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THE CRYOSCOPY OF MILK¹

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In 1896, as a result of numerous cryoscopic investigations on organic liquids, including serums prepared from blood, bile, gastric juice, milk, etc., Winter² announced the general conclusion that the serums of blood and milk have the same freezing temperature and that this temperature is a physiological constant. This fact was fully verified by investigations carried out on numerous species of animals. Stoecklin³ states that milk is isotonic with blood and in consequence that the freezing point of the two fluids will range from -0.550° to -0.560° C. This conclusion, with certain restrictions, was confirmed by many exact determinations. The prediction on the part of Atkins⁴ that the freezing point of milk would never lie below that of the blood of the animal producing it was borne out by the examination of a hundred samples extending over a period of 2 mo. References relative to the freezing point of cow's blood are inadequate, but various investigators give figures ranging from -0.550° to -0.590° C. Pliester⁵ cites an instance of serum prepared from blood of a sick cow as having a freezing point of -0.601° , while Atkins gives the freezing point of normal cow's blood at -0.620° . The freezing-point figure is commonly cited as a gage of osmotic pressure, and the cryoscopic determination is a well-known convenient method of measurement. A general conception regarding the various factors entering into the freezing point of a sample of milk may be obtained by inspection of the following classification given by Alexander:⁶

CONSTITUENTS OF MILK—COLLOID CHEMICAL CLASSIFICATION	
Crystalline dispersion	{ Salts (such as NaCl, etc.) Sugar (lactose)
Colloidal dispersion	{ Casein—an unstable or irreversible colloid Lactalbumin—a stable or reversible colloid
In suspension	Milk fat

It will appear from the above outline that the osmotic pressure of milk is due chiefly to the sugar

(lactose) and soluble salts which it contains. The fat has no effect and the influence of the proteins is either negligible or too small for cryoscopic measurement. It can be seen that variations in the proportion of one of the soluble constituents, for example, the lactose, will be accompanied by such corresponding variations in the soluble salts as will be necessary to maintain a proper osmotic pressure. In other words, an increase in the amount of lactose will be accompanied by a decrease in the total number of salt molecules and ions which are normally present in milk. It is also apparent that the alkali chlorides, on account of their relatively low molecular weights and highly dissociated state, contribute very largely to the total osmotic pressure. Coste and Shelbourne¹ have illustrated the osmotic pressure of average milk as follows:

OSMOTIC PRESSURE OF AVERAGE MILK			
Constituents	Per-centage	Osmotic Pressure Atmospheres	Δ
Lactose.....	4.7	3.03	0.250°
Alkali chlorides { Cl ions..... } { Na or K ions..... }	0.1	1.33	0.110°
Other salts and ions.....	...	2.42	0.200°
TOTAL.....	...	6.78	0.560°

Following these general considerations various questions naturally arise. What is the freezing point of milk? Is the freezing-point figure fairly constant or does it vary within certain maximum and minimum extremes? What are the conditions giving rise to these variations? Is it possible to lay down a safe rule to serve as a guide in judging a sample of milk on the basis of a freezing-point determination? Among the twenty-five or more investigators who have published articles on this subject there appears to be a considerable diversity as regards conclusions. A few individuals seem inclined to lay down hard-and-fast rules, while others attempt broad generalizations, such as we find expressed by Stoecklin,² somewhat as follows: Milk, freshly drawn, from any variety of cow, whether of high or low breed, from whatever region of the country, from animals in stable or in pasture, whether poorly or substantially fed, whether drawn during a period near or remote from parturition, in winter or in summer, morning or evening, and whether the yield be scant or abundant, has a definite freezing point which varies but little around -0.550° , although under various influences the chemical composition changes in enormous proportions.

One single condition is indispensable—that the animal must be healthy, that the pathological conditions of milk secretion must be in equilibrium with the osmotic properties of the body fluids which in consequence modify the freezing point.

Stoecklin states as a result of tests applied on 2500 samples in 4 yrs. that under all conditions milk does not vary in freezing point outside the limits of -0.545° and -0.565° . Other writers have expressed similar conclusions regarding the comparatively

¹ Based on report submitted as Referee on Dairy Products at Convention of Association of Official Agricultural Chemists, Washington, D. C., November 16, 1920.

² *Arch. gen. Physiol.*, **8**.

³ *Ann. fals.*, **4**, 232.

⁴ *Chem. News*, **97**, 241.

⁵ *Chem. Weekblad*, **12**, 354.

⁶ "Colloid Chemistry," p. 57.

¹ *Analyst*, **44**, 158.

² *Loc. cit.*, p. 234

narrow range in freezing-point variations on samples of normal milk.

Henderson and Meston¹ give the extreme variations at -0.540° to -0.560° for the mixed milk of herds with an average on all samples of -0.550° , but make no definite statement regarding the number and character of samples tested by themselves.

MacLaurin,² as a result of examination of 270 authenticated samples obtained from individual cows and herds, places the extremes at -0.545° and -0.565° , with an average of -0.550° .

Forty-nine samples tested by Winter,³ and representing individual cows and herds, yielded results ranging from -0.540° to -0.570° , with a general average of -0.555° . Winter and Parmentier state that the extremes are the exceptions, whereas the intermediate results constitute the general rule.

Ducrose and Imbert⁴ give results on a number (not stated) of samples yielding values from -0.533° to -0.575° . It is, however, explained that the lowest extreme (-0.533°) was obtained on milk drawn probably from a sick cow and that the highest value (-0.575°) occurred in the case of only two samples, one being 2 days old and the other thickened.

Gooren⁵ concludes that, as a rule, whole milk will not give a freezing point higher than -0.540° ; that a higher freezing point indicates an abnormal milk, citing as an instance a sample testing -0.515° . Regarding the 20 samples tested by Gooren, in the absence of any direct statement as to their origin, we are left to infer that all were of known purity.

Hummelinck⁶ gives, as a result of investigations covering several years on a number of samples, variations in freezing point from -0.542° to -0.570° .

Van Raalte⁷ reports a freezing-point range of -0.540° to -0.570° on 155 known-genuine milks. A like general range is given by Filippo⁸ and by Bomstein.⁹

Keister¹⁰ tabulates analyses of 31 authentic samples obtained from complete milkings, including one sample of herd milk, showing freezing-point figures from -0.541° to -0.574° . Similar maximum and minimum variations are reported by Reicher¹¹ and by Lam.¹²

As compared with these results, Atkins¹³ and Monier-Williams¹⁴ report exceptional extremes, but, owing to the unusual character of the values published by these investigators, it will be necessary to discuss them separately in sections dealing with apparatus and methods.

