters whence πR^2 equals the area, and y equals the factor necessary to transform the number of bacteria found in one field of the microscope into terms of bacteria per gram. Substituting we get $\frac{1 \times 100}{3.1416 \times (.08)^2} \times \frac{100}{1} = 500{,}000$. Since a hundred or more fields from each sample were counted, it was necessary to find the average per field and multiply this average by the factor. This was done as follows:

$\frac{500{,}000}{n} \times m =$ Number of bacteria per gram, when n = the number of fields counted and m the total number of bacteria found in n fields.

An explanation is necessary of the terms "individuals" and "groups". By the term "individuals" is meant the total number

of bacteria including isolated single bacteria and the individual bacteria in groups (any organism in the process of division was counted as two individuals); thus the count under the column labeled individuals represents a total of all the bacterial cells in an average of 100 fields times the factor. The column labeled "groups" represents a somewhat different meaning than is usually ascribed to this term and should be thoroughly understood by anyone wishing to interpret the data correctly. By this term is meant those bacteria, either individuals or groups, so located in the microscopic field that in the opinion of the observer would, if alive and plated on suitable medium, form one bacterial colony. Thus a single individual if sufficiently isolated would count as a "group" or several individuals if sufficiently close to each other would constitute a "group". In the process of counting many objects were encountered about which there might be a reasonable doubt as to whether they were bacteria or not. In all such cases, they were not counted as bacteria.

RESULTS.

The results of the experiment are set forth in the tables that follow. In table I a comparison is made of the data secured from the plate count and the direct microscopic count. In the second column under plate count are listed the number of bacteria found in the ice cream mix before it was homogenized, but after it had been pasteurized at 145°F for thirty minutes. In the third column are listed the numbers of bacteria found on the plates after the ice cream mix had passed through the homogenizer under 2000 pounds pressure.

In column four are listed the counts of individual bacteria of the same mix under the same conditions as in column two above except that the number of bacteria have been determined by the modified direct microscopic method. In column five are listed the "groups" of bacteria of this same mix as determined by the modified microscopic method.

In columns six and seven are listed respectively the counts of the same mix as listed in column three above except that the "individuals" and "groups" have been determined by the direct microscopic method slightly modified. In other words the same mix under identical conditions has been analyzed by two different bacteriological methods both before and after the mechanical operation of homogenizing to determine the effect this process had on the bacterial content of the mix. A comparison of the plate count and direct microscopic count before and after homogenizing are set forth in table I which follows:

TABLE I.

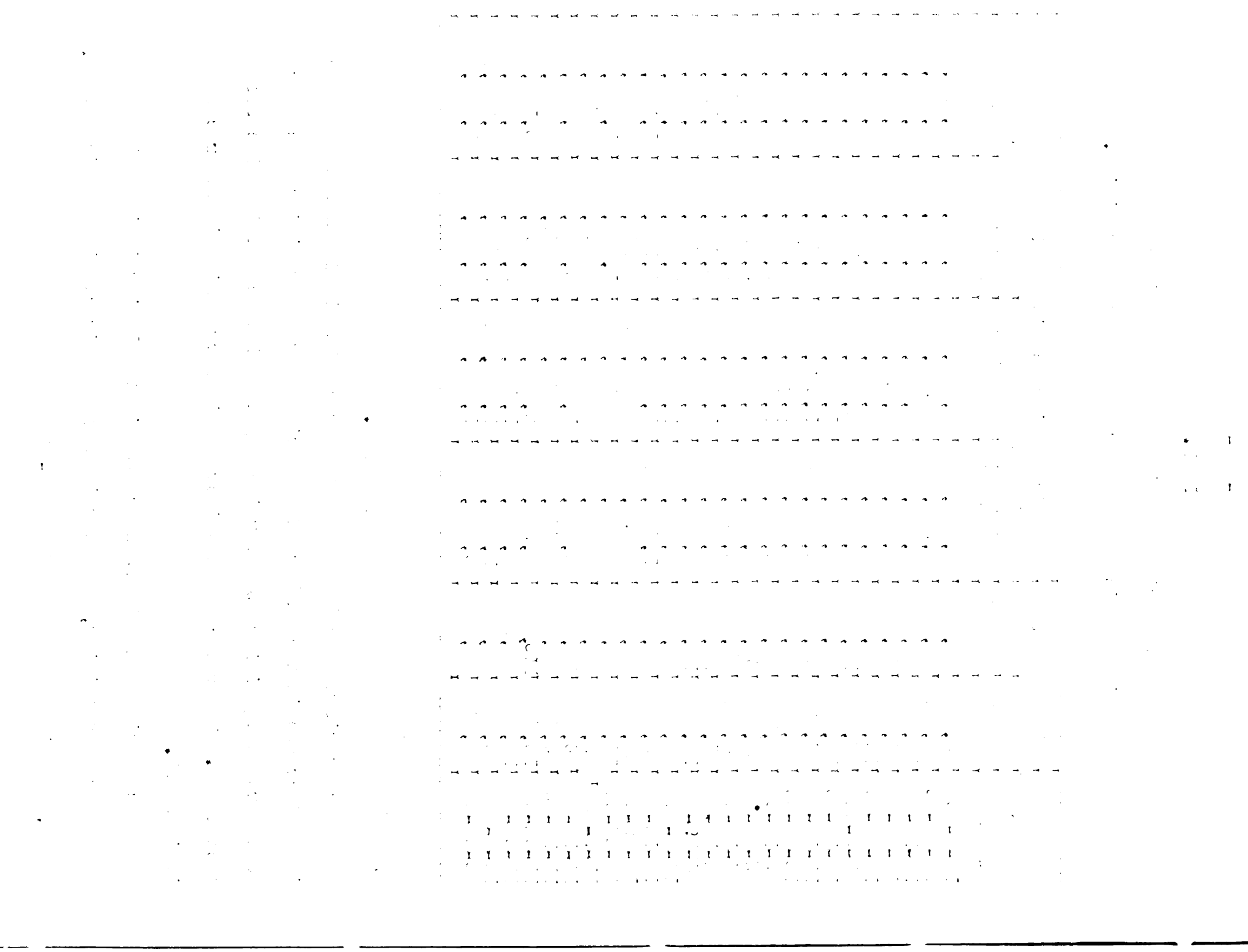
Comparison of Plate Count and Direct Microscopic Count Before and After Homogenizing.

Date	Plate Count		Direct Microscopic Count			
			Before		After	
	Before	After	Individuals	"Groups"	Individuals	"Groups"
10-3-22	5,800	10,000	1,980,000	1,160,000	3,330,000	3,110,000
10-10-22	17,000	18,000	1,950,000	945,000	2,200,000	2,000,000
10-17-22	10,000	25,000	1,450,000	1,100,000	7,335,000	7,120,000
10-23-22	46,500	71,000	2,400,000	1,890,000	3,000,000	2,900,000
10-30-22	12,500	31,000	2,865,000	2,200,000	3,825,000	3,450,000
11-3-22	7,600	16,000	3,400,000	2,500,000	4,100,000	3,800,000
11-10-22	18,000	30,000	2,780,000	1,650,000	4,350,000	4,000,000
11-15-22	6,500	23,000	2,800,000	1,650,000	2,700,000	2,320,000
12-20-22	19,000	88,000	2,500,000	1,560,000	4,315,000	4,000,000
3-16-23	8,100	4,600	2,400,000	1,550,000	3,725,000	3,230,000
3-21-23	23,000	67,000	3,340,000	1,880,000	3,885,000	3,600,000
4-10-23	4,500	8,000	2,330,000	1,700,000	3,000,000	2,565,000
4-18-23	5,500	15,000	2,530,000	1,585,000	2,680,000	2,195,000
4-19-23	12,000	18,000	3,000,000	2,600,000	2,720,000	2,340,000
10-8-23	16,000	38,000	1,700,000	1,560,000	1,850,000	1,700,000
10-12-23	3,000	3,800	1,600,000	1,300,000	1,900,000	1,700,000
10-19-23	24,000	83,000	490,000	455,000	465,000	435,000
10-25-23	14,000	60,000	850,000	750,000	1,600,000	1,500,000
11-9-23	69,000	75,000	545,000	450,000	720,000	600,000
11-13-23	6,500	19,000	2,000,000	1,500,000	2,200,000	2,000,000
11-15-23	3,600	6,500	665,000	570,000	570,000	555,000
11-22-23	12,500	170,000	3,965,000	2,000,000	2,420,000	2,160,000
11-28-23	11,000	45,000	2,100,000	1,600,000	3,500,000	3,200,000
12-3-23	2,500	300,000	1,850,000	1,450,000	3,500,000	3,200,000
2-20-24	4,400	6,000	2,365,000	1,440,000	2,700,000	1,750,000

DISCUSSION.

