

Attachment of Bacteria to Teats of Cows

S. NOTERMANS*, RUTH FIRSTENBERG-EDEN¹ and M. VAN SCHOTHORST

Laboratory for Zoonoses and Food Microbiology,
 National Institute of Public Health,
 P.O. Box 1, Bilthoven, The Netherlands

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ABSTRACT

The mechanism of attachment of bacteria to meat skin is of great importance in slaughter hygiene and sampling methods. In this study teats of cows were chosen as the meat surface for attachment experiments. Both the attachment rate and strength of attachment were determined. Results show that bacteria attached themselves readily to the surface of the teat. After the initial attachment the strength of attachment increased. This increase appeared to be faster at higher storage temperatures, and an optimum was reached after 2 h at 20 C and after 3 h at 12 C. After longer periods of storage the strength of attachment decreased, probably due to formation of colonies.

Attachment of bacteria to solid surfaces has been studied extensively. These investigations involved both biological surfaces (9,12), and non-biological surfaces (11,13-15,20) and were usually done to elucidate the predomination of some specific groups of bacteria on certain surfaces (1,7,8,10). Besides the just mentioned studies there have also been studies regarding the contribution of bacterial appendages, like flagella, fimbriae and spinae (15,19), and bacterial polymers (5) to the attachment.

In spite of all these investigations there has only been a small number which deals with attachment of bacteria to surfaces of meat, which is of great importance in improvement of slaughter-hygiene. In this respect the mechanism of attachment and detachment is of special interest, but knowledge about this is also of value as regards sampling methods for estimation of bacterial counts from meat. Studies by Notermans et al. (18) and Notermans and Kampelmacher (16), on attachment of bacteria to the skin of broiler chickens show this clearly. This attachment caused an important difference between bacterial counts determined with rinsing broiler carcasses with peptone-saline solution, and counts determined by macerating a portion of the carcass.

Blankenship (3) demonstrated that spores of *Clostridium perfringens* attach to skin of broiler chickens. He

also stated that knowledge of microbial attachment characteristics may be useful in devising processing decontamination techniques. Up to now it was not known whether the attachment of bacteria and bacterial spores, as studied by Notermans and Kampelmacher (16), and by Blankenship (3), occurred on substances other than chicken skin. Therefore another kind of meat skin (teats of cows) was used for these investigations to study aspects of bacterial attachment. Besides the attachment rates of different bacteria to the skin, studies were also made of the strength of the attachment.

MATERIALS AND METHODS

Bacterial strains and their counting media

In these experiments different bacteria were used. Some of them were the same as used in an earlier study by Notermans and Kampelmacher (16). (a) *Escherichia coli* K12 - naladixic acid-resistant, peritrichous flagella. Growth medium: Brain-Heart-Infusion broth (Oxoid) with incubation at 37 C for 23 h. Counting medium: Violet-Red-Bile agar (Oxoid) +200 ppm naladixic acid, incubation for 20 h at 37 C. (b) *Klebsiella* sp. Non-motile, slime-producing, resistant to naladixic acid. The same growth and counting media as for *E. coli* were used. (c) *Pseudomonas* EBT/2/143 - Lophotrichous polar flagella, arginine-positive, non-pigmented, growth at 1 C, isolated from the skin of a turkey. Growth medium: Brain-Heart-Infusion broth (Oxoid) with incubation at 18 C for 18 h with rotary shaking (150 rpm). Counting medium: Brain-Heart-Infusion agar (Oxoid) with surface inoculation and incubation for 14 days at 1 C. (d) *Staphylococcus aureus* - Produces enterotoxin type C, isolated from skin of teats. Growth medium (Brain-Heart-Infusion Broth (Oxoid) with incubation for 20 h at 37 C on a rotary shaker (200 rpm). Counting medium: Baird-Parker (Oxoid) agar, surface inoculated, incubated for 24 h at 37 C. (e) *Salmonella typhimurium* II-505 and x-201 - Two strains isolated from different sources. Growth medium: Brain-Heart-Infusion broth (Oxoid), incubation for 20 h at 37 C. Counting medium: Brilliant-Green agar (Oxoid), with surface inoculation and incubation at 37 C for 20 h.

Teats

Teats of cows were obtained from a local slaughter-house. They were cleaned and deep-frozen till experimentation. The teats were thawed by holding them at 4 C for 15 h before attachment experiments.