Attention is directed to the paragraph given by Leach¹⁵ on the determination of the freezing point of milk. In spite of the fact that there are safely anywhere from twenty-five to thirty important references available in the literature on the cryoscopy of milk, we find in this paragraph citations from only six authors, as follows: Beckmann,¹⁶ Grüner,¹⁷ Pins,¹⁸ Stutterheim,¹⁹ Gooren,²⁰ and Keister.²¹ It has not been possible to obtain access to the original publications of Grüner and Pins, but an explanation should be made with reference to the remainder of the refer-

ences cited. It is true that while Beckmann does not report a very wide range in results, his values do not tend in the main to indicate any very low freezing points. Also, there should be borne in mind the fact that Beckmann advocated certain correction formulas which in recent years have been under controversy, chiefly on the part of Dekhuijzen and Lam. The results reported by Stutterheim were obtained on only eight cows, which appear to have been selected for investigation owing to the fact that they were known to be sick or poorly fed. The conclusion, based on results which range from -0.520° to -0.560° , that 8 per cent or less of added water cannot be detected, is manifestly unwarranted. The results of Gooren's investigations have already been alluded to and on proper interpretation are favorable toward the acceptance of a narrow range in freezing-point values. However, these investigations covered only a limited number of samples, concerning which no definite claim was made as to purity. It is further stated in the paragraph that Gooren finds that homogenizing, pasteurizing, and sterilizing have the effect of lowering the freezing point. As relates to pasteurizing, this statement is not based on the actual results. The conclusion was to the effect that pasteurizing sometimes changes the freezing point and sometimes does not. Attention is also called to the expression which occurs in the second sentence of the paragraph, that—"most of the later investigators find -0.580° is none too high for the minimum limit." This statement is not justified, owing to the fact that only one or two investigators have reported freezing-point depressions as low as -0.580° and that these results are liable to obvious criticisms. Also the further statement, "Mixed herd milks appear seldom to fall outside the limits of -0.570° to -0.530° ," while entirely unfounded, is nevertheless more closely in conformity with facts. The actual variations reported for herd milks occur within much narrower limits.

It is apparent from the foregoing discussion that there has doubtless been a lack of uniformity as regards the conditions under which freezing-point determinations have been carried out. While it is true in the main that these conditions have not been overlooked and in a number of instances have been given serious and painstaking attention, nevertheless many investigators have published articles of more or less importance which fail to take into account the essential factors which determine a freezing-point result. Broadly speaking, the various conditions which are recognized as being vital in a freezing-point determination may be covered by the following headings:

- 1—Freezing-point apparatus—cryoscopes
- 2—Thermometers
- 3—Methods of procedure

FREEZING-POINT APPARATUS—CRYOSCOPES

The apparatus employed by most investigators in making freezing-point determinations appears to be modeled generally on the principle of the Beckmann cryoscope. There are, however, a number of modifications, as may be judged from the comparatively limited number of adequate descriptions which have been published. Very few writers seem to be satisfied with the original Beckmann apparatus and have attempted modifications for the purpose of correcting manifest experimental errors.

The simplest device which has been employed in milk testing is the one described by Henderson and Meston.¹ This model hardly embodies many of the features of the typical Beckmann apparatus. There is a well-insulated cooling bath, but no arrangement for maintaining a uniform freezing mixture, no control thermometer, and only a single test tube in which is held the sample to be frozen. The authors do not appear to place

¹ *Chem. News*, **110**, 259, 275, 283.

² Dominion Laboratory, New Zealand, *47th Annual Report*, 1914.

³ *Rev. gen. Lait*, **3**, 193, 217, 241, 268; **4**, 505.

⁴ *Bull. sci. pharmacol.*, **7**, 65.

⁵ *Centr. Bakt., Parasitenk.*, **35**, 641.

⁶ *Chem. Weekblad*, **11**, 207.

⁷ *Ibid.*, **11**, 206.

⁸ *Ibid.*, **11**, 204.

⁹ *Russki Vrach*, **3**, 90.

¹⁰ *THIS JOURNAL*, **9** (1917), 862.

¹¹ *Chem. Weekblad*, **11**, 323.

¹² *Ibid.*, **11**, 84.

¹³ *Chem. News*, **97**, 241.

¹⁴ *Analyst*, **97**, 241.

¹⁵ "Food Inspection and Analysis," 4th Edition, 1920, 153.

¹⁶ *Milch Ztg.*, **23**, 202.

¹⁷ *Ann. Inst. Agric.*, **6**, 27.

¹⁸ Inaugural Dissertation, Leipzig, 1910.

¹⁹ *Pharm. Weekblad*, **54**, 458.

²⁰ *Centr. Bakt., Parasitenk.*, **35**, II, 625.

²¹ *THIS JOURNAL*, **9** (1917), 862.

¹ *Chem. News*, **110**, 283.

any great emphasis upon details of construction, and appear only to have in mind an elementary form of apparatus that can be conveniently manipulated.

Bordas¹ makes severe criticisms of the Beckmann apparatus and calls particular attention to the imperfect contact of freezing mixture with milk test tube, liability of test tube to breakage while being inserted in the freezing mixture, loss of time involved in manipulation, and the necessity of frequently changing the mixture of crushed ice and salt. The writer states further that it is difficult to maintain a refrigerating mixture of ice and salt at a relatively constant temperature, and that the warming of the mass by conduction and radiation limits the number of operations and necessitates undue haste in making observations. Attention is also directed to the great discrepancies among results obtained by different authors. He proposes the use of a d'Arsonval-Dewar flask as an insulating container for a cooling mixture consisting of acetone reduced in temperature by means of carbon dioxide snow.

A number of investigators, notably Winter and Parmentier,² appear to use an apparatus modeled on the Beckmann principle, including a mechanical stirring device, but use only the single test tube holding the sample.

Stoecklin³ appears not to be satisfied with the construction of the customary Beckmann apparatus and devises a special make of cryoscope, using for the purpose a Dewar flask in which is prepared the freezing mixture of ice and salt. He employs a special kind of stirring device and gives much attention to the importance of using a double test tube, but does not appear to use a control thermometer.

Dekhuyzen⁴ describes a cryoscope of his own design, including among other features a Dewar tube, but uses a freezing mixture of ice and salt and provides special arrangements for avoiding the effects of conduction and radiation; and Schoorl⁵ considers it important that the temperature of the freezing bath be even throughout and that the surface of the milk sample be a number of centimeters below that of the freezing mixture.

There is a marked tendency toward the increased application of the Dewar flask (known to some investigators as the d'Arsonval) as a substitute for the various insulating devices which have been attempted by way of improvement of the Beckmann apparatus. A number of cryoscopes, in fact, have been designed on this principle, using as a refrigerating medium either a mixture of ice and salt or other compound, or in some instances a volatile refrigerating fluid, such as ether or acetone, either with or without addition of solidified carbon dioxide.

Atkins⁶ claims to have used an ordinary type of Raoult cryoscope, but gives no description of the apparatus. Monier-Williams⁷ gives a description of an elaborately constructed cryoscope based on the original principle of Raoult, but uses a Dewar flask for holding the refrigerating fluid. The plan of this apparatus appears to have in view chiefly the purpose of obtaining freezing-point results under conditions approximately ideal, that is to say, by eliminating as far as practicable well-known sources of experimental error, chief among which are the temperature of the cooling bath and the consequent supercooling of the sample under test.

The discrepancies noted among the results published by a good many collaborators may doubtless be explained on account of the various freezing-point arrangements which have been employed. There does not appear to be any fairly defined uniformity in respect to design and construction of apparatus, and even in cases in which the ordinary Beckmann

cryoscope has been used, there are notable differences as regards the conditions under which the determinations are carried out.

THERMOMETERS

The special Beckmann type of thermometer has been in general use. Various modifications have been described, but there is lacking in the literature the necessary descriptive matter relating to the construction of thermometers.

Henderson and Meston¹ first used a thermometer constructed on the Beckmann principle. They mention the trouble of having occasionally to readjust the column of mercury, and in order to avoid this inconvenience recommend the use of a special type of instrument graduated to 0.01°, each degree covering 8.5 cm. on the stem.

Stoecklin² uses a thermometer of special design, with scale +3° to -2° C., each degree about 7 cm. in length and divided into 0.01°. Monier-Williams³ describes the special make of thermometer employed by him as having a total range of about 1° C., divided into intervals of 0.005°, and each 0.005° division about 0.4 mm. in length. With this instrument, it is stated, the correct reading was reached in less than one minute, and the observed freezing point remained constant for an indefinite period, provided that the bath temperature and the speed of the stirrer did not alter.