A comparison of columns two and three of table I shows that there is an increase in the number of bacteria after homogenizing as compared to before homogenizing as determined by the plate count in all cases except one. In some cases the increase is large while in other cases it is small.

A comparison of columns four and six tabulating individuals before and after homogenizing, as determined by the direct microscopic method, shows, that in twenty out of twenty-five or 80 percent of the samples



there is an increase; while in five out of twenty-five or 20 percent of the samples, there is a decrease in the number of individuals after as compared with before homogenizing. The same relationship as just stated for individuals also holds true for "groups" tabulated in columns five and seven. It will thus be seen that, in the majority of cases, there is an increase in the bacterial count, as determined by both the plate and direct microscopic method, after the mix has passed through the homogenizer.

RELATIONSHIP BETWEEN INDIVIDUALS, "GROUPS" AND GROUP SIZE.

The individuals, "groups", and group size are recorded in table II. What is meant by individuals and "groups" has been defined earlier in this paper. However, it was thought advisable to make further tabulations concerning the number of groups and group size, since, we are concerned primarily in trying to determine the cause of the increase of the number of bacteria as determined by the plate count and as reported by previous investigators.

For this reason a record was made of the number of groups and the size of each group as the count was made. In column seven are tabulated all the groups of two and in column eight all the groups of three or more are listed in column six while the average size of groups of two or more are listed in column nine. The complete record of this detailed information follows in table II.

TABLE II.

Tabulation of Individuals, "Groups" and Group Size.

Date		Number of Individual Bacteria	Number of "Groups"	Average size of "Groups"	Total Groups of two or more	Number of Groups of two	Number of Groups of three or more	Average size of Groups of two or more
	B = Before Homogenizing A = After Homogenizing							
10-3-22	B	1,980,000	1,160,000	1.70	39	19	20	3.8
	A	3,330,000	3,110,000	1.07	28	17	11	3.0
10-10-22	B	1,950,000	945,000	2.06	55	26	29	4.7
	A	2,200,000	2,000,000	1.10	39	34	5	2.3
10-17-22	B	1,450,000	1,100,000	1.31	30	11	19	3.0
	A	7,335,000	7,120,000	1.03	14	4	10	2.9
10-23-22	B	2,400,000	1,890,000	1.27	44	16	28	5.0
	A	3,000,000	2,900,000	1.04	5	3	2	2.4
10-30-22	B	2,865,000	2,200,000	1.32	78	31	47	3.0
	A	3,825,000	3,450,000	1.17	40	27	13	2.5
11-3-22	B	3,400,000	2,500,000	1.36	65	19	46	3.8
	A	4,100,000	3,800,000	1.06	41	21	20	2.4
11-10-22	B	2,780,000	1,650,000	1.70	106	34	72	3.5
	A	4,350,000	4,000,000	1.08	35	16	19	2.8
11-15-22	B	2,800,000	1,650,000	1.70	104	33	71	4.0
	A	2,700,000	2,320,000	1.16	23	10	13	3.0
12-20-22	B	2,520,000	1,560,000	1.42	92	45	47	3.1
	A	4,315,000	4,000,000	1.07	38	20	18	2.6
3-16-23	B	2,400,000	1,550,000	1.54	95	32	62	3.1
	A	3,725,000	3,230,000	1.15	58	30	28	2.3
3-21-23	B	3,340,000	1,880,000	1.77	78	40	38	3.3
	A	3,885,000	3,600,000	1.08	42	29	13	2.4
4-10-23	B	2,330,000	1,700,000	1.43	76	44	32	3.0
	A	3,000,000	2,565,000	1.17	60	39	21	2.4
4-18-23	B	2,530,000	1,585,000	1.69	89	40	49	3.2
	A	2,680,000	2,195,000	1.22	36	27	9	3.1