Attachment suspension

Teats were dipped in physiological saline solution (8.7 g of NaCl/liter) containing phosphate buffer (0.01 M) of pH 7.2. The

¹Guest-worker from the Haifa Technion, Israël.

attachment suspension contained ca 10^5 bacteria/ml for those bacteria counted by plate pouring and ca 10^6 bacteria/ml for bacteria counted by surface inoculation.

Counting methods

From every teat two skin samples (each with a surface area of 4.83 cm^2) were cut with a sterile cutting cylinder. One sample was counted using the blending method while the other was counted by the rinse method.

The blending method as described by Avens and Miller (2) was used. For this purpose the skin sample was cut, using scissors, into a laboratory blender containing 100 ml of 0.1% peptone water, and then the blender was operated for 1 min. Fluid was then diluted and/or plated in the appropriate medium. It was assumed that using this method all the bacteria present on the skin tissue were counted.

Using the rinse method, the skin sample was introduced to a sterile glass-jar containing 100 ml of 0.1% peptone water. The sample was shaken on a rotary shaker (250 rpm) for 1 min. The fluid was diluted and plated in the appropriate medium. This method enumerated only bacteria which were not attached or which are attached insecurely.

Attachment to skin of bacteria in the suspension

The teats were dipped in a bath containing 12 liters of attachment suspension. The attachment suspension was mixed by forced aeration and maintained at 20 C. After holding the teats for the appropriate time, they were removed from the bath. To count only attached bacteria, teats were washed by gently moving them in sterile physiological saline. This washing was repeated three times using fresh physiological saline.

Attached bacteria were regarded as those which were transferred from the attachment suspension to the skin, and which remained on the skin after washing.

The attachment rate was the number of bacteria which attached per minute to the skin with a surface area of 4.83 cm^2 .

The difference between the logarithm of bacterial numbers obtained by the blending method and those obtained by the rinse method is expressed as the S-value.

Incubation after attachment

Teats were dipped in the attachment suspension for 20 min and then washed in sterile physiological water, as described above. They were then transferred to sterile glass jars which were firmly closed to prevent the skin from drying out. Jars were transferred to an incubator at either 20 or at 12 C. After various storage times the numbers of bacteria were estimated by using the blending method and the rinsing method.

RESULTS

Attachment of bacteria to teats in a suspension

The attachment rates of five different strains of bacteria to teat skin were determined by the blending method and are reported in Fig. 1. From the results it becomes clear that all the bacterial strains tested show a marked attachment. *Ps. EBT/2/143* showed the fastest rate of attachment, and *S. typhimurium* II-505 the slowest rate. This slow rate was shared by another *Salmonella* strain (*S. typhimurium* x-202).

The attachment of some bacterial strains (*S. aureus*, *E. coli* K12, *S. typhimurium* II-505) was slow at the beginning, but reached a constant value after the teats had been in the attachment suspension for a few minutes.

To determine the strength of attachment, the number of bacteria was determined using both the rinse, and the blending methods. The numbers of *Ps. EBT/2/143* on the teat skin after immersion in the bacterial suspension for various periods of time are shown in Fig. 2. From data in

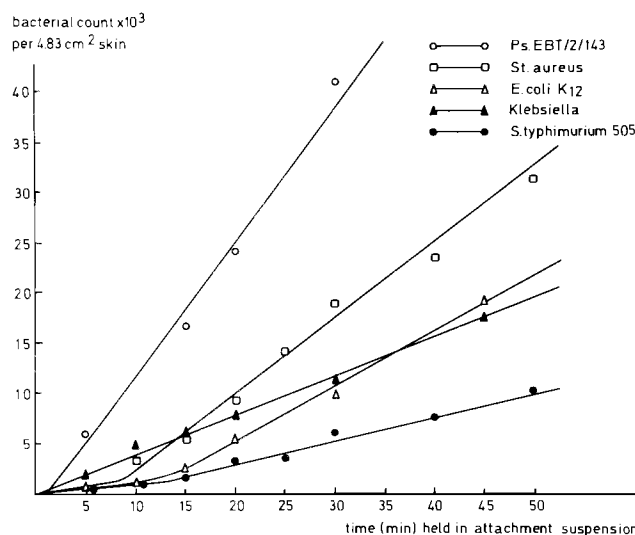


Figure 1. Attachment rates of different bacteria to teat skin at 20 C, based on a bacterial concentration of 10^5 /ml of attachment suspension.

