EFFECTS OF TIME AND TEMPERATURE OF GRADE-A RAW MILK SAMPLE STORAGE ON BACTERIAL TEST RESULTS[1]

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Abstract

Milk produced on 30 grade-A farms was analyzed bacteriologically after the following storage treatments: less than 2 hr, 3.3 and 7.2 C for 1, 2, and 3 days, and 3.3 C for 54 hr followed by preliminary incubation at 12.8 C for 18 hr. The effects of these storage treatments were determined with the following bacterial tests: Standard Plate, thermoduric, coliform, total, psychrophilic, and enterococcus counts.

The Standard Plate and total counts showed essentially the same response to sample storage. Preincubation and storage at 7.2 C for 3 days were the only storage treatments that caused a marked change in the counts; with these treatments, the numbers more than doubled. The coliform count response to storage was similar to that of the Standard Plate and total counts, except coliforms decreased when the sample was stored at 3.3 C. The psychrophilic count showed the most marked increase, of any of the tests, to sample treatments. After storage for 1 day, the psychrophiles increased, especially at 7.2 C; there was more than a 10-fold increase during storage at 7.2 C for 3 days and during preliminary incubation. The thermoduric and enterococcus counts did not change a statistically significant amount during sample storage.

These results emphasize the importance of maintaining milk at temperatures of 3.3 C or below and not attempting to hold milk too long. The potential spoilage problem that psychrophiles may present is shown.

At present, milk is handled almost exclusively in bulk tanks. This practice has introduced important parameters (temperature and time of bulk tank storage) that affect bacterial populations. Several tests are described in *Standard Methods for the Examination of Dairy Products (1)* for estimating the bacteriological quality of raw milk. There is no information, however, regarding the effect of storage temperature or age of sample on results. This study was undertaken to discern the ability of different groups of microorganisms, as determined by plating procedures, to multiply during storage. Such information would provide guide lines in fixing the best temperature and length of milk storage.

Methods

Collection of samples

Milk produced on 30 grade-A farms was sampled after milking, as described in a previous paper *(8)*. After immediate refrigerated transportation to the laboratory, each sample was promptly dispensed by using a 10-ml manual continuous syringe, into 8 sets of sterile tests tubes (Fig. 1). After all 8 series of test tubes were filled, 4 sets were placed in a 3.3 C air incubator, and three others were placed in a 7.2 C air incubator. The remaining set was analyzed immediately. As shown in Fig. 1, one set of test tubes was removed for analysis from each incubator after 1, 2, and 3 days of storage. The series of test tubes intended for preliminary incubation (PI) was removed from the 3.3 C incubator after 54 hr and placed in a 12.8 C air incubator for 18 hr before being analyzed.

Analysis of samples

After appropriate storage, the following bacterial tests were

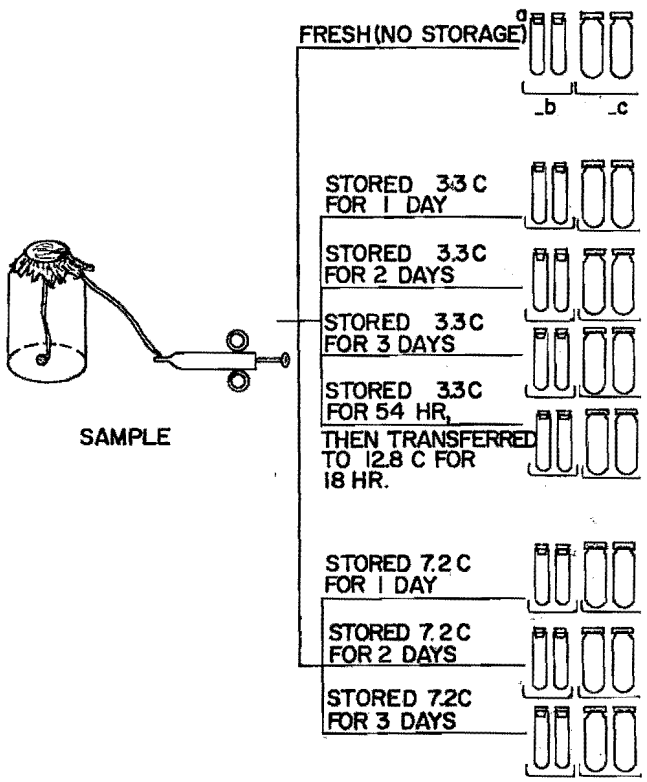

Figure 1. Distribution of sample into test tubes. [a]Samples were analyzed within 2 hr of milking. [b]15 x 125 rubber-stoppered culture tubes for thermoduric bacterial counts. [c]20 x 125 mm screw-capped test tubes for bacterial counts and resazurin tests.