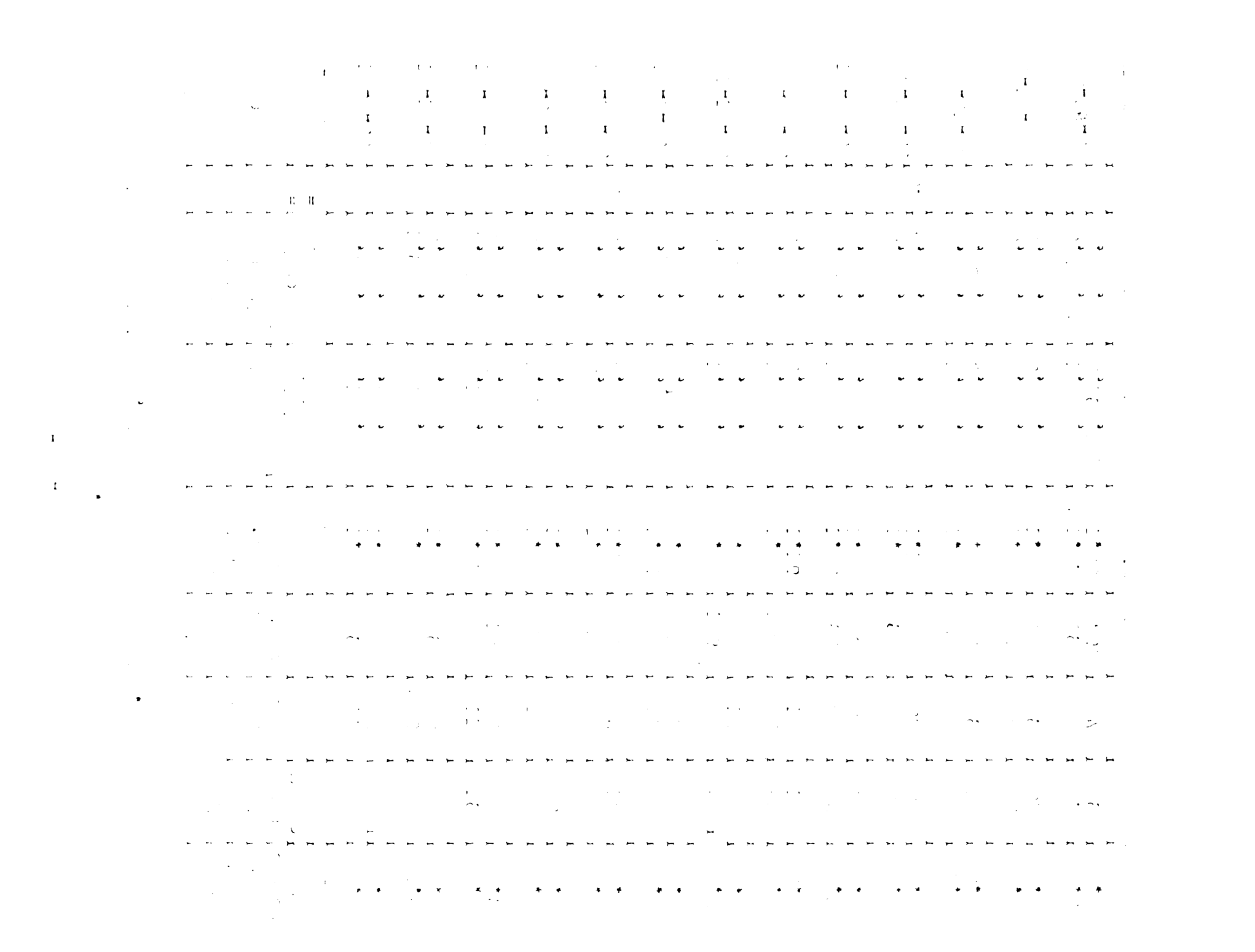
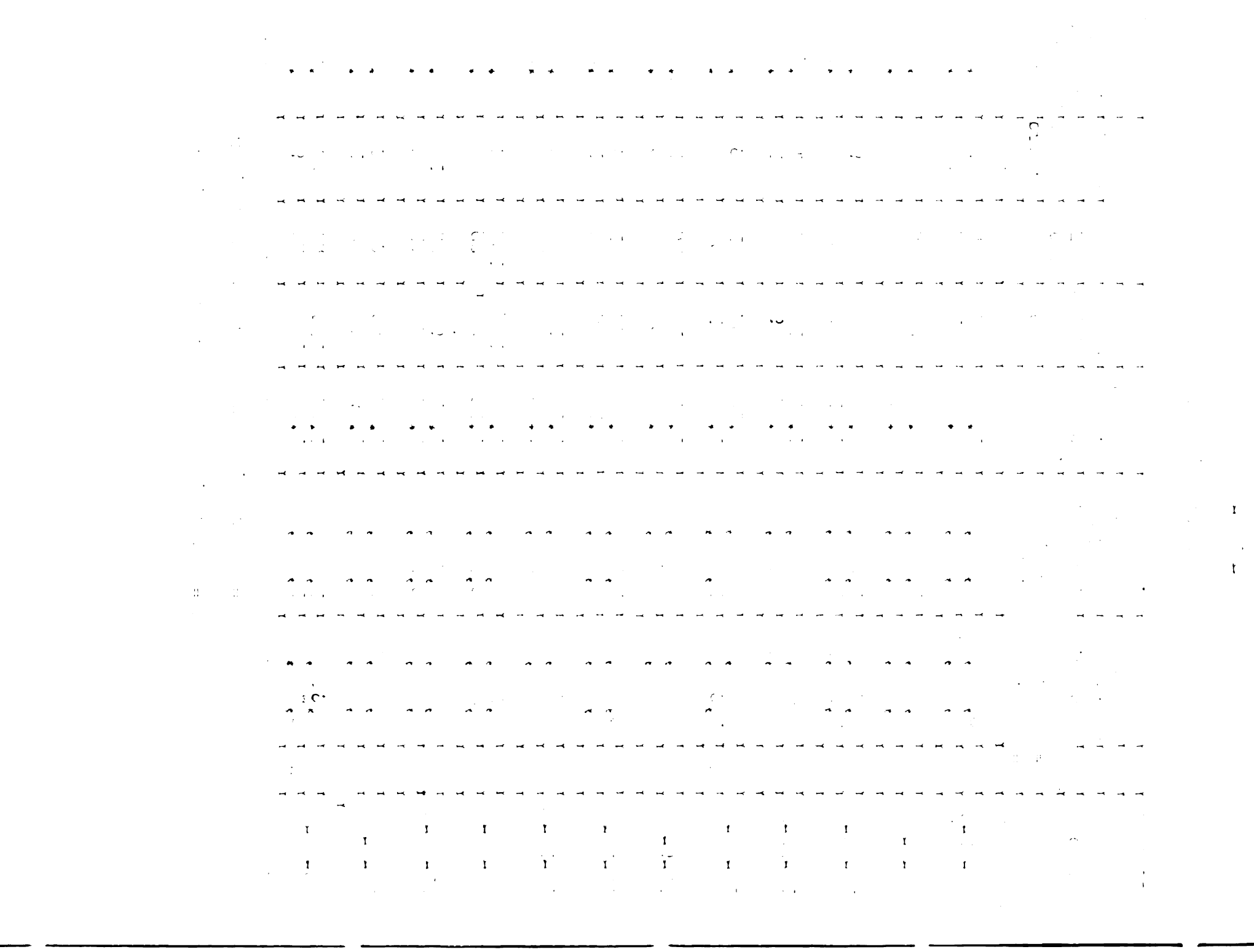


TABLE II (continued)

Date		Number of Individual Bacteria	Number of "Groups"	Average size of "Groups"	Total "Groups" of two or more	Number of Groups of two	Number of Groups of three or more	Average size of Groups of two or more
	B = Before Homogenizing A = After Homogenizing							
4-19-23	B	5,000,000	2,600,000	1.15	46	27	19	3.0
	A	2,720,000	2,340,000	1.11	51	37	14	2.5
10-8-23	B	1,700,000	1,560,000	1.09	30	27	3	2.2
	A	1,850,000	1,700,000	1.08	23	20	3	2.1
10-12-23	B	1,600,000	1,300,000	1.23	44	35	9	2.2
	A	1,900,000	1,700,000	1.11	29	24	5	2.1
10-19-23	B	490,000	455,000	1.07	17	16	1	2.2
	A	465,000	435,000	1.06	9	8	1	2.1
10-25-23	B	850,000	750,000	1.13	27	21	6	2.3
	A	1,600,000	1,500,000	1.06	21	21	0	2.0
11-9-23	B	545,000	450,000	1.21	18	17	1	2.1
	A	720,000	600,000	1.20	25	23	2	2.0
11-13-23	B	2,000,000	1,500,000	1.33	67	61	6	2.4
	A	2,200,000	2,000,000	1.10	37	36	1	2.0
11-15-23	B	665,000	570,000	1.17	15	13	2	2.5
	A	570,000	555,000	1.02	20	15	5	2.2
11-22-23	B	3,965,000	2,000,000	1.98	173	127	46	4.3
	A	2,420,000	2,160,000	1.12	33	33	0	2.0
11-28-23	B	2,100,000	1,600,000	1.31	99	87	12	2.1
	A	3,200,000	2,950,000	1.06	63	61	2	2.0
12-3-23	B	1,850,000	1,450,000	1.27	72	66	6	2.1
	A	3,500,000	3,200,000	1.09	44	43	1	2.0
2-20-24	B	2,665,000	1,440,000	1.85	112	76	36	3.1
	A	2,700,000	1,750,000	1.54	105	76	29	2.4

B = Before Mix Passed through Homogenizer

A = After Mix Passed through Homogenizer



DISCUSSION.

The size of groups most common above two are three, four and five in the order mentioned. Groups of eight are not infrequent while the size of a few groups ran as large as sixty or more. However, the larger groups occurred rather infrequently in the ice cream mix.

It will be noticed that there is a considerable difference between the average size of "groups" in column five and the average size of groups of two or more in column nine. The average size of groups in column nine being considerably larger than in column two. This is readily understood when the method of computing these averages is explained.

The method of computing the average size of the "group" in column five was by dividing the total number of individuals by the total number of "groups"; while the method used in computing the average size of the groups of two or more in column nine was by finding the total number of individuals occurring in the groups alone (not including separate individuals) and dividing this sum by the total number of actual groups. Both methods of computation show that there is a decrease in the size of groups after the mix has passed through the homogenizer.

Since all these differences are of the same sign, it is safe to conclude that homogenizing reduces the average size of the clumps of bacteria in the mix.

A STUDY OF THE INFLUENCE OF HOMOGENIZING ON THE SIZE OF THE BACTERIAL CLUMPS IN MILK.