Schoorl⁴ emphasizes the requirement that every thermometer be standardized and states that not all thermometers are reliable on account of thermic changes in the glass. Gooren⁵ expresses the same general opinion, and in order to insure reliability recommends that all thermometers be tested at the Physikalisch-Technischen Reichsanstalt; and Dekhuyzen⁶ devotes a good deal of attention to various details relative to their standardization and calibration.

Aside from the authors who have been mentioned, there are only a few who appear to lay any great emphasis upon the importance of accurately constructed thermometers, and there is little in the literature on the cryoscopy of milk which has to do with proper methods of standardizing.

METHODS OF PROCEDURE

Obviously, of primary importance is the method according to which a freezing-point determination is carried out. While a number of investigators undoubtedly base their procedure on well-established rules, there are many, on the other hand, who appear to ignore or neglect certain first principles underlying the subject of cryoscopy. The conditions which are considered essential in a proper cryoscopic procedure may be comprised under the following headings:

- 1—Temperature of the cooling bath
- 2—Convergence temperature—supercooling
- 3—Volume of the sample to be tested
- 4—Rate of manipulation of the stirring device
- 5—Method of adjusting and observing the position of the mercury column

The cryoscopic method described in the Codex-Alimentarius, and which for some time has been in use in Holland, prescribes a cooling-bath temperature ranging from -2° to -4° C., and a supercooling of sample not less than 1° and not over 1.5°. Filippo⁷ criticises this cooling-bath temperature and proposes a range from -2.5° to -3.5°; Reicher⁸ concludes in favor of a bath temperature of -2.8° and a supercooling of ±1°; Dekhuyzen⁹ urges that the cooling bath must have a constant temperature at not lower than -2.5°, and does not favor more than

¹ *Ann. fals.*, **4**, 301.

² *Rev. gen. Lait*, **3**, 193.

³ *Ann. fals.*, **4**, 232.

⁴ *Chem. Weekblad*, **11**, 126.

⁵ *Ibid.*, **12**, 220.

⁶ *Chem. News*, **97**, 241.

⁷ *Analyst*, **40**, 258.

¹ *Chem. News*, **110**, 284.

² *Loc. cit.*

³ *Analyst*, **40**, 261.

⁴ *Chem. Weekblad*, **12**, 220.

⁵ *Centr. Bakt., Parasitenk.*, **35**, II, 641.

⁶ *Chem. Weekblad*, **11**, 91.

⁷ *Ibid.*, **11**, 204.

⁸ *Ibid.*, **11**, 323.

⁹ *Ibid.*, **11**, 91.

1° supercooling. A committee of investigators composed of Van Eck, Filippo, Lam, Van der Loan, Van Raalte and Reicher¹ recommends a bath temperature of -2° to -3° and a supercooling of 0.8° to 1.3°.

Other investigators who do not base their procedure upon the Codex method show differences of opinion regarding the temperature of the cooling bath. Hummelinck supercools 1° and carries out his tests in a bath temperature of -4°; Atkins employs a bath temperature of -5° to -6°, implies a general caution against too great an extent of supercooling, and makes all measurements on a rising column of mercury; Stoecklin mentions -4° to -5° as the cooling-bath temperature, and supercools about 0.5°; Keister makes no definite mention of bath temperature but leaves an inference favoring -4°, and advocates a supercooling from 1° to 1.2°; Monier-Williams endeavors to carry out his determinations with bath and supercooling temperatures very close to the final freezing point of the sample, ranging somewhere within 0.25° to 0.5° C., the aim being apparently to obtain results under supposedly ideal conditions and thus avoid the necessity of corrections which have been much under controversy. Winter and Parmentier make no mention of bath temperature and apparently give no attention to supercooling, and the same statement is apparently true regarding the procedure adopted by Henderson and Meston, Gooren, and others.

Great variations are noted regarding the quantity of sample to be used in carrying out the test, ranging all the way from 12 cc. to 60 cc. Many investigators advocate a 50-cc. sample, chiefly on the ground that a comparatively large volume contributes somewhat toward diminishing experimental errors.

The rate of stirring is also given considerable attention, some persons advocating a very low rate of 10 or 12 strokes per minute, others a rapid rate of 1 to 2 per second. Monier-Williams sets a constant speed of about 1300 r. p. m. as the rate of movement for the mechanically operated, spiral, glass stirrer employed by him.

Dekhuyzen displays not only critical skill in the construction of his apparatus as well as in the arrangement of conditions for carrying out his tests, but also adds a number of refinements in the way of correction factors based largely on the ideas of Beckmann. A controversy was carried on, chiefly during the years 1911 to 1912, between Dekhuyzen and Lam, owing to differences in views regarding practical methods of cryoscopic examination and the application of correction factors. Results reported by Dekhuyzen were obtained after the application of corrections, whereas Lam, on the other hand, was disposed so to arrange conditions that corrections would be practically eliminated. Schoorl and Spanjaard² were able to show that the corrections applied by Dekhuyzen and others were in fact erroneous under various conditions of supercooling.

Several investigators have endeavored to devise apparatus and methods which, when practically applied, would obviate the necessity for corrections. The method of Winter and Parmentier involves a number of details having this object in view and is therefore peculiar in a number of respects. The sample taken is from 40 to 50 cc. The bath temperature is lowered well below the freezing point of the sample until it is stationary (temperature lowering immaterial), then the frozen sample withdrawn and warmed in the hand, in the meantime stirring until the greater portion of the ice crystals melt. The tube is then inserted in the freezing bath and the observation made on a falling column of mercury, the lowest depression of the mercury being read off as the true freezing point. Henderson and Meston cool the sample in the freezing tube until crystal formations are well developed, then remove the tube and warm in the hand until a rise of about 0.2° is observed. The frozen mass is stirred

and the reading taken when the mercury falls and becomes stationary for about 2 min. Monier-Williams employs a 60-cc. sample, lowers the temperature of the freezing bath by means of a current of air drawn through the apparatus, which, as previously stated, is constructed on the Raoult principle. The apparatus is so manipulated as to maintain a relatively high temperature of cooling bath (not above -0.25°) with a supercooling initially at 0.5° and ultimately approximating that of the bath. The question may be raised in criticism of the above modes of procedure as to whether there does not repeatedly arise a confusion as regards the actual condition or phase of the sample which is under test; that is to say, whether at the time of the freezing-point observation there exists a predominating crystal formation or the reverse change from the crystalline to the liquid phase.

For practical purposes, Monier-Williams proposes to simplify the freezing-point procedure by dispensing with the determination of the 0 point given by distilled water, and, instead, comparing the freezing point of the milk sample with that of a solution of 9.495 g. of pure sucrose in 100 g. of water, which solution is said to freeze at "exactly -0.5345° C." Therefore, if the two determinations, viz., the freezing point of the sucrose solution and the freezing point of the sample of milk, are determined in precisely the same manner, the "difference between the two results will indicate fairly accurately the true freezing point" of the sample. It is stated that the bath may be of ice and salt and the temperature as low as -5° C., the important point being that the temperature be kept approximately the same in both determinations, and that the degree of supercooling, rate and manner of stirring, etc., be maintained alike. Whether the results reported by Monier-Williams on 141 samples of genuine milk were all obtained by means of the above procedure or by means of the method described in the preceding paragraph of his article is not stated. At any rate, the values found, ranging from -0.558° to -0.514°, are said to have been subjected to "all the necessary corrections," which corrections were doubtless embodied mainly in the following formula attributed to Raoult:

$$K = \frac{C' - C}{CS},$$

where C' is the observed depression of the freezing point, C the true depression, S the degrees of supercooling, and K a constant, which for milk is 0.017. Attention is also given to the correction for the emergent column of mercury, and mention is made of other sources of error arising chiefly from the mechanical production of heat by the stirrer and too low a temperature of the freezing bath. It is stated, however, that these errors "may be eliminated if the temperature of the bath be so regulated that the freezing point of the solution coincides with the so-called temperature of convergence, i. e., the point at which the solution is at exact heat-equilibrium with its surroundings, the amount of heat abstracted from the solution in unit of time by the freezing bath being exactly equal to the heat imparted by the stirrer, by radiation from the outside, etc."