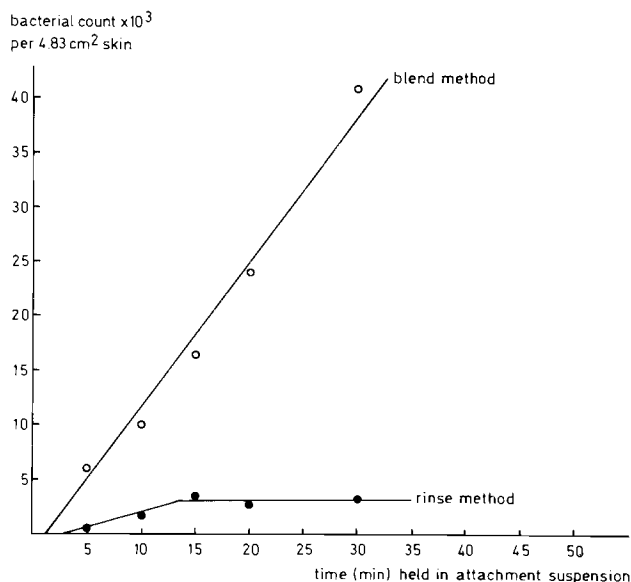


Figure 2. Comparison of rinse and blend methods to estimate the numbers of *Ps. EBT/2/143* attached to teat skin at 20 C, based on a bacterial concentration of 10^5 /ml of attachment suspension.

this graph it is evident that the numbers obtained by the blending method rose with time, whereas those obtained by the rinse method showed hardly any increase. For *E. coli* K12 (Fig. 3), however, a different type of behavior was observed. In this instance the numbers of *E. coli* K12, determined with the rinse method, rose in a slow but constant rate.

The attachment rate as well as the S-values of different bacterial strains are summarized in Table 1. From the results it is evident that a high attachment rate did not always result in a high S-value. Furthermore the S-value increased considerably after a longer immersion time of the teats in the bacterial suspension.

Incubation after attachment

In these experiments bacteria were allowed to attach to

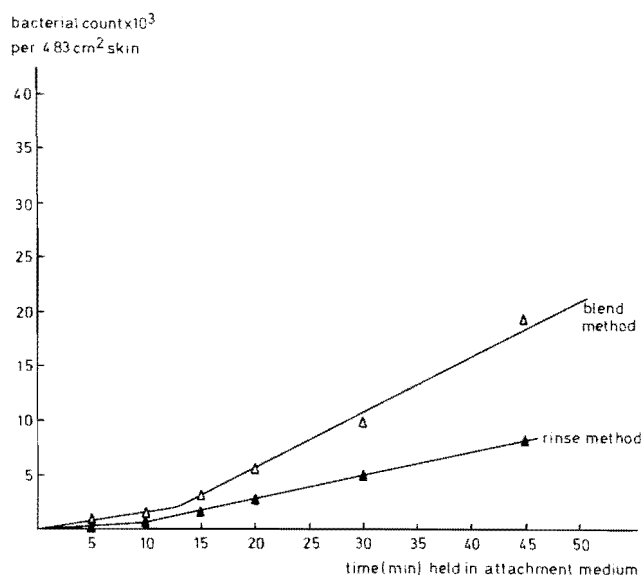


Figure 3. Comparison of rinse and blend methods to estimate the numbers of *E. coli* K12 attached to teat skin at 20 C, based on a bacterial concentration of 10^5 /ml of attachment suspension.

teat skin in a bacterial suspension at 20 C for 20 min. After washing the teats in sterile physiological saline, the teats were stored at 20 or at 12 C. The numbers of bacteria present after different storage times were estimated using both the blending and the rinsing methods. Results are in Fig. 4 and 5, and show clearly that the S-value increased during the first hours of storage, although the actual numbers of bacteria increased only in a moderate way during this time. Differences between the counting methods have a maximum after 1.5-3 h of storage at 20 C, and after 2.5-3.5 h of storage at 12 C. After longer storage times, the S-value decreased and at the same time the number of bacteria was higher for both methods. One-way analysis of variances was done to test whether the differences between the S-values were significant (6). The null-hypothesis in this case was that there is no difference in S-value obtained after different storage times. The results of this test are in Table 2. The differences were significant.