When the work had been completed showing the influence of homogenizing on ice cream, it was thought that a similar study of a few samples of milk might prove interesting and throw some light on the problem. Accordingly five samples of milk were studied with this in view. At first it was thought advisable to secure samples of fresh unpasteurized milk having large numbers of bacteria present.

Samples of milk coming into the dairy were analyzed until one was found with a high bacterial plate count and then run but it was not found to be satisfactory for this work as will be seen later. A composit sample of milk as delivered at the dairy was found most satisfactory and was used in the other four cases.

PROCEDURE.

A ten gallon can of fresh unpasteurized milk containing milk from many different farms was taken from the vat at the College dairy. This can of milk was then dumped into the starter can leading to the homogenizer and a sample taken. It was then passed through the homogenizer without pressure. The homogenizer pressure was then raised to 1000 pounds, 1500 pounds, 2000 pounds and 2500 pounds pressure per square inch respectively and samples taken as it was leaving the homogenizer at these various pressures. These samples were then taken immediately to the laboratory and analyzed according to the direct and plate methods as previously described. Previous to homogenization, the homogenizer was rinsed with sterile water and a sample of this rinse water was

collected and analyzed by the plate method.

RESULTS.

The results of this experiment are recorded in tables III to VII. In table III are recorded the results obtained by using the sample of milk containing a large number of bacteria. Only twenty-five fields were counted because the bacteria were so numerous. In this sample there were so many large clumps and the organisms so many that the number of bacteria in the large clumps had to be estimated. For this reason it was thought best to work with milk containing fewer bacteria. This would enable a more accurate counting of the bacteria in the groups.

In tables IV, V, VI, and VII are recorded the results from the composite samples of milk. The numbers of bacteria by the plate count and the direct microscopic count are recorded the same as for the ice cream mix. The same information as to individuals, "groups", average size of "groups", etc. are also contained in the tables.

The operations are lettered and have the following meaning: Operation "A", fresh unpasteurized milk after being placed in the starter can and just before passing through the homogenizer. Operation "B" the same milk after passing through the homogenizer without pressure. Operation "C" same milk after passing through the homogenizer at 1000 pounds pressure. Operation "D" same milk after passing through the homogenizer at 1500 pounds pressure. Operation "E" same milk after passing through the homogenizer at 2000 pounds pressure. Operation "F" same milk after passing through the homogenizer at 2500 pounds pressure.

A detailed record of the results are set forth in tables III to VII that follow.

INFLUENCE OF HOMOGENIZING ON THE SIZE OF THE BACTERIAL CLUMPS IN MILK.

TABLE III. Sample No. 1, 2-28-24.

Operation	Plate Count	Number of Individual Bacteria	Number of "Groups"	Average Size of "Groups"	Remarks
A	3,500,000	** 50,000,000	2,520,000	19.84	25 fields
B	10,000,000	**166,300,000	Bacteria spread evenly over entire field		
C	14,000,000	"	"	"	"
D	26,000,000	"	"	"	"
E	23,000,000	"	"	"	"
F	*35,400,000	"	"	"	"

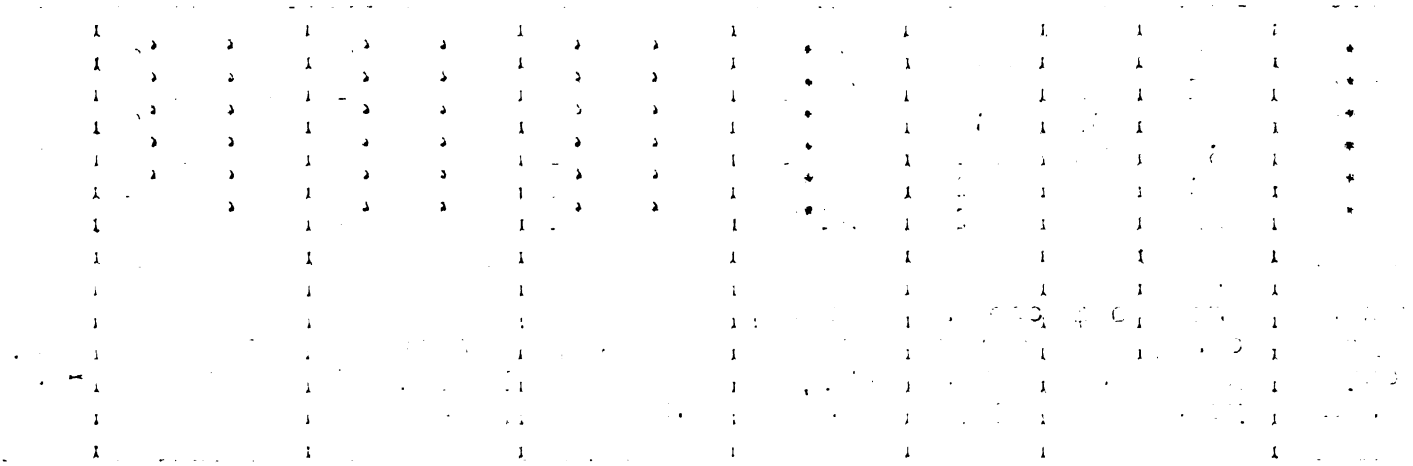
Water from Homogenizer 1500 bacteria per c.c.

** 25 fields counted. * only one plate counted.

TABLE IV. Sample No. 2, 3-13-24.

Operation	Plate Count	Number of Individual Bacteria	Number of "Groups"	Average Size of "Groups"	Total Groups of two or more	Number of Groups of two	Number of Groups of three or more	Average size of Groups
A	840,000	2,700,000	1,240,000	2.17	142	85	57	2.8
B	1,000,000	4,500,000	1,580,000	2.84	154	64	90	5.0
C	2,115,000	2,570,000	1,800,000	1.43	101	72	29	2.5
D	2,300,000	2,400,000	1,330,000	1.80	97	69	28	2.5
E	2,600,000	1,830,000	1,380,000	1.32	78	64	14	2.2
F	2,800,000	2,500,000	1,450,000	1.73	77	54	23	2.4

Water from Homogenizer 6 bacteria per c.c.



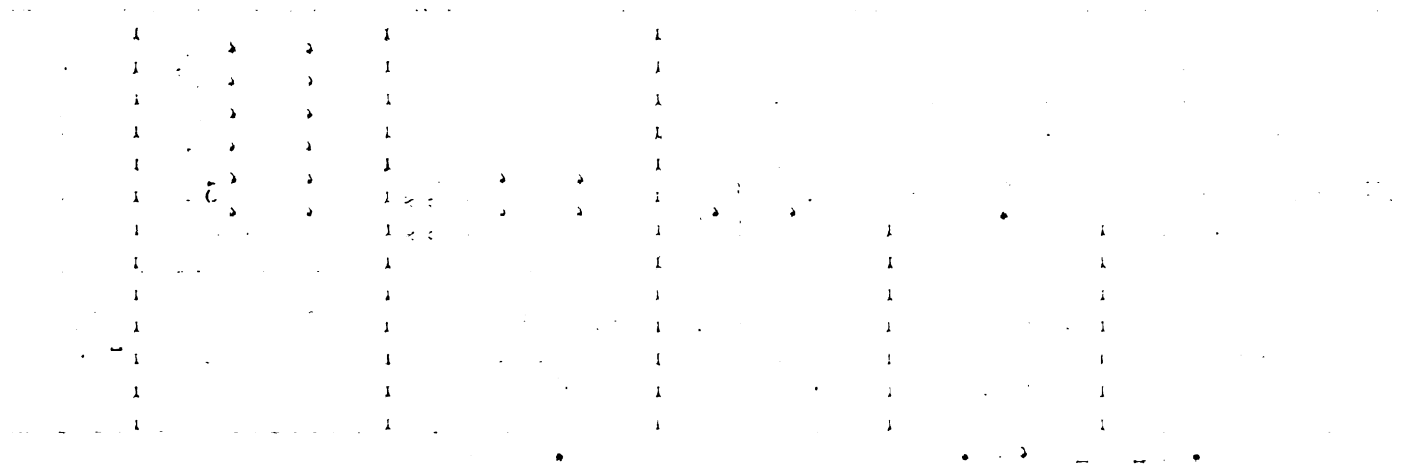


TABLE V. Sample No. 3, 3-20-24.

