less used in the preparation of fluid milk or fluid milk products; and further

K. Authorize necessary appropriations for the Surgeon General to carry out his responsibilities under the legislation.

The official statement of the Association of State and Territorial Health Officers containing these recommendations and principles was submitted to the appropriate committees of the Congress, to individual members of the Congress who had previously introduced bills for sanitary control of interstate milk, to the Secretary of Health, Education and Welfare, and to other interested parties. As a result H.R. 3840, S. 988 and nineteen identical bills, all of which were titled "National Milk Sanitation Act," were introduced in the first session of the 86th Congress (1959). These bills embodied all of the principles and recommendations contained in our Association’s official report on the need and principles of Federal milk sanitation legislation. I should like to emphasize that although these new bills carried the same title as those previously opposed by the Association i.e., "National Milk Sanitation Act," they contained a completely different approach to deal with the problem.

In 1960 hearings were held on H.R. 3840 and S. 988 by the appropriate committees of the Congress. Our Association testified at both hearings in favor of this form of Federal legislation. Neither of these bills was voted upon by the 86th Congress prior to the end of the second session and thus these bills "died." The Association, however, is pleased to note that similar bills, embodying its recommendations and principles, have already been introduced in this new session of the Congress. (H.R. 50, 51, 52, 53, 54, 55, 56, 57, 58, 59 and 60, H.R. 1825 and S. 212). When hearings are held on these bills our Association proposes to testify in favor of their enactment.

I appreciate this opportunity to present to you the views of our Association on this important matter.

THE MEANING AND SIGNIFICANCE OF THE FREEZING POINT OF MILK

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The freezing point of milk is an indirect measure of the concentration of water soluble substances in the aqueous phase. It is independent of the concentration of water insoluble substances. By diluting milk with water the concentration of water soluble substances is reduced and consequently the freezing point is changed. The freezing point of water is higher than milk and therefore, the addition of water to milk raises the freezing point. The magnitude of the rise is approximately proportional to the amount of added water. This general relationship has been known for over sixty years, yet there is still some disagreement about the interpretation of freezing point data.

The disagreement centers around the question - What is the freezing point of undiluted milk? This question raises another question - Does all undiluted milk have the same freezing point? Obviously, we cannot answer either of these questions unless we have an accurate method of determining the freezing point.

Before 1921, a number of cryoscopic methods and techniques were used. Since there is very little information concerning the accuracy of these earlier methods they will not be included in this discussion. In 1921 Hortvet (7) described a new cryoscopic method which he had developed because he felt that the methods used prior to that date lacked standardization. Hortvet pointed out that freezing point determinations are empirical, i.e., the results are dependent on the technique used. For this reason he gave a very detailed description of his apparatus and procedure. On the basis of his ability to obtain reproducible results in his own laboratory, Hortvet believed that his method was sufficiently accurate. Collaborative studies conducted by Robertson in 1956 (10) and Shipe in 1958 (12) and 1960 (13) indicated that in most cases analysts could reproduce their own observations. However, there were significant differences between the freezing points reported by the different laboratories for identical milk samples. These observations emphasize the empirical nature of the Hortvet method. By using a consistent procedure, an analyst may be able to reproduce his own results, but this does not ensure that he will get the same results as other analysts. To obtain comparable results, all analysts must use comparable procedures. The lack of agreement between analysts

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could have been due to failure to follow Hortvet's directions or perhaps Hortvet's directions were not explicit enough.

Although the Hortvet method has been universally accepted as the standard freezing point method, numerous modifications of the Hortvet apparatus and procedure have been suggested. These modifications have been designed to either simplify the procedure or to improve the accuracy or both. Attempts to simplify the procedure have met with considerable success, however, attempts to improve the accuracy have not been as successful. Although quick, easy methods are desirable they are not necessarily more accurate.

In recent years cryoscopes have been developed which use thermistors in the place of mercury in glass thermometers. Freezing points can be determined much more easily and rapidly with these new cryoscopes than with the Hortvet samples. The results of two collaborative studies conducted in 1958 (12) and 1960 (13) indicate that these new cryoscopes give results which are comparable to the Hortvet results. These studies also revealed that the magnitude of variations between samples and analysts were about the same for both types of cryoscope. The major limitation regardless of the type of cryoscope, is undoubtedly the analytical procedure. This in turn is dependent on the training and conscientiousness of the analyst.

In light of all of this, what can we say about the accuracy of cryoscopic methods? First, there is no doubt that an analyst can obtain reproducible results. Secondly, good agreement can be obtained between different analysts provided they all follow the same procedure. Unfortunately, as is true with any analytical method, the fact that accurate results can be obtained, does not prove that all results will be accurate.