Obviously, the results reported by Monier-Williams must be interpreted in the light not only of the apparatus and method employed by him, but also after making allowances for corrections which were doubtless applied in all cases. Whatever criticisms may be raised in regard to these results and the methods whereby they were obtained, the fact remains to be noted that the writer regards the freezing point as "the most constant of any of the properties exhibited by milk," and one that "may, in certain circumstances, be applied with advantage, as a confirmatory test, to the detection of added water and to the approximate estimation of the amount present." The qualified doubt expressed in conclusion as to whether the freezing-point method is capable of general application for purposes of milk control may well be anticipated, owing largely to the obvious

¹ Chem. Weekblad, 12, 108.

² Ibid., 11, 648.

experimental difficulties involved in the apparatus and plan of procedure employed by the writer in making his investigations.

Such meager details as are available relating to the apparatus and method employed by Atkins have already been given. It may, however, be pointed out that results of tests made on samples "bought daily from various Dublin dairies" and including "daily morning and evening milks from the same cows," lead to the conclusion that:

The mean freezing point of cow's milk is -0.55°C. , and the value does not usually vary by more than -0.03°C. above or below. Occasionally it may fall to -0.61°C. but not below -0.62°C. , the freezing point of cow's blood; the extreme variation on the other side met with was -0.49°C. , which is 0.06°C. above the mean, just as much as the lower limit observed differs from it.

Nevertheless, in spite of these exceptionally and unaccountably wide limits, Atkins concludes that:

The determinations of the freezing point and specific gravity of a sample of milk are sufficient to show, on the one hand, whether it has been diluted with water, and, on the other, whether the fat has been removed * * * With a little practice the freezing-point determination can be carried out very rapidly; four samples were examined in 50 min. on one occasion, two of the determinations being repeated a second time.

It follows from the foregoing summary of the literature that there is a demand for uniformity in regard to the essential conditions involved in the cryoscopic method as applied to the testing of milk. Aside from the Codex-Alimentarius method and modifications based thereon, there appears to be nowhere anything approximating the objects which have been pointed out. Whether it is possible to unify these conditions by means of various designs of cryoscopes may seriously be questioned, but most important of all is uniformity regarding the method of procedure. There have been for many years certain well-established methods which will be found described in standard texts on practical physical chemistry, but these methods appear to have been given little or no attention on the part of many individuals who have attempted cryoscopic investigations on samples of milk.

As stated by Findlay,¹ the conditions affecting the temperature of the liquid in the freezing-point tube are the following:

- 1—Abstraction of heat by the cooling bath.
- 2—Addition of heat from outside by conduction through stirrer, thermometer, etc.
- 3—Addition of heat (latent heat of fusion) by the solidifying solvent.

The resultant of (1) and (2) is the convergence temperature or its equivalent degree of supercooling below the freezing point of the liquid. When solidification (freezing) takes place, latent heat of fusion is added to the liquid with a resultant rise of temperature. Hence, the observed temperature will not be the true freezing point, being a resultant of factors which may be summarized as follows:

- 1—Rate at which heat is withdrawn from the liquid, depending on difference between observed temperature and convergence temperature.
- 2—Rate at which heat is given to the liquid, depending on latent heat of fusion and velocity of crystallization.

These important factors are embodied in the following formula:

$$T = t + \frac{k}{K} (t - t'),$$

where T equals the true freezing point, t the observed freezing point, t' the convergence temperature, k a constant depending on rate of heat abstraction, and K a constant depending on rate of addition of latent heat.

The velocity of crystallization being proportional to the degree of supercooling, it is apparent that the factor $(t - t')$ should be made small in order to diminish the correction $k/K (t - t')$.

Combined with practical considerations, the conclusions derived from the foregoing formula are stated by Findlay as follows:

- 1—The temperature of the cooling bath must not be too low. It should not exceed 3° below the freezing point of the liquid.
- 2—The amount of supercooling should not exceed 0.3° to 0.5° .
- 3—The stirring should not be too rapid and should be as uniform as possible. A rate of about once per second is regarded as sufficient.
- 4—The thermometer should be tapped repeatedly before taking a reading.

Only one practical exception may be made to the conditions above stated, *viz.*, the amount of supercooling, especially in testing a sample of milk. It will be recalled that Keister recommends a supercooling of 1° to 1.2° , while others place the supercooling temperature in the neighborhood of 1° . For practical reasons these conclusions seem to be right. Unless a much lower supercooling than 0.5° is secured, it will be found that in the testing of milk the rise of the mercury column immediately after the freezing has been initiated is not sufficiently pronounced, and that there is more or less uncertain wavering and oftentimes difficulty in deciding upon the exact point at which the top of the column becomes stationary. In order to satisfy practical requirements, an attempt has been made to unify the conditions which have been outlined by giving attention chiefly, first, to the construction of a suitable cryoscope and thermometer, and, second, to the method of manipulation. The cryoscope which has been designed to serve the purposes under discussion is described as follows:

CRYOSCOPE

A cylindrical-shaped Dewar flask of 1 liter capacity and 28 cm. internal depth, surrounded by a metal casing, is tightly closed by means of a large cork of about 4 cm. thickness. Through the center of the cork is tightly fitted a medium thin-walled glass tube, 250 mm. in length by 33 mm. outside diameter. At one side of the cork is inserted a narrow metal inlet tube, the lower end of which is formed into a perforated loop near the bottom of the flask. At the opposite side is a metal tube of T-shape construction and 6-mm. internal diameter, intended to afford escape for vapors, and also for introducing volatile fluid into the apparatus. At the back portion of the cork is fitted a control thermometer having a scale range of $+20^{\circ}$ to -30°C. , and with bulb extending nearly to the bottom of the flask. The freezing test tube is of thin glass, about 240 mm. in length by 30 mm.

¹ "Practical Physical Chemistry," p. 124.

outside diameter, and fits closely into the larger tube which is sealed into the cork. In the rubber stopper of the freezing tube is fitted the standard thermometer. The thermometer is constructed of such length as to enable insertion of the bulb to near the bottom of the freezing test tube and at the same time allow complete exposure of the scale above the stopper. At the right side of the thermometer a stirring device made of non-corrodible low conductivity metal is fitted into the stopper through a short section of thin-walled metal tubing. The lower end extends to near the bottom of the test tube and is provided with a loop, around the outside of which are a number of pointed projections. At the left of the thermometer is a freezing-starter attachment inserted through an opening in the stopper formed by means of a short section of metal tubing.