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performed: Standard Plate (SPC), thermoduric (TdBC), coliform (CC), total (TC), psychrophilic (PBC), and enterococcus (EC) counts. The procedures used, which conformed to the recommendations in *Standard Methods for the Examination of Dairy Products (1)* unless otherwise noted, are described in a previous paper *(8)*. The bacterial tests were performed on duplicate tubes of milk. Milk in the two 15 x 125 mm rubber-stoppered test tubes was laboratory pasteurized for the TdBC. Milk in the two 20 x 125 mm screw-capped test tubes was used for the remaining bacterial tests.

Statistical analysis

For statistical analysis, counts of <1 were recorded as 0. Counts were transformed by taking the $\log_{10}$ (count + 1). Because of changes in procedure after the experiment was started, the statistical treatment of PI test results does not include the first 6 samples. A complete least-square analysis of the data, however, adjusted the effects of the slightly unequal subclass numbers.

TABLE 1. SIGNIFICANCE OF SAMPLE-STORAGE COMPARISONS ON BACTERIAL TEST RESULTS OF 30 GRADE-A RAW MILK SAMPLES

Treatment comparison	SPC	TdBC	CC	TC	PBC	EC
Low temp vs. high temp	**	NS[a]	**	**	**	NS
Linear effect of time	**	*	**	**	**	NS
Time x temp interaction	**	NS	**	**	**	NS

[a]Not significant.
*Significant at P <0.05.
**Significant at P <0.01.

RESULTS AND DISCUSSION

The bacterial counts that would be expected when a "typical" sample of milk is subjected to the 8 storage treatments are presented graphically in Fig. 2 and 3. These results were computed with regression coefficients calculated from the data. The "typical" sample represents the average of the 30 farms surveyed in this experiment. Table 1 lists the statistical significance of three of the comparisons included in the regression analysis.

Standard Plate Count

There was a statistically significant difference in the SPCs of the aliquots subjected to the storage treatments. There also was a statistically significant interaction between temperature and time of sample storage; Fig. 2 shows the response to sample storage. When an aliquot was stored at 3.3 C, the SPC declined slightly. When stored at 7.2 C, however, it declined slightly the first 2 days, and then more than doubled between the second and third day. The significant effects of both temperature and time resulted from growth at 7.2 C between the second and third day of storage. The SPC on the PI aliquot was almost as high as on the portion stored at 7.2 C for 3 days. Although the SPC on the aliquot stored at 7.2 C did not change appreciably after 1 or 2 days of storage, it increased rapidly after 2-days storage, which emphasizes that 7.2 C is too high for prolonged holding of milk. During storage at 7.2 C, psychrophiles were undoubtedly increasing fast