Although the limitation of cryoscopic methods must be considered in interpreting freezing point data, it is possible to draw certain conclusions. The published results clearly prove that the freezing point of milk does vary, although within relatively narrow limits. This author has occasionally heard the statement that the freezing point of normal milk is a constant. Of course, anyone, who is familiar with the literature on freezing points, knows that such a statement is false. However, this idea was unquestionably fostered by the fact that for years the per cent of added water in milk was computed by using a freezing point of -0.550°C as a base point for calculation. Some people assumed that the freezing point of all normal milk was -0.550°C.

The use of the value of 0.550°C as a base point for calculating added water apparently was originally suggested by Winter (15). He published a table which has been accepted by some as indicating the exact relationship between freezing points and percentage of added water. A literal interpretation of this table, leads one to assume that the freezing point of undiluted milk is constant and that the correct value is -0.550°C. The published freezing point data provide ample evidence to refute this assumption.

In 1923, when the Association of Official Agricultural Chemists (8) officially adopted the Hortvet cryoscopic method, they recommended that -0.550°C be considered the average freezing point of normal milk, with the allowance of a 3% tolerance. In other words milk having a freezing point higher than -0.533°C was considered illegal. The 3% tolerance figure was apparently chosen because most of the freezing points determined in the original A.O.A.C. collaborative study (2) fell within 3% of -0.550°C. However, it is worth noting that the highest freezing point observed in this study was -0.530°C. In view of this, it is not clear why the tolerance was not extended to -0.530°C.

The results of numerous studies conducted since 1923 reveal that most of the freezing points fall between -0.530° and -0.570°C. In a few cases values have been reported outside of this range. In general, the observed ranges depend on the source and number of samples. The maximum variations have been observed when individual milkings from individual cows have been tested, whereas, samples from bulk supplies exhibit the least variations.

The causes for variations have been studied by several investigators. Some of the variations have been attributed to seasonal effects, feed, water intake, breed of cow, and time of day (i.e. morning versus evening milk). In some cases the effects of these factors have been shown to be interrelated. For example, the differences between the freezing point of morning and evening milk have been shown to be affected by the time of feeding and watering. Differences have been observed between the freezing points of milk from different geographical areas. Such differences might be explained on the basis of differences in the breeds of cows and feeding practices. In spite of the reported variations, results obtained in England (14), Australia (11), India (4, 5) and the United States (2, 6, 9) show approximately the same range of values.

Variations in the freezing point have also been attributed to the methods of handling milk. It has been reported that storing samples at low temperatures, or freezing them, may raise the freezing points. Of course, the freezing points of samples which have undergone microbial decomposition will be lowered as a result of the production of such water-soluble constituents as lactic acid. Some analysts claim that heat treatment affects the freezing point slightly,
whereas other workers have not observed any change. Vacuum treatment of milk has been reported to raise the freezing point slightly. The effect of vacuum treatment has been attributed primarily to the removal of carbon dioxide.

There has been considerable controversy concerning the average freezing point of milk. Most of the averages reported in the literature fall between -0.540 and -0.550°C. The fact that not all averages were the same could easily be explained on the grounds that the different analysts did not necessarily have truly representative samples. Of course, the differences between different analysts could also have been due to differences in cryoscopic techniques.

At the beginning of this talk, I asked the question - "Does all undiluted milk have the same freezing point?"* The answer to that question is an unqualified "No"! Obviously, then, a single value can not be given in answer to the question - "What is the freezing point of undiluted milk?"* It is evident from the data cited in this discussion that any fixed arbitrary standard is not equally valid for all milks. In recognition of this fact, the Association of Official Agricultural Chemists has revised its recommendations for interpreting freezing point data. These revised recommendations, as published in the 1960 edition of Official Methods of Analysis (1), are essentially as follows:

1. The presence of added water is indicated if the freezing point is above -0.530°C.

2. It should not be assumed that milk with a freezing point below -0.530°C is necessarily free of added water. In fact, samples representing large mixed lots of milk will probably have freezing points below -0.540°C. Such milk having a freezing point above -0.540°C, or showing large fluctuations in freezing points from day to day should be regarded with suspicion.

3. If desired, the "minimum percentage of added water" can be computed, using the following formula: \( W = \left(100 - TS\right) \left(T - T'\right) / T, \) where \( T = -0.530°C, \) \( T' = \) the freezing point of the sample, and \( TS = \) percentage of total solids.

These revised recommendations are similar to those adopted by the British Standards Institution (3) in 1959.

These recommendations call attention to the fact that freezing points do vary and that this variability should be considered in interpreting results. The use of the term, "minimum percentage of added water" calls attention to the fact that it is not possible to calculate the exact percentage of added water. To calculate results by the suggested formula it is necessary to determine the percentage of total solids. This requirement increases the accuracy of the estimation and, incidentally, provides supplementary information concerning the sample.

In spite of the variations between samples, analysts, and interpretations, the freezing point of milk is the most reliable index of added water. As with any index, its usefulness is limited by one's understanding of its meaning.

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