THE HORTVET CRYOSCOPE

This device consists of a noncorrodible metal rod, at the lower end of which is a 10-mm. length opening for the purpose of carrying a small fragment of ice. At one side of the cryoscope is installed an air-drying arrangement which consists of a Folin absorption bulb inserted through a tightly fitting stopper and extending to near the bottom of a large-sized test tube. A short section of glass tubing is inserted through a second opening in the stopper and is connected to the vaporizing tube which enters the cryoscope. Sulfuric acid is poured into the drying tube to a level slightly above the inner bulb. At the opposite side of the apparatus is arranged a drain tube for the purpose of conducting vapors away from the operator. By means of a pressure and suction pump dry air may be forced into the apparatus at a suitable rate and the

mixed vapors conducted out through the base of the drain tube into the sink. An adjustable lens is mounted in a suitable position in front of the thermometer for the purpose of magnifying the scale.

THERMOMETER

The standard thermometer designed especially for testing milk is a solid-stem instrument measuring a total length of 58 cm., with a scale portion measuring 30 cm. The total scale range is 3°C ., from $+1^{\circ}$ to -2° , each degree division subdivided into tenths and hundredths. The length of a degree division approximates to one decimeter, thus making the smallest subdivisions of such magnitudes as to enable easy observation and readings estimated to 0.001° . The thermometer should be carefully standardized and calibrated in comparison with a U. S. Bureau of Standards tested instrument.

The *control thermometer* should be tested in a bath of melting crushed ice for the purpose of determining whether the 0 mark on the scale is fairly correct to within a small fraction of a degree.

PROCEDURE

Insert a small caliber funnel-tube into the vertical portion of the T-tube at one side of the apparatus and pour in 400 cc. of ether previously cooled to 10°C . or lower. Close the vertical tube by means of a small cork and connect the pressure pump to the inlet tube of the air-drying attachment. Adjust the pump so as to pass air through the apparatus at a moderate rate, as may be judged by the agitation of the sulfuric acid in the drying tube. Continuous vaporization of the ether will cause a lowering of the temperature in the flask, from ordinary room temperature to 0°C . in about 8 min. Continue the temperature lowering until the control thermometer registers near -3°C . At this stage, by lowering a narrow-gage, graduated glass tube into the ether bath, then closing the top by means of the forefinger and raising to a suitable height, an estimate can be made as to the amount of ether necessary to pour in for the purpose of restoring the 400-cc. volume. When the apparatus has once been cooled down to the proper temperature an additional 10 to 15 cc. of ether is on an average sufficient for each succeeding determination. Measure into the freezing test tube 30 to 35 cc. of boiled distilled water, cooled to 10°C . or lower. Enough water should be measured in fairly to submerge the thermometer bulb. Insert the thermometer together with the stirrer and lower the test tube into the larger tube. A small quantity of alcohol, sufficient to fill the space between the two test tubes, will serve to complete the conducting medium between the interior of the apparatus and the liquid to be tested. A sufficiently tight connection between the inner and outer tubes is afforded by means of a narrow section of thin-walled rubber tubing. Keep the stirrer in steady up-and-down motion at a rate of approximately one stroke each 2 or 3 sec., or even at a slower rate, providing the cooling proceeds satisfactorily. Maintain passage of air through the apparatus until the temperature of the cooling bath reaches -2.5°C ., at which time the top of the mercury

thread in the standard thermometer usually recedes to a position in the neighborhood of the probable freezing point of water. Maintain the temperature of the cooling bath at $-2.5^{\circ}\text{C}.$, and continue the manipulation of the stirrer until a supercooling of sample of 1.2° is observed. As a rule, by this time the liquid will begin to freeze, as may be noted by the rapid rise of the mercury thread. Manipulate the stirrer slowly and carefully three or four times as the mercury column approaches its highest point. By means of a suitable light-weight cork mallet tap the upper end of the thermometer cautiously a number of times, until the top of the mercury column remains stationary a couple of minutes. Taking necessary precautions to avoid parallax, observe the exact reading on the thermometer scale and estimate to $0.001^{\circ}\text{C}.$ When the observation has been satisfactorily completed, make a duplicate determination, then remove the thermometer and stirrer and empty the water from the freezing tube.

Rinse out the test tube with about 25 cc. of the sample of milk, previously cooled to 10° or lower, measure into the tube 35 cc. of the milk, or enough fairly to submerge the thermometer bulb, and insert the tube into the apparatus. Maintain the temperature of the cooling bath at 2.5° below the probable freezing point of the sample. Make the determination on the milk, following the same procedure as that employed in determining the freezing point of water. As a rule, however, it is necessary to start the freezing action in the sample of milk by inserting the freezing starter, carrying a fragment of ice, at the time when the mercury column has receded to 1.2° below the probable freezing point. A rapid rise of the mercury column results almost immediately. Manipulate the stirrer slowly and carefully two or three times while the mercury column approaches its highest point. Complete the adjustment of the mercury column in the same manner as in the preceding determination, then, avoiding parallax, observe the exact reading on the thermometer scale and estimate to 0.001° . The algebraic difference between the reading obtained on the sample of water and the reading obtained on the sample of milk represents the freezing-point depression of the milk.

For deducing the percentage of added water from the determined freezing point, use Winter's table,¹ or use the scale accompanying the cryoscope. The percentage of added water (W) may also be calculated as follows:

$$W = \frac{100(T - T')}{T},$$

where T represents the freezing point of normal milk (average -0.550°) and T' the observed freezing point on the given sample.

As stated by Keister² it is essential that the cryoscopic test be applied only to reasonably fresh milk, owing to the fact that the development of acidity to the extent of 0.10 per cent beyond normal for fresh milk (0.15 per cent) lowers the freezing point about

0.25 to 0.30 per cent. Therefore, a sample of milk in which a considerable amount of acidity has developed should not be subjected to test. The correction factor proposed by Keister (0.003° for each 0.01 per cent increase in acidity) appears to be about as practical as any that has so far been suggested, but owing to insufficient investigations regarding this point, it is not deemed advisable, excepting in extreme cases, to test samples when it is apparent that a correction factor will be necessary. In actual practice, as a rule, by applying the above correction, results have turned out substantially as would be anticipated from the other analytical figures. There is, however, very seldom any real occasion for applying a correction, owing to the fact that it is entirely practicable to obtain fresh samples, put up in proper condition, and get them to the laboratory in time to avoid a marked development of acidity.

PRACTICAL APPLICATIONS

During the years 1919 to 1920 analyses were made of seventy-five samples of known-genuine milks taken from individual cows and from herds. A general summary of the results of these analyses is given in Table I.

TABLE I—SUMMARY OF RESULTS ON KNOWN-GENUINE MILKS, 1919 TO 1920

	Sp. Gr. 60° F.	Fat %	S-N-F %	Freezing point $-0^{\circ}\text{C}.$
KNOWN-GENUINE MILKS—INDIVIDUAL COWS				
<i>Minn. State Farm and State Fair, 1919-1920—18 Samples</i>				
Maximum.....	1.0346	7.30	9.83	0.560
Minimum.....	1.0285	3.20	8.00	0.535
Average.....	1.0320	4.01	8.95	0.544
<i>Local Dairy Farms, 1919-1920—17 Samples</i>				
Maximum.....	1.0350	6.70	10.15	0.561
Minimum.....	1.0287	3.25	8.31	0.535
Average.....	1.0322	4.41	9.10	0.549
<i>Holstein-Friesian Guaranty Sale, June 1920—25 Samples</i>				
Maximum.....	1.0343	4.70	9.39	0.562
Minimum.....	1.0262	2.80	7.37	0.534
Average.....	1.0317	3.57	8.72	0.547
KNOWN-GENUINE HERD MILKS				
<i>Local Dairy Farms, 1919-1920—15 Samples</i>				
Maximum.....	1.0330	5.50	9.27	0.562
Minimum.....	1.0305	3.10	8.48	0.545
Average.....	1.0319	4.15	8.95	0.551
ALL SAMPLES—75				
Maximum.....	1.0350	7.30	10.15	0.562
Minimum.....	1.0262	2.20	7.37	0.534
Gen. average....	1.0319	4.03	8.93	0.548