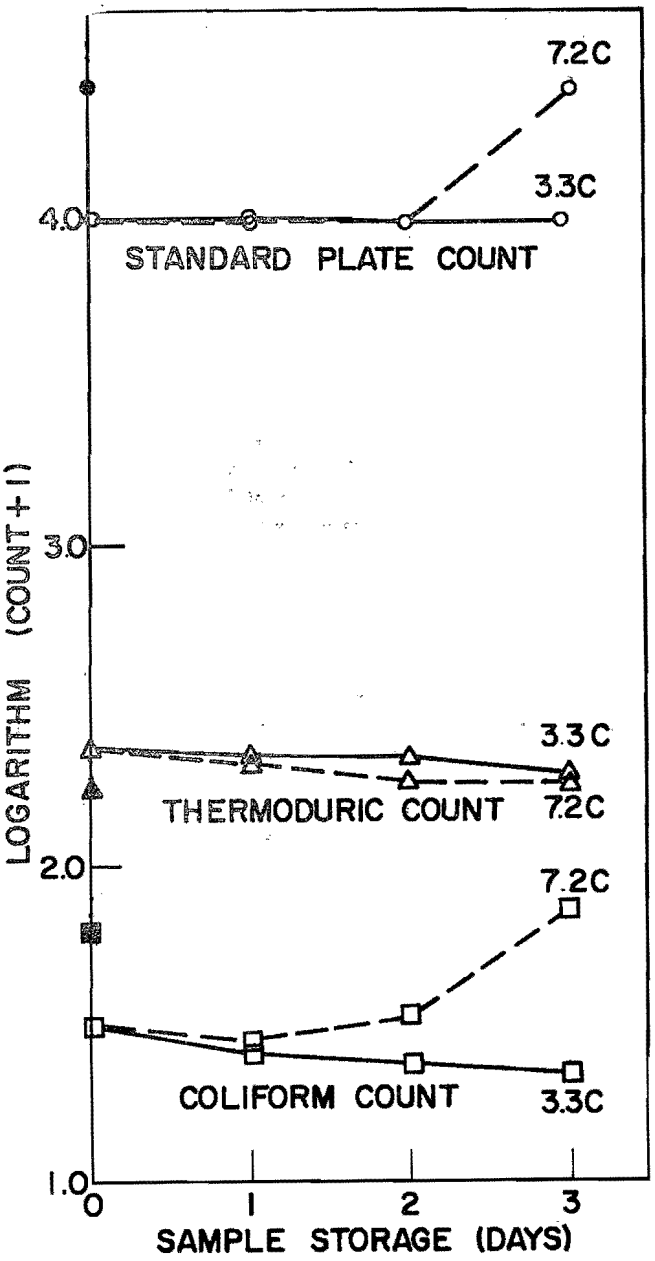

Figure 2. Trends of Standard Plate, thermoduric, and coliform counts of a "typical" grade-A raw milk sample receiving specified storage treatments. (_____Storage at 3.3 C, ... Storage at 7.2 C, ○ Standard Plate Count, △ Thermoduric bacterial count, □ Coliform count. The shaded symbols ●, ▲, and ■ represent the respective counts on the preliminary incubation sample which was stored at 3.3 C for 54 hr and then at 12.8 C for 18 hr.)

Regression coefficients were computed from results of six bacterial tests performed on 30 grade-A raw milk samples which had been subjected to eight storage treatments. These regression coefficients were then used to predict the effect of the eight storage treatments on bacterial counts of a "typical" theoretical grade-A raw milk sample.