DISCUSSION

From earlier investigations by Notermans and Kampelmacher (16), it was already clear that bacteria

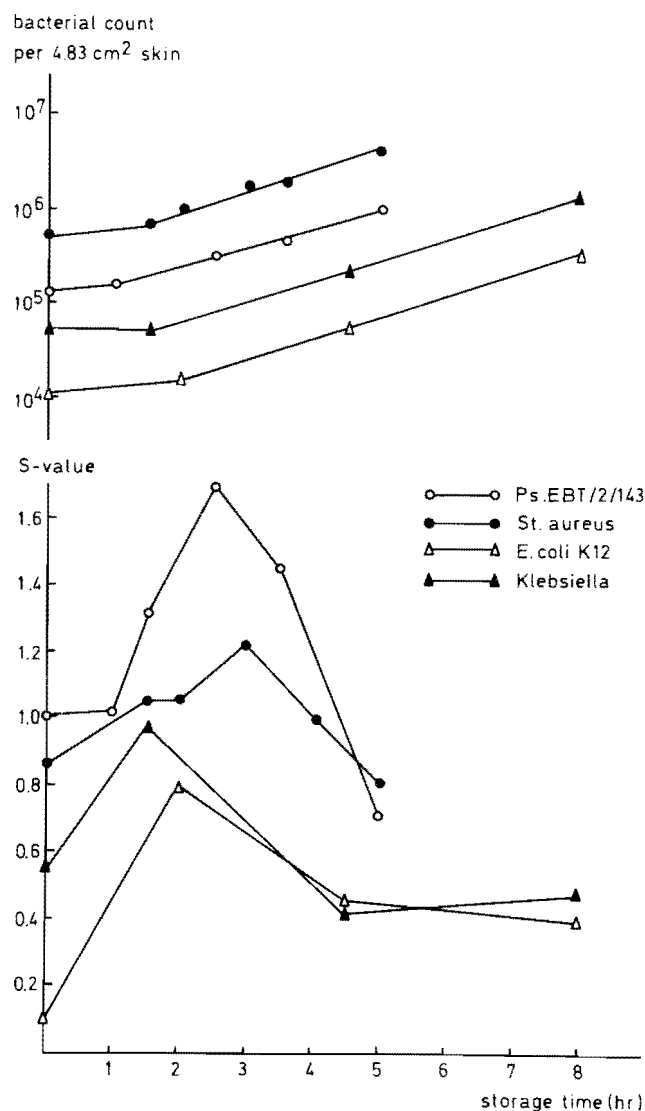


Figure 4. Effect of storage time at 20 C on growth and on the difference between counting methods. $S\text{-value} = \log_{10} (\text{bacterial count blend method}) - \log_{10} (\text{bacterial count rinse method})$.

can attach to skin of broiler chickens. From the results described here it is evident that this can also happen on the skin of cows' teats. The observed attachment rates of the different bacteria are in the same order as those obtained with the skin of broiler chickens. This fact underlines the dominant role of the bacteria in the mechanism of the attachment. The experiments of Notermans and

TABLE 1. Attachment of different bacterial strains at 20 C, based on 10^5 bacteria/ml of attachment suspension.

Bacterial strain	Attachment rate ^a		S-value ^b	
	Bacterial number attached/min.	Regression coefficient	After 10 min.	After 30 min.
Ps. EBT/2/143	1450	0.988	0.78	1.07
<i>S. aureus</i>	720	0.996	0.59	0.99
<i>E. coli</i> K12	550	0.998	0.10	0.35
<i>Klebsiella</i>	390	0.998	0.55	0.67
<i>S. typhimurium</i> II-505	230	0.988	0.14	0.70

^aNumber of bacteria which attach per minute to the skin with a surface area of 4.83 cm².

^bS-value = $\log_{10} (\text{bacterial count blending method}) - \log_{10} (\text{bacterial count rinse method})$.