Operation	Plate Count	Number of Individual Bacteria	Number of "Groups"	Average size of "Groups"	Total Groups of two or more	Number of Groups of two	Number of Groups of three or more	Average size of Groups
A	25,000	*450,000	320,000	1.40	5	4	1	3.6
B	68,000	550,000	300,000	1.83	19	15	4	2.5
C	124,000	360,000	290,000	1.24	10	9	1	2.3
D	78,000	340,000	295,000	1.15	8	7	1	2.1
E	77,000	350,000	315,000	1.11	7	7	0	2.0
F	86,000	*350,000	300,000	1.16	3	3	0	2.0

Water from Homogenizer 125,000 bacteria per c.c.

* 50 fields counted.

TABLE VI. Sample No. 4, 3-26-24.

Operation	Plate Count	Number of Individual Bacteria	Number of "Groups"	Average size of "Groups"	Total Groups of two or more	Number of Groups of two	Number of Groups of three or more	Average size of Groups
A	435,000	1,870,000	675,000	2.77	38	23	15	6.8
B	2,300,000	3,500,000	1,500,000	2.33	182	119	63	3.1
C	2,200,000	4,200,000	2,400,000	1.75	194	123	71	2.8
D	2,600,000	4,500,000	2,400,000	1.87	220	135	85	2.9
E	2,800,000	3,000,000	2,320,000	1.29	145	102	43	2.5
F	3,400,000	2,300,000	1,460,000	1.57	120	67	43	2.5

Water from Homogenizer 140,000 bacteria per c.c.

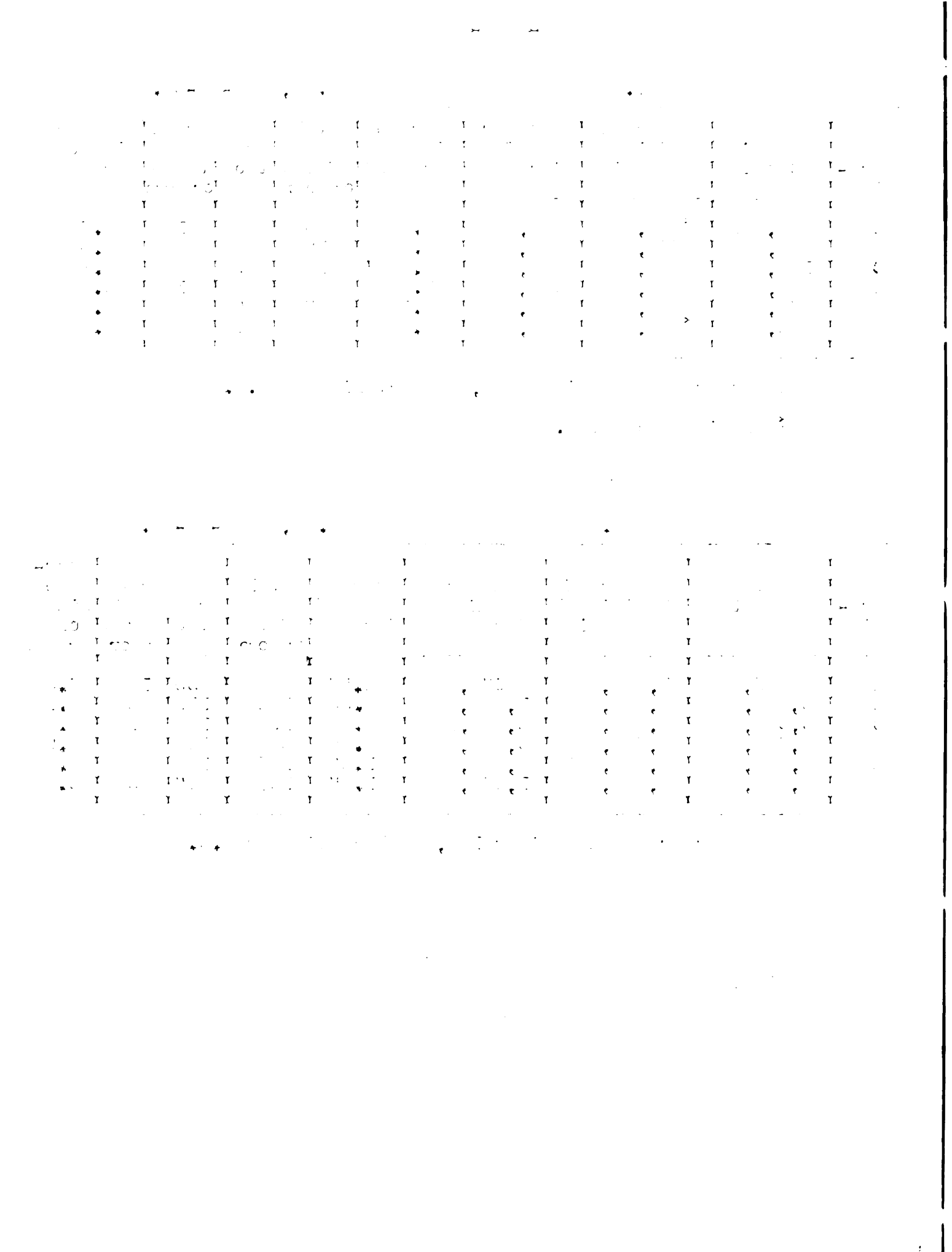


TABLE VII. Sample No. 5, 3-27-24.

Operation	Plate Count	Number of Individual Bacteria	Number of "Groups"	Average size of "Groups"	Total Groups of two or more	Number of Groups of two	Number of Groups of three or more	Average size of groups
A	250,000	1,950,000	780,000	2.5	39	21	18	7.0
B	400,000	2,400,000	800,000	3.0	89	53	36	4.9
C	72,000	1,700,000	875,000	1.94	71	40	31	3.2
D	105,000	1,400,000	830,000	1.70	69	54	15	2.5
E	285,000	1,400,000	950,000	1.47	56	37	19	2.6
F	560,000	1,900,000	1,000,000	1.70	56	34	22	2.1

Water from Homogenizer 1800 Bacteria per c.c.

Explanation of letters for all tables.

Operation A = Fresh unpasteurized milk from vat.

" B = Same milk passed through homogenizer without pressure.