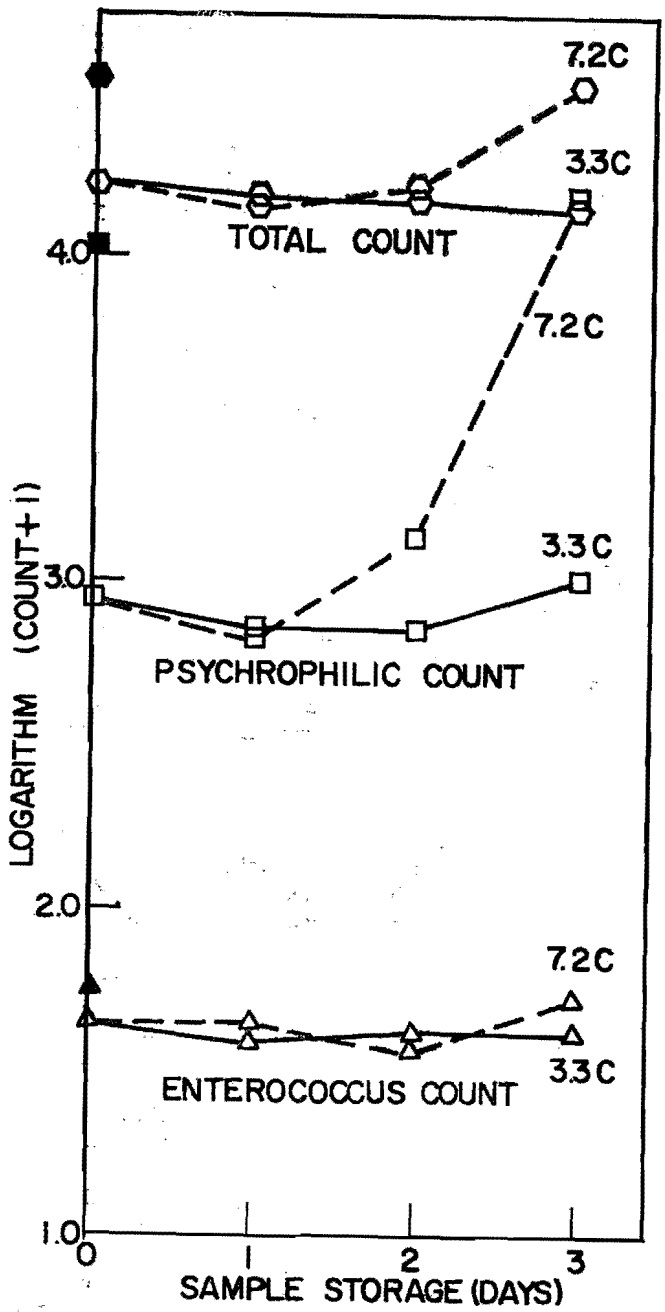

Figure 3. Trends of total, psychrophilic, and enterococcus counts of a "typical" grade-A raw milk sample receiving specified storage treatments. (_____Storage at 3.3 C, ...Storage at 7.2 C, ◯ Total count, □ Psychrophilic bacterial count, Δ Enterococcus count. The shaded symbols ●, ■, and ▲ represent the respective counts on the preliminary incubation sample which was stored at 3.3 C for 54 hr and then at 12.8 C for 18 hr.)

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enough to maintain the bacterial population. After 2 days, the flora contained a higher proportion of psychrophiles, and so the count began to increase rapidly.

The effects of temperature and period of sample storage observed in this experiment agree with results of other workers. Marth and Frazier *(12)* reported little increase in SPCs in most samples when high-count raw milk samples were stored at 2.2 C. Ayers, Cook, and Clemmer *(3)* demonstrated that with milk produced with good sanitation, the count decreased slightly when stored at 4.4 C for 24 hr and then increased slightly when stored for an additional 24 hr. Atherton and Bradfield *(2)* found significantly higher SPCs on raw milk samples stored at 5 C than at 3.3 C for 3 and 5 days. Provided the temperature is low enough, raw milk can be satisfactorily held for several days.

Thermoduric count

The TdBC was relatively insensitive to the sample-storage treatments. The only statistically significant effect shown in Table 1 was the linear effect of time. Figure 2 shows that the TdBC decreased when the aliquot was stored at either 3.3 or 7.2 C. The lack of significant interaction indicates there was no statistically significant difference between the rate of decrease at both storage temperatures. The TdBC decreased slightly more when the aliquot was stored at 7.2 C than at 3.3 C. The TdBC also decreased when the aliquot was preincubated; this decrease may result from death of the thermoduric microorganisms during storage, increased sensitivity of the cells to laboratory pasteurization after storage, clumping of cells, or growth of competitive organisms at 7.2 C.

These results agree with reports by Clegg et al. *(6)*, Marth and Frazier *(12)*, and Atherton and Bradfield *(2)*, who also observed that the thermoduric count did not increase when milk samples were stored at low temperatures. Johns and Berzins *(10)*, and Orr, McLarty, and Baines *(15)* found that thermoduric organisms did not increase in raw milk during PI.

Coliform count

The CC was responsive to sample storage; Table 1 shows that all three treatment comparisons were significant. Figure 2 shows that in aliquots stored at 3.3 C, the CC decreased with each additional day of storage. When held at 7.2 C, however, the count decreased slightly the first day, increased slightly the second day, and then more than doubled between the second and third day of storage. The count of the PI aliquot was almost as high as that of the one stored at 7.2 C for 3 days.

An overall review of the literature reveals conflicting reports on the relationship between sample storage and the coliform count. Ayers and Clemmer

(4), Finkelstein (7), and Thomas (20) reported little or no increase in coliform count when raw milk samples were held at 10 C or less. Skelton and Harmon (18) found that cultures of *Escherichia coli* and *Aerobacter aerogenes* decreased in numbers when stored at 0 and 4 C. McKenzie and Robinson (14) reported that pure cultures of coliforms did not grow at 4 C, but considerable multiplication occurred in raw milk stored at 10 C for 24 hr. Thomas et al. (21) and Panes and Thomas (16) reported up to 1000-fold increases in the coliform count of some milk samples stored at 3-5 C for 3 days. Strain differences could account for these conflicting reports.

Total count

All treatment comparisons shown in Table 1 were statistically significant for the TC. When a portion was stored at 3.3 C, the TC decreased slightly but continually (Fig. 3). At 7.2 C, the TC decreased slightly the first day, increased slightly the second, and then almost doubled between the second and third day of storage. The TC of the PI aliquot was slightly higher than of the sample stored at 7.2 C for 3 days.

