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OBSERVATIONS ON MASTITIS IN AN EXPERIMENTAL HERD*

E. G. HASTINGS, B. A. BEACH AND MILDRED JOHNSON

There is probably no aspect of dairying that interests so many diverse groups as does mastitis of the dairy cow; producers, consumers and manufacturers of any milk product meet the varied questions it presents as to the quantity, the quality and the healthfulness of milk and its products. The disease has been studied in the usual herd rather than under experimental conditions. While much can be learned regarding many aspects of the trouble by studying it in the field, it is probable that other aspects can be studied only under experimental conditions. Few herds have been maintained primarily for such a purpose; the most ambitious of such experimental attempts is that started in 1935 by the Commonwealth of Australia, on which a preliminary report has appeared (1).

During the past decade the research work on animal nutrition at this station offered opportunity for the study of mastitis in three groups of milking cows under experimental conditions. Reports on two such groups have been made (2, 3, 4). The record of the third deviates sufficiently from the others to justify a report thereon, which is herein presented.

There seems to be no question concerning the significance of *Streptococcus agalactiae,* in both chronic and acute mastitis. The prevalent opinion is that, while udder disturbances due to other agents are noted, these agents are of minor significance either because they do not cause material injury, or because they are not transmissible as is *Streptococcus agalactiae.* This conclusion may be faulty since almost all studies of mastitis have been of herds in which *Streptococcus agalactiae* was present, and thus the role of other agents made difficult or impossible of detection. The difficulty in producing mastitis by intentional transfer of *Streptococcus agalactiae* has caused many to believe that some predisposing condition in the udder is essential for its establishment therein. Many things point to the validity of this conclusion, especially the much higher incidence of the infection in the older than in the younger cattle (8), an observation not easily explained by greater opportunity for infection due to the longer period.

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There is still question as to whether herds can be kept free from *S. agalactiae,* especially if additions are made from herds of unknown condition, since it has been shown that infection of the udder can occur during calfhood, primarily by the feeding of milk containing *S. agalactiae* to calves and their suckling each other soon after feeding (10). Those who think such infection during calfhood is common believe the assembly of a herd which would prove to be free from this organism difficult of attainment. Plastridge and associates (9) found that not only could a herd free from *S. agalactiae* be established but that infected herds could be freed from *S. agalactiae* and thereafter kept free. Our experience with the first two groups studied confirms the findings of Plastridge.

In order to orient the reader as to the findings with these two groups and their relation to those of the third group a brief statement is made as to the nature of the herds previously studied and the findings thereon.

PREVIOUS STUDIES

The first herd consisted of 44 Holstein-Friesian heifers purchased before their first conception. They were placed on a farm on which there had been no cattle for 8 months. The stables had been cleaned and disinfected. It was thought that both environment and animals might prove free from *S. agalactiae.* No additions, other than a sire, were to be made to the herd, and there was to be no contact with other cattle. The heifers of this isolated herd came to their first lactation period about 1 year after they were assembled. Approximately 100 samples of fore milk from each animal were taken in the first lactation period and were studied in detail with reference to chemical and bacteriological composition.

The first report (2) presented the detailed records of 31 of the 44 cows which seemed most significant. Twelve of the 31 cows were available for study during their second lactation period. Their record and that of one animal not discussed in the first report formed the second report (3).

The herd proved to be free from *S. agalactiae* during the two lactation periods, but not from chronic mastitis of a low degree of intensity. Approximately 25 per cent of the herd showed no evidence of udder trouble during the first period. The remainder did reveal evidence of abnormal conditions for intervals of varying length.

The animals were classified as "normal," "questionable" and "abnormal." The questionable and abnormal groups made up about 75 per cent of the herd in the first period, and a somewhat higher percentage in the second. The boundary line between "normal" and "questionable" is of course quite indefinite since both extent and duration of change must be considered. Therefore in the reports discussion was confined to the "normal" and to the "abnormal" cases. One of the cows that was normal in the first period became abnormal in the second, and two that were questionably nor-

mal in the first became abnormal in the second. No case of change from abnormal to normal was noted.

The low intensity of the inflammatory process was shown by the absence of gross abnormalities in the milk, such as flocs and by the absence of loss of quarters. Since production of milk is influenced not only by the condition of the udder but by the total physiology of the animal, data as to yield are difficult of interpretation. An increase in production in the second period over the first is normally expected. The 3 cows classed as normal in each period produced 21,791 pounds of milk in the first period and 28,246 in the second, an average increase of 2,151 pounds. Three cows that were normal in the first period and abnormal in the second produced 26,814 and 26,610 in the first and second periods, a decrease of 68 pounds per cow. Seven cows abnormal in each period produced 42,757 pounds of milk in the first period and 53,697 in the second, an average increase of 1,590 pounds. The variation in this group was from a decrease of 33 pounds to an increase of 3,099.

No definite conclusion could be drawn regarding the cause of the disturbances of the udders other than that it was not *S. agalactiae.*

The second group of 18 Holstein-Fresian heifers was assembled previous to their first lactation period. The group was not as completely isolated as the first since they were kept in the same barn as the University dairy herd, but on a separate floor with different attendants. There was no contact with other cattle outside the stable. The observations were more detailed than in the case of the first group. A greater variety of culture media was used in the hope of discovering the causal agent of the chronic mastitis which, in its general nature, was similar to that observed in the first group and which occurred in the absence of *S. agalactiae.* Eleven of the 18 cows were available for study during their first three lactation periods.

TABLE 1

Percentage changes in the production of milk in successive lactation periods by cows classified as normal, slightly abnormal, and very abnormal

Classification	Number	Increase (+) or decrease (−) in production from	
		First to second period	Second to third period
		Per cent	*Per cent*
Normal cows	4	+16	+24
Slightly abnormal cows	5	+ 4	+12
Very abnormal cows	2	− 3	+ 2

The recognition of physiological disturbances in the udder was more certain than in the case of the first group. We had come to recognize that the most valid reason for judging a particular cow to be abnormal is a variation in composition of milk from different quarters, especially when one

quarter at least is still producing milk of a composition similar to that which it produced when first it came to milk production. Each animal was milked on 1 day of each week with a machine so arranged as to keep the product of each quarter separate from that of the other quarters. The data thus obtained were helpful in judging the intensity of the inflammatory process since quantity and quality could then be considered, and the composition of the entire milk differentiated from that of the fore milk of any quarter. The general effect of the disturbances in the udder on milk production in the case of the 11 cows is presented in table 1. The data need no discussion.

THE THIRD HERD

A third herd was assembled for nutritional studies. Its composition was as follows:

13	animals	in	the	first	gestation	period
6	"	"	"	second	"	"
4	"	"	"	third	"	"
1	animal	"	"	fourth	"	"

The animals were purchased only after a careful physical examination, and after samples collected from each quarter, were examined in the laboratory as to abnormalities in composition and as to the presence of *S. agalactiae*.

Most of the cows freshened within one month of the beginning of the experiment and in two cases about eight weeks before. Clinical observations, which were absent in the cases of the two groups on which reports have previously been made, supplied data in the present case to supplement those obtained from a study of the milk as to its chlorine and catalase content and as to the types of bacteria of the fore milk. The Hotis test and various culture media were used to detect streptococci and other possibly significant organisms. While the frequency of examination of milk samples was much less than in the two previous trials, it is believed that a reasonable picture of the condition of the udder of each animal was obtained.

The samples examined for chlorine and catalase were the first 30 cc. drawn from a quarter; those examined by the plate method or by the Hotis test were so largely composite samples from all the quarters that the data do not enable us to relate any specific organism to the condition of a specific quarter as would have been possible had quarter samples been used for all bacteriological as well as for chemical examinations. One objective was to see if this herd repeated the record of the other two with reference to the absence of *S. agalactiae* and as to the presence of mastitis. The nutrition was also far from the usual, since each cow, during a part of the period of observation, was fed urea to replace a part of the protein of the usual feed. It was thought this might be a factor of some significance as regards the condition of the udders, since if those animals receiving urea should show, as

regards the condition of the udders, a significantly different record than those on the usual rations, the variations might be ascribed to the urea, and thus form an objection to its practical use. The record of the herd supplies no evidence of an effect of the urea on the udder, and the record is to be considered apart from the nutrition of the animals.

No animal was removed from the herd during the first two lactation periods of the trial. One cow was injured in the sixth month of the third period of the trial and was slaughtered soon thereafter. Another lost one quarter in the third period.

The identification of any isolation of a streptococcus from the bovine udder is usually accomplished by determining whether it conforms to certain laboratory criteria. The most significant criterion is rarely used, namely is the organism able to invade the udder and to cause inflammation therein. The isolation of an organism satisfying the usual criteria for *S. agalactiae* from the udder during a considerable period in the absence of those clinical symptoms characteristic of *S. agalactiae* raises the question as to the validity of the answer supplied by the laboratory tests.

A streptococcus was found in the milk of one member of the third group in her first and second lactation periods as a member of the herd. The composition of the fore milk in these periods indicated the presence of an inflammation process of low intensity since during the first six months of the third period the fore milk was normal in composition and since the production of milk was not unfavorably influenced. The animal had had one lactation period before purchase; in her first period in the herd she produced 6,534 pounds of milk in 321 days, 8,998 pounds in 289 days in the second period, and 7,001 in 191 days of the third period in the herd, a record that is not indicative of deterioration of the udder.

The fact that the organism did not spread to other cows in the group indicated a low invasive power and raises further question concerning the validity of the laboratory identification.

The authors believe it is relatively safe to conclude that this third herd like the other two was and remained free from *S. agalactiae* until a very invasive strain of streptococcus was, in some manner, brought into the herd. This organism conformed to the laboratory tests for *S. agalactiae* and was highly invasive and virulent since within 3 months of its first discovery it had spread to 6 other cows and in the next four months to 6 additional animals. In addition to the 13 certainly infected, 2 others were possibly infected. Seven of the infected cows were observed for 8 months in which interval 10 quarters out of 28 were lost in contrast to the loss of no quarter out of 96 in the first and second lactation periods.

Since the record of the herd is much the same as that of the groups previously studied, it seems unnecessary to discuss it from the standpoint of mastitis in general. During the first two lactation periods six of the 24

animals seemed wholly free from any disturbance in the udder as judged by the composition of the milk. Three of six were in their first and second periods of lactation, one in her second and third, one in her third and fourth and one in her fourth and fifth.

In order to give the reader an idea of that which is considered a normal animal, the record of Cow 5 is presented. The maximum catalase value noted in any sample of fore milk from a quarter during the first and second periods and during 245 days of the third was 40; the minimum value on the same date was 30. The maximum per cent of chlorine in the fore milk was 0.20; the minimum on the same date 0.14. The maximum catalase value in the total milk produced at a milking was 12; the maximum chlorine 0.14. The curdling properties with rennet were normal as was the ratio of casein nitrogen to total nitrogen. The milk production was as follows: first period, 287 days, 5,218 pounds; second period, 295 days, 8,387 pounds; third period, 313 days, 10,253 pounds.

In the case of a cow classed as abnormal, the maximum catalase value of fore milk was 750; minimum value at same milking 40; maximum per cent of chlorine 0.37; minimum of the same milking 0.16. The catalase values of three samples of pail milk taken on the 26th, 40th and 78th day were 100, 130 and 140. The curdling was abnormal; the ratio of casein nitrogen to total nitrogen normal. The milk production was 7,096 pounds in 287 days, 7,689 pounds in 305 days and 4,191 pounds in 293 days in the three lactation periods, respectively. One quarter was lost in the middle of the third period. By such records the cows were judged normal or abnormal for the first two periods.

Normality and Milk Production

The production records of six normal and six abnormal animals are given in table 2.

The normal group was slightly handicapped with reference to the abnormal by three cows that had had one, two and three lactation periods, respectively, before the trial was begun, while the abnormal group contained only cows that were in the first period at the beginning of the experiment. The normal group was also handicapped by a longer average third lactation period; 275 days for the normal and 254 for the abnormal group.

Normality and Bacterial Content of Milk

From the entire record of each cow, each of the 24 animals was classed as producing normal or abnormal milk and thus the herd divided into two parts. The record of the bacterial content of the milk of each animal, as determined by the standard plate method of the American Public Health Association for milk control, was then reviewed and the data are here expressed in terms of frequency of occurrence.

TABLE 2

The influence of disturbances in the udder on milk production in successive periods of lactation

Cow	First period		Second period		Third period	
	Days	Pounds per day	Days	Pounds per day	Days	Pounds per day
			Normal cows			
5	287	18.1	295	28.4	313	32.8
8	238	18.2	259	24.9	238	28.5
13	252	29.3	361	33.2	305	31.1
14	252	17.1	275	25.3	234	29.1
20	252	25.6	375	23.1	280	31.0
23	266	22.5	271	24.7	279	32.3
	1547	21.8	1836	26.6	1649	30.8
			Abnormal cows			
1	328	19.9	326	18.7	250	31.3
7	307	25.9	319	26.5	305	23.4
10	287	24.8	305	25.2	293	14.3
11	245	18.8	278	27.3	232	24.1
15	273	19.6	313	22.6	241	28.9
18	252	14.8	237	14.4	203	20.7
	1692	20.6	1788	22.4	1524	23.8

The normal animals present a more favorable picture as regards bacterial content of the pail milk than do the abnormal. This observation is in agreement with that of Murphy (5) who found rigid standards of biochemical normality were satisfied only when the content of strict fore milk in staphylococci alone or in combination with non-hemolytic udder diphtheroids did not exceed 200 per cc.

Aerobacter aerogenes

An exceptional condition was noted; namely, the presence of an organism, identified as *Aerobacter aerogenes,* in the udders of certain of the animals. *A. aerogenes,* a normal inhabitant of the bovine digestive tract, is a constantly potential invader of the udder. In various phases of research and control work on milk, the authors have examined the milk of many hun-

TABLE 3

Frequency distribution of milk samples on the basis of the bacterial content of each sample

Bacteria per cc.	Normal cows	Abnormal cows
	Per cent	*Per cent*
0– 5,000	55.1	36.7
5–10,000	17.1	11.6
10–20,000	13.8	18.0
20–30,000	5.5	10.3
30,000 +	8.2	23.1

dred cows and have not found this organism in such numbers or with such constancy as to show it had established itself in the udder. Some contamination of milk samples with *A. aerogenes* may occur unless excessive precautions are taken; therefore, its intermittent detection is not likely to attract the attention of the analyst, nor should it. In the present instance, its repeated discovery both by the Hotis test and by plate cultures leaves no doubt of its presence in the udders of some of the animals in question. In the detection of *A. aerogenes* in the Hotis test, reliance must be placed on the amount of gas evolved as free gas or made evident by a gassy curd in case of accompanying acid production. The infrequency with which aerogenes has been reported as an inhabitant of the udder in spite of its constant presence in the environment of the cow leads one to suspect that in the present instance the variety involved differs from those commonly found in the stable and in milk. However, the study of the strain present in the herd has not differentiated it from the common isolations made from milk. The source of the organism is unknown. The only certainty is that is was present in the udders of certain of the animals for long periods in detectable numbers. Its role as a cause of the disturbances noted will be discussed in reference to certain animals.

A. aerogenes and other members of the colon group have been found, apparently as the causal agent, in cases of acute mastitis. We have found no report of its presence in cases of chronic mastitis, other than the recent one of Murphy and Hanson (6) who found 79 cases of infection of the bovine udder with coliform bacteria in a three-year study of the milk and udders of a herd of 120 cows. The infections existed for from 1 day to 22 months and the degree of irritation ranging from negligible to severe acute mastitis. Approximately 60 per cent of infecting organisms were identified as *Aerobacter aerogenes,* 26 per cent as "inter-mediate" types, and the remainder as *Escherichia coli.* Observers seem to agree that such agents are not transmitted from cow to cow. It is unusual for infection to occur with organisms constantly present in the environment of the cow and considered as constantly potential invaders of the lactating udder. Thus it is necessary to invoke some special condition in the udder that permits their occasional establishment therein. Possibly now and then an infrequently occurring strain, able to establish itself in the udder, may be present in a herd, as seems true in the present instance.

Aerogenes and Production

The significance of *A. aerogenes* in the udders of certain of the cows has been studied by comparing the records of six cows, table 4, that had a record of freedom or relative freedom from the organism with the records of 10 animals that had shown the organism in the milk consistently over long periods. The Aerobacter probably was of some significance in reducing

production. The number of quarters of the animals in either group harboring the organism is not known since quarter samples were not taken frequently enough to be of value. Some of the animals infected with this organism, on the basis of composition of fore milk, were classed as normal; others abnormal. Here again the failure to examine quarter samples of fore milk makes it impossible to relate in a definite manner the organism to the disturbances noted in the udders of certain of the animals.

TABLE 4

The influence of A. aerogenes in the udder on milk production

Cow	First period		Second period		Third period	
	Days	Pounds per day	Days	Pounds per day	Days	Pounds per day
			Aerogenes absent			
2	321	21.5	257	38.4	.....	.....
6	259	14.9	305	22.0	186	43.0
8	238	18.2	259	24.9	238	28.5
15	273	19.6	313	22.6	241	28.9
20	252	25.5	375	23.1	280	31.0
21	280	25.8	354	23.4	273	24.9
	1623	20.9	1863	25.7	1218	31.2
			Aerogenes present			
1	328	19.9	336	18.7	250	31.3
3	307	21.3	285	35.0	.....	.....
4	245	18.5	368	28.2	274	38.8
7	307	25.7	319	26.5	305	23.4
10	287	24.7	305	25.2	293	14.3
12	287	16.6	397	21.6	149	30.9
17	524	22.7	366	25.3	44	25.0
22	323	20.2	413	25.2	113	44.0
23	266	22.5	271	24.7	279	32.3
24	328	19.5	457	22.6	58	53.4
	3202	21.1	3517	25.3	1765	32.6

A physical examination of the udders of the entire group in the last half of the third period was made and the results studied with reference to the record as regards the presence or absence of *A. aerogenes*. Of the seven cows that had shown the organism consistently over long periods of time, five showed definite abnormality and two showed no physical evidence of past udder disturbances. Four cows were considered to have been free from aerogenes; two of these showed physical abnormalities and two none.

The bacteriological examinations made in the latter half of the third lactation period did not disclose the organism constantly or in sufficient numbers to be of practical significance except in the case of one cow, in the udder of which the organism was well established, and apparently was the cause of the mastitis then clinically and chemically evident.

The data do not warrant any dogmatic statement as to the role or roles *A. aerogenes* played in determining the record of the herd. It certainly had little influence on production of milk. An udder free from this organism is undoubtedly preferable to one in which it is present. It is to be emphasized that in the two and one-half lactation periods, all of the 96 quarters continued to function.

Invasion by and Spread of S. agalactiae

One of the perplexing questions of chronic mastitis caused by *S. agalactiae* concerns the difficulty of causing the disease by intentional introduction of the organism into the milk conducting system of the udder, the path which is supposed to be the important one, if not the sole one, in spontaneous spread of the trouble. This difficulty has led to the supposition that the udder must be prepared by some agent for its invasion by *S. agalactiae.* A few infection experiments on cows in the groups previously studied, which had shown udder disturbances in the absence of any recognized causal agent, supplied some support for the theory that such disturbances favor a later invasion by *S. agalactiae* (7). The abnormalities which had been noted in the 1st and 2nd lactation periods of the cows as members of the group and the presence of aerogenes in the udders of a part of the cows might have created a condition favoring the spread of *S. agalactiae* in the herd.

About the middle of the third lactation period for most of the cows as members of the herd an invasive variety of *S. agalactiae* was found in the herd. It was earlier stated that a streptococcus had been present in the udder of one member of the herd for at least two years before a streptococcus was found in the milk of any other member of the herd. It seems probable that the invasive streptococcus came in with an animal from another herd rather than that the one found earlier had become more invasive than it had previously been.

The invasive organism was first found in Cow 10 on the 120th day of her third period. In the next three months it had spread to six other cows and in the next four months to six additional animals. In addition to the 13 certainly invaded, two others were possibly infected. Seven of the infected animals were still members of the herd eight months after the streptococcus was first found. Ten of the 28 quarters of the seven animals were apparently lost.

The effect of the presence of *A. aerogenes* in the udder relative to its subsequent invasion by streptococci has been considered. Of 10 cows that had shown aerogenes consistently, six became infected with streptococci and four did not. Of seven that were classed as free from aerogenes, four remained free from streptococci and three became infected.

The predisposing effect of the disturbances in the udder revealed by abnormalities in the milk relative to subsequent invasion by streptococci was

also studied. Seven of the twelve animals classed as abnormal became infected; five did not. Of the 12 normal animals, five became infected; seven did not. The record is slightly in favor of the normal group. The significance of the data may be decreased by two conditions: the invasive nature of the streptococcus, and to some let down in sanitary precautions due to labor conditions.

DISCUSSION

During the past seven years, three herds have been studied as to the incidence of *S. agalactiae* in the udders of their members, and as to the incidence of mastitis as indicated by the composition of samples of fore milk of individual quarters.

The 44 members of the first herd were purchased when one year old, and were placed in an environment believed to be free from *S. agalactiae* and kept from contact with other cattle. The second group of 18 animals was purchased before their first calving. The degree of isolation was less perfect than in the case of the first group. The details of the third group have been presented herein. A part of the first group was observed during the second lactation period. Most of the second herd were observed during the second period and a part during the third.

The complete freedom of two groups from *S. agalactiae* and the probable freedom of a third herd for over two lactation periods, leads us to believe that the establishment of herds free from this organism is not so impossible as some have indicated. Indeed, our experience indicates that one is likely to meet with success in such an endeavor if reasonable precautions are taken in selection of the cows and in the management of the herd.

In the absence of *S. agalactiae* chronic mastitis occurs. The intensity of the process is usually low and rarely is its presence manifested by gross changes in the milk, and rarely does it cause permanent loss of quarters. The manner of prevention of such disturbances of the function of the udder of the cow cannot now be suggested since the knowledge of causes is so incomplete.

A strain of *Aerobacter aerogenes* was found in the udders of a part of the animals of the third group studied. Its significance is uncertain since some of the cows infected with it produced milk of abnormal composition and others milk of normal composition. It probably was of some significance in reducing production.

The third herd was invaded at about the middle of the third lactation period of the herd by a very invasive and virulent strain of *S. agalactiae* which in 7 months infected 15 cows and apparently caused the loss of 10 quarters of 7 cows that were observed for a period of 8 months in contrast to the loss of none of 96 quarters in the first two years of the herd's history.

CONCLUSIONS

It seems possible to assemble groups of cows that will be free from *S. agalactiae* by purchase of heifers during the first gestation period or earlier.

Trouble due to chronic mastitis in such groups will be relatively small in amount as measured by abnormalities in the quality of milk, in lessened production, and in loss of quarters.

An adequate approach to chronic mastitis from the point of view of prevention must await the accumulation of knowledge regarding the significance of all phases of management of dairy cows by the study of herds kept under controlled conditions. It cannot be gained by observations on the usual farm herd.

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RAPID METHODS FOR ESTIMATING THE QUALITY OF BULL SEMEN

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Certain methods have been used for making judgments of the potential fertilizing capacity of semen before it is used for actual breeding. The methods include the determination of (a) the duration of spermatozoan motility at a standard low temperature (9); (b) the glyolytic power of spermatozoa (1); (c) the oxygen consumption of spermatozoa (14); and (d) the resistance of spermatozoa to temperature shock (6), or to a 1.0 per cent sodium chloride solution (7).

Recent work in this laboratory has been directed at similar objectives. New techniques for testing quality of bull semen have been developed which may be employed in field artificial insemination with the simplest of equipment.

I. MOTILITY DURATION AT HIGH TEMPERATURES

A study was undertaken to determine the relation between the duration of spermatozoan motility in a nutrient-buffer diluter at a standard low temperature and the duration of spermatozoan motility from the same semen ejaculations in the same diluter at higher temperatures. To be most useful the high-temperature or incubation test should not require more than an hour.

The desirable range of temperature for this purpose was established by an experiment in which semen, collected by means of the artificial vagina, from fertile bulls, was diluted at the rate of 1 : 4 with yolk-citrate (11), and was examined for motility at 15, 30, and 45 minutes, when stored at 2.5° C. intervals from 37.5° C. up to 47.5° C. The temperature of the water in the bath was controlled by mercury thermoregulators, mercury relays, and immersion heating coils. Ten ejaculations of semen were diluted and divided into 15 portions of about 1.0 ml. each. Three portions of each ejaculation, one for each motility examination, were stored at each of the five different temperatures. The motility estimations were made to the nearest 10 per cent, making 10 general classes of motility from 90 to 0. Willett and Salisbury (15) have shown this method of estimating motility to give highly repeatable results, and unpublished work of the present authors show the method to be highly accurate when compared with a more objective method of counting the number of motile spermatozoa in a dilute sample.

The mean results are shown in figure 1. The data indicated that temperatures between 45.0° C. and 47.5° C. should be employed if the duration

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of motility was not to extend beyond the period of an hour or so; a time interval which appeared to be most nearly suitable for such a duration of motility test.

Specific temperatures for incubation. In order to more specifically identify the optimum temperature for short-time motility duration studies an investigation was set up to compare the duration of motility at temperatures of 46.5°, 47.0°, 47.5°, and 48.0° C., for semen samples diluted at the rate of 1:4 with yolk-citrate and examined at 15-minute intervals for one hour.

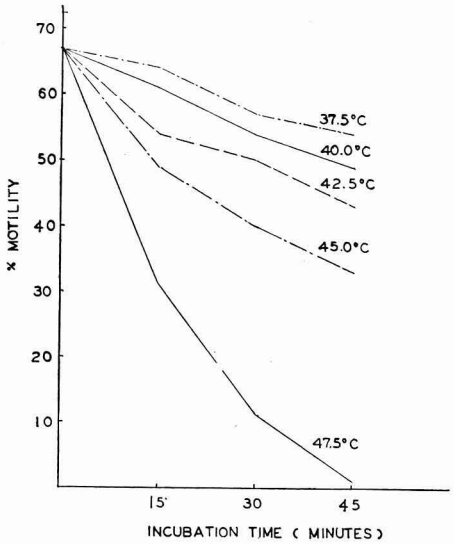

FIG. 1. Influence of incubation temperature on duration of motility.

Seven different semen collections were used in this experiment except for the 47.5° C. temperature where 14 collections were studied at this temperature and at 5° C. storage. In addition to the studies on duration of motility at incubation temperatures, 1 ml. portions of these same ejaculations diluted at the same rate were stored at 5° C. for 2, 4, 6, 8, and 10 days before they were examined for motility. These samples were cooled to the storage temperature at the rate of a 5° C. drop each 10 minutes. This storage temperature and this cooling rate have been shown to be nearly optimum for field use (15).

The mean motilities for each interval of storage for the several experimental temperatures are shown in figure 2. Superimposed over these curves is the curve showing the decrease in motility when 7 samples of the same semen were stored at 5° C. for 2, 4, 6, 8, and 10 days.

Correlation coefficients were calculated between the total reduction in motility during 10-day storage at 5° C., and the total reduction in motility

at the four incubation temperatures after several of the intervals of storage. As the number of observations in this experiment was small only the most pertinent correlations are given below:

Correlations between total reduction in motility during 10-day storage at 5° C. and:

1. Total reduction in motility when incubated at 46.5° C. for 1 hour = 0.9088**
2. Total reduction in motility when incubated at 47.0° C. for 45 minutes = 0.8979**
3. Total reduction in motility when incubated at 47.5° C. for 30 minutes = 0.6731**

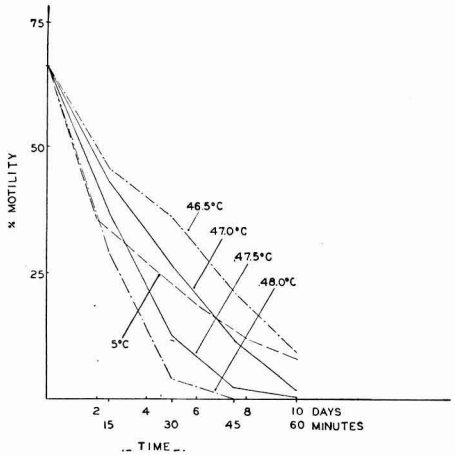

FIG. 2. Relation between duration of motility at high temperatures for short periods and at 5° C. for 2, 4, 6, 8, and 10 days.

These correlations are mathematically highly significant**, notwithstanding the fact that only seven pairs of observations were used in the calculations of the first two coefficients and fourteen for the last. These data are not conclusive proof of the quantitative relationships between the duration of motility under the two different sets of conditions, but are sufficient to indicate that much of the information now obtained from long-time storage in research laboratories and in artificial breeding circuits might be obtained in less time.

More recently, the writers have placed a flat-sided culture flask on the microscope stage to be used as an incubated microscope slide. Circles of petrolatum were made on the flask, the diluted semen to be studied was placed within the circle, and a cover-glass was tightly pressed down on the circle of petrolatum so as to seal off the semen from the air and to prevent drying. Water at 46.5° C. was siphoned into the flask and out again at a fairly rapid rate so that the walls of the flask were only slightly lower in temperature than the water of the bath. By such an arrangement it was possible to study five or six different treatments of the same semen under standard temperature conditions until the spermatozoa were dead under

each cover-glass. By such means four different diluters have recently been compared; two of which enabled the spermatozoa to live for more than 10 days at 5° C. with an average rate of motility of about 30 per cent after the 10-day period. Spermatozoa in the other two diluters ceased to live somewhere between 2 and 4 days' storage at 5° C. By the short method, the spermatozoa in the first two diluters lived for 62 and 65 minutes, while for the latter two diluters motility continued for only 14 to 33 minutes.

II. THE METHYLENE BLUE REDUCTION TEST

Because of its property of losing its deep blue color when reduced by the addition of two atoms of hydrogen, methylene blue has been widely used by biologists as a tool in studies of cellular metabolism. Methylene blue as a hydrogen acceptor has been most often used to demonstrate the presence or absence of enzyme systems in certain biological oxidations involving the, so-called, dehydrogenases. Methylene blue has been used in Thunberg experiments by Lardy and Phillips (5), on two semen samples for which they have reported data, to demonstrate the presence of succinic acid dehydrogenase activity of spermatozoa. Also, it has been used by Klein and Saroka (4), who reported recently that human semen would reduce methylene blue much faster at 37° C. than at 20° C. The technique has been simplified in this laboratory for work in the field to test the relative activity of bull semen samples. As a result of the study of data from several hundred ejaculations of semen it appeared to offer possibilities as a test for quality of semen, especially if combined with the test reported above.

Method. Fifty mg. of methylene blue was dissolved in 100 ml. of the buffer used for making the yolk diluent. The buffer used was 4.76 gm. crystalline sodium citrate ($2\ Na_3C_6H_5O_7 \cdot 11\ H_2O$) per 100 ml. water distilled over glass. In these experiments the semen to be assayed for quality was first examined for motility, and the concentration was then determined by the visual determination of opacity rank, recently described by Salisbury, Beck, Elliott and Willett (10). Two-tenths ml. of the semen was then diluted with 0.8 ml. of the yolk-citrate diluent, and placed in a small test tube of approximately 10.0 mm. outside diameter and of 3.5 to 4.0 ml. capacity. One-tenth ml. of the methylene blue solution was measured accurately into the 1.0 ml. of diluted semen, and the contents of tube thoroughly mixed. The tube was sealed by the addition of a one-half inch layer of mineral oil, and then placed in a constant-temperature water bath where the reduction proceeded.

The mineral oil was added to decrease the rate of diffusion of oxygen from the air into the upper level of diluted semen. As it does not completely prevent this exchange some allowance for the fact must be made in reading the end point of the reduction time.

Apparently most of the substrates required by spermatozoa to perform their proper function in artificial insemination are supplied by the semen and the yolk-citrate or the yolk-phosphate (8) diluents. The test has been used successfully with either diluter.

Effect of methylene blue on spermatozoa. If methylene blue, at the concentration used, was toxic to spermatozoa its value in a test for quality would be questionable. An experiment was designed to determine whether or not methylene blue was toxic. Ten different collections of fresh semen were diluted 1:4 with yolk citrate. The diluted material was divided into 1.0 ml. portions and placed in small culture tubes. To one-half of these tubes from each ejaculation was added 0.1 ml. of the methylene blue solution. One pair of samples was then placed in each of five different water baths at 37.5°, 40.0°, 42.5°, 45.0°, and 47.5° C. for 45 minutes and the motility esti-

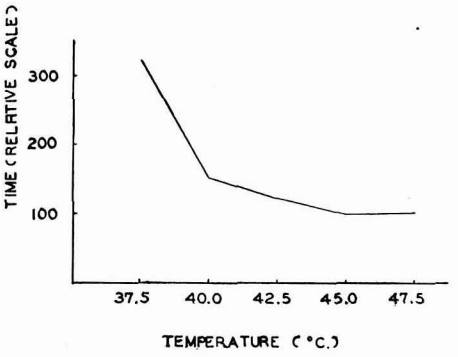

FIG. 3. Effect of temperature on methylene blue reduction time.

mates made. An analysis of variance of the data shows a highly significant difference in motility at these temperatures in favor of the diluted semen to which methylene blue had been added.

An equal number of paired samples, one-half of which received 0.1 ml. of the methylene blue solution before storage, from the same semen was stored at 5° C. after having been cooled to storage temperature at the rate of a 5° C. drop each 10 minutes. These samples were examined after 2, 4, 6, 8, and 10 days of storage. No significant difference in livability was indicated by the results. It is concluded that for either short-time incubation or for long-time storage methylene blue has no toxic effect on livability of bull spermatozoa.

Conditions for running the test. It has seemed desirable to make these determinations rapidly. Therefore, an investigation was conducted to determine the temperature for the optimum speed of methylene blue reduction. Eight ejaculations of semen were used and a reduction test run for each sample at 37.5°, 40.0°, 42.5°, 45.0°, and 47.5° C. The results giving

the mean for the eight samples expressed as a percentage of the fastest time are shown in figure 3. The fastest reduction time resulted from the use of a bath held at 45.0° C., though at 47.5° C. the reduction required but little longer, and not significantly so.

In the Thunberg technique oxygen and other gases are evacuated from the specially designed tubes. In the case of the test under discussion there is no means of getting rid of the air but its influence could be studied. In one case the tests were run as described, but for other samples of the same ejaculations bubbles of air were forced through the diluted semen for 2 minutes. Eight ejaculations of semen were used in this comparison. No difference in reduction time of the two series could be demonstrated statistically; the mean for those samples receiving no treatment was 8.38 minutes, and for those receiving air the mean was 8.45 minutes. Furthermore, the

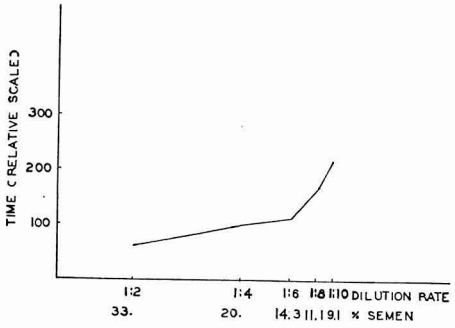

FIG. 4. Effect of dilution rate on methylene blue reduction time.

two series of observations could be considered as almost exact duplicates. It is believed that the thorough shaking required to mix the methylene blue solution into the diluted semen thoroughly saturated the contents of the test tube with air. While the entrapped oxygen was undoubtedly a factor in prolonging the reduction time it apparently was constant for all samples.

Though it was earlier stated that a standard dilution rate of 1:4 was used in the test, this rate has been adopted primarily as a matter of convenience. However, the rate of dilution did have an important effect on the time required to reduce 0.1 ml. of the standard methylene blue solution, as is seen in figure 4. Ten different semen ejaculations were used in this study. One ml. portions of diluted semen from each collection at each dilution rate were used for the test.

It is obvious that some standard rate of dilution is necessary if different samples of semen are to be compared, for the rate of dilution had an important influence on the reduction time. Apparently, this effect was not due to the reduction in the number of spermatozoa present only, but to other factors as well, for the increase in reduction time was not in direct relation

to the decrease in spermatozoan numbers. The reduction time becomes progressively longer with each rate of dilution above 1:6. In this connection, Winchester and McKenzie (16) have reported that increasing the dilution rate of boar spermatozoa leads to an increase in oxygen uptake per unit number of spermatozoa.

It would appear that if a different rate of dilution than recommended here were to be used, appropriate changes in the concentration of the methylene blue solution should be made.

Effect of spermatozoan concentration and motility on methylene blue reduction time. In cooperation with the New York Artificial Breeders' Cooperative a study of a total of 383 collections of semen from 30 different bulls has recently been completed. The methylene blue test was run at 47.5° C. on the fresh semen diluted at the rate of 1:4 with yolk-citrate.[2] Spermatozoan concentration and motility were estimated by the methods previously described.

These data were studied by the multiple regression methods outlined by Snedecor (13). The analysis showed that a large portion of the variation in the observed methylene blue reduction time was associated with variations in concentration and motility of the spermatozoa. The pertinent statistics are given below:

	X_1	X_2
Methylene blue reduction time Y	*concentration*	*motility*
Correlation of Y with X's	−0.6532**	−0.6577**
Standard regression of Y on X's	−0.4460	−0.4543
$r_{x_1x_2} = 0.4561$**	$R^2 = 0.5901$	$R = 0.7682$**

** Significant at the 1 per cent level of probability (highly significant).

When the means for the individual bulls were used for the analysis a highly significant correlation between concentration and motility of 0.8829 was revealed, indicating that the bulls varied in their ability to produce semen, and that motility tended to be poor for the spermatozoa of those bulls producing semen of low concentration. In this same analysis highly significant correlations of −0.8169 and −0.8252 were obtained between mean methylene blue reduction time and mean concentration and mean motility respectively. These coefficients would indicate that a large proportion of the difference between the mean methylene blue reduction times was associated both with concentration and motility, with about equal weight given to variations in each.

However, for any one particular bull the data indicated that variations in concentration of semen samples were not necessarily associated with similar variations in motility of the spermatozoa.

The results of the analysis were interesting, for they indicated that fresh semen containing no spermatozoa or fresh semen containing unlimited num-

[2] The writers are indebted to Maurice Johnson, technician at the Cooperative's Syracuse, New York, laboratory for much of the data.

bers of dead spermatozoa would not be able to reduce methylene blue under the conditions of this test. Such was the case, for in a series of experiments centrifuged plasma of fresh semen diluted with the yolk-citrate diluent would not reduce the methylene blue, nor would semen samples of high concentration, the spermatozoa of which had been rendered immotile by the addition of small quantities of toluene, ether, chloroform, or distilled water.

Effect of ascorbic acid concentration on methylene blue reduction time. Since the discovery by Phillips *et al.* (9) that spermatozoan production of certain poor-breeding bulls was stimulated by subcutaneous injections of ascorbic acid, this laboratory has been interested in the function of the vitamin in spermatozoan physiology. Experiments were undertaken to determine the relationship between the inherent level of ascorbic acid in fresh semen samples on methylene blue reduction time under the conditions of the test.

First of all, the effect of removal of the ascorbic acid by ascorbic acid oxidase was studied. Crude preparations of the enzyme were prepared from cucumbers after the method described by Sharp, Hand and Guthrie (12). All preparations were active when tested before use. To 0.2 ml. of semen diluted with yolk-citrate at the rate of 1:4 was added 0.1 ml. of the enzyme preparation. After shaking, the mixture was allowed to stand for 10 minutes at room temperature, then methylene blue reduction tests were run on these samples as well as control samples of normal semen. Fourteen semen ejaculations were run in this fashion. The mean reduction time for the untreated samples was 11.3 minutes, and for the treated samples the mean was 12.1 minutes. The difference was highly significant.

For a series of eight ejaculations tested in the same manner, but to which air had been bubbled through both samples before the test, the results were similar and the difference highly significant. The mean time was 8.5 minutes for the untreated, and 9.7 minutes for the treated samples. A series of nine other ejaculations of obviously poor quality, where the mean reduction time was about 20 minutes, failed to show a significant difference in reduction time between treated and untreated samples. These data, showing that destruction of the ascorbic acid in semen samples of good quality slowed reduction time, were suggestive that the level of ascorbic acid in semen might play some part in the methylene blue reduction test. However, it was interesting to note that no significant difference in motility was found between the semen to which ascorbic acid oxidase had been added and the untreated semen after incubation for 45 minutes at 47.5° C. in these experiments. At the time the authors had not seen the necessity for determining the ascorbic acid content of each semen ejaculation and interpretation of the quantitative aspects of the data was impossible.

Fortunately, the methylene blue reduction test had been used in connection with another experiment in this laboratory where the ascorbic acid

content of fresh semen samples was also obtained. The ascorbic acid determinations were made on 0.1 ml. samples by standard titration procedure just before the methylene blue tests were carried out. Data were obtained on 73 semen ejaculations from 15 different bulls in this experiment. The mean data are given in table 1.

TABLE 1

*Summary of information on semen samples for which data on ascorbic acid content were available**

No. of bull	No. of samples	Mean concentration sperm/mm³.	Mean motility	Mean ascorbic acid content	Mean methylene blue reduction time
		thousands	*per cent*	*mg./100 ml.*	*minutes*
1	2	1405	70.0	5.23	3.50
2	1	1400	75.0	7.22	3.50
3	2	1160	70.0	7.55	5.25
4	2	1200	82.5	6.14	5.25
5	3	1300	71.6	5.33	4.25
6	7	1177	71.4	5.18	5.68
7	6	1382	78.3	6.73	3.46
8	1	1020	80.0	10.34	4.00
9	1	800	60.0	4.68	8.50
10	13	1185	69.6	7.62	5.96
11	5	1468	74.0	7.57	3.55
12	7	1038	45.0	4.37	20.68
13	6	1342	70.0	5.66	4.71
14	11	930	61.8	5.46	13.77
15	6	553	31.6	3.76	40.92

* The writers are indebted to Irvine Elliott for these data.

The data were studied by multiple regression and point out several interesting facts which had not heretofore been apparent. Between concentration of spermatozoa per mm³. and motility there was a highly significant positive correlation of 0.6720, higher than was obtained with the larger sample of 383 ejaculates, but between concentration and ascorbic acid content the correlation was 0.1752; so small that it was below the level of significance at even the 5 per cent level of probability. However, between ascorbic acid content and motility the correlation coefficient was 0.4973 and highly significant. Davis and Cole (2) have markedly influenced the motility of the spermatozoa of one stallion by alternately adding and removing ascorbic acid from his diet. These facts would seem to point to the conclusion that the ascorbic acid content of fresh semen is intimately concerned with the motility of the spermatozoa. However, the failure of Lardy and Phillips (5) to prolong motility by addition of ascorbic acid and the results reported here on the continuance of motility at incubation temperatures in the absence of reduced ascorbic acid suggests that the two are not connected in a cause and effect manner.

The statistics calculated from the data are presented below:

Methylene blue reduction time Y	X_1 *concentration*	X_2 *motility*	X_3 *ascorbic acid content*
Correlation of Y with X's	− 0.7131**	− 0.7460**	− 0.3818**
Standard regression of Y on X's	− 0.4127	− 0.4209	− 0.1002

$r_{x_1x_2} = 0.6720^{**}$; $r_{x_1x_3} = 0.1752$; $r_{x_2x_3} = 0.4973^{**}$

$R^2 = 0.6465$ $R = 0.8041^{**}$

** Significant at the 1 per cent level of probability.

It is seen that a large portion of the variation in methylene blue reduction time was due to the concentration of the spermatozoa and to the motility of the spermatozoa in the samples. However, the ascorbic acid content of the semen apparently had a part to play in methylene blue reduction. Later it was found that the yolk-citrate diluter alone plus ascorbic acid would reduce methylene blue under the conditions of this test, providing the ascorbic acid was added in sufficient quantities. The amounts required were from ten to twenty times the quantities found in bull semen. Lardy and Phillips (5) have shown that addition of ascorbic acid to semen leads to an increase in oxygen uptake in amounts readily explained by the oxidation of ascorbic acid to dehydroascorbic acid.

The effects of the kinds and the numbers of bacteria on the reduction test for fresh semen and for semen stored at 5° C. for ten days has been studied[3] simultaneously with the experiments reported here. These data will be published later. Preliminary examination of the data indicates that the kinds and numbers of bacteria found in semen have little effect on the reduction test of fresh semen providing the usual precautions are taken to collect a clean sample of semen (3). For stored semen the test should be applied with caution.

CONCLUSIONS

The decrease in motility of the spermatozoa in bull semen samples, diluted with yolk-citrate and stored 10 days at 5° C. after having been brought to storage temperature in steps of a 5° C. drop each 10 minutes, was positively and significantly correlated with the decrease in motility for similar samples stored in water baths for one hour at 46.5° C., for 45 minutes at 47.0° C., and for 30 minutes at 47.5° C. The correlation coefficients were 0.9088, 0.8979, and 0.6731, respectively. Such an incubation test was suggested for use in determining relative livability of the spermatozoa in semen samples under the same and different handling techniques.

A test for quality of semen in which the rate at which semen diluted with

[3] By I. C. Gunsalus and J. J. R. Campbell, Laboratory of Bacteriology, Cornell University.

yolk-citrate diluent will reduce a dilute solution of methylene blue was suggested for use, especially by operators of artificial breeding circuits. The quantitative relationships between this test and several factors affecting it were presented. Under standard conditions the test was shown to be largely dependent upon the concentration of spermatozoa, the motility of the spermatozoa, and the concentration of ascorbic acid in the semen.

The two tests may be combined for each semen sample. Thus information may be rapidly obtained on initial motility, duration of motility, and relative metabolic rate as influenced mainly by spermatozoan concentration and motility, and ascorbic acid content of the semen. All of these factors now appear to be important as indications of the potential fertilizing capacity of semen.

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INFLUENCE OF SEVERAL FACTORS UPON THE AMOUNT AND STABILITY OF CAROTENOIDS IN FROZEN CREAM AND ITS RELATIONSHIP TO THE METAL-INDUCED OXIDIZED FLAVOR*

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Since Anderson (1) suggested that carotene is the constituent of milk most likely responsible for the inhibition of the development of the oxidized flavor in milk, many investigations have been undertaken to determine the factors affecting its presence in milk and its possible association with stability of flavor. The studies in many cases involved only the factors affecting the carotene values of the feeds to which the cows were given access.

On the other hand, the researches of Whitnah, Martin and Beck (13), Tucker, Garrett and Bender (12), Garrett, Tucker and Button (7), Garrett, Hartman and Arnold (8), and Brown, VanLandingham and Weakley (4) concerned in large part with flavor, demonstrated the relationship which existed between the carotene content of milk and its stability toward the development of the copper-induced oxidized flavor. Later Brown, VanLandingham and Weakley (5) (6) concluded that the amount of carotene in the fat may not be the substance responsible for the reduced susceptibility of milk to oxidized flavor development but suggested that substances associated with it probably had a greater effect than the carotene itself.

That carotene is quite stable in frozen milk products was shown by Olson and co-workers (10) who concluded that milk could be stored for considerable periods without affecting the carotene or vitamin A content.

Inasmuch as carotene was found to be a stable anti-oxidant in milk even in the frozen state, it seemed desirable to ascertain its stability and anti-oxidizing effects in frozen high-testing cream during a prolonged period of storage. Consequently, carotenoid determinations were made on monthly samples of high-test cream throughout the year, the cream being treated in many ways to ascertain the effects upon oxidized flavor development. The data reported herein were obtained from a study of such factors as season of the year, pasteurization exposure, homogenization, copper contamination, additions of sugar and type of container as well as frozen storage at 0° to −10° F. for six and twelve months.

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EXPERIMENTAL

The cream used in this study was secured from the separation, at 100° F., of fresh, raw mixed milk either from the College Creamery or from a local dairy. The fat content ranged from 45 to 60 per cent and averaged 51.75 per cent throughout the twelve-month period.

The cream, divided into three lots, was pasteurized as follows: Lot 1 was heated to 150° F. and held for 30 minutes; Lot II was heated to 165° F. for 15 minutes; and Lot III was heated to 185° F. for 5 minutes. In handling and processing the cream, precautions were taken against copper contamination throughout, the pasteurization being accomplished in a 25-gallon, stainless-steel vat.

Each lot was then subdivided into three portions which were homogenized at 0, 1500, and 3000 pounds pressure respectively, after which the cream frequently stirred, was cooled to 50° F. in ice water.

Each portion thus pasteurized and homogenized was again subdivided into four parts, one serving as a control; a second to which was added 1 ppm. copper in the form of anhydrous copper sulfate; a third to which was added ten per cent sugar; and a fourth having had both sugar and copper at the rates specified above.

Immediately following cooling and treatment, approximately one-pint samples were put into each of three different types of containers, namely, a glass, mayonnaise-type jar with a water-proof, inner-lining screw cover; a pint, paper Sealright carton; and third, a No. 2 "C"-enamel tin can. Separate sets were put up for examination at six and at twelve months' storage. When a lot was packaged, the packages were taken at once to a −10° F. room for freezing, where they remained until date of examination. In all, approximately 30 gallons of cream were required for each monthly series during the twelve-month period of the study.

Since Baldwin (2), Trelogan and Combs (11), and Baldwin and Doan (3) showed that cream containing above 25 to 30 per cent fat froze homogeneously and that it might be reliably sampled in the frozen state, samples for the carotenoid determinations were weighed while the cream was in a completely frozen condition. The procedure followed in making the carotenoid analysis of cream was a modification of the method developed by Moore (9) for the determination of blood plasma carotene.

RESULTS

Seasonal variation in carotenoids of cream. Carotenoid determinations were made on samples of cream secured near the middle of each month throughout the year. The data show that the cream varied widely in its carotenoid content throughout the year, reaching and maintaining a high level from June to October, inclusive, and its lowest level from January to April, inclusive (figs. 1, 3, 4 and 6). These periods roughly correspond to

those of green and dry feeding. Although determinations were not made on the fresh March and April samples, the data on the frozen samples for those months indicate that the carotenoid level was at its lowest in April when the cows were yet on dry feed. Cows were being turned to pasture approximately the first week in May during the year in which these studies were made. Fall rains made excellent pasturage which undoubtedly accounts for the high carotenoid value in October cream.

The influence of the pasteurization exposure on the carotenoids of cream. The samples of cream secured monthly throughout the year intended for frozen storage were pasteurized at exposures of 150° F. for 30 minutes, 165° F. for 15 minutes, and 185° F. for 5 minutes. Analyses were made on these creams to determine what effect the pasteurization exposure had on the carotenoid content.

The data, presented in figure 1, show that the pasteurization exposures had no significant effect upon the carotenoid content of the cream. The carotenoids of cream pasteurized at the higher temperature apparently were as stable as those in cream pasteurized at the lower exposure.

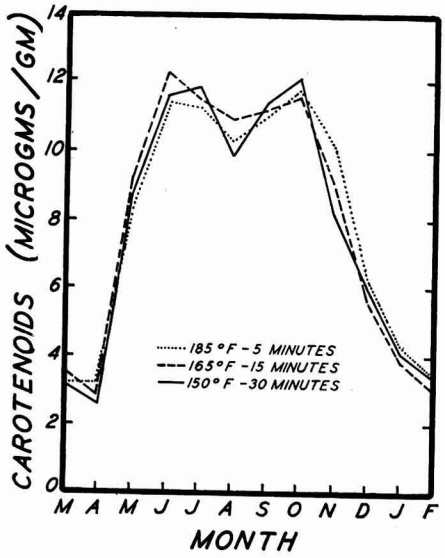

FIG. 1. The carotenoid content of 6-month-old cream as influenced by pasteurization.

The influence of homogenization on the carotenoids of cream. Inasmuch as homogenization was known to retard the development of copper-induced oxidized flavor in milk, and that carotene had been shown to have a similar effect, studies were made to determine the effect of homogenization upon the stability of carotenoids of frozen cream even when one part per million copper was added to the cream prior to storage.

The data presented in figure 2 show that homogenization had no significant effect upon the stability of carotenoids in cream, the amount present being approximately constant regardless of the pressures of homogenization. The presence of one ppm. copper in the homogenized cream appeared to exert no influence on the stability of the carotenoids.

Stability of carotenoids in frozen cream. Carotenoid determinations made on monthly samples of cream throughout the year when fresh and after

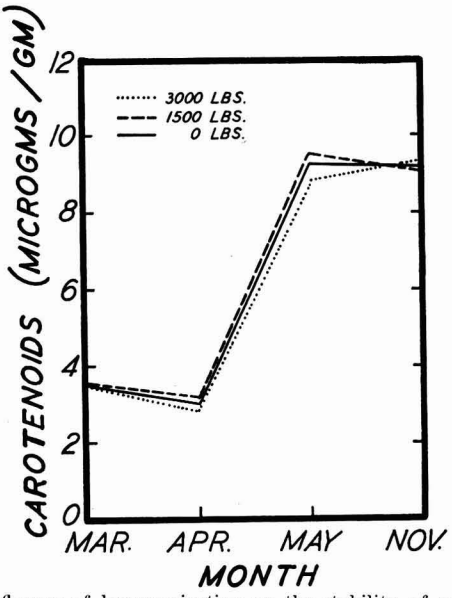

FIG. 2. The influence of homogenization on the stability of carotenoids in cream stored 6 months.

six and after twelve months of frozen storage show that the length of storage, under conditions of the experiment, had no detrimental influence on the carotenoid content of cream (fig. 3).

The influence of copper on the stability of carotenoids in frozen cream. As carotene has been shown to have a stabilizing effect on the flavor of milk against copper-induced oxidation, analyses were made of the cream samples to determine what effect the presence of copper might have on the carotenoid content of the cream when stored in the frozen state.

The data, presented in figure 4, show little difference between the carotenoid contents of the control and of the copper-contaminated cream samples. In view of the close relationship which existed between the carotenoid values of the control and copper-contaminated samples, it should be pointed out that oxidized flavors always developed in the copper-contaminated samples, whereas the control lots were largely without flavor criticisms.

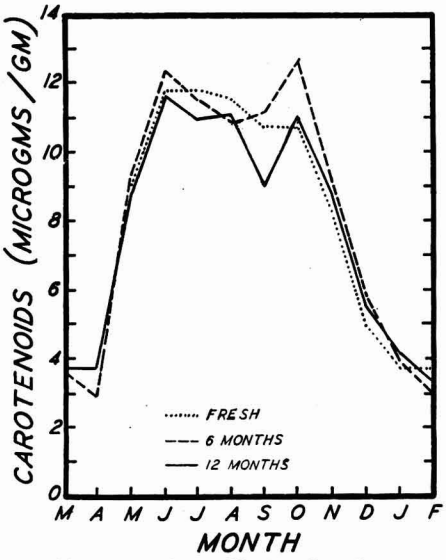

FIG. 3. The carotenoid content of monthly samples of cream as affected by period of frozen storage.

The influence of the type of container on the carotenoid stability of frozen cream. The type of the container whether paper, tin or glass, had

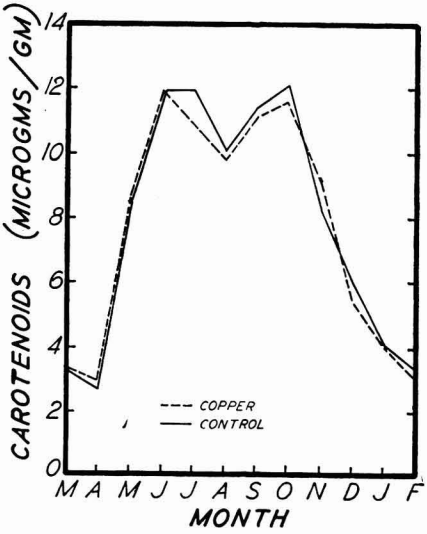

FIG. 4. The carotenoid content of monthly samples of cream as influenced by addition of one part per million of copper. (Cream pasteurization at 150° F. for 30 min. and stored 6 months.)

no significant influence on the carotenoid values of cream, upon frozen storage (fig. 5). While close study of the data revealed some slight variance in the carotenoid content between the creams stored in the various containers, these differences may be attributed largely to experimental errors. No definite trends were found which favored any one type of container over another so far as carotenoid values were concerned.

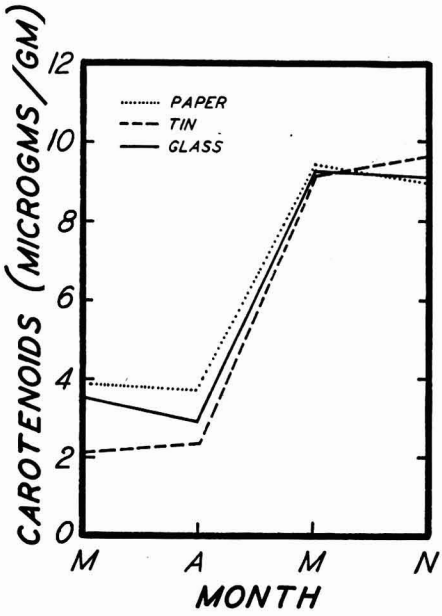

FIG. 5. The carotenoid content of cream stored 12 months in paper, tin and glass.

The influence of sucrose on the carotenoid stability of cream. Carotenoid analyses were made on frozen stored cream containing 10 per cent of sucrose added prior to freezing with and without the addition of one ppm. of copper.

No significant effects resulting from the addition of sucrose were noted. Specific data for one month's sample of cream carried throughout the experiment showed no definite trend as a result of the addition of sucrose alone or of the addition of sucrose and copper to the cream (Table 1).

The influence of the carotenoid content on the flavor of frozen cream. No correlation was found to exist between the intensity of oxidized flavor and the carotenoid content of the cream (figs. 6 and 7). While there appeared to be no significant differences between the carotenoid levels of cream with and without copper, there were marked differences in the flavors of the creams with and without added copper. Apparently the carotenoids

TABLE 1

The carotenoid content of November cream after various treatments

EXPERIMENTAL DATA

Freezing and Storage of Sweet Cream

Sample November 20,1940 Factor studied Carotenoids (micrograms / gm. fat) Date examined 5 - 23 - 41 / 11 - 12 - 41

Original Cream	Heat Treatment	Storage in	Treatment: 0# Control	0# 1 ppm Cu	0# 10% Sucrose	0# Cu + Sucrose	1500# Control	1500# 1 ppm Cu	1500# 10% Sucrose	1500# Cu + Sucrose	3000# Control	3000# 1 ppm Cu	3000# 10% Sucrose	3000# Cu + Sucrose
RAW (Separated at 100-110°F.)	150°F.- 30 min.		After 6 months at 0°F.											
		Glass	8.20	9.15	8.59	9.33	8.81	8.86	9.17	8.86	9.03	8.49	8.86	9.14
		Paper	9.50	8.86	9.33	8.86	9.86	9.79	8.63	8.86	lost	9.27	9.33	8.86
Tests:		Tin	9.99	9.27	9.17	8.86	9.38	9.33	9.02	8.86	9.32	9.35	8.86	9.14
			After 12 months at 0°F.											
Fat 52.0%		Glass	8.80	8.90	10.12	10.45	9.23	8.80	9.79	9.46	9.40	8.93	9.86	9.62
Acid.		Paper	9.71	8.93	10.12	10.12	8.93	9.71	10.12	9.46	9.71	8.93	9.62	9.96
Flav.		Tin	8.90	8.93	10.12	9.79	8.93	9.40	10.45	9.79	9.40	9.40	10.45	9.79
Caro- 9.60 / tene 9.99 / 9.40	Fresh / 6 Mos. / 12 Mos.		After 6 months at 0°F.											
		Glass	9.10	9.70	8.86	8.92	9.03	9.03	8.86	9.02	9.21	8.80	8.46	8.92
Bact.		Paper	9.03	9.07	9.17	8.92	8.80	9.99	10.07	9.45	8.54	9.84	9.33	9.23
Misc.	165°F.- 15 min.	Tin	9.70	9.70	9.01	8.92	8.81	8.54	9.17	9.23	9.03	8.91	9.08	8.92
			After 12 months at 0°F.											
		Glass	8.80	8.80	9.46	10.12	8.93	8.80	9.46	9.62	8.80	8.93	9.62	9 46
		Paper	9.71	9.40	10.45	9.79	9.40	8.80	9.79	10.12	9.71	9.71	10.12	10.45
		Tin	8.93	9.40	10.45	9.46	9.40	8.93	10.45	9.79	8.93	8.80	10.12	9.46
			After 6 months at 0°F.											
		Glass	9.99	8.98	9.11	8.88	9.38	9.44	9.86	9.70	8.51	8.98	9.99	9.37
		Paper	9.38	9.93	9.39	9.08	9.70	9.44	9.24	9.11	9.84	9.93	8.80	9.11
	185°F.- 5 min.	Tin	9.38	9.64	9.39	9.11	8.74	8.75	9.27	9.11	9.84	8.98	9.11	9.11
			After 12 months at 0°F.											
		Glass	8.80	8.90	9.79	9.46	8.90	8.80	9.46	9.46	8.93	8.80	9.46	9.46
		Paper	9.40	9.40	10.45	10.45	8.93	9.71	10.12	9.79	9.71	9.40	9.46	9.79
		Tin	9.42	9.23	10.45	10.45	8.80	9.40	9.79	9.46	9.42	9.71	10.12	9.46

in cream are not present, even during the summer months when the cows are on green feed, in sufficient quantities to stabilize the fatty constituents of high-fat cream against copper-induced oxidation.

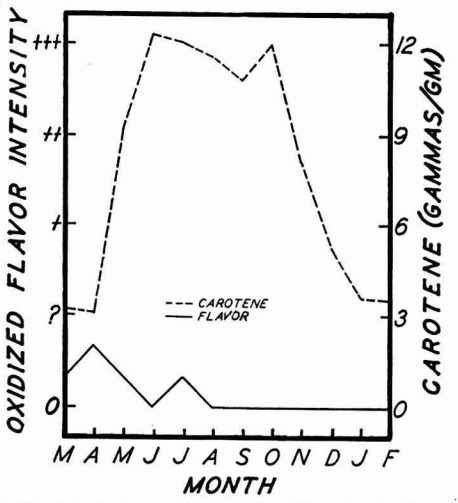

FIG. 6. The relationship between the carotenoid content of cream and the intensity of oxidized flavor. Cream was pasteurized at 165° F. for 15 minutes and stored for 6 months at 0° to – 10° F. (Carotenoids expressed as carotene.)

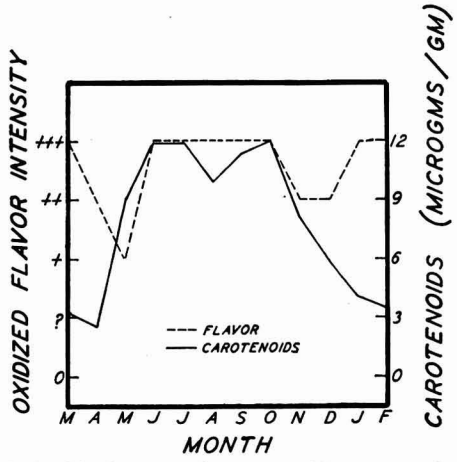

FIG. 7. The relationship between the carotenoid content of cream stored for 6 months at 0° to – 10° F. and the intensity of the oxidized flavor. The cream was pasteurized at 150° F. for 30 minutes, after which 1 ppm. of copper was added.

DISCUSSION

While the carotenoid content of butterfat in cream produced at different seasons of the year varied widely from a low in the late winter months to a high in the early summer months, the amount present apparently exerted little influence on the storability of cream in the frozen state so far as inhibition of the development of the copper-induced oxidized flavor is concerned. June cream high in carotenoids, to which one ppm. of copper in the form of copper sulphate was added, developed the oxidized flavor upon storage as readily as late winter cream, similarly treated, which was quite low in carotenoids. Apparently, therefore, should conditions warrant, winter cream might be stored frozen at 0° to −10° F. for six months or longer as well as June cream.

Since manufacturing processes, such as pasteurization, homogenization, and freezing were found to have no appreciable destabilizing influence on the carotenoids of butterfat, cream might be safely stored for reasonable periods of time with the assurance that the carotenoid content would be maintained.

SUMMARY

The carotenoid values of high-fat cream, determined monthly throughout the year, varied from a low during the winter months when the cows were on dry feed to a high during the summer months when the cows had access to green feeds.

Pasteurization, homogenization, addition of sucrose, addition of one ppm. copper, type of container, freezing, and storage for twelve months at 0° to −10° F. had no appreciable effect upon the carotenoid content of high-fat cream.

The relatively high levels of carotenoids present in cream during the summer months did not stabilize the cream against the development of copper-induced oxidized flavor when one ppm. of copper was added after pasteurization.

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RELATIONSHIP OF CONSUMPTION OF PEPPERGRASS BY COWS TO THE FLAVOR AND INDOL CONTENT OF BUTTER

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A rather characteristic flavor defect of butter is designated by many butter judges as peppergrass flavor and often is considered to be due to consumption of peppergrass (*Lepidium virginicum*) by the cows producing the cream. The defect varies greatly in intensity; it may be pronounced or it may be so slight that its identity is difficult to determine.

Butter with peppergrass flavor is produced principally in certain areas during a definite part of the year. It has been encountered especially in sections of North Dakota, South Dakota, Nebraska and some of the nearby states; it may begin late in April or early in May and continue until late June or early July. The defect is much more serious in certain years than in others; lack of rainfall, particularly over a period of more than a year, seems to result in many weeds (including peppergrass) developing in pastures and in considerable butter having peppergrass flavor. During certain of the recent drouth years peppergrass flavor in butter sometimes was such a serious defect that the butter was almost unsalable, and creameries in the affected sections suffered heavy losses. In some areas at the peak of the peppergrass season it was almost impossible to find milk that did not have the defect to some degree.

Although peppergrass flavor commonly is associated with butter from certain states, there is evidence that it occasionally is present to some degree in butter from other sections. For the most part this butter is not recognized as having peppergrass flavor, and the defect is described as weed or feed flavor. Since peppergrass is widely distributed throughout the United States, the defect would be expected in butter produced in various sections.

LITERATURE REVIEW

Although the relationship of consumption of peppergrass by the cows to a specific defect of butter is accepted by many butter judges, others believe that the peppergrass flavor of butter is due entirely or in part to plants other than peppergrass. Frenchweed (*Thlaspi arvense*) has been considered especially in this connection.

Feeding trials carried out by Olson (5) involved a plot seeded to peppergrass; an alfalfa pasture containing some Frenchweed; force feeding of peppergrass, Frenchweed and onions; stall feeding of peppergrass; and stall feeding of mixtures of peppergrass and Frenchweed and of alfalfa and Frenchweed. Cows were allowed their freedom on the plot seeded to pepper-

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grass which also contained other weeds, including Frenchweed; the cows preferred to feed on the peppergrass. Frenchweed in the alfalfa pasture was not eaten until all of the alfalfa had been consumed, or about 3 weeks after the cows were turned in the pasture. Cows were fed 40 pounds of peppergrass daily for 4 days and Olson reported slight to pronounced weed flavors in the milk, which were considered to be the most pronounced of any of the trials. When peppergrass was force-fed only 9 per cent of the milk samples were reported to have a weed flavor. Results of force feeding Frenchweed were not reported. Milk from tests in which Frenchweed was mixed with peppergrass and alfalfa had a weed flavor after the first trial, and the intensity increased with successive feedings. Olson concluded that the objectionable flavor attributed to peppergrass was due to small quantities of some other weed, such as Frenchweed, and not to peppergrass.

Frenchweed and peppergrass were used in feeding trials by Downs (2); the cows were fed by drenching about one hour before milking. Downs reported that when fed in this manner peppergrass did not produce any off flavor in the milk, while Frenchweed produced a slight garlic flavor. He noted that cows will not eat Frenchweed if anything else is available.

The end products of digestion of Frenchweed and blue glass by bacteria and enzymes were studied by Ingle (4). He reported that when Frenchweed to which only water had been added was allowed to stand at 100° F. for 12 hours, the aqueous portion had an indol content of 26.6 μg. per gram of dry weed. Another sample of Frenchweed, partially spoiled in transit, was held under the same conditions and the indol content after the holding was 38.2 μg. per gram of dry weed; when the holding time was lengthened to 18 hours the indol content had risen to 70.5 μg. per gram of dry weed. With blue grass held for 18 hours in water, the indol content was 11.5 μg. per gram of dry grass. When the Frenchweed and blue grass were mixed with water and toluene or chloroform and enzymes added (types not reported), only traces of indol were found. Presumably the toluene or chloroform used to inhibit bacterial growth inhibited the enzymes or the enzymes would not change the indol forming compounds to indol. Ingle concluded that, on the basis of his tests, Frenchweed was potentially able to break down into much greater amounts of indol than blue grass and might easily furnish enough indol to cause its appearance in the milk.

Eckles, Combs and Derby (3) stated that butter defects reported to them and apparently due to Frenchweed were confined to the winter months when dry feed was used but that statements in the literature indicated the difficulty was most frequently experienced during the grazing season. These investigators found that each of eight cows had an extreme dislike for Frenchweed, even when the cows were not getting any other green forage. They found that when 300 grams of the green plant was fed to the cows 3 hours before milking no off flavor could be detected in the milk but that

when 610 grams was fed the flavor was readily detected. When Frenchweed seed was fed to cows, a minimum of 125 grams per day was necessary to produce an off flavor in the milk. The description of the flavor produced by these seeds was that it resembled the flavor of oil of mustard and oil of garlic.

GENERAL EVIDENCE OF RELATIONSHIP OF PEPPERGRASS TO THE DEFECT

In sections supplying butter having peppergrass flavor, the defect is conspicuous in the milk or cream from certain farms; in these products the flavor is essentially the same as it is in butter. Often the farmers cannot use the milk they produce because of the intensity and objectionable character of the off flavor and obtain milk from a neighbor. Many of these farmers definitely believe that the off flavor in the milk occurs when peppergrass has made a heavy growth in the pastures and that it is due to consumption of the peppergrass by the cows. Operators of small butter plants who know conditions on the farms supplying them with cream also commonly consider that the characteristic defect they encounter at certain seasons is due to the consumption of peppergrass by the cows.

Many people familiar with conditions in the areas supplying peppergrass butter believe that cows will not eat Frenchweed as long as there is anything else available. Evidence to support this idea is the fact that many of the pastures have abundant growth of Frenchweed, but there is little indication that any of it has been touched by the animals. It is generally recognized that cows readily eat peppergrass, often apparently preferring it to various other plants growing in the pastures.

OBSERVATIONS IN A PEPPERGRASS AREA

In a season when peppergrass flavor in butter was very common in a certain area, observations were made on the grazing habits of the cows and on the pasture conditions in that area. Nearly all milk, cream and butter produced there had a pronounced peppergrass flavor which in many instances was so objectionable that farmers could not use the dairy products produced on their own farms. During this time much of the food prepared with milk, cream or butter had a peppergrass flavor. Coffee to which the off-flavored cream had been added showed a conspicuous flavor defect.

At a farm producing milk with a pronounced peppergrass flavor a careful search of the pasture failed to reveal the presence of any Frenchweed, but it contained an abundance of peppergrass. Since there were no normal pasture grasses, the cows had only peppergrass and other weeds for forage, and they ate the peppergrass readily.

The six cows in a herd producing cream having a conspicuous peppergrass flavor were followed by three persons from the time they were turned

on pasture in the morning until they were returned to the barn at evening. There was some Frenchweed in the low areas of the pasture, but none of it was eaten, and none of the plants showed any evidence of having been grazed. On various occasions a cow turned away from a Frenchweed plant that she approached in her grazing. The cows spent most of the day on some low hills along one side of the pasture and ate steadily of the peppergrass that grew abundantly there. On their way to the barn at evening they crossed some low ground having a rank growth of various weeds including Frenchweed; here they ate only the heads of volunteer oat plants.

In the area at the time, a farmer was distributing milk that was generally recognized as the one supply that was free of peppergrass flavor. The cows were using a rye pasture and an examination of the pasture failed to reveal either peppergrass or Frenchweed. One of the cows in this herd was kept in the barn and offered Frenchweed; she refused to eat it although no other feed was given her during 24 hours.

The general unpalatability of Frenchweed was emphasized when the plants were offered hogs being kept in a dry lot. Althought they would eat all other weeds offered, they refused Frenchweed.

INDOL CONTENTS OF PEPPERGRASS BUTTER

The odor of peppergrass butter suggests, in a general way, the odor of a dilute solution of indol or skatol. Experimental butter made from good-flavored cream treated with a small amount of one of these compounds had a flavor somewhat resembling the flavor of peppergrass butter. Many butter judges make a sharp distinction between this flavor in butter and the flavor they attribute to Frenchweed, the latter flavor being more "musty."

Following the disclosure by Clarke *et al.* (1) that butter made from decomposed cream showed high values for indol, a study was made of the indol content of various lots of commercial butter, including peppergrass butter. The method of determination used was the extraction procedure described by Clarke *et al.* (1), and the results are expressed as micrograms (μg.) of indol per 50 ml. of milk fat.

Table 1 presents results of indol determinations on commercial butter having pronounced peppergrass flavor and, for comparison, results on good quality butter definitely free of peppergrass flavor. The samples of peppergrass butter had indol contents ranging from 45 to 1100 μg. per 50 ml. of milk fat. These results cover the general range of values obtained on peppergrass butter. Most of the samples having the off flavor had indol contents ranging from 40 to 100 μg. per 50 ml. of milk fat. When the flavor was less pronounced, however, the values frequently were considerably lower. The good quality butter was produced in plants not in peppergrass areas; the indol contents were far below those of peppergrass butter and are in agreement with the lowest values reported by Clarke *et al.* (1) for normal butter.

Cans of commercial cream from peppergrass areas, all of which had a pronounced peppergrass flavor, were churned individually and indol determinations made on the resulting lots of butter. The following values were obtained: 404, 204, 1070, 1000, 1100, 500, 300, 1020 and 640 μg. indol per 50 ml. fat.

TABLE 1

Indol contents of butter having pronounced peppergrass flavor and of good quality butter free of the defect

Butter with pronounced peppergrass flavor		Good quality butter free of peppergrass flavor	
Sample No.*	Indol μg./50 ml. fat	Sample No.†	Indol μg./50 ml. fat
1	153	1	2
2	150	2	3
3	66	3	2
4	1100	4	3
5	91	5	3
6	45	6	2
7	640	7	2
8	71	8	3
9	500	9	3
10	77	10	2

* Samples from various butter plants in peppergrass areas.
† Samples from widely distributed butter plants not in peppergrass areas.

During a season when peppergrass cream was very common in a certain area, analyses were made on first and second grade butter from a plant in that area; the data are given in table 2. The values are much higher on the second grade butter than on the first grade butter. Probably much of the cream used in the second grade product was put there only because of the peppergrass flavor and was otherwise satisfactory.

TABLE 2

Indol contents of first and second grade butter from a plant in a peppergrass area during the peppergrass season*

Grade of butter	Indol μg./50 ml. fat
1	13
1	9
1	4
1	5
1	10
2	1070
2	1100
2	1000
2	500
2	300
2	1020
2	640

* All samples of second grade butter had peppergrass flavor.

When the observations were being made in the peppergrass area, small churnings of butter were prepared in glass churns and samples taken to the laboratory for indol determinations. Some of the data are given in table 3. High indol values regularly were obtained on butter from cream having a pronounced peppergrass flavor, while the butter from cream having no peppergrass flavor had low values; the latter came from the herd fed on rye pasture. In general, as the intensity of the abnormal flavor increased, the indol values increased.

During the investigation an attempt was made to determine the lowest indol contents which accompanied the off flavor in butter. From the results obtained it appeared that the flavor could first be detected by experienced judges at an indol level of about 20 μg. per 50 ml. of fat. The effect of indol on the flavor depended to some extent on whether the butter was salted since salt tends to bring out various flavors in butter.

TABLE 3

Indol contents of small lots of experimental butter made in the peppergrass area

Cream used			Indol μg./50 ml. fat
Source		Flavor	
Butter plant		Pronounced peppergrass	166
Farm 1	A.M.	No peppergrass flavor	2
	P.M.	No peppergrass flavor	4
Farm 2	A.M.	Pronounced peppergrass	202
	P.M.	Pronounced peppergrass	404
Farm 3	A.M.	Pronounced peppergrass	113
	P.M.	Pronounced peppergrass	173

INDOL CONTENTS OF VARIOUS TYPES OF BUTTER

Samples of butter were collected outside peppergrass areas in an attempt to obtain information on the indol contents of various types of this product. Results of some of the analyses are presented in table 4. Samples 1 to 5 are from the same plant, with samples 1 and 2 being from onion flavored cream. While one of the samples of butter from onion flavored cream was higher in indol than might be expected, this probably was caused by factors other than the onion flavor. Samples 6 to 11 illustrate the difference that may exist between the indol contents of butter from good flavored cream (samples 6, 7 and 8) and butter from fair flavored cream (samples 9, 10 and 11). Since the butter came from an area where peppergrass had not been a problem, it is probable that the high indol values were caused by some other factor, such as action of organisms in the cream. Samples 12 to 20 are representative of sour cream butter from a number of sections where peppergrass flavor has not been a problem. Sample 21 shows the high indol content of butter made from experimental cream held until it was badly decomposed. Actually the cream could not be churned, and the fat for the indol

determination was recovered by heating the cream; the acidity of the cream also shows the extensive changes that had gone on in it.

TABLE 4

Indol contents of butter made outside peppergrass areas

Sample No.	Cream used		Indol μg./50 ml. fat
	Flavor	Acidity	
1	Onion	0.68	15
2	Onion	0.42	6
3	.....	0.39	9
4	.....	0.36	4
5	.....	0.50	3
6	Good	.....	6
7	Good	.....	8
8	Good	.....	6
9	Fair	.....	33
10	Fair	.....	25
11	Fair	.....	13
12	.....	.....	5
13	.....	.....	5
14	.....	.....	6
15	.....	.....	3
16	.....	.....	10
17	.....	.....	12
18	.....	.....	6
19	.....	.....	10
20	.....	.....	8
21	Poor	2.04	420

DISCUSSION

Evidence collected during the observations in an area in which much peppergrass butter was being produced indicated that cows found peppergrass palatable and preferred it to other weeds growing in the pastures. Since there were no normal pasture grasses, peppergrass often provided the main feed for animals on pasture. Cows grazing on a pasture with an abundance of peppergrass, but which was free of Frenchweed, produced milk with a distinct peppergrass flavor.

Frenchweed has been suggested as responsible for the off flavor in dairy products that is commonly described as peppergrass flavor. However, observations in the peppergrass area and observations reported by various investigators indicate that cows commonly refuse to eat Frenchweed. It appears that cows may eat it when other feed is lacking, but this condition apparently is not frequently encountered. The fact that the peppergrass flavor is present in milk when there is much peppergrass on which the cows are feeding, as well as Frenchweed which they are not eating, points to the former as the probable cause of the flavor defect. The common belief among farmers in peppergrass areas that peppergrass is responsible for the flavor defect is based on extensive observations, often over periods of many years.

Green Frenchweed has a musty odor while crushed seeds have a strong onion or garlic flavor. References to the off flavor produced by Frenchweed (2, 3) describe it as an onion or garlic flavor. Eckles, Combs and Derby (3), noted that the complaints reaching them, and apparently due to Frenchweed, were concerned with off flavors encountered during the months that the cows were on dry feed. Frenchweed seeds can easily get into the feed of cows from dried Frenchweed plants in the hay or from mill feed.

It is interesting to note that some experienced judges make a distinction between peppergrass flavor and Frenchweed flavor. Apparently the former closely resembles the flavor of indol or skatol while the latter suggests the flavor of onions or garlic. It is not uncommon to find butter, made in the winter in areas where Frenchweed is prevalent, with a flavor suggestive of onions or garlic.

Assuming that peppergrass is responsible for the peppergrass flavor of milk and other dairy products, there probably is no other feed flavor of milk so different from the flavor of the feed involved. Peppergrass itself has a hot peppery taste and has no suggestion of the indol-like flavor present in the off-flavored milk. Feed flavors caused by such materials as onions, alfalfa and silage all resemble the flavors of the causative feeds.

Although feeding trials with peppergrass have not been particularly successful in producing peppergrass flavor in milk, various factors may partially explain this. A considerable feeding period may be necessary before the defect appears in the milk and this would be expected since the flavor produced in the milk is so different from that of the plant; perhaps indol formation must be built up to the point where the natural elimination processes can no longer remove it and some of it is eliminated in the milk. Since the reported feeding trials have run for relatively short periods this point may not have been reached. Again it may be that the plant produces the objectionable flavor in milk only when it is in certain stages or has grown under certain soil or climatic conditions. Certainly peppergrass is present in pastures before the defect becomes serious in the milk, and it is also present after the flavor disappears, although it is largely dried.

Peppergrass flavor is one of the abnormal flavors of milk which can be measured chemically. In a general way the intensity of the flavor can be correlated with the indol content of milk fat.

Since butter with peppergrass flavor has a high indol content, it is evident that a high indol value on butter does not necessarily indicate that the butter was made from cream in which there had been extensive microbiological changes.

CONCLUSIONS

1. In an area in which peppergrass butter was being produced the following observations were made:

(a) Essentially the same objectionable flavor that was evident in the butter was present in much of the milk and cream.

(b) The defect was conspicuous in cream produced by cows grazing in a pasture in which peppergrass was prevalent but which was free of Frenchweed.

(c) In a pasture used by cows producing peppergrass cream, the animals ate peppergrass but not Frenchweed. The cows throughout the area ate peppergrass readily.

(d) A cow held in a barn refused to eat Frenchweed even when other feed was withheld for 24 hours. Hogs in a dry lot ate many different weeds that were offered them but refused Frenchweed.

2. The peppergrass flavor resembled the flavor of a dilute solution of indol or skatol.

3. Butter with peppergrass flavor regularly had an indol content higher than that of normal butter.

4. The intensity of peppergrass flavor in butter was related to the indol content of the fat.

5. The indol content of fat from peppergrass butter ran as high as 1100 μg. per 50 ml. of fat. It appeared that a peppergrass flavor could be detected by experienced judges when the indol content was as low as 20 μg. per 50 ml. of fat.

6. The indol content of butter may be very high when the butter is made from decomposed cream.

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SOME FACTORS AFFECTING THE STABILITY OF CERTAIN MILK PROPERTIES. VII. THE EFFECTS OF METALS AND OF ASCORBIC ACIDS ON THE OXIDATION–REDUCTION POTENTIAL*

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Oxidation-reduction systems and potentials in milk have received the attention of a considerable number of investigators. Lack of space does not permit a comprehensive review of the subject. The following systems, however, have been reported as occurring in milk: glutathione (thiol)—glutathione disulfide, cysteine-cystine, levoascorbic acid-dehydroascorbic acid, riboflavin—reduced riboflavin. The oxygen-hydrogen peroxide and hydrogen peroxide-hydroxyl systems probably should be included.

Recently, considerable attention has been devoted to a study of oxidation-reduction potentials in connection with the processing and handling of dairy products. The widespread study of oxidation and reduction reactions involved in the destruction of vitamins and in the development of undesirable flavors undoubtedly has been responsible for this attention.

Gebhardt and Sommer (1) found the oxidation-reduction potential to be related to the rate of solution of copper from metal surfaces. The presence of dissolved copper caused an increase in the potential. Thurston (6) reported that stannous chloride, stannic chloride, and aluminum chloride caused a decrease in the oxidation-reduction potentials of milk, whereas iron powder and ferrous, ferric, and cupric salts caused increases. The increase due to the ferrous salt was only temporary. Swanson and Sommer (5) showed that there was a rapid increase in oxidation-reduction potential shortly after the addition of copper but that the addition of ferrous ions caused a decrease. Ferric ions caused the potential to increase slowly during storage of the milk. When copper was added, the potential did not increase until virtually all of the reduced ascorbic acid was oxidized. These investigators also added crystalline ascorbic acids to milk and observed a decrease in the oxidation-reduction potential.

Results have been published by several investigators which indicate an inverse relationship between the magnitude of the oxidation-reduction potential and the keeping quality of milk products. In 1932, Morris and Sommer (4) obtained results which showed that the keeping quality of cream was poorest in samples having the highest potentials. Tracy, Ramsey, and Ruehe (7) concluded that oxidation-reduction potentials are related to

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fat oxidation in dairy products. Webb and Hileman (8) asserted that, "the development of oxidized flavors in milk by the addition of copper is due to or accompanied by an increase of the oxidation-reduction potential of the milk to a point sufficiently high to bring about a change in some milk constituent," and concluded that the measurement of oxidation-reduction potentials affords a means of detecting the source of copper contamination. Greenbank's (2, 3) results and interpretations indicated that there is a direct relationship between the potential and the tendency of a milk to develop oxidized flavors. He showed a variation in the poising action of milks toward oxidation-reduction; milks that readily developed the flavor increased in potential upon the addition of a small amount of copper. This investigator has proposed the measurement of the increase in oxidation-reduction potential after the addition of a standard amount of copper as a means of predicting the susceptibility of milk to the development of oxidized flavor. Swanson and Sommer (5) concluded from their studies, however, that the oxidation-reduction potential of the medium did not seem to inhibit or accelerate the development of oxidized flavor.

It appears from the various reports in the literature that the oxidation-reduction potential of milk is characterized chiefly by its complexity. The potential at any given time is actually a measure of a number of interacting forces. Under certain conditions one system may predominate over the others. For instance, when bacteria are allowed to grow and multiply freely, dissolved oxygen is greatly reduced and consequently the potential is lowered. On the other hand, when the growth of bacteria are inhibited and soluble copper is present, there is an increase of the positive value of the potential.

The significance of oxidation-reduction potential changes in milk is not clearly understood. In seems reasonable, however, that the destruction of ascorbic acid and perhaps of riboflavin and the development of oxidized flavor in milk may be, in some way, related to actual changes in potential or to the poise or stability of this potential.

EXPERIMENTAL METHOD

The apparatus used in the studies reported here consisted of a number of bright platinum electrodes, a saturated calomel half cell (reference electrode), a Leeds and Northrup Type K potentiometer, a Leeds and Northrup thermionic vacuum tube amplifier, and a Leeds and Northrup Type R2500 reflecting galvanometer. The platinum electrodes were used in pairs, and duplicate measurements were obtained by switching from one member of the pair to the other. The accuracy of all electrodes was determined by checking the potential of a standard buffer solution containing freshly added hydroquinone the potentials of which had previously been determined with a glass electrode.

Accurate measurements of the potential were obtained by using electrodes that had been cleaned thoroughly by boiling in 1:1 nitric acid and rinsing with water, dilute ammonia, and again with water, by "ageing" the platinum electrodes in a control milk sample for several hours, and by holding the reference electrode, when not in use, at the same temperature employed in making the measurements. The potassium chloride solution used for replenishing the supply in the calomel half cell also was kept at this temperature.

The potential measurements were made at the same temperature as that used for storage of the samples, which was approximately 4.5° C. The electrodes were shifted from sample to sample while a series of determinations was being made. The electrodes were not rinsed with water when they were transferred. This procedure was satisfactory, provided the electrodes were allowed to reach equilibrium before the voltage reading was taken. If the different milks exerted nearly the same voltage on the electrodes, the time required to reach equilibrium was usually less than five minutes. If, however, there was wide variation in the potentials of the milks, additional time was required.

EFFECT OF DISSOLVED METALS ON THE OXIDATION-REDUCTION POTENTIAL OF MILK

Soluble copper (copper sulfate solution) was added to six samples of milk from different individual cows (two Holsteins, one Jersey, one Ayrshire, one Brown Swiss, one Guernsey) so as to give copper concentrations of 0, 10^{-6}, 10^{-5}, and 10^{-4} moles per liter of milk. Periodic determinations of the oxidation-reduction potential were made during 48 hours while the samples were being held in storage. The effect of the different concentrations of copper upon the magnitude and trend of the potential of the milk samples is shown by the plotted curves in figure 1. Use of average values for the six samples in plotting the curves seemed justified, since the variations between samples were slight. Had curves for each sample been plotted they would have agreed closely with the average curves shown in figure 1. It is conceivable, however, that another set of samples would have given curves of slightly different characteristics.

The potential of the control samples, which contained no added copper, dropped slightly during the 48 hours, the decrease being most rapid during the first eight hours. The potential of the samples containing 10^{-6} moles (0.064 ppm.) of copper decreased slightly during the first eight hours and then increased at the end of 48 hours of storage to an E_H of about 0.011 volt above the initial potential. The potentials of the samples containing 10^{-5} (0.64 ppm.) and 10^{-4} (6.4 ppm.) moles of copper began to increase immediately upon the addition of copper, reached maxima, and maintained these levels during the remainder of the storage period. The maximum

potential and the speed with which it was reached were dependent upon the concentration of added copper. These results are in good agreement with those reported by Swanson and Sommer (5), with the exception that their maximum levels were somewhat higher than the ones reported here. This might be attributed to a difference in the poising action of the milks used.

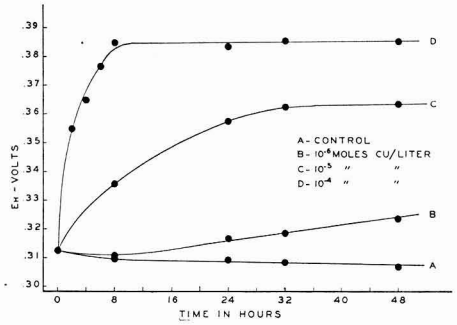

FIG. 1. Effect of concentration of copper on the oxidation-reduction potential of milk.

The initial E_H value, although in good agreement with those reported by Swanson and Sommer (5), is much higher than those observed by Greenbank (2, 3).

Ferrous iron (freshly prepared ferrous sulfate solution) was added at the rate of 0, 10^{-5} (0.56 ppm.), 4×10^{-5} (2.24 ppm.), and 10^{-4} (5.6 ppm.) moles per liter to some of the same samples of milk used in the copper experiments. The average E_H values were plotted against time as shown in

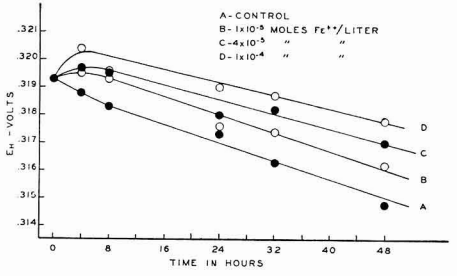

FIG. 2. Effect of concentration of ferrous iron on the oxidation-reduction potential of milk.

figure 2. It is evident from these graphs that the ferrous iron in the concentrations used here had only a slight effect on the oxidation-reduction potential, namely, to keep the potential at a level slightly above the control sample. All samples had a lower E_H value at the end of the 48-hour storage period than the initial potential.

These results are not in agreement with those reported by Swanson and Sommer (5), which showed that the addition of ferrous iron greatly reduced the potential. They worked with higher concentrations of iron than those used in this experiment, with the exception that their lowest concentration was approximately equivalent to the highest concentration used here. For some unexplained reason, they obtained the greatest decrease in potential with the lowest concentration of iron, whereas the second highest of the four concentrations used gave the least decrease. The results reported here seem to agree with those given by Thurston (6).

Data, not presented in this paper, showed that nickel sulfate and ferric, vanadium, aluminum, manganese, chromium, and stannous chlorides, when added in concentrations of 10^{-5}, 4×10^{-5}, and 10^{-4} moles per liter of milk, did not significantly alter the oxidation-reduction potential from that of the

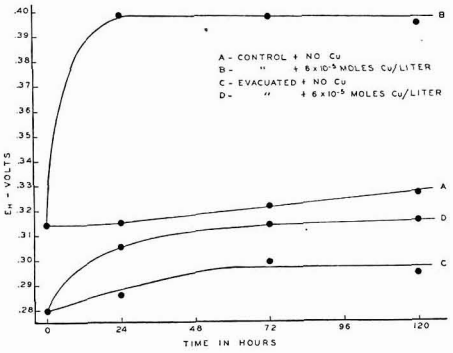

FIG. 3. Effect of evacuation on the oxidation-reduction potential of milk.

control sample. The results on ferric iron do not agree with those of either Thurston (6) or Swanson and Sommer (5). The results on stannous and aluminum chlorides are not in agreement with Thurston's (6) results.

Most of the oxygen was removed from a sample of pasteurized milk by evacuation for 30 minutes with a high vacuum pump. After addition of copper at the rate of 5×10^{-5} moles per liter to some of this milk and to an unevacuated control portion, the samples were stored in such a manner that only very small amounts of oxygen could be absorbed by the milk. Periodic measurements of the oxidation-reduction potential were made, the results of which are shown in figure 3.

The results show that the removal of oxygen caused a decided decrease in the potential of the sample containing no copper and definitely limited the rise in potential of the sample containing copper. It seems likely that, had all the oxygen been removed, the increase in potential of the evacuated samples during storage would have been nil or considerably less than that obtained. It appears that this milk was poorly poised, since there was a

slight gradual rise in the potential of the unevacuated control sample and the maximum potential reached in the unevacuated sample containing copper was definitely greater than those obtained in the first experiment reported in this paper.

Hydrogen gas was slowly bubbled through a sample of pasteurized milk for 15 minutes. Copper, at the rate of 2×10^{-5} moles per liter, was added to a portion of the hydrogen-treated milk and to a portion of similar milk that had not been treated with hydrogen. The samples were stored in glass milk bottles plugged with paper caps. Periodic measurements of the oxidation-reduction potential were made, the results of which are shown graphically in figure 4.

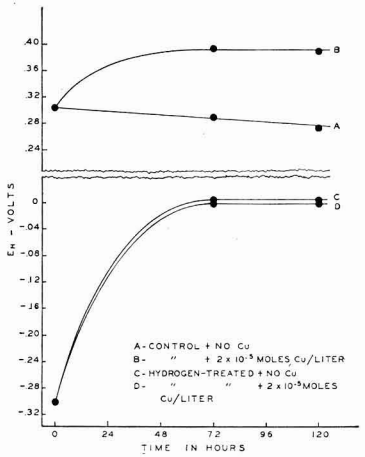

FIG. 4. Effect of bubbling hydrogen through milk on the oxidation-reduction potential.

The hydrogen caused a great decrease in the potential. Part of this decrease probably was due to the partial sweeping out of oxygen. The increase of the potential of the hydrogen-treated samples during storage probably was due to escape of hydrogen through the plug cap until a condition of equilibrium was reached.

INFLUENCE OF SYNTHETIC ASCORBIC ACIDS ON THE OXIDATION-REDUCTION POTENTIAL OF MILK

Synthetic crystalline levo-ascorbic acid was added to sub-samples of pasteurized milk at the rate of 0, 25, 50, 75 and 100 mgs. per liter. Each sub-sample was divided into two parts; part 1 received no copper and part 2 received 10^{-5} moles (0.64 ppm.) of copper per liter. The oxidation-reduction potentials of all samples were determined periodically during 72 hours storage. The change in potential with time is shown in figure 5.

The experiment was repeated, with the exception that the concentration of copper was 6×10^{-5} moles (3.84 ppm.) per liter and the storage period was only 48 hours. The results are shown in figure 6.

The addition of synthetic ascorbic acid caused a large decrease in oxidation-reduction potential, which is in agreement with the observation of

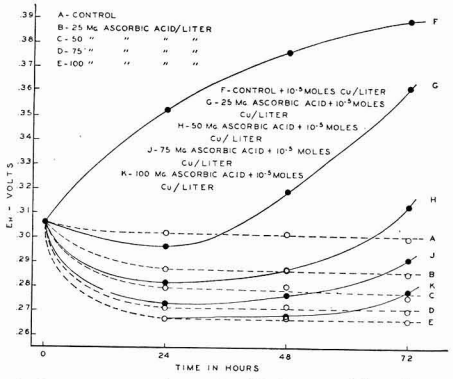

FIG. 5. Effect of the concentration of added crystalline levo-ascorbic acid and of copper (10^{-5} moles per liter) on the oxidation-reduction potential of milk.

Swanson and Sommer (5). The drop in potential increased with an increase in the amount of ascorbic acid added. When both copper and ascorbic acid were added the initial fall in potential, due to the added ascorbic acid, was followed by a rise. In these experiments, the rate of

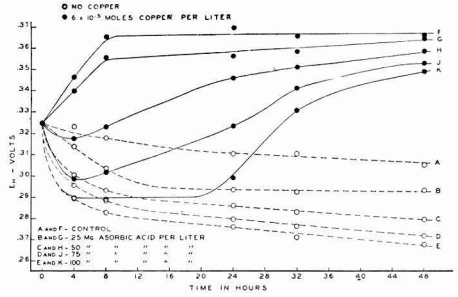

FIG. 6. Effect of the concentration of added crystalline levo-ascorbic acid and of copper (6×10^{-5} moles per liter) on the oxidation-reduction potential of milk.

increase in potential and its eventual magnitude were dependent upon the concentration of both the added copper ions and the added synthetic ascorbic acid. In no case did the potential of the samples containing both added copper and ascorbic acid reach as low a level as in the corresponding samples to which only ascorbic acid had been added. This is in direct

disagreement with the results of Swanson and Sommer (5), who found that the potentials were lower in samples containing both added copper and synthetic ascorbic acid than in the corresponding samples containing no copper. Swanson and Sommer's results, however, showed no decided increase in the samples containing added ascorbic acid to which copper was added, such as were obtained in the experiments reported here.

These experiments were repeated using synthetic dextro-isoascorbic acid instead of levo-ascorbic acid. The results were so nearly alike that it seems unnecessary to present them in detail. Since Swanson and Sommer's (5) results showed that dextro-isoascorbic acid caused a greater decrease in potential than an equivalent amount of levo-ascrobic acid, however, the graphs in figure 7 are presented to show that insignificant differences were obtained with the two ascorbic acids in our experiments.

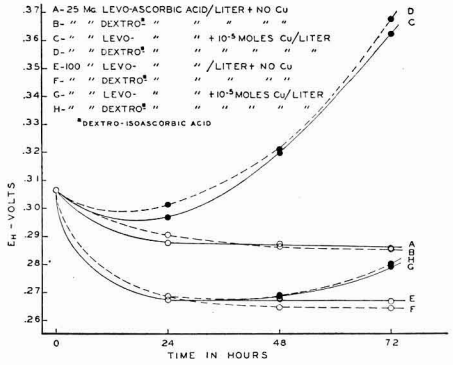

FIG. 7. Comparison of the effects of added levo-ascorbic acid and dextro-isoascorbic acid on the oxidation-reduction potential of milk.

SUMMARY

The effect of soluble metals, oxygen, hydrogen, and ascorbic acids on the oxidation-reduction potential of milk has been studied.

The addition of copper (copper sulfate solution) to milk caused an increase in the oxidation-reduction potential, the speed of increase and the magnitude reached depending upon the concentration of copper. With the higher concentration of copper the maximum potential was reached in a relatively short time, and this level was maintained throughout the storage period.

Milks to which ferrous iron was added had lower potentials at the end of 48 hours than at the beginning, but the ferrous iron maintained a potential slightly above that of the control sample. This observation is not in agreement with reports of other investigators.

Nickel sulfate and ferric, vanadium, aluminum, manganese, chromium,

and stannous chlorides, when added in concentrations of 10^{-5}, 4×10^{-5}, and 10^{-4} moles per liter of milk, did not alter significantly the potential from that of the control sample.

Partial removal of dissolved oxygen by evacuation lowered the potential of milk to which no copper was added and definitely limited the increase in potential of milk to which copper was added.

Bubbling hydrogen gas through milk greatly lowered the potential. On storage, however, the potential increased to an E_H of about 0, at which point equilibrium was apparently established. Addition of copper to the hydrogen-treated milk did not significantly affect the potential.

The addition of synthetic crystalline ascorbic acids greatly decreased the potential, the amount of decrease depending upon the concentration added. Addition of both soluble copper and synthetic ascorbic acids to the milks caused a subsequent rise in potential, the magnitude and rate of increase depending upon both the concentration of acid and the concentration of copper added. The milks containing the greater concentration of ascorbic showed a lesser rise in potential upon the addition of a given amount of copper. Very little difference was observed between the influence of levo-ascorbic acid or of dextro-isoascorbic acid on the potential either in the presence or the absence of added soluble copper.

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THE RELATION OF COPPER AND ASCORBIC ACID TO OXIDIZED FLAVOR IN MARKET MILK[1]

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INTRODUCTION

It has been shown repeatedly that copper induces the development of oxidized flavor in milk. Likewise, it has been demonstrated that copper contamination results in the destruction of ascorbic acid. Many data have been compiled to substantiate these findings (2).

Faulty or worn tinned copper equipment may cause the milk to be contaminated with copper. The role that such contamination plays during the processing and during the time until it reaches the consumers' refrigerator has not been fully understood. Literature records few data on the actual amount of copper and its relation to the ascorbic acid content of milk. Apparently, the lack of a suitable method for the accurate determination of minute amounts of copper has been a factor. Obviously, data on these factors should afford a better understanding of this catalytic action on the oxidized flavor defect in milk.

REVIEW OF LITERATURE

Swanson and Sommer (7) found that contamination of milk with 2 ppm. of copper caused the destruction of vitamin C in 5 to 14.5 hours. For the same milk not contaminated with copper the average loss of vitamin C during the holding time of 26 hours was 22.3 per cent.

Henderson and Roadhouse (5) found that when 0.1 ppm. cupric ion was added to milk from individual cows, 30 per cent of the ascorbic acid was oxidized in the pasteurized sample and 45 per cent was destroyed after one day storage.

Garrett (4) has shown that when 0.1 mole of copper sulphate was added per million parts of milk all of the ascorbic acid had been destroyed at the end of 24 hours while in the same milk without the addition of the copper salt only 30.9 per cent of the ascorbic acid was oxidized.

Sharp, Trout and Guthrie (6) found that the addition of 0.13 mg./l (0.13 ppm.) copper resulted in a reduction of the ascorbic acid of cold milk to 1.7 mg. per liter in three days.

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EXPERIMENTAL PROCEDURE

The method used for the determination of copper in milk was a modification of the authors' procedure recommended for butter (3). In determining the copper content in milk, the hydrolyzed sample was filtered while hot through a wetted quick-acting filter paper and then washed with hot glass-distilled water until there was no yellow color in the residue containing the butterfat. In the wet ashing digestion, after a greater portion of the organic matter was decomposed, 0.5 – 0.7 ml. of 72 per cent perchloric acid was added cautiously. The digestion was continued until colorless and a greater portion of the sulfuric acid was distilled off. Care was exercised to prevent complete dryness of the sample. The final volume should be about 5 ml., since this amount of sulphuric acid results in a more soluble calcium salt. The remainder of the procedure for the determination of copper in the milk was the same as recommended for butter (3).

The Bessey and King (1) titration method was used for the ascorbic acid determination. Fresh solution of 6 per cent metaphosphoric acid was used for flocculating the protein in the milk. The 2–6 dichlorobenzenone indophenol (purified) was standardized against Merck's "Cebione" (ascorbic acid). The "Cebione" was compared with iodine, and the latter standardized against sodium thiosulphate and this in turn against a standard solution of copper. The resulting dye value was determined for each set of titrations which involved both the pasteurized and the storage milk samples.

EXPERIMENTAL

The purpose of these experiments was to determine the rate of destruction of ascorbic acid and the amount of copper: first, in individual herd milk and mixed herd milk at various stages of processing and their effect on the oxidized flavor; second, in milk from individual cows throughout their lactation period, and third, in milk pasteurized in various metal containers.

Experiment 1. The milk for this experiment was secured from a local market-milk plant and the University dairy herd. The equipment in the milk plant was of modern design and consisted largely of stainless steel. Fittings such as valves and threaded connections were of bronze and nickel alloy respectively. Most of the minor installations were of tinned copper, and some of these showed scratches and worn surfaces resulting in a small amount of copper being exposed.

Samples of milk for each of the analyses were taken from the mixing vat, from the preheater, and from the bottler. These are designated as raw, preheated, and pasteurized milk. All samples were scored immediately for oxidized flavor and again at the end of a 24-hour storage period.

Experiment 2. The milk from two cows of each of the following breeds, Holstein, Jersey and Guernsey from the University Herd were used. The cows all freshened between December 20 and January 31. Milk samples

from each cow were obtained by hand milking into glass containers and were delivered to the laboratory for the immediate determination of ascorbic acid and copper. Samples were also held in a refrigerator at 40° F. for 24 hours, when the ascorbic acid was again determined. Samples were taken every 15 days throughout the lactation period.

Experiment 3. The samples of the plant milk and herd milk were pasteurized in various metal containers. For this purpose metal tubes, 1½ inches in diameter and 5–6 inches long, consisting of the following metals were used: (1) Stainless steel plus an alloy fitting, (2) tinned copper, (3) copper and (4) stainless steel. The stainless steel tube plus an alloy fitting consisted of a stainless steel tube to which was soldered a threaded union ferrule made of copper-nickel alloy and fitted with a cap of the same alloy. The tinned copper tube was badly worn and considerably scratched on the interior. The copper tube showed very little coating of tin on the inside. The stainless steel tube which had been used in a milk plant seemed in fair condition, free from tarnish or scratches. For the control a glass cylinder was used. A constant temperature water bath, electrically controlled and insulated with transit, was used for the pasteurization. To each of the various containers, holding about 150 ml. of milk, a slow rotating motor supporting a glass rod was fitted for stirring the milk sample while undergoing pasteurization. The temperature used was 142° F. for 30 minutes.

At the end of the pasteurization period each sample was immediately removed and the contents poured in duplicate glass bottles, cooled and placed in a refrigerator at a temperature of 40° F. Copper and ascorbic acid were determined in the samples immediately after pasteurization and the latter was again determined at the end of 24 hours. All the samples were tasted for oxidized flavor.

EXPERIMENTAL RESULTS

The results of Experiment 1 are summarized in table 1. The experiment was conducted over a period of one year in order that seasonal conditions for both milks could be observed. These results were averaged with respect to positive and negative oxidized flavor score. The individual herd milk developed an oxidized flavor in 15 of the 21 samples while 10 of the 23 samples of the mixed herd milk showed this defect. The oxidized flavor defect developed in most cases in the pasteurized samples held in storage for 24 hours.

All the raw milk samples contained from 0.21 – 0.24 ppm. of copper. This would seem to indicate that the amount of copper in raw milk was fairly constant. However, in considering the ascorbic acid averages, it is obvious that the many causes for its oxidation may result in considerable variation in ascorbic acid content before such milk reaches the plant. The effect of processing, especially when milk is pasteurized, showed definitely that the amount of copper has been increased in the milk samples. Samples

which developed an oxidized flavor had an increase of copper practically equal to that of the original copper content of the raw milk.

The data showing the per cent loss of ascorbic acid due to pasteurization revealed that there was a greater loss of ascorbic acid in the samples showing oxidized flavor. The total loss of ascorbic acid in the samples was also greater in the milk that developed the oxidized flavor than in those samples with no oxidized flavor. With conditions greatly reduced for oxidation due

TABLE 1

The influence of copper on ascorbic acid in milk at various processing stages and the development of oxidized flavor

Sample	Individual herd milk					
	No oxidized flavor*			With oxidized flavor†		
	Copper	Ascorbic acid per l.		Copper	Ascorbic acid per l.	
		Fresh	After 24 hrs. storage		Fresh	After 24 hrs. storage
	ppm.	*mg.*	*mg.*	*ppm.*	*mg.*	*mg.*
Raw	0.23	20.6	14.4	0.24	19.7	15.1
Preheated	0.28	17.4	12.3	0.30	16.7	11.7
Pasteurized	0.36	15.3	8.2	0.49	10.9	4.9
Per cent loss	.....	25.7	34.5	.....	44.7	30.4
Total loss	.....	.....	60.2	.....	.....	75.1
	Mixed herd milk					
	No oxidized flavor‡			With oxidized flavor§		
Raw	0.21	18.9	13.7	0.24	18.0	14.0
Preheated	0.27	15.9	10.5	0.29	15.6	9.8
Pasteurized	0.33	13.5	8.0	0.45	11.2	6.6
Per cent loss	.....	28.6	29.1	.....	37.8	25.5
Total loss	.....	.....	57.7	.....	.....	63.3

* Average of 6 samples.
† Average of 15 samples.
‡ Average of 13 samples.
§ Average of 10 samples.

largely to a lowering of temperature, the various increases of copper do not materially affect the total loss of ascorbic acid in the 24 hour storage period.

The analyses of copper and ascorbic acid in table 2 represent averages for each three months of the lactation period. Since metallic contact had been excluded, it was evident that the copper contents were indicative of the amount normally contained in the milk. The results showed that there was a higher copper content in the milk of all cows at the beginning of the lactation period. For the remainder of the lactation period the amount of copper secreted gradually decreased. The average for all cows showed very little variation in the amount of copper contained in the milk.

The average ascorbic acid, while showing considerable variation, nevertheless tended toward lower values at beginning of the lactation period. For the three breeds the milk from the Holstein cows showed lower ascorbic acid for all three periods of lactation, although the number of observations are too few to determine whether this is a true breed difference. In general, the averages for the 24-hour storage represented a fair approximation of the ascorbic acid contained in milk as it is received at the plant.

TABLE 2

The relation of copper and ascorbic acid in milk from individual cows during the loctation period

Cow No.	Breed	Copper in milk at different stages of lactation			Ascorbic acid per l. in milk when fresh and after 24 hrs. storage at different stages of lactation					
					When fresh			After 24 hrs. storage		
		1 to 3 mos.	4 to 6 mos.	7 to 9 mos.	1 to 3 mos.	4 to 6 mos.	7 to 9 mos.	1 to 3 mos.	4 to 6 mos.	7 to 9 mos.
		ppm.	*ppm.*	*ppm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
43A	Hols.	0.28	0.22	0.19	20.3	21.3	22.2	18.0	17.9	18.0
69A	Hols.	0.25	0.24	0.21	18.8	19.1	19.2	16.6	16.2	15.3
337	Jer.	0.29	0.25	0.20	21.3	21.8	23.2	18.3	18.8	20.5
338	Jer.	0.31	0.23	0.19	22.7	21.8	22.3	17.9	18.7	18.8
536	Guer.	0.32	0.23	0.16	20.6	22.7	21.4	18.0	18.3	19.2
541	Guer.	0.27	0.21	0.17	21.7	24.3	27.9	18.2	21.9	23.8
Average		0.29	0.23	0.19	20.9	21.8	22.7	17.8	18.6	19.3
Average per cent loss of ascorbic acid due to storage								14.7	13.9	15.1

The results of pasteurizing the herd milk and plant milk in contact with various metals are shown in table 3. From the results, it was evident that the various metal containers were the primary cause for the variation in copper, ascorbic acid and oxidized flavor in the milk. As expected, the copper container proved to be the greatest source of copper contamination for both samples and resulted in almost complete destruction of the ascorbic acid. This was also accompanied with a positive oxidized flavor in the milk at the end of pasteurization. The stainless steel with alloy fitting was similar in its effect on the milk, but with considerably less dissolved copper. Despite this decrease in copper content, the ascorbic acid was almost completely oxidized, and the milk had a positive oxidized flavor. The results for the tinned copper container were nearly the same as those obtained in Experiment 1. It is interesting to note, however, that the oxidation of ascorbic acid proceeded at a fairly uniform rate in both the pasteurized and storage samples. The results for the glass container were highly significant, in that, with no additional copper except that normally found in milk, the oxidation of the ascorbic acid was greatly retarded. This fact was evident in both the pasteurized and the storage samples. Here the total loss of

ascorbic acid was comparatively small, and corresponded favorably with the results for the raw milk samples when held in storage. The results obtained from pasteurizing milk in stainless steel metal compared favorably with that pasteurized in glass.

DISCUSSION

The contamination of milk with copper from the processing equipment had an adverse effect on its quality. The dissolving action of copper was found to increase with each succeeding stage of the processing. In the ex-

TABLE 3

The pasteurization of milk in contact with various metals and their effect on the copper, ascorbic acid and oxidized flavor in milk

Milk pasteurized in contact with	Copper content after past.	Loss of ascorbic acid in the milk					Intensity of oxidized flavor present after storage*	
		After pasteurization		After 24 hours storage		Per cent total loss		
		Mg. per l.	Per cent loss	Mg. per l.	Per cent loss		3 hrs.	24 hrs.
Milk used from one herd†								
	ppm.							
Tinned copper	0.54	12.7	36.7	3.6	44.7	81.4	–	–
Stainless steel with alloy fitting	1.60	6.6	67.3	1.3	26.0	93.3	–	+++
Copper	4.00	2.1	89.0	0.8	6.0	95.0	+	+++
Stainless steel	0.26	15.6	22.3	13.5	10.7	33.0	–	–
Glass	0.22	15.9	21.0	13.9	9.7	30.7	–	–
None	Raw	.....	.....	14.2	28.0	28.0	–	–
Milk used from milk plant‡								
Tinned copper	0.62	11.1	40.3	3.9	38.7	79.0	–	–
Stainless steel with alloy fitting	0.98	10.5	43.5	2.3	44.5	88.0	–	+++
Copper	3.00	1.6	91.0	0.5	5.0	96.0	++	+++
Stainless steel	0.28	13.9	25.2	12.2	9.3	34.5	–	–
Glass	0.25	14.2	23.8	12.6	8.4	32.2	–	–
None	Raw	.....	.....	13.5	27.4	27.4	–	–

* No oxidized flavor –; Slight oxidized flavor +; Oxidized flavor ++; Strong oxidized flavor +++.

† Raw herd milk contained 20.1 mg. per l. of ascorbic acid.

‡ Raw milk from milk plant contained 18.6 mg. per l. of ascorbic acid.

periment shown in table 1, the average amount of copper in the pasteurized samples varied from 0.33 ppm. for the milk not oxidized to 0.49 ppm. for the milk that developed an oxidized flavor. This variation in copper content was reflected in the increased destruction of ascorbic acid and was also accompanied with an oxidized flavor in the milk. This is in agreement with Sharp, Trout and Guthrie (6), since they found that the addition of 0.13 mg./l. (0.13 ppm.) of copper to the normal amount contained in milk produced an oxidized flavor.

The raw milk from individual cows was highest in the copper for the first three months of the lactation period. For the remainder of the lactation period there was a gradual decrease. The ascorbic acid content of the fresh milk from the individual cows studied varied from 18.8 mgs. to 27.9 mgs. per liter. There was an averaged loss of 14.6 per cent of the ascorbic acid for the storage period of 24 hours. The milk from the Holstein cows was the lowest in ascorbic acid, while the milk from the Jersey and Guernsey cows was found to be higher although with greater variation. Similar findings have been reported by Swanson and Sommer (7).

Dissolved copper in milk resulting from pasteurization in various metal containers was the highest for the copper container. This resulted in almost complete destruction of the ascorbic acid together with an intense oxidized flavor of the milk.

The milk pasteurized in stainless steel with the alloy fitting showed that the milk contained considerable dissolved copper and there was a high loss of ascorbic acid. The oxidized flavor developed at the end of the storage period. These results are in agreement with the findings of Henderson and Roadhouse (5).

When the milk was pasteurized in a tinned copper container, the copper and ascorbic acid values in the milk were substantially the same as those obtained in Experiment 1. However, oxidized flavor for both samples remained negative.

The stainless steel and the glass (control) containers used for pasteurizing the two milks yielded results that were in close agreement. The copper content of the milk was about the same as obtained from individual cows. Similarly the loss of ascorbic acid in the milk resulting from pasteurization as well as that for the storage period was uniformly low. The milk pasteurized in these containers showed no oxidized flavor defect. The total loss of ascorbic acid for both samples of pasteurized milk averaged 33.7 per cent for the stainless steel container and 31.5 per cent for the glass. For the holding period of the raw samples a loss of 27.7 per cent was obtained. It is evident from these trials that in the absence of copper contamination, pasteurization and storage of the milk caused little more destruction of the ascorbic acid than did the holding period of the raw samples.

SUMMARY AND CONCLUSIONS

The copper and ascorbic acid content of milk and their relation to the development of oxidized flavor in the milk were studied.

The increased temperature during the processing of the milk caused the greatest copper contamination.

The development of oxidized flavor in market milk was found to vary greatly even though the same equipment was used for the processing.

The amounts of copper and ascorbic acid in the milk from individual

cows varied inversely throughout the lactation period. The copper content was the highest at the beginning of the lactation period while the ascorbic acid was at a maximum at the end. The gradual decrease of the ascorbic acid in the milk did not affect the loss resulting from the 24-hour storage period.

The pasteurization of milk in the presence of different metals resulted in various degrees of copper contamination, oxidation of ascorbic acid and the development of oxidized flavor. The milk pasteurized in the copper tube had the highest copper content, lowest ascorbic acid and developed an oxidized flavor shortly after pasteurization. The milk pasteurized in the stainless steel tube with alloy fitting had the next highest copper content and almost as much reduction of ascorbic acid. The milk pasteurized in the tinned copper container had less copper contamination with no development of oxidized flavor and the ascorbic acid oxidized at a slower rate than either the milk pasteurized in the copper or stainless steel with the alloy fitting.

The loss of ascorbic acid due to pasteurizing the milk in stainless steel and glass was only slightly more than the same milk held for 24 hours without pasteurization.

These studies indicate that stainless steel or glass equipment used for the processing of milk will result in low copper and a relatively high ascorbic acid content of the milk.

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A STUDY OF THE GENUS MICROBACTERIUM

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Orla-Jensen (6) described seven strains of rod-shaped bacteria, occurring chiefly in dairy products, which manifested sufficient peculiar properties in common to be placed in a separate genus to which he gave the name *Microbacterium.* Since that time but little information has been added regarding these organisms, and their relationships to other more well-defined groups have remained questionable. In Bergey's Manual (2) this genus was placed in the family *Bacteriaceae,* a "heterogeneous collection of genera whose relationships to each other and to other groups are not clear."

The cultures of Orla-Jensen (6) were small rods, weak acid-formers in milk; they reduced nitrates to nitrites, and usually split hydrogen peroxide. He believed they constituted a genus entirely separate from the other lactic acid rod forms which were discussed; *i.e., Thermobacterium, Streptobacterium,* and *Betabacterium.* Three species, viz., *Mbm. lacticum, Mbm. flavum,* and *Mbm. mesentericum,* were definitely recognized and less definitely a fourth, *Mbm. liquefaciens.*

Robertson (7) described several thermoduric cultures from pasteurized milk and concluded that they were probably identical with *Microbacterium lactis,* (probably *Mbm. lacticum* of Orla-Jensen), although some of their properties were different from those described by Orla-Jensen for this species. These organisms were the most heat resistant of a number of non-spore-forming species isolated by him from pasteurized milk. He was not convinced that *Lactobacillus thermophilus* described by Ayers and Johnson (1) was not actually *Microbacterium lacticum.*

Wittern (9) considered the genus *Microbacterium* and the tetracocci as marking the boundary of the lactic acid bacteria on one side as do the coli-aerogenes group on the other. Wittern was able to isolate the three species originally described by Orla-Jensen, but as was true with Robertson's cultures, the properties of her organisms did not conform entirely with the properties described by Orla-Jensen. Wittern was particularly concerned with *Mbm. mesentericum* which showed numerous similarities to the *Actinomycetes* and the genus *Mycobacterium.* After a detailed comparison of the properties of these various groups, she concluded that *Mbm. mesentericum* showed decidedly more similarities to the genus *Mycobacterium* than to the lactic acid bacteria and should more rightfully be considered a member of that genus.

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Jensen (5) believed that the morphology of the microbacteria warranted their being considered as corynebacteria and mycobacteria. He designated *Mbm. lacticum* as *Corynebacterium lacticum, Mbm. flavum* as *Mycobacterium flavum,* and *Mbm. liquefaciens* as *Corynebacterium liquefaciens.* Jensen pointed out that physiological properties of *Mbm. lacticum* and *Mbm. flavum* are considerably different from those of the true corynebacteria and mycobacteria, but believed that the morphological similarities of these groups should receive precedence in classification studies.

The present study is an effort to define more clearly the genus *Microbacterium,* and to study the relationships of these organisms to other well-defined groups.

EXPERIMENTAL AND RESULTS

Isolation and Sources of Cultures Studied

Many of the organisms were first encountered as contributing materially to high thermoduric plate counts of milk. Subsequent studies showed that these organisms were common on milking equipment. Milk stone contained large numbers of organisms belonging to the genus *Microbacterium,* and this material may be considered the immediate source of those which appeared in milk in particularly large numbers. The *Microbacterium* cultures used in this study were isolated from the following sources: milk pasteurized in the laboratory at 161° F. for 16 seconds, 1 sample, 9 cultures; milk pasteurized in the laboratory at 145° F. for 30 minutes, 1 sample, 3 cultures; milk pasteurized in a dairy plant at 161° F. for 16 seconds, 1 sample, 4 cultures; raw milk as delivered by farmers to the dairy, 8 samples, 8 cultures; dairy farm strainer, 1 sample, 1 culture; milking machines, 3 samples, 14 cultures; milk stone on a milk pail, 1 sample, 8 cultures; cheddar cheese, 3 samples, 25 cultures.

Isolations from equipment were made by rinsing the particular piece with sterile skimmilk. A sample of the rinsing was pasteurized at 161° F. for 16 seconds and plated on tryptone-glucose-meat-extract-skimmilk agar. Raw milk arriving at the dairy was pasteurized and plated as were the rinsings of the equipment. The pasteurized samples were also plated and incubated in the same manner. Milk stone was removed from pails by a cotton swab moistened with sterile skimmilk. The swab was pressed out in 5 ml. of sterile skimmilk and the milk was then heated at 161° F. for 2 minutes. Plates were poured with tryptone-glucose-meat-extract-skimmilk agar and incubated at 30° C. for 3 days.

Isolations from cheese samples were made by triturating 1 g. of ripe cheddar cheese and 0.1 g. of sodium citrate in about 2 ml. of sterile water. Finally 8 ml. more water were added and mixed with the dissolved cheese. This solution was then heated at 161° F. for 2 minutes and plated with tryptone-glucose-meat-extract-skimmilk agar and incubated at 30° C. for 3 days.

In the majority of the samples it was found that 3 days at 30° C. should be allowed for the development of *Microbacterium* colonies. The colonies may not be visible or they may be easily overlooked after only 2 days' incubation, specially at 37° C. For this reason the microbacteria may be overlooked when samples are incubated for this time and temperature in routine milk plate counts.

In the present investigation 48 cultures representing all of the sources listed previously were studied. In addition, two cultures, Nos. 8180 and 8181, which were received from the American Type Culture Collection, Washington, D. C., were studied. These cultures were believed to represent two original cultures of Orla-Jensen.

After a culture was purified it was carried in litmus milk, or on agar slants of the following composition: Bacto proteose-peptone 0.5 g.; beef extract 0.30 g.; glucose 0.1 g.; K_2HPO_4 0.4 g.; KH_2PO_4 0.125 g.; distilled H_2O 100 ml.; agar 1.5 g.; final pH approximately 7.0. A clear broth which supported good growth of the microbacteria could be obtained by omitting the agar from the formula of the foregoing agar medium.

Morphology

The morphology of the microbacteria has been described extensively by Orla-Jensen (6), Wittern, and Jensen, but several further observations have been made in the present investigation. The appearance of *Microbacterium* organisms in milk when stained by the Newman-Lambert stain may often be misleading as to their actual morphology. In such preparations the irregular staining of the cells and their frequent grouping often make them appear as small cocci in groups. But when grown on agar slants and stained by the Gram method the cells usually stained evenly and were gram positive with the exception of one culture, the cells having characteristic angular and palisade arrangements. Occasionally some cells would show a darker polar staining giving the appearance of short-chained streptococci. When grown on tryptone-glucose-skimmilk-meat agar for 3 days at 30° C. and stained by the Gram stain, the individual cells measured 0.4–0.5 × 0.6–1.0 μ, the average being about 0.4 × 0.8 μ.

None of the cultures was found to form spores and all were non-motile.

The colonies on standard tryptone-glucose-skimmilk-meat-extract agar were very small and smooth, the surface colonies round, and the sub-surface ones lens-shaped. After 7 days' incubation at 30° C. on this medium, the colonies averaged about 0.7 mm. in diameter, although individually they varied from 0.2 mm. to 1.0 mm. Frequently the colonies would be hardly more than visible after 3 days at 30° C. on this medium.

Oxygen Relationships

Orla-Jensen (6) observed that the microbacteria were aerobes and were able to split hydrogen peroxide. Wittern (9) found that all cultures of

the genus *Microbacterium* which she tested were able to grow in broth when the atmosphere was reduced to 14–15 mm. mercury pressure. In a study of the rod-shaped lactic acid bacteria, Hansen (4) found that *Mbm. lacticum* and *Mbm. flavum* utilized oxygen much more rapidly in the presence of glucose or lactate than did organisms of the *Thermobacterium, Streptobacterium,* and *Betabacterium* genera. The strong inhibiting effect of hydrocyanic acid on the *Microbacterium* cultures indicated that they differed from other lactic acid rods not only by having catalase but in their hemin content.

All of the microbacteria in this study were characteristic aerobes as they grew well on agar slants and produced catalase. The ability of the organisms to grow in anaerobic conditions was determined by inoculating the cultures into tubes of the proteose-peptone-broth medium which had been heated in the steamer for 20 minutes just prior to inoculation to aid in eliminating dissolved oxygen from the broth. The cultures were then incubated in a vacuum oven and the temperature held at 32° C. Air was evacuated from the oven and replaced with CO_2. Evacuation and replacement with CO_2 was repeated several times and finally the oven was filled with CO_2 and the cultures were incubated for 5 days. The cultures grew under these conditions, although the growth was not so abundant as under aerobic conditions, which indicated that the microbacteria are facultatively anaerobic.

Acid Produced in Milk

Although many of the microbacteria were relatively weak acid formers in milk, a number of the cultures formed sufficient acid in 7 days at 30° C. to produce a typical acid curd in the milk. It remained to learn to what extent lactic acid was responsible for the acidity, since it was only assumed previously that it was lactic acid, as based on Orla-Jensen's observation that d-lactic acid was usually formed.

Eighteen representative cultures were used to study the amount of lactic acid produced in milk. The cultures were inoculated from a 3 day litmus milk culture into 75 ml. of sterile skimmilk and incubated at 30° C. usually for 7 days. The initial acidity and total acidity were determined by titrating a 9 g. sample with N/10 NaOH using 3 drops of 1 per cent phenolphthalein (in 50 per cent alcohol) as indicator. The lactic acid was determined by the method of Troy and Sharp (8) in which a 25 g. sample was used for assays in this investigation. This method is particularly adapted for determining lactic acid in milk. The procedure consists of the precipitation of interfering substances by copper hydroxide at 45° C., direct oxidation of the filtrate with potassium permanganate after acidifying with a sulfuric acid-manganese sulfate mixture, distillation of the acetaldehyde into sulfite solution, and titration of the bound sulfite with iodine.

The results of these analyses showed that the acid formed by most of the cultures was predominantly lactic acid (Table 1). Culture 342S1,

TABLE 1

Acid production of Microbacterium cultures in skimmilk

Culture	Total acidity after growth	Developed acidity	Lactic acid	Lactic acid in developed acidity	Condition of milk
	per cent	*per cent*	*per cent*	*per cent*	
78–1*	0.64	0.43	0.33	82.2	Curdled
F6	0.56	0.35	0.26	74.3	do
S1*	0.48	0.27	0.25	93.4	Slight curd
H3*	0.42	0.21	0.19	92.2	do
S7	0.50	0.30	0.27	83.1	do
30M1	0.51	0.31	0.23	74.5	do
S4	0.46	0.26	0.23	87.3	do
F3	0.31	0.11	0.113	102.5	Not curdled
M1	0.56	0.36	0.25	70.7	Curdled
S3	0.47	0.27	0.21	78.4	Slight curd
S8	0.48	0.30	0.24	81.1	Curdled
D1–1	0.28	0.10	0.058	58.7	Not curdled
H1	0.48	0.30	0.21	71.1	Curdled
8180	0.31	0.12	0.118	98.3	Not curdled
CC10	0.45	0.26	0.225	86.7	Slight curd
M2	0.51	0.32	0.259	80.8	Curdled
D1–3†	0.40	0.22	0.159	72.3	Slight curd
342S1†	0.24	0.06	0.0239	39.8	Not curdled

* Incubated 12 days at 30° C.
† Incubated 14 days at 30° C.

which showed very weak acid production and produced but little lactic acid, possessed other properties which were different from most of the cultures studied and will be discussed later. The percentage of the acid as lactic acid, produced by these cultures in milk, is similar to that found by Troy and Sharp (8) to be produced by *Lactobacillus bulgaricus* and *Leuconostoc paracitrovorus* when grown in milk. It would seem that the microbacteria should be considered as lactic acid bacteria as regards the kind of acid produced.

Thermal Resistance

Orla-Jensen (6) and Wittern (9) used the unusually high termal resistance of the microbacteria in isolating these organisms from various products. Robertson (7) has also noted this property in the *Microbacterium* cultures which he isolated from pasteurized milk. In the present study the organisms were first encountered as contributing to high counts of pasteurized milk, and it became obvious that the high heat-resistance was one of the most outstanding characteristics of the microbacteria. It seemed desirable to determine the thermal resistance of a number of these cultures and ascertain whether they could be eliminated from a product by any reasonable heat treatment.

Ten representative cultures were selected and grown in sterile litmus milk for 4 days at 30° C. Tubes containing 5 ml. of sterile litmus milk at room temperature were inoculated with one drop of the 4-day culture immediately before being subjected to each heat treatment. The tubes were then immersed in a water-bath with the water level about 2–3 inches above that of the milk. The tubes were shaken continuously during the time required to reach temperature, and less frequently during the holding period. One and one-half minutes were required to reach 62.5° C. and approximately 2 minutes to reach 71.6° C. and 85° C.; the holding period was begun only after the tubes reached the proper temperature. After heating the tubes were cooled quickly to about 30° C. and incubated at 30° C. to determine the viability of the cultures.

All of the cultures withstood 62.5° C. for 30 minutes and all but one (342S1) survived 71.6° C. for 10 and 30 minutes. These results indicate that the microbacteria would survive in milk pasteurized by the usual holder-process or by the high-temperature-short-hold process, as was found to be true during the earlier part of this investigation, when a number of the cultures were first isolated. All but culture 342S1 withstood 85° C. for 2½ minutes, and seven survived heating to 85° C. for 10 minutes, while none survived 85° C. for 30 minutes. In a separate experiment, two of the cultures remained viable in skimmilk for approximately 12 hours at 62.5° C., although no multiplication occurred during this time. Like the true lactic acid bacteria, the microbacteria have been found to be non-pathogenic (Wittern (9)) and their occurrence in milk and dairy products should be no public health menace.

Production of CO_2

The microbacteria do not produce sufficient gas when grown in carbohydrate media to be detected by visual inspection. Since it is recognized that the amount of carbon dioxide production is of considerable value in characterizing the propionic acid bacteria and certain of the lactobacilli, it seemed desirable to measure the CO_2 produced by the microbacteria.

The amount of CO_2 produced by 15 typical *Microbacterium* cultures was determined in improvised Eldredge tubes. Into one tube was placed 20 ml. of broth containing: glucose, 1 per cent; yeast extract, 1 per cent; proteose-peptone (Difco), 0.5 per cent; K_2HPO_4, 0.4 per cent; KH_2PO_4, 0.1 per cent; the pH was adjusted to 7.0. To the other tube was added 25 ml. N/10 $Ba(OH)_2$ to absorb the CO_2 produced. In another series of tubes skimmilk was substituted for the glucose broth.

The tubes were incubated at 30° C. for 7 days in a slanting position to give a maximum absorption surface to the $Ba(OH)_2$. The amount of $Ba(OH)_2$ converted into $BaCO_3$ by the CO_2 produced was determined by titrating the residual $Ba(OH)_2$ with N/10 HCl (Table 2).

The microbacteria produced relatively small amounts of CO_2 from both the milk and glucose broth, although the volume produced from the glucose broth was slightly larger than that from the milk. Owing to the small amount of CO_2 produced, further studies on the relationship of sugar fermented and CO_2 produced were considered unnecessary. The fact that the cultures were grown under a limited oxygen supply probably accounted in some measure for the limited CO_2 production, although the growth in all the cultures was abundant. For comparative purposes, however, it can be concluded that the microbacteria, with a limited oxygen supply, produce markedly less CO_2 than do the propionic acid bacteria.

TABLE 2

Carbon-dioxide production by Microbacterium cultures

Culture	Gm. CO_2 per 20 ml. medium	
	Skimmilk	Glucose broth
8180	0.002	0.010
H3	0.002	0.013
342S1	0.010	0.019
CC10	0.004	0.011
S4	0.003	0.012
78–1	0.008	0.011
F6	0.013	0.010
30M1	0.010	0.010
Ca1	0.010	0.006
F6	0.011	0.006
S8	0.004	0.005
D1–3	0.007	0.009
M2	0.009	0.010
S3	0.006	0.006
Cb1	0.010	0.011

General Biochemical Characteristics

Litmus milk. All of the cultures produced an acid reaction in litmus milk, although several cultures were decidedly weaker acid producers than were the majority. Eighteen cultures produced sufficient acid in 7 days at 30° C. to curdle the milk, a typical acid curd being produced. A number of the cultures were found to curdle the milk after prolonged laboratory cultivation in milk, whereas this property was lacking when the culture was freshly isolated. Litmus milk was a very satisfactory medium for carrying the cultures, as they usually remained viable for several months in this medium when stored in the refrigerator.

Action on gelatin. The organisms were grown in the proteose-peptone medium to which was added 4 per cent Bacto-Gelatin. None of the cultures liquefied the gelatine after incubation at 30° C. for about 14 days.

Catalase production. The cultures were grown in the proteose-peptone medium for 7 days at 30° C. After this period 3 ml. of 1 per cent H_2O_2 was added to each tube. All of the cultures produced catalase, usually a

considerable amount. Catalase was formed in much larger amounts in this medium than in sterile skimmilk.

Nitrate reduction. There are conflicting reports describing the ability of the microbacteria to reduce nitrate. Orla-Jensen (6), Jensen (5), and Bergey's Manual (2) describe the organisms as able to reduce nitrate to nitrite, while Robertson (7) found that his cultures usually failed to reduce nitrates. In the present investigation the cultures were grown in broth containing 0.5 per cent Bacto-proteose-peptone, 0.3 per cent meat extract, and 0.1 per cent KNO_3 (pH adjusted to 7.0–7.2) for 10–14 days at 30° C. Forty-four of the cultures failed to reduce nitrate, while five were able to reduce it, indicating that, in general, the microbacteria are unable to reduce nitrate.

Starch hydrolysis. The organisms were streaked onto starch agar plates, the medium containing 0.5 per cent Bacto-proteose-peptone, 0.3 per cent meat extract, 0.2 per cent soluble starch, and 1.5 per cent agar (pH adjusted to 7.0–7.2). After 10–14 days at 30° C., 48 of the cultures had hydrolyzed the starch, while one culture failed to attack it. This culture, 342S1, also possessed other peculiar characteristics which will be mentioned later. These results are in accord with those of Dull (3), who isolated, from the intestines of adult persons, cultures of *Mbm. lactis* which produced diastase.

Fermentation of sugars. The sugars which have been described by others as valuable for distinguishing between species of microbacteria were used in this work (*i.e.*, glucose, maltose, and raffinose). The media used contained 0.5 per cent proteose-peptone, 0.3 per cent meat extract, brom thymol blue, and the pH adjusted to 7.0–7.2. This was tubed and sterilized, and then sufficient 10 per cent sterile sugar solution (sterilized by filtration through a Seitz filter) was added to give approximately 1 per cent concentration of the sugar. Prior to inoculation, the tubes were incubated for 24 hours to ascertain their sterility. All of the cultures produced acid from glucose and maltose; all failed to ferment raffinose except culture 342S1.

With the exception of its ability to ferment raffinose, culture 342S1 possessed the properties of *Mbm. flavum.* The key to the species of the genus *Microbacterium* in Bergey's Manual (Bergey *et al.* (2)) designates the failure of *Mbm. flavum* to ferment maltose as an important identifying characteristic of this species. In the present investigation the *Mbm. flavum* cultures was found to ferment maltose. Likewise, Wittern (9) found 13 out of 21 cultures of *Mbm. flavum* fermented maltose. These data indicate that the inability to ferment maltose is not a constant characterisic of *Mbm. flavum* and should not be used as an identifying characteristic of this species.

DISCUSSION

The microbacteria form a well-defined group of bacteria. They have some properties in common with the genus *Propionibacterium,* but differ

mainly in that they produce chiefly lactic acid, are more aerobic, and produce relatively little carbon-dioxide from carbohydrates in an atmosphere containing a limited oxygen supply. This is the genus to which the microbacteria show the closest relationship, both morphologically and physiologically. Differing chiefly from the lactobacilli by producing catalase, the microbacteria possess some similarities to this group, such as producing chiefly lactic acid in milk, being non-proteolytic, gram positive, and nonmotile, and usually failing to reduce nitrates to nitrites. Members of the genus *Microbacterium* are found in human and animal feces, milk, dairy products, and dairy farm equipment, along with the true lactic acid bacteria. Morphologically, the microbacteria show similarities to organisms of the genus *Corynebacterium,* particularly the "diphtheroids," but differ greatly from them physiologically. To place the microbacteria in the genera *Corynebacterium* and *Mycobacterium,* on the basis of morphology as proposed by Jensen (5), would undoubtedly prove confusing. The characteristics shown by the microbacteria in the present investigation seem to support the view that the genus *Microbacterium* shows close relationships to the genera *Propionibacterium* and *Lactobacillus,* and should be placed close to these genera in a system of classification.

The genus *Microbacterium* seems to contain two well-defined species; viz., *Mbm. lacticum* and *Mbm. flavum. Mbm. lacticum* forms much more acid in milk, chiefly lactic acid, does not ferment raffinose, hydrolyzes starch, usually does not reduce nitrate to nitrite, and forms no distinct pigment on agar. *Mbm. flavum* (culture 342S1 in this study) is somewhat larger in size (approximately $0.5 \times 1-2\ \mu$), forms relatively small amounts of acid in milk, of which lactic acid forms a smaller part, fails to hydrolyze starch, reduces nitrate to nitrite, forms a yellow pigment on agar, and is less heat resistant than *Mbm. lacticum.* Although Orla-Jensen (6) included in the genus *Mbm. mesentericum,* Wittern (9) demonstrated that this organism by its morphology, its ability to use petroleum as a carbon source, and by other differences from the microbacteria, should be allocated more properly to the genus *Mycobacterium.* No culture of this organism was encountered in the present investigation.

The distinct differences between *Lactobacillus thermophilus* and any members of the genus *Microbacterium* should be emphasized. Robertson (7) suggested that this organism was possibly the same species as *Mbm. lacticum. L. thermophilus,* however, does not form catalase, is a true thermophile in that it grows best from 55° C. to 63° C., has a lower thermal death time (*i.e.,* killed immediately when heated to 82.2° C.) and is distinctly different morphologically (cells measure $0.5–1.0 \times 2.5–6\ \mu$) from *Mbm. lacticum.* From the data available it would appear that *L. thermophilus* is, as proposed by Ayers and Johnson (1), a true lactobacillus, and that its properties separate it entirely from the genus *Microbacterium.*

One of the outstanding characteristics of the *Microbacterium* cultures is their high heat resistance. The organisms evidently have their origin primarily in the intestines and feces of animals (see Orla-Jensen (6), Dull (3), and Wittern (9)) and gain entrance into milk by means of dairy equipment. Their ability to withstand high temperature enables them to remain on dairy farm equipment between milkings if the equipment is not thoroughly cleaned with water above 200° F., or if it is not chemically sterilized. In this manner they can contribute materially to high thermoduric plate counts. Once the organisms have gained entrance into the milk, the usual pasteurization temperatures for market milk and cooking temperatures for cheeses permit them to survive in these products.

CONCLUSIONS

Organisms belonging to the genus *Microbacterium* may be characterized as short, gram positive, non-motile, non-sporulating, diptheroid-like rods which form catalase, produce predominantly lactic acid in milk, fail to liquefy gelatine, usually fail to produce nitrite from nitrate, and are very thermoduric.

Two well-defined species, *Microbacterium lacticum* and *Microbacterium flavum* are recognized in this genus.

The microbacteria gain entrance into milk chiefly by means of dairy farm milking equipment. The high heat resistance of these organisms enables them to occur in pasteurized milk and milk products. The metabolism of the microbacteria suggests that their role in these products is very similar to that of the true lactic acid bacteria. Owing to their presence in dairy products in smaller numbers, their activity probably represents only a small part of the microbiological activity occurring in these products.

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DRY MATTER DETERMINATION IN GREEN PLANT MATERIAL AND IN SILAGE[1]

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Losses of 10 to 20 per cent or more of the dry matter of the ensiled crop, exclusive of visible spoilage at the top or elsewhere, have commonly been reported by investigators of silage problems. Losses of such magnitude have been difficult to explain on the basis of known chemical changes occurring in the ensiled material. They have also been difficult to harmonize with the observed efficiency of silage as a feed for livestock.

A certain amount of loss is encountered in any method of handling or storing crops, and the various methods of crop storage may be considered as more or less in competition as to which can show the smallest loss. It is, therefore, important that a method of crop storage which is as convenient and satisfactory in most respects as silage-making should not be charged with large losses due to hidden errors and faulty methods of analysis.

LITERATURE AND DISCUSSION

Good reviews of the earlier literature regarding ensiling losses are given in other readily available publications and need not be repeated here. Shaw *et al.* (10) review more particularly the early American work, while Watson and Ferguson (12) give a fuller review of the British and European work. Newlander *et al.* (6) include a good review of modern work. Two references (5 and 13) are included because they seem typical of the earlier work at American experiment stations.

Various conditions tend to complicate such silage studies. Farm silos or experimental silos of comparable size are rarely emptied at one time. Removal of the silage goes on over a feeding period of several months; hence it is seldom practical to make direct comparisons of the weights of crop and silage. Representative sampling is also complicated by the slow removal and by the rather extensive movement of soluble nutrients within the silo (7, 8, 10, 16). Many studies, particularly those of British or European origin, are complicated by considerable losses of liquids or juice from the silos (10, 12, 15) due to the ensiling of excessively wet or immature crops or failure to provide roofs for the silos.

When a direct comparison of weights is attempted by totaling the weights of silage as removed for feeding, the total weight so obtained is always much less than the weight of the crop ensiled. A considerable but unknown pro-

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[1] Some of this material was presented in different form before the Division of Agricultural and Food Chemistry of the American Chemical Society at Buffalo, N. Y., Sept. 10, 1942.

portion of this apparent loss of weight is doubtless due to the continuous exposure of a moist surface of silage to evaporation during the entire feeding period. This explanation is supported by work at the Missouri Agricultural Experiment Station on small silos (4, 9) from which the entire contents have been removed and weighed at one time and the complication of top spoilage also avoided. A portion of these results is shown in table 1. The weight losses are considerably smaller than those commonly reported.

TABLE 1

Loss of dry matter in the silo
(Ragsdale and Turner*)

Crop	Number of silos	Dry matter loss, average per cent
Normal corn	20	4.01
Shock corn plus water	13	9.95
Oats and peas	4	6.90
Grass crops	6	18.06
Legume crops	9	2.12

* Missouri Agricultural Experiment Station Research Bulletin 65.

Unpublished small-scale silage experiments at the Ohio Agricultural Experiment Station have also shown weight losses which are impressively small in comparison with those which are commonly reported. Some of these are shown in table 2. Most attempts to study the loss of dry matter as distinct from the total loss of weight have likewise indicated losses much greater than could be explained on the basis of changes known to occur in

TABLE 2

Percentage fermentation loss (including some evaporation) of various crops in silo presses

Crop	Number of silos studied	Percentage loss	
		Range	Average
Soybeans	5	3.32 5.12	4.20
Red clover	5	3.27 4.92	3.99
Alfalfa	4	3.04 5.85	4.27
Alsike	2	3.20 4.12	3.66
Timothy	2	3.45 3.72	3.58
Lawn clippings	4	2.37 5.53	4.45
Green oats	3	4.30 4.95	4.71
Sweet corn stover	6	2.95 5.70	4.08
Corn	18	1.66 7.10	4.22

ensiled crops. The method of dry matter determination almost universally followed in such studies has been to dry weighed portions of the silage to constant weight at about the boiling temperature of water. The dried residue has been considered as the dry matter and the weight lost on drying as water. It has long been known that silage contains volatile materials other than water, but this fact does not seem to have been taken into account fully

in most silage studies, though some workers have introduced various corrections designed to overcome this difficulty (8, 11, 13, 14).

A considerable increase in the apparent water content of many experimental silages over that of the corresponding crop was frequently observed in previous work. The determinations were made by the oven-drying method, and often no addition of water from outside sources was possible. The writer was led by the frequent recurrence of such results to question the reliability of the customary method of water- and dry-matter determinations as applied to silage.

EXPERIMENTAL

Accordingly the usual method of water determination in which the loss of weight on drying is considered to be water was compared with a newer but also officially approved method in which the water is recovered as such and measured volumetrically (1, 2, 3). This is accomplished by the distillation of weighed samples of the material with an excess of a volatile solvent such as toluene B.P. 110. Xylene has also been similarly used (3). The water of the sample is vaporized and the vapors carried to the reflux condenser in mixture with the toluene vapors. On condensation the liquid phases of these materials separate promptly and completely. Water being heavier is collected in an appropriate trap while the toluene automatically returns to the distillation flask. In this work the form of trap introduced by Dean and Stark (3) was employed. At first the volume of water was read directly in the traps. Later it was transferred to a graduated tube of small bore to secure greater precision of reading. Methods of this type are usually somewhat lacking in precision as compared with gravimetric methods. The results however are at least not confused by considering as water other volatile materials which may be driven off during the drying or distillation. Added water is 100 per cent recovered by this method.

The two methods were compared on several green crops which are frequently used in silage making; also on a series of silages made from the same or similar crops. The materials to be compared were finely chopped in the Hobart food chopper whereby a fine state of subdivision was obtained even on wet materials without any separation or loss of liquid. Triplicate 10-gram samples were weighed into previously dried and weighed porcelain dishes. These samples were then dried in an electrically heated oven maintained at 100° C. for several hours to approximately constant weight. Three other 10-gram samples were transferred to 200-ml. Erlenmeyer flasks and covered with about 100-ml. of toluene. They were then ready for distillation as previously described. Boiling was continued on a hot plate for some time after all the water appeared to have been removed from the sample. The results of this work are shown in tables 3 and 4.

DISCUSSION

On the green silage crops (table 3), results by the two methods were identical within the limits of precision attainable by these methods. This finding tends to show the correctness and reliability of both methods for

TABLE 3

Water determinations by oven-drying and by toluene distillation
Green plant material

Crop	Per cent of water				Dry matter	
	Oven-drying (1)	Distillation with toluene (2)			(1)	(2)
Green corn	80.8	81	81	.....	19.2	19.0
Alfalfa	74.9	75	75	75	25.1	25.0
Timothy	64.7	65	65	64	35.3	35.0
	75.0	75	75	75	25.0	25.0
Bluegrass	65.3	65	65	.....	34.8	35.0
	72.0	72	72	.....	28.0	28.0
Orchard grass	76.8	76	76	.....	23.2	24.0
	76.5	76	77	.....	23.5	23.5

fresh green plant material which does not contain large amounts of volatile substances other than water. Not all green plant materials are of this type, however. In table 5 are compared results obtained by both these methods on several green materials which because of their strong odor were judged

TABLE 4

Water determination in silage by oven-drying and by toluene distillation

Kind of silage	Water as determined by	
	Loss of weight on oven-drying	Direct measurement after distillation
	Per cent	*Per cent*
Corn silage (1)	71.8	69.7
" " (2)	71.1	70.0
" " (3)	72.9	71.0
Mixed legume, untreated	73.6	71.8
" " + corn (1)	72.2	70.0
" " + " (2)	71.1	70.0
Alfalfa, dry, fine cut	56.7	56.0
Alfalfa, wet, " "	72.7	70.0
Timothy (1)	77.7	76.0
" (2)	78.3	75.7
" (3)	77.2	75.0

to possess a high content of volatile matter. The results, while variable show marked differences between results by the two methods in some cases. In the case of silage, as shown in table 4, the apparent amount of water was always greater by the method of oven-drying than by the toluene distillation procedure.

The explanation is, of course, that the volatile materials other than water which are formed in the process of silage-making are driven off along with the water in the drying process and are thus included with the water in calculating the results by this method. In the toluene distillation process, on the other hand, the water is recovered as such and its amount volumetrically determined. The other volatile materials in this case are included with the dry matter if the same method of calculation, that of subtracting water percentage from 100, is followed. It may be suggested at this point that the simultaneous application of these two methods of dry matter determination will practically amount to a determination of the amount of volatile products produced in the silage under study.

TABLE 5

Water determination indirectly by drying and by direct measurement after distillation

Material (leaves of the following plants)	Per cent water by	
	Indirect method, oven-drying	Direct measurement after distillation
Peach	63.82	62.8
Cedar	59.41	59.2
Wild cherry	58.15	56.5
Hardy chrysanthemum	78.63	76.0
Spearmint	80.17	77.5

Since some of the volatile materials in question are known to contribute to the feeding value of the silage and others of them may be reasonably assumed to do so, it seems logical that they belong with the dry matter rather than with the water. On this basis, there can be little doubt that the toluene distillation method is more reliable than oven drying for making water and dry matter determinations on materials such as silage. The amount of difference in dry matter between these two methods of determination, or the amount of volatile matter other than water occurring in the silage, is not the same for all the kinds of silage studied; but ranges from 0.7 per cent to 2.7 per cent on the wet or fresh basis. If the observed differences are figured as percentages of the dry matter itself rather than of the wet silage, these figures become much more impressive; they then range from about 3 per cent to a maximum of more than 10 per cent of the total dry matter of the silage.

There is nothing to indicate that these values represent the extremes that would be found in studying larger numbers of silages. The fact that wide variations in this respect exist among the silages studied would indicate that different amounts of volatile matter are found in silage, depending on the crop ensiled and the conditions under which the silage is made. Numerous suggestions of this kind may be found in the literature but sufficient exact information is lacking, however, to justify much in the way of detailed statements regarding the influence of specific factors in controlling silage quality.

It has been suggested that some of the volatile fatty acids of the silage would probably be present in the water distilled from the sample by the toluene procedure and that they would increase the volume of this distillate to a noteworthy extent. This possibility was checked as follows:

Ten-ml. portions of the distillate from a series of six silage samples were titrated with 0.2 N NaOH solution to the end point of phenolphthalein. Titrations of 7.5, 6.8, 6.7, 5.5, 5.0, and 4.3 ml. were obtained. Assuming that the volatile acid is principally acetic the 6–8 ml. of distillate recovered in the usual determination reported in the study would contain at most, $0.8 \times 0.012 \times 7.5$ g. = .072 g. of acid or about the same number of ml. This amount is less than the smallest division on the apparatus, but should be detectable.

The distillates from duplicate samples treated with an excess of magnesium oxide before distillation were alkaline to phenolphthalein and of perceptably smaller volume to about the extent of the calculated volume of acid.

Instead of explaining the difference in results between the two methods under discussion, however, correction of the volume of distillate for the amount of acid it contains, only serves to intensify the difference in results between distillation with toluene and oven drying as shown earlier in this work.

SUMMARY

Oven-drying has been the method of water determination generally employed in silage studies. The large losses of dry matter usually reported in such studies have often been partly unexplainable though the issue has been confused by juice and evaporation losses and sampling difficulties.

The presence of different volatile materials in silage has long been known, but has failed to gain appropriate recognition in the methods of analysis commonly employed. The present study compares the usual indirect method of water determination by oven drying with a direct method in which the water is recovered and determined volumetrically. On the usual green silage crops, results by the two methods are in good agreement; but on silage the results are often widely divergent. This is explainable on the grounds that in drying the volatile matter other than water of the silage is included with the water; while in the direct or distillation method this nonaqueous volatile matter is included with the dry matter where it seemingly belongs.

Simultaneous application of both these methods to silage analysis should give a reliable measure of the volatile products in the silage. These are doubtless affected both as to character and amount by the kind of crop and the conditions under which the silage is made, and probably have a definite relationship to the quality of the silage. Details in this line, however, are at present unknown.

General use of the drying method of water determination has resulted in overestimating the dry matter losses of silage-making and in underesti-

mating the feeding value of silage as determined by analysis. Widespread application of a direct method, such as the one described, to silage studies may call for a review of existing figures regarding the average composition and comparative feeding value of silage.

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ASCORBIC ACID STIMULATION IN THE BLOOD PLASMA OF DAIRY CATTLE PRODUCED BY THE INGESTION OF CHLOROBUTANOL*

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Some of the experimental work reported in this paper was initiated originally for the purpose of determining the variations that could be produced in the blood plasma ascorbic acid level of dairy cattle by feeding certain organic compounds. Recent investigations, however, have shown that the level of ascorbic acid in the blood plasma is closely associated with fertility in dairy cattle. The results obtained by Phillips *et al.* (11, 12) with ascorbic acidotherapy on sterile bulls and "hard to settle" cows indicate that more knowledge of the factors affecting the ascorbic acid metabolism of dairy cattle is of major importance. The results presented in this paper are of additional interest in this connection because of the lack of information in the literature on the stimulation of ascorbic acid synthesis produced by the feeding of chlorobutanol to dairy animals.

Considerable work has been described in the literature concerning various factors affecting the metabolism and excretion of ascorbic acid in different subjects. Sutton, Kaeser and Hansard (20) observed that bulls which had low blood ascorbic acid values and poor breeding performance were usually fed poor quality roughage and that cows which were fed excellent roughage had a higher average level of ascorbic acid in the plasma than cows receiving poor quality roughage. Phillips, Lundquist and Boyer (13) showed that low plasma ascorbic acid values could be increased by the addition of vitamin A to the ration of calves. Erb and Andrews (5) reported that the injection of 1,000 to 2,250 rat units of gonadotropin into dairy bulls produced a 42 to 67 per cent decrease in the plasma ascorbic acid level within 24 hours. Cows which had received an equivalent amount of the hormone showed decreases of only 20 to 50 per cent during the same time. The injection of 20 units of insulin into normal dogs caused a decrease in the vitamin C content of the blood plasma and in the urinary excretion of this vitamin which lasted for several hours (14).

Svirbely (21) concluded that the rat is able to synthesize vitamin C irrespective of the composition of the diet although adequate amounts of the vitamin B factors are essential to obtain normal vitamin C values in certain tissues. Sure and coworkers (19) reported that deficiencies of vitamins A, B_1 and riboflavin caused a decrease in the ascorbic acid values of certain rat

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tissues. Sutton *et al.* (20) found that a limited vitamin A intake produced a significant decrease in the level of plasma ascorbic acid in the rat. Rudra (16, 17) postulated that mannose, glucose and galactose acted as precursors for vitamin C but that manganese was a determining factor in the synthesis of this vitamin. Drake and coworkers (3) found that the administration of 500 mg. of sodium diphenyl hydantoinate (Dilantin sodium) per kilo of body weight increased the urinary excretion of ascorbic acid and decreased the concentration of ascorbic acid in the liver, brain, muscle, adrenal glands and blood of rats. They (4) reported also that the daily administration of 13 mg. of dilantin sodium per kilo of body weight to guinea pigs produced a rapid and progressive decrease in the plasma ascorbic acid level whereas the control animals maintained their normal level. Both groups received an ascorbic acid-free diet but they received 5 mg. of ascorbic acid, subcutaneously, daily. The investigations of Merritt and Foster (9) showed no appreciable difference between the vitamin C content of the blood plasma of human beings who had taken dilantin sodium for two years for the treatment of epilepsy and those who had never received medication.

Longenecker and associates (6, 7) found that terpene-like cyclic ketones and other organic compounds produced a marked increase in the ascorbic acid excretion of rats. Ritz *et al.* (15) reported that the liver and blood plasma of rats receiving carvone had consistently higher ascorbic acid values than control rats whereas rats receiving sodium salicylate or acetylsalicylic acid lost ascorbic acid from the brain and liver soon after the administration of the drugs. The urinary output of ascorbic acid increased in both experimental groups. Vacuum-distillable fractions from the unsaponifiable matter of halibut liver oil, oat, grass-leaf and alfalfa-leaf oils were shown by Musulin and coworkers (10) to cause high urinary excretions of vitamin C in the rat. Similar results were obtained by Sutton *et al.* (20) from the feeding of chloretone to rats. They reported also, that chloretone-fed rats with the gonads, pituitary and adrenal glands removed were still capable of synthesizing vitamin C.

Preliminary reports of our studies have been presented on the effects produced by the ingestion of chlorobutanol on the breeding efficiency of bulls and on the level of ascorbic acid in the blood plasma of dairy cattle (1, 18). The data presented in this paper deal specifically with the changes produced in the concentration of plasma ascorbic acid by feeding various amounts of chlorobutanol to calves, heifers, cows and bulls.

EXPERIMENTAL

A total of 4 calves from 3 to 9 weeks of age, and 7 heifers, 11 cows and 7 bulls of all ages were fed various amounts of chlorobutanol. The administration of chlorobutanol to the mature animals varied from a single 40-gram dose given in a gelatin capsule to a 5-gram dose, daily, fed with the grain.

Two animals received the chlorobutanol each day for 60 days and 3 animals received three doses per week for 105 days. Three of the calves received the chlorobutanol by capsule and the fourth one received it in the milk.

No attempt was made to feed a special ration but almost all of the animals received an alfalfa hay ration with various supplements. The calves received a standard calf ration in amounts adequate for maintenance and growth.

Blood samples were collected several times each week prior to, during and following the chlorobutanol feeding period and analyzed for ascorbic acid according to a procedure previously outlined (2).

Dillseed oil which contained 50 to 60 per cent *d*-carvone was also fed by capsule in amounts up to 20 ml. per day to two 400-pound heifers.

RESULTS

Results with dairy cows. The experimental data showing the variations in the concentration of ascorbic acid in the plasma of dairy cows receiving various amounts of chlorobutanol are presented in table 1. The largest single dose of chlorobutanol given to any animal was a 40-gram dose given to cow 269. Two hours after the administration of this dose she became drowsy and was unable to walk without falling down. The anesthetic effect continued for 48 hours. During that time she went off feed and decreased in milk production. The level of ascorbic acid in the blood plasma began to increase on the day following the massive dose of chlorobutanol and reached its peak value of 0.87 mg. per 100 ml., on the seventh day. By this time the cow had apparently returned to normal in every respect except for the high level of ascorbic acid in the blood. Seven days later the level of ascorbic acid also returned to normal.

Two cows, D5 and A19, were each given 20 grams of chlorobutanol in gelatin capsules daily for a 5-day period. On the fourth day D5 went off feed, decreased in milk production and had some difficulty in walking. The amount of ascorbic acid nearly doubled in the blood plasma after she had received the second dose but the peak was not reached until 12 days after the administration of the first dose. Twenty days after the last dose of chlorobutanol was given the plasma ascorbic acid returned to normal. A19 (non-lactating) seemed drowsy after receiving the fourth and fifth doses of chlorobutanol but she did not go off feed. The amount of ascorbic acid reached a peak value of 1.03 mg. per 100 ml. of plasma on the day following the first 20-gram dose and continued high above the pre-feeding level for more than four weeks.

Four cows (66, A21, A29 and 264) received daily doses of chlorobutanol at the 10-gram level for 5 to 10 days. A mild anesthetic effect was produced in only two of the cows. Cow 66, a small Jersey, decreased in feed intake and in milk production during the 6-day experimental period. After an

hour or two following each dose of chlorobutanol she appeared drowsy and wobbly on her feet. The plasma ascorbic acid level did not increase during the feeding period but the concentration was doubled on the ninth day after the initial dose. The chlorobutanol did not produce an anesthetic effect on cows A21 and A29 but it doubled the amount of ascorbic acid in the plasma over the pre-feeding level. Cow 264 did not show an increase in the level of ascorbic acid in the plasma after receiving seven 10-gram doses of chlorobutanol. The concentration of ascorbic acid in her blood plasma decreased during the feeding period and for two weeks following. She exhibited the anesthetic effects of the chlorobutanol however.

TABLE 1

The effect of feeding chlorobutanol to dairy cows on the ascorbic acid content of the blood plasma

Cow No.	Daily dose	Days fed	Weeks of feeding				Weeks after feeding				Peak value†	Days to reach peak
			0*	1	2	3	1	2	3	4		
	gm.		*mg. per 100 ml.*				*mg. per 100 ml.*					
269	40	1	0.42	.....	.....	.....	0.65	0.46	0.40	0.43	0.87	7
D5	20	5	0.38	0.65	.....	.....	0.91	0.42	0.16	.....	0.92	12
A19	20	5	0.20	0.67	.....	.....	0.59	0.47	0.34	0.42	1.03	1
66	10	6	0.42	0.39	.....	.....	0.69	0.37	0.39	.....	0.86	9
A21	10	10	0.47	0.93	0.80	.....	0.49	0.43	0.50	0.39	0.96	5
A29	10	5	0.38	0.60	.....	.....	0.53	0.39	0.51	0.41	0.73	8
264	10	7	0.54	0.43	.....	.....	0.33	0.39	.....	.....	0.56	1
76	5	10	0.36	0.73	0.61	.....	0.43	0.42	0.35	0.51	0.80	7
264‡	5	15	0.36	0.53	0.88	.....	0.42	0.39	0.53	.....	1.10	8
267	5	15	0.44	0.64	0.62	.....	0.69	0.38	0.36	0.43	0.81	15
285	5	16§	0.35	0.52	0.61	0.64	0.51	0.39	0.33	0.28	0.72	17
289	5	18¶	0.33	0.46	0.53	0.60	0.61	0.49	0.31	0.21	0.77	22

* Average value obtained for the 10-day period prior to the experimental period.
† Highest single value obtained.
‡ Started 11 days after the last 10-gram dose.
§ Fed 15 grams on the last day of the experiment.
¶ Fed 10 grams daily for last 3 days.

Five milking cows (76, 267, 285, 289 and 264) were fed 5-gram doses of chlorobutanol for periods of 10 to 18 days. None of the cows showed any of the untoward effects obtained from feeding the larger doses and all of the cows showed marked increases in plasma ascorbic acid similar to those obtained by feeding 10-gram doses. The only difference noted was that three of the five cows required a longer period of time to manifest maximum ascorbic acid levels. It is of interest to note that cow 264 reached her peak value of 1.10 mg. per 100 ml. only eight days after the second feeding trial began.

Results with dairy heifers. The results obtained from feeding chlorobutanol to heifers are shown in table 2. These data indicate that three consecutive 5-gram doses of chlorobutanol will increase the concentration of ascorbic acid in the blood plasma significantly for a short time but the high

level will not be sustained (A33, A34). The two heifers (C424 and A32) which received five grams of chlorobutanol per day for 60 days exhibited a marked increase in plasma ascorbic acid during the first week of feeding. Their peak values were obtained on the sixteenth day and continued at a high level for the entire feeding period. The plasma levels were still above the pre-feeding level for four weeks after the experimental period. It should be pointed out that heifers A34 and A37 practically maintained the same plasma ascorbic acid levels while receiving three 5-gram doses of chlorobutanol per week as they did when receiving 5-gram doses per day.

TABLE 2

The effect of feeding chlorobutanol to dairy heifers on the ascorbic acid content of the blood plasma

Animal No.	A33*	A34*	128	C424	A32	A34‡	A37
Daily dose, gm.	5	5	5	5	5	5	5
Days fed	3	3	51†	60	60	73§	73§
	mg. per 100 ml.						
Av. value 10 days prior	0.49	0.52	0.25	0.30	0.29	0.42	0.32
Av. value 1st week	0.74	0.60	0.49	0.70	0.70	0.44	0.49
" " 2nd "	.....	.....	0.52	0.71	0.87	0.51	0.57
" " 3rd "	.....	.....	0.50	0.74	1.01	0.61	0.67
" " 4th "	.....	.....	0.61	0.68	0.84	0.53	0.43
" " 5th "	.....	.....	0.66	0.62	0.73	0.96	0.83
" " 6th "	.....	.....	0.48	0.71	0.75	0.77	0.78
" " 7th "	.....	.....	0.53	0.71	0.66	0.84	0.61
" " 8th "	.....	.....	0.65	0.63	0.69	0.52	0.68
" " 9th "	.....	.....	.....	0.79	0.67	0.42	0.70
" " 10th "	.....	.....	.....	.....	.....	0.75	0.72
" " 11th "	.....	.....	.....	.....	.....	0.59	0.68
" " 1st " after	0.30	0.53	0.63	0.59	0.60	0.45	0.49
" " 2nd " "	0.48	0.44	0.51	0.58	0.53	0.23	0.39
" " 3rd " "	0.50	0.44	0.55	0.34	0.74	0.46	0.61
" " 4th " "	0.46	0.40	0.43	0.40	0.63	0.39	0.54
Peak value	0.92	0.72	0.76	0.80	1.02	0.96	0.83
Days to reach peak	4	4	31	16	16	35	35

* Had received one 5-gram dose 15 days prior to this experiment.
† Fed daily for first 5 days, then 3 times per week for balance of experiment.
‡ Had received chlorobutanol 5 months previous but none since that time.
§ Fed daily for 6 weeks, then 3 times per week for balance of the experiment.

The feeding of 20 ml. of dillseed oil per day for 16 days did not produce any appreciable change in the level of ascorbic acid in the blood plasma of two 400-pound heifers. The data are not presented.

Results with dairy calves. Table 3 shows the effect of chlorobutanol on the concentration of ascorbic acid in the blood plasma of calves. The ascorbic acid increased in the plasma of two calves (C460 and C495) similar to that shown by the cows and heifers. The peak values for the other two calves (C506 and C508) were elevated significantly and they maintained a consistently higher level during the experimental period than the average normal value reported for calves (2). The difference in response obtained for the calves may have been due to the difference in concentration of ascorbic acid

in the plasma prior to the experimental period. The ascorbic acid values of calves C506 and C508 were much higher during the pre-feeding period than for calves C460 and C495.

Results with bulls. The results obtained from feeding chlorobutanol to bulls are shown in table 4. Six out of seven bulls responded favorably to the ingestion of chlorobutanol. It was observed in most cases that the bulls did not respond as rapidly as the cows and heifers responded to chlorobutanol

TABLE 3

The effect of feeding chlorobutanol to calves on the ascorbic acid content of the blood plasma

Calf No.*	C460		C495		C506		C508	
	Intake†	Mg.%‡	Intake	Mg.%	Intake	Mg.%	Intake	Mg.%
Av. value 10 days prior	0.0	0.18	0.0	0.27	0.0	0.43	0.0	0.43
" " 1st week	0.2	0.21	4.0	0.45	2.0	0.34	6.0	0.48
" " 2nd "	4.9	0.34	.....	.....	2.0	0.16	6.0	0.48
" " 3rd "	7.0	0.44	.....	.....	4.0	0.31	6.0	0.64
" " 4th "	8.5	0.51	.....	.....	6.0	0.41	2.0	0.35§
" " 5th "	10.5	0.36	.....	.....	6.0	0.35	6.0	0.38
" " 6th "	4.0	.....	.....	.....	9.0	0.53	4.0	0.51
" " 7th "	.....	.....	.....	.....	9.0	0.46	6.0	.....
" " 8th "	.....	.....	.....	.....	9.0	0.46	6.0	0.40
" " 9th "	.....	.....	.....	.....	9.0	0.49	.....	.....
" " 10th "	.....	.....	.....	.....	9.0	0.47	.....	.....
" " 11th "	.....	.....	.....	.....	6.0	0.51	.....	.....
" " 12th "	.....	.....	.....	.....	9.0	.....	.....	.....
" " 13th "	.....	.....	.....	.....	9.0	0.44	.....	.....
" " 1st " after	.....	0.41	.....	0.66	.....	.....	.....	.....
" " 2nd " "	.....	0.23	.....	0.20	.....	.....	.....	.....
" " 3rd " "	.....	.....	.....	0.35	.....	.....	.....	.....
" " 4th " "	.....	0.28	.....	.....	.....	.....	.....	.....
Peak value	.....	0.51	.....	0.74	.....	0.60	.....	0.63
Days to reach peak	23	.....	12	.....	42	.....	20	.....
Total intake, gm.	35.1	.....	4.0	.....	89.0	.....	42.0	.....

* Age of calves at beginning of experiment: 9 weeks, 4 weeks, 3 weeks and 4 weeks, respectively.

† Intake of chlorobutanol per week.

‡ Milligrams of ascorbic acid per 100 ml.

§ Calf went off feed and scoured.

and that very large doses were required to produce a significant increase in plasma ascorbic acid. The peak values obtained were consistently lower than those obtained for cows and heifers but the percentage increases were significant. Two of the bulls, Patty and Wells, received 330 and 300 grams of chlorobutanol respectively before significant changes in plasma ascorbic acid were observed. During the sixth week these bulls each received three 30-gram doses which produced definite anesthetic effects after the third dose. The chlorobutanol was discontinued and both animals returned to normal within four days. After one week the feeding of chlorobutanol was resumed at a lower level and the plasma ascorbic acid reached a peak. The higher

TABLE 4

The intake of chlorobutanol per week and the effects produced in the concentration of ascorbic acid in the blood plasma of bulls

	Wells		Patty		Patty		Dane		Cesor		1840		1645		1645		Shorthorn		Shorthorn	
	Gm.*	Mg.% †	Gm.	Mg.%	Gm.	Mg.%	Gm.	Mg.%	Gm.	Mg.%	Gm.	Mg.%	Gm.	Mg.%	Gm.	Mg.%	Gm.	Mg.%	Gm.	Mg.%
Av. value 10 days prior	0	0.25	0	0.22	0	0.19	0	0.20	0	0.26	0	0.39	0	0.28	0	0.36	0	0.24	0	0.28
" " 1st week	40	0.30	40	0.28	40	0.24	50	0.22	10	.....	50	0.30	40	0.40	40	.....	70	0.09	40	0.26
" " 2nd "	30	0.17	35	0.41	30	0.35	90	0.28	10	0.38	30	0.48	35	0.43	35	.....	60	0.32	30	0.38
" " 3rd "	30	0.33	35	0.51	30	0.34	30	0.33	15	0.49	10	0.48	25	0.43	35	0.39	50	0.22	20	0.32
" " 4th "	60	0.23	35	0.27	60	0.26	20	0.33	15	0.52	.....	.....	.....	.....	35	0.50	.....	.....	.....	.....
" " 5th "	50	0.25	35	0.40	50	0.24	30	0.47	15	0.55	.....	.....	.....	.....	35	0.49	.....	.....	.....	.....
" " 6th "	90	0.30	30	0.35	90	0.39	20	0.41	15	0.49	.....	.....	.....	.....	5	0.44	.....	.....	.....	.....
" " 7th "	0	0.42	.....	.....	0	0.37	20	0.33	10	0.41	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 8th "	30	0.31	.....	.....	30	0.24	20	0.37	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 9th "	30	0.48	.....	.....	30	0.48	10	0.39	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 10th "	20	0.44	.....	.....	20	0.40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 11th "	30	0.43	.....	.....	30	0.43	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 12th "	20	0.58	.....	.....	20	0.49	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 13th "	20	0.41	.....	.....	20	0.34	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 14th "	20	0.47	.....	.....	20	0.37	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 15th "	20	0.35	.....	.....	20	0.37	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
" " 1st " after	0	0.31	0	.....	0	0.42	0	0.40	0	0.38	0	0.55	0	0.52	0	.....	0	0.24	0	0.21
" " 2nd " "	.....	0.36	.....	0.08	.....	0.41	.....	0.26	.....	0.38	.....	0.51	.....	0.31	.....	.....	.....	0.20	.....	0.20
" " 3rd " "	.....	0.20	.....	0.12	.....	0.18	.....	0.14	.....	0.38	.....	0.59	.....	0.36	.....	.....	.....	0.22	.....	0.22
" " 4th " "	.....	0.31	.....	.....	.....	0.25	.....	0.26	.....	0.27	.....	0.43	.....	.....	.....	0.34	.....	0.30	.....	.....
Peak value	.....	0.63	.....	0.51	.....	0.57	.....	0.50	.....	0.64	.....	0.63	.....	0.52	.....	0.50	.....	0.32	.....	0.38
Days to reach peak	79	.....	20	.....	61	.....	34	.....	19	.....	20	.....	25	.....	22	.....	14	.....	12	.....
Total intake, gm.	490	.....	210	.....	490	.....	290	.....	90	.....	90	.....	100	.....	185	.....	180	.....	90	.....

* Intake of chlorobutanol per week.

† Milligrams of ascorbic acid per 100 ml.

levels were then maintained as long as chlorobutanol was fed. The Shorthorn bull did not respond significantly to the ingestion of chlorobutanol during the two experimental periods at the level fed. This may have been due to the failure to feed a sufficient amount of chlorobutonal.

DISCUSSION

From the nature of the results reported in this paper it is evident that the feeding of chlorobutanol will increase the plasma ascorbic acid level in calves, heifers, cows and bulls although the physiological significance of this phenomenon is not clear. The length of time required to increase the plasma ascorbic acid level and the length of time required to reach a maximum value seemed to be determined not only by the size of the dose but also by the individual response of the animal to the ingestion of chlorobutanol. The latter factor appeared to be the more important one.

Doses of 10 grams or more of chlorobutanol produced a mild anesthetic effect in some of the mature animals. This was accompanied by a marked reduction in feed intake and in milk production. The larger dosages (10 grams or more) served to shorten the length of time necessary to produce a marked increase in the plasma ascorbic acid level and reduced the length of time required to reach a peak value. The feeding of 5 grams of chlorobutanol per day to heifers and cows was sufficient to produce maximum changes in plasma ascorbic acid without any untoward effects. The feeding of 5 grams of chlorobutanol per day for extended periods of time did not produce any observable harmful effects. Doses several times this size were fed without any permanent deleterious effects so it is not likely that the proper feeding of chlorobutanol will involve any danger. The only advantage obtained in feeding more than 5 grams per day would be to increase the concentration of ascorbic acid more rapidly. The results obtained with heifers that have reached breeding age indicate that 15 grams per week are sufficient to maintain the plasma ascorbic acid at a high level.

The extreme sex difference and individual response to the amount of chlorobutanol required to stimulate ascorbic acid synthesis are particularly evident in the case of the bulls. It would appear from the results obtained that bulls require much more chlorobutanol than cows to produce a comparable change in ascorbic acid synthesis. It is evident that the amount of chlorobutanol required to stimulate ascorbic acid synthesis is not proportional to body weight.

Due to the fact that only a few calves were used in this work, the results obtained are somewhat variable. Age may be an important factor in the interpretation of the results. Lundquist and Phillips (8) observed that the plasma ascorbic acid diminished in calves from birth to the third week of life and then gradually returns to a normal level of approximately 0.30 mg. per 100 ml. Certain of the changes noted in our calves may have been due to normal changes at this particular age period but all of the calves which

received chlorobutanol maintained an ascorbic acid level higher than the average values reported in the literature (2, 8). It should also be noted that the ascorbic acid level of two of the calves was reduced markedly after the feeding of chlorobutanol was discontinued.

SUMMARY

1. Four calves, 7 heifers, 11 cows and 7 bulls were fed various amounts of chlorobutanol for as long as 105 days as a supplement to a standard alfalfa hay ration.

2. The ingestion of chlorobutanol served to elevate ascorbic acid in the blood plasma of all of the animals.

3. Doses of 10 grams or more of chlorobutanol produced mild anesthetic effects in the animals but the maximum concentration of ascorbic acid in the blood plasma was obtained within a few days.

4. No deleterious effects were observed when 5-gram doses were administered for long periods of time.

5. A sex difference was noted in the amount of chlorobutanol required to produce a significant increase in plasma ascorbic acid.

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ABSTRACTS OF LITERATURE

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MILK AND MILK PRODUCTS

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State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BACTERIOLOGY

213. Beta Hemolytic Streptococci Isolated from Public Room Floors. WILLIAM G. WALTER AND G. J. HUCKER, N. Y. State Agr. Expt. Sta., Geneva. Jour. Infect. Dis., *71*, No. 3: 237–240. Nov.–Dec., 1942.

One hundred thirteen sweepings collected from 37 rooms in 6 schools, a dormitory, a theater, and a hotel, were examined for beta hemolytic streptococci. Isolation of the streptococci was accomplished by successive enrichment in potassium tellurite broth and blood broth and finally by plating on blood agar. Twenty-two of the 37 rooms yielded beta hemolytic streptococci in at least one examination. Isolations were accomplished more readily during the period from February to May than during the summer months. Seventeen representative cultures were studied physiologically and serologically. On the basis of this study, 7 of the 17 cultures were classified as Lancefield's group A, 2 as group B, and one as group C. The remaining 7 cultures not falling into these three groups were classed by their physiological reactions alone as belonging to Lancefield's group G. Thirty-nine other cultures not tested serologically were grouped on the basis of their physiological reactions as follows: 24 group A, 3 group B, and 12 group G. The public health significance of these findings is discussed briefly. J.F.C.

214. A Study of the Bacteriostatic Activity of Fluoro and Bromo De-Derivatives of Some Organic Acids. GEORGE P. HAGER AND THOMAS C. GRUBB, Univ. Md., School of Phar., Baltimore. Jour. Infect. Dis., *71*, No. 3: 228–231. Nov.–Dec., 1942.

"A study was made of the comparative bacteriostatic activity of the o-, m- and p-fluoro substitution derivatives of benzoic, phenylacetic, cinnamic and mandelic acids. The parabromo analog of the above acids was also included for comparative purposes.

"The bacteriostatic tests were conducted by inoculating *Staphylococcus aureus* or *Eberthella typhosa* into various dilutions of the acids in nutrient broth adjusted to pH 5.0. Bacteriostasis was indicated by the highest dilutions which inhibited growth after incubation at 37° C. for one week.

"The oil-water distribution coefficient of each acid was determined and an attempt made to correlate these coefficients with the relative bacteriostatic activity of the various acids.

"The inclusion of fluorine in the aromatic nucleus caused a slight increase in bacteriostatic activity, being most evident in the para or meta isomers.

Nuclear bromine in the para-position produced a much greater increase in activity, especially against Staph. aureus." Author's summary. J.F.C.

215. The Significance of the Incubation Temperature of Recovery Cultures in Determining Spore Resistance to Heat. O. B. WILLIAMS AND J. M. REED, Res. Lab., Natl. Canners Assoc., Washington, D. C. Jour. Infect. Dis., *71*, No. 3: 225–227. Nov.–Dec., 1942.

Spore suspensions of *Clostridium botulinum*, types A and B, and of an unidentified putrefactive anaerobe were heated at lethal temperatures and samples for plating to determine percentage of survival were removed at varying intervals of time during the heating. Plates were made in replicate and plates from each sampling were incubated at 37, 31, 27, and 24° C. Greater thermal death times, based on 99.99% destruction, were apparent for spores of all 3 strains when the plates for recovery culture were incubated at 24 or 27° C. than when incubated at 31 or 37° C. J.F.C.

216. Hyodesoxycholic Acid Media for the Estimation and Enumeration of Coliform Bacteria in Milk and Water. EINAR LEIFSON, Univ. S. D. Jour. Bact., *45*, No. 1: 45. Jan., 1943.

Of the bile acid media developed for the purpose of detecting and enumerating coliform bacteria in milk and water none is perfect of those heretofore published since the growth of a considerable proportion of the coliform bacteria is inhibited. Both liquid and agar media are described, having the provisional formula: peptone 1%, lactose 1%, sodium hyodesoxycholate 0.1%, pH 6.7 to 6.9. To the agar medium is added 1/50,000 neutral red. Colonies of coliform bacteria in the agar medium are very similar to those in desoxycholate agar. In the liquid medium the coliform bacteria produce gas rapidly and abundantly. D.P.G.

217. The Accelerating Effect of Sublethal Heat upon the Germination of Aerobic Mesophilic Spores. F. R. EVANS AND H. R. CURRAN, Bur. Dairy Indus. U.S.D.A. Jour. Bact., *45*, No. 1: 47. Jan., 1943.

With each of six species of aerobic, mesophilic bacteria preincubation heating of the spores between 65° and 95° C. increased the number of spores which became heat-labile during the subsequent incubation period. Greatest stimulation was usually greater when spores were heated and incubated in extract broth or evaporated milk than when preheated in distilled water and subcultured in extract broth. D.P.G.

218. The Lactic Acid Fermentation of Streptococci. PAUL A. SMITH AND J. M. SHERMAN, Cornell Univ. Jour. Bact., *43*, No. 6: 725–731. June, 1942.

In a total of 286 tests made with 151 cultures representing the better known groups and varieties of streptococci, washed cells were suspended in a phosphate-buffered glucose solution. The average per cent lactic acid from glucose fermented for each group was: Pyogenic streptococci, 81.8 to 89.7; viridans streptococci, 90.2 to 93.6; lactic streptococci, 93.7 and 96.6; enterococci (group D), 90.8 to 96.6.

"The slightly lower average efficiency of the pyogenic streptococci is of possible significance." D.P.G.

219. Industrial Importance of Bacteriophage. ELIZABETH MCCOY, Univ. Wis. Jour. Bact., *45*, No. 1: 75. Jan., 1943.

A discussion of the nature and importance of outbreaks of bacteriophage in industrial ferementations including lactic starters and butyl alcohol fermentations. Controls proposed include sterilization of equipment with rigorous exclusion of new phage, use of mixed cultures and increased size of inoculum, and use of specially prepared phage-resistant strains. D.P.G.

220. Growth of Thermophiles in Pasteurizing Equipment. M. L. ISAACS AND A. GORDON. Yeshiva Col., New York, N. Y. Jour. Bact