" C = " " " " " at 1000 "

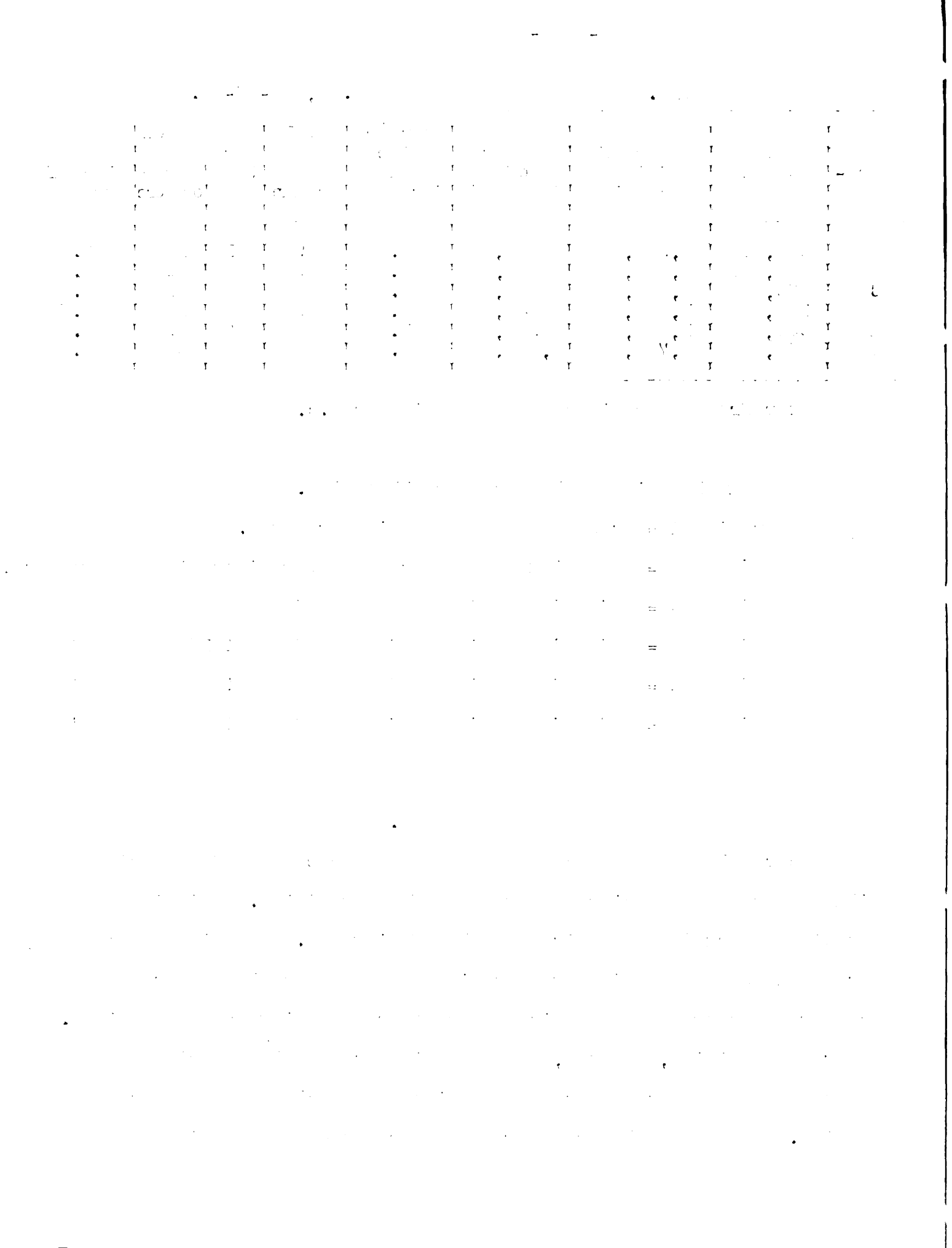
" D = " " " " " at 1500 "

" E = " " " " " at 2000 "

" F = " " " " " at 2500 "

DISCUSSION.

A careful study of the data shows that the clumps of bacteria in milk are broken up during the process of homogenizing. This is especially marked in the first sample of milk run. In this sample large clumps of bacteria together with many smaller clumps were scattered throughout the entire smear before the milk was homogenized. After homogenizing, however, the bacteria were evenly distributed throughout the entire smear and the size of the clumps greatly reduced in size. The same held true for the other samples homogenized but



to a less degree because there were fewer large groups and not nearly as many organisms present. In all cases where the milk passed through the homogenizer under pressure there is a decrease in the size of the groups.

The data also shows that the homogenizer is a source of contamination for in all cases there is an increase in both the plate and microscopic counts after the milk has passed through the homogenizer. It is true that a part of this increase might be attributed to the breaking up of clumps and no doubt was to a slight extent but the large increase should then be maintained throughout which is not the case. Another interesting thing is that the average size of the "groups" increases in most cases after the milk has passed through the homogenizer, which would indicate contamination from the homogenizer. The average size of groups decreases slightly in most cases with an increase of pressure. The decrease is gradual which would indicate that as the groups get smaller they are harder to break up.

The above data shows that homogenizing has the same influence on the bacterial groups in milk as it does on the bacteria groups in the ice cream mix.

A STUDY OF THE TYPES OF BACTERIA FOUND IN THE HOMOGENIZER AND IN THE ICE CREAM MIX BEFORE AND AFTER HOMOGENIZING.

In making a bacteriological study of the homogenizing process milk-powder agar was used. By the use of this medium interesting data as to the types of bacteria present in the homogenizer were obtained. By using this medium it was thought that some data could

also be obtained as to the types of bacteria present in the mix before and after homogenizing and also the types of bacteria present in the homogenizer.

TYPES OF BACTERIA PRESENT.

The medium was made according to directions and the bacterial colonies counted and recorded according to the system described by Ayers and Mudge (4) on p. 579 of their article, viz:

1. A total count was made.

2. The strong acid-forming colonies were then counted. The strong acid-forming colonies are defined as, "Those with a cloudy zone about them or a slight hazy edge."

3. Weak acid-forming colonies were next counted. All colonies showing acid but without a cloudy zone or a slight hazy edge about them were considered as weak acid-forming colonies.

4. The plate was then flooded with a 5 percent solution of acetic acid and all colonies showing the characteristic clearing about the colony were counted as peptonizers.

5. The difference between the sum of the strong acid-forming, the weak acid-forming, and the peptonizing colonies and the total count were classified as alkali formers and inert colonies.

By the use of milk-powder agar and classifying the colonies according to the above scheme, one is able to obtain a pretty good bacteriological picture of the types of colonies present.

RESULTS.

Thy types of colonies found in the water from the homogenizer are set forth in table VIII. The homogenizer was thoroughly rinsed

with sterile hot water by passing several gallons through it and collecting the last part of the water and plating it on the milk-powder agar. The results show a predominance of alkaline and inert colonies with weak acid colonies next in number with nearly as many peptonizers. The strong acid-forming colonies are few in number as compared to the rest.

TABLE VIII.

Type of Colonies of Bacteria Found in Water from Homogenizing.

Date	Total Count	Strong acid		Weak acid		Peptonizers		Alkaline and Inert Bacteria	
		Number	%	Number	%	Number	%	Number	%
1922									
10-3	8	-	-	-	-	6	75.00	2	25.00
10-10	2,800	700	25.00	600	21.43	600	21.43	900	32.14
10-17	2,200	150	6.82	1,000	45.45	500	22.72	550	25.00
10-23	5,000	500	10.00	2,000	40.00	1,500	30.00	1,000	20.00
10-30	3,900	-	-	360	9.23	350	8.97	3,190	81.80
11-3	8,000	500	6.25	5,000	62.50	1,900	23.75	600	7.50
11-10	450	-	-	100	22.22	200	44.44	150	33.33
11-15	15	-	-	-	-	10	66.66	5	33.33
12-20	7,500	-	-	4,500	60.00	2,000	26.66	1,000	13.33
1923									
3-16	No water saved	-	-	-	-	-	-	-	-
3-21	No water saved	-	-	-	-	-	-	-	-
4-10	8,500	-	-	-	-	500	5.88	8,000	94.12
4-18	300	18	6.00	6	2.00	80	26.66	196	65.33
4-19	2,000	10	.50	-	-	10	.50	1,980	99.00
10-8	8,500	-	-	4,000	47.05	1,000	11.76	3,500	41.17
10-12	5	-	-	4	80.00	1	20.00	0	-
10-19	5,000	500	10.00	3,500	70.00	800	16.00	200	4.00
10-25	4,000	-	-	500	12.50	2,500	62.50	1,000	25.00
11-9	43,000	3,000	6.97	18,000	41.86	10,000	23.25	12,000	27.90
11-13	Spreader	-	-	-	-	-	-	-	-
11-15	650	-	-	85	13.07	400	61.54	165	25.38
11-22	2	-	-	2	100.00	-	-	-	-
11-28	18,000	-	-	3,500	19.44	5,000	27.77	9,500	52.77
12-3	140,000	10,000	7.14	70,000	50.00	45,000	32.14	15,000	10.71
1924									
2-20	315	-	-	85	26.98	130	41.27	100	31.74
Average			3.58		32.90		29.50		34.02

In table IX are recorded the results of plating out a sample of the mix just after it had been pasteurized and before it passed through the homogenizer. The procedure followed in the College dairy is to put the materials for the mix in a starter can and pasteurize and mix it at once and the same time after which it is passed through the homogenizer. The data in table IX are collected from samples taken as the mix is ready to pass on to the process of homogenizing.