Psychrophilic count

The PBC gave statistically significant responses to all treatment comparisons (Table 1) and showed the greatest response of any of the bacterial counts to sample storage (Fig. 3). When stored at 3.3 C, the count decreased the first 2 days and then showed a moderate increase between the second and third day of storage. Although the PBC on the portion stored at 7.2 C decreased after 1-day storage, it showed a dramatic increase on further storage. The large increase in count at 7.2 C was responsible for the significant interaction. The PBC of the PI portion was almost as high as of the sample stored at 7.2 C for 3 days. Rapid growth at 7.2 C beyond 1-day storage reemphasizes the necessity of maintaining a low holding temperature for milk coupled with reducing psychrophilic contamination.

There are many literature references substantiating the observed ability of psychrophiles to grow at milk storage temperatures (2, 12, 19). Prouty (17) found that, when raw milk samples from bulk tanks were held between 2.8-3.9 C, facultative psychrophilic bacteria, as determined by the plate count at 17 C, increased more rapidly than did the microorganisms enumerated by the SPC. Marth and Frazier (12) reported that a storage temperature of 7.2 C permitted too much psychrophilic growth in bulk tank milk. In low-count milk, psychrophiles grew faster than in high count milk after 2 days of incubation (13).

Enterococcus count

None of the treatment comparisons were statistically significant with the EC, as shown in Table 1, and it did not show a definite trend when the milk was stored (Fig. 3). When an aliquot was held at 3.3 C, the EC decreased slightly the first day, increased slightly the second day, and then decreased slightly by the third day. The trend of the EC was reversed on those stored at 7.2 C. The EC increased only slightly on the PI aliquot. Although enterococci are known to grow at 10 C (5), the lack of response to storage treatment at 7.2 C indicated that their growth below 10 C is slow. Data presented by Higginbottom (9) show that enterococci gradually die at 5 C or below.

The preceding results show "typical" bacterial test responses to the effects of sample storage temperature and time. They also reemphasize the need for maintaining low milk-storage temperatures on the farm as well as for samples to be examined microbiologically.

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REFERENCES

1. American Public Health Association. 1960. Standard Methods For the Examination of Dairy Products. 11th ed. American Public Health Association, New York. 448 p.

2. Atherton, H. V., and A. Bradfield. 1957. An evaluation of milk quality as influenced by daily vs. every-other-day pick-up of milk cooled in farm bulk tanks. J. Milk Food Technol. 20:223-229.

3. Ayers, S. H., L. B. Cook, and P. W. Clemmer. 1918. The four essential factors in the production of milk of low bacterial content. U. S. Dep. Agr. Bull. 642. 63 p.

4. Ayers, S. H., and P. W. Clemmer. 1918. The significance of the colon count in raw milk. U. S. Dep. Agr. Bull. 739. 35 p.

5. Breed, R. S., E. G. D. Murray, and N. R. Smith. 1957. Bergey's Manual of Determinative Bacteriology. 7th ed. Williams and Wilkins Co., Baltimore. 1094 p.

6. Clegg, L. F. L., J. W. Egdell, S. B. Thomas, D. A. McKenzie, and W. A. Cuthbert. 1950. Thermoduric organisms in milk. Part II. Preliminary studies on testing techniques. Soc. Appl. Bacteriol., Proc. 13:124-131.

7. Finkelstein, R. 1919. Occurrence of the colon-aerogenes group of organisms in raw and in pasteurized milk, and its significance. J. Dairy Sci. 2:460-481.

8. Hartley, J. C., G. W. Reinbold, E. R. Vedamuthu, and W. S. Clark, Jr. 1968. Bacterial test results of grade-A raw milk samples as a measure of farm production conditions. J. Milk Food Technol. 31:388-392.

9. Higginbottom, C. 1962. Bacteriological studies on raw milk before and after the adoption of bulk milk cooling on the farm. 16th Int. Dairy Congr. (Copenhagen), Proc. (A): 426-432.

10. Johns, C. K., and I. Berzins. 1959. The value of preliminary incubation in bacteriological tests for milk. 15th Int. Dairy Congr. (London), Proc. (3):1293-1302.

11. Lawton, W. C., and F. E. Nelson. 1954. The effect of storage temperatures on the growth of psychrophilic or-

ganisms in sterile and laboratory pasteurized skimmilks. J. Dairy Sci. 37:1164-1172.

12. Marth, E. H., and W. C. Frazier. 1957. Bacteriology of milk held at farm bulk cooling tank temperatures. I. Effect of time and temperature during storage, farm source of milk, and season of year. J. Milk Food Technol. 20:72-78.