It will be observed that these tabulated results comprise milks exhibiting a very wide range in composition. No information of special value would be added by tabulating the analytical results on all samples in full. The table includes samples representing any imaginable type of milk, ranging anywhere from 2.20 to 7.30 per cent in fat, and from 7.37 to 10.15 per cent in fat-free solids. At the same time, the freezing-point figures are seen to vary over a narrow range, from -0.534° to -0.562° . Also, as may be expected, the general range for herd milks is within much narrower limits than the range shown for milks drawn from individual cows, being in the case of the former -0.545° to -0.562° , and in the case of the latter from 0.534° to -0.562° . It may further be pointed out that cryoscopic tests have also been applied to a large number of samples which, while not strictly vouched for as genuine or unadulterated, could nevertheless for practical purposes be safely classed with samples of known purity.

¹ *Chem. News*, 110, 283.

² *THIS JOURNAL*, 9 (1917), 862.

These samples were obtained from producers and distributors concerning whom there was little or no reason for suspicion and whose products throughout a long period of time have customarily tested normal. There have been subjected to cryoscopic tests somewhat over 1300 samples during the past 2 yrs., beginning in February 1919. This number includes all samples, *viz.*, samples purchased from distributors, producers' samples (many of which were of safely assumed known-genuine grade), and the seventy-five authentic samples included in the foregoing tabulation. While it is true that a large proportion of these samples were diluted by means of added water as indicated by freezing-point elevations (that is, results above -0.535°), it is a significant fact that in no case has there been found a sample having normal acidity (near 0.15 per cent) and testing lower than -0.562° . Care has been exercised in all cases before applying the cryoscopic test to ascertain whether the sample was "off" in acidity, and the above statements do not cover samples which were distinctly soured or shown to be abnormal by the customary lactic acid titration. Owing to the observed natural variations in freezing points of genuine milk obtained from healthy cows, properly fed and kept, it has been deemed advisable to adopt a 3 per cent tolerance in passing judgment on market samples. This tolerance figure is plainly very liberal owing to the fact that the high freezing-test results (-0.534° and -0.535°) were obtained on milks from individual cows, while the range noted in the case of milks from herds is much narrower and includes no results higher than -0.545° .

During the past 20 yrs. much attention has been given in various state and federal food control laboratories to the development of methods of examination of the milk serum as a means of detecting adulteration with water. The analyst of the Massachusetts State Board of Health¹ gives the following statement relative to these methods:

The detection of added water in milk depends upon being able to show abnormal chemical or physical constants, which can be explained only by the addition of water, there being no test which will distinguish between the water which may be added to the milk and the water naturally present. It is incumbent, therefore, upon persons engaged in the chemical examination of milk to become familiar with the chemical and physical properties of milk of known purity * * *. If we depend upon the solids, fat or proteins to indicate added water, it is evident that considerable adulterated milk will escape detection, but if a minimum figure is employed for ash, solids not fat or sugar, more adulterated milk will be discovered. The most successful methods for the detection of added water are based upon the milk sugar content, and for this purpose it is usual to prepare a milk serum, because the most variable constituents (the fat and the proteins) remain in the curd, while the serum will contain the sugar and the ash, which are the least variable.

Based on results obtained on the copper serums prepared from samples of milk systematically watered, also on results of examination of a large number of samples of known purity, the analyst states the following conclusion:

¹ Report upon Food and Drug Inspection, year ending November 30, 1910, pp. 18-44.

A study of the above table shows that each 5 per cent of added water lowers the refraction by one scale division, and, therefore, in order to detect 10 per cent of added water in milk the milk before watering must give a serum refracting below 38. The question now arises as to what are the probabilities of this happening. Of 221 samples of known purity milk, 124, or 56 per cent, gave sera refracting below 38, and of these samples 107, or 48 per cent, were below 12.78 per cent in total solids, and 114, or 51 per cent, were below 8.77 per cent in solids not fat. The average of the samples of milk collected by the State Board of Health in 1909, exclusive of samples which could be declared skimmed, watered or cream, was 12.78 per cent in solids and 8.77 per cent in solids not fat. It is fair to presume from these figures that the average of the 1909 collection of milk in Massachusetts would refract below 38, and, therefore, 10 per cent of water could be detected if it were added to the average milk sold in this state. Probably 40 per cent of the samples collected would have given sera refracting above 38, and in these cases 15 per cent of added water could have been detected if the sample had been adulterated to that extent.

As stated by Nurenberg,¹ a comparative study of methods of examination of milk serum leads to the following conclusions:

There is no relation between the refraction of the sour serum and the sour serum ash, since these figures depend upon different milk constituents. When both of these figures fall below the lowest limits established for pure milk (38.3 and 0.730) it is absolute proof of the presence of added water, and all possibility of the sample being abnormal milk from a sick cow is removed * * *. In all doubtful cases the sour serum ash has served as a court of last resort.

The following limits are applied in interpreting results obtained on various serums prepared from milk:

ACETIC SERUM—A refractometer reading below 39 indicates added water; between 39 and 40, the addition of water is suspected. An ash result below 0.715 g. per 100 cc. indicates added water.

SOUR SERUM—A refractometer reading below 38.3 and an ash result below 0.730 g. per 100 cc indicate added water.

COPPER SERUM—A refractometer reading below 36 indicates added water.

COMPARATIVE STUDY OF METHODS

During the summer of 1920 a study was made of the cryoscopic method as compared with the refractometer method when applied to samples of known-genuine milk and to mixtures of milk containing known percentages of added water. The known pure samples selected for the investigation were of representative types, two obtained from individual cows and three from mixed milk of herds. Each sample was used as the basis for a series of mixtures containing added water in definite proportions, beginning with 6 per cent and continuing at 2 per cent intervals up to 14 per cent. For reasons which will be apparent, the fifth series of mixtures includes an additional sample watered up to 16 per cent.

Sample 18-S was taken from a 5 yr. old registered Holstein cow, well fed and kept, with a recorded good daily yield of average-test milk.

¹ J. Assoc. Official Agri. Chemists, II, 159.