We find a predominance of alkali and inert colonies, 42.82 per-cent, with weak acid-forming colonies coming next, 34.75 percent, with peptonizers next, 17.41 percent and strong acid-forming colonies last with 5.07 percent.

TABLE IX.

Type of Colonies of Bacteria Before Homogenizing.

Date	Total Count	Strong acid		Weak acid		Peptonizers		Alkaline and inert bacteria	
		Number	%	Number	%	Number	%	Number	%
1922									
10-3	5,800	450	7.76	3,800	65.51	500	8.62	1,050	18.11
10-10	17,000	2,200	12.94	2,700	15.88	1,200	7.05	10,900	64.12
10-17	*10,000	3,000	30.00	-	-	2,000	20.00	5,000	50.00
10-23	46,500	-	-	25,000	53.76	6,500	13.98	15,000	32.25
10-30	*12,500	-	-	6,300	50.40	4,000	32.00	2,200	17.60
11-3	* 7,600	-	-	4,400	57.90	500	6.58	2,700	35.52
11-10	18,000	-	-	13,000	72.22	2,900	16.11	2,100	11.66
11-15	6,500	50	0.77	2,500	38.46	800	12.30	3,150	48.46
12-20	19,000	-	-	6,000	31.58	4,500	23.68	8,500	44.73
1923									
3-16	8,100	-	-	100	1.23	450	5.55	7,550	93.21
3-21	23,000	2,300	10.00	650	2.82	4,000	17.39	16,050	69.79
4-10	4,500	-	-	-	-	700	15.55	3,800	84.45
4-18	5,500	1,400	25.45	450	8.18	1,500	27.27	2,150	39.09
4-19	12,000	450	3.75	300	2.50	1,400	11.66	9,850	82.08
10-8	16,000	800	5.00	11,000	68.75	1,800	11.25	2,400	15.00
10-12	3,000	800	26.66	200	6.66	800	26.66	1,200	40.00
10-19	24,000	-	-	11,000	45.83	4,000	16.66	9,000	37.50
10-25	14,000	-	-	2,500	17.85	5,000	35.71	6,500	46.44
11-9	*69,000	-	-	20,000	29.00	9,000	13.00	40,000	58.00
11-13	6,500	-	-	2,500	38.46	1,500	23.07	2,500	38.46
11-15	3,600	-	-	1,300	36.11	1,100	30.55	1,200	33.33
11-22	12,500	-	-	9,300	74.40	1,600	12.80	1,600	12.80
11-28	11,000	500	4.54	7,500	68.18	2,000	18.18	1,000	9.09
12-3	2,500	-	-	1,000	40.00	300	12.00	1,200	48.00
2-20-24	4,400	-	-	1,900	43.16	700	15.90	1,800	40.90
Average			5.07		34.75		17.41		42.82

* Only one plate counted.

Table X shows the type of colonies prevailing in the mix after it has passed through the homogenizer. It will be noticed that the difference between the number of colonies in the alkali and inert group, 36.43 percent, and weak acid-forming group, 35.69 percent is not so great as in table IX. It will be further noted that the number of colonies of peptonizers has increased from 17.41 percent in table IX to 21.81 percent in table X while the number of strong acid-forming colonies shows but a slight increase.

TABLE X.

Type of Colonies of Bacteria After Homogenizing.

Date	Total Count	Strong acid		Weak acid		Peptonizers		Alkaline and Inert bacteria	
		Number	%	Number	%	Number	%	Number	%
1922									
10-3	10,000	1,900	19.00	4,900	49.00	800	8.00	2,400	24.00
10-10	18,000	3,900	21.66	9,100	50.55	550	3.05	4,450	24.72
10-17	*25,000	1,000	4.00	15,000	60.00	4,000	16.00	5,000	20.00
10-23	71,000	3,000	4.22	30,000	42.25	9,000	12.66	29,000	40.84
10-30	31,000	2,000	6.45	14,000	45.16	1,500	4.83	13,500	43.54
11-3	*16,000	600	3.75	10,000	62.50	1,600	10.00	3,800	23.75
11-10	30,000	-	-	25,000	83.33	1,500	5.00	3,500	11.66
11-15	23,000	5,000	21.74	8,000	34.77	3,000	13.04	7,000	30.43
12-20	88,000	500	.57	45,000	51.13	22,000	25.00	20,500	23.30
1923									
3-16	4,600	150	3.26	700	15.21	1,600	34.78	2,150	46.74
3-21	67,000	1,300	1.94	300	.44	1,500	2.24	63,900	95.37
4-10	8,000	550	7.00	-	-	450	5.62	7,000	87.50
4-18	15,000	3,000	20.00	1,800	12.00	4,500	30.00	5,700	38.00
4-19	18,000	2,500	13.88	900	5.00	1,100	6.12	13,500	75.00
10-8	38,000	1,400	3.68	21,000	55.26	1,900	5.00	13,700	36.05
10-12	3,800	-	-	1,800	47.37	1,500	39.47	500	13.15
10-19	83,000	5,000	6.02	28,000	33.73	22,000	26.50	28,000	33.73
10-25	*60,000	-	-	10,000	16.66	40,000	66.66	10,000	16.66
11-9	*75,000	2,000	2.66	16,000	21.33	16,000	21.33	41,000	54.66
11-13	19,000	500	2.63	4,500	23.68	7,500	39.47	6,500	34.21
11-15	6,500	-	-	2,000	30.77	3,000	46.15	1,500	23.07
11-22	170,000	2,000	1.18	40,000	23.53	45,000	26.47	83,000	48.82
11-28	45,000	4,000	8.88	29,000	64.44	500	1.11	11,500	25.55
12-3	300,000	5,000	1.66	85,000	28.33	170,000	56.66	40,000	13.33
2-20-24	6,000	-	-	2,000	33.33	2,400	40.00	1,600	26.66
Average			6.16		35.69		21.81		36.43

* Only one plate counted.

STATISTICAL ANALYSIS OF THE SIGNIFICANCE OF CHANGES DUE TO HOMOGENIZING.

In table XI is set down the total number of bacteria in the mix before and after homogenizing. Also the number of each of the different types before and after the homogenizing process. This comparative data permits one to analyze statistically the significance of the changes in the number of each type before and after homogenizing. Table XI follows:

TABLE XI.

