STUDIES ON THE CLARIFICATION OF MILK

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DAIRY SECTION

AMES, IOWA
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By B. W. Hammer.

The desirability of producing a perfect milk is now generally recognized. The production of milk in which there is only an extremely small amount of dirt is, however, an expensive process and one which ordinarily necessitates an increased price. The desire to supply milk of a good quality at a moderate price has resulted in the use of various methods of handling milk. The centrifugal separator has been recognized since its introduction as a means of removing a part of the undesirable elements from milk; however, objections have been raised to the use of these machines and recently centrifugal machines designed especially for the removal of foreign material from milk, known as milk clarifiers, have been put upon the market.

Milk clarifiers, if their increasing use can be taken as an index, are destined to hold an important place in the modern milk plant. The desire to secure additional information regarding the effect of clarification on the bacterial content of milk, the cell content, and the bacterial content where pasteurization is used, as well as to secure data on the bacterial and cell contents of the clarifier slime, has led to the work herein reported.

HISTORICAL

The action of the centrifugal separator on milk has been studied by a number of investigators, both as regards the effect on the bacteria and as regards the action on the contained sediment. It seems inadvisable to review the large amount of work done, much of which is contradictory, and only some of the work which bears most closely on the problem under consideration will be mentioned.

In 19001 "clarification" was reported to be a comparatively new term, meaning, in its commonly accepted sense, "simply running the milk through a centrifugal separator for the purpose of removing the impurities, the spouts being so arranged that both empty into one vat where the milk and cream are mixed before bottling." At that time clarification was apparently being used rather extensively by certain milk plants and condenseries.

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1 This work was done with a No. 100 DeLaval clarifier.
2 Clarification of Milk. Hoard's Dairyman. 31: 90. Mr 1900.
Eckles & Barnes (1901)² in each of seven experiments found fewer organisms after separation than before, the differences varying from 15 to 51%. They collected corresponding amounts of skim milk and cream and mixed them in order to secure the samples representing milk that had been through the separator.

Grotenfelt³ reported fewer organisms in either the skim milk or cream than in the original whole milk and concluded that separation will decrease the bacterial content.

Fleischmann⁴ states that Hueppe recommends that milk be submitted to the action of the centrifugal separator and asserts that most of the organisms remain behind in the residue and that such treatment of milk renders it much more easily sterilized. Fleischmann doubts that centrifuging has this effect.

Severin (1905)⁵ found that the number of bacteria was always larger after separation than before.

Heinemann, Luckhardt & Hicks (1910)⁶ studied 48 sets of samples and found average bacterial contents as follows: Milk before separating, 738; separator milk, 2,130; and separator cream, 132 per c. c. The calculated value for the number of bacteria that would have been present if the separator milk and cream had been mixed was 1,987 per c. c. or an increase of 169%.

Harrison, Savage & Sadler (1914)⁷, in studying the milk supply of Montreal, made counts on the milk in some of the large plants at various stages in its handling. Results on clarified milk (stated in the first case to have been run through a separator) showed that there had been an increase in the bacterial counts in three cases and a decrease in one case when agar was used for plating and a decrease in two cases and an increase in two cases when gelatin was used.

Pease (1914)⁸ in a recent hearing before the chief of the bureau of chemistry, United States Department of Agriculture, mentioned “the well know fact that the milk passed through a clarifier when the milk contains large numbers of bacteria, and, assuming that the bacteria have grown there, and that they are present in the milk in clumps, always possesses larger numbers of bacteria than were in the milk before going into the clarifier.”

Stiles⁸ stated that in work with a clarifier on a dairy farm a reduction in the bacteria was secured.

Although in the past the term clarification has been applied

³The Principles of Modern Dairy Practice. 183. 1905. (3rd edition Woll translation.)
⁴The Book of the Dairy. 98. 1896. (Aikman & Wright translation.)
⁸Rpt. of Hearing on Ice Cream before Dr. C. A. Alsberg. 166. Je 1914. (Published by “The Nat’l Assn. of Ice Cream Mfrs.” Je 1914.)
to the process of running milk through a centrifugal separator and mixing the cream and skim milk, at present it seems desirable to limit the term clarification to the process of running milk through a milk clarifier and to apply the term "separator clarification" to the older process.