13. Marth, E. H., and W. C. Frazier. 1957. Bacteriology of milk held at farm bulk cooling tank temperatures. II. Effect of numbers of bacteria in the original milk. J. Milk Food Technol. 20:79-83.

14. McKenzie, D. A., and K. Robinson. 1962. The growth of mesophilic coli-aerogenes organisms in milk at 4° and 10°. J. Appl. Bacteriol. 25(3):V.

15. Orr, M. J., R. M. McLarty, and S. Baines. 1965. Further investigations on alternate-day collection of bulk milk. Dairy Ind. 30:278-284.

16. Panes, J. J., and S. B. Thomas. 1959. The multiplication of coli-aerogenes bacteria in milk stored at 3-5°. J. Appl. Bacteriol. 22:272-277.

17. Prouty, C. C. 1955. Observations on the presence of facultative psychrophilic bacteria in milk produced under the farm tank system. J. Milk Food Technol. 18:250-253.

18. Skelton, W. R., and L. G. Harmon. 1963. Growth rate of coliform organisms in skimmilk at various temperatures. J. Dairy Sci. 46:601.

19. Smillie, D. M., M. J. Orr, and R. M. McLarty. 1958. Bulk milk collection in Wigtownshire. Bacterial counts at 6 and 30 hours. Dairy Ind. 23:560-565.

20. Thomas, S. B. 1955. Coli-aerogenes bacteria in raw milk. J. Appl. Bacteriol. 18:331-357.

21. Thomas, S. B., P. M. Hobson, and E. R. Bird. 1959. Psychrophilic bacteria in milk. 15th Int. Dairy Congr. (London), Proc. 3:1334-1340.

NEED OBJECTIVES TO FOR NEW ASSOCIATION

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(Editor's Note: The following article was prepared by Mr. Roger Lewis, president of the National Association of Sanitarians (NAS) and appeared in the Journal of Environmental Health, Volume 31, pages 217-218. It is reproduced below with the permission of both Mr. Lewis and Dr. A. H. Bliss, editor of the indicated journal. Since the possible amalgamation of IAMFES and NAS has been a subject of much discusion, viewpoints expressed by Mr. Lewis should be of interest to all IAMFES members.)

Inquiries concerning the status of the proposed amalgamation have been directed to me. A review of the issues and the proposals of the National Association of Sanitarians are in order.

The proposed amalgamation of sanitarians' organizations has much to offer the sanitarians; one national organization representing all of the sanitarians of the United States, one annual educational conference, one national office to support, one publication to circulate. The increased membership could produce greater impact on legislative and administrative bodies, could result in more efficient operation, could produce more services. The energies expended in association competition and rivalry could be redirected into constructive channels.

Why aren't we progressing toward unification? After six proposed bylaws revisions and much discussion by the Unification Committee, the Board of Directors, during the 32nd A. E. C. of the National Association of Sanitarians, referred the seventh draft of the proposed bylaws to its Unification Committee for further consideration.

Lack of success in the recent efforts indicates the need to re-examine the proposal. The original proposal would establish a new organization with a name acceptable to both organizations. Bylaws, drafted by the Unification Committee were to be submitted to the board of each organization for review and, if approved, to the membership of each association. Members would accept or reject by mail ballot. The present associations members were to be absorbed into the new organization within sections. Present personnel and officers would be retained and serve alternate terms until replaced through attrition. Executive directors and national offices would be continued and assigned definite areas of responsibility and activity. Two publications would be continued for the present, and eventually phased into one monthly publication. The bylaws would be revised at some later date to establish the organization structure acceptable to the combined membership.

The procedures outlined were compromises and postponed decision on the issues until some later date.

On June 22 and 23 (1968) our Board of Directors, by referral to the Unification Committee, rejected the proposed bylaws. The Unification Committee has been instructed to re-examine the seventh draft of the proposed bylaws and the suggested procedures for combining the two associations. The committee was also instructed to first determine the objectives of an association that would be satisfactory to the membership of N. A. S. and the structure best suited to accomplish these objectives. Once these steps are taken, the membership should be informed and the proposed bylaws reviewed and revised, if necessary, to provide procedures to establish the structure and attain the objectives. The Unification Committee is composed of experienced and competent members: John McHugh, president-elect and chairman; John Todd, past president; William Walter, past president; Verne Reierson, second vice president; and Harry R. Pool, Jr., president, New York Association of Sanitarians.

Any new organization must be successful. To be successful, we must insure against a substantial portion of the membership separating from the new organization and setting up a splinter organization because a new association fails to represent their objectives.