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THE FAT METABOLISM OF THE MAMMARY GLAND[1]

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Certain aspects of the fat metabolism of the mammary gland have been given considerable attention for a number of years. In 1912, Foa (6) using the perfusion technique concluded that milk fat was formed from neutral fat. When olive oil was added to the perfusion solution, the fat in the fluid obtained from the gland had a lower iodine number than that of the olive oil. The work of Meigs, Blatherwick and Cary (17) was accepted as proof that milk fat was formed from blood phospholipids until Blackwood and Sterling (2) showed that this work was invalidated by a difference in the concentration between the jugular and the mammary venous bloods. Lintzel (15) working with goats in which arterial blood was obtained by heart puncture demonstrated the loss of neutral fat to the mammary gland. Graham, Jones and Kay (9) in a similar study with cows in which the arterial blood was obtained from the internal iliac by rectal puncture concluded that, in the main, milk fat is derived from the non-phosphatide fatty acids of the blood. The use of neutral fat by the active gland was also demonstrated by Maynard *et al.* (16) in a series of experiments in which the arterial blood was obtained from the internal pudic artery through the vaginal wall. Later, Graham *et al.* (8) reported that the respiratory quotient of the lactating gland of the goat exceeded unity suggesting that fat was possibly being synthesized from some carbohydrate material. A study of the relative amount of blood fat used by the mammary gland of the cow has been in progress in the Minnesota laboratory for some time and some preliminary notes have been published, Shaw and Petersen (20, 21).

Using the Evelyn-Salter (5) method for the determination of hemoglobin, Shaw and Petersen (24) found that with any excitation or disturbance of the animal there were invariably large blood volume changes in the mammary gland regardless of the rapidity with which the samples were taken. These changes did not occur in the mammary gland of the com-

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[2] Now at the University of Connecticut.

pletely undisturbed animal. Corrections of arteriovenous differences on the basis of blood volume changes produced values which were obviously untenable.

These findings not only account for many of the unexplained variations in arteriovenous differences found in the data of all workers, but serve as a very valuable tool in determining whether or not the arteriovenous differences can be expected to represent the normal metabolism of the gland.

In a previous communication, Shaw and Petersen (21) reported that the quantity of blood fat used by the mammary gland increased with the increase in time following milking. This paper deals with the explanation of this and other phenomena associated with the fat metabolism of the mammary gland.

EXPERIMENTAL

For the arteriovenous studies the venous blood was drawn from the subcutaneous abdominal vein. In most of the experiments, the skin was anesthetized at the point of venipuncture with ethyl chloride since any disturbance to the animal is usually due to the venous and not the arterial puncture. The arterial blood was obtained by rectal puncture from either the prepudic or internal iliac arteries. The arteriovenous data unless otherwise reported includes only those experiments in which there were no detectable blood volume changes and in which the animals showed no sign of disturbance.

The following chemical techniques were used: hemoglobin, Evelyn-Salter (5); blood fat, Allen (1); blood glucose, Shaffer and Somogyi (19); plasma calcium, Clark-Collip (3); and, plasma phosphorus, Fiske and Subbarow (7). The obstetrical pituitrin used, being predominately oxytocin, will be referred to as such.

More than 200 arteriovenous blood fat differences have been determined since the study of the amount of blood fat used by the lactating gland was initiated in 1936. However, we were unable to obtain an orderly picture until the relationship of excitation to blood volume changes in the gland was established. Practically all of the data reported in this communication were obtained from the Holstein herd at the University of Minnesota.

In 52 of the analyses of blood fat differences, there were no measurable blood volume changes in the gland. This data is presented in figure 1. As will be observed, there is little or no blood fat lost to the gland immediately after milking. In two cases there was actually a passage of fat back into the blood. Following the period immediately after milking, there was a slowly increasing uptake of blood fat by the gland for a period of about four hours after which the fat was used at a more constant rate, although the three highest values are to be found nine and ten hours after milking. In two cases, the cows were not milked out for a period of more than 15 hours. The

results of blood samples taken at this time show that the passage of fat into the gland had almost ceased. The failure of the gland to use fat at this particular time is undoubtedly due to the pressure built up in the gland.

The passage of blood calcium into the lactating gland presented a similar picture. It will be seen from figure 2 that milking retarded the uptake of calcium considerably. The building up of considerable pressure in the gland at the end of 15 hours also almost completely stopped the use of blood calcium. However, blood glucose continued to be used by the gland at this time in normal amounts, the arteriovenous change being 14.6 and 11.4 mg. per cent in the two experiments cited.

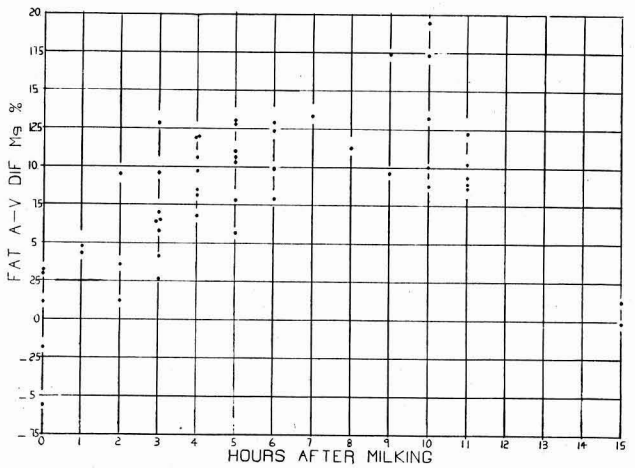

FIG. 1. Arteriovenous differences in blood fat in relation to time after milking.

The use of blood calcium by the gland is considered as the best indirect measure of the volume of blood passing through the gland per unit volume of milk. The more direct measures have not been considered because of the accompanying excitation and because the analyses are necessarily too limited to give any adequate picture of the loss of the various substances to the mammary gland. From the great variations in individual animals in arteriovenous differences and in the rate of blood flow as shown by the uptake of oxygen (18), it is apparent that balances on the mammary gland can not be considered significant unless large numbers of data are available from experiments in which blood volume changes have not occurred. The errors involved become minimized as the number of good observations increase. Calcium, therefore, was used in an attempt to determine what per cent of the milk fat was derived from blood fat. The average loss of fat to the gland in the data presented in figure 1 was 9.0 mg. per cent. The average calcium loss to the gland in the data presented in figure 2 was 0.29 mg. per cent. The values obtained 15 hours after milking were not in-

cluded because the glands were abnormally distended at that time. On the basis of 120 mg. per cent of calcium in the milk approximately 410 volumes of blood plasma would be required to provide milk calcium. Similarly, on the basis of 3500 mg. per cent of fat in the milk, the volume of blood plasma required to produce the milk fat is approximately 390. Even allowing for considerable error in these calculations, it is apparent that most of the milk fat is derived from the blood fat. Recently some observations have been made which further favor the conclusion that most of the milk fat is derived from blood fat. In a series of operations on the glands of cows and goats, it was observed that the flow of lymph was very large and indicated that any attempt to conduct a balance of the mammary gland would have to include lymph. An analysis of the lymph demonstrated that while considerable calcium was removed from the gland by the lymph, very little fat was carried away in this manner. These data will be presented elsewhere in a later communication.

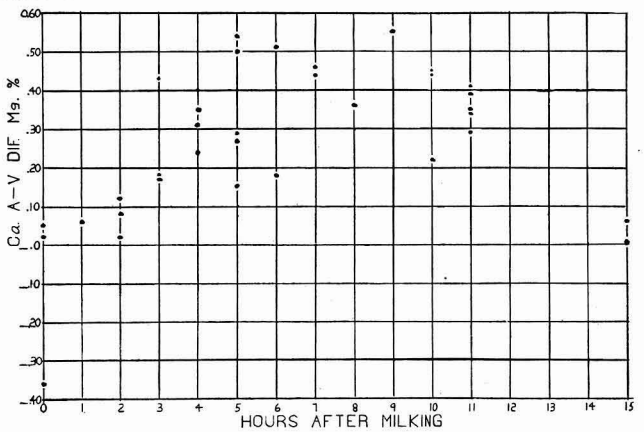

FIG. 2. Arteriovenous differences in blood calcium in relation to time after milking.

In the method used for fat determination neither phospholipids nor free fatty acids are recovered. The relatively large amount of fat shown to be used by the gland by this method is therefore limited to neutral fat, cholesterol and cholesterol ester fractions.

Several experiments were conducted to further study the peculiar effect of milking upon the uptake of blood substances by the lactating gland. One half of the udder was milked out, leaving the other half distended with milk. Arterial blood samples and right and left venous samples were then taken simultaneously. The results of a series of such experiments are presented in table 1. Unfortunately, in several of these experiments, hemoglobin determinations were not made. The data are presented, however, because the striking effect noted in these cases occurs consistently. Only a small quantity of blood fat was taken up by the gland on the side which had

TABLE 1

The effect of milking out one half of the udder upon the use of blood substances by both right and left glands

	No. of observations	Arterial	Left venous	Right venous (side milked out)
Blood Fat mg. %	1*	239.7	235.6	238.5
	2	233.5	219.5	229.7
	3	201.9	187.1	195.6
	4	162.2	153.3	158.6
	5	214.2	200.0	209.9
	6	199.5	188.9	198.3
	7*	182.7	173.4	179.2
Calcium mg. %	8*	10.00	9.32	9.95
	9	8.20	7.98	8.20
	10	9.66	9.49	9.60
Phosphorus mg. %	11*	.....	4.92	5.47
Amino-Acids mg. %	12	4.41	4.00	4.00
	13	3.90	3.62	3.58
Glucose mg. %	14	64.0	55.6	56.0
	15	64.8	58.2	58.0
	16	74.0	56.6	56.6
	17*	50.0	44.6	43.4

* No blood volume changes.

been milked out. Blood fat continued to pass into the unmilked side rather freely, however. It, thus, became apparent that the presence of milk in the gland was necessary to facilitate the normal transfer of fat from the blood to the secretory tissue of the gland. The same appeared to be true of both calcium and acid soluble phosphorus. In observation number 11, no arterial sample was drawn. That the milked side, however, used less phosphorus than the unmilked side is shown by the fact that in the former case the level of venous blood phosphorus was higher, indicating that less phosphorus had been removed. Both sides continued to use glucose and amino acids in equal amounts which was to be expected from previous findings in which it was shown that the arteriovenous differences of glucose and amino acids were not affected materially by the time interval after milking, Shaw, Boyd and Petersen (22), Shaw and Petersen (23).

These results were quite surprising inasmuch as it was thought that if the milking out had any effect it would be to increase the arteriovenous differences. With the release of the pressure in the gland by the removal of the milk, it might be expected that there would be an increased passage of certain substances into the gland. Such however, is not the case. The determination of hemoglobin shows that it is not merely a matter of concentration of the blood at this time.

Another series of experiments dealing with the effect of oxytocin upon arteriovenous differences serves to still further explain many of these phenomena. Since the work of Ely and Petersen (4) had demonstrated that oxytocin or an oxytocic-like principle must be responsible for the ejection of

milk, it was suspected that the failure of the gland to remove fat and other substances from the blood following milking was associated with the effect of this principle upon the gland.

Accordingly, the effect of injections of oxytocin upon the use of blood fat by the mammary gland was studied. A number of cows were injected with 10 I.U. of oxytocin after arteriovenous blood samples had been drawn, following which arteriovenous blood samples were again drawn. Usually the oxytocin was injected into the mammary vein into which a hypodermic needle had already been placed in obtaining the initial venous blood sample. In table 2, four such experiments are recorded in which no significant blood volume changes occurred. Samples were drawn from Cow Number 612 im-

TABLE 2

The effect of injections of oxytocin upon the use of blood fat by the mammary gland

		Hemoglobin %	Plasma fat mg. %	Remarks
Cow No. 612 Immediately after milking	Arterial	12.18	298.7	Before injection of oxytocin
	Venous	12.18	300.9	
	Arterial	12.18	294.8	After injection of oxytocin
	Venous	12.18	299.9	
Cow No. 615 2 hours after milking	Arterial	12.18	277.0	Before injection of oxytocin
	Venous	12.18	270.6	
	Arterial	11.87	277.2	After injection of oxytocin
	Venous	11.87	276.5	
Cow No. 449 10 hours after milking	Arterial	13.29	306.2	Before injection of oxytocin
	Venous	13.29	288.9	
	Arterial	13.29	301.3	After injection of oxytocin
	Venous	13.29	298.6	
Cow No. 577 Right side milked out	Left venous	12.95	184.4	Before injection of oxytocin
	Right venous	12.95	195.8	
	Arterial	12.92	192.5	After injection of oxytocin
	Left venous	12.97	191.2	
	Right venous	12.95	190.9	

mediately after milking. An increase of 2.2 mg. per cent in venous blood fat was observed. Following the injection of oxytocin, there was an increase in the venous blood fat of 5.1 mg. per cent. This procedure was repeated with Cow Number 615 two hours after milking. The arteriovenous fat difference of 7.6 mg. per cent decreased to 0.7 mg. per cent after the injection of oxytocin. The procedure was then repeated with Cow Number 449 ten hours after milking. The fat loss of 17.3 mg. per cent was decreased to 2.7 mg. per cent by the injection of oxytocin.

In the experiment with Cow Number 577, the right half of the gland was milked out and venous samples were taken simultaneously from both the

right and left mammary veins. With the needles remaining in the veins, oxycotin was injected and both right and left venous blood samples were drawn simultaneously with an arterial sample. Since the level of venous blood fat prior to the injection of oxytocin was higher on the side milked out than on the unmilked side, it was apparent that the unmilked side was using more blood fat. Following the injection of oxytocin, neither side used any appreciable quantities of blood fat. From the results of these experiments, it was apparent that intravenous injections of oxytocin almost completely inhibited the uptake of blood fat by the lactating mammary gland even in glands filled with milk. This was unexpected since it had previously been shown that the inhibiting effect of milking upon the use of blood fat by the gland did not occur until after the milk had been removed. It is believed, however, that the two effects are both due to oxytocin, and that the effect of injections of oxytocin upon the unmilked gland are due to the use of this principle in excess of physiological dosage.

In the experiments with the undisturbed animal in which there were no blood volume changes in the gland, the use of blood calcium and blood phosphorus appears to follow that of the fat. During excitation, however, the calcium and phosphorus presents an extremely varied picture and the arteriovenous differences are unpredictable. This is not true of blood fat. In an earlier communication, it was demonstrated that with a concentration of the blood in the gland excessive amounts of fat passed into the gland while with a dilution of the blood in the gland the fat often passed back into the venous blood. In a number of experiments, observations were made of the effect of oxytocin upon the arteriovenous fat differences in the gland of the excited cow. It will be observed in a typical experiment reported below with Cow Number 446 that the expected variations did not occur.

Cow 446	*Hemoglobin* %	*Plasma fat* mg. %	
Arterial	14.25	255.0	After injecjtion of oxytocin.
Venous	14.50	259.7	Cow excited.

With the concentration of the blood of 1.1 per cent, it was expected from previous experience that considerable blood fat would be retained in the gland. On the contrary, however, due to the effect of oxytocin, blood fat was concentrated in the venous blood to approximately the same extent as hemoglobin, indicating that here were no interchanges of fat between the gland and the blood plasma at this time. The injection of oxytocin also hindered the passage of calcium and phosphorus into the gland of the excited cow from time to time, but the effect was not as marked as in the case of fat.

DISCUSSION OF RESULTS

Following milking very little blood fat was taken up by the gland and, in some cases, immediately after milking, there was actually a passage of fat back into the venous blood. With the increase of the time interval following milking, blood fat was used in increasing amounts until about four hours after milking, after which there was a more constant uptake of fat by the gland. In some cases, the amount of fat used by the gland continued to increase for several hours longer.

The decreased use of blood fat by the lactating gland following milking was found to be associated with the removal of the milk from the gland. The stimulus of the "letting down" of milk did not in itself prevent the passage of blood fat into the gland. Injections of oxytocin, in apparently greater than physiological amounts, usually completely prevented the uptake of blood fat by the gland regardless of the time interval after milking.

Blood calcium and blood phosphorus were influenced in the same direction but with any excitation or changes in blood volume in the gland the results were more unpredictable. The uptake of glucose and amino acids, however, did not materially change.

In dealing with the transfer of substances from the blood to the gland, and from the gland to the blood, it appears that two phases must be reckoned with. Consideration must be given not only to the equilibrium between the blood plasma and the tissue fluid, but also to the equilibrium existing between the tissue fluid and the secretory cells of the gland. It can be assumed, as suggested by Starling, that the blood pressure decreases progressively along the capillary, being greatest at the arterial and least at the venous end. At the arterial end of the capillary, the hydrostatic pressure exceeds the colloidal osmotic force and fluid is forced from the capillary. At the venous end, the colloidal osmotic force exceeds the blood pressure and fluid passes from the tissue spaces into the capillary. The capillaries in the mammary gland of the lactating cow are apparently quite permeable to plasma fat as well as water, salts and a certain amounts of plasma proteins. It is believed that there is a continuous and relatively large flow of fluid between the blood plasma and the tissue spaces of the gland containing considerable plasma fat and other substances. A temporary change in blood pressure, pressure within the gland, or changes in the permeability of the capillaries would tend to alter, at least momentarily, the normal equilibrium existing between the plasma and the tissue fluid. These changes undoubtedly account at least in part for the variations in the arteriovenous differences in excited cows reported in an earlier communication (24). The mechanism by which oxytocin causes fat and calcium to increase in the venous blood after the gland has been milked out may be explained as being due to a greater momentary pressure in the tissue spaces following the constriction of the smooth muscle of the gland. This would

tend to offset the blood pressure in the venous end of the capillary more completely and possibly even exceed the force of the blood pressure at the arterial end of the capillary.

The effect of injections of oxytocin in preventing the passage of certain blood substances into the secretory tissue of the gland may be explained in part on the same basis. Indeed, it may well be that the complete contraction of the smooth muscle can not occur until the milk has been removed from the alveoli. However, it has been shown by Hammond (10) and others that the pressure is greatest following stimulation of the letting down of milk when the gland is still filled with milk. More significant, however, is the fact that fat is not used in normal amounts until three to four hours after milking and in some cases the increase in the use of fat continues for several more hours. It is highly improbable that the contraction of the smooth muscle of the gland by oxytocin would continue this length of time.

It is believed that during the process of milking the alveoli collapse rather completely due to oxytocin which results in a decrease in the permeability of the basement membrane of the secretory cells to blood fat and possibly other substances. With the gradual engorgement of the cells and the filling of the alveoli with milk, the basement membrane becomes increasingly permeable to the lipides in the fluid which is circulating between the plasma and the tissue spaces. The more freely diffusible substances, such as glucose and amino acids, are apparently not materially affected by these changes. The passage of substances from the interstitial fluid to the cells must be governed not only by the permeability of the basement membrane but also by the rate at which they are used for synthesis in the secretory cells. The fact that calcium and phosphorus are affected in a similar manner to that of fat may mean that they are taken in, in part, by combination with other materials such as calcium proteinate and in connection with fat phosphorylation in the transport of fat across the basement membrane of the secretory cells of the gland.

The work of Kelly (13) and Kelly and Petersen (14) has shown that there is considerable lipase in the lactating gland and that only free fatty acids are present in the basal part of the cell. This suggests the possibility that the action of lipase may be necessary in the transport of fat across the basement membrane. However, it is possible that the fat is not acted upon by lipase until it passes into the cell and that the action of lipase is so rapid in the basal portion of the cell that the fat is hydrolyzed before the material can be fixed and stained and therefore could not be detected in the cells of the excised gland.

The effect of oxytocin in preventing the loss of fat to the milk-filled gland is probably due to a partial collapse of the alveoli and the building up of excessive pressures in the gland which occurs following the injection of oxytocin.

The increasing uptake of blood fat by the gland with the increase in the time interval after milking would be explained by the gradual distension of the alveoli and the engorgement of the cells which makes the basement membrane increasingly permeable to the fat.

It is apparent from the observations herein reported that most of the milk fat is derived from the blood fat. These results are not in agreement with the suggestion of Smith and Dastur (25) that oleic acid is synthesized from carbohydrate and that the short chain fatty acids are by products of this synthesis. They found that during inanition there was a decrease of about 80 per cent in the original content of the lower fat acids, a deficiency which was almost entirely made up by an increase in oleic acid. If oleic acid were synthesized from carbohydrate material, it would be extremely difficult to explain why the gland used such large quantities of blood fat since oleic acid makes up about 32 per cent of the total milk fat.

On the basis of the quantity of fat used by the gland, it appears more likely that this apparent relationship between oleic acid and the short chain acids is due to a breakdown of oleic glycerides as suggested by Hilditch and Thompson (12), Hilditch and Paul (11) and Shaw and Petersen (20). If this is true, then some other explanation must be found for the high respiratory quotient reported by Graham *et al.* The data of Lintzel is of little help in this regard since his blood samples were drawn shortly after milking at which time the arteriovenous fat differences would be expected to be small. In fact, if the milk fat of the cow and the goat is produced from similar blood precursors, the arteriovenous fat differences in goats should be about twice that found in cows because the volume of blood per unit volume of milk as reported by us here and elsewhere is about double that reported for goats.

CONCLUSIONS

1. Very little blood fat is taken up by the gland immediately after milking. With the increase of the time interval following milking, blood fat is used in increasing amounts until about four hours after milking, after which time the fat is used in more constant amounts. Calcium appears to present a similar picture. The absorption of fat and calcium ceases about 15 hours after milking. Glucose continues to be used in normal amounts, however.

2. The passage of blood fat into the gland can be prevented by intravenous injections of oxytocin.

3. The passage of blood fat into the lactating gland and to a lesser extent of calcium and acid soluble phosphorus is associated with the distension of the alveoli and the filling of the secretory cells with milk. Blood glucose and amino acids are not similarly affected but continue to be used in fairly constant amounts. A hypothesis is developed on the basis of the observations to account for the passage of blood substances into the glandular tissue.

4. The quantity of blood fat used by the gland is sufficient to account

for all of the milk fat and justifies the conclusion that but little milk fat is produced from other substances.

5. The average use by the lactating gland of the cow of 9.0 mg. per cent of plasma fat is limited to neutral fat and/or cholesterol fractions.

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THE EFFECT OF STORAGE TEMPERATURES UPON CERTAIN CHARACTERISTICS OF BOVINE SEMEN

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Motility of spermatozoa has been widely studied and at one time motility was identified with fertilizing capacity. While this determination was easily made, some workers, (3, 12, 13) believed that motility was not necessarily a criterion of fecundity of spermatozoa. Williams and Savage (15, 16) in their investigations of bulls, showed that no great dependability could be placed upon the motility of spermatozoa. Lagerlöf (11) found that in sterile bulls and in those of reduced fertility, there was great variability in the motility of spermatozoa. Donham *et al.* (8) found a definite correlation between the conception rate of cows and motility of the spermatozoa, and they stated that semen which contained less than 90 per cent of active spermatozoa should be regarded as abnormal, since it did not insure satisfactory fertilization. While a high percentage of active motility of spermatozoa does not guarantee the fecundity of the semen, it is, therefore, likely, according to the workers cited, that reduced motility would indicate reduced fertility or even sterility.

Various other criteria have been set up for the evaluation of the fecundity of bovine semen specimens. Volume, concentration of spermatozoa, and abnormalities of spermatozoa have been suggested as important factors related to fecundity of semen. The standards formulated were the result of studies of semen from bulls with disturbed fertility (1, 2, 15, 16). An analysis of semen samples of a group of fertile bulls would seem to furnish a better basis of appraisal of fertilizing capacities of sperm cells. In a previous study of fresh semen samples obtained from 11 fertile bulls (6), it was shown that there were certain relationships between volume of semen sample, percentage of progressive motility, concentration of spermatozoa, and pH value of the semen. The fertilizing capacity of the semen appeared to be dependent upon a combination of these factors rather than a single one. It might be mentioned that the mean number of spermatozoa per mm.^{3} in the samples from the 11 fertile bulls was 734,000 with a range of from 8,000 to 1,997,000 in a total of 266 ejaculates in studies of the first, second, and third successive ejaculates (6). The mean number of the 168 samples

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of the first ejaculate was 826,000. Anderson (1, 2) concurrently reported a mean concentration of 873,000 per mm.^{3} with a range of from 510,000 to 1,875,000 in samples obtained from six fertile bulls. The number of ejaculates studied was not mentioned nor which ejaculate. Anderson showed also that the percentage of abnormal spermatozoa in fertile bulls was 8.1; in bulls of reduced fertility 13.1; and in sterile bulls 17.6 per cent. Unpublished data at the University of Nebraska dairy herd have revealed that atypical spermatozoa are of normal occurrence in the case of fertile bulls. Hotchkiss *et al.* (10) in a detailed analysis of 200 fertile men whose wives were in the first half of gestation arrived at similar conclusions regarding characteristics and fecundity of semen samples. They observed further that atypical spermatozoa were not so great a factor affecting fecundity as had been concluded by some investigators since these abnormal cells were found in the semen of the 200 fertile men. From these observations it may be concluded that abnormal cell count, at least when the proportion is relatively small, is of minor importance in evaluating the fecundity of a semen sample.

In this study the influence of four temperatures during storage upon the motility and the pH values of semen samples obtained from 11 fertile bulls and grouped according to the initial percentage of progressive motility of spermatozoa, concentration of spermatozoa per mm.,^{3} and pH, is presented. The characteristics of the semen of the bulls from which the samples were obtained are presented and conceptions resulting from insemination with both fresh and stored semen are listed.

PROCEDURE AND METHODS

The semen samples were collected from 11 fertile bulls of the Jersey, Guernsey, Ayrshire, and Holstein breeds over a period of approximately nine months. The bulls ranged in age from one year and two months to eight years (table 4). All were free from contagious diseases. For housing, the bulls were kept in a semi-closed shed and each was allowed to run in a paddock for a half day, each day. A limited quantity of alfalfa hay was fed daily and grain was fed at the rate of between three-fourths of a pound and one pound for each 100 pounds of live weight. The grain mixture contained about 14 per cent of digestible protein and one per cent each of steamed bone meal and iodized salt.

The regular procedure was to take a semen sample every third day but sometimes only one sample was taken during a week. Semen samples were taken with the Cambridge type artificial vagina (14) and the ejaculating bull was allowed to mount either a protected cow or another bull. Each semen sample collected was kept separate and the second and third ejaculates were taken immediately after the first. Care was taken to wash the prepuce and irrigate the sheath of the bull with warm water before the first

semen sample was taken. Aseptic precautions were followed carefully in the collection, handling, and storing of semen samples. All equipment coming in contact with semen was first rinsed with diluter. The diluter used was made according to a Russian formula (14) by dissolving 13.6 grams of sodium sulfate, 12 grams of anhydrous glucose, and 5.0 grams of salt-free peptone in one liter of distilled water and sterilizing at a steam pressure of 13 pounds for 30 minutes in an autoclave. No diluter was added to any sample of semen. Each ejaculate was emptied, as soon as possible after collection, into a sterile cotton-stoppered test tube and this in turn was placed in a larger test tube where it rested on a cork at the bottom. The double test tube was then placed in a pail of water at a temperature between 50° and 60° F. except for samples to be stored at 70° F. This method cooled the semen sample at the rate of about one degree F. per minute and cooling was completed in thermostatically controlled storage boxes.

A microscope fitted with a low-power objective and equipped with a slide and coverslip preparation in a stage incubator at a temperature of 102° F. was used to determine the motility which was estimated to the nearest 10 per cent of progressive motion of spermatozoa across the field. Initial motility was determined within an hour after the semen sample was taken. The determination of pH values was made at a temperature of 77° F. (25° C.) with a potentiometer equipped with a quinhydrone gold electrode. The concentration of spermatozoa per mm.^{3} was determined by the use of a haemocytometer using standard procedure. The ejaculates of semen varied in volume and each ejaculate was divided into one-cc. samples which were stored at various temperatures until observed. The storage chambers were equipped with thermostatic switches and maintained temperatures with a variation of one degree F. The observations recorded were made only on semen samples which showed spermatozoa in progressive motion. The procedure for insemination consisted of the use of a glass or stainless steel inseminating tube which was inserted about an inch into the cervix before the semen sample was delivered. The amount of semen used for each insemination was one cc.

EXPERIMENTAL

Semen samples that were examined varied in the percentage of initial progressive motility from 10 to 100. More than 90 per cent of the samples ranged from 50 to 100 per cent in initial motility and only those were included since samples showing an initial progressive motility of less than 50 per cent may have been due to faulty technique in collecting the samples.

In table 1 the semen samples were divided into two groups, namely, those having an initial progressive motility of 50 to 70 per cent, and those having a motility ranging from 80 to 100 per cent. The pH values of the fresh samples and at various periods during storage, when stored at 35°, 40°, 50°,

TABLE 1

Influence of storage temperature upon pH values of semen samples grouped according to percentage of initial motility

Storage period	Storage at							
	35° F.		40° F.		50° F.		70° F.	
	Samples	pH	Samples	pH	Samples	pH	Samples	pH
	hrs.	*no.*	*mean*	*no.*	*mean*	*no.*	*mean*	*no.*	*mean*
Range in initial motility 50–70 per cent								
Fresh	21	7.01	24	7.05	85	7.16	21	7.28
24– 28	2	6.78			22	6.54	13	6.31
48– 52	5	6.79	1	6.10	52	6.43	8	6.02
72– 76	7	6.77	5	6.70	19	6.22	1	6.39
96–100	10	6.90	10	6.90	28	6.25		
120–124	2	7.03	2	6.31	8	6.14		
144–148	5	6.66	7	6.59	9	6.21		
168–172			3	6.62	1	6.85		
192–196	8	6.85	7	6.67				
216–220	3	6.64	2	6.40	1	5.92		
240–244	1	6.97	6	6.54				
264–268								
288–292	9	6.85	5	6.34				
312–316			2	6.97				
336–340			1	6.18				
360–364	1	7.05						
384–388	9	6.70	4	6.97				
408–412	2	6.73	2	6.06				
432–436	1	7.14						
456–460								
480–484			1	6.69				
Range in initial motility 80–100 per cent								
Fresh	46	6.82	55	7.03	141	6.93	12	6.84
24– 28	6	6.53	1	6.64	52	6.38	7	5.96
48– 52	31	6.78	14	6.68	118	6.36	2	5.80
72– 76	6	6.59	6	7.05	37	6.11		
96–100	21	6.72	34	6.71	66	6.04		
120–124	9	6.62	4	6.72	29	6.03		
144–148	21	6.70	15	6.64	32	6.05		
168–172	2	7.32	3	6.86	9	6.44		
192–196	11	6.73	27	6.52	5	6.26		
216–220	5	6.64	4	6.39	2	6.21		
240–244	1	7.01	5	6.59	2	5.99		
264–268	2	6.78	1	6.88				
288–292	6	6.68	19	6.48				
312–316								
336–340	2	6.83	1	6.77				
360–364	4	6.57	1	6.24	2	5.84		
384–388			11	6.32				
408–412			1	7.11				
432–436	1	7.27						
456–460			3	6.11				
480–484			2	6.36				

and 70° F., are presented in the various columns of the table. All values reported represent means if more than one sample is represented. The

initial pH values for the group 50 to 70 per cent motility are 7.01, 7.05, 7.16, and 7.28, while the values for the group 80 to 100 per cent motility were

TABLE 2

Influence of storage temperature upon motility of spermatozoa in semen samples grouped according to pH values

Storage period	Storage at							
	35° F.		40° F.		50° F.		70° F.	
	Samples	Motility	Samples	Motility	Samples	Motility	Samples	Motility
hrs.	*no.*	*mean*	*no.*	*mean*	*no.*	*mean*	*no.*	*mean*
Range in initial pH—6.40–6.99								
Fresh	47	81	44	76	125	80	17	75
24– 28	12	60	2	60	62	61	10	35
48– 52	33	53	15	44	107	48	7	13
72– 76	10	47	9	39	37	38	1	10
96–100	25	39	24	37	67	28		
120–124	9	41	5	28	30	24		
144–148	20	30	16	29	35	20		
168–172	3	13	1	30	6	22		
192–196	13	26	19	29	5	20		
216–220	9	17	5	24	2	1		
240–244			7	17	2	5		
264–268	2	11						
288–292	7	19	13	18				
312–316								
336–340			2	6				
360–364	3	13	1	10	2	1		
384–388	2	10	8	7				
408–412	2	20	2	10				
432–436								
456–460								
480–484								
Range in initial pH—7.00–7.60								
Fresh	20	75	37	78	117	67	16	67
24– 28								
48– 52			3	30	21	50	10	28
72– 76	7	44	6	35	69	39	3	13
96–100	3	30	6	35	14	33		
120–124	9	29	20	24	26	30		
144–148	2	30	2	25	7	23		
168–172	8	30	9	27	8	23		
192–196			5	28	2	15		
216–220	8	21	15	17				
240–244	2	30	1	20	1	1		
264–268	1	20	4	28				
288–292			1	20				
312–316	8	15	10	12				
336–340			2	20				
360–364	2	10						
384–388	2	10						
408–412	7	10	6	14				
432–436	2	10						
456–460								
480–484			3	7				

respectively 6.82, 7.03, 6.93, and 6.84. It will be apparent from table 1 that storage at 70° F. causes a very rapid decrease in the pH value and that storage at 50° F. showed a rapid decline for the first 24 hours and a slower decline thereafter. At storage temperatures of 35° and 40° F. there was a very slow rate of decline in pH values for all periods of storage, the least shift occurring at 35° F. It is apparent that untreated semen tends to become more acid upon storage and that higher temperatures of storage tend to increase the rapidity of decline in pH. Semen stored at the higher temperatures reached lower pH values than that stored at 35° and 40° F.

Table 2 presents the data arranged according to the initial pH values of semen samples as measured in motility initially and after storage. In this table, the semen samples that ranged in initial pH values from 6.40 to 6.99 were grouped, and those that showed a range of 7.00 to 7.60 were grouped. In the first mentioned group, the initial mean percentages of progressive motility were 81, 76, 80, and 75 for the samples that later were stored respectively at 35°, 40°, 50°, and 70° F. The corresponding initial mean percentage of progressive motility for the semen samples showing a range of 7.00 to 7.60 pH value were 75, 78, 67, and 67. In every case there was a large drop in percentage of motility for samples stored 24 hours and a smaller relative decline during storage for longer periods. There was comparatively little difference between the storage temperatures 35° and 40° F. as to the keeping quality of the semen as measured by motility, although the lower temperature appeared to be slightly better. At 50° F. the rate of decline in motility was more rapid than at the lower storage temperatures and the survival was shorter.

Table 3 presents a study of semen samples during storage as measured by pH values. Initially the samples were divided into two groups, namely, those where the concentration per mm.^{3} ranged from 1 to 999,000, actually from 8,000 to 999,000, and those which ranged higher, namely, from 1,000,000 to 1,999,000. It will be noted that the mean pH values for fresh samples of the lower concentration group showed 6.98, 7.15, 7.18, and 7.29 while for the higher concentration group, the respective values were 6.74, 6.76, 6.70, and 6.73. Storage at 70° F. always showed a marked drop in pH value during the first 24 hours and at slower decline thereafter. The 50° F. storage temperature showed the same general trend, but the drop in pH during the first 24 hours was not so great and the samples showed motility after longer storage periods. The samples stored at 35° and 40° F. showed the best keeping quality. Not only was the rate of decline slower, but the total decline in pH values was less than for the higher storage temperatures.

In table 4, the characteristics of fresh semen from the 11 fertile bulls are presented together with the fecundity of fresh and stored semen. The figures presented are mean values for volume, motility, pH value, and concentration for all samples of successive ejaculates obtained from the bulls

TABLE 3

Influence of storage temperature upon the pH value of semen samples grouped according to concentration of spermatozoa per mm.³

Storage period	Storage at 35° F.		40° F.		50° F.		70° F.	
	Samples	pH	Samples	pH	Samples	pH	Samples	pH
	Concentration of spermatozoa per mm.³ 1–999,000							
hrs.	*no.*	*mean*	*no.*	*mean*	*no.*	*mean*	*no.*	*mean*
Fresh	40	6.98	55	7.15	178	7.18	23	7.29
24– 28	5	6.76			53	6.55	14	6.30
48– 52	21	6.96	13	6.91	121	6.57	9	5.99
72– 76	5	7.07	8	6.99	38	6.24	1	6.39
96–100	18	6.97	34	6.86	64	6.20		
120–124	5	6.76	3	7.11	20	6.09		
144–148	13	6.89	16	6.77	26	6.17		
168–172	2	7.62	4	6.89	6	6.51		
192–196	8	6.94	24	6.66	3	5.88		
216–220	5	7.13	3	6.59	2	6.02		
240–244	1	7.01	8	6.85	1	5.73		
264–268	1	6.75	1	6.88				
288–292	7	6.89	18	6.49				
312–316			2	6.96				
336–340	1	6.93	1	6.77				
360–364	2	6.93			1	5.92		
384–388	5	6.66	13	6.57				
408–412			2	6.04				
432–436	2	7.20						
456–460			2	6.12				
480–484			4	6.60				
	Concentration of spermatozoa per mm.³ 1,000,000–1,999,000							
Fresh	27	6.74	26	6.76	63	6.70	10	6.73
24– 28	3	6.31			20	6.07	6	5.91
48– 52	17	6.51	5	6.36	50	5.94	1	5.86
72– 76	8	6.45	4	6.68	17	5.93		
96–100	13	6.54	11	6.41	30	5.91		
120–124	6	6.63	4	6.28	18	6.08		
144–148	13	6.49	7	6.33	11	5.88		
168–172	1	7.04	1	6.51	4	6.51		
192–196	10	6.64	10	6.33	3	6.43		
216–220	5	6.45	3	6.20	1	6.30		
240–244			4	6.17	1	6.25		
264–268	1	6.81						
288–292	7	6.63	6	6.33				
312–316								
336–340	1	6.72	1	6.18				
360–364	3	6.48	1	6.24				
384–388	3	6.66	3	6.26				
408–412	2	6.73	1	6.14				
432–436								
456–460								
480–484								

during the study. The ranges for all samples were as follows: volume—0.5 to 12.0 cc.; motility—10 to 100 per cent; pH value—6.18 to 8.31; and concentration—8,000 to 1,997,000 mm.³ (6). The semen used for insemina-

TABLE 4

Characteristics and fecundity of semen when fresh and after storage at different temperatures

Name of bull	Breed	Average age years months	Fresh semen						Stored semen							
			Volume	Initial motility of spermatozoa	Initial pH value	Concentration of spermatozoa M/mm.³	Inseminations	Conceptions	35° F. stored 24–47 hours		40° F. stored 24–99 hours		50° F. stored 24–59 hours		All samples stored 24–99 hours	
									Inseminations	Conceptions	Inseminations	Conceptions	Inseminations	Conceptions	Inseminations	Conceptions
			mean	mean	mean	mean										
	*		*c.c.* **	*per cent* **	**	*no.* **	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*	*no.*
Beta	A	3y 3m	(43) 5.8	(24) 76	(24) 6.74	(24) 1008	6	5								
Bon Jamie	A	2y 0m	(33) 4.3	(43) 61	(40) 7.19	(43) 477										
Hankey	A	8y 0m	(79) 4.3	(53) 79	(47) 6.78	(50) 1024	9	8			2	1	3	1	5	2
Benedict	G	3y 6m	(82) 4.2	(44) 77	(40) 7.14	(43) 656	20	15			2	2			2	2
Marose	G	7y 1m	(16) 3.1	(7) 70	(7) 6.79	(7) 700	2	1								
Caliph	H	2y 6m	(89) 4.6	(27) 84	(20) 7.06	(26) 692	40	29			1	1	3	2	4	3
Garbon	H	1y 2m	(38) 3.4	(17) 67	(12) 7.33	(17) 371	26	18			2	1	1	0	3	1
Quanto	H	4y 7m	(33) 4.8	(9) 73	(9) 6.70	(9) 967	13	9	1	1	3	0	1	1	5	2
Quinter	H	3y 4m	(50) 5.8	(27) 77	(27) 6.88	(27) 515	14	7	1	0			2	0	3	0
Fon 86 C	J	2y 4m	(30) 2.7	(14) 71	(9) 7.46	(14) 714	3	2					1	0	1	0
Radiance	J	3y 3m	(78) 2.4	(8) 65	(2) 6.70	(6) 833	21	18			1	1	4	0	5	1
All ejaculates			(571) 4.2	(273) 74	(237) 6.99	(266) 734	154	112	2	1	11	6	15	4	28	11

* A—Ayrshire.
G—Guernsey.
H—Holstein.
J—Jersey.

** No. samples.

tion was taken from these samples. Thus the general character of the semen can be judged with reference to inseminations and conceptions. Samples were not stored successfully for insemination at 70° F. Mechanical difficulties in the storage chambers prevented the use for insemination of many samples stored at 35° F. Most samples of fresh semen were used for insemination within four hours from the time of collection. For this study, however, fresh semen was considered to be any sample that was stored less than 24 hours. While there were no inseminations reported in this study for the bull Bon Jamie, he was proved previously to be a fertile bull. Of the 154 inseminations of fresh semen, 112 resulted in conceptions, or a conception percentage of 72.7, that is, 1.375 inseminations per conception. These figures are more favorable than were obtained in a previous study with massage obtained semen (5) when 181 inseminations resulted in 107 conceptions. This was a percentage of 59.1 or 1.69 inseminations for each conception. The table shows the length of storage, storage temperature and inseminations and conception using stored semen. The numbers are small and are merely indications. Grouping the samples stored at 35° and 40° F. together, there were 13 inseminations which resulted in seven conceptions or 53.8 per cent. The 15 samples stored at 50° F. resulted in four conceptions, a percentage of 26.7. For all stored samples, 28 inseminations resulted in 11 conceptions or 39.3 per cent.

DISCUSSION

It has been shown previously in table 1 that variations in initial pH and in the percentage of progressive motility for the two groups of samples stored at various temperatures appeared to have little or no effect upon the pH value during storage. Since all samples upon which pH determinations were made showed motility of spermatozoa it may be concluded that initial variations in motility within the ranges studied did not have an appreciable effect upon the keeping quality of spermatozoa as measured. It appears also that slight initial variations in the pH values have no appreciable effect upon the pH values developed during storage. However, the rate of decline in pH values developed during storage apparently is proportional to the temperature of storage; namely, the lower the storage temperature the slower the decline in pH of the semen, with but little difference being exhibited between the samples stored at 35° and 40° F.

When the samples of semen were sorted according to pH values and tabulated in terms of percentage of progressive motility as in table 2, it was found that initially, the semen samples with higher pH values showed slightly lower mean percentages of motility, which is a confirmation of the data in table 1. When measured by motility, the initial variation of pH values appeared to have no significant effect upon the samples after storage, since there was an approximately equal decline in the percentage of motility for the groups stored at the same temperature.

A study of table 3 where the semen samples were grouped according to concentration of spermatozoa per mm.^{3} indicates a definite relationship between high concentration and lower initial pH value. There was apparently no different effect after storage between the two general groups. Here again, the storage temperature seemed to be the critical factor as affecting the rate of decline in pH value with the lower temperatures of storage giving the best results.

From these studies it appears that fresh semen samples which possess a high degree of motility tend to have a high concentration of spermatozoa per mm.^{3} and pH values slightly on the acid side. Based on a population study it is apparent that these factors are definitely associated with fecundity, since the values of the various criteria of the 182 semen samples used for insemination must have been close to the mean (table 4). While it is true that there are considerable variations in the characteristics of successive ejaculates of individual bulls (6, 7), the data in table 4 indicate that the mean values of the semen characteristics of the individual bulls varied little from the mean of the population. Since the volume and the motility of the fresh semen samples used for insemination were substantially the same (volume 1 cc., motility 70–90 per cent) it does not seem likely that any deleterious influence on the fecundity or fertilizing capacity of the semen could have been exerted by these factors in the cases discussed. Whether it is the pH value or the concentration of spermatozoa which influenced the fertilizing capacity of the fresh semen samples of the individual bulls is not apparent since there are no great differences in the percentage of conception between the individual bulls. Apparently, the mean concentration of spermatozoa from individual bulls may vary a maximum of 363,000 and the pH value 0.34 unit from the population mean, without having any appreciable effect on fecundity.

Obviously, the fecundity of stored semen was inferior to that of fresh semen. Since volume and concentration were usually the same, and motility was but slightly lower, the decrease in fecundity of the stored semen may therefore be sought in biological changes as manifested by a decline in the pH value. It has been shown in tables 1 and 3 that there is a rapid shift in the pH value of semen at various temperatures during storage and that the shift in pH was greater with the higher temperatures. The least change in pH took place during storage at 35° and 40° F. The fecundity of the stored semen corresponds to this finding. Combining the semen samples stored at 35° and 40° F., which were used for insemination, a conception percentage of 53.8 was obtained; whereas, those samples stored at 50° F. resulted in a conception per cent of only 26.7. It is reasonable to believe that it is not the pH *per se* which affects the fecundity, but that the shift in pH values of the semen is an expression of certain catabolic processes which take place during storage and which are detrimental to spermatozoa although the sperm cells are inactivated by lowering the temperature.

Decreased fecundity of stored semen has been observed by other workers.

Using motility as a criterion of survival, Hatziolos (9) found that the average length of life for bovine sperm was 27 hours when stored at 32°–37° F. and 22 hours when stored at 37°–43° F. The fecundity of semen stored at these temperatures was very low. Of 20 cows inseminated only two became pregnant, one conceived from a 24-hour-old sample and one from a sample 48 hours of age. Chabibullin (4) found great differences between the motility percentage and the conception percentage of stored sheep sperm. Thus the motility following 24 hours of storage was 67–70 per cent while the corresponding conception rate was 31–36 per cent. He believed that a storage temperature of 46°–50° F. was the important one for storing sheep sperm.

Although motility is necessary for fertilization and a lowering of motility is commonly accepted as being associated with reduced fertility, it would seem that motility should be used with caution as a single criterion of fertilizing capacity of stored semen. At best the microscopic determination of motility is only an estimate, and chemical changes detrimental to the fecundity of spermatozoa are likely to occur before any change in motility is manifested. In a study of the effect of storage upon semen, a determination of the motility of spermatozoa taken in connection with the decline in pH due to natural causes, may possibly offer a laboratory test that can be correlated with fecundity.

SUMMARY

Semen samples obtained by means of the artificial vagina from 11 fertile bulls were grouped according to motility, pH values, and concentration, and the effects of storage at 35°, 40°, 50°, and 70° F. were studied.

When the semen samples were grouped according to percentage of initial progressive motility, the higher group showed lower initial pH values but no apparent difference was noticeable between the groups during storage. The pH values declined during storage, the lower the temperature the slower the decline.

Grouping semen samples according to initial pH values indicated that the lower pH value was associated with higher progressive motility. During storage there was no apparent difference between the groups but there was a decline in percentage of progressive motility and the higher the temperature the faster the decline.

Semen samples, when grouped according to concentration of spermatozoa per mm.,^{3} showed that the higher concentration had the lower pH values. When stored at the various temperatures there was no difference in rate of decline in pH values between the groups.

Average semen characteristics for 11 fertile bulls, based upon several hundred samples, together with the number of inseminations and conceptions for both fresh and stored semen are presented. A total of 154 inseminations with fresh semen resulted in 112 conceptions, a conception percentage of 72.7, or 1.375 inseminations per conception. Grouping all stored

samples, 28 inseminations resulted in 11 conceptions, a conception percentage of 39.3, or 2.545 inseminations per conception. Fifteen inseminations from samples stored at 50° F. resulted in four conceptions or 26.7 per cent, while 13 inseminations from samples stored at 35° and 40° F. resulted in seven conceptions or 53.8 per cent.

A storage temperature of 35° F. was found to be the most advantageous for storing semen, based upon motility and pH values, since at that temperature the least change occurred. The fecundity of semen is best preserved when stored at the lower temperatures.

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Milk--Bacteriology

A COMPARISON OF THE RESULTS OBTAINED FROM INCUBATING BACTERIOLOGICAL PLATES AT 32°C. AND 37° C. ON THE BACTERIAL COUNTS OF MILK

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H. P. Hood & Sons, Inc., Boston

As a result of a ruling of the Boston Board of Health stipulating the use of 32° C. instead of 37° C. as the incubation temperature for making bacterial counts on milk, several series of parallel determinations were made using various grades of milk. Appropriate dilutions of the milk sample to be tested were made and two plates were inoculated from the same dilution blank. Tryptone-glucose-beef-extract milk agar (A.P.H.A. 7th edition) was poured into each plate and one of the plates incubated at 37° C., the other at 32° C., for forty-eight hours.

The data presented in tables 1 to 4 inclusive show scattered diagrams of the distribution of counts on the two media. Of particular interest are the results on grade A raw milk purchased for pasteurization and sale as grade A pasteurized milk. This milk is purchased on a basis of full premiums for milk with a bacterial count below 10,000 per ml. and a sliding scale of partial premiums for milk with bacterial counts between 10,000 and 25,000 per ml.

Obviously, if the incubation of plates at 32° C. materially increased the bacterial counts, a producer might receive partial premiums or perhaps no premium on milk which would have earned full premium if the plates had been incubated at 37° C. If the effect of such a change in the incubation temperature proved serious in this regard, it would leave but two alternatives; first, to change the premium bases in keeping with the increased counts, and second, to improve the quality of the milk (which is, of course, the objective of the Board of Health in changing the requirements). If, on the other hand, the increases in counts do not seriously affect the premium scale, or if the normal variations of the plating procedure overshadow the increases in counts induced by the lower incubation temperature, no change in the premium schedule need be made.

A study of the data in table 1 based on 253 samples of grade A raw milk as sampled at the receiving station leads to the general conclusion that the lower temperature of incubation has not seriously affected the premium schedule and that the effect is partly offset by the factor of normal variation of the plating procedure.

In table 2 are shown the results based on 777 samples of market milk which were pasteurized in the laboratory by holding for 33 minutes at 143.5° F. in a thermostatically controlled water bath. These counts are used by the field men as an important part of the quality control program. An arbitrary

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Bacteria, Effect of temperature on

standard of 5,000 is used as a criterion of the need of immediate farm inspection. The extent to which these counts are increased by the lower incubation temperature has no effect on the price paid to the producer. An essentail feature of any quality control program is the extent to which it enables the detection of the sources of poor quality milk. If the lower temperature of incubation more effectively throws a spotlight on the less desirable producer, the change in procedure would be beneficial to the central objective of the field man's efforts. It remains to be seen if the lower temperature of incubation will yield counts that check more closely with field inspection.

A study of the data in table 2 indicates that only a relatively small percentage of the counts were materially increased by the 32° C. incubation and that this effect was particularly noticeable in the samples in the higher brackets of counts. This, of course, suggests that the incubation of plates at 32° C. tends to accentuate the very conditions which a quality control program is designed to detect.

Tables 3 and 4 show the distribution of counts at 32° C. and 37° C. based upon samples taken from tank car shipments. In table 3 are presented the counts of the raw milk and in table 4 the "heat-resistant" counts on the same milk after pasteurization in the laboratory. From these data, as in previous tables, it seems logical to conclude that the lower temperature of incubation will not necessitate any material reorganization of the quality control program.

It should be emphasized, however, that all of these samples were "winter samples" collected between the months of January and March. Whether or not the bacterial flora of summer milk will be sufficiently different to alter the results cannot be determined from these data.

TABLE 1

A comparison of the results of incubating 253 raw milk samples from grade A dairies at 37° C. and 32° C. Tabular values refer to the numbers of samples

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.						
		0 to 5,000	5,001 to 10,000	10,001 to 15,000	15,001 to 25,000	25,001 to 50,000	50,001 to 100,000	Over 100,000
0 to 5,000	129	93	26	6	1	3		
5,001 to 10,000	72	21	30	14	4	3		
10,001 to 15,000	26	2	5	10	7	1		1
15,001 to 25,000	16	2	1		10	3		
25,001 to 50,000	9		1	1		5	1	1
50,001 to 100,000	1						1	
Total	253							

Observations from table 1

1. Of the 253 samples, 201 were in the full premium class (below 10,000) when the plates were incubated at 37° C. Of these 201 samples, 25 (12 per cent) were moved into the partial premium class (10,000 to 25,000) and 6 (3 per cent) were moved out of the premium class (above 25,000).
2. Of the 42 samples in the partial premium class (10,000 to 25,000) when the plates were incubated at 37° C., 27 (64 per cent) remained in the partial premium class,

10 (25 per cent) were moved into a full premium class, and 5 (12 per cent) were deprived of premium by incubating the plates at 32° C. In one of these the count at 32° C. was sufficiently increased to exceed the state legal standard of 100,000.

3. Due in part to normal variation in plate counting, one-third as many producers (12) were favored as were penalized (36) by the lower temperature incubation. Normal variation undoubtedly was responsible also for some of the penalties, that is, if the parallel plates had been incubated at the same temperature no doubt there would have been some "penalties."

TABLE 2

A comparison of the results of incubating parallel plates from 777 laboratory pasteurized market milk samples at 37° C. and 32° C. Tabular values refer to the numbers of samples

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.				
		0 to 1,000	1,001 to 2,500	2,501 to 5,000	5,001 to 10,000	Over 10,000
0 to 1,000	618	519	68	21	6	4
1,001 to 2,500	64	24	22	11	2	5
2,501 to 5,000	33	9	2	10	6	6
5,001 to 10,000	22	5	1	4	5	7
Over 10,000	40	2			5	33
Total	777					

Observations from table 2

1. Of the 777 samples, 715 gave counts below 5,000 when incubated at 37° C. Of these 715 samples, only 29 (4 per cent) gave counts in excess of this arbitrary standard when incubated at 32° C.
2. A study of the data indicates that a greater percentage of those samples in the higher brackets (37° C.) were increased by 32° C. incubation than was observed among the samples in the lower brackets of counts (below 2,500).

TABLE 3

A comparison of the results of incubating parallel plates at 37° C. and 32° C. from 140 samples of raw market milk from tank car shipments. Tabular values refer to the numbers of samples

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.			
		0 to 100,000	100,001 to 200,000	200,001 to 300,000	300,001 to 400,000
0 to 100,000	96	81	15		
100,001 to 200,000	33	6	24	3	
200,001 to 300,000	9		2	6	1
300,001 to 400,000	2	1			1
Total	140				

Observations from table 3

1. Of the 140 samples, 129 conformed to the arbitrary standard of 200,000 or below when the plates were incubated at 37° C.
2. Of these 129 samples, only 3 (2 per cent) were caused to exceed the arbitrary standard by incubating the plates at 32° C.

CONCLUSIONS

1. Grade A milk (253 samples, raw milk counts): As a result of incubating plates at 32° C., 12 per cent of the samples were moved from full to

TABLE 4

A comparison of the results of incubating parallel plates at 37° C. and 32° C. from 142 samples of market milk from tank car shipments pasteurized in the laboratory. Tabular values refer to the numbers of samples

Ranges of counts at 37° C.	No. of samples	Ranges of counts at 32° C.				
		0 to 2,500	2,501 to 5,000	5,001 to 10,000	10,001 to 20,000	Over 20,000
0 to 2,500	92	66	21	5		
2,501 to 5,000	32	10	16	4	2	
5,001 to 10,000	17		6	8	2	1
10,001 to 20,000	1			1		
Total	142					

Observations from table 4

1. Of the 142 samples, 124 were below the standard of 5,000 when incubated at 37° C. Of these 124, there were 11 (9 per cent) which were caused to exceed the arbitrary standard when the plates were incubated at 32° C.
2. Of the 18 samples which were above the 5,000 standard when incubated at 37° C., 12 (66 per cent) were also above the standard when incubated at 32° C.

partial premium class, and 3 per cent were deprived of premiums. Of the samples in the partial premium class (at 37° C.), 12 per cent were deprived of premium. To offset this, however, 25 per cent were moved back into a full premium class. (table 1.)

2. Market milk (777 samples, heat-resistant counts): On a basis of a 5,000 standard at 37° C., 4 per cent of otherwise acceptable counts were caused to exceed the standard when plates were incubated at 32° C. (table 2.)

3. Tank car samples (140 raw milk counts): On a basis of 200,000 standard (37° C.), 2 per cent of otherwise satisfactory samples were caused to exceed the standard by incubating the plates at 32° C. (table 3.)

4. Tank car samples (142 heat-resistant counts): On a basis of a 5,000 standard (37° C.), 9 per cent of otherwise satisfactory samples were caused to exceed the standard by incubating the plates at 32° C. To offset this, 33 per cent of the 18 samples which exceeded the 5,000 standard at 37° C. gave counts below that standard when incubated at 32° C. (table 4.)

5. The producers of grade A milk were not sufficiently penalized by the use of the lower temperature of incubation to warrant readjustment of the premium bases. It would seem more logical to correct this situation by intensified field work with the relatively few indicated producers, thereby benefiting by the change of methods, rather than loosening the requirements on all grade A producers.

6. Since the fundamental purpose of making most bacterial analyses on milk is to point the way toward improvement of milk supplies, it seems reasonable to conclude that the lower temperature of incubation facilitates the attainment of that objective.

METHOD FOR DETERMINING LOSSES OF BUTTER FAT IN THE CREAMERY*

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Creamery patrons and directors, and even some buttermakers, have only a vague understanding of what should be considered as a fair overrun to be obtained in the manufacture of butter. Some states, as Iowa, have laws specifying the maximum overrun that may be obtained. In such cases the creamery directors generally consider that a buttermaker who cannot obtain an overrun within a small fraction of the maximum legal overrun lacks in ability and they frequently look for another operator.

The current overrun trends have been studied at the Iowa Experiment Station for some time, with the cooperation of a group of carefully selected Iowa creamery operators. From the results thus obtained it has been possible to evolve a system by which it will be not merely possible to determine with a reasonable degree of accuracy the correct overrun, but also to locate various existing irregularities and losses of butter fat.

In this study each creamery cooperating with the Experiment Station submits samples from one churning monthly. These samples which are analyzed chemically, consist of a sample of the cream before pasteurization, another after pasteurization and one after the cream is in the churn. A sample is taken from the can rinsings, one from the buttermilk, one from the starter and three samples from the butter; the butter samples are taken from different locations in the churn. Forms have been prepared for recording results and a copy is mailed to each operator monthly giving results obtained. The items listed on this form with a sample set of determinations accompanies this report.

Calculation of loss and overrun data from chemical analyses and operator's reports.

The entries to be made under "Analysis for Fat" and "Salt" need no explanation. The entries to be made under "Lbs. Cream in Vat When Sample is Taken" and "Lbs. Cream in Churn" require explanation.

If the can rinsings were not added to the experimental vat, the amount of cream weighed in, that is not placed in the vat, *i.e.*, the rinsings, should be determined and substracted from the pounds of cream received for the experimental churning.

Assume that 2,000 pounds of cream containing 40 per cent butter fat are received and that 100 pounds of can rinsings, testing 25 per cent butter fat,

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Items listed on creamery record form, with a sample set of determinations

Name of Creamery ..... Name of Operator ..... Date .....

Analysis for fat		Butter	
Butter	80.63	Lbs. butter made	1064.62
Cream		Lbs. butter sold	1058.62
vat	32.53	Lbs. fat in butter made	858.40
churn	31.38	Overrun	
Can rinsings	12.17	From vat test	21.92
Starter	3.80	From churn test	22.38
Buttermilk	.562	Based on fat losses	21.92
Lbs. can rinsings	110.00	Total per cent reduction, all losses	3.08
Salt		Distribution of fat losses	
Analysis	2.50	Buttermilk	
Lbs. in butter	26.62	lbs. buttermilk	1588.00
Lbs. added	27.0	lbs. fat lost	8.92
Per cent lost	1.41	lbs. butter lost	11.15
Lbs. cream in vat when sample is taken		per cent total fat received	1.03
Lbs. cream received for vat	2703.00	per cent reduction in overrun	1.28
Less cream in can rinsings	41.00	Overweight	
Plus lbs. neturalizer and rinsings added before pasteurization		lbs. fat lost	4.8
Total lbs. cream in vat	2662.00	lbs. butter lost	6.00
Lbs. cream in churn		per cent of fat received	0.55
Plus lbs. can rinsings		per cent reduction in overrun	0.69
Plus lbs. neutralizer and rinsings added after vat sample taken	40.00	Composition	
Plus lbs. starter	62.00	lbs. fat lost	6.71
Less lbs. evaporation during pasteurization	7.3	lbs. butter lost	8.38
Total lbs. cream in churn	2756.7	per cent of fat received	0.77
Lbs. fat		per cent reduction in overrun	0.97
Can rinsings		Miscellaneous	
Starter	2.36	lbs. fat lost	0.99
Lbs. fat by vat test plus fat added	868.31	lbs. butter lost	1.24
Lbs. fat by churn test	865.05	per cent of fat received	0.12
		per cent reduction in overrun	0.14
		Remarks	

are obtained. The amount of original cream in the can rinsings is then equal to $100 \times 25/40 = 62.5$ pounds, which amount is subtracted from the 2,000 pounds of cream received.

If, on the other hand, the can rinsings are added to the cream received, and the sample of cream is taken before the rinsings and neutralizer are added, the pounds of water contained in the can rinsings must be included in the second column under "Lbs. Cream in Churn."

If the can rinsings are added to the cream received before the sample of cream is taken from the vat for analysis, the test obtained represents the

test of the original cream received + amount of water in the can rinsings. The amount of water in the can rinsings is determined by the following formula:

$$X = \tfrac{1}{2}\left[(R - C) + \sqrt{(C + R)^2 - 4CRt/T}\right]$$

This formula is derived from the following equations:

$$(C + X)T = CT_1$$
$$Rt = (R - X)T_1$$

C represents lbs. cream received
R represents lbs. can rinsings
T represents test of cream + water in can rinsings
t represents test of can rinsings
X represents lbs. water in can rinsings
T_1 represents test of original cream

The following problem will serve as an illustration: "2000 pounds of cream have been received. One hundred pounds of rinsings testing 25 per cent butter fat are obtained. The rinsings are added to the cream before the sample is taken from the vat for testing and the cream plus the water in the rinsings contains 40 per cent butter fat. Determine the pounds of cream held in the vat at the time the sample is taken."

Answer: $$X = \tfrac{1}{2}\left[(100\text{–}2000) + \sqrt{(2000 + 100)^2 - \frac{4 \times 2000 \times 100 \times 25}{40}}\right]$$

$$= 38.6 \text{ pounds of water.}$$

This should be added to the amount of cream received. The vat, therefore, contains 2000 + 38.6 pounds of cream at the time when the cream sample was taken from the vat for testing.

Operators handling sour cream will frequently add the rinsings and then neutralize before the vat sample is taken for testing. In that case the vat will contain "Pounds cream received + Pounds water in rinsings + Pounds neutralizer solution," and the amount of water in the rinsings may be determined from the following equations:

$$(C + Y + N)T = CT_1$$
$$Rt = (R - Y)T_1$$

Then: $$Y = \tfrac{1}{2}\left[C + N + R + \sqrt{(C + N + R)^2 - 4CRt/T}\right] - (C + N)$$

In this formula the symbols are the same as in the former formula except that Y stands for pounds of water in the can rinsings, N for pounds of neutralizer solution added and T for test of (cream + water in can rinsings + neutralizer solution).

When the cream is weighed in a weigh tank and the can steamings are run into the weigh can and are weighed with the cream, the total amount

of fat received is contained in the vat and the only water added is that which is required to rinse the spout and the amount in the neutralizer solution.

The pounds of cream in the churn are calculated by adding to the total amount of cream, as calculated for the vat, the amount of rinsings and starter added and subtracting the amount of water lost due to evaporation during the process of pasteurization.

The amount of water lost by pasteurization is determined by the following formula:

$$W = C - CT/T_2$$

W represents pounds of water lost
C represents total weight of cream in the vat when pasteurization is started
T represents fat test of cream before pasteurization
T_2 represents fat test of cream after pasteurization

Illustration: A vat of 2,400 pounds cream and 50 pounds of water tests 35 per cent fat. Determine the amount of water lost during pasteurization if the cream tested 35.3 per cent fat after pasteurization.

$$2{,}450 - \frac{2{,}450 \times 35}{35.3} = 20.8 \text{ pounds.}$$

The entries made under the heading "Lbs. Fat" require no explanation, except possibly the third column, "Lbs. fat by vat test plus fat added." By "fat added" is understood the fat in the starter or any other source of butter fat added after the vat sample has been taken.

The amount of buttermilk obtained is determined by the following formula by Mortensen[1].

$$B = \frac{C(100 - 11 - T)}{100 - 11 - a}$$

B represents pounds of buttermilk obtained in free form
C represents pounds of cream churned
T represents test of the cream
a represents test of the buttermilk

The amount of butter taken from the churn less the amount allowed for overweight, is equal to amount of butter sold.

A fat content of butter above 80 per cent reduces the overrun from the theoretical 25 per cent.

The miscellaneous losses are determined from the following formula:

Lbs. butter fat in vat – (Lbs. fat in butter + Lbs. fat lost in buttermilk) = Miscellaneous losses.

The following problem will explain the method employed in determining the effect of each of the various fat losses on the final overrun.

[1] Mortensen, M. Management of Dairy Plants. The Macmillan Co., 1938.

"A vat of cream contains 2500 lbs. of 30 per cent cream from which are produced 915 lbs. of butter testing 80.6 per cent fat. The test of the buttermilk is 0.6 per cent and 4 lbs. of butter are allowed for overweight. Determine the per cent overrun."

The overrun determined by the regular method of figuring is equal to:

$$\frac{(915-4)-750}{750}\times 100 = 21.46\%$$

That system of figuring is correct if the weights and tests are correct, but if errors have been made in weighing or testing the overrun should be determined from the losses occurring during the process of manufacturing. This is done in accordance with the method outlined in the following:

Buttermilk Losses

$$2500\times\frac{100-11-30}{100-11-0.6} = 1669 \text{ lbs. buttermilk}$$

$1669\times .006 = 10$ lbs. fat lost in the buttermilk

$10\times 100/80 = 12.5$ lbs. 80% butter lost in buttermilk

$2500\times .30 = 750$ lbs. fat in vat (no starter added).

$\frac{12.5}{750}\times 100 = \underline{\underline{1.67\%}}$ reduction in overrun.

Miscellaneous Losses

$750-(915\times 80.6+10) = 2.51$ lbs. fat as Miscel. loss

$2.51\times 100/80 = 3.137$ lbs. butter

$\frac{3.137}{750}\times 100 = \underline{\underline{0.42\%}}$ reduction in overrun.

Composition Losses

$$\frac{915\times(80.6-80)}{100} = 5.49 \text{ lbs. fat}$$

$5.49\times 100/80 = 6.86$ lbs. butter

$\frac{6.86}{750}\times 100 = \underline{\underline{0.91\%}}$ reduction in overrun.

Overweight

$$4\times\frac{80.6}{100} = 3.224 \text{ lbs. fat}$$

$3.224\times 100/80 = 4.03$ lbs. 80% butter

$\frac{4.03}{750}\times 100 = \underline{\underline{0.54\%}}$ reduction in overrun.

The final per cent of overrun

$$= 25-(1.67+0.42+0.91+0.54) = 21.46\%.$$

Although the study to which this system has been applied is yet limited, the work so far seems to indicate that it has been of considerable value to some of the operators who have cooperated in the project.

Milk -- Flavor & odor

STUDIES ON THE SOURCE-ORIGIN OF ACTIVATED FLAVOR IN MILK

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bibliography

INTRODUCTION

Milk unduly exposed to radiation, either natural or artificial, will acquire a flavor defect called activated, sunshine, or burnt. This defect is to be differentiated from that referred to as oxidized flavor. Homogenized milk is known to acquire the flavor more readily than unhomogenized milk. Excessive or improper exposure of milk to artificial radiation is a frequent cause for occurrence of the flavor. The ultimate prevention of the development of the flavor, however, necessitates further information concerning its origin and method of development. In the study presented here, milk or milk products, and other substances were exposed for prolonged periods to radiation so as to accentuate the intensity of the flavor, and permit its ready identification and isolation. Reviews of the literature on the activated flavor of milk have been included in previous reports (4, 8). It was pointed out that the flavor originates with the protein fraction of milk, and it was indicated that the flavor which results from undue exposure to ultra-violet radiation is identical or very similar to the burnt or sunshine flavor caused by exposure of milk to sunlight. Radiation is known to cause destructive effects upon proteins in general and to produce disagreeable flavors and odors. Casein and lactalbumin have been shown to develop the typical activated flavor of milk. The present study is concerned with ascertaining the specific source of the flavor.

EXPERIMENTAL PROCEDURE

Two general procedures were followed in determining the specific source of the activated flavor of milk. These were a study of the effect of radiation on the nitrogen distribution of milk, and an investigation of the effects of radiation on the flavor of milk protein hydrolysates, amino acids, and other selected materials.

The source of ultra-violet radiation was a Hanovia quartz mercury-vapor arc placed at a distance of 76.5 cm. above the various materials. The intensity of the radiation was approximately 700 microwatts per square centimeter per second, at the surface of the materials, and was maintained by means of a

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Ultra violet rays -- Biological effect

Milk, Irradiated

Hanovia-Englehard meter photocell circuit. The milk and other fluid products were irradiated after being placed in stainless steel pans 18 cm. square and 3 cm. deep. Two hundred ml. portions were usually used. Generally, the pans were placed on a platform which was given a steady rocking motion through eccentric drive, by means of an electric motor. The materials which were exposed in dry form were spread on parchment paper beneath the arc.

A recently recommended (5) semi-micro Kjeldahl method for the determination of the total-, casein-, albumin-, globulin-, proteose-peptone-, and non-protein nitrogen was used for a study of the effect of radiation upon the nitrogen distribution of milk. The milk was irradiated for 15 minutes so that it had a very strong activated flavor and odor.

Van Slyke amino nitrogen determinations also were made of excessively irradiated milk, skimmilk and cream, and compared with similar determinations of untreated samples. Since exposure to radiation was continued for four hours in these experiments, the materials were weighed before and after the exposure, and the moisture lost by evaporation replaced with distilled water. In some cases determinations also were made after heating the samples to 87.8° C. and cooling, a procedure which intensifies the activated flavor.

In order to determine whether riboflavin or other dialysable substances of milk have a role in production of the flavor, milk in collodion bags was dialysed with distilled water for 24 hours. Fresh milk in new bags was then placed in the water and the dialysing continued, this being repeated four times in order to increase the concentration of dialysable substances in the water. The riboflavin of milk is said to be about 90 per cent dialysable (2). The water had the yellow-green pigmentation characteristic of riboflavin. For further concentration this solution was condensed under reduced pressure to about 20 per cent its original volume. The temperature did not exceed 60° C. during the concentration. Samples of the original and of the concentrated solutions were exposed to the radiation and examined for flavor and odor. A sample of milk which had been dialysed for four days with daily changes of water was also exposed to the radiation and its flavor compared with that of an unexposed non-dialysed sample.

For preparation of the protein hydrolysates, casein was precipitated from skimmilk with acetic acid, washed with distilled water, suspended in 7 normal hydrochloric acid, and autoclaved for eight hours at 15 pounds steam pressure. After shaking the hydrolysate with charcoal and filtering, much of the hydrochloric acid was boiled off at reduced pressure. Water was added and the evacuation repeated several times. The acid hydrolysate and a neutralized portion of it were then exposed to the radiation. A pepsin hydrolysate of milk was prepared by acidifying 200 ml. milk to approximately pH 2.0 with hydrochloric acid, adding one gram of pepsin, and incubating for five days at 37° C. This hydrolysate in the acidic form and after being neutral-

ized was then irradiated. Bacteriological peptone and tryptone in dry form and dissolved in distilled water, and a commercial sample of soft curd milk prepared by enzymatic treatment (Enzylac Process) were subjected to action of radiation and compared with non-treated samples for presence of activated flavor and odor.

Amino acids, either chemically pure or of high quality,[1] in dry form, in distilled water, or in M/10 phosphate buffer at pH 7.0 were irradiated for one and one-half hours. Similarly, commercial granular gelatin in dry form and as a one per cent solution in distilled water was irradiated for two hours.

RESULTS OF EXPERIMENTS

A. Effect of Radiation on the Nitrogen Distribution of Milk.

Table 1 shows the nitrogen distribution in normal samples of milk. It also shows the nitrogen distribution in milk which had a strong activated flavor and odor caused by exposure to radiation for 15 minutes. Determinations made on three separate lots of milk failed to show any appreciable effect of radiation upon the total-, casein-, or albumin plus globulin-, nitrogen content of the milk. The proteose-peptone nitrogen was somewhat greater in the irradiated sample in two out of three examinations while the non-protein nitrogen was slightly less in all three cases. Although the exposure to radiation in these trials was prolonged enough to produce a very strong activated flavor and odor, the data fail to indicate any drastic effects upon the various nitrogenous fractions comparable with the observed effects on flavor. Previous reports have indicated no (1) significant influence of irradiation upon

TABLE 1

*Effect of radiation upon the distribution of nitrogen of milk**

Fraction	Nitrogen as per cent of the milk							
	Trial							
	I		II		III		Average	
	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated	Unirradiated	Irradiated
Total Nitrogen	0.5578	0.5488	0.5152	0.5118	0.5348	0.5404	0.5359	0.5337
Casein Nitrogen	0.4547	0.4457	0.4051	0.4004	0.4275	0.4345	0.4291	0.4269
Albumin + globulin Nitrogen	0.0611	0.0598	0.0644	0.0644	0.0639	0.0639	0.0631	0.0627
Proteose-peptone Nitrogen	0.0140	0.0158	0.0178	0.0231	0.0154	0.0150	0.0157	0.0180
Non-protein Nitrogen	0.0280	0.0276	0.0284	0.0245	0.0280	0.0270	0.0281	0.0264

* Milk exposed to radiation from quartz mercury-vapor arc for 15 minutes; intensity of radiation approximately 700 microwatts per square centimeter at surface of fluid.

[1] Obtained from Pfanstiehl Chemical Company.

the content of casein or albumin in milk, but has been found (7) to delay rennet coagulation of milk.

TABLE 2

*Effect of radiation upon the Van Slyke amino nitrogen content of milk, cream, and skimmilk**

Material	Milligrams amino nitrogen in 5 milliliters			
	Unheated		Heated	
	Unirradiated	Irradiated	Unirradiated	Irradiated
Milk**	1.01	0.96	1.01	1.04
	0.98	1.01	1.03	1.01
	1.02	0.96	1.02	1.02
Average	1.00	0.98	1.02	1.02
Cream**	1.31	1.25	1.35	1.29
	1.29	1.29	1.34	1.34
	1.26	1.21	1.35	1.32
Average	1.29	1.25	1.35	1.32
Skimmilk	0.89	0.87		
	0.89	0.88		
	0.88	0.88		
Average	0.89	0.88		

* Products exposed to radiation from a quartz mercury vapor arc for 4 hours, intensity 700 microwatts per square centimeter at surface.

** Determinations also made on these samples after heating to 87.8° C.

The results of Van Slyke determinations of amino nitrogen of excessively irradiated milk, cream, and skimmilk are shown in table 2. The data obtained from triplicate determinations indicate no appreciable difference in amino nitrogen content of exposed and non-treated samples. They show that even with the prolonged four-hour exposure to radiation there was no significant breakdown of the proteins that could be measured by the Van Slyke amino nitrogen method. This is especially significant, since during commercial irradiation milk is exposed for periods of only a few seconds at the most. Formol titrations made as preliminary work to the Van Slyke studies also failed to show any difference between samples of milk and cream exposed for long and short periods to radiation. These results indicate that the methods employed were not sufficiently sensitive to measure the protein breakdown believed responsible for the flavor production.

B. Effect of Radiation on the Riboflavin and other Dialysable Substances of Milk.

It has been indicated (3) that the burnt flavor resulting from the action of sunlight on milk has its origin in the casein-free albumin-free serum of the milk, and the coincidence between development of this flavor and the fading

in color of the serum pigment riboflavin was noted. Dilute and vacuum-concentrated solutions of riboflavin and other dialysable substances obtained from milk were exposed to the quartz mercury-arc radiation. The treatment resulted in a disagreeable flavor and odor and a bleaching of the riboflavin pigmentation. The flavor and odor definitely were not typical of the activated flavor and odor of excessively irradiated milk. Addition of this irradiated solution to normal milk resulted in an off-flavored product, but which was not similar to the activated flavor observed in milk unduly exposed to radiation.

Milk which had been continually dialysed for four days with daily changes of distilled water developed the typical activated flavor and odor observed in normal milk when similarly exposed to radiation. This shows that removal of a large proportion of the dialysable substances of milk does not alter its susceptibility to development of the flavor and odor.

C. Production of Flavor and Odor in Protein Hydrolysates Exposed to Radiation.

It seemed of interest to investigate whether the typical activated flavor and odor of milk could be produced by irradiation of partially hydrolysed protein, which would indicate whether unaltered protein is necessary for production of the flavor and odor. Irradiation of acid hydrolysed casein, either acidic or neutralized, caused a change in its flavor and odor. The flavor and odor were not typical of that of milk subjected to prolonged exposure to the radiation. Irradiation of the acidic or neutralized pepsin hydrolysate of milk caused a change in its flavor and odor. The flavor and odor were not typical of that of milk similarly treated. The appearance of the digest suggested extensive protein hydrolysis and was of such character that accurate flavor and odor comparisons could not be made.

Irradiation of bacteriological peptone in dry form for two hours resulted in an off-flavor that was not typical of that of irradiated casein or milk. The irradiation of bacteriological tryptone in dry form for two hours resulted in a burnt flavor which was slightly similar to the flavor observed when casein or skimmilk powder is irradiated under similar conditions. Irradiation of peptone or tryptone in distilled water for periods up to two hours caused an off-flavor and odor. The flavor and odor produced in the tryptone were slightly similar to but not really typical of that of milk exposed to radiation. Little or no similarity in flavor and odor of irradiated peptone and casein could be noted. No difference in flavor and odor could be observed between enzyme treated soft curd milk and non-enzyme treated milk when both were exposed to radiation. The slight protein hydrolysis of the soft curd milk was therefore without effect on the susceptibility of the milk to development of activated flavor and odor.

These studies show that irradiation of proteins which have undergone extensive hydrolysis causes changes in flavor and odor, although these

changes are not typical of those occurring in similarly irradiated casein or milk.

D. Development of Flavors in Amino Acids Exposed to Radiation.

Because the results obtained in the study of irradiated protein hydrolysates were inconclusive, attention was given to the effect of radiation on amino acids. The results obtained by irradiating the amino acids in dry form are given in table 3. The data show that cystine, cysteine, methionine,

TABLE 3

*Effect of radiation from quartz mercury vapor arc on the flavor of amino acids in dry form**

Amino acid	Flavor	
	Before irradiation	After irradiation
l-cystine, C.P.	No flavor	Burnt hair, strong sulfur
cysteine HCl, C.P.	Acid, smoky	Strong burnt, smoky
dl-methionine, C.P.	Sweet	Very disagreeable, similar to irradiated casein
dl-b-phenylalanine, C.P.	Sweet, medicinal	Sweet, medicinal
l-tryptophane, C.P.	Slightly bitter	Strong, similar to irradiated casein, sulfur-like
l-tyrosine, C.P.	No flavor	No flavor
l-histidine HCl, C.P.	Acid, salty	Strong, similar to irradiated casein, sulfur-like
glycine, C.P.	Sweet	Sweet
dl-alanine, C.P.	Sweet	Sweet
dl-serine, C.P.	No flavor	Slightly sweet
dl-threonine	No flavor	Slightly sweet
dl-valine, C.P.	Slightly sweet	Sweet
dl-norleucine	No flavor	Slightly sweet
dl-isoleucine, C.P.	No flavor	Nut-like
l-leucine, C.P.	No flavor	Burnt
l-aspartic acid, C.P.	Slightly acid	Slightly burnt
d-glutamic acid, C.P.	Acid	Slightly burnt, smoky
d-arginine	Bitter	Bitter
d-lysine 2HCl, C.P.	Acid	Bitter, nut-like
l-proline, C.P.	Sweet	Sweet
l-hydroxyproline, C.P.	Sweet	Sweet, bitter

* Exposed to radiation 90 minutes, intensity approximately 700 microwatts per square centimeter at surface of material.

tryptophane, histidine, leucine, aspartic acid and glutamic acid developed at least a slight burnt flavor somewhat similar to that noted in irradiated dry casein or skimmilk powder. Irradiated cystine, methionine, tryptophane, and histidine possessed especially strong flavors which indicated that these amino acids may be important contributors to the flavor of casein or milk exposed to radiation. When a small amount of each of these four irradiated amino acids was mixed with unirradiated dry casein, the mixture was found to have practically the same burnt flavor as irradiated casein. The flavor of irradiated cysteine was also strong and suggestive of the flavor of casein and milk exposed to radiation.

Irradiation of the amino acids in distilled water and in phosphate buffer of pH 7.0 gave less conclusive results than were obtained by irradiation of the amino acids in dry form.

The development of peculiar odors and flavors is known to occur in various proteins exposed to radiation. Casein and albumin have been shown to possess the typical odor and flavor of milk similarly subjected to radiation. Because its different amino acid content made study of the source of activated flavor convenient, gelatin was included in these studies. Irradiation of dry granular gelatin for two hours failed to produce any appreciable amount of the typical burnt activated flavor observed in casein after similar treatment. Irradiation for two hours of a one per cent solution of gelatin in distilled water caused a slight change in flavor and odor, but the typical flavor and odor of irradiated casein and milk were not evident. Since these results are in contrast to previously reported findings (4), they were carefully repeated and verified.

Since gelatin either lacks or has a much lower content of tryptophane, methionine, and histidine (6) than does casein or lactalbumin it is tempting to hypothesize that this is the explanation for the absence of the typical activated flavor and odor when it is exposed to radiation. Both gelatin and casein have much lower contents of cystine than does lactalbumin.

DISCUSSION

The fact that ultraviolet radiation has destructive effects upon proteins and may produce unpleasant odors and flavors is in accord with the evidence that the activated flavor of milk originates with the proteins. However, the applied analytical procedures were not sufficiently sensitive to show that exposure of milk to radiation has a measurable destructive effect upon the proteins comparable with the effects on the flavor.

The failure of the irradiation of riboflavin and other dialysable substances of milk to produce any semblance of the typical activated flavor eliminates these substances as possible precursors of this specific flavor.

Irradiation of the various protein hydrolysates was carried out in order to discover whether the same flavor changes would result as are produced from the unaltered protein, which would indicate whether the flavor arises from certain molecular groupings. The inconclusive results obtained with the acid hydrolysate may have been due to removal of flavor precursors by the purification procedure necessary. The various hydrolysates have inherently such disagreeable odors and flavors that accurate flavor determination could not be made. Tryptone developed a flavor somewhat similar to the typical activated flavor, showing that this partially hydrolysed product of casein retains some of the flavor producing characteristics of casein.

The results of irradiation of the amino acids indicated that cystine, methionine, tryptophane, and histidine may be important contributors to

the activated flavor of milk. The importance of methionine, tryptophane, and histidine was also indicated by the lack of flavor production by irradiation of gelatin, in view of the low content of these amino acids in gelatin as compared to casein and lactalbumin.

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ISOLATION OF SUBSTANCES RESPONSIBLE FOR THE ACTIVATED FLAVOR OF MILK

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INTRODUCTION

The prevention of development of activated flavor in milk (7, 8) is at present limited to control of the conditions under which milk is exposed to artificial radiation, and avoidance of exposure of milk and milk products to solar radiation. The isolation and identification of the substance or substances responsible for activated flavor have yet to be completed in order that information on better control practices may be acquired. It is the object of this report to present methods for isolation and qualification of the substances responsible for activated flavor in milk.

The possible rôle of sulfur compounds as the cause of the activated flavor of milk has been suggested (2, 7). It was observed that exposure of a dilute sodium chloride elution product of casein to ultra-violet radiation resulted in an odor which resembled that of milk excessively irradiated and more particularly that which results from exposure of human skin to ultra-violet light. This was interpreted as suggesting the mobilization of SH bodies (2). Irradiation is known to affect the sulfur residues of proteins. For example, egg albumin irradiated by a mercury vapor lamp in an atmosphere of nitrogen gave a positive nitroprusside reaction indicative of the presence of sulfhydryl groups (R–SH) (9). Irradiation of skin was found to result in formation of sulfhydryl compounds (10).

Sulfur compounds are known to be responsible for various disagreeable flavors. The cooked flavor of milk heated to high temperatures has been associated with liberation of sulfides (5) and sulfhydryls (6). It appeared significant that the temperature (about 76–78° C.) at which the cooked flavor of milk becomes evident and at which begins appreciable formation of sulfides and sulfhydryls (5, 6) is near the temperature (82.2° C.) at which the activated flavor is definitely intensified when milk exposed to radiation is heated.

EXPERIMENTAL PROCEDURE

The source of ultra-violet radiation was a Hanovia quartz mercury-vapor arc placed at a distance of 76.5 cm. above the milk or other selected materials. The intensity of the radiation was approximately 700 microwatts per square centimeter per second at the surface of the substances exposed,

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and was maintained by means of a Hanovia-Engelhard meter, photocell circuit. The milk or other fluids were exposed to the radiation after being placed in stainless steel pans attached to a platform given a steady rocking motion by an eccentric drive, attached to an electric motor. Materials which were exposed in dry form were spread on parchment paper beneath the arc.

The problem of isolating the compounds responsible for the activated flavor of milk was complicated by the fact that the measure of progress depended upon the senses of taste and smell. The methods employed in the work were necessarily chosen with this fact in mind.

Isolation of Flavor Substance: Various methods of isolating the flavor compounds were attempted. The method finally adopted utilized steam distillation, in the apparatus shown in figure 1. The apparatus consisted

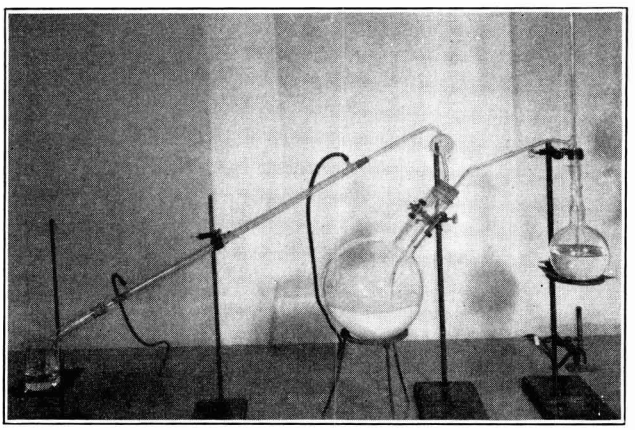

FIG. 1. Apparatus for separating from milk and casein suspensions by steam distillation, substances responsible for activated flavor.

of a pyrex flask, steam inlet and outlet tube, a Kjeldahl connecting bulb, condenser and adapter. Steam was prepared from distilled water. Fused glass and ground-glass joints were used where possible to minimize loss of volatile substances. While distillation of milk or cream was investigated, the use of an intensely irradiated (exposure, two hours) suspension of locust bean-gum-precipitated casein in distilled water (1) was found more satisfactory. The suspension could be distilled without difficulty of foaming, and the distillate possessed a strong activated odor. The casein assumes a colloidal suspension similar to that found in milk, when dispersed in water.

Approximately two liter samples of the casein suspension were distilled for a period of about 30 minutes. The flavor and odor materials recovered in the distillate were adsorbed with activated charcoal, followed by elution with ether. The distillate was collected beneath an aqueous suspension of

charcoal, which prevented loss of volatile substances. The charcoal and aqueous mixture were separated by suction filter. Prior to its use, the charcoal was washed with hot water, filtered, dried and extracted with ether in a Soxhlet apparatus. The charcoal was used for recovery of volatile material from several portions of various distillates of volatile substance. Practically all of the volatile odor material was taken up by the charcoal.

The ether used for elution was previously purified by shaking with concentrated sulfuric acid to remove alcohol, and with 10 per cent ferrous sulfate to remove peroxides, in order to prevent possible excessive oxidation of the volatile odor substances. The ether was then washed several times with water and distilled.

Elution of the flavor substances from the charcoal with the ether was conducted in a Soxhlet apparatus, using a ground glass joint between the ether flask and extraction tube. Only small volumes of ether were used.

The ether was removed from the elution product by distillation from a flask connected by a ground joint to a vertical Glinsky fractionating column. The ether escaping from the fractionating column was passed through a condenser into a receiver. It was impossible to remove the last traces of ether without excessive loss of the volatile odor material. The water which remained was frozen out and the ether solution decanted. The final ether solution having a volume of about 6–8 ml., obtained from around three kilograms of casein, had a very strong odor typical or identical to the characteristic odor of milk and milk proteins excessively irradiated. It was necessary to store the solution in a ground glass stoppered flask in a refrigerator to prevent loss of volatile odor material. When the flask was opened for a few seconds, the room was rapidly filled with the odor.

Estimation of Sulfhydryls in Milk; Steam Distillation: A recently recommended procedure (3) was employed for the quantitative determination of the volatile sulfhydryls of milk exposed to radiation. The method depends upon the formation of methylene blue from p-aminodimethylaniline by distillation of the sulfhydryls into a hydrochloric acid solution of the reagent in the presence of added nitric acid solution of ferric chloride. The color formed is stable in light. In the reaction, each sulfur atom distilled as a sulfhydryl enables the formation of one molecule of methylene blue. The details of the procedure as recommended (3) were followed except that steam and vacuum distillation were employed instead of flame heat distillation. Duplicate sets of distillation apparatus were used permitting simultaneous distillation of different samples of milk. In figure 2 is shown the equipment used for the steam distillation of the sulfhydryls. The apparatus was made entirely of Pyrex tubing and flasks, the connections being either fused or of ground joints. The apparatus consisted of a distilling flask, a Kjeldahl connecting bulb, condenser and a vacuum connection receiving flask.

From fifty ml. of milk, forty-five ml. of distillate were collected in the

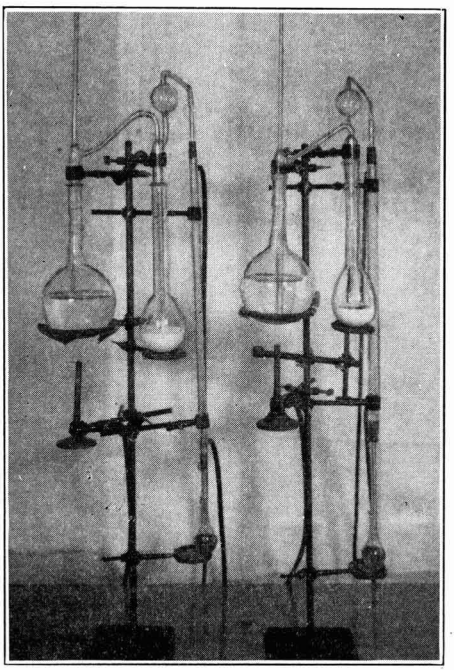

FIG. 2. Apparatus for separating from milk by steam distillation a distillate containing sulfhydryls.

receiving flask beneath the surface of the reagents. Color comparisons of the distillate and standard methylene blue solutions by use of an Evelyn Photoelectric Colorimeter were promptly made.

The reagents were 0.5 per cent p-aminodimethylaniline in concentrated hydrochloric acid and a ferric chloride solution which consisted of 80 ml. of 10 per cent boiled-out nitric acid, 40 ml. normal ferric chloride and 40 ml. water. In all determinations, one ml. of the p-aminodimethylaniline solution, one ml. of a one to ten dilution of the ferric chloride solution, and three ml. water were placed in the receivers prior to distillation.

In order to limit the effect of heat in forming sulfhydryls in the milk, the distillation was carried out as rapidly as possible without excessive foaming. The difficulty of foaming during distillation was largely prevented by addition of a few milligrams of lecithin to the milk. The time of active distillation was approximately 8 to 10 minutes.

As approximate color standards, suitable dilutions were prepared from methylene blue chloride (86 per cent dye content, National Aniline and Chemical Company). A concentration curve was established from readings promptly obtained using the photoelectric colorimeter. The concentration

of methylene blue formed in the distillates from the various samples of milk was determined from the colorimeter-concentration curve of the standard methylene blue solutions.

Estimation of Sulfhydryls in Milk; Vacuum Distillation: Distillation under reduced pressure was also employed to minimize the effect of heat in forming sulfhydryls from the milk and to determine the distillation temperature at which appreciable sulfhydryl liberation would occur from milks given different degrees of exposure to ultra-violet radiation.

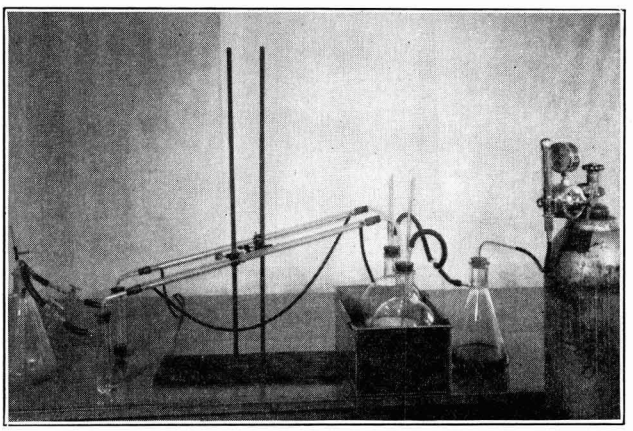

FIG. 3. Apparatus for separating from milk by vacuum distillation a distillate containing sulfhydryls.

The equipment used for the vacuum distillations is shown in figure 3 and consisted of a source of nitrogen, alkaline pyrogallol wash bottle, distilling flask, condenser, receiver and vacuum flask.

Sufficient nitrogen was drawn through the milk to insure vigorous agitation and uniform heating. In the various determinations the total time of heating and distilling was limited to 35 minutes. Approximately 45 ml. of distillate from 350 ml. samples of milk were collected beneath the surface of the methylene blue reagents previously described, and the colorimeter determinations made with a Wilkens-Anderson KWSZ Photometer.

RESULTS OF EXPERIMENTS

A. *Characteristics of the activated flavor concentrate.*

The concentrate of material having an intense activated flavor prepared by ether elution of the adsorbate on charcoal from the distillate of casein appeared to be a homogeneous solution having a slight yellow color. Addition of a very small amount of this material imparted to milk a flavor and odor very similar to that of milk exposed to radiation. This indicated

the concentrate contained at least a large proportion of the compounds responsible for the activated flavor of milk.

When the material was held at – 18° C. for 24 hours followed by several days' storage at about 4.5° C., a few small white crystals were formed. Analysis of the solution was not carried further than to show that it gave a strong nitroprusside test after treatment with potassium cyanide, and a strong sulfhydryl test with the methylene blue reagents after a few milligrams of zinc dust were added to the acid solution. Neither of these tests was positive without the reduction accomplished by addition of cyanide and zinc respectively, indicating the presence of disulfides.

B. *Liberation of sulfhydryls by steam distillation from milk exposed to radiation.*

Preliminary trials were made with skimmilk, milk and cream which had been exposed to the radiation produced by a quartz mercury-vapor arc for periods up to two hours. Rough color comparisons of the methylene blue formed in the distillates invariably indicated more sulfhydryl liberation from the milk exposed to radiation than from the milk not exposed to radiation.

The quantitative sulfhydryl-methylene blue procedure was applied to seven different lots of milk processed in a commercial, quartz mercury-vapor arc milk irradiator. The milk contained 400 U.S.P. units of vitamin D per quart. The raw milk was selected at random from mixed herd deliveries at the Department of Dairy Industry of the University. The samples were examined critically and found to possess only a slight degree of activated flavor.

The amount of sulfhydryls in the distillates of the various samples, as determined by methylene blue formation, is given in table 1. The values

TABLE 1

Sulfhydryl content of distillates obtained by steam distillation from untreated milk and milk exposed to radiation from a quartz mercury-vapor arc lamp

Lot No.	From milk exposed to radiation		From milk not exposed to radiation	
	Methylene blue chloride*	Sulfur	Methylene blue chloride*	Sulfur
	milligrams	*milligrams*	*milligrams*	*milligrams*
1	0.116	0.0115	0.069	0.0068
2	0.101	0.0100	0.081	0.0081
3	0.093	0.0092	0.065	0.0064
4	0.101	0.0100	0.030	0.0030
5	0.103	0.0102	0.078	0.0077
6	0.119	0.0118	0.087	0.0086
7	0.114	0.0113	0.091	0.0090

* Methylene blue chloride formed and its sulfur equivalent in 45 ml. distillate from 50 ml. of milk.

are expressed as methylene blue chloride and as sulfur. It may be observed that in every case appreciably more sulfhydryl liberation was obtained from the milk exposed to radiation than from the milk not exposed to radiation. This indicates that even the limited exposure given milk in commercial units increases the lability of the sulfur of the milk and further that sulfur compounds may be responsible for activated flavor.

It was noted in a previous report (4) that gelatin failed to acquire significant amounts of the typical activated flavor when exposed to radiation. A two per cent solution of gelatin was exposed to mercury-vapor arc radiation for two hours. One hundred ml. of each of the untreated and exposed samples was steam distilled into the methylene blue reagents according to the procedure used for milk. No perceptible blue color could be noted after 45 ml. of either distillate was collected, thus giving a negative test for sulfhydryl liberation.

Fresh egg white was diluted with distilled water and a portion exposed to the radiation for one hour. Steam distillation separately, of an untreated and an exposed sample into the methylene blue reagents resulted in greater blue color in the distillate from egg white which had been irradiated than in the distillate from untreated egg white.

In these experiments the gelatin developed practically none of the typical activated flavor or odor, whereas the egg white solution did develop the typical flavor and odor, providing further indication that sulfur may be involved in the development of activated flavor.

C. *Liberation of sulfhydryls by vacuum distillation from milk exposed to ultra-violet radiation.*

Because of the small amount of sulfhydryls liberated by the vacuum distillation procedure, and in order to accentuate the effect of the radiation, the milk used in these experiments was exposed to radiation for 90 minutes. The moisture which evaporated was replaced with distilled water. The milk possessed an intense activated flavor. The amount of sulfhydryls obtained from the samples of milk by distilling at various temperatures is given in table 2, again expressed as methylene blue chloride and as sulfur. The results show that sulfhydryl liberation occurred at appreciably lower temperatures from the milk exposed to the radiation, than from the milk not exposed to the radiation. At the distillation temperature (75° C. and greater) which caused sulfhydryl liberation from untreated normal milk, significantly more sulfhydryl material was obtained from the milk exposed to the radiation. At the lowest distillation temperatures used (47–58° C.) no sulfhydryl liberation from either samples of milk was detected by the test. These results are in agreement with those obtained using distillates derived from milk by steam distillation, and indicate that exposure to radiation increases the lability of sulfur of milk.

TABLE 2

*Sulfhydryl content of distillates obtained by vacuum distillation from untreated milk and milk exposed to quartz mercury-vapor arc radiation**

Distillation temperature	Bath temp.	From milk exposed to radiation		From milk not exposed to radiation	
		Methylene** blue chloride	Sulfur	Methylene blue chloride	Sulfur
		milligrams	*milligrams*	*milligrams*	*milligrams*
77–79° C.	95° C.	0.132	0.0131	0.036	0.0036
74–75	90	0.071	0.0070	0.011	0.0011
68–70	85	0.037	0.0037	No blue color	
64–66	80	0.019	0.0019	No blue color	
58–60	75	questionable blue color		No blue color	
56–58	70	No blue color		No blue color	
47–48	60	No blue color		No blue color	

* Irradiation for 90 minutes with quartz mercury vapor-arc.

** Methylene blue chloride formed, and its sulfur equivalent in 45 ml. distillate from 350 ml. of milk.

DISCUSSION AND CONCLUSIONS

A method was developed for isolating and concentrating a highly volatile flavor and odor material believed responsible for the typical activated flavor produced in milk exposed to solar or artificial sources of radiation. Analysis of the material so isolated was not possible because of the small yield of product obtainable. Distillates from milk containing the flavor yielded a positive test for sulfhydryls, whereas the concentrate prepared according to the method described yielded only a strongly positive disulfide test. It is believed this is due to the oxidation of sulfhydryls during isolation of the flavor concentrate material. The chemical similarity of sulfhydryls and disulfides indicates the similarity of the flavor bearing products, and the rôle of sulfur bearing compounds in the source of the flavor.

The sulfur of milk exposed to ultra-violet radiation is more heat labile than that of milk not so exposed to radiation. The sulfur of egg white which acquires a typical activated flavor when exposed to radiation is more labile than the relatively small sulfur content of gelatin which fails to acquire the flavor when exposed to radiation. This is further evidence of the rôle of sulfur compounds in the production of activated flavor.

Milk exposed to radiation may be differentiated from milk not exposed to radiation by use of a colorimetric test for sulfhydryls.

The increased lability of the sulfur of milk exposed to radiation also appears to be good evidence of the possible rôle of sulfur compounds in formation of the activated flavor. Although a limited heat treatment was necessary to remove sulfhydryls from milk exposed to radiation, the liberation occurred with less heating than was required for milk not so treated.

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COMPOSITION OF GOAT MILK OF KNOWN PURITY

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There are more than three hundred registered goat breeders in Massachusetts. Information was received that the Goat Breeders' Association intended to introduce legislation pertaining to the sale of goat milk. It was deemed advisable to obtain samples of goat milk for analysis, the results of which would be of value to the legislative committee considering the proposed bill, which recommended standards for total solids and fat lower than those usually reported.

The reports of the Division of Livestock Disease Control of the Massachusetts Department of Agriculture made by the animal inspectors of the cities and towns in the state show the following number of goats in the respective years: 1934, 1,200; 1936, 1,598; 1937, 2,181; 1938, 2,527.

At this rate of increase there should have been 3,088 goats in Massachusetts at the close of 1939. It is probable that not all of the goats are necessarily reported by the local animal inspectors. For this reason Dr. Harrie W. Peirce of the Division of Livestock Disease Control has estimated that at the close of 1939 there were nearly 4,000 goats in Massachusetts. Of this number only 246 have been "abortus tested," all of which were negative (1). Although this number of goats is altogether too small to produce even a minute portion of the two million quarts of fluid milk necessary for daily consumption as such, yet the number is sufficiently large to be of public health and legal significance.

A review of the literature of the past ten years shows considerable work pertaining to the composition of the fat of goat milk; but with one exception, nothing was found relating to variance in the solids, fat, proteins, etc., of goat milk.

König (2) quotes 111 analyses; Richmond (3) quotes König, Moser and Soxhlet, Fleishmann, Pizzi, Richmond, Piccardi, Steinegger, and Bosworth. Associates of Rogers (4) quote Frahm (5), giving the average of 326 samples by 18 investigators. These figures are shown in the table on p. 1098.

In March, 1939, after this study was started there appeared Technical Bulletin 671 of the United States Department of Agriculture on the subject of goats' milk by J. A. Gamble, N. R. Ellis, and A. K. Besley (6). That bulletin gives the results of a study extending over a period of three years. The goat milk used in that study was obtained from one herd of from twenty to thirty-five goats. The analyses reported are mostly of herd milk, but the data do not contain any figures relating to milk serum constants or to the phosphatase content.

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Source	Solids	Fat	Lactose	Proteins			Ash
				Casein	Albumin		
	%	%	%	%	%	%	%
König Highest	17.98	9.38	5.72		6.59		1.36
Lowest	9.84	2.29	2.80		2.34		0.35
Average	13.12	4.07	4.44		3.76		0.85
Richmond quotes							
König	14.29	4.78	4.46	3.20		1.09	0.76
Moser and Soxhlet	13.52	4.43	4.56	3.44		0.30	0.79
Fleishmann	14.50	4.80	4.00	3.80		1.20	0.70
Pizzi	13.25	5.35	3.60		3.64		0.67
Richmond	13.24	3.78	4.49		4.10		0.87
Piccardi	17.54	6.10	3.95	5.56		1.01	0.93
Steinegger	12.60*	3.25	4.80		3.92		0.63
Bosworth	.....	3.80	4.50		3.10		.....
Associates of Rogers quotes Frahm	12.86	4.09	4.20		3.71		0.78

* This figure is quoted as 88.40 per cent moisture. It is obviously erroneous, since the sum of the fat, proteins, lactose, and ash is 12.60 and not 11.60.

In the absence of specific information relative to the source of the milk, the chemist making the analysis must assume that the sample may have been obtained from an individual animal and must base his conclusions as to whether the milk is normal or adulterated by comparison with analyses of milk obtained from individual animals rather than from herds. This is more important in dealing with goats' milk than with cows' milk because the goat herds are small, usually from three to five goats each, and if any abnormality exists in the milk of any one of these goats, it will not be effectively lost in the mixture as is usually the case with cows' milk where the herds are larger.

EXPERIMENTAL

The figures reported herein may be of interest since they represent milk obtained mostly from individual goats and include figures relating to the serum constants and also pertain to a study of the phosphatase content of goats' milk. Most of the goats were stall-fed with but little pasture. In a few instances alfalfa pastures were available. All but fifty of the samples used in this study were collected by Dr. G. L. Drury, a Veterinary Inspector with this Department. Each sample except one commercial sample was milked in the presence of an inspector of the Department, the inspectors personally delivering the samples to the chemists who made the analyses. The first samples were collected late in December, 1938, and it was then ascertained that there would be but little goat milk available until February. Samples, however, were obtained during January, February, March, May, June, July, August, and September.

The methods of analysis are as follows: Total solids were determined by drying a five-gram sample in a flat-bottomed platinum dish for two hours at the temperature of boiling water. The ash was determined by burning

with nitric acid the residue from the total solids. The fat was determined by the Babcock method. The total nitrogen, casein, lactose, freezing point, copper serum refraction, and acetic serum ash were determined by the official methods of the A. O. A. C. The phosphatase was determined by the Scharer method. (7) The calcium was determined upon the ash of the sour milk serum of the goat milk. This should give results as correct as if the determination were made upon the ash of the milk itself as was done on the samples of cow's milk. The method of analysis is as follows: After weighing the ash it was dissolved in dilute hydrochloric acid, the solution was nearly neutralized, sodium acetate and acetic acid were added, and the calcium was precipitated from the boiling solution by potassium oxalate. The precipitated calcium oxalate after filtering and washing was dissolved in hot dilute sulphuric acid and was titrated hot with permanganate.

Table 1 gives the summary of the analyses of these samples, the highest figure, the upper quartile, the median, the average, the lower quartile, and the lowest figure being given in each instance. Table 2 gives similar figures for samples of herd milk. The thirteen samples collected in the last week of December are included with the January samples, and May, June, and July samples are compiled together. No samples were collected during April. The samples collected in December and January were from seventy-seven individual goats and from one herd. The quantity given by each goat was small, and the average time since kidding was 6.24 months. Only a few goats had kidded recently, and many had been in lactation for more than a year. The milk collected during this period is characterized by high total solids. The goats supplying the samples collected in February showed an average of eight months since kidding, and the milk averaged in composition about the same as that of the samples collected in January. The lactation period for March averaged 2.1 months, yet the average composition of the milk varied only slightly from that produced in January or February. The samples collected during May, June, and July averaged much lower in total solids than the other samples. The average stage of lactation for these samples was 3.4 months, which was longer than that of the goats supplying the milk collected during March. Eighty-seven more samples were collected in August, the average period of lactation being nearly five months. This is considerably longer than the lactation period for the samples collected during May, June and July, and it averages nearly as long as that for those for January and February. Notwithstanding this advance in the lacation period, the solids content of the milk was on the average the lowest of any collected.

Table 3 gives the average composition reported by Gamble *et al.* (6, p. 14), together with the averages reported in table 1. Both figures show a high solids concentration in the milk produced in February and a low solids concentration in that produced in August.

TABLE 1

Summary of Analyses of Samples of Milk from Individual Goats

	Total Solids	Fat	Solids Not Fat	Lactose	Proteins	Casein	Ash	Freezing Point Depression	Copper Serum Refraction	Acetic Serum Ash
	%	%	%	%	%	%	%	°C.		
Samples collected Dec. 21, 1938 to Jan. 26, 1939.										
Highest	21.06	9.80	11.90	5.50	6.08	.....	1.20	0.618	41.9	1.260
Upper quartile	15.50	5.75	9.80	5.05	4.39	.....	0.89	0.591	39.3	0.980
Median	14.72	5.00	9.53	4.80	3.88	.....	0.84	0.576	38.6	0.920
Average	14.50	5.08	9.42	4.78	3.99	.....	0.85	0.579	38.6	0.937
Lower quartile	13.12	4.25	8.89	4.45	3.38	.....	0.80	0.564	37.5	0.870
Lowest	11.70	2.95	8.07	3.80	3.15	.....	0.68	0.537	35.9	0.775
No. of Samples	77	77	77	77	77		64	73	64	64
Samples collected Feb. 1, to Feb. 28, 1939.										
Highest	19.92	9.00	11.23	5.65	5.62	4.35	1.04	0.611	42.2	1.105
Upper quartile	16.04	6.05	9.94	5.20	4.13	3.18	0.90	0.586	39.7	0.985
Median	14.38	4.95	9.34	4.85	3.91	3.01	0.86	0.576	38.6	0.935
Average	14.56	5.13	9.43	4.87	3.97	2.98	0.85	0.577	38.8	0.943
Lower quartile	13.12	4.30	8.89	4.60	3.59	2.69	0.80	0.568	3.75	0.905
Lowest	10.72	2.40	8.82	4.20	3.03	1.98	0.70	0.550	36.3	0.815
No. of samples	62	62	62	62	62	54	62	62	62	62
Samples collected Mar. 1, to Mar. 9, 1939.										
Highest	16.50	6.45	10.45	5.50	4.61	3.68	0.92	0.587	41.6	1.080
Upper quartile	14.90	5.40	9.57	5.25	4.00	2.89	0.82	0.578	39.6	0.920
Median	14.24	5.00	9.14	5.10	3.62	2.65	0.78	0.573	38.8	0.880
Average	14.08	4.80	9.28	5.03	3.74	2.74	0.76	0.571	38.8	0.876
Lower quartile	13.06	4.20	8.98	4.75	3.47	2.55	0.70	0.563	38.1	0.830
Lowest	11.44	2.45	8.37	4.45	3.09	2.28	0.60	0.547	36.2	0.780
No. of samples	35	35	35	35	35	34	35	35	35	35
Samples collected May 23, to July 6, 1939.										
Highest	16.62	6.65	9.97	5.10	4.67	.....	0.90	0.616	39.0	1.030
Upper quartile	13.06	4.05	8.92	5.00	3.78	.....	0.82	0.604	38.4	0.876
Median	11.74	3.55	8.24	4.60	3.42	.....	0.76	0.596	37.2	0.844
Average	12.24	3.79	8.45	4.66	3.34	.....	0.77	0.594	37.2	0.850
Lower quartile	11.38	3.20	8.02	4.45	2.76	.....	0.72	0.586	36.7	0.805
Lowest	10.30	2.70	7.60	4.15	2.44	.....	0.68	0.563	35.6	0.740
No. of Samples	49	49	49	15	15		15	15	15	41

TABLE 1—(*Continued*)

	Total Solids	Fat	Solids Not Fat	Lactose	Proteins	Casein	Ash	Freezing Point Depression	Copper Serum Refraction	Acetic Serum Ash
	%	%	%	%	%	%	%	°C.		
Samples collected Aug. 1, to Aug. 31, 1939.										
Highest	15.80	7.00	9.46	5.20	4.20	.....	0.88	0.646	38.4	0.960
Upper quartile	12.32	4.10	8.56	4.50	3.28	.....	0.82	0.613	36.8	0.884
Median	11.04	3.15	7.99	4.30	2.91	.....	0.80	0.598	36.2	0.840
Average	11.47	3.37	8.10	4.32	2.99	.....	0.78	0.599	36.3	0.839
Lower quartile	10.44	2.50	7.68	4.15	2.67	.....	0.76	0.588	35.8	0.790
Lowest	9.12	1.90	7.16	3.56	2.30	.....	0.68	0.567	34.6	0.720
No. of samples	87	87	87	87	87		47	49	87	76
Samples collected Sept. 20, to Sept. 26, 1939.										
Highest	15.04	6.00	9.12	5.05	3.60	.....	0.86	0.621	38.9	0.940
Upper quartile	12.92	4.50	8.72	4.80	3.43	.....	0.82	0.598	37.5	0.900
Median	12.22	3.90	8.32	4.40	3.16	.....	0.80	0.586	37.0	0.856
Average	12.29	3.98	8.31	4.49	3.16	.....	0.79	0.589	37.0	0.857
Lower quartile	11.50	3.25	8.04	4.25	2.98	.....	0.76	0.579	36.3	0.820
Lowest	10.94	2.60	7.68	4.05	2.66	.....	0.72	0.563	35.5	0.768
No. of samples	25	25	25	25	25		25	25	25	25
Samples collected Feb. 7, 1940, to April 30, 1940.										
Highest	20.68	9.80	10.88	.....	.....		.....	.....	42.5	.....
Upper quartile	15.62	6.40	9.34	.....	.....		.....	.....	39.6	.....
Median	14.40	5.40	8.90	.....	.....		.....	.....	38.8	.....
Average	14.54	5.55	8.99	.....	.....		.....	.....	38.8	.....
Lower quartile	13.06	4.50	8.60	.....	.....		.....	.....	38.0	.....
Lowest	10.72	2.90	7.58	.....	.....		.....	.....	36.2	.....
No. of samples	104	104	104						104	

TABLE 2

Analyses of Herd Samples of Goats' Milk

Total solids	Fat	Solids not fat	Lactose	Proteins	Casein	Ash	Freezing point depression	Copper serum refraction	Acetic serum ash
%	%	%	%	%	%	%	°C.		
14.74	5.90	8.84	4.25	3.54	.....	0.84	0.596	37.3	0.948
14.36	5.25	9.11	4.70	3.80	2.88	0.80	0.579	38.2	0.935
13.58	5.00	8.58	4.55	3.46	.....	0.80	0.591	37.1	0.900
13.34	4.40	8.94	4.80	3.90	.....	0.80	0.586	37.7	0.885
12.92	4.25	8.67	.....	.....	.....	.....	.....	.....	0.888
12.60	4.40	8.20	4.50	3.05	.....	0.82	0.579	36.7	0.904
12.54	4.00	8.54	4.70	3.19	.....	0.78	0.578	37.2	0.884
12.30	4.05	8.25	4.70	3.39	.....	0.78	0.610	37.7	0.850
12.30	3.90	8.40	4.35	3.18	.....	0.76	0.615	37.0	0.792
12.14	3.85	8.29	4.65	2.98	.....	0.76	0.585	37.2	0.840
12.08	4.10	7.98	.....	.....	.....	.....	.....	.....	0.876
11.92	3.60	8.32	4.30	3.15	.....	0.78	0.583	36.4	0.840
11.80	4.05	7.75	4.25	2.83	.....	0.76	0.585	36.0	0.860
11.72	3.70	8.02	4.25	3.15	.....	0.80	0.590	36.0	0.876
11.58	3.30	8.28	4.50	3.09	.....	0.76	0.600	37.2	0.852
11.56	3.40	8.16	.....	.....	.....	.....	.....	.....	0.820
11.52	3.50	8.02	.....	.....	.....	.....	.....	.....	.....
11.46	3.50	7.96	4.25	3.00	.....	0.78	0.586	36.0	0.804
11.40	3.50	7.90	4.35	2.91	.....	0.76	0.611	36.4	0.840
10.12	2.40	7.72	4.35	2.71	.....	.....	.....	36.2	0.780
9.94	2.50	7.44	4.25	2.71	.....	.....	.....	35.9	0.780
Samples collected February 7, 1940, to April 30, 1940, as follows:									
16.02	6.80	9.22	.....	.....	.....	.....	.....	39.0	.....
15.36	5.80	9.56	.....	.....	.....	.....	.....	38.9	.....
15.12	6.00	9.12	.....	.....	.....	.....	.....	38.9	.....
14.92	6.00	8.92	.....	.....	.....	.....	.....	39.3	.....
14.90	5.80	9.10	.....	.....	.....	.....	.....	38.3	.....
14.56	5.60	8.96	.....	.....	.....	.....	.....	39.6	.....
14.12	5.20	8.92	.....	.....	.....	.....	.....	39.3	.....
14.10	5.20	8.90	.....	.....	.....	.....	.....	39.0	.....
13.74	5.60	8.14	.....	.....	.....	.....	.....	38.2	.....
13.60	4.60	9.00	.....	.....	.....	.....	.....	38.6	.....
13.34	4.30	9.04	.....	.....	.....	.....	.....	38.3	.....
13.30	4.50	8.80	.....	.....	.....	.....	.....	39.0	.....
12.96	4.00	8.96	.....	.....	.....	.....	.....	39.2	.....
12.56	4.40	8.16	.....	.....	.....	.....	.....	37.7	.....
11.94	3.70	8.24	.....	.....	.....	.....	.....	38.0	.....

TABLE 3

Herd Milk Analyses of U. S. Department of Agriculture (6) and Average of Analyses Reported Here

Month	Total solids	Fat	Solids not fat	Lactose	Proteins	Ash	Protein-fat ratio
	%	%	%	%	%	%	%
February	13.05	4.30	8.75	4.91	3.39	0.82	0.79
March	12.45	4.10	8.35	4.46	3.34	0.79	0.79
April	11.82	3.70	8.12	4.68	2.98	0.76	0.80
May	11.49	3.50	7.99	4.62	2.87	0.76	0.82
June	11.19	3.50	7.69	4.34	2.88	0.77	0.82
July	11.06	3.30	7.76	4.49	2.79	0.75	0.84
August	10.78	3.10	7.68	4.40	2.77	0.77	0.89
September	10.80	3.10	7.70	4.44	2.87	0.77	0.92
October	11.85	3.50	8.35	4.50	3.22	0.80	0.92
November	11.91	3.20	8.71	4.38	3.61	0.85	1.13
December	12.36	3.50	8.86	4.75	3.53	0.85	1.00
Average	11.71	3.50	8.21	4.55	3.10	0.79	0.89
Average from Table 1, individual goats.							
December and January	14.50	5.08	9.42	4.78	3.99	0.84	0.78
February	14.56	5.13	9.43	4.87	3.97	0.85	0.78
March	14.08	4.80	9.28	5.03	3.74	0.76	0.80
May, June and July	12.24	3.79	8.45	4.66	3.34	0.77	0.86
August	11.44	3.37	8.07	4.32	2.99	0.78	0.89
September	12.29	3.98	8.31	4.49	3.16	0.79	0.82

The analyses reported by Gamble show on the average a lower total solids and fat percentage than do those in table 1 and are less variable than those shown in table 2.

Most of the goats producing milk used in this study were of the Saanen and Toggenburg breeds. The analyses of the samples obtained from the Saanen and Toggenburg goats have been tabulated. During December, January, February, and March eighty samples were obtained from Toggenburg goats with an average solids of 14.18 per cent, fat of 4.97 per cent, and solids not fat of 9.21 per cent. During the same period samples were obtained from sixty Saanen goats, giving milk with an average solids of 14.24 per cent, fat of 4.95 per cent, and solids not fat of 9.29 per cent. During May, June, and July samples from thirteen Toggenburg goats were obtained with an average solids of 12.20 per cent, fat of 3.73 per cent, solids not fat of 8.47 per cent. During the same period samples with an average solids of 12.14 per cent, fat of 3.66 per cent, and solids not fat of 8.48 per cent, were obtained from twenty-seven Saanen goats. It appears from these figures that there is but little difference in the quality of the milk produced by these two breeds of goats. Several goat breeders stated that the Nubian goats gave richer milk than did the goats of the Saanen and Toggenburg breeds. Only eighteen Nubian goats were among those from which samples were obtained. The total solids of eight such samples collected during January, February and March varied from 15.32 per cent

to 18.40 per cent with an average of 17.13 per cent. Similar figures for the fat were 5.35 per cent, 9.00 per cent and 6.63 per cent respectively. The ten samples collected during June and August contained total solids from 11.38 per cent to 14.48 per cent with an average of 13.37 per cent and fat from 3.35 per cent to 5.50 per cent with an average of 4.45 per cent. These figures average higher than the results of the total samples collected, which for the 174 samples collected in January, February and March was 14.44 per cent total solids, 5.04 per cent fat and for the 136 samples collected in May, June, July and August was 11.74 per cent total solids, 3.52 per cent fat.

TABLE 4

Comparison of Copper Serum Refraction and Serum Ash of Goats' and Cows' Milk

Copper serum refraction	834 samples of cows' milk	174 samples of goats' milk collected Dec., Jan., Feb., Mar.	128 samples of goats' milk collected May, June, July, Aug., Sept.
20° C.	*Per cent of total samples*	*Per cent of total samples*	*Per cent of total samples*
34.0 to 34.9	.....	.....	3.1
35.0 to 35.9	.....	1.1	20.3
36.0 to 36.9	14.8	7.5	46.1
37.0 to 37.9	35.8	24.2	20.3
38.0 to 38.9	40.7	26.4	9.4
39.0 to 39.9	8.1	22.5	0.8
40.0 to 40.9	0.6	13.8	.....
41.0 to 41.9	.....	4.0	.....
42.0 to 42.2	.....	0.5	.....
50% of samples between	37.4 and 38.4	37.6 and 39.5	35.9 and 37.1

Serum ash	Sour serum ash, 371 samples of cows' milk	Acetic serum ash, 150 samples of goats' milk collected Dec. to Mar.	Acetic serum ash, 120 samples of goats' milk collected May to Sept.
	Per cent of total samples	*Per cent of total samples*	*Per cent of total samples*
0.72	.....	0.7	.....
0.73 to 0.76	31.4	1.3	7.5
0.77 to 0.80	41.4	15.3	22.5
0.81 to 0.84	17.7	17.3	21.7
0.85 to 0.88	7.3	25.3	25.0
0.89 to 0.92	1.4	18.0	15.0
0.93 to 0.96	0.8	14.0	7.5
0.97 to 1.00	.....	1.3	.....
1.01 to 1.04	.....	2.7	.8
1.05 to 1.08	.....	.....	.....
1.09 to 1.12	.....	2.7	.....
1.13 to 1.16	.....	.....	.....
1.17 to 1.20	.....	0.7	.....
1.21 to 1.24	.....	.....	.....
1.25 to 1.26	.....	0.7	.....
50% of samples between	0.759 and 0.804	0.822 and 0.910	0.793 and 0.878

As in the cows' milk the fat percentage is the most variable, the per cent of fat being higher during the winter months than it is during the summer. This seasonal variation is much greater in goats milk than in cows milk. The milk sugar does not differ greatly from that of cows' milk but averages somewhat lower. The proteins of goats' milk are, however, higher than those of cows' milk and are characterized by a lower casein content. The protein-fat ratio so useful in detecting skimming in cows' milk is of but little value when applied to goats' milk. The protein-fat ratio calculated from the figures reported by Gamble *et al.* (6) varies from 0.79 to 1.13. The figures reported here average from 0.76 to 0.89, with individual figures varying from 0.49 to 1.55.

Table 4 gives a comparison of the acetic serum ash of 270 samples of goat milk with the sour serum ash of 271 samples of cows' milk (8). In comparing these figures it should be understood that the acetic serum ash is 2 per cent lower than that of the sour serum. The same table also shows the comparison of the copper serum refraction of 302 samples of goat milk with that of 834 samples of cows' milk. Many years experience with these figures obtained from cows' milk shows but little seasonal variance. A recent compilation from records of this department shows an average copper serum refraction of 37.8 from 233 samples of known purity cows' milk collected in the winter, and of 37.6 from 385 samples of known purity cows' milk collected in the summer. The same figures from goats' milk show a marked seasonal variance and consequently in table 4 the results obtained from samples collected from December to March have been compiled separately from those collected from May to September. The serum ash of goats' milk varies more than that of cows' milk, and particularly in the winter is characterized by a higher figure. It is unusual to find serum ash figures above 0.88 in cows' milk but 40.1 per cent of the goat milk samples collected in the winter and 23.3 per cent collected in the summer gave sera with higher figures. It is unusual for the copper serum refraction of cows' milk to be above 40.0 or below 36.0, but 18.3 per cent of the goat milk collected in the winter gave sera above that figure as did 10.5 per cent of those collected in the summer. Few of the samples of goat milk gave copper sera with refraction below 36 in the winter, but 10.7 per cent of the samples gave sera of this character in the summer.

This is difficult to understand, unless it is due to a high albumin content in the winter and a low albumin content in the summer. Unfortunately, in this study the casein was determined only in the winter, and the same statement is also true to similar results reported by Gamble *et al.* (6) The average protein content of the 174 samples collected in the winter was 3.93 per cent and of the 127 samples collected in the summer was 3.07 per cent, representing a drop of 21.9 per cent which exceeds that of any other constituent except the fat. The non-casein proteins coagulable by heat have a greater influence on the concentration of goat milk serum than of cow

milk serum. Sixty-two samples were examined in February and March, 1940. The copper refraction plotted against the proteins of the serum shows a well-marked zone, the lower portion from 36.5 refraction 0.50 protein to 39.5 refraction 1.10 protein; the upper portion from 39.0 refraction 0.50 protein to 41.8 refraction 1.00 protein. The copper serum of each sample was heated for five minutes in a boiling water bath, filtered, and the refractive index again determined. The figures show a drop of from 0.8 to 3.8, average 2.08. Similar figures on known purity cows' milk show a drop of from 1.2 to 1.7 in copper serum refraction after heating. The freezing point depression in goats' milk is greater than in cows' milk. The amount of added water in cows' milk is computed from a freezing point depression of 0.55° C. The average of this figure for goats' milk varied from 0.57° C. to 0.59° C. The percentage of calcium in the ash is shown in table 5; it averaged 16.13. Four samples of herd milk from pure bred cows were obtained during July, and the calcium and ash were determined on each, using twenty-five-gram samples, each figure representing the average of three determinations. The percentage of calcium in the ash of the milk obtained from pure bred Jersey cows was 18.74; from pure bred Guernsey

TABLE 5

Per cent of Calcium in the Acetic Serum Ash of Fifty Samples of Goats' Milk

Lowest	13.11
Lower quartile	14.90
Median	16.13
Average	16.08
Upper quartile	17.02
Highest	19.95

cows, 18.49; from pure bred Ayrshire cows, 17.24, and from pure bred Holstein cows was 16.20.

Experiments were undertaken to ascertain if proper pasteurization of goats' milk would inactivate the phosphatase. Preliminary experiments indicated that if goats' milk were pasteurized at 142° F. for thirty minutes the phosphatase would be inactivated. In order to be sure that no idiosyncrasy of an individual goat could be responsible for opinions to the contrary, thirty-nine samples of milk obtained from individual goats were pasteurized in the laboratory. The Scharer modification of the phosphatase test was used, the reagents were prepared in the laboratory, and the incubation period was ten minutes. Of the thirty-nine raw samples collected one gave the deep blue color characteristic of the presence of phosphatase and similar in extent to the reaction given by raw cows' milk. Five samples gave a medium blue color, eleven gave a light blue color, ten a faint blue color, seven were indeterminate, and five gave negative reactions. These samples were pasteurized in the laboratory in test tubes at a temperature of 142° F. After five minutes at this temperature thirty-three gave negative phosphatase reactions, and in six samples the reaction was indeterminate.

After ten minutes of pasteurization thirty-six were negative, and three were indeterminate, but in fifteen minutes all were negative. Scharer (10) states regarding the modified method, "Chocolate drink, Vitamin D milk, condensed milk, goat milk and the like need no special treatment other than a control determination." He gives no figures relating to the thermal destruction of the phosphatase of goat milk.

In consideration of this phase of the subject the bacterial count may be of interest. During February, March and April, 1940 bacterial counts have been made upon goat milk. The goats were milked in the evening, the milk was refrigerated, and the plates were made the following day, corresponding to usual commercial conditions, using the new standard media of the American Public Health Association (11). Astonishingly low results were obtained. Of the 133 samples examined, one sample gave a count of less than 10, and the highest count was 85,000. The lower quartile was 131; the median was 575; the geometric mean was 586; the arithmetic mean was 1,130, and the upper quartile was 1,830. The geometric mean of the bacterial counts of 88 samples of raw certified milk examined during 1939 was 2,389.

Besley (12) has stated that the average bacterial count over a period of a year was 1,300 colonies per cubic centimeter. He collected samples aseptically from 23 goat udders and reports that 40 per cent of the milk did not show any bacteria, thus accounting for the low count of market goat milk.

SUMMARY AND CONCLUSIONS

Goat milk of known purity from 335 individual goats and 21 herds has been collected and analyzed. Determinations of solids, fat, total proteins, casein, lactose, ash, freezing point, and serum constants have been made. The per cent of calcium in the ash has been determined on fifty samples and has been compared with similar determinations on herd milk from pure bred cows. The rate of inactivation of the phosphatase of goat milk has been determined.

The watering and skimming of goat milk can be practiced to a greater extent with less chance of detection than is the case with cows' milk due to the greater variance in the serum constants of goat milk as well as of the freezing point.

The variation in the total solids and the fat is greater than is the case with cows' milk. In this work 85 per cent of the samples were obtained from Saanen and Toggenburg goats giving milk as nearly alike as could be obtained from Jersey and Guernsey cows, yet the individual variance is more marked than that found between Jersey and Holstein cows (9).

The goat milk produced in the summer months is inferior in solids content to the average market milk sold during the same period, and in order to obtain the same food value as that of cows' milk its consumption must be increased by 10 per cent.

Although the per cent of calcium in the ash of goat milk is substantially the same as in cows' milk, yet because of the higher ash content it is a valuable food when an excess of calcium is desired in the diet.

A study of the data indicates that the seasonal variation in composition is more marked than the variation with the lactation period.

The daily production per goat is small and in the 178 cases where a record was obtained it varied from 1 to 11 pounds with an average of 4.95 pounds per goat.

The phosphatase test is of little value in determining if goat milk is pasteurized as defined by law. If the phosphatase has not been inactivated the milk is raw milk, but it will be inactivated at pasteurization temperature considerably before the expiration of the legal holding time.

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EFFECT OF THE PROPERTIES OF THE FAT AND OF THE FAT GLOBULE SURFACE ON LIPOLYTIC ACTIVITY IN MILK

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INTRODUCTION

It may safely be assumed that all normal, raw, cow's milk contains lipase. It has been demonstrated that the velocity of lipase action on milk fat can be greatly accelerated by the so-called "activation" methods such as (1) homogenization (6, 13); (2) shaking (5, 10); and (3) cooling, warming and cooling (9). It is reasonable to assume that the quantitative effects obtained are dependent on (1) the amount of enzyme, (2) the properties of the fat, and (3) the properties of the interfacial layer.

This paper presents the results of some experiments which show that the properties of the milk fat as well as the conditions of the fat-plasma interface influence the lipolytic hydrolysis of milk fat.

EXPERIMENTAL

Effect of fat content

In order to determine the effect of concentration of substrate upon lipolysis, samples containing increasing amounts of fat were prepared by recombining the cream and skimmilk separated from fresh, raw milk. These samples were activated by cooling to 2° C., warming to 30° C. and recooling to 2° C. The increases in acidity of the plasma and of the fat were determined. The results presented are the differences in acidities obtained by subtracting the value of the pasteurized control after all samples had been held for 24 hours at 2° C. The acidity of the fat was determined by titrating 5 grams of fat with 0.05N alcoholic NaOH. The results are expressed in acid degrees, that is, cc. of 1N alkali per 100 grams of fat (7).

The data presented graphically in figure 1 show that the acidity of the total fat phase on the basis of the product increased with increasing fat content up to 35–45 per cent of fat, but that the acidity per unit of fat increased only up to about 8 per cent fat and from this point on the acidity per unit of fat slowly declined. The titratable acidity of the plasma phase, expressed as lactic acid, increased with increasing fat content up to about 6 per cent; however, at increasing levels of fat the titratable acidity of the plasma phase remained approximately constant. These results show the influence of the ratio of fat to plasma phase on the rate and character of milk fat lipolysis. At the higher levels of fat content the acid degree of the fat may be less, even though the total acids produced in the cream may be greater. At the fat percentage where the acid degree of the fat starts its decline, the acidity

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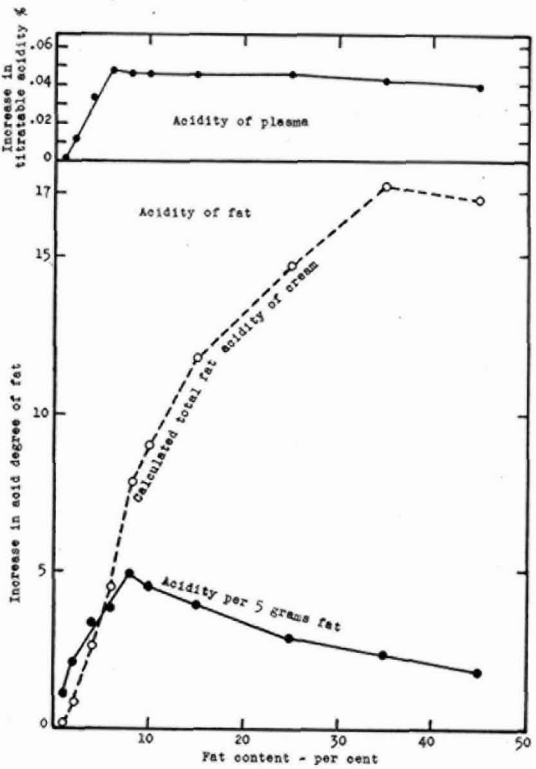

FIG. 1. Effect of increasing fat content on lipolysis after activation by cooling, warming and cooling. Values represent increase in acidity as a result of holding for 24 hours at 2° C.

of the plasma phase becomes constant. This interrelation indicates a depressing effect of the water-soluble fatty acids, produced by lipase activity, on the further activity of the lipase itself, and the establishment of a partition of the fatty acids between the plasma and the fat which reduces the hydrolysis of the glycerides which produce the water-soluble acids. The lipase continues to hydrolyze glycerides which do not produce water-soluble acids. It is intended to investigate this point further.

Separation of milk fat fractions

Chevreul (4) showed in 1823 that milk fat was a mixture of glycerides which he succeeded in separating into three fractions on the basis of their relative solubilities in alcohol. Much later Amberger (1, 2), using fractional crystallization from ether, alcohol and acetone, separated a number of fractions based on melting points, and claimed by this method to have

isolated small amounts of triolein and tristearin. Pizzi (11) separated milk fat into six fractions by removing the crops of crystals resulting from stepwise cooling. He found that the iodine number and Reichert-Meissl number of the fractions increased as the temperature at which the crystalline fractions were removed decreased.

The method of fractional crystallization was used to prepare a series of fractions of milk fat obtained from butter churned from fresh pasteurized cream. The water phase of the butter was separated from the fat by centrifuging in cups in a warmed centrifuge. The clear, supernatant oil was filtered through filter paper in a hot water funnel. The mixed glycerides were separated into a number of fractions by removing the crystals produced by stepwise cooling. The oil was first held for a week or two at a constant temperature of 30° C., the crystals formed were removed by filtration through a Büchner funnel, and the oil filtrate was then adjusted to a lower temperature for an additional period of holding, the second crop of crystals was removed, etc. A final oil was obtained in which no crystals were formed as a result of holding for several months at 4° C. Sharp separation of chemical entities is of course not produced by this single series of fractionations; however, the products thus obtained have sufficiently different properties to indicate trends. The yields of the various fractions are shown in figure 2.

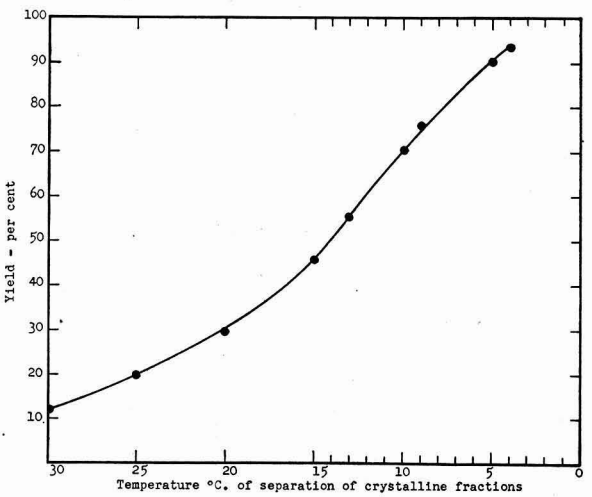

FIG. 2. Yield of crystalline milk fat resulting from stepwise cooling to a series of temperatures.

Attention should be called to the possibility that the presence or absence of at least certain portions of one fraction may affect the solubility of the other fractions in the residual mixture. Crystallization temperatures above 30° C., although not used in this instance, will produce small crops of crystals, as figure 2 indicates.

A number of the fat constants were determined on the various fractions using the A.O.A.C. (3) methods. The results are presented graphically in figure 3.

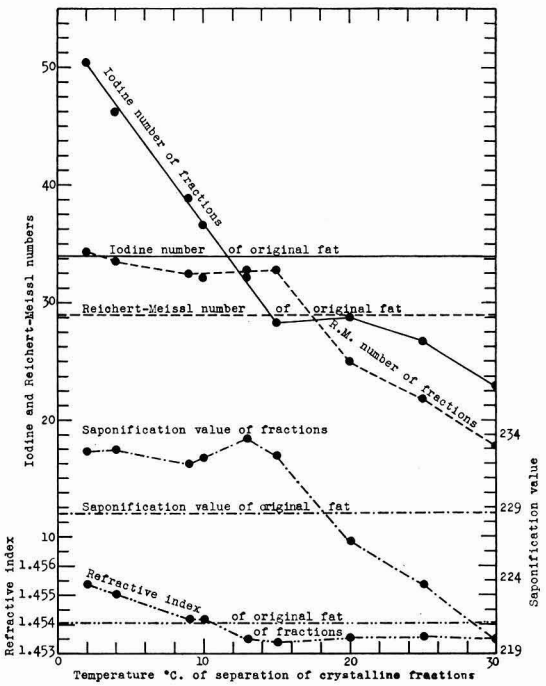

FIG. 3. Fat constants of various milk fat fractions separated by stepwise cooling.

The iodine numbers of the fractions obtained at temperatures lower than 15° C. show a marked increase, while the iodine numbers of the fractions separated above this temperature show a relatively slight decline. The data for refractive index show the same general trend. The curves for saponification value[1] and Reichert-Meissl number are similar in shape, the values tending to decrease in fractions above 15° C. and to remain constant in fractions obtained below this temperature. In general, figure 3 indicates that the fractions removed at the higher temperatures yield filtrates which are progressively richer in the shorter chain, low-molecular-weight acids and slightly richer in unsaturated acids down to 15° C. Below this temperature fractionation produced little change in molecular weight, but did markedly enrich the filtrate in its content of unsaturated fatty acids. The progressive crystallization of the various fat fractions is determined by their solubility in the uncrystallized glycerides remaining in solution at the various temperatures. It is apparent that the mutual solubility of the individual frac-

[1] The saponification values were kindly determined by Professor B. L. Herrington.

tions decreases with a lowering in temperature and the glycerides separate successively to the extent of their lowered solubility and the volume of solute available.

Lipolysis of milk fat fractions

Re-emulsified creams of 35 per cent fat content were prepared using pasteurized skimmilk and the following milk fats: unfractionated milk oil and fat fractions which were crystallized at 30, 25, 13, 10, and 4° C., and the oil remaining uncrystallized at 4° C. Re-emulsification was produced by homogenization. The attempt was made to produce fat emulsions in which the fat globules approximated in size those in normal milk. The adsorption layer on redispersed fat globules is of different composition and properties from the material present on the natural fat globules. We have used the term "resurfaced" to designate such globules. Each cream was diluted with portions from the same lot of raw skimmilk to produce a 3 per cent fat milk.

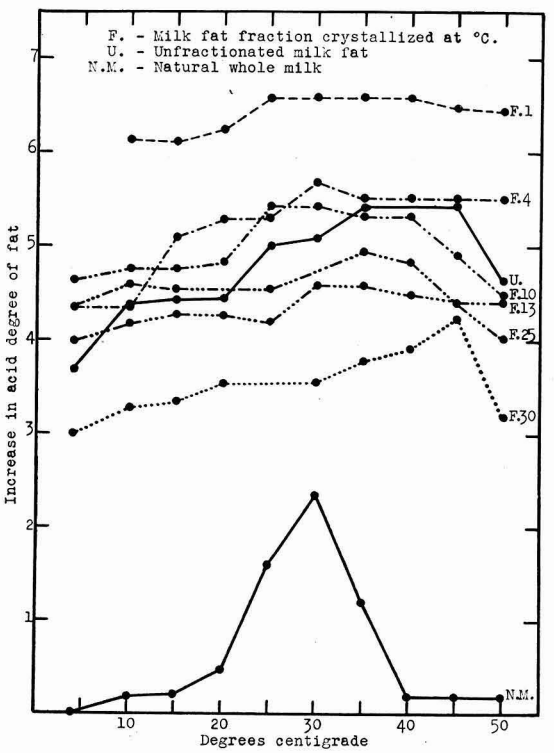

FIG. 4. Effect of preheating to various temperatures on the increase in acidity due to lipolysis resulting from 24 hours of holding at 2° C. Curves represent natural raw milk and reconstituted milk prepared from raw skimmilk and homogenized cream prepared from various fat fractions.

These milks, as well as a sample of the normal, whole, raw milk were cooled to below 5° C. and were then subjected to temperature activation. The milks were warmed gradually and aliquot samples were taken at 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50° C. and recooled. The acidities of the fats were determined after 24 hours of holding at 2° C. The increases in acidity as a result of holding are presented in figure 4. The data reveal that resurfacing of the fat as a result of emulsification with skimmilk renders the fat more susceptible to attack by lipase. Furthermore, since the lipase was already in the active state as a result of the resurfacing of the milk fat, it failed to respond in any appreciable degree to the activating influence of cooling, warming and cooling which exerts such a pronounced effect on normal, raw, whole milk with its naturally surfaced fat globules. The data in figure 4 show clearly that lipolysis was progressively greater with the fractions of progressively lower crystallization temperatures. About twice as much acid was produced during 24 hours holding at 2° C., in the fraction which did not crystallize at 4° C., as compared with the fraction which crystallized at 30° C. Attention should be called to the probability that the samples heated to 50° C. in figure 4 were partially inactivated by heat and oxygen, and this probably accounts for the slightly lower values obtained with some samples heated to that temperature.

The conditions at the fat-plasma interface as influencing the temperature coefficient of lipase action in milk

It was previously observed (13) but not reported at that time, in connection with cream separated at 85° F. from previously cooled milk, that the titratable acidity increased more rapidly the lower the temperature of holding. It was shown that the titratable acidity of homogenized milk increased with the temperature of holding. It was also shown (9) that the warming of cold, natural, raw milk to 30° C. greatly accelerates the lipolysis when the milk is recooled. However, the maximum effect does not appear unless the milk is cooled below 15° C. (59° F.). The above observations, and the data presented in figure 4, we interpreted as showing the marked contrast between natural and resurfaced fat globules.

A series of experiments was performed to show that the negative temperature coefficient for lipase activity was associated with the natural material on the surface of the fat globules, and that the expected normal positive coefficient was obtained when the fat was resurfaced. Reconstituted 35 per cent fat cream was prepared by homogenizing at 50–60° C. aliquots of the same pasteurized skimmilk with the following fat fractions: fraction crystallizing at 30° C., fraction not crystallizable at 4° C., pure triolein (m.p. – 17° C.), pure trielaïdin (m.p. 38° C.),[2] and unfractionated milk fat. These

[2] The sample of trielaïdin was a preparation kindly furnished by Dr. Gordon Ellis of the Laboratory of Animal Nutrition of Cornell University.

creams, cooled to 37° C., were then diluted to 3 per cent fat with aliquots of the same raw skimmilk, also adjusted to 37° C. The five lots of milk described above, as well as one from the original whole milk taken at the time of milking, were placed in ice water. Samples were taken from each lot as the temperature fell to 25, 20, 15, 10 and 5° C. The samples removed were maintained at these same temperatures for 24 hours. In addition one lot of original whole milk was activated by cooling and warming, and aliquots were taken at these same temperatures during the second cooling.

Figure 5 shows the increase in the acidity of the fat as the result of 24 hours holding at the different temperatures. Lipolysis proceeded faster, the

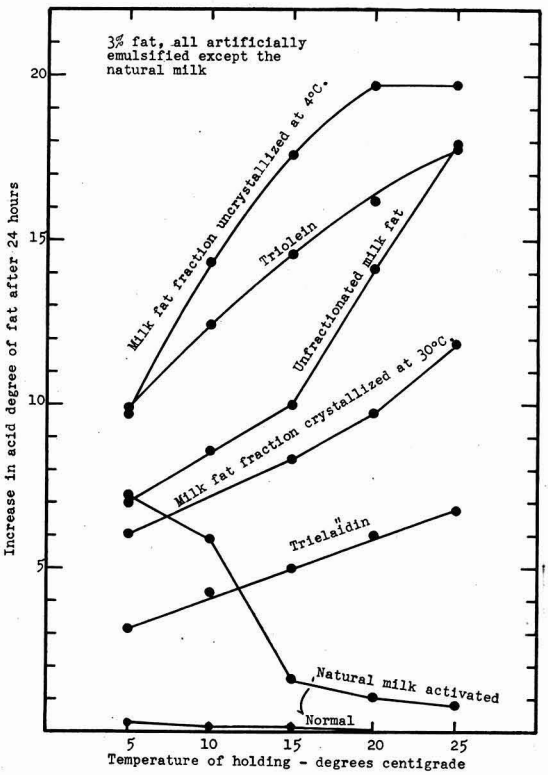

FIG. 5. Effect of temperature on lipolysis of normal surfaced and resurfaced milk fat and of fat fractions.

higher the temperature, in the case of all reemulsified fats, whereas lipolysis was faster the lower the temperature in the case of the fat globules with natural surfaces; this effect is shown most strikingly with the natural milk after activation. This experiment shows very clearly the difference in lipolytic susceptibility of fat globules with natural surfaces as contrasted with resurfaced fat globules.

As in figure 4, also in figure 5, lipolysis is greater the lower the melting point of the fat. A sharp break in the curve for the reemulsified, unfractionated fat occurs at 15° C. Near this temperature occurs a marked softening of the fat as shown by specific heat (12) determinations. Triolein and its isomer, trielaïdin, were used for comparative purposes because their melting points are more definite, and widely different, whereas the "melting" points of milk fat and to a lesser extent of milk fat fractions are more or less indefinite because they are mixtures. Lipolysis of the trielaïdin proceeded more slowly than of the triolein, as was expected from the difference in physical state. The curves for resurfaced fat globules indicate that the velocity of the lipolytic reaction and its final equilibrium are influenced by the physico-chemical properties of the fat.

Effect of natural variations in fat, enzyme content, and the conditions at the fat-plasma interface on lipolytic activity

The marked effect of the properties of the fat and the conditions at the fat-plasma interface raises the question as to the possibility of some of these variations occurring naturally. The following experiments were carried out to test this point. Milk from 9 individual Holstein and 8 individual Jersey cows was separated. The skimmilks were used to prepare standardized, 4 per cent fat milk, using in one series the same Holstein cream and in the other the same Jersey cream. In addition the lipolytic activity of the natural, unseparated milks was tested. The lipase in all milks was activated by cooling, warming and cooling, and the increase in acidity of the fat as a result of holding for 24 hours at 2° C. was determined. The results are expressed graphically in figure 6.

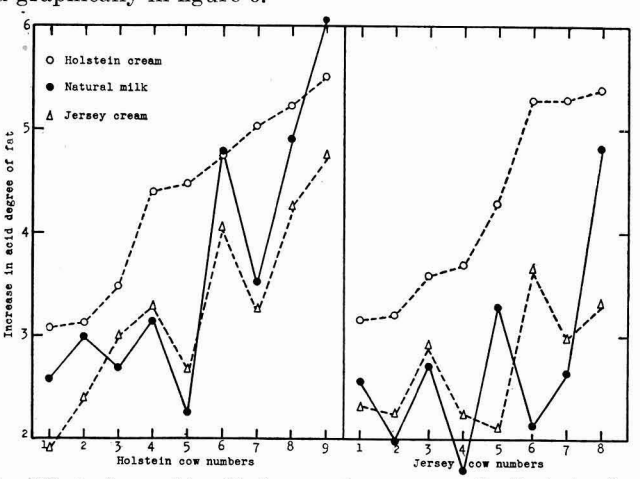

FIG. 6. Effect of raw skimmilk from various cows on the lipolysis of natural fat globules when natural whole and standardized milks were activated by cooling, warming and cooling. Increase in acidity as a result of holding for 24 hours at 2° C.

The samples are arranged in ascending order of the lipolysis of the milk standardized with Holstein cream. In general, the other two curves show a trend upward from left to right, indicating a variation in enzyme content in spite of the marked irregularities. Attention is called to the fact, however, that the lipolytic activities of the series of skimmilks standardized with Holstein and with Jersey cream do not exactly parallel each other, although all samples contain the same amount of fat, the samples in each series containing the same kind of fat and fat globules, and the pairs made from the same skimmilk contain the same amount of lipase. The chance differences between the two kinds of fat or cream probably explains why more lipolysis occurred in the Holstein fat. We are inclined to explain the irregular variations between the milks standardized with Holstein and Jersey creams as being due to differences in the interrelations of the various factors at the fat surface. The lipolytic activity of the natural milk when activated shows additional dissimilarities from the curves for the standardized milks. This result should be expected because of the additional influence of variations in fat content (substrate concentration) as well as the effect of the variability in properties of the individual fats and their surfaces.

CONCLUSIONS

The rate of lipolysis of milk fat is influenced by the activation procedure, the amount of enzyme present, the fat (substrate) concentration, the fat-plasma ratio, the physico-chemical properties of the fat, and the conditions of the fat-plasma interface.

Cooling, warming and cooling is effective in increasing lipolytic activity in raw cream as well as in raw milk.

Total lipolytic action increases with fat content up to 35–45 per cent, but acidity per unit of fat and the acidity per unit of plasma increases with fat content up to 8–10 per cent of fat and then remains constant or decreases.

Crystalline fractions separated from milk fat by stepwise cooling show a broken trend toward higher iodine, Reichert-Meissl and saponification numbers.

The lower the temperature required for crystallization of the fat fractions, the greater the increase in acidity when used as a substrate for milk lipase, which indicates that the rate of lipolysis is dependent upon the melting point of fat or upon the degree of solidification of fat at a given temperature. The degree of solidification is determined by the mutual solubility properties of individual glycerides of which milk fat is composed.

Lipolysis of milk fat is accelerated by resurfacing the fat globules.

Resurfaced fat globules show no further increase in lipolysis due to cooling, warming and cooling.

The rate of lipolysis of resurfaced fat globules increases with increasing temperature (showing a normal temperature coefficient), whereas the rate

of lipolysis of fat globules with the original normal surface increases as the temperature is lowered (showing a negative temperature coefficient).

The experiments demonstrate the influence of the conditions at the fat-plasma interface on lipolytic activity in milk and cream.

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Milk -- Enzyme content

INACTIVATION OF MILK LIPASE BY DISSOLVED OXYGEN

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INTRODUCTION

It has long been known that certain heavy metal salts inactivate or "poison" many enzymes. Davies (1) found that dissolved copper, in concentrations of 2 and 10 parts per million, as well as some other metal salts, retarded the action of milk lipase. He interpreted his results as indicating the susceptibility of the lipase to break down under conditions of low active oxygen concentration in the presence of an activating catalyst such as heavy metal salts. He correlated the depressing effect of metals on milk lipase with their catalytic power in inducing oxidation of milk fat, and suggested that destruction of lipase was catalyzed by heavy metal salts according to their varying powers of activating oxygen. Herrington and Krukovsky (4) reported that 0.2 and 0.4 parts per million of copper reduced lipase activity by about 20 per cent and that smaller amounts had less effect. Hellerman, Perkins and Clark (3) found that urease was inactivated by oxygen in the presence of dissolved copper.

We performed experiments to test Davies' explanation of the destructive action of copper on lipase and to determine whether dissolved oxygen must be present for this destructive action to occur in normal milk. The hypothesis that lipase is inactivated by dissolved oxygen as well as by heat has a bearing on the fact that most of the lipolytic activity in milk is inhibited by heating to relatively low temperatures. To test this idea, a comparison was made of the heat stability of lipase in normal and in deaerated milk, with and without added copper.

EXPERIMENTAL

The importance of dissolved oxygen as one of the primary reactants in the inactivation of milk lipase in the presence of dissolved copper was demonstrated. Whole raw milk was divided into two parts and one part was deaerated. The oxygen-free milk was produced by subjecting milk previously heated to 46° C. to low pressure so that the temperature was caused to fall about 20° C. by evaporation of water (2, 7). A series of aliquots containing increasing amounts of dissolved copper (added as copper sulfate) was prepared using each lot of milk. After preparation the lipase in the aliquots was activated by the cooling, warming and cooling method described by Krukovsky and Herrington (5). The milk was held for various periods of time at 2° C. and the increases in acidity of the fat were determined by titration. The results presented are the differences in acidities

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obtained by subtracting the value of the pasteurized control after all samples had been held for various times at 2° C. The acidity of the fat was determined by titrating 5 grams of fat with 0.05N alcoholic NaOH. The results are expressed in acid degrees, that is, cc. of 1N alkali per 100 grams of fat (4).

The results of a typical experiment are presented in figure 1. The destruction of lipase in the presence of dissolved copper was almost entirely

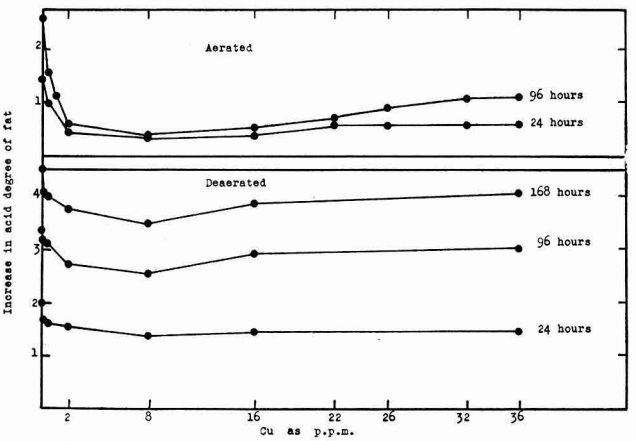

FIG. 1. Effect of increasing amounts of copper on the lipolysis of normal (aerated) and deaerated whole raw milk held at 2° C. for various periods of time.

prevented by the removal of the dissolved oxygen, whereas in the presence of dissolved oxygen the destruction increased with increased copper content up to 2 to 8 mg. per liter. This experiment confirms Davies' general hypothesis and shows that dissolved oxygen is necessary for the destructive action. There is some indication that with the larger amounts of copper a second factor enters which produces a slightly greater acidity of the fat with time. The data also indicate that in the absence of added copper more lipase activity was found in the deaerated than in the normal milk. This can be interpreted as an indication of the presence of a small amount of catalytically active copper or other catalyst in normal milk, or merely that the destructive effect of dissolved oxygen is proceeding normally during the measurement of lipase action.

The slight decrease in development of acid in the deaerated samples upon the addition of the smallest amounts of copper indicates that a trace of oxygen still remained in the milk. With this exception, figure 1 shows that in the absence of dissolved oxygen, dissolved copper exerts no destructive action on lipase even when present in concentrations up to 36 parts per million.

The effect of the removal of oxygen in lessening the destruction of lipase by heat was demonstrated by suitable experiments. The results of one of these are presented graphically in figure 2. Aliquots of raw whole milk,

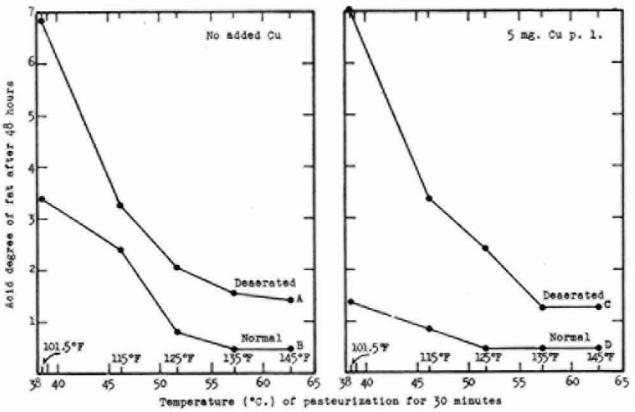

FIG. 2. Effect of temperature on the inactivation of lipase in normal and deaerated milk, with and without added copper. Lipase activated by cooling-warming-cooling, and activity measured by the increase in acidity of the fat as a result of holding for 48 hours at 2° C.

deaerated and normal, and with and without added copper, were heated for 30 minutes at various temperatures. Markedly less destruction of the lipase took place when the oxygen-free samples were heated, as contrasted with the normal milk which had not been deaerated; compare curves A and B. The difference is even more marked when the experiment was performed with added copper; compare curves C and D. Curves A and C are almost identical and confirm our other results by indicating that dissolved copper exerts no destructive action on milk lipase in the absence of dissolved oxygen. A comparison of curves B and D shows that in the presence of dissolved oxygen, copper exerts an accelerating effect on the destruction of lipase.

DISCUSSION

Following the lead given by Davies, it was shown that the lipase in normal milk may be inactivated by a reaction with dissolved oxygen in which dissolved copper acts as a catalyst. The results obtained indicate that normal milk contains enough dissolved copper or other catalyst to cause the inactivation of lipase by dissolved oxygen at relatively low temperatures of heating. The relatively low temperature of destruction of lipase in normal milk is the resultant of two processes: (1) the effect of the increased temperature in accelerating the inactivation of lipase by dissolved oxygen, and (2) the general destructive effect of heat, which is typical of most true enzymes.

Variations in the amount of dissolved oxygen and in the copper content of the milk, as a result of contact with equipment containing copper, may account for some of the variation in results obtained on the inactivation of lipase when milk is subjected to intermediate temperatures (100 to 135° F.). These results indicate that limited destruction of lipase in oxygen-free products may be expected at the low temperatures which may occur during concentration by vacuum evaporation. The inactivation of lipase should occur prior to such treatment, since some activation and lipolysis may result from the agitation incident to evaporation (6).

CONCLUSIONS

1. Dissolved copper causes no inactivation of lipase in normal whole milk in the absence of dissolved oxygen.

2. Normal milk lipase is susceptible to inactivation by dissolved oxygen, and this inactivation reaction is accelerated by heat and by dissolved copper.

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THE "IN VITRO" CONVERSION OF INORGANIC NITROGEN TO PROTEIN BY MICROORGANISMS FROM THE COW'S RUMEN*

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INTRODUCTION

Recent work at this station (Hart *et al.* (1)) has shown that growing dairy calves are able to use inorganic nitrogen in the form of urea or NH_4HCO_3 as a substitute for part of the protein in the ration. The most probable explanation given for this utilization is the production of protein from this nitrogen by the growth of bacteria in the rumen and later digestion of these bacterial cells in the fourth stomach and intestines. Species other than herbivora have failed to demonstrate an appreciable use of urea (2, 3, 4) due probably to the absence of the polygastric type of stomach. With this view in mind the following "in vitro" experiments were set up to test the above hypothesis of inorganic nitrogen utilization in ruminants via rumen bacteria.

EXPERIMENTAL

Preliminary trials were inaugurated in which we attempted to duplicate the conditions found in the rumen. These experiments consisted in adding urea to rumen contents and following the fate of the inorganic nitrogen. All samples were incubated at 37° C. Results in this trial were negative since the level of inorganic nitrogen did not decrease.

The determination of ammonia nitrogen was made by placing an aliquot of the medium into a Kjeldahl flask to which 300 cc. of H_2O were added followed by 5 grams of MgO. The ammonia was then determined by distilling into standard acid. Foaming was prevented by adding liquid paraffin. The urea nitrogen was estimated by treating an aliquot of the medium, neutralized to methyl red, in a Kjeldahl flask with 10 cc. of a 1 per cent solution of urease which also had been neutralized to methyl red. This was incubated for 1 hour at room temperature and then treated as described above for the ammonia nitrogen. The inorganic nitrogen includes the urea and ammonia nitrogen.

In the next trial the animal's ration was used as the medium to which urea was added. This was inoculated with rumen liquid. The rumen liquid, which was obtained from a paunch fistula, was secured by expressing the

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* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This research is part of the larger problem of the utilization of inorganic nitrogen by polygastric animals. We are grateful to E. I. DuPont de Nemours and Company for financial assistance in the study of this problem.

rumen contents. Results were again negative. It was found that a short time after incubation the pH of the medium became acid (4.0–4.5). It has been shown at this station and by others (5) that the reaction of the cow's rumen ranges from a pH of 6.0 to 7.5. With this fact in mind experiments were started in an attempt to maintain an optimum pH. Using sodium phosphate buffer solutions (pH 7.5), 0.15 to 0.5 M, the pH could not be controlled for a sufficient length of time to permit a maximum bacterial growth.

When stronger phosphate buffer solutions were used (1 M) activity was depressed possibly due to the hypertonicity of the solution. However, with the 0.15 or 0.5 M buffers, during the period of optimum pH, disappearance of inorganic nitrogen was observed. The first indication of a conversion (decrease in NH_3-N) was obtained on a synthetic medium. The medium consisted of 600 cc. of water, 1.1 gram of urea, 15 cc. of molasses, 100 cc. of 1 M Na_2HPO_4, and was inoculated with 20 cc. of rumen liquid.

The results obtained follow:

Hours of incubation	mg. NH_3-N/100 cc. medium	pH
0	74.8	7.50
24	68.0	7.35
48	63.0	6.40
72	49.5	4.80

An examination of the above data shows that the phosphate buffer was not able to maintain an optimum pH.

Further trials with $CaCO_3$ as the buffer showed that it was possible to maintain the medium at a pH of 5.8 to 6.5. An excess of $CaCO_3$ was always added, so that a part of it remained undissolved. Since this pH approximates the reaction of the rumen, in all subsequent trials $CaCO_3$ was used as the buffer. In all these experiments uninoculated samples were also run as controls.

Carbohydrate studies

The carbohydrates used included corn molasses, cerelose (commercial glucose), starch and cellulose. The source of carbohydrate in the medium with the exception of cellulose all supported bacterial growth as measured by the NH_3-N disappearance. Cellulose failed as a source of energy for the organisms used. These experiments were all carried on in synthetic media.

Nutrient salts for bacteria were found to be essential in the synthetic medium containing cerelose or starch. The salt mixture had the following composition:

KH_2PO_4	—10	grams
K_2HPO_4	—10	grams
$MgSO_4 \cdot 7H_2O$	— 4	grams
NaCl	— 0.2	gram
$MnSO_4$	— 0.2	gram
$FeSO_4$	— 0.2	gram

The mixture was dissolved and made up to a volume of 1 liter. Ten cc. of this solution per liter of medium were used when necessary.

The extent of disappearance of inorganic nitrogen, regardless of the source of carbohydrate (cellulose excepted), depended on the amount of carbohydrate in the media. The data secured follow:

Hours of incubation	mg. NH_3-N/100 cc. medium	Sugar added
0	83.0	30 cc. corn molasses
24	74.5	0
48	50.0	0
72	46.0	20 cc. corn molasses
96	3.5	0

$\frac{83.0-3.5\times 100}{83.0}$ = 95.7 per cent disappearance of NH_3-N.

As can be seen from the above table there was a large decrease in NH_3-N up to 48 hours. At this point the rate of disappearance decreased. On the addition of more corn molasses the rate of disappearance again increased markedly.

Nitrogen studies

It was observed that 24 hours after inoculation of the media the amount of urea present was negligible. At the same time there was an increase in NH_3-N that was comparable to the decrease in urea nitrogen. This seemed to indicate that there was an initial hydrolysis of urea to NH_3 followed by disappearance of the NH_3-N. This led us to compare disappearance of NH_3 using NH_4HCO_3 *vs.* urea as the inorganic nitrogen source. No difference in the rate of disappearance was observed.

Data illustrating the use of these two materials follow:

Hours of incubation	mg. NH_3-N/100 cc. medium	
	NH_4HCO_3-N	urea – N
0	74	74
24	56	54
48	24	19
72	12	3

The question arises as to the fate of the inorganic nitrogen that disappeared in these fermentations. Was it lost from the media, or converted to protein? The first possibility was eliminated by finding that there was no loss of total nitrogen in the media; the Kjeldahl method was used to determine total nitrogen. At the same time there was a disappearance of NH_3-N. Illustrative data follow:

Hours of incubation	mg. NH_3-N/100 cc. medium	mg. total N/100 cc. medium
0	133	139
96	79	132
difference	54	7

$\frac{139-7\times 100}{139}$ = 95 per cent of total N recovered.

The second possibility, namely that there was conversion of NH_3-N to protein, was investigated. This involved the removal of the protein from the medium (bacterial cells) by filtering the medium through a filter cell. The filter cell used was made of finely powdered silica. The filter was prepared by forming a quarter inch pad of filter cell over a filter paper in a Büchner funnel. Ammonia nitrogen was run on one aliquot and the protein (filter cell) nitrogen determined by Kjeldahl on another aliquot. The following data were obtained:

Hours of incubation	mg. NH_3-N/100 cc. medium	Residual – N/100 cc. medium
0	60	2.9
72	42	19.2
difference	18 mg. NH_3-N lost	16.3 mg. residual N gained.

$\frac{16.3 \times 100}{18} = 90.6$ per cent recovery of NH_3 nitrogen as non-filterable N.

The recovered non-filterable material was presumably bacterial cells which contained the NH_3-N lost, which was also the residual nitrogen gained.

Poor conversion was observed in samples using the cow's ration as the medium to which NH_4HCO_3 was added. The ration consisted of equal parts of corn and oats, timothy hay, and corn silage, and was added at a 15 per cent level. Since the greatest difference between the ration and the synthetic medium seemed to be the protein, the effect of varying the protein content of the medium was studied. The basal medium consisted of 350 cc. of water, 25 grams of cerelose, 20 grams $CaCO_3$, 50 cc. rumen inoculum, 3 cc. nutrient salts, 2.6 grams NH_4HCO_3, to which varying amounts of casein were added. The following results were secured:

Sample	Supplement	mg. NH_3-N remaining/100 cc. medium			
		0 hours	24 hours	48 hours	72 hours
1	No casein	105	49	26	3
2	2.5 gms. casein	104	88	74	53
3	5.0 " "	105	91	93	96
4	10.0 " "	101	93	98	100

The above data indicate that only on samples "no casein added" and "2.5 grams casein level" was there conversion; the other two levels of casein added to the medium were negative. A probable explanation is that the bacterial flora used protein nitrogen in preference to NH_3-N, or that the bacterial proteolytic enzymes masked the drop in the NH_3-N by forming NH_3 from the protein as fast as the NH_3 was built into bacterial cells.

Since the conversion over a 24-hour period represents merely an average of that period, hourly NH_3-N determinations were made during the peak of activity of the bacterial growth in the media. This was done in order to

determine the maximum rate of conversion calculated on a 24-hour basis. The data secured follow:

Hours of incubation	mg. NH_3-N/100 cc. medium	Conversion per 24 hrs./ 100 cc. medium
20	86	0.0 mg. N
22	85	12.0 " "
24	82	36.0 " "
26	79	36.0 " "
28	73	72.0 " "
30	66	84.0 " "
32	57	108.0 " "
34	50	84.0 " "

The maximum conversion calculated in this manner is much greater (108 mg. NH_3-N per 24 hours per 100 cc. medium) than that found when determinations were made every 24 hours, which were never found to be over 50–60 mg. NH_3-N/100 cc. medium, since they were an average of the 24-hour period. This suggests what could be expected in the rumen of the animal assuming optimum conditions existed in the paunch at all times.

The buffering action of cow's saliva is recognized as an important factor in maintaining the reaction of the rumen at a near neutral point. Since large amounts of starch are normally present in the ration the presence of amylase in the saliva could be readily invoked as a means of forming soluble sugars which would promote bacterial growth. The literature indicates that cow's saliva has no diastatic properties. Amylase activity of cow's saliva was determined on a starch medium adjusted to pH 6.8 and incubated at 37° C. The saliva was collected by having the cow chew on a sponge; the liquid in the sponge was expressed into a flask at short intervals. To 100 cc. of saliva that was saturated and covered with toluene, 10 cc. of a 3 per cent starch solution were added, and the reducing sugars determined with Fehling's solution by measuring at frequent intervals the Cu_2O precipitated. The results indicated only a slight amylolytic activity.

Because of this slight amylolytic property the addition of cow's saliva to a synthetic starch medium inoculated with rumen liquid might enhance conversion due to an increase of available fermentable sugars for the bacteria. However, when cow's saliva was added to an inoculated starch medium, no increase in conversion was produced. Using maltose in place of starch the maltase activity of saliva was found to be negative. The possibility of diastatic activity in the rumen through bacterial action directly presents itself. Preliminary data on chemical changes in the rumen of the cow indicate a distinct diastatic action.

As evidence for this hypothesis, the diastatic action of rumen liquid was determined using the same technique as described above for saliva. In comparison to saliva, diastatic action of rumen juice is decidedly greater and

could very likely account for the hydrolysis which must occur in the breakdown of starch and possibly other polysaccharides.

Proteolytic activity of the saliva on a casein medium was also tested. Both tryptic and peptic enzymes were found to be absent. This finding indicated that there was no contamination of the cow's saliva we collected since considerable proteolysis occurs in the rumen.

DISCUSSION

The foregoing results have demonstrated that the conversion of inorganic nitrogen to protein may be obtained by inoculation of appropriate media with microorganisms contained in liquid from the cow's rumen. No attempts were made to inoculate the media with pure cultures of bacteria from the rumen since a multiplicity of different kinds undoubtedly exists in the paunch. For the same reason contamination was not considered as an important factor in this work.

In reviewing these results several criticisms appear obvious. This is especially true if an attempt is made to relate these findings to what actually happens in the rumen of the cow. In the rumen a maximum bacterial flora is always present while in "in vitro" experiments this flora must first develop. In this intervening time chemical changes such as proteolysis may be going on in the medium (using the ration as medium) which do not have time to occur in the rumen. This will lead to non-comparable results since two reactions are working in opposite directions.

$$NH_3 - N \underset{\text{Bacterial Proteolytic Enzymes}}{\overset{\text{Bacterial Growth}}{\rightleftharpoons}} \text{Protein}$$

All that is attempted in this work is to show how some of these factors influence the conversion. Through a fistula in the cow, studies on conversion in the rumen are now in progress. The food in the rumen is a continually moving mass, part of it being removed and new material being constantly added. This condition cannot be duplicated "in vitro." Further, the products of fermentation may have an adverse effect on conversion. In the rumen these products are continually being removed. From the above statements it also can be surmised why the results on a synthetic medium cannot be correlated closely with those obtained using the animal's ration as the medium.

However, the main fact obtains, namely, that given optimum conditions, which approach those in the rumen of the cow, conversion of inorganic nitrogen to protein can be demonstrated in "in vitro" experiments.

SUMMARY

1. Evidence is presented through "in vitro" experiments that conversion

of inorganic nitrogen to protein can occur through the use of bacteria from the cow's rumen.

2. Bacterial activity, and hence conversion, is dependent on the pH of the media, the optimum range being 5.5 to 7.

3. The carbohydrates used in the media were of equal efficiency in influencing conversion, with the exception of cellulose which was not an acceptable carbohydrate for these studies.

4. NH_4HCO_3 is as efficient as urea in the rate of utilization by rumen organisms.

5. The decrease in NH_3 can be accounted for by an increase in protein nitrogen.

6. The level of protein in the media has a negative influence on the decrease in $NH_3 - N$.

7. Amylolytic activity of rumen liquid has been demonstrated, with only slight activity in the saliva itself.

8. Proteolytic activity of cow's saliva is absent.

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Milk -- Vitamin C content

THE EFFECT OF COMMERCIAL PRACTICES ON ASCORBIC ACID AND DEHYDROASCORBIC ACID (VITAMIN C) IN MILK*[1]

biology tabs

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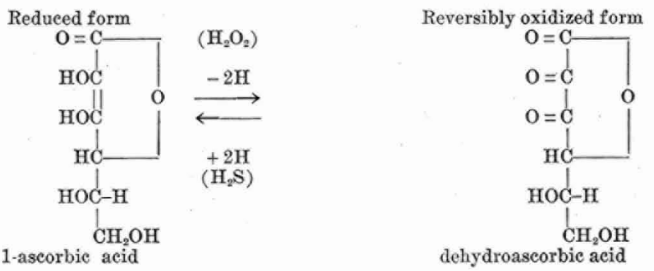
Ascorbic acid, analogous to vitamin C, exists in two chemical forms, reduced and reversibly oxidized, which possess equal biological activity for the prevention of scurvy (2). The reversibility of the relationship is indicated by the following equation:



The influence of processing methods on the stability of the two forms of vitamin C has not been clearly established. During the progress of this investigation Gjessing and Trout (1) reported on the stability of vitamin C in milk pasteurized at different temperatures and for varying intervals using the indophenol titrimetric technique to determine only the ascorbic acid.

The object of this study was to determine the influence of commercial practices on the two forms of vitamin C, ascorbic and dehydroascorbic acids. Little attention has been given to the practibility of producing a vitamin-C fortified milk on a commercial scale. Hence it was deemed advisable to study the production of such a product, for if it were available it should prove of significant value for general use in welfare work, in maternity wards, and in general malnutrition.

EXPERIMENTAL

The literature contains numerous procedures for the determination of ascorbic acid in milk but only very few for the determination of the impor-

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tant, equally biologically active dehydroascorbic acid. Kon and Watson (3) have suggested a procedure for the estimation of dehydroascorbic acid in milk but Woessner, Elvehjem and Schuette (4, 5) have shown that the use of a photoelectric colorimeter is essential for such a determination because it eliminates the interference due to other substances which are formed when the milk is treated with hydrogen sulfide. Their method which was used for this research is specific for ascorbic and dehydroascorbic acids.

The apparatus and reagents were identical with the photoelectric technique described by Mindlin and Butler (6) except that the potassium oxalate, cyanide, and metaphosphoric acid solutions were replaced by a modified Willberg (7) reagent (0.6 g. $H_2C_2O_4$ $2H_2O$, 4.8 g. NaCl and 6.5 g. HPO_3 in one liter of water) prepared accurately to insure proper pH in the colorimeter tube.

Determination of ascorbic acid. In the absence of strong light 25 ml. of milk are pipetted into a 125-ml. Erlenmeyer flask containing 75 ml. of modified Willberg reagent. The protein precipitate is removed by filtering through paper of quality similar to Whatman No. 42. Five ml. of the filtrate are measured into a colorimeter tube and 10 ml. of the indophenol solution are added. The contents are stirred and examined immediately. Since it is not always possible to obtain a filtrate that is crystal-clear, it is recommended that a small quantity of ascorbic acid be added after the original reading has been made, whereupon the correction due to the turbidity is determined. Thus, the true reading is equal to the original reading plus 100 minus the reading after the crystal of ascorbic acid has been added.

Determination of dehydroascorbic acid. After the addition of a few drops of dibutyl phthalate to the milk to prevent foaming, wet hydrogen sulfide is bubbled through the milk for exactly 20 minutes. Then as rapidly as manipulation will permit, 25 ml. of the hydrogen-sulfide-saturated milk are added to 75 ml. of modified Willberg reagent, and the whole is shaken well to break into small particles the curd which forms. The hydrogen sulfide is removed immediately by passing a vigorous stream of wet oxygen-free nitrogen through the mixture for 20 minutes. After the curd is removed by filtration, 5 ml. of the filtrate are measured into one of the colorimeter tubes. Then with the simultaneous start of a stop watch, 10 ml. of the dye acetate solution are added to the tube by means of a rapid delivering pipette, and the galvanometer readings at 15 and 30 seconds are recorded. The galvanometer reading corresponding to zero seconds can be considered equal to the difference of the galvanometer readings at 15 and 30 seconds substracted from the galvanometer reading at 15 seconds.

Calculations are the same as those described by Mindlin and Butler (6); K under the conditions prescribed has a value of 0.166 ∓ 0.003.

Milk supply and plant equipment. The milk used for these studies was of the regular plant supply as received daily at the Department of Dairy

Industry of the University. It was handled by either one of two different procedures. In the manner of handling which shall be referred to later for convenience, as the A process, the milk was received at the intake in a stainless steel weighing tank and then dropped into a stainless steel holding tank. Next it was passed through a short piece of partially worn tinned-copper piping, a stainless steel positive-feed pump, an air-tight separator-clarifier and finally through a short piece of partially worn tinned copper piping into a vertical cylindrical spray-vat pasteurizer. In the B process, the milk was handled as in the A process until it was passed through the pump whence it was passed through a tubular preheater (90° F.) and thence through the separator-clarifier. From the clarifier the milk was passed through forty feet of well worn tinned-copper piping into a stainless steel horizontal spray-vat pasteurizer.

THE INFLUENCE OF ELEVATED TEMPERATURES

The clarifier and tubular preheater used were found to exert no destructive or oxidative effect on ascorbic or dehydroascorbic acids that could in any way be considered significant.

In table 1 is summarized the effect of holder pasteurization on the ascorbic acid content of several normal milks and on the ascorbic acid content of milks containing added ascorbic acid. Lots I to V inclusive were handled by the A process and Lots VI and VII by the B process. Since the A process milks were to be homogenized they were pasteurized at a higher temperature (150° F.) than the B process milks (145° F.).

From the data (table 1) it is observed that the A process milks show an average destruction of ascorbic acid of 11.4 per cent and what may be considered complete destruction of dehydroascorbic acid. The overall destruction of the vitamin by this process is 20.2 per cent. This latter figure is in excellent agreement with values previously cited in the literature.

However, Kon and Watson (3) found that the loss in total ascorbic acid originally present in the milk is more than three times that suffered by the reduced form. Hence they claimed it is chiefly the reversibly oxidized form that is destroyed. The data (table 1) indicate that the loss in total ascorbic acid is never much greater than the loss of ascorbic acid itself and thus the results are not in agreement with those of Kon and Watson (3).

The quantity of dehydroascorbic acid remaining after pasteurization is less than the quantity present before pasteurization and is also less than the quantity of ascorbic acid lost during pasteurization. This indicates that part of the dehydroascorbic acid originally present in the milk which was formed from ascorbic acid by normal exposures of the milk to light, as well as that formed from the ascorbic acid during the pasteurization holding process undergoes a very rapid destruction. It appears that the dehydroascorbic acid is destroyed as rapidly as it is formed from the ascorbic

TABLE 1

The effect of vat pasteurization on the forms of ascorbic acid in milk

Milk lot	Source of sample	Ascorbic acid per liter			Total per cent loss
		Reduced	Oxidized	Total	
		mgs.	*mgs.*	*mgs.*	
I[1]	From vat before pasteurization	17.4	1.6	19.0	
	After pasteurization for 30 min. at 150° F.	15.9	0.0	14.9	21.5
II	From vat before pasteurization*	40.0	3.2	43.2	
	After pasteurization for 30 min. at 150° F.	38.4	1.1	42.0	2.7
III	From vat before pasteurization	14.4	6.1	20.5	
	After pasteurization for 30 min. at 150° F.	12.5	2.4	14.9	27.3
IV	From vat before pasteurization*	38.7	4.7	43.4	
	After pasteurization for 30 min. at 150° F.	33.0	0.7	33.7	22.3
V	From vat before pasteurization	17.1	2.8	18.9	
	After pasteurization for 30 min. at 150° F.	14.2	0.0	14.2	24.8
VI[2]	From vat before pasteurization	15.4	5.8	21.2	
	After pasteurization for 30 min. at 145° F.	10.5	4.9	15.4	12.6
VII	From vat before pasteurization*	39.9	10.3	50.2	
	After pasteurization for 30 min. at 145° F.	25.7	12.7	38.4	23.3

* Fortified with added ascorbic acid.

[1] Lots I to V—Stainless steel vertical spray vat pasteurizer.

[2] Lots VI and VII—Stainless steel lined horizontal spray vat pasteurizer. Copper content 0.13 to 0.29 p.p.m.

acid. The limiting factor in destruction of total vitamin C, therefore, is not the rate of destruction of dehydroascorbic acid but rather the transformation of ascorbic acid to dehydroascorbic acid by heat. The reversibly oxidized form (dehydroascorbic acid) may be considered as being chiefly destroyed only if it is originally present in a larger quantity than the amount of ascorbic acid which is normally lost during the thirty-minute holding period. It is for this reason that statements in the literature which claim the flash pasteurization procedure to be less destructive than the holding process are believed to be sound. The destruction of the ascorbic acid appears to be more dependent on the time it is held at the elevated temperature than on the temperature itself; in other words, the rate of the conversion of ascorbic acid to dehydroascorbic acid has a small temperature coefficient whereas the decomposition of dehydroascorbic acid has a high temperature coefficient.

This conclusion is supported by the results obtained when handling milk by the A process including the experimental lots I to V (table 1). A somewhat different result was obtained when the milk was handled by the B method, including lots VI and VII (table 1). The processing of the milks in this instance involved considerably more copper piping which introduced

an average of 0.21 p.p.m. of copper into the milk. As a result dehydroascorbic acid was very rapidly formed from ascorbic acid and the percentage loss of ascorbic acid was as high as 35.6 per cent. The results of the measurement of dehydroascorbic acid shows that the copper present caused the formation of dehydroascorbic acid as rapidly as heat destroyed it. This can be more readily understood by comparing the total loss of ascorbic acid during the pasteurization with the quantity of dehydroascorbic acid originally present in the milk. The two figures are about equal. Copper, therefore, can be considered as accelerating the conversion of ascorbic to dehydroascorbic acid, a reaction ordinarily quite slow at elevated temperatures in the absence of light, so that it equals the rate at which dehydroascorbic acid is thermally destroyed. Thus an explanation is provided for the instability of ascorbic acid in the presence of copper in milk.

In the presence of copper the conversion of ascorbic to dehydroascorbic acid evidently has a high temperature coefficient. That the thermal decomposition of dehydroascorbic acid has a high temperature coefficient in the absence of copper is demonstrated by the fact that the dehydroascorbic acid produced by exposing copper-free milk to light can only be quantitatively recovered if the sample is kept cool; short heating to 100° C. will destroy completely the dehydroascorbic acid. Hence it is assumed that copper does not accelerate the irreversible oxidation of dehydroascorbic acid but only speeds the conversion of the heat-stabile ascorbic acid to the heat-labile dehydroascorbic acid.

TABLE 2

*The relative destruction of the forms of ascorbic acid in milk during vat pasteurization**

Milk lot	Temperature at which sample was taken	Holding time	Ascorbic acid per liter		
			Reduced	Oxidized	Total
		Minutes	*mgs.*	*mgs.*	*mgs.*
I**	80° F.		17.1	2.8	18.9
	100° F.		16.0	.....	.....
	120° F.		16.0	.....	.....
	130° F.		16.2	2.0	18.2
	140° F.		15.8	.....	.....
	150° F.	0	16.0	1.1	17.1
	150° F.	30	14.2	0.0	14.2
II	140° F.		18.0	0.0	18.0
	150° F.	0	17.6	0.0	17.6
	150° F.	10	17.1	0.0	17.1
	150° F.	20	16.7	0.0	16.7
	150° F.	30	15.8	0.0	15.8

* Vertical cylindrical stainless steel pasteurizer.

** The time of heating from 80° F. to 150° F. was 16 minutes. Approximately 3 minutes elapsed between each temperature indicated.

In order that the manner in which the loss of ascorbic acid occurs in the vertical spray-vat pasteurizer (process A) might be still better understood measurements were made during the entire preheating and holding period of the milk while in the vat. The results of such an investigation are summarized in table 2. The data obtained were not influenced by the presence of copper in the milk.

During the preheating period a nine per cent loss in ascorbic acid was observed. It also appears that there was a general diminution of the dehydroascorbic acid so that by the time (16 minutes) the milk reached 150° F. there was little or no dehydroascorbic acid remaining. During the holding period the data denote there was a ten per cent loss of the ascorbic acid, the dehydroascorbic acid being destroyed as rapidly as it was formed. This observation confirms the reasoning which has already been presented.

Since nine per cent of the total destruction occurred during the short preheating period of 12 minutes, it is pertinent to note that most of the loss was due to the destruction of the dehydroascorbic acid already present in the raw milk. The loss during the thirty-minute holding period consisted wholly of ascorbic acid by way of the mechanism postulated. Thus it is reasonable to suppose that the superiority of the flash process over the holder method comes mainly from the fact that the milk is held at the higher temperature for a shorter period of time and not because of a rapid preheating period. The dehydroascorbic acid originally present in the milk no doubt is destroyed in the flash process but the ascorbic acid is probably little affected because of its low temperature coefficients coupled with the fact that it remains at the elevated temperature for such a short period of time.

From the foregoing discussion it can also be concluded that if a sample of milk is subjected to thermal treatment sufficient to cause a diminution of the ascorbic acid present there will also be a total destruction of the dehydroascorbic acid, including that present before the heat is applied and that formed during the time the milk is held at the elevated temperature.

THE EFFECT OF HOMOGENIZATION

The milk for these experiments was processed by the A method and was then passed from the pasteurization vat through stainless steel piping to the homogenizer. The homogenization was effected at 150° F. and 2000 lbs. pressure after which the milk was passed over a newly tinned copper cooler (40° F.). The cooler was protected from light with metal covers.

In table 3 are summarized the results of homogenizing 6 different lots of milk. Inspection of the data of both the normal and vitamin C fortified lots discloses that homogenization does not cause any destruction of ascorbic acid or formation of dehydroascorbic acid.

It also may be concluded from table 3 that passing milk over a tinned-copper cooler in good condition causes no loss of ascorbic acid or formation of dehydroascorbic acid.

TABLE 3

The effect of homogenization on the forms of ascorbic acid of milks[1]

Sample taken	Ascorbic acid per liter					
	Reduced	Oxidized	Total	Reduced	Oxidized	Total
	mgs.	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*
	I			II[2]		
From vat before pasteurization	17.4	1.6	19.0	40.0	3.2	43.2
After 30 minutes at 150° F.	15.9	0.0	14.9	38.4	1.1	39.5
After homogenization[3]	14.5	0.0	13.6	39.5	0.0	39.5
After cooling	14.7	1.4	16.1	38.9	1.3	40.2
	III[2]			IV[2]		
After 30 mintues at 150° F.	35.7	2.5	38.2	33.0	0.7	33.7
After homogenization[3]	35.0	3.7	38.7	32.4	2.6	35.0
After cooling	35.7	1.3	37.0	33.0	0.0	32.6
	V			VI		
After 30 minutes at 150° F.	14.2	0.0	14.2	15.8	0.0	15.8
After homogenization[3]	.....	.....	.....	.....	.....	.....
After cooling	12.8	2.3	15.1	15.8	0.0	15.8

[1] Vertical stainless steel pasteurizers and stainless steel homogenizer (2000 lb. pressure).

[2] Ascorbic acid added to milk before pasteurization.

[3] Sample taken while hot before the milk was passed over the cooler.

THE FEASIBILITY OF PRODUCING A VITAMIN C-FORTIFIED MILK

Reedman (8) and Kroker (9) suggested the feasibility of preparing a vitamin C-fortified milk. Kroker (9) suggested the addition of ascorbic acid to milk directly after it is flash-pasteurized and, because of the destructive effect that light has on the vitamin, he also suggested that the milk be marketed in dark glass bottles.

The fortification of milk handled by the A and B processes was studied. In the absence of significant quantities of copper these experiments which were conducted on a commercial scale (600 gallons) proved that such fortification is practical. The only limiting factors from the commercial standpoint are the present cost of ascorbic acid and the necessity of affording protection from light and exposed copper.

In series 1 (table 4) the milk was handled by the A process and divided equally into two identical vertical spray pasteurizer vats. The fortification with ascorbic acid was accomplished by dissolving the vitamin in 100 ml. of sterile copper-free water and pouring the solution slowly into the vat containing the milk; this operation was followed by a short counter flow mixing with a stirring rod to insure rapid distribution of the vitamin throughout

the milk. The addition of ascorbic acid had no froward effects upon the flavor of the milk.

TABLE 4

The stability of the forms of ascorbic acid in vitamin C fortified milk

Sample taken	Ascorbic acid per liter					
	Unfortified			Fortified[3]		
	Reduced	Oxidized	Total	Reduced	Oxidized	Total
	mgs.	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*
	Series I[1]					
From vat before pasteurization	17.4	1.6	19.0	40.0	3.2	43.2
After 30 minutes pasteurization at 150° F.	15.9	0.0	14.9	42.0	0.0	42.0
After 50 minutes pasteurization at 150° F.	14.7	0.5	15.2	38.4	1.1	39.5
After homogenization[4]	14.5	0.0	14.5	39.5	0.0	39.5
After cooling[4]	14.7	0.0	14.7	38.9	1.3	40.2
	Series II[2, 5]					
From vat before pasteurization	15.4	5.8	21.2	39.9	10.3	50.2
After 30 minutes pasteurization at 145° F.	10.5	4.9	15.4	25.7	12.2	38.4
After cooling	5.7	7.8	13.5	25.7	10.2	35.9
After 24 hrs. storage 40° F.	0.0	3.8	3.8	0.0	14.8	14.8

[1] A process.
[2] B process.
[3] 20 gms. ascorbic acid added to 730 quarts of milk in vat before pasteurization.
[4] Sample removed after being held for 30 minutes at elevated temperature.
[5] On analysis these samples contained 0.13 to 0.29 p.p.m. of copper.

The data (table 4) indicate that the losses of ascorbic acid in both the unfortified and the fortified milks upon pasteurization are normal. It may be seen, however, that a definite antiscorbutically better product can be produced at will. By adjusting the initial fortification, a milk could be easily produced that would be on a par antiscorbutically with human milk (50 to 70 mg. per liter). If the milk should become contaminated with copper from the pipe lines or bottling equipment, an attempted fortification would be impractical as the vitamin C would almost disappear in 24 hours.

Regulations permitting, a more economical fortification is possible if the vitamin is added at the close of the holding period. This fact is illustrated by the data of table 5. Consequently, the suggestion of Kroker (9) is the ideal method of fortification as flash pasteurization conserves the maximum amount of the natural vitamin already present in the milk.

THE EFFECT OF LIGHT

Kon and Watson (3), Houston, Kon and Thompson (10) and Hender-

TABLE 5

*The stability of the forms of ascorbic acid added to milk before and after pasteurization**

Sample taken	Ascorbic acid per liter		
	Reduced	Oxidized	Total
	mgs.	*mgs.*	*mgs.*
	I		
From vat before pasteurization	14.4	6.1	20.5
After 30 minutes pasteurization at 150° F.	12.5	2.4	14.9
After 30 minutes pasteurization at 150° F., *ascorbic acid added* (16 gms. per 730 quarts)	35.7	2.5	38.2
After homogenization	35.0	3.7	38.7
After cooling	35.7	1.3	37.0
After 24 hr. storage in dark	29.0	3.4	32.6
	II		
From vat before pasteurization	14.4	7.0	21.4
From vat before pasteurization, *ascorbic acid added* (16 gms. per 730 quarts)	38.7	4.7	43.4
After 30 minutes pasteurization at 150° F.	33.0	0.7	33.7
After homogenization	32.4	2.6	35.0
After cooling	33.0	0.0	32.6
After 24 hr. storage in dark	26.3	3.3	29.6

* Vertical stainless steel spray pasteurizers and stainless steel homogenizer used (2000 # pressure).

son, Foord and Roadhouse(11) have studied the effect of light and the effect of different containers on the antiscorbutic activity of milk. The results of this investigation, as shown in table 6 confirm their qualitative observations that brown glass and wax-impregnated cartons protect the ascorbic acid from actinic rays of light considerably more than the ordinary clear glass milk bottle. While a difference in the actinic effect on the vitamin C was observed by using different bottles or containers, it should be noted that exposure of any of these bottles or containers to direct sunlight for sufficient period caused the formation of the labile dehydroascorbic acid. Milk in plain glass milk bottles which were carried on the regular delivery route (enclosed truck delivery) showed no losses in excess of those normally encountered when the milk is stored in the dark. This indicates that in the ordinary delivery of the milk serious exposure to light probably does not occur. The significant loss will occur, therefore, not while the milk is being processed (assuming no copper contamination) including preheating, pasteurization, homogenization and cooling but rather when the milk is exposed to sunshine after its delivery. The benefits of fortification would be nullified if subsequent protection of the milk from light is not provided.

THE EFFECT OF OTHER COMMERCIAL PRACTICES

The stability of the two forms of vitamin C in normal and vitamin

TABLE 6

The value of containers in preventing losses of ascorbic acids in milk exposed to light[1]

	Ascorbic acid per liter		
	Reduced	Oxidized	Total
	mgs.	*mgs.*	*mgs.*
I. Analyzed immediately:			
Unfortified Milk			
Unexposed	17.0	0.0	17.0
Clear glass bottle, exposed to light 1 hr.	12.1	3.9	16.0
Brown glass bottle, exposed to light 1 hr.	17.0	0.0	17.0
Wax container, exposed to light 1 hr.	17.0	0.0	17.0
Fortified (Added C) Milk			
Unexposed	40.6	0.0	40.6
Clear glass bottle, exposed to light 1 hr.	32.0	7.1	39.1
Brown glass bottle, exposed to light 1 hr.	40.4	0.0	40.4
Wax container, exposed to light 1 hr.	39.8	0.6	40.4
II. Exposed and then stored in dark 8 hrs. at 40° F. before analysis			
Unfortified, clear glass bottle, exposed to light 1 hour	5.8	3.1	9.9
C-fortified, clear glass bottle, exposed to light 1 hour	23.0	5.0	28.6
C-fortified, brown glass bottle, exposed to light 1 hour	28.6	4.9	33.5

[1] A Process—homogenized milks. Overcast but bright day; no direct sunlight. Quart containers were used.

C-fortified milk processed by various methods was not in any way altered when further fortified by the addition of vitamin D concentrates processed in stainless steel equipment.

The use of sodium metaphosphate to lower the curd tension as recommended by Schwartze, Jones, Mack and Vance (12) was found neither to accelerate the oxidation of ascorbic acid or protect it from oxidative catalysis of copper (0.15 p.p.m.). Identical results were obtained when a pancreatic enzyme concentrate (Armour and Co.) was used (1 part in 25,000). However, it is pertinent to note that although the enzyme concentrate lowered the curd tension in addition to preventing copper-induced oxidized flavor it did not prevent the rapid oxidation of ascorbic acid. Thus it appears that ascorbic acid is not related to copper-induced oxidized flavor in milk.

CONCLUSIONS AND SUMMARY

Pasteurization by the holder methods was found to cause a 20 per cent loss of total ascorbic acid. After pasteurization no dehydroascorbic acid was found in the milk. The advantage of the flash pasteurization process in preserving the vitamin C content of milk seems dependent upon the fact that the destruction of the ascorbic acid appears to be more dependent on the time that it is held at the elevated temperature than on the temperature

itself; in other words, the rate of the conversion of ascorbic acid to dehydroascorbic acid has a small temperature coefficient whereas the decomposition of dehydroascorbic acid has a high temperature coefficient.

The most serious losses in antiscorbutic activity of milk during its processing and delivery were caused by contamination by copper and exposure to light. It is practical to produce a vitamin C-fortified milk on a commercial scale but the rigid exclusion of copper and protection from light are essential if the fortified milk is to be marketed through normal channels.

Tubular preheating (90° F.), clarification, homogenization, cooling and protected delivery individually, or collectively, were found to cause no loss of ascorbic acid or dehydroascorbic acid in milk. Likewise, no loss was caused by the addition of vitamin D concentrate followed by homogenization. Use of sodium metaphosphate or pancreatic enzyme were found to have neither a protective or detrimental effect on the vitamin C. The enzyme concentrate prevented copper-induced oxidized flavor but did not inhibit the usual rapid disappearance of ascorbic acids.

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THERMODURIC BACTERIA IN PASTEURIZED MILK. A REVIEW OF LITERATURE

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During recent years there has been a growing interest in the study of thermoduric bacteria in pasteurized dairy products. Among the factors which have been operative in stimulating this interest, the following four have been important.

1. Increasing emphasis on the part of certain Departments of Health on low bacteria counts.
2. The distinctly higher counts obtained on agar plates when the medium is enriched, as by the change from the old standard nutrient agar to the tryptone glucose extract milk agar adopted as official in the 1939 edition of Standard Methods for the Examination of Dairy Products (1). It was pointed out by Sherman in 1916 (2) that as simple a change in the old standard nutrient agar as the addition of lactose would give much higher counts. Since the adoption of the tryptone glucose extract milk agar as standard, data has been accumulating showing that higher counts are obtained with the new as compared with the old medium (3). In addition, numerous articles were published previously showing the increases in count obtained on other enriched media similar to, but differing slightly in composition from the one finally adopted as standard (4, 5, 6, 7, 8).
3. The much higher counts obtained when the temperature of incubation is lowered. When the American Public Health Association was considering the change in the composition of the agar, referred to above, it was also considering a reduction from 37° C. to 32° C. (98.6° F. to 89.6° F.) in the temperature of incubation. Studies made and published in connection with this proposal showed that counts on all dairy products except dry milk are increased by such a reduction in the incubation temperature (3, 5, 6, 9, 10).
4. The rapid development in recent years of equipment for pasteurizing milk by the high-temperature, short-hold method. This method has decided advantages over the low-temperature, long-hold method from the standpoint of engineering and cost efficiency (11, 12, 13). Moreover, it is safe from the standpoint of destruction of pathogenic bacteria (11, 13, 14, 15, 17); phosphatase is destroyed (13, 16); flavor is not affected (13, 16); and cream volume is not reduced (13, 14, 16). However, bacteria counts are apt to be higher unless thermoduric organisms are eliminated from the supply, a point that will be discussed more in detail later in this review.

A. BACTERIA SURVIVING LOW-TEMPERATURE PASTEURIZATION

When pasteurization first came into general use, it was assumed, especially by the medical bacteriologists, that most, if not all, of the organisms surviving would be spore-formers (18, 19), although Russell and Hastings (20) had reported in 1901 the discovery of a micrococcus which could survive heating to 76° C. (168.8° F.)

The classical studies on the bacteriology of pasteurized milk by Ayers and Johnson (21, 22, 23, 24, 25) in 1913–1916 established several important facts, as listed below.

1. The relative proportions of acid-producing, inert, alkali-producing and peptonizing bacteria are about the same in milk pasteurized at 145° F. (62.8° C.) for 30 minutes as in raw milk (21, 22, 25).

2. In milk pasteurized at 145° F. (62.8° C.) for 30 minutes (22), the percentage of each class of organism was as given in table 1.

TABLE 1

Organisms surviving pasteurization at 145° F. for 30 min.

Class of organism	Grade A	Grade B
Acid without coagulation	61.87	34.82
Acid with coagulation	17.91	31.89
Inert	9.06	24.00
Alkali producing	9.77	5.63
Peptonizing	1.39	3.59
Total acid producers	79.78	66.71

3. As the temperature of pasteurization increases (with 30 minute holding), the proportion (but not the total number) of acid-producers and alkali-producers decreases, the proportion of inert organisms increases somewhat, and the proportion of peptonizers increses markedly (21, 22, 25).

4. Of a group of 139 cultures of acid-producers which were studied, 127, or 96.20 per cent, were cocci.

5. Of 43 alkali-producers, 31, or 72.09 per cent, were cocci.

6. Of 50 peptonizers, 34, or 68 per cent, were cocci.

7. The members of the inert group were not classified morphologically.

8. Of the three groups of acid-producers, alkali-producers and peptonizers, comprising 232 cultures, 192, or 82.71 per cent, were cocci (22).

9. Heating at 145° F. (62.8° C.) for 30 minutes in milk had the effect shown in table 2 on 139 cultures of streptococci isolated from four sources (23).

TABLE 2

The effects of heating at 145° F. for 30 minutes on 139 cultures of streptococci from 4 different sources

Source	Number of cultures tested	Number of cultures surviving	Per cent of cultures surviving
Feces	45	9	20.00
Udder	40	7	17.50
Mouth	36	13	36.11
Milk	18	17	94.44
Total	139	46	33.07

10. Colon bacilli are largely killed by heating to 145° F. (62.8° C.) for 30 minutes (24).

11. Bacteria do not multiply faster in pasteurized milk than they do in raw milk (21).

Most of the studies made by Ayers and Johnson were on milk of relatively high bacteria count. More recently three studies of the effect of pasteurization on the flora of low-count milk have been published (26, 27, 28), and the results differ from those of Ayers and Johnson. Instead of the percentage of acid-producing, inert, alkali-producing and peptonizing bacteria remaining unchanged by the pasteurization, as reported by Ayers and Johnson, the more recent work showed that the percentage of acid-producers was reduced when low-count milk was pasteurized, so that when spoilage occurred it did not consist of a typical acid coagulation, but rather a development of off-flavors (26), rennet coagulation at low acidities (27), and other similar types of spoilage (28). None of these three investigations showed peptonizing bacteria over-growing other types in this low-count pasteurized milk. However, it is obvious that the surviving flora and the consequent type of spoilage (when spoilage occurs) are far more desirable in the higher-count milks studied by Ayers and Johnson than in the lower-count milks studied by these more recent workers. Such an unfavorable change in the type of the surviving flora and in the type of spoilage may generally be expected where counts are forced down to the point where but few organisms, aside from the udder flora, get into the milk.

In 1923 Robertson (29) reported the presence on agar plates made from pasteurized milk of many punctiform colonies, most of which were micrococci, with a few rods and streptococci. Most of these organisms failed to curdle milk.

Robertson, Yale and Breed (30) reported in 1926 finding, on microscopic examination of pasteurized milk from certain plants, large numbers of spore-forming rods, belonging to nine species, as listed in table 3.

TABLE 3

Spore forming bacilli isolated from pasteurized milk

Name	Number of cultures	Per cent
B. subtilis	48	34.2
B. mesentericus	29	20.7
B. vulgatus	22	15.7
B. circulans	21	12.0
B. albolactis	10	7.2
B. lacterosporus	2	1.5
B. panis	1	0.7
B. cereus	1	0.7
B. mycoides	1	0.7
Not identified	5	3.6
Total cultures	140	100.0

Most of them had been killed by the pasteurization, so that plate counts were not high. The source of the spores was found to be "milk stone" on the equipment, due to improper cleaning.

Robertson (31) in 1927 isolated a group of thermoduric organisms by holding raw milk samples at pasteurizing temperatures and making plates at hourly intervals up to seven hours. Cultures were also obtained from plates sent to him from pasteurizing plants in various cities. Only those cultures showing 90 per cent survival on being pasteurized in milk were studied. The species found to meet these conditions, in the order of their frequency were: *Sarcina lutea, Streptococcus thermophilus, Microbacterium lacticum, Micrococcus conglomeratus* and *Sarcina rosea.* The decreasing order of thermal resistance of these cultures was found to be: *Microbacterium lacticum, Sarcina lutea, Streptococcus thermophilus, Sarcina rosea* and *Micrococcus conglomeratus.*

In 1927 Fay (32) studied certain thermoduric organisms (55 cultures) surviving thirty-minute pasteurization at 143° F. (61.6° C.). Sugar was necessary for their growth on agar, and they would grow on standard agar only if the dilution was not greater than 1:100. Most of the cultures were very short rods (*Streptococcus lactis* type) or cocci growing in short chains, up to 10 cells long. Only about 10 per cent were long rods.

Brannon and Prucha (33) in 1927 submitted two-to-five-hour-old cultures of 47 unidentified non-spore-forming organisms to a temperature of 144.5° F. (62.5° C.) for 35 minutes in milk and found two surviving. Of these, one was not examined further, and the other was a micrococcus. In another series of cultures, the identity of which was known, treated in the same way, *Sarcina lutea* and the following four spore-formers survived: *B. subtilis, B. ramosus, B. butyricus* and *B. glaligu.*

Hammer and Trout (34) reported in 1928 that yellow micrococci commonly survive pasteurization. These organisms produced only slowly any change they brought about in milk.

Hucker (35) in 1928 studied 180 strains of cocci surviving pasteurization. Of these, 76, or 42.2 per cent, were micrococci, the most frequently occurring species of which were: *M. epidermidis, M. candidus, M. varians* and *M. luteus.* Of the streptococci, *S. thermophilus* was more common than any other species of cocci. Other lactic acid streptococci found were *S. faecium* and *S. liquifaciens. Streptococcus lactis* did not survive pasteurization temperatures. Hucker found that holding raw milk at 50° F. (10° C.) as compared with 68° F. and 86° F. (20° C. and 30° C.) for four hours prior to pasteurization did not greatly affect the total number of surviving cocci, but that the higher temperatures of pre-holding (68° F. and 86° F.) caused the percentage of *S. thermophilus* in the pasteurized milk to increase at the expense of the other species of cocci. Pasteurization was at 142° F. (61.1° C.) for 30 minutes.

Pricket (36) in 1929 studied 480 cultures of thermoduric bacteria forming "pin point" colonies, isolated from raw and pasteurized milk, from materials collected from farms producing milk that contained thermoduric

bacteria, from milk powders, media that had been sterilized in the autoclave, and from pea-blanche liquor. Seven types of organisms were observed as follows: Spore-forming rods, Non-spore-forming rods, Streptococci, Micrococci, Sarcinae, Actinomyces and Yeast.

Of these, only the spore-forming rods and certain Actinomyces were thermophilic.

The thermoduric micrococci were tentatively identified as: *M. candidus, M. epidermidis, M. luteus* and *M. albus.*

The streptococci were: *S. thermophilus, S. glycerinaceous* and *S. liquefaciens.*

The thermophilic spore-formers were: *B. subtilis, B. terminalis* var. *thermophilus, B. michaelisii, B. calidus, B. thermoalimentophilus, B. aerothermophilus, B. thermoliquefaciens, B. nondiastaticus, B. calidolactis* and *B. kaustophilus.*

Examination of a collection of cultures labelled *B. subtilis* showed two types. The *B. subtilis* Cohn type as described by Ford grew luxuriantly at 122° F. (50° C.) but the other type, resembling *B. cereus* Frankland did not grow at that temperature.

The name *B. kaustophilus* was proposed for a new species isolated.

Sherman and Pauline Stark (37) studied, in 1931, 294 cultures of streptococci from milk and other sources which grow actively at 113° F. (45° C.). All survived heating for 30 minutes in milk at 145° F. (62.8° C.). The most prevalent types were: *S. thermophilus, S. bovis, S. inulinaceus, S. fecalis, S. glycerinaceus, S. liquefaciens* and *S. zymogenes.* The authors raise the question as to whether *S. inulinaceus, S. glycerinaceus* and *S. zymogenes* should be considered as varieties of *S. bovis, S. fecalis* and *S. liquefaciens,* rather than as separate species.

McRady and Langevin (38) state that organisms of the coli-aerogenes group are seldom found in 1 cc. of properly pasteurized milk.

Minett and Pullinger (39) examined 49 samples of commercially pasteurized milk from six plants in England for the presence of *S. agalactiae,* the organism associated with the commonest form of bovine mastitis. Although this organism is very common in raw milk, they could demonstrate it in only one of their 49 samples of pasteurized milk.

Sherman (40) reported in 1936 that nine per cent of the samples of pasteurized milk examined contained hemolytic streptococci, but that the maximum number per cubic centimeter was 50, and that no pathogenic organisms could be found.

Macy (41) in 1939 reported on a study of high bacteria counts in pasteurized milk. He stated that the bactericidal efficiency of pasteurization is greater in summer than in winter. He studied 81 cultures isolated from 37° C. standard agar plates made from pasteurized milk, and classified them as shown in table 4. He found that dirty farm utensils were the usual sources of thermoduric bacteria.

TABLE 4

Reactions in litmus milk of cultures isolated from agar plates of pasteurized milk

Type of Organism	Cultures		Caseolytic		Acid, no coagulation		Acid, coagulation	
	Number	Per cent of total	Number	Per cent of group	Number	Per cent of group	Number	Per cent of group
Spore-forming rods	13	16.05	13	100.00	7	53.84	?	?
Short, non-spore-forming rods	23	28.39	0?	0?	0	0	0	0
Streptococci	19	23.46	0?	0?	1	5.26	18	94.74
Micrococci	24	29.63	2	8.33	19	79.17	0	0
Sarcinae	2	2.47	0?	0?	1	50.00	0?	0?
Total	81	100.00	15	18.51	28	34.57	18	22.22

Taylor (42) in 1924 was apparently the first one to use laboratory pasteurization of the milk from individual farms in controlling high counts in commercially pasteurized milk. He reported that improperly sterilized "milk contact surfaces" on the farm or in the milk plant were common sources of the thermoduric organisms, and that some farms are apt to give trouble continually.

Hussong and Hammer (43) in 1931 pasteurized morning's milk from individual farms in test-tubes at 142° F. (61.1° C.) making plate counts both before and after pasteurization. Large variations occurred in the percentage of organisms killed, both in samples from different farms and in different samples from the same farms. In the case of one farm having high counts in the pasteurized milk, a change in methods of caring for utensils and equipment on the farm resulted in lower initial counts and higher pasteurization efficiencies, and when initial counts increased again, due to hot weather, the higher efficiencies persisted.

A group of workers from United Dairies, London, England (Anderson and Meanwell, 1931; Davies, 1931; and Meanwell, 1939) (44, 45, 46) have published articles from which the following conclusions may be drawn.

1. Standard agar enriched with 0.5 per cent sterile milk is a satisfactory plating medium for the control of pasteurized milk.

2. There is a great variation in the number of heat-resisting organisms present in raw milk from different sources.

3. Under ordinary farm conditions and without utensil sterilization, more heat-resistant organisms are present in machine-produced milk than in hand-produced milk.

4. There is no constant relationship between the number of organisms present in raw milk and in the same milk after pasteurization.

5. Non-cooling of milk at the farm frequently encourages the development of heat-resisting organisms.

6. Heat-resisting organisms frequently originate from the surfaces of unsterilized utensils.

7. Daily sterilization of milking vessels resulted in nearly eliminating the heat-resisting organisms from the milk.

8. A simple quantitative plate count of raw milk affords little information as to the suitability of the milk for pasteurization.

It should be pointed out that this English group was the first to use laboratory pasteurization of the milk from individual farms, with a plate count of the pasteurized milk, as a routine method on a large scale in the control of high counts in pasteurized milk, although Taylor (42) and Hussong and Hammer (43) had both done it on a smaller scale prior to that time. Despite the good results reported, the method was ignored by dairy bacteriologists in this country until after the advent of high-temperature pasteurization and

the adoption of milk agar, both of which tend to show up the presence of thermoduric bacteria, forced the industry to adopt the method. The findings of these workers that thermoduric bacteria are associated with improperly cared-for farm utensils has been confirmed, as will be seen later in this review. The use of milk agar by the English group, several years before its official adoption in this country, was probably partly responsible for their recognition of the importance of the problem of the thermoduric bacteria.

The findings reported by various investigators (41, 42, 43, 44, 45, 46) that dirty farm utensils are the principal source of thermoduric bacteria is not surprising, since it has been shown that stable air (47) and unsanitary stables in general (48) have little effect in increasing the original bacterial contamination of milk, while utensils (49) and especially milking machines (50) supply most of this original contamination.

Moreover, at least one report has been published showing that thermoduric organisms grow readily in non-cooled milk (44).

B. BACTERIA SURVIVING HIGH-TEMPERATURE, SHORT-HOLD PASTEURIZATION

It seems to be generally conceded by investigators who have published work on high-temperature, short-hold pasteurization that bacteria counts are higher than in milk pasteurized by the low-temperature, long-hold method (11, 13, 51, 52, 53, 54, 55, 56, 57). However, some of these reports give no data to show the extent of the difference in count (11, 13, 52, 55, 57). Dotterer (51) presents considerable data showing that the high-temperature, short-hold method gives materially higher counts than does the low-temperature, long-hold method. He used single lots of milk divided into two portions, which were pasteurized by the two different methods. Parfitt (53) gives some data that seems to indicate that the high-temperature short-hold method gives, in certain plants, *lower* counts than does the other method, but his data must be discounted somewhat because it appears that his comparisons were made, not on single lots of milk divided into two parts and pasteurized by the two methods, but on different lots of milk, although the statement is made that the milk did all come from "a common source". Moreover, Parfitt intimates that the high-temperature short-hold method of pasteurization gives the higher counts, since he says in his conclusions "that a closer control of thermoduric organisms is necessary for low bacterial counts in milk pasteurized by the high-temperature short-hold method."

Quin and Burgwald (54) state that laboratory pasteurization shows little difference in the bactericidal results of holding 15 seconds at 160–162° F. (71.1°–72.2° C.) or 30 minutes at 143° F. (61.7° C.), but that commercial pasteurization gave an average count of 50,271 for high-temperature and 35,087 for low-temperature pasteurization. Yale (56) found that 18 lots of milk pasteurized in commercial apparatus at 143° F. (61.7° C.) for 30 minutes gave an average count of 17,200, whereas the same lots of milk pasteur-

ized in commercial apparatus at 160° F. (71.1° C.) for 15 seconds gave a count of 20,600. The same comparison for laboratory pasteurization was 6,490 and 12,200 per cc.

As in low-temperature, long-hold pasteurization, there seems to be little relationship between counts before and after pasteurization by the high-temperature, short-hold method (53).

Laboratory pasteurization tends to give lower counts than does pasteurization in commercial apparatus, regardless of the temperature and time used (53, 54, 56).

Several of the investigators who have published reports on high-temperature short-hold pasteurization have adopted the method of controlling high counts in the finished product that was first suggested by Taylor (42) and was extensively used by the United Dairies group in London, England (44, 45, 46). Samples of milk from individual farms are pasteurized in the laboratory and plate counts made. Those farms sending in milk the count of which is high after pasteurization are visited by an inspector, who attempts to find and correct the cause of the high count. There is uniform agreement among reports of such work that the thermoduric organisms originate principally in dirty farm utensils, and that cleaning up these utensils results in reduction of the count (11, 42, 43, 44, 45, 46, 51, 53, 55). Milking machines seem to be especially fertile sources of the offending organisms (11, 53, 55, 57, 58). Parfitt (53) shows a log average count of 630 for fifty hand-milking farms, 8,500 for fifty machine-milking farms. Prucha and Parfitt (57) presented the data in table 5 showing the relationship between the number of thermoduric organisms per cubic centimeter in milk from individual farms and the use of milking machines.

TABLE 5

Relationship between the number of thermoduric organisms in milk and the use of milking machines

Thermoduric count per cc.	Number of farms with milking machines	Number of farms without milking machines
5,000	0	20
10,000	0	7
20,000	38	1
30,000	30	0
40,000	21	0
50,000	16	0
Over 50,000	14	0
Total	119	28

Cans may also be a serious source of these thermoduric organisms (58). Krueger, of the Chicago Department of Health (11), recommends that milking machine tubes be stored, when not in use, in 0.5 per cent sodium hydroxide solution, that they be boiled in such a solution occasionally, that

all milk stone be kept off of all farm equipment, and that routine cleaning practices on the farm be good, if thermoduric organisms are to be held in check.

The technique of laboratory pasteurization of large numbers of samples as a control procedure seems to have been very generally the low-temperature long-hold method. The English group used that method (44, 45, 46), as would be expected, since they were also using it in their plant operations. Parfitt (53) used 144° F. (62.2° C.) for 30 minutes and his results in controlling high counts in commercial high-temperature pasteurization were apparently satisfactory. Theoretically, it might be better to use the high-temperature method for laboratory work in controlling a commercial high-temperature operation. Two German workers have described a laboratory high-temperature pasteurizer (59), but it is extremely complicated. Quin and Burgwald (54) also used such an apparatus, but it would be difficult to clean and also difficult to use for routine pasteurization of large numbers of small samples. A satisfactory method has supposedly been developed in this country (60). The difficulty in laboratory high temperature short-hold pasteurization lies in obtaining the very rapid heating and cooling necessary to be strictly comparable with the commercial equipment.

The claim has been made that clarification of the milk after the regenerative stage of heating, with the milk at a temperature of about 125° F. (51.7° C.), will materially reduce the count (51, 52), but this has been denied by other workers (53, 55).

There seems to have been very little work done on the identity of the organisms surviving high-temperature short-hold pasteurization. One German worker (61) reported that *E. coli* survived a high-temperature pasteurization, and that the difficulty was eliminated when the low-temperature long-hold system was installed. However, no details of temperature and time are given, and the date of the experiments was 1924–25, so that it is probable no sufficiently accurate controls of time and temperature of heating were available for commercial equipment. No recent work on the incidence of *E. coli* in milk pasteurized commercially by the high-temperature short-hold method appears to have been published, but in view of the emphasis placed on the absence of this organism from pasteurized milk by public health authorities in this country, the mere fact that survival of the organism is not mentioned in the literature is fairly conclusive evidence that its presence in milk pasteurized by this high-temperature method is not a serious problem.

The only other work published on the survival of a definite group of organisms in milk pasteurized by the high-temperature short-hold method is the observation of Eglinton and Yale (58) that yellow, heat-resistant micrococci, which they state are common in milking machines and milk cans, often appear on agar plates made from pasteurized milk, especially where the high-temperature method of pasteurization is used.

C. THE BACTERIAL FLORA OF DAIRY UTENSILS

Inasmuch as the principal source of thermoduric bacteria seems to be farm utensils, it appears that any literature available on the bacterial flora of these utensils should be of interest in this connection.

In 1924 Whiting (62) studied 357 cultures isolated from milk cans. The distribution of these cultures is shown in table 6.

TABLE 6

The distribution of cultures isolated from milk cans

Type of organism	Number of cultures	Per cent of cultures
None-spore-forming rods	216	60.5
Micrococci	105	29.4
Sporeforming rods	36	10.1
Total	357	100.0

The species of micrococci present, in the probable order of their abundance, were: *M. aureus, M. conglomeratus, M. varians, M. luteus, M. flavus* and *M. cinnebareus.*

The first three are heat-resistant (31, 35, 36).

In the same year Robertson (63) studied 721 cultures from milking machines. He found that, when brine-hypochlorite solutions were used for sterilizing the machines, the white, Gram-positive cocci were the commonest organisms. The Gram-negative rods and *Streptococcus lactis* were quite common under all conditions, but they formed a larger proportion of the total flora as the condition of the machines became less sanitary. The alkali-forming rods appeared to be associated with a treatment in which the tubes were submerged in cold water or in old sterilizing solutions of inadequate strength. A few cultures of the colon-aerogenes group were isolated. Sporeformers were rare. Molds (primarily *Oidium lactis*) and yeasts were found in accumulations of old milk in the tubes, stanchion hose and moisture traps. Actinomyces were found in small numbers in machines well-sterilized with hot water, and were regarded as dust contamination.

Of the 721 cultures isolated, 265, or 36.7 per cent, were micrococci. Of these, 78 or 10.8 per cent of the total of 721 were white heat-resistant micrococci, and 54 or 7.5 per cent were yellow heat-resistant forms.

The next year Robertson studied in detail these 265 cultures of micrococci (64). They seem to be able to survive sterilization with sodium chloride brine or with sodium or calcium hypochlorite, or chloramines, and therefore they are the commonest organisms present when these sterilizing compounds are used. Eleven species were identified. In the order of their probable abundance they are: *M. candidus, M. freudenreichii, M. casei, M. conglomeratus, M. epidermidis, M. varians, M. flavus, M. aurantiacus, M. luteus, M. albus* and *M. aureus.*

The first four species were sufficiently common to be regarded as a part of the normal bacterial flora of the milking machines. The other species are probably somewhat accidental contaminants.

After nine months without transferring, an attempt was made to revivify them. The attempt was successful with 49 cultures, and the species most common among these 49 were: *M. conglomeratus, M. casei* and *M. freudenreichii.* Apparently these three species were able to withstand drying better than *M. candidus* and other species.

As has been already shown, six of these species of micrococci are able to survive low-temperature pasteurization (see references 31, 35, 36).

Eglinton and Yale, in a paper previously referred to (58), found that the yellow, heat-resistant micrococci common on agar plates made from pasteurized milk, especially where the high-temperature method of pasteurization was used, were found to originate in milking machines and to a lesser extent in milk cans. While he did not identify any cultures, the species of micrococci producing yellow pigment (65) are: *M. conglomeratus, M. citreus, M. flavus, M. variens* and *M. luteus.*

It has already been noted that all of these but *M. citreus* and *M. flavus* have been shown to be heat-resistant (31, 35, 36). *M. conglomeratus* is apparently one of the most common organisms in both milking machines and milk cans (62, 64). It is heat-resistant (31).

The question now arises: Where do these heat-resistant micrococci come from? The fact that they are apparently able to resist heat and also killing by sodium chloride brines and by chlorine sterilizers acts as a means of selective enrichment, since they readily withstand the commonest methods of sterilizing farm utensils. But how are they introduced into the utensils? The answer is readily available in three studies on the udder flora of cows. In 1913 Harding and Wilson (66) studied the udder flora of cows. In 900 samples of aseptically drawn milk, they found 71 groups of organisms, none of which were sporeformers. About 75 per cent were micrococci. Fifteen years later, Alice Breed (67) studied the micrococci present in the normal cow's udder. The species she found, as well as her classification of the species isolated by Harding and Wilson, are listed in table 7.

It is especially significant that Robertson (31), Hucker (35) and Pricket (36) have found six species of micrococci to be heat-resistant. These species are listed as follows with the reference showing them to be heat-resistant: *M. albus* (36), *M. candidus* (35, 36), *M. conglomeratus* (31), *M. epidermidis* (35, 36), *M. luteus* (35, 36) and *M. varians* (35).

The studies of Harding and Wilson (66) and of Alice Breed (67), just described, show that these heat-resistant micrococci comprise better than 40 per cent of the micrococci present in the udder.

Evans (68) in 1916 studied the bacteria found in milk freshly drawn from normal udders, and found micrococci in 58.8 percent of 192 samples

TABLE 7

Classification of the micrococci present in the normal cow's udder

Species	Breed		Harding & Wilson		Combined	
	Number	Per cent	Number	Per cent	Number	Per cent
M. aureus	33	18.8	6	12.0	39	17.2
M. aurantiacus	24	13.6	1	2.0	25	11.1
M. freudenreichii	23	13.1	2	4.0	25	11.1
**M. albus*	21	11.9	10	20.0	31	13.7
**M. candidus*	20	11.4	3	6.0	23	10.2
**M. epidermidis*	13	7.4	3	6.0	16	7.1
M. citreus	10	5.7	3	6.0	13	5.7
**M. virians*	10	5.7	6	12.0	16	7.1
M. flavus	8	4.5	2	4.0	10	4.4
**M. conglomeratus*	4	2.3	5	10.0	9	4.0
**M. luteus*	3	1.7	3	6.0	6	2.6
M. casei	2	1.1	0	0.0	2	0.9
Not identified	5	2.8	6	12.0	11	4.9
Total	176	100.0	50	100.0	226	100.0
*Total heat-resistant	71	41.5	30	68.0	101	44.7

* Indicates heat resistant.

drawn from 161 cows of five different herds in two widely distant sections of the country. Although she stated that the majority of these organisms, while non-virulent, resembled the pyogenic staphlococci (*M. aureus*), she also found *M. caseolyticus* and *M. luteus,* the latter being one of the heat-resistant forms listed above.

From these three studies (66, 67, 68) it seems evident that heat-resistant micrococci make up a significant proportion of the udder flora of normal cows. While their total numbers in the milk in the udder are doubtless small, the farm utensils are constantly inoculated with them. It seems probable that the long tubes of milking machines receive most of their outside contamination from the milk itself. Since these micrococci will withstand heat sterilization unless it is more efficient than is usually the case on farms, and since they will also survive sterilization by chlorine sterilizers and salt brines (63), most farm utensils and many milk cans contain them after they have supposedly been sterilized. If conditions (moisture, nutrients, etc.) are such that growth can subsequently occur, then the utensils will quickly become rich sources of these thermoduric organisms. This accounts, therefore, for their having been found in cans (62) and milking machines (63, 64), and also for the commonly-reported fact that thermoduric organisms are associated with dirty farm utensils, especially milking machines (11, 41, 42, 43, 44, 45, 46, 51, 53, 55, 57, 58).

Those who may be interested in more detailed information concerning the characteristics of the various species of the micrococci are referred to Hucker's "Studies on the Coccaceae", particularly to numbers I, II, III, IV, VIII and IX (69, 70, 71, 72, 35, 65). The staphylococci or parasitic group are considered by Hucker to be species of the genus micrococcus (69). In

his latest classification he lists nineteen species (65). Only one, *M. caseolyticus,* causes proteolysis in milk (72).

D. ENVIRONMENTAL FACTORS TENDING TO PROMOTE HEAT-RESISTANCE IN BACTERIA

The ability of a given species of bacteria to resist high temperatures is largely a specific characteristic. However, there are certain environmental factors which tend to promote ability to resist heat (and other unfavorable circumstances also), and these will be briefly discussed.

Robertson in 1927 reviewed rather completely the literature on "the thermal resistance of microorganisms" (73). His conclusions are that the thermal resistance of micro-organisms seems to depend to a great degree on the moisture content of the cell. Thus, the moisture content of a spore is lower than that of a vegetative cell, and the cell wall of a spore is less permeable to moisture. Moreover, cells subjected to desiccation survive longer if encapsulated than if not encapsulated. Sarcinae survive desiccation better than certain other species, and they also survive higher temperatures than the micrococci and the common non-spore-forming rods, with the exception of *Microbacterium* lacticum and *Lactobacillus thermophilus.* It is probable that, as suggested by the literature, certain bacteria, when gradually subjected to increasing temperatures, have a faculty of adaptibility. This doubtless depends on the elimination of water from the cell contents. Also, suspending cells in distilled water, with lower osmotic pressure than the cell contents and a consequent tendency for water to migrate into the cell, lowers thermal resistance, whereas the reverse is true if cells are suspended or grown in solutions of increasing sucrose concentration. The effect of desiccation by the concentrated sucrose solution is doubtless enhanced by the presence of capsules formed when some species grow in fairly concentrated sucrose solutions.

The effect of either acclimatization or concentration, or both, may be operative in increasing the thermal resistance of bacteria in dairy utensils. Moreover, storage of milking machine tubes in brines may very well serve to strengthen the heat resistance of the organisms by lowering their moisture content.

In another paper in 1927, Robertson (74) reports that cultures of *Microbacterium lacticum, Sarcina lutea* and *Streptococcus thermophilus* are more susceptible to heat in the accelerative growth stage than in the resting stage. This doubtless accounts for the observation of Macy (41) that the bactericidal efficiency of pasteurization is greater in summer than in winter. In summer, due to higher temperatures, the organisms may reach the accelerative growth stage before pasteurization, whereas in winter they are doubtless maintained in the resting stage in many cases.

To summarize briefly, it may be stated that certain thermoduric micrococci are normal inhabitants of the bovine udder, so that the milk itself con-

tinually seeds the farm utensils with these organisms. If the utensils are not properly washed and sterilized, these organisms will grow in them, and certain inefficient sterilizing procedures may tend to enhance their thermal resistance. These udder micrococci form a significant proportion (one-third to one-half or even more) of the organisms surviving low-temperature long-hold pasteurization. Moreover, there is reason to believe that they are just as important in milk pasteurized by the high-temperature short-hold method, since at least one paper reports that heat-resistant yellow micrococci are especially abundant on plates made from milk pasteurized by the high-temperature method, and since there seems to be practically universal agreement that cleaning up dirty farm utensils, especially milking machines (which contain large numbers of micrococci), results in a marked decrease in the number of bacteria surviving high-temperature pasteurization. The species of micrococci known to occur in milk and to be heat-resistant are: *M. albus, M. candidus, M. conglomeratus, M. epidermidis, M. luteus* and *M. varians.*

Many other species of heat-resistant bacteria have been shown to occur in milk, but their origin is in general not so clearly known as is the origin of the micrococci. Among these organisms may be mentioned the following species, although many of the Bacilli are rather rare: *Bacillus aerothermophilus, Bacillus albolactis, Bacillus butyricus, Bacillus calidolactis, Bacillus calidus, Bacillus cereus, Bacillus circulans, Bacillus glaligu, Bacillus kaustophilus, Bacillus lacterosporus, Bacillus mesentericus, Bacillus michaelisii, Bacillus mycoides, Bacillus nondiastaticus, Bacillus panis, Bacillus ramosus, Bacillus subtilis, Bacillus terminalis* var. *thermophilus, Bacillus thermoalimentophilus, Bacillus thermoliquefaciens, Bacillus vulgatus, Lactobacillus thermophilus, Microbacterium lacticum, Sarcina lutea, Sarcina rosea, Streptococcus bovis, S. faecium, S. fecalis, S. glycerinaceus, S. inulinaceus, S. liquifaciens, S. thermophilus* and *S. zymogenes.*

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American Dairy Science Association Announcements

RESULTS OF ELECTION

The results of the election of officers on October 1, were as follows:

Vice-President: HENRY F. JUDKINS, Sealtest Inc., New York, New York.

Directors to serve for three years each: HOWARD B. ELLENBERGER, University of Vermont, Burlington, Vermont; ARTHUR C. DAHLBERG, Agriculture Experiment Station, Geneva, New York.

Annual Meeting—1941—at University of Vermont, Burlington, Vermont, June 23–27.

Many of our members are now making plans along with their families to attend the Annual Meeting next June. Those members who will present papers should write to the Chairman of the Program Committee, Dr. E. S. Guthrie at Cornell University and inform him that you desire to present a paper. Those of you who have been in Vermont will be sure to want to go again. Those who have never been there cannot afford to miss this opportunity.

The Association now has available all the Journals that have been published. You will find the price list for all back Journals in one of the advertising pages.

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JOURNAL OF DAIRY SCIENCE

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ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

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JOURNALS

American Butter Review
American Milk Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics

Biochemical Journal
Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Canadian Public Health Journal
Certified Milk
Cornell Veterinarian

Dairy Industries
Dairy World
Deutsche Molkerei Zeitung

Endocrinology

Food Industries
Food Manufacture
Food Research

Ice and Refrigeration
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Ice Cream Industry
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Ice Cream Trade Journal
Industrial and Engineering Chemistry

Journal of Agricultural Research
Journal of Agricultural Science
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Proceedings of Society of Experimental Biology and Medicine

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Scientific Agriculture

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Zentralblatt für Bacteriologie
Züchtungskunde

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copehagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS TO APPEAR IN THE JOURNAL OF DAIRY SCIENCE

549. Avenized versus Standard Parchment for Wrapping Print Butter. W. B. COMBS, S. T. COULTER AND DANA W. WHITMAN, University of Minnesota, St. Paul.

Samples of print butter from a large number of churnings were wrapped in standard parchment wrappers and wrappers that had been treated with oat flour (Avenex). The butters were stored for varying periods. The surface deterioration was measured organoleptically and by means of the fat aldehyde test. The results indicate that parchment paper treated with oat flour had a very slight effect in retarding the deterioration of the surface of butter. The treated parchment proved of most value when used on butter made from neutralized cream and of a "90" score.

550. Some Factors Affecting the Stability of Certain Milk Properties. IV. Interrelation of Certain Metals and Metallic Ions and the Development of Oxidized Flavor in Milk. O. F. GARRETT, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

Contamination of milk with copper or ferrous iron is known to catalyze the oxidation reaction which produces oxidized flavor in milk. When divalent manganese was added to milk in molar concentration equal to or greater than either copper or iron the development of oxidized flavor was completely inhibited or greatly retarded for periods up to 96 hours. The manganese had a similar effect when strips of copper metal were placed in the milk. Pieces of manganese metal acted in a manner similar to the manganese salt.

Divalent manganese added to milk after the development of copper-induced oxidized flavor had begun arrested further development of the flavor. Trivalent aluminum ($AlCl_3$) ions had no retarding effect on the development of copper-induced oxidized flavor.

The addition of manganese to uncontaminated or copper-contaminated milk had no effect on the oxidation rate of reduced ascorbic acid nor on the magnitude of the oxidation-reduction potential.

551. Some Factors Affecting the Stability of Certain Milk Properties. V. A Comparison of Seven Different Roughages on the Color and Flavor in Milk. O. F. GARRETT, R. B. ARNOLD AND G. H. HARTMAN, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

The results of three separate experiments are reported. The first experi-

ment showed that milk produced on molasses alfalfa silage was almost equal in yellow color to milk produced on spring pasture (6.3 lactochrometer units and 6.4 lactochrometer units, respectively), was slightly better in flavor score than pasture milk, and resisted equally well with pasture milk the development of copper-induced oxidized flavor.

The second experiment showed that dried citrus pulp impregnated with molasses and dried beet pulp were about equally poor in producing yellow color, flavor and resistance to the development of copper-induced oxidized flavor in milk. Both types of pulp were greatly inferior to molasses grass silage with respect to these factors in milk.

The third experiment showed that molasses grass silage and phosphoric acid grass silage were about equal in producing milk of high yellow color, good flavor and high resistance to copper-induced or spontaneous development of oxidized flavor. Both types of grass silage were definitely superior to corn silage with respect to those factors.

552. The Time of Ovulation in Cattle. C. L. COLE AND J. E. BREWSTER, Dept. of Animal Husbandry, Michigan State College, East Lansing, Mich.

The increased use of artificial insemination has brought about a greater need for definite information relative to the time of ovulation in cattle. The results reported in the literature relative to the time of ovulation are variable and incomplete.

This study was carried out on both dairy and beef cattle. Seventy-three rectal examinations were made on 47 cows. Nine cows were slaughtered immediately after ovulation to check the results obtained by palpation.

Ovulation was found, in all except three instances, to occur within the first day after estrus. One cow ovulated on two occasions before she went off estrus. Another cow ovulated 26 hours after the end of estrus. The average time of ovulation from the end of estrus was 13.57 ± 0.68 hours.

No significant difference in time of ovulation was noted between breeds, types of cattle, or time of day. Heifers ovulated on the average of 3.04 hours sooner than did cows that had calved previous to these studies.

Both ovaries produced follicles with equal frequency and there was no apparent order in which they functioned in any one animal.

553. The Relation of Certain Factors to the Drying of Whey Mixtures on the Atmospheric Drum Drier. E. L. JACK AND A. J. WASSON, University of California.

When whey alone is dried on the double drum atmospheric drier, a gummy mass results that is difficult to remove from the machine and which hardens when cool so that grinding is necessary to put it into useable condi-

tion. For the formation of a continuous sheet of dry material it is necessary to add a film-forming substance to the whey. Various materials have been used, including skimmilk solids, either in liquid or dry form, and cereal products. This study has been concerned with the properties of different combinations of whey and drying agents which yielded a satisfactory sheet when scraped from the drum.

It was found that when liquid skimmilk was used as the film-forming material it required about one and one-half parts skimmilk solids to one part whey solids at low acidities to form a satisfactory sheet. This represents about one part milk protein to two parts lactose. As the acidity increases the amount of skimmilk solids required increases also. When condensed skimmilk was used approximately equal parts of skimmilk solids and whey solids in the mixture formed a satisfactory drying combination. Increasing acidity again required that more milk solids be used. Mineral acids gave substantially the same results as developed or added lactic acid. Ground cereal products were also used. Approximately one part cereal product to two parts whey solids gave satisfactory results. Those found to be useable were flour, corn starch, ground oats (sifted), and ground barley (sifted). The amount of cereals required was not much affected by different degrees of acidity. The lactose : nitrogen ratios and the pH relationships have been determined.

BOOK REVIEW

554. Industrial Microbiology. S. C. Prescott and C. G. Dunn. 541 pages, illustrated, price $5.00. Published by McGraw-Hill Book Company, New York, N. Y.

The authors have treated the subject of industrial microbiology from the standpoint of the investigation and control of those fermentations that are of industrial importance because of their end-products or their effect in altering the quality or composition of certain substrates such as foods.

The book is divided into four parts. Part I discusses the characteristics, methods of handling, and industrial applications of yeasts. It includes production of industrial alcohol; mechanism of the ethyl alcohol fermentation; the brewing, wine and distilling industries; commercial yeast manufacture; and production of glycerol and fat. Part II includes the acetone-butanol; acetone-ethanol; butyl alcohol-isopropyl alcohol; acetic acid; commercial lactic acid and propionic acid fermentations; as well as certain fermentations important in the food industry. Part III is devoted to molds; industrial fermentations in which molds are utilized; mold enzyme preparations; and production of fat by molds. Part IV reviews the microbiology of wood and textiles. Also included are two useful appendices, one on detergency, disinfection and sterilization, the other on treatment and disposal of industrial microbiological wastes.

Discussions which are well written throughout cover not only the established industrial fermentations, but also some of the more recently discovered fermentations that offer possibilities for industrial application in the future. Applications of industrial microbiology as related to the manufacture of sera and related products and to certain phases of agriculture and dairy manufacture are omitted. This is unfortunate, but the breadth of the subject presents an overwhelming task to anyone attempting a thorough treatment of all phases of industrial microbiology.

Throughout the book, both in the discussions and at the end of each chapter, numerous references are supplied to guide the reader to further information on specific processes and general reviews of the subject. These aid materially in providing a book that should prove valuable, particularly for courses in industrial fermentations or food technology and microbiology.

P.R.E.

BACTERIOLOGY

555. Further Studies on Development of *Clostridium botulinum* in Refrigerated Foods. F. W. TANNER, P. R. BEAMER AND C. J. RICKHER. Dept. of Bacteriology, Univ. of Illinois, Urbana, Ill. Food Res., *5:* 4, 323. July-Aug. 1940.

It was found that samples of food artificially inoculated with strains of *Clostridium botulinum* and frozen, were not toxic when thawed and held at 5° C. (41° F.) for 14 days. Similar samples thawed and held at higher temperatures were in general toxic, particularly when the pH of the food was higher than 4.5. The authors state that frozen foods, if properly handled and kept frozen until used, should be as safe and as satisfactory as similar fresh foods.

F.J.D.

556. New Media for Bacterial Counts. H. G. HARDING, Akron Pure Milk Co., Akron, Ohio. Dairy World, *19:* 4, 28. Sept. 1940.

A brief discussion of the effects of the new media adopted by the A.P.H.A. on bacterial counts and on compliance with milk ordinances. The author stresses the fact that the milk industry and control agencies are less dependent on plate counts as quality indications since the use of special tests is becoming more general. Such tests as the methylene blue, the resazurin, the direct microscopic examination, laboratory pasteurization and the phosphatase test are mentioned.

F.J.D.

BREEDING

557. Reproductive Efficiency in Dairy Cattle. F. E. HULL, W. W. DIMMOCK, FORDYCE ELY AND H. B. MORRISON, Univ. of Kentucky, Lexington, Ky. Bull. 402, 28 pages. May 1940.

The breeding efficiency of the University of Kentucky dairy herd was

studied from 1900 to 1939, inclusive, in relation to health conditions and control measures. During the period 1900 to 1927, inclusive, there was no organized program for Bang's disease control or other diseases except tuberculosis. From 1928 to 1932, inclusive, there was an intensive health supervision program during which Bang's disease was eradicated. From 1933 to 1936, inclusive, the herd was free from Bang's disease. From 1937 to 1938, inclusive, trichomoniasis infection occurred. A total of 482 individuals was involved. A summary of the results is presented in the following table:

Period inclusive	Calving interval per cow	Breeding efficiency	Abortions in terms of pregnancy	Per cent of pregnancies calves born dead	Calves born alive but died in 6 months	Per cent of pregnancies that grew to maturity
	months	%	%	%	%	%
1900–1927	17.2	69.8	15.6	3.1	12.1	71.5
1928–1932	18.0	66.7	12.4	10.5	3.7	74.2
1933–1936	14.2	84.5	9.3	8.0	0.7	82.1
1937–1938	15.7	76.4	7.3	7.3	5.7	80.5
1939	12.7	94.5	2.6	10.3	11.8	76.9
The whole period	16.6	71.8	13.6	5.1	9.3	73.7

W.E.P.

558. Directions for the Ascorbic Acid Therapy of Slow-breeding Bulls. PAUL H. PHILLIPS, Dept. of Biochemistry, Univ. of Wisconsin, Madison, Wis. J. Am. Vet. Assn., *97:* 165–166. 1940.

Subcutaneous injection of ascorbic acid caused marked recovery in 65 to 75 per cent of impotent bulls. Approximately 5 mg. ascorbic acid should be injected per kilogram of body weight every 3 or 4 days over a period of 5 or 6 weeks. One gram of ascorbic acid is dissolved in 5 ml. of a buffer solution. The buffer solution is prepared by dissolving 0.1 gram monobasic potassium phosphate and 0.4 gm. sodium phosphate (U.S.P. dried) in 50 ml. distilled water. W.E.P.

BUTTER

559. The Relation of Carbon Dioxide Gas to the Keeping Quality of Butter. W. B. COMBS, Dept. of Dairy Husbandry, Univ. of Minn., St. Paul, Minn. Ice and Refrig., *97:* 3. 1939.

This paper outlines the procedure to be used in an experiment to determine the value of an atmosphere of carbon dioxide for preventing the development of stale and oxidized flavors on the surface of high quality butter. L.C.T.

560. Iron and Copper Content of Butter. G. M. MOIR AND E. D. ANDREWS. N. Z. J. Sc. and Techn., *21:* 249A–265A. 1940.

In a new filtration method for estimating iron, 10 grams of butter are melted in 15-ml. centrifuge tubes; 1 ml. of 5 per cent Sod. hydro-sulphite is added, shaken and left over night at 35–40° C. After shaking with 1 ml. of 20 per cent trichloroacetic acid the tubes are stood 30 min. at 40–50° C., centrifuged, and the melted fat siphoned off. The aqueous layer is shaken with 5 drops of 10 per cent sodium tungstate and the later filtered cold through papers just previously washed with 5 per cent nitric acid, the filtrate is shaken with 0.5 ml. of saturated pot. persulphate, followed by addition of 2 drops each of nitric acid and hydrogen peroxide. After adding 1 ml. of 30 per cent ammonium thiocyanate, all tubes including standards are filled up to the same level. To extract the color 2 ml. of amyl alcohol are added and shaken. Standards and blanks are prepared with all the reagents included. To clear the amyl alcohol layer, the tubes are cooled in ice-water prior to the final color comparisons. Many results thus obtained have been compared with those yielded by an improved dry-ashing method.

For copper 25 gram samples are melted at 40–50° C. and shaken with 0.5 ml. conc. hydrochloric acid, 5 drops of 3 per cent hydrogen peroxide, 2.5 ml. of 20 per cent trichloroacetic acid, and 5 drops of 10 per cent sodium tungstate. After keeping warm 30 minutes the samples are centrifuged and the fat layer sucked off. The tubers are cooled prior to filtering through papers freshly washed with acid. One washing of the precipitate with 5 per cent nitric acid is followed by others with 5 per cent trichloroacetic acid. To the filtrate are added 2 ml. of 20 per cent sodium citrate, a few drops of phenolphthalein, and sufficient strong ammonia dropwise to make alkaline. A few mg. of powdered sod. diethyl-dithiocarbamate are added to each tube, the volumes equalized, and 5 ml. amyl alcohol added. The tubes are shaken, allowed to stand three or four hours, and shaken again. When clear the amyl alcohol colors are compared with similarly prepared standards and blanks, using if possible a Klett colorimeter with a blue filter.

For copper the wet-ashing method of Williams as modified by Koppejan and Van der Burg has been further improved. In a large centrifuge tube 25 grams of butter are warmed with 8 ml. of glass distilled nitric acid. The water-bath is raised gently to near boiling-point and effervescence dispersed by stirring. After an hour or more the fat is removed by centrifuging and sucking off, followed by two similar treatments with 5 ml. of high-boiling petrol. The acid liquid is washed out into a 200 ml. Kjeldahl flask and gently evaporated almost to dryness. When cool, 2 ml. of pure sulphuric acid are added, and after further heating small amounts of conc. nitric acid are added as required; later a few drops of perhydrol may be required to complete the oxidation. The residue is washed into a large test-tube, neu-

tralized, and other reagents, citrate, etc., added as in the filtration method. The original paper contains useful details about purifying reagents, etc.

Author's Abstract.

FEEDS & FEEDING

561. Silage from Hay Crops. Making It—Feeding It. S. T. DEXTER AND C. F. HUFFMAN, Michigan State College, East Lansing, Mich. Circ. Bull. 173, 8 pages. July, 1940.

A popular treatment of the problems in preparing and feeding grass silage. W.E.P.

ICE CREAM

562. Stimulating Carry-Home Sales of Ice Cream. ANONYMOUS. Ice Cream Rev., *23:* 12, 24. July, 1940.

An unlimited increase in the per capita consumption of ice cream is possible by stimulating carry-home sales. Suggestions given for increasing these sales include: giving the consumer a greater knowledge on the preparation of sundaes, etc., in the home; using insulated bags to keep ice cream hard under adverse conditions; the use of flat refrigerator-type packages; and point-of-sale advertising suggesting specifically the "carry home" idea. J.H.E.

563. A Change in Vanilla Nomenclature. ROBERT ROSENBAUM, David Michael and Co., Philadelphia, Pa. Ice Cream Rev., *23:* 12, 52. July, 1940.

Conforming to international rules the U. S. Department of Agriculture has adopted *Vanilla fragrans* (Salisb.) Ames as its official technical name for the source of our commercial vanilla beans. For years the beans were referred to as *Vanilla planifolia* Andrews. This terminology is now to be dropped. This new designation may have a bearing on the commercial products now being offered on the world markets. For instance, there is a question as to whether Tahiti beans are truly *Vanilla fragrans* and whether their use in products can be labelled as "vanilla." J.H.E.

564. Factors Affecting Mix Viscosity. A. J. HAHN, Dept. of Dairy Husbandry, Univ. of Illinois, Urbana, Ill. Ice Cream Field, *36:* 2, 26, 34, 35, 36. Aug., 1940.

Defining viscosity as "the ability of a liquid to resist flow" the author points out the necessity of distinguishing between "apparent" and "basic" viscosities. He further states that "fluidity" is the ability of any fluid to flow without the application of an exterior force; while "plasticity" is

the ability of any liquid to flow only after the application of an external force.

A brief discussion is given of the influence of the following factors upon ice-cream viscosity: (1) temperature, (2) mix composition and (3) methods of processing and cooling mixes.

It is stated that the use of unsweetened frozen cream in mixes results in about the same viscosity as that obtained with the use of fresh cream. It is further claimed that mixes made with concentrated milk, vacuum roll, or spray skimmilk powder and evaporated milk will not vary much in viscosity, but atmospheric roller powder, superheated condensed milk and sweetened condensed skimmilk will result in an increase in mix viscosity. Increasing the stabilizer content has a greater influence on mix viscosity than increasing other mix components.

It is claimed that as long as the acid content is not great enough to precipitate the proteins the viscosity of the mix will decrease with increased acidity, and further, that calcium and magnesium ions increase viscosity, whereas citrate and phosphate ions ordinarily decrease mix viscosity.

It is stated that homogenization causes a marked increase in mix viscosity especially if it is accomplished at 120° F. to 140° F.

A table is given showing the influence of the various factors considered upon viscosity and whipping ability of mixes and body, texture and flavor of ice cream. W.C.C.

565. Ice Cream Sales Index. ANONYMOUS. Spec. Bull. of the Statistical and Accounting Bureau, Int. Assn. of Ice Cream Manufacturers, Washington, D. C. July, 1940.

This publication contains an analysis of ice cream sales for the first four months of 1940. During this period the sale of ice cream in the United States was 4.35 per cent higher than for the same period in 1939. Canadian sales showed an increase of 17.89 per cent when sales for the same periods were compared.

A supplement to the bulletin contains the following data: (1) Ice cream production in gallons by months by states, 1938; (2) Percentage of ice cream production by months by states, 1938; and (3) Percentage of ice cream production by months by states—ten year average, 1929–1938. M.J.M.

566. New Rulings on Ice Cream under the Food and Drug Law. R. C. HIBBEN. Ice Cream Trade J., *36:* 7, 14. 1940.

Five new interpretations from the Federal Food and Drug Administration regulating ice cream shipped in interstate commerce are presented. These rulings cover the labeling of "coated ice cream," "ice cream sandwich," and "retail pails and cartons." The common name of chocolate ice cream and the regulation on the color declaration on labels of ices and sherbets are also discussed. W.H.M.

567. Vanilla in a Changing World. R. C. SCHLOTTERER. Ice Cream Trade J., *36:* 7, 10. 1940.

The effect of the European war upon the productions, procurement and price of vanilla is discussed. It is pointed out that Madagascar, the great vanilla producing center, is a French possession and that its transfer to Germany might bring out new economic problems. Due to the present disturbance of shipping and foreign exchange the normal market indices of supply and demand have lost their importance, therefore prediction of price or supply in this country is not possible. The Mexican beans, while higher in price, usually follow the supply and demand curve of the Madagascar product. W.H.M.

568. Do Small Stops Pay? VINCENT M. RABUFFO. Ice Cream Trade J., *36:* 7, 8. July, 1940.

The Diamond Company with headquarters in Jersey City, New Jersey, is doing an annual ice cream distribution of $400,000 and finds that the small stops pay if properly managed. The secret is in keeping the stops close together, keeping waste and expense at a minimum, and not attempting to furnish dealers with supplies other than ice cream cabinets and very closely related materials. Heavy merchandising campaigns are not carried on because the small type of accounts do not warrant it. The Diamond Company does not manufacture ice cream but only maintains storage houses for keeping the ice cream prior to distribution. The head of the company estimates that 70 per cent of his sales are in packages and novelties; about 30 per cent in bulk. He has approximately 1450 dealers to which deliveries are made. W.H.M.

569. The Use of Fruits in Ice Cream. B. I. MASUROVSKY. Ice Cream Trade J., *36:* 8, 31. August, 1940.

Some of the latest developments in the use of fruits in ice cream are discussed. New possibilities are suggested. The author cites a paper by Dr. D. G. Sorber[1] containing the following directions for packing fruit for ice cream purposes.

"1. Select full flavored fruit of predetermined suitable varieties.

"2. Precool as an aid to controlling oxidation.

"3. Wash the fruit thoroughly.

"4. Coarsely crush or puree fruit in such a way as to avoid beating air into the product.

"5. Add a predetermined amount of sugar or syrup and thoroughly mix to further aid in preventing enzymatic alteration of flavor and color.

[1] "The Preparation of Frozen Fruit Pulp and Their Use in Ice Cream and Related Products," by Dr. D. G. Sorber, U. S. Dept. of Agriculture. (Report of Proceedings of the 39th Annual Convention, Int. Assoc. Ice Cream Mfgrs., Production and Laboratory Council, Vol. 2, 1939.)

"6. Pack in tightly sealed enamel-lined tin cans, preferably closed under vacuum.

"7. Rapid freezing at sub zero temperatures.

"8. Storing at a temperature of 0 degrees F. or colder." W.H.M.

MILK

570. Crystal Form of Ice Packing Appeals to Shippers of Perishable Foods. ANONYMOUS. Ice and Refrig., *97:* 341. 1939.

A brief article which among other things points out the advantages of crystal form of ice packing for milk crates. This crystal ice may be briquetted by use of 17 tons pressure. L.C.T.

571. Installation of FlakIce Equipment. ARTHUR ADAMS, FlakIce Corp., N. Y. and R. E. MILLER, York Ice Machinery Corp. Ice and Refrig., *96:* 281. 1939.

This paper consists essentially of a story of the FlakIce installation in the N. Y. Sheffield Farms Dairy Plant, but it is of general interest because it includes a description of the equipment and operating characteristics. The machines resemble in general appearance double drum milk dryers. They are cooled with brine at from 0 to 14° F. and 8 to 10 lbs. per sq. inch pressure. The usual temperature rise is 2° F. The brine is maintained at a pH of 7.5 to 8.0. The ice leaves the rolls at about 20° F. and the storage rooms or bins are maintained at not higher than 22° F. The hoppers are so arranged that a predetermined amount of ice can be deposited on each crate of milk. Six to twelve pounds are generally used. A list of advantages of FlakIcing is given. L.C.T.

572. A Study of Concentration and Freezing as a Means of Preserving Fluid Whole Milk. R. T. CORLEY AND F. J. DOAN, Pennsylvania State College, State College, Pa. Food Res., *5:* 4, 369. July-Aug., 1940.

High temperature pasteurization (180° F. (82.2° C.) for 15 minutes) of fluid milk before condensation, homogenization and freezing retarded oxidation, lessened the tendency toward irreversible coagulation of protein and increased the possible storage period in the frozen condition as compared with low temperature pasteurization (145° F. (62.8° C.) for 30 minutes). Homogenization after condensing proved more effective in stabilizing the milk than when applied before condensing. Any copper contamination invariably caused tallowy flavors during storage. A storage period up to 15 weeks was possible. The reconstituted stored milk exhibited higher *in vitro* digestibility than similar normal milk. F.J.D.

573. **Rancidity—Its Effects and Control.** K. G. WECKEL, Dept. of Dairy Industry, Univ. of Wisconsin, Madison, Wis. Dairy World, *19:* 4, 16. Sept. 1940.

A brief discussion of the chemistry and biology of rancidity (hydrolytic) in milk and some milk products with descriptions of methods of measuring lipolytic activity and means of controllng the reaction. F.J.D.

PHYSIOLOGY

574. **Artificial Insemination.** C. L. COLE, Michigan State College, East Lansing, Mich. Ext. Bull. 207, 4 pages. June, 1940.

A brief consideration of the advantages and limitations of artificial insemination together with methods of application and organization problems. W.E.P.

575. **Influence of Uterine and Ovarian Nerves on Lactation.** JOHN S. LABATE, Depts. of Anatomy and Obstetrics and Gynecology, New York Univ. Endocrinology, *27:* 342. 1940.

An attempt was made to demonstrate the role played by the autonomic nerves supplying the ovaries, uterus, and hypophysis in the initiation and maintenance of lactation. Three control rabbits were bred and on the 25th day of gestation a Caesarian section was performed. The onset and duration of lactation was noted. Two rabbits were sympathectomized by removing all the known sympathetic pathways to the uterus, tubes and ovaries. They were then bred and treated as the control rabbits. Section was performed 27 and 32 days following sympathectomy. No difference in the onset and duration of lactation was observed between the two groups and both groups showed normal reproductive instincts. R.P.R.

576. **Experimental Superfecundity with Pituitary Gonadotropins.** HERBERT M. EVANS AND MIRIAM E. SIMPSON, Institute of Experimental Biology, Univ. of California. Endocrinology, *27:* 305. 1940.

Female rats from 26 to 34 days of age were injected with various levels of the follicle stimulating hormone alone and in conjunction with the principle in human pregnancy urine. Animals were usually sacrificed 12 or 22 days after breeding and implantation sites were counted. Supernumerary implantations were produced by the injected gonadotropins. The maximum number was 34, the average number was 17 which exceeded by at least 7 the number of implantation sites observed in normal rats. It was noted that a surprising number of embryos perish and undergo intrauterine resorption and that instances of prolongation of the span of gestation were common. R.P.R.

MISCELLANEOUS

577. Trained Employees Build Good Will. FRED E. KUNKEL. Ice Cream Review., *23:* 12, 58. July, 1940.

Foremost Dairies, of Jacksonville, Florida, believe very strongly in the importance of good customer contacts. This article gives the plan the company has worked out to make employees realize that their contact with customers either helps or hinders their business. J.H.E.

578. The Theory of Atmospheric Cooling Tower Operation. F. F. STEVENSON, Ice and Refrig., *98:* 4, 273; *98:* 348. 1940.

This paper consists of a technical discussion of the operation of cooling towers. The relationship of wet and dry bulb temperatures to the operation and size of cooling towers is explained. The various types of cooling towers are described. Factors affecting the performance of the towers are discussed. L.C.T.

579. Notes on Corrosion Control in Refrigeration Condensers. K. M. HOLADAY, Chemical Eng., Anheuser-Busch Inc. and A. VON GONTARD, Vice Pres. and Chief Eng., Anheuser-Busch Inc. Ice and Refrig., *98:* 286. 1940.

In this paper the authors report on the results of experimental work in connection with corrosion of condensers. The electrolytic and galvanic system of corrosion prevention is discussed, and it is shown that neither one has been effective to date, although the experiments have not yet been concluded. The use of sodium silicate in dosages sufficient to impart 8 parts of silica per milllion of water has not been found very effective. Further experiments under controlled pH conditions are desirable. The carbonate balance system showed considerable promise. The average requirement was approximately 0.10 lb. caustic soda per ton of refrigeration. A pH between 9.2 and 10.1 is most desirable. The use of paint has value if properly selected and applied. Some differences were noted in the rate of corrosion of various materials used in the construction of the pipes. L.C.T.

580. Cost of Operation and Maintenance of Diesel Engines in Refrigeration Plants. J. R. WATSON. Ice and Refrig., *97:* 143. 1939.

The author points out the importance of a good operating engineer for keeping costs low. In his discussion he shows the differences in cost per ton of ice resulting from the use of various drives, *e.g.*, V-belt, flat belt, electric drive using direct connected generator and direct connected synchronous motor, for electric drive using direct connected generator and belted synchronous motor, and for electric drive using direct connected generator and belted induction motor.

A table giving Diesel operating costs for four ice plants follows:

	Tons ice	Fuel oil cost	Lub. oil costs	Cost per ton
1	90	4½¢	50¢	23.6¢
2	34	4½¢	50¢	23.4¢
3	28	4½¢	50¢	23.6¢
4	23	6¢	60¢	27.0¢

L.C.T.

581. Some Problems in the Preparation, Processing and Distribution of Frozen Food Products. W. E. GUEST, W. E. Guest and Co., Chicago. Ice and Refrig., *96:* 339. 1939.

This paper consists of a rather extensive abstract of a talk presented at the Dairy Manufacturers Conference at the University of Wisconsin, March 15, 1939.

Information on the preparation of vegetables, fruits, poultry, and meats for quick freezing are given. A brief discussion of packing and packaging material is included. Short descriptions of the direct contact, cold air circulation, brine or sirup spray, and immersion methods of quick freezing plant is estimated at $25 or $35 per pound of product per hour. A plant to handle 2000 pounds of product per hour would cost from $50,000 to $70,000.

L.C.T.

582. Business Factors Affecting the Use of Cold Storage Lockers in Illinois. E. N. SEARLS, Univ. of Illinois, Dept. of Agr. Econ. Ice and Refrig., *96:* 249. 1939.

A brief history of the development of locker plants indicates that the first known locker plant was installed by a creamery in Crete, Nebraska, in 1910. A creamery in Walla Walla, Washington, built a locker room in 1927. An independent creamery in Minnesota built a 48 locker plant in 1924 but the second plant in Minnesota was not started until 1935. In 1938 it was estimated that there were 2,000 locker plants operating in the U. S. F. A. Gougler, of the Illinois Agricultural Association, 608 South Dearborn Street, in a survey made in 1938 of 13 Illinois locker plants showed that the total cost per locker ranged from $22.60 to $35.98. The average cost was $30.33. A list of expense items is included. Many of these may be overlooked by the average owner. Factors involved in the management of a locker plant are listed. Additional suggestions for attaining successful operation include:

1. Securing an adequate amount of capital.
2. Locating the plant in a territory that justifies its existence.
3. Providing an operating income sufficient to meet operating expenses.

4. Setting up an accounting procedure that shows an accurate picture of the business operations.
5. Providing an informed and intelligent management.
6. Keeping directors informed monthly of the operations of the business.

L.C.T.

583. Building Complete Refrigerated Locker Systems. GEO. C. FOERSTNER, Mgr. Electric Dept., The Amana Society, Amana, Iowa. Ice and Refrig., *96:* 161. 1939.

The article is of interest not only because of the organization involved but also because of the floor plan which is included, as well as construction details which are given. Neglecting some of these details may mean the difference between satisfactory operation and dissatisfaction. L.C.T.

584. Refrigeration as Applied to Air Conditioning. JOHN R. HERTZLER, York Ice Machinery Corp. Ice and Refrig., *96:* 105. 1939.

A brief description of electric, gas, coal, and oil operated units is included. Advantages and disadvantages are briefly listed. L.C.T.

585. Maintaining Refrigerating Plant Efficiency. H. L. LINCOLN, Gen. Plant Mgr., Union Ice Company, San Francisco, Calif. Ice and Refrig., *98:* 368. 1940.

The author presents a convenient check sheet for making monthly operating comparisons. In making comparisons of one plant with another it is, of course, important to take into consideration such factors as water and air temperatures as well as any other items which might make a difference in operating conditions. Adjustments must therefore necessarily be made.

Graphs are included to show refrigeration required in ice storage under various conditions, ratio of tons of refrigeration to tons of ice where different raw water temperatures are used, refrigeration requirements for cold storage with varying outside temperatures, and a graph showing KW-hrs. per ton of ice at 20 lbs. suction pressure used by a belt-driven horizontal ammonia compressor when the temperature of the water to the condenser varies and when different raw water temperatures prevail. L.C.T.

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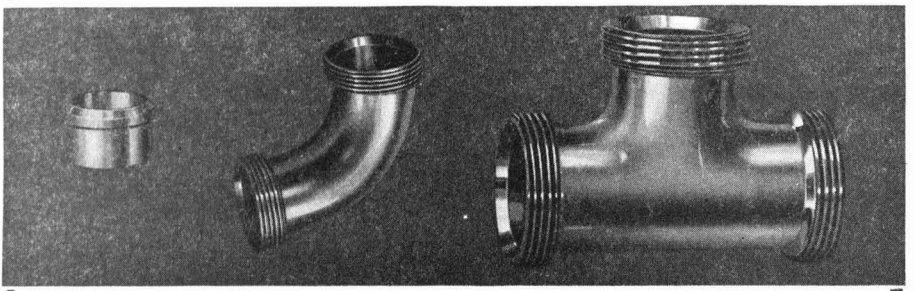

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