TABLE II—MILKS CONTAINING KNOWN PERCENTAGES ADDED WATER

DESCRIPTION OF SERIES	Sp. Gr. 15.6° C.	Fat Per cent	S-N-F Per cent	IMMERSION REFRACTOMETER READINGS 20° C.				—ACETIC SERUM—		—CRYOSCOPIC EXAMINATION—		
				Sour Serum	Acetic Serum	Copper Serum	Added Water Indicated	Ash G. in 100 Cc.	Sp. Gr. 15° C.	Freezing Point —0° C.	—Added Water—	
											Per cent (T-T')/100	Per cent (0.550-T')/100
Samples from Individual Cows												
18-S Holstein	1.0301	3.50	8.37	39.20	40.53	36.34	None	0.7560	1.0278	0.535	None	2.72
Added water Per cent												
6	1.0286	3.25	7.95	37.90	38.50	35.05	Present	0.6948	1.0265	0.503	5.98	8.54
8	1.0284	3.20	7.88	37.23	38.00	34.75	Present	0.6760	1.0257	0.492	8.00	10.54
10	1.0275	3.15	7.66	36.50	37.64	34.44	Present	..	1.0244	0.478	10.65	13.09
12	1.0265	3.10	7.39	35.83	37.14	34.19	Present	..	1.0241	0.468	12.52	14.91
14	1.0265	3.00	7.37	35.20	36.40	33.80	Present	..	1.0237	0.459	14.20	16.54
309-P Guernsey	1.0328	5.00	9.34	43.17	44.40	38.59	None	0.8000	1.0320	0.552	None	None
Added water Per cent												
6	1.0310	4.80	8.85	41.33	42.63	37.35	None	0.7600	1.0306	0.519	5.97	5.63
8	1.0308	4.62	8.75	40.73	42.03	36.93	None	0.7476	1.0300	0.509	7.79	7.45
10	1.0303	4.50	8.62	40.11	41.40	36.50	None	0.7354	1.0293	0.494	10.50	10.18
12	1.0296	4.40	8.42	39.47	40.73	35.99	None	..	1.0285	0.486	11.95	11.64
14	1.0285	4.35	8.13	38.86	40.09	35.60	Probable	..	1.0280	0.474	14.13	13.82
Samples from Herds												
51-S 100 Cows, Common	1.0322	4.65	9.13	41.11	41.55	37.73	None	0.7920	1.0305	0.559	None	None
Added water Per cent												
6	1.0305	4.50	8.67	39.39	40.00	36.55	None	0.7404	1.0288	0.527	5.72	4.18
8	1.0299	4.39	8.50	38.75	39.47	36.15	None	0.7268	1.0280	0.514	8.05	6.54
10	1.0294	4.23	8.34	38.42	38.95	35.69	Probable	0.7145	1.0274	0.502	10.20	8.73
12	1.0285	4.12	8.10	37.72	38.39	35.33	Present	0.7022	1.0263	0.491	12.16	10.72
14	1.0278	4.04	7.91	37.17	37.75	34.93	Present	0.6920	1.0252	0.481	13.95	12.54
100-S 44 Cows, Grade	1.0305	3.90	8.55	39.69	40.22	36.32	None	0.7972	1.0283	0.562	None	None
Added water Per cent												
6	1.0290	3.62	8.12	38.22	38.67	35.12	Probable	0.7500	1.0262	0.528	6.05	4.00
8	1.0283	3.58	7.93	37.70	38.17	34.81	Present	0.7340	1.0255	0.515	8.36	6.36
10	1.0279	3.53	7.83	37.22	37.65	34.40	Present	0.7160	1.0252	0.502	10.67	8.73
12	1.0274	3.45	7.68	36.77	37.12	34.02	Present	0.6988	1.0248	0.492	12.45	10.54
14	1.0268	3.40	7.53	36.15	36.62	33.67	Present	0.6828	1.0242	0.482	14.23	12.36
184-S 11 Cows, 6 Guernsey, 5 Jersey	1.0321	5.50	9.27	42.96	43.87	38.22	None	0.8060	1.0310	0.559	None	None
Added water Per cent												
6	1.0302	5.19	8.73	41.20	42.09	37.00	None	0.7760	1.0295	0.523	6.44	4.91
8	1.0296	5.10	8.56	40.50	41.43	36.65	None	0.7480	1.0290	0.511	8.58	7.09
10	1.0291	5.00	8.41	39.95	40.56	36.17	None	0.7268	1.0285	0.501	10.37	8.91
12	1.0284	4.90	8.22	39.38	40.17	35.72	None	0.7060	1.0275	0.491	12.16	10.72
14	1.0280	4.79	8.10	38.61	39.55	35.34	Probable	0.6888	1.0265	0.480	14.13	12.73
16	1.0271	4.68	7.86	37.96	38.80	34.96	Present	0.6700	1.0260	0.468	16.27	14.91

TABLE III—MILKS CONTAINING ADDED WATER—MARKET SAMPLES

Laboratory Number	Sp. Gr. 15.6° C.	Fat Per cent	S-N-F Per cent	IMMERSION REFRACTOMETER READINGS 20° C.				—ACETIC SERUM—		—CRYOSCOPIC EXAMINATION—		
				Sour Serum	Acetic Serum	Copper Serum	Added Water Indicated	Ash G. in 100 Cc.	Sp. Gr. 15° C.	Freezing Point —0° C.	Per cent (0.550-T')/100	Per cent (0.550-T')/100
4431	1.0315	3.50	8.72	40.40	41.50	37.40	None	..	..	0.527	4.2	4.2
4432	1.0303	3.90	8.50	38.40	40.00	37.20	None	..	..	0.502	8.7	8.7
4502	1.0305	3.00	8.38	37.25	39.13	35.45	Present	0.6888	1.0263	0.490	10.9	10.9
4602	1.0282	3.00	7.78	38.52	..	35.78	Present	0.6976	1.0230	0.477	13.3	13.3
4612	1.0290	3.30	8.05	..	39.48	35.92	None	..	..	0.500	9.1	9.1
4644	1.0307	3.30	8.47	37.52	39.76	35.75	None	0.7520	1.0280	0.506	8.0	8.0
4663	1.0294	4.40	8.38	38.18	40.41	36.52	None	0.7696	1.0280	0.523	4.9	4.9
4714	1.0304	3.25	8.40	39.53	40.27	36.00	None	0.7488	1.0275	0.506	8.0	8.0
4747	1.0300	2.60	8.16	37.57	38.34	35.48	Present	0.7308	1.0270	0.481	12.5	12.5
4752	1.0279	4.30	7.98	37.95	38.46	35.43	Present	0.6928	1.0255	0.485	11.8	11.8
4766	1.0306	3.40	8.47	..	41.67	37.75	None	0.7320	1.0282	0.519	5.6	5.6

Sample 309-P was taken from a 3 yr. old Guernsey cow, well fed and kept, with recorded large daily yield of rich milk.

Sample 51-S is representative of mixed milk from a herd of 100 cows of common grade, well kept and fed under good pasturage conditions.

Sample 100-S is representative of a milking from 44 common-grade cows, poorly kept and fed under poor pasturage conditions.

Sample 184-S is representative of milk from a small group of 11 cows, including 6 Guernseys and 5 Jerseys, all kept under excellent conditions and scientifically fed.

The plan of investigation included the routine determinations of specific gravity, fat and solids, immersion refractometer readings on serums prepared

in various ways, ash and specific gravity determinations on the acetic serums, and cryoscopic examination of all samples. The specific gravity results on the whole and mixed samples were obtained by means of an accurate Quevenne lactometer; fat was determined by the Babcock method, the measurements being estimated closely to the second decimal, although fully realizing the limits of accuracy inherent in the method; solids were calculated from the results for specific gravity and fat; and the specific gravity of the acetic serums was determined by means of a Westphal balance. The serums were prepared and examined, following closely in all details the A. O. A. C. Methods of Analysis.¹ The freezing-point determinations were made by means of the apparatus and pro-

¹ A. O. A. C., 21 (1920), 16.

cedure described in this paper. For the efficient work done in carrying out the determinations above outlined, the writer wishes to express his appreciation to the assistant chemists in the Minnesota Dairy and Food Department Laboratory—to Mr. Henry Hoffmann, who handled the cryoscopic determinations, many of which were made in duplicate and involved much painstaking checking of thermometers and careful control of conditions; to Mr. Otto Kueffner, who prepared the accurately mixed samples and made all determinations of specific gravity, fat and solids; and to Mr. Donald F. Mitchell, who handled the refractometric work and the serum ash determinations. The chemists' results are included in Table II and are submitted for careful examination and study. Also, there follows (Table III) a tabulation showing results obtained on a number of market milks purchased during August and September 1920.

DISCUSSION OF RESULTS

Samples 18-S and 100-S are similar as regards general composition. Following the accepted rules of interpretation of results of immersion refractometer readings, it will be seen that the added water indications are similar in both series based on these samples. It will be noted, however, that the two series fail to yield any resemblances on the basis of the sour serum refractometer readings and the acetic serum ash results. Results of the cryoscopic tests applied to both series yield consistent and uniformly agreeing results and indicate conclusively adulteration with water from the lowest percentage to the highest. Owing to the fact that the freezing-point result on Sample 18-S is near the maximum so far obtained on an authentic sample of milk, a discrepancy is shown, as may be expected, between the results tabulated in the last two columns. Results calculated on the basis of the known freezing point correspond closely with the actual percentages of added water, whereas results calculated on the basis of the average freezing point of pure milk (-0.550°) involve a discrepancy amounting to 2.72 per cent throughout the entire series.

Samples 309-P and 184-S are also very similar as regards general composition. The immersion refractometer readings taken in conjunction with the ash results on the acetic serum fail to indicate added water as high as 12 per cent in both series, while the conclusion is doubtful at the 14 per cent limit, and in the case of Sample 184-S is positive at only 16 per cent. On the other hand, freezing-point results afford positive indications of added water throughout both series, and are concordant, and show fairly uniform gradations throughout. However, in the case of Sample 184-S, owing to the comparatively low freezing-point result, the added water calculations shown in the last column involve a discrepancy amounting to approximately 1.5 per cent as compared with results calculated on the basis of the known freezing point of the sample. The general contrasts between the series headed 18-S and 100-S on the one hand, and the series headed 184-S and 309-P on the other, may be anticipated after an inspection of the results for fat-free solids in the four

unmixed samples, which in the former two are low (8.37 and 8.55, respectively) and in the latter two are high (9.34 and 9.27, respectively). It may be assumed that corresponding relationships would also have been revealed by a determination of lactose on all samples.

Sample 51-S is intermediate as regards general characteristics. Results obtained on the various serums fail to indicate, conclusively, added water under 10 per cent. At the 10 per cent limit the indication is probable, followed with positive indications at the higher limits. On the other hand, freezing-point tests yield results which afford conclusive indications of added water, which, when calculated on the basis of the known freezing point of the authentic sample, are in close agreement with the actual known amounts added. Results calculated on the basis of the average freezing point for pure milk involve, however, a discrepancy amounting to approximately 1.5 per cent.

CONCLUSIONS

1—A large majority of investigators publish freezing-point results which range approximately from -0.540° to -0.570° ; a small number report lower results in the neighborhood of -0.575° ; and the very few results reported outside of the above limits may probably be accounted for by taking into consideration peculiarities or defects as regards apparatus and methods employed, or by noting the application of correction factors.

2—There is revealed a great want of uniformity in respect to the construction of cryoscopes. The principal designs range in general from various modifications of the Beckmann apparatus to a few models suggested by the apparatus originated by Raoult.

3—There exists also a great diversity regarding design and use of thermometers. In many instances there is found no definite statement descriptive of the thermometer employed, and many investigators give little or no attention to standardization and calibration.

4—There are also noted radical differences regarding the procedure followed in making freezing-point tests. Attention to essential conditions appears to be given by only a minority of investigators, and very great differences are noted in actual practice in the observance of such essential conditions as temperature of cooling bath, supercooling, amount of sample, rate of stirring, etc.

5—It is conclusively shown that the cryoscopic method as applied to the examination of milk is in need of being standardized; in other words, it is necessary that uniformity be secured respecting conditions involved, chiefly in the following, *viz.*, the construction of the cryoscope, methods of testing the thermometer, and the method of procedure.

6—The application of correction factors may for all practical purposes be avoided by means of a carefully standardized method of procedure.

7—Results so far obtained by means of the apparatus and procedure described in this paper indicate a narrow range of freezing-point values as a characteristic property of milk.

8—The cryoscopic test is reliable as a method for the determination of added water in amounts far below 10 per cent. When the freezing point of the original whole milk is known, results are obtainable to within an error not far from 0.5 per cent, and when the freezing point of the original milk (*e. g.*, a herd milk) is unknown, the addition of water may safely be reported in an amount as low as 3 per cent.

THE FORMATION OF ANTHRACENE FROM BENZENE AND ETHYLENE

[PRELIMINARY PAPER]

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In his classic researches on pyrogenetic reactions Berthelot¹ found that when benzene and ethylene are passed through "red hot tubes," anthracene is one of the products obtained. He stated that the reaction takes place in two steps:

The ethylene combines with one volume of benzene vapor to form styrolene, which in turn combines with another volume of benzene to form anthracene. No indication of the yield of anthracene available is given by the author. He found that the main product of the reaction which distilled at 270° to 280° C. was diphenyl.

Graebe² treated toluene similarly and obtained anthracene among other products. His main reaction product was also diphenyl.

Van Dorp³ passed *o*-benzyltoluene through a tube heated to incipient red heat. He filtered the condensed liquid and treated the residue with glacial acetic acid, from which he obtained yellow crystals of anthracene, melting at 213° C. The presence of anthracene was confirmed by the formation of the characteristic crystals of anthracene picrate by treatment with picric acid.

A careful search of the literature fails to show any other work that has been done on the formation of anthracene by the pyrogenetic reaction of hydrocarbons. In connection with other work undertaken in this laboratory on pyrogenetic relations of hydrocarbons, it seemed of interest to study the anthracene formation from a quantitative standpoint and to study the temperature relations of this reaction.

THEORETICAL

The formation of anthracene from benzene and ethylene is an endothermic reaction:

¹ *Ann. chim. phys.*, **142**, 254.

² *Ber.*, **7**, 48.

³ *Ibid.*, **5**, 1070.

This value represents, however, only a minimum value, since the heat of formation of anthracene is known only to the solid phase, and no data exist as to its latent heat of fusion and heat of vaporization which would make such a correction possible.

We can at best obtain an approximation to this value by using Trouton's rule, according to which the molecular latent heat of evaporation is approximately 21 times the absolute boiling temperature. Since anthracene boils at 351° the molecular heat of evaporation lies in the neighborhood of 13.1. This would make the heat of formation 18.3 Cal. + X, where X is the latent heat of fusion and has of course a negative value. The reaction is therefore strongly endothermic and should be favored by high temperatures. As, however, all hydrocarbons become extremely unstable at temperatures in the neighborhood of 1000°, the decomposing tendency begins to manifest itself and the decomposition to carbon and gas becomes so rapid that there can no longer be any anthracene formed. The formation of anthracene from hydrocarbons by pyrogenic reactions can at best give only small yields, for it will not form at low temperatures and it will decompose above 900°.

FIG. 1—ACTUAL BENZENE ON BASIS OF TOTAL BENZENE

These conclusions are, as far as this work has proceeded, fully confirmed by our results and likewise by the occurrence of anthracene in coal tar in only very slight amounts, since coal distillation takes place below even the optimum temperature for the formation of anthracene.

¹ Calculated from the following data of Berthelot:

$\text{C}_6 + \text{H}_2 \rightarrow \text{C}_6\text{H}_6 - 22.6$, $2\text{C}_2 + 2\text{H}_2 \rightarrow \text{C}_2\text{H}_4 - 14.6$, and $\text{C}_{14} + \text{H}_{10} \rightarrow \text{C}_{10}\text{H}_{14} - 42.4$. ("Thermochemie," Vol. II, pp. 403, 416, 436.)