A Comparison of the Types of Colonies

Before and After Homogenizing. (Plate count - Milk-Powder Agar)

Date	Total Count		Strong acid		Weak Acid		Peptonizers		Alkaline and Inert		Water from Homogenizer
	Before	After	Before	After	Before	After	Before	After	Before	After	
1922											
10-3	5,800	10,000	450	1,900	3,800	4,900	500	800	1,050	2,400	8
10-10	17,000	18,000	2,200	3,900	2,700	9,100	1,200	550	10,900	4,450	2,800
10-17	*10,000	*25,000	3,000	1,000	-	15,000	2,000	4,000	5,000	5,000	2,200
10-23	46,500	71,000	-	3,000	25,000	30,000	6,500	9,000	15,000	29,000	5,000
10-30	*12,500	31,000	-	2,000	6,300	14,000	4,000	1,500	2,200	13,500	3,900
11-3	7,000	*16,000	-	600	4,400	10,000	500	1,600	1,700	3,800	8,000
11-10	18,000	30,000	-	-	13,000	25,000	2,900	1,500	2,100	3,500	450
11-15	6,500	23,000	50	5,000	2,500	8,000	800	3,000	3,150	7,000	15
12-20	19,000	88,000	-	500	6,000	45,000	4,500	22,000	8,500	20,500	7,500
1923											
3-16	8,100	4,600	-	150	100	700	450	1,600	7,550	2,150	-
3-21	23,000	67,000	2,300	1,300	650	300	4,000	1,500	16,150	63,900	-
4-10	4,500	8,000	-	550	-	-	700	450	3,800	7,000	8,500
4-18	5,500	15,000	1,400	3,000	450	1,800	1,500	4,500	1,150	5,700	300
4-19	12,000	18,000	450	2,500	300	900	1,400	1,100	9,850	13,500	2,000
10-8	16,000	38,000	800	1,400	11,000	21,000	1,800	1,900	2,400	13,700	8,500
10-12	3,000	3,800	800	-	200	1,800	800	1,500	1,200	500	30
10-19	24,000	83,000	-	5,000	11,000	28,000	4,000	22,000	9,000	28,000	5,000
10-25	14,000	*60,000	-	-	2,500	10,000	5,000	40,000	6,500	10,000	4,000
11-9	*69,000	*75,000	-	2,000	20,000	16,000	9,000	16,000	40,000	41,000	43,000
11-13	6,500	19,000	-	500	2,500	4,500	1,500	7,500	2,500	6,500	-
11-15	3,600	6,500	-	-	1,300	2,000	1,100	3,000	1,200	1,500	650
11-22	12,500	170,000	-	2,000	9,300	40,000	1,600	45,000	1,600	83,000	2
11-28	11,000	45,000	500	4,000	7,500	29,000	2,000	500	1,000	11,500	18,000
12-3	2,500	300,000	-	5,000	1,000	85,000	300	170,000	1,200	40,000	140,000
1924											
2-20	4,400	6,000	-	-	1,900	8,000	700	2,400	1,800	1,600	315

* Only one plate counted.

In the computations percentages were used instead of numbers of bacteria because it was believed that this would give more accurate results.

The arithmetical mean of the differences in percentages of the different types of bacteria before and after homogenizing was determined. The standard deviation of these differences was computed and "Students" method (9) of determining the significance of the difference was used. In table XII the results of these computations are set down.

TABLE XII.

Tabulation of the Results of Computation.

Type	Number of Experiments	Average Difference	Standard Deviation of Differences	Z	Odds
Strong acid	25	+1.93	8.68	.2223	5:1
Weak acid	25	- .83	20.50	.0404	1:1
Peptonizers	25	+2.46	14.90	.1650	2:1
Alkali and Inert	25	-6.46	17.20	.3750	1:2

In statistical analysis odds of less than 30:1 are not considered highly significant. In the light of this fact we can not reliably state that the percentage of any type of bacteria is changed by homogenizing. This simply indicates that even though bacteria may be added to the ice cream mix from the homogenizer that the different types are added in the same relative ratio as they already existed in the mix before homogenizing. This is not hard to understand since the bacterial contamination in the homogenizer is due mostly to inadequate or improper cleaning of the machine after the mix has passed through.

Another point brought out by the computation is the fact that during the process of homogenizing that the groups of the different types are broken up in about the same relative proportion.

GENERAL DISCUSSION.

In carrying out this work an attempt was made to follow as nearly as possible factory conditions so that an actual picture would be obtained of the bacteriological changes during the process of homogenizing. In this way it was hoped to throw some light on the questions raised by the author in a previous paper and also by other workers in this field.

A close study of the data would seem to indicate that there is no definite relationship between the number of colonies on milk-powder agar and the number of bacteria found by the direct microscopic count in the ice cream mix. The type of organisms predominating in the sample might be one cause for this. If a sample had a large number of heat resistant bacteria present, a greater number would show on the plate count. A majority of the samples showed an increase in the number of colonies by the plate method after homogenizing. The same was true with the direct microscopic method. This held true both for the individuals and "groups". From a study of this data it would seem that there was some contributing cause to the increases shown.

A further study of table II begins to throw some light upon the cause for the increase after homogenizing. In every single case the "group" size is less. It is true that in some cases the difference is negligible. However, if one considers that the mix has been continuously agitated from 45 minutes to an hour at a temperature of

around 145°F, he will readily understand that during this agitation many of the larger clumps will have been broken up before the mix has passed through the homogenizer. That a further breaking up of clumps does occur in the homogenizer, however, is clearly indicated by the data.

Another indication that the clumps are broken up during the process of homogenizing is indicated by the total number of groups of two or more before and after homogenizing. In 18 or 72 percent of the samples there is a decrease, in 5 or 20 percent of the samples there is an increase while in 2 or 8 percent of the samples there is no change in the total number of the groups of two or more after homogenizing as compared with the total number of groups of two or more before homogenizing. When the calculations are carried still further and the average size of the groups of two or more is calculated, we again find, as in the case of the average of "groups", that there are fewer bacteria per clump after the mix has passed through the homogenizer than before. This of course indicates that there has been a breaking up of the clumps.

When milk is passed through the homogenizer the same thing holds true. There is a gradual reduction in the size of the groups as the pressure is increased. Although the reduction is slightly irregular for the few samples run yet the sum total of the results indicates that there is a reduction in the size of the groups. The results from the few samples studied, - also indicate that the homogenizer is a source of contamination as shown by the increase in the counts by both methods.

A study of the types of bacteria present in the homogenizer and present before and after homogenizing presented some interesting data. It was found that the peptonizers run considerably higher in the water from the homogenizer than they did in the mix. This no doubt accounts

for at least a part of the 5.12 percent increase of the peptonizers found in the mix after homogenizing. The only other notable change in the percentages was in the case of the alkali and inert groups where there was a decrease after as compared to before homogenizing. The strong acid-forming and the weak acid-forming types also showed an increase after homogenizing. This may have been due partly to the breaking up of clumps and partly to the contamination from the homogenizer.

However, a statistical analysis of the significance of the slight changes in the percentages of the different types before and after homogenizing shows that they are not to be taken too seriously since the odds are not sufficient to make them highly significant.

CONCLUSIONS.

1. In a majority of the cases, homogenizing increased the number of bacterial colonies in the ice cream mix as determined by the plate count using milk-powder agar.

2. In a majority of the cases, homogenizing increased the number of individuals and "groups" as determined by the direct microscopic method.

3. The average size of the "groups" and also the average size of groups of two or more in the ice cream mix are decreased. In view of the fact that all these differences are of the same sign, it is safe to conclude that homogenizing reduces the average size of bacterial groups in the ice cream mix.

4. The bacterial clumps in milk are decreased in size by passing through the homogenizer. Increasing the pressure has the general tendency to decrease the average size of the groups.

5. Milk passed through the homogenizer without pressure showed an increase in colonies by the plate method and an increase in individuals and "groups" by the direct microscopic method.

6. Sterile rinse water from the homogenizer contained from 2 to 140,000 bacterial colonies per c.c.

7. The type of colonies found in the ice cream mix were strong acid-forming, weak acid-forming, peptonizers and alkali and inert.

The general conclusion that may be drawn from the data as a whole is, that, the increase in the bacteria content after homogenizing is due to two causes; First, the breaking up of bacterial clumps and, second, contamination from the homogenizer. It would therefore, seem that a part of the increase noted in the previous papers was partly apparent and partly real.

ACKNOWLEDGMENT.

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