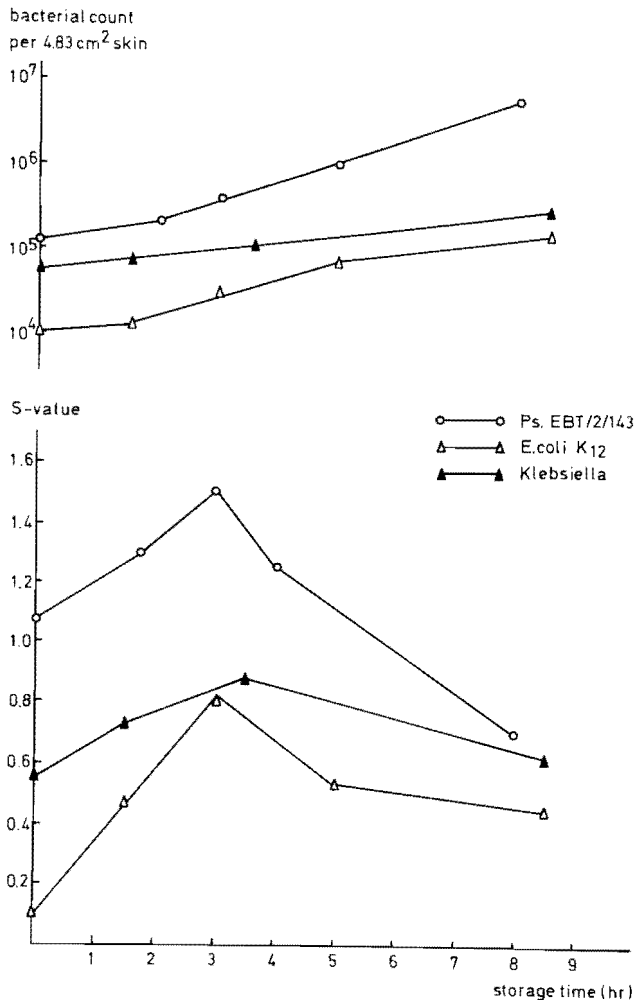


Figure 5. Effect of storage at 12 C on growth and on the difference between counting methods. $S\text{-value} = \log_{10}(\text{bacterial count blend method}) - \log_{10}(\text{bacterial count rinse method})$.

Kampelmacher (16) implicated flagella in the attachment of microorganisms to the skin of broiler chickens. The flagellated *E. coli* K12 had a higher attachment rate than *Klebsiella*. The same was found in these experiments. Now it becomes clear in using the rinse method that relatively fewer *Klebsiella* were determined than *E. coli* K12. This could mean that *Klebsiella* are attached more strongly to the surface than *E. coli* K12. In all probability this is due to slime production by the *Klebsiella* sp.

When the teats were stored after contamination in a

bacterial suspension, the difference in the counts obtained by the blending method and the rinsing method increased with length of storage. This increase in difference could serve to indicate the strength of bacterial attachment.

This increase also appears to be faster at higher storage temperatures, and is probably due to formation of extra-cellular substances produced earlier when the bacteria have a higher rate of metabolism (4,13). These substances, often composed of acidic polysaccharides (5), can play a role in the strength of attachment. The decrease in the difference between the blending and the rinsing methods after longer storage times could be due to formation of colonies by the bacteria. As the numbers of bacteria increase, more and more are probably attached to each other and not to the skin, and these may be easier to remove by the rinse method. It would be valuable to gain more information about this by using a scanning electron microscope.

From the results described here it is evident that attachment is a time-dependent process, and bacteria once attached are difficult to remove. This has important consequences for improving both hygiene and determination of bacterial counts in meat processing plants. For example, it will be important to prevent fecal contamination during slaughtering, and should it occur the bacteria should be removed as quickly as possible, by say, spray-cleaning (17).

From the results described here it can be seen that bacterial counts of surface contamination (e.g. from meat tables etc.) could be significantly different depending on the sampling method used.

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TABLE 2. Variance analysis of $S\text{-value}^a$ after different times.

Bacterial strain	Storage temperature (C)	Number of time point	Total number of samples	F value ^b
<i>Klebsiella</i>	12	4	44	6.6
	20	4	34	14.2
<i>E. coli</i> K12	12	5	48	18.1
	20	4	37	25.8
Ps. EBT/2/143	12	5	21	12.1
	20	6	18	23.8
<i>S. aureus</i>	20	6	36	7.5

^a $S\text{-value} = \log_{10}(\text{bacterial count blending method}) - \log_{10}(\text{bacterial count rinse method})$.

^bAll F values in this table are above 0.1% level of significance.