**METHODS**

The samples of milk used for the various tests were taken from the machine running in the market milk laboratory, the idea being to make the tests on samples representing essentially practical rather than laboratory conditions. The examinations were not made on successive days and in many instances the operator of the machine did not know that tests were to be made until the samples were being taken. The bowl, tank and piping were thoroughly steamed in order to eliminate them as a source of contamination. The unclarified samples were taken from the tank of the machine after the contained milk had been thoroughly agitated with a carefully steamed stirrer, while the clarified samples were collected as the milk flowed from the machine. In the great majority of the tests the crank of the machine made the advised number of revolutions per minute, but in a few instances there were slight variations from this speed. The temperature of the milk was taken in the tank, the thermometer being simply carefully washed before its introduction.

The bacterial counts represent the average of two plates poured with the agar recommended by the American Public Health association for bacterial milk analysis and incubated at 37° C. for two days. All fat tests were made by the Babcock method.

**RESULTS OBTAINED**

1. Influence of Clarification on the Bacterial Content of Milk.

In discussing the influence of clarification on the bacterial content of milk, increases in the numbers of colonies developing on the plates will be spoken of as increases in the bacterial content, although it is recognized that these are only apparent increases; the causes for such apparent increases will be discussed later.

The data obtained on the influence of clarification on the bacterial content of milk are presented in tables I, II and III, the division of the data being made on the basis of the bacterial content of the unclarified samples. Table I deals with the samples containing less than 100,000 organisms per c. c., table II with those showing from 100,000 to 500,000 per c. c and table III with samples showing over 500,000 per c. c.
Fifty-one comparisons were made on samples showing less than 100,000 organisms per c. c. (table I); in 3 cases (6%) the bacterial content before and after clarification was the same, in 14 cases (27%) there was a decrease during clarification varying from 2 to 24% and averaging 12%, while in the remaining 34 cases (67%), there was an increase during clarification varying from 2 to 256% and averaging 41%. If the total 51 samples are considered there was an average increase of 24%.

Twenty-seven comparisons were made on samples containing from 100,000 to 500,000 bacteria per c. c. in the unclarified milk (table II); 9 comparisons (33%) showed a decrease during clari-

**TABLE I. BACTERIA PER C. C. BEFORE AND AFTER CLARIFICATION. ORIGINAL COUNT UNDER 100,000 PER C. C.**

<table>
<thead>
<tr>
<th>Temp. of Milk</th>
<th>% Fat in Milk</th>
<th>Bacteria per c. c. before Clarification</th>
<th>Bacteria per c. c. after Clarification</th>
<th>Percentage change in Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td></td>
<td>61,500</td>
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<td>-5</td>
</tr>
<tr>
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<td>15,400</td>
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<td>32</td>
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<td>97,500</td>
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</tr>
<tr>
<td>57</td>
<td>4.2</td>
<td>59,000</td>
<td>63,000</td>
<td>7</td>
</tr>
</tbody>
</table>
fication varying from to 2 to 36% and averaging 12% while 18 comparisons (67%) showed increases varying from 3 to 187% and averaging 43%. Considering all of the samples there was an average increase of 25%.

Fourteen comparisons were made on samples containing more than 500,000 bacteria per c. c. in the unclarified milk (table III); only 3 comparisons (21%) showed a decrease during clarification, one of 5, one of 27, and one of 40% (average 24%) while 11 comparisons (79%) showed increases varying from 4 to 102% and averaging 29%. There was an average increase of 18%, when the total 14 samples are considered.

**TABLE II. BACTERIA PER C. C. BEFORE AND AFTER CLARIFICATION. ORIGINAL COUNT FROM 100,000 TO 500,000 PER C. C.**

<table>
<thead>
<tr>
<th>Temp. of Milk</th>
<th>% Fat in Milk</th>
<th>Bacteria per c. c. before Clarification</th>
<th>Bacteria per c. c. after Clarification</th>
<th>Percentage change in Number</th>
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</thead>
<tbody>
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<td>605,000</td>
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<td>56</td>
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</table>

**TABLE III. BACTERIA PER C. C. BEFORE AND AFTER CLARIFICATION. ORIGINAL COUNT OVER 500,000 PER C. C.**

<table>
<thead>
<tr>
<th>Temp. of Milk</th>
<th>% Fat in Milk</th>
<th>Bacteria per c. c. before Clarification</th>
<th>Bacteria per c. c. after Clarification</th>
<th>Percentage change in Number</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
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<td>3.7</td>
<td>545,000</td>
<td>785,000</td>
<td>44</td>
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</table>
The results presented in tables I, II and III show that plates poured from clarified milk commonly, although by no means constantly, show larger numbers of colonies than plates poured from unclarified milk. In view of the fact that the clarifier slime contains large numbers of bacteria, it is evident that the increase is only an apparent one. Heineman, Luckhardt & Hicks\(^9\) considered that their results substantiated the theory that clumps and aggregates of bacteria which are in the milk are broken up by the violence of the process of centrifuging. Pease\(^10\) has stated that for an increase in the numbers of bacteria present in milk during clarification, the milk must have clumps in it. Power ice cream freezers in which there is a rapid agitation of the contained material commonly increase the apparent numbers of bacteria in the materials run through them under conditions which, quite evidently, do not admit of a growth of microorganisms. Moore\(^11\) cites Scheurlen as having shown that anthrax bacilli and their spores, as well as the organisms of typhoid and Asiatic cholera present in milk, usually go into the cream on centrifugal separation, while the organism of tuberculosis is thrown into the slime. Moore\(^11\) found that most of the organisms of tuberculosis are thrown into the separator slime, but that separation can not be relied upon to free milk from these germs. It seems probable then that whether there will be a decrease or an apparent increase during clarification depends upon the types of organisms present in the milk and also upon the presence of clumps. The data given in tables I, II and III indicate that there is no definite relationship between the effect of clarification on the one hand and such factors as the original count, the temperature of the milk or the percentage of fat on the other. The slight variation in speed that, as has already been mentioned, occasionally occurred, also had no apparent influence on the effect of clarification on the bacterial count.

2. Influence of Clarification on the Cell Content of Milk.

The fact that a certain number of the cellular elements present in milk can be sedimented by centrifuging has long been known, and furthermore these cells are recognized as one of the important constituents of separator and clarifier slimes. The influence of clarification on the cell content has been studied on 52 samples of milk and the data are given in table IV. The determinations of the number of cells were made by the Doane-Buckley method, the samples being heated before centrifuging.

The data show that the percentage of cells eliminated varied from 7 to 73% and averaged 39%. The percentage of cells

\(^9\) C. 51.
\(^10\) C.
eliminated appeared to bear no relation to the original number of cells present, the temperature of the milk or the percentage of fat. The work done on the Doane-Buckley method has shown, that unless the milk is subjected to heat in order to break up the fat globule clusters, many cells are carried up into the cream layer and it seems probable that the fat globule clusters are in a large part responsible for the retention of the cells during clarification; the nature of the clusters very likely determines to a large extent the number of cells retained.

The cell counts on the unclarified milk varied from 120,000

<table>
<thead>
<tr>
<th>Temp. of Milk</th>
<th>% Fat in Milk</th>
<th>No. Cells per c. c. before Clarification</th>
<th>No. Cells per c. c. after Clarification</th>
<th>Percentage of Cells thrown out</th>
</tr>
</thead>
<tbody>
<tr>
<td>58</td>
<td>3.9</td>
<td>266,000</td>
<td>206,000</td>
<td>23</td>
</tr>
<tr>
<td>59</td>
<td>3.6</td>
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</tr>
<tr>
<td>56</td>
<td>3.9</td>
<td>441,000</td>
<td>280,000</td>
<td>65</td>
</tr>
<tr>
<td>66</td>
<td>3.8</td>
<td>376,000</td>
<td>227,000</td>
<td>65</td>
</tr>
<tr>
<td>46</td>
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<tr>
<td>64</td>
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<td>193,000</td>
<td>49</td>
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<tr>
<td>44</td>
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<td>193,000</td>
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<td>54</td>
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<td>48</td>
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</tr>
<tr>
<td>64</td>
<td>3.7</td>
<td>405,000</td>
<td>225,000</td>
<td>45</td>
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<td>64</td>
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<td>194,000</td>
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<td>60</td>
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<td>216,000</td>
<td>23</td>
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<tr>
<td>52</td>
<td>4.1</td>
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<td>7</td>
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<td>59</td>
<td>4.4</td>
<td>109,000</td>
<td>102,000</td>
<td>45</td>
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<tr>
<td>69</td>
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<td>72,000</td>
<td>41</td>
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<td>69</td>
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<td>324,000</td>
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<td>62</td>
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<td>3.8</td>
<td>308,000</td>
<td>167,000</td>
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<td>67</td>
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<td>64</td>
<td>4.0</td>
<td>287,000</td>
<td>206,000</td>
<td>28</td>
</tr>
<tr>
<td>64</td>
<td>4.1</td>
<td>267,000</td>
<td>184,000</td>
<td>31</td>
</tr>
<tr>
<td>68</td>
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<td>68</td>
<td>4.0</td>
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<td>4.6</td>
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<td>68</td>
<td>4.1</td>
<td>216,000</td>
<td>89,000</td>
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<td>77</td>
<td>4.5</td>
<td>288,000</td>
<td>149,000</td>
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<tr>
<td>72</td>
<td>3.6</td>
<td>226,000</td>
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<td>61</td>
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<td>28</td>
</tr>
<tr>
<td>61</td>
<td>3.9</td>
<td>120,000</td>
<td>95,000</td>
<td>21</td>
</tr>
<tr>
<td>64</td>
<td>4.2</td>
<td>393,000</td>
<td>212,000</td>
<td>46</td>
</tr>
<tr>
<td>64</td>
<td>4.2</td>
<td>421,000</td>
<td>316,000</td>
<td>25</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>297,481</td>
<td>177,442</td>
<td>39</td>
</tr>
</tbody>
</table>
to 832,000 per c. c. and averaged 297,481 while the cell counts on the clarified milk varied from 52,000 to 489,000 and averaged 177,442. On the average 120,039 cells per c. c. were thrown out of the milk by the process of clarification.

3. The Numbers of Bacteria and Cells present in Clarifier Slime.

Slime from the clarifier bowl was studied regarding its content of bacteria and cells. At first 1 c. c. portions of the clarifier slime were employed but, as the pipettes could be emptied only with extreme difficulty, 1 gm. samples were used in the later work. The gm. samples were weighed on a small platform scale instead of on a chemical balance because of the desire to weigh out the samples very quickly. It seemed desirable to sacrifice accuracy in weighing in order to minimize the loss of water and the contamination from the air. Each sample was weighed on a sterile paper which was supported by a larger sterile paper and was transferred to the dilution flask by transferring the smaller paper. The cell counts were made on the diluted material by the use of a Thoma-Zeiss blood cell counting apparatus. In the majority of the instances, the number of pounds of milk passing the clarifier was recorded and the amount of clarifier slime was roughly determined.

Table V presents the data dealing with the bacteria per c. c. in 11 samples of clarifier slime, and, for some of the tests, data regarding the bacterial content of the milk used. The large variations in the counts made on the same run are due to the fact that milk was added to the clarifier tank from cans as needed, and the milk was commonly of different ages or from different sources. The bacteria per c. c. of slime range from 31,000,000 to 1,445,000,000 per c. c., the majority of the counts running between 400,000,000 and 900,000,000 per c. c. Little relationship can be seen between the number of organisms per c. c. in the clarifier slime and the number per c. c. in the milk passed through the machine; although the highest count on the slime was secured with the milk running the lowest in bacteria (as judged by the two counts made) and the next to lowest count on the slime with the milk running the highest in bacteria (as judged by the two counts made) the other results do not follow this inverse relationship with any consistency. The data also show that on the same run very different results, as regards the change in the number of bacteria in the milk were secured at different times. Although this may be in part due to different batches of milk, one of the runs shows these differences even when the original counts were much the same, indicating milk from the same source.

Table VI shows the number of cells per c. c. in three samples of
TABLE V. BACTERIA PER C. C. IN CLARIFIER SLIME.

<table>
<thead>
<tr>
<th>Pounds of Milk Clarified</th>
<th>c. c. Slime</th>
<th>Bacteria per c. c. in Slime</th>
<th>Tests on Milk Passing Clarifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>635</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>837</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>725</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1150</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>918</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1169</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>953</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1249</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1147</td>
<td>250</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1356</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1241</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>830,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31,000,000</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1,445,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>710,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>790,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>860,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>455,000,000</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>278,000,000</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>680,000,000</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,885,000,000</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1,050,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56,500,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7,150,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>131,000,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>73,500,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>480,000,000</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,885,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,050,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7,150</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>131,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>73,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>480,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,885,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,050,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>56,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7,150</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>131,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>73,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>480,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TABLE VI. CELL PER C. C. OF CLARIFIER SLIME.

<table>
<thead>
<tr>
<th>Pounds of Milk Clarified</th>
<th>c. c. of Slime</th>
<th>Cells per c. c. of Slime</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1249</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>1147</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>1356</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>1,120,000,000</td>
<td>850,000,000</td>
</tr>
<tr>
<td></td>
<td>830,000,000</td>
<td></td>
</tr>
</tbody>
</table>

clarifier slime. The numbers run from 830,000,000 to 1,120,000,000.

Inasmuch as it seemed desirable to get an idea of the variations in the number of both the bacteria and cells in different parts of the layer of slime, a number of tests were made in which three samples were taken from the slime layer, one at the top, one at the bottom, and one at a point approximately equidistant from the two and called the middle. Bacterial counts and cell determinations were made in all cases and the data are presented in table VII.

The data presented show that bacteria are not constantly present in greater numbers in any one part of the slime layer. Although some large variations are encountered, in general the results for various parts of the bowl are surprisingly uniform, when the much larger amount of dirt in the bottom of the bowl is taken into consideration. These data show the high bacterial count of 20,000,000,000 per gm. and from here the values vary down to 103,500,000.

The cells per gram likewise show a surprising uniformity for the various parts of the bowl and in addition considerable uniformity from one test to the next. The smallest number of cells observed was 565,000,000 and the highest 1,295,000,000 per gram. No part of the bowl constantly showed the smallest or the largest number of cells.

From table VII as well as from tables V and VI the ratio between the slime and the milk is seen to be extremely variable.
<table>
<thead>
<tr>
<th>Pounds of Milk Clarified</th>
<th>c. c. Slime</th>
<th>BACTERIA PER GM.</th>
<th>CELLS PER GM.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Top</td>
<td>Middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,925,000,000</td>
<td>1,700,000,000</td>
</tr>
<tr>
<td>980</td>
<td>100</td>
<td>240,000,000</td>
<td>183,500,000</td>
</tr>
<tr>
<td>737</td>
<td>75</td>
<td>186,500,000</td>
<td>135,500,000</td>
</tr>
<tr>
<td>1021</td>
<td>140</td>
<td>505,000,000</td>
<td>420,000,000</td>
</tr>
<tr>
<td>987</td>
<td>110</td>
<td>665,000,000</td>
<td>510,000,000</td>
</tr>
<tr>
<td>1007</td>
<td>130</td>
<td>620,000,000</td>
<td>490,000,000</td>
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<td>986</td>
<td>110</td>
<td>255,000,000</td>
<td>490,000,000</td>
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<td>1023</td>
<td>65</td>
<td>720,000,000</td>
<td>3,600,000,000</td>
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<tr>
<td>634</td>
<td>80</td>
<td>147,000,000</td>
<td>103,500,000</td>
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<td></td>
<td></td>
<td>20,000,000,000</td>
<td>9,000,000,000</td>
</tr>
<tr>
<td>908</td>
<td>85</td>
<td>160,000,000</td>
<td>112,500,000</td>
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<tr>
<td>733</td>
<td>90</td>
<td>690,000,000</td>
<td>615,000,000</td>
</tr>
</tbody>
</table>
This is to be expected since the milk used came from various sources, which ordinarily means varying amounts of care in the production.

4. Influence of Clarification on the Numbers of Bacteria Present after Pasteurization.

In order to get an idea of the influence of clarification on pasteurization, samples of both clarified and unclarified milk were collected, as usual, in thoroughly steamed bottles and the bottles capped with crown seals. The seals were used directly from the capping machine. The capped bottles were then immersed during one of the regular runs in the pasteurizing vat used in the market milk laboratory. After cooling, the milk was plated either at once or after holding several hours in ice water; the data obtained are presented in table VIII.

In 7 (33%) of the 21 comparisons, the bacterial content of the clarified pasteurized sample was lower than that of the unclarified pasteurized sample, while in the remaining 14 comparisons (67%) the lower count was obtained with the unclarified pasteurized sample. In some of the tests the comparative counts are so close that experimental error is likely responsible for the differences. Each method shows both large and small advantages and there is apparently no relationship between the original count and the method giving the lower final count. Since clarification, in the majority of the experiments carried out, gave an apparent increase in the number of bacteria, it would be expected that, in certain tests, larger counts would be obtained on clarified pasteurized milk than on unclarified pasteurized milk. If the organisms in clumps were resistant to pasteurization, a higher

TABLE VIII. EFFECT OF CLARIFICATION ON PASTEURIZATION.

<table>
<thead>
<tr>
<th>Bacteria per c. c. in Unclarified Pasteurized Milk</th>
<th>Bacteria per c. c. in Clarified Pasteurized Milk</th>
<th>Influence of Clarification</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,700</td>
<td>3,150</td>
<td>-550</td>
</tr>
<tr>
<td>5,200</td>
<td>5,650</td>
<td>450</td>
</tr>
<tr>
<td>106,500</td>
<td>148,500</td>
<td>42,000</td>
</tr>
<tr>
<td>6,400</td>
<td>8,000</td>
<td>35</td>
</tr>
<tr>
<td>275</td>
<td>310</td>
<td>-350</td>
</tr>
<tr>
<td>5,400</td>
<td>3,350</td>
<td>1,550</td>
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<tr>
<td>500</td>
<td>750</td>
<td>250</td>
</tr>
<tr>
<td>210</td>
<td>230</td>
<td>-20</td>
</tr>
<tr>
<td>185</td>
<td>100</td>
<td>-35</td>
</tr>
<tr>
<td>13,900</td>
<td>16,050</td>
<td>2,150</td>
</tr>
<tr>
<td>161,000</td>
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<td>-31,000</td>
</tr>
<tr>
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</tr>
<tr>
<td>900</td>
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<td>6,450</td>
</tr>
<tr>
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<td>12,250</td>
<td>1,300</td>
</tr>
<tr>
<td>9,050</td>
<td>13,100</td>
<td>4,050</td>
</tr>
<tr>
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<td>225</td>
<td>-2,075</td>
</tr>
<tr>
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<td>240</td>
<td>-1,260</td>
</tr>
<tr>
<td>1,000</td>
<td>230</td>
<td>-770</td>
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</table>

- = a decrease, no sign = an increase.
count on the clarified pasteurized milk than on the unclarified
pasteurized milk would in all probability follow, especially where
the pasteurization did not involve agitation as is the case with
the bottle method. Of the 21 comparisons made, clarification
caused a decrease in 33%.

The bacterial content of the milk before clarification was de­
termined in a number of instances and in the great majority of
these was under 500,000 per c. c. In table I (original count
under 100,000 per c. c.) clarification caused a decrease in 27%,
of the tests and in table II (original count from 100,000 to
500,000 per c. c.) clarification caused a decrease in 33% of the
test; these percentages agree closely with the percentage re­
ported for table VIII and indicate that clarification is responsible
for the larger counts obtained on the clarified pasteurized milk
in certain cases. Where there are no clumps present in milk
to be broken up by centrifuging, clarified pasteurized milk un­
doubtedly gives a lower count than unclarified pasteurized milk.

5. The Clarifier Slime.

In part 3, the numbers of bacteria and cells present in clari­
fier slime have been considered and the data presented indicate
that large numbers of both are commonly present. The bottom
of the layer of material thrown against the bowl in all cases
showed a dark color and close examination made it evident that
the color was due to the presence of considerable amounts of
dirt. Dirt was present in the bowl after running through the
machine only milk that was produced under conditions for above
the average. The removal of this material, which can not be
kept out except with a great deal of labor entailing considerable
extra expense and then only in part, must be looked upon as an
essentially desirable procedure. Although every one admits
the desirability of keeping out this material, the added cost
makes it impossible for the great bulk of the milk supply of
today and accordingly the removal of as much of this material
as possible followed by efficient pasteurization must be con­
sidered a commendable substitution.

Another condition that has been occasionally met with is the
deposition of red blood cells in the clarifier bowl. Although con­
siderable quantities of red blood cells in milk can be detected
by the appearance of the milk, and smaller quantities by allow­
ing the milk to stand, there can be no doubt that small quantities
of blood can be present in milk without its being detected by the
ordinary means. Blood has been found in the clarifier bowl
where the milk passed through the machine has been produced
under very careful conditions. The cells reported in part 3 are
made up in large part of polymorphonuclear leucocytes. Al­
though it would be very difficult to prove any harmful effect
from the ingestion of these cells, their presence in large numbers in infected areas makes their presence in milk in excessive numbers objectionable.

The removal from milk of a certain number of these various cells by centrifuging, either in a separator or clarifier, must be looked upon as a distinct advance from the standpoint of supplying better milk. Although it would be extremely difficult to prove any of them dangerous, this removal must be looked upon as desirable.

CONCLUSIONS

I. The results obtained show that plates poured from clarified milk commonly, although by no means constantly, show larger numbers of colonies than plates poured from unclarified milk. Since clarifier slime contains large numbers of bacteria, and contamination was practically excluded, the increases in the number of colonies developing on plates are only apparent increases due to the breaking up of clumps of organisms by the centrifuging.

II. There is no definite relationship between the effect of clarification on the one hand and such factors as the original count, temperature of the milk, or the per cent of fat on the other. Whether there will be an increase or a decrease in the apparent number during clarification probably depends on the types of organisms and on the presence of clumps.

III. Fifty-one comparisons of the bacterial content of clarified and unclarified milk were made on samples showing less than 100,000 organisms per c. c. In 3 cases (6%) the bacterial content was not influenced by clarification, in 14 cases (27%) it was decreased from 2 to 24% (Av. 12%) and in 34 cases (67%) it was increased from 2 to 256% (Av. 41%). Considering the 51 samples there was an average increase of 24%.

IV. Twenty-seven comparisons of the bacterial content of clarified and unclarified milk were made on samples showing from 100,000 to 500,000 organisms per c. c. In 9 cases (33%) clarification caused a decrease of from 2 to 36% (Av. 12%) and in 18 cases (67%) it caused an increase of from 3 to 187% (Av. 43%).

V. Fourteen comparisons of the bacterial content of clarified and unclarified milk were made on samples showing over 500,000 per c. c. In 3 cases (21%) the bacterial content was decreased by clarification from 5 to 40% (Av. 24%) and in 11 cases (79%) it was increased from 4 to 102% (Av. 29%).

VI. In 52 comparisons of the cell content of clarified and unclarified milk, clarification caused a decrease of from 7 to 73% (Av. 39%). The average cell content of the unclarified milk was 297,481 and of the clarified milk 177,442 per c. c. The
percentage of cells thrown out showed no relationship to the original cell content, the percentage of fat or the temperature of the milk.

VII. Large numbers of bacteria were found in all the samples of slime studied. In 11 tests on clarifier slime, using a c. c. sample, the counts ran from 31,000,000 to 1,445,000,000 per c. c., while on 36 samples of 1 gm. each, the counts ran from 103,500,000 to 20,000,000,000 per gm.

VIII. The cell content of clarifier slime also was constantly high. In 3 tests using a c. c. sample there were from 830,000,000 to 1,295,000,000 per c. c. while in 36 samples of 1 gm. each there were from 565,000,000 to 1,295,000,000 per gm.

IX. Neither the bacteria nor the cells were constantly present in greater numbers in any part of the slime.

X. Clarified pasteurized milk gave larger numbers of colonies on agar plates than unclarified pasteurized milk in 14 cases (67%) out of the 21 tried while in the remaining 7 cases (33%) the unclarified pasteurized milk gave the higher counts. The larger numbers of colonies from the clarified samples are undoubtedly due to the breaking up of the clumps as a result of the clarification.

XI. The clarifier slime showed a certain amount of dirt, even when the milk clarified was produced under conditions that must be regarded as much above the average.

XII. Masses of red blood cells were occasionally found in the slime even when the milk was produced under careful conditions.

XIII. The ratio between the pounds of milk clarified and the amount of slime was very variable; this is explained, to a certain extent, by the fact that the milk came from various sources and presumably was produced under very different conditions.

Clarification from the Standpoint of the Bacteriological Control of Milk Supplies.

Since in the majority of cases clarification (either with or without pasteurization*) causes an increase in the apparent numbers of bacteria in milk, it is necessary that in the bacteriological control of milk supplies this fact be taken into consideration. The increase may be a large one but, since it is only an apparent and not a true increase, high counts on clarified milk should not be considered as serious as approximately the same counts on unclarified milk. Serious contamination from a clarifier is not an impossibility and must be considered in dealing with clarified milk, but high counts on clarified milk evidently have a different significance than approximately the same counts on unclarified milk, due to the breaking up of the clumps during centrifuging.

*Altho our experiments were carried out with the bottle method of pasteurization it seems that our results are also applicable to other methods.