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Interim Report Farm Methods Committee

(Editor's note: Several subcommittees of the Farm Methods Committee provided few details for this report, as detailed material has either been contained in previous reports or will appear in future reports. Thus, these reports will not be provided here. These subcommittees are: Antibiotics, Pesticides, and other Adulterants Subcommittee, Cleaning and Sanitizing of Farm Milk Equipment Subcommittee, Education Subcommittee, Plastics Task Subcommittee, Animal Waste Management Task Subcommittee, Standardization of Milking System Installations Subcommittee, Standardization of Procedures for Uniform Inspection and Recommendation for Mastitis Prevention and Control Subcommittee, and Farm Sanitation Chemical Advisory Subcommittee.)

Precooling Raw Milk on the Dairy Farm Subcommittee

In many dairy operations the milk cooling equipment is inadequate because of changes in cooling requirements, increased milking rates, loss of efficiency in the condensing unit(s) and/or milk pick up schedules that no longer allow sufficient time to cool milk in refrigerated milk tanks. Consequently, the dairyman is faced with a decision to install a complete new milk cooling system or to improve his existing system.

Every year an increasing number of dairymen choose the latter alternative and install a precooler. As the name implies, a precooler cools the milk before it enters the milk tank. Sanitary heat exchangers designed to clean in-place with the milking system cool the milk in the portion of the pipeline used to convey the milk to the tank. The heat exchanger may be designed to remove a small portion of the heat from the milk or to cool it to a safe storage temperature. The amount of

cooling accomplished is usually determined by the need (how bad is the existing system) and/or the amount of coolant available.

Precoolers are not limited to retro-fitting. Large producers find it possible to reduce the operating cost of milk cooling systems if part of cooling is accomplished in a precooler. The reduced load on the milk tank condensing unit(s) permits the use of smaller condensing units. The reduced cost of the smaller condensing unit(s) and/or the reduced running time will, in part, offset the cost of the precooler.

Although a precooler cannot improve the quality of the milk being cooled, two problems associated with refrigeration milk tanks, churning and rancidity, are minimized because cooling is rapid and without agitation.

Sanitary heat exchangers using well water as the cooling medium serve a dual purpose. The well water is "preheated" enroute to conventional water heating equipment. The resulting cost reduction is partially responsible for the increasing popularity of precooling milk and "preheating" of water on dairy farms.

In an ideal situation, no water is wasted. The heat exchanger is sized to gain maximum cooling based on the normal water flow during the milking.

3-A Approved Equipment Only Guidelines for Precooler Installation

1. Openings to the heat exchanger should be in the milkroom for adequate environmental protection for cleaning, sanitizing and drainage.
2. Heat exchangers should be installed so that adequate space is provided for disassembly and inspection.
3. Milkways shall be self-draining following the cooling cycle.
4. Waterways in tubular heat exchangers shall be self-draining following the cooling cycle to prevent lowering of cleaning solution temperatures.

5. Milk filtering shall be between the milk receiver group and the heat exchanger.
6. Tubular or plate heat exchangers must be installed in a manner that permits ease of disassembly for visual inspection of the milkways. (Milkways designed for cleaning in-place are not required to be accessible for visual inspection if the milkway is cleaned as a continuous tube.)
7. The refrigeration unit(s) used on tanks in connection with a precooler may be sized so that the *combined* cooling effect of the precooler and the refrigeration unit(s) meets or exceeds applicable cooling requirements.
8. It is recommended that recording thermometers be used with refrigerated milk tanks that are equipped with minimum refrigeration and with holding tanks that have no refrigeration. (It is recommended that all raw milk holding tanks have sufficient refrigeration for maintaining the milk at safe storage temperatures.)
9. In lieu of built-in refrigerated surface in milk holding tanks, it is recommended that means be provided for recirculating milk through the precooler.

Sampling of Milk in Transport Tanks Subcommittee

Some progress has been noted in the sampling of milk in transport tanks. Jay Boosinger, Florida Department of Agriculture and Consumer Services, reported at the Farm Methods Committee Meeting held in conjunction with the National Mastitis Council on February 20, 1978, in Louisville, Kentucky, on trials conducted by Elbert Cammack, Chief, Bureau of Dairy Laboratories, Tallahassee, Florida. High speed agitation in the over-the-road tanker for five minutes was adequate in obtaining a representative sample. The interval fat testing was used as an index for getting a representative sample that then could be used for compositional, bacteriological and other testing.

con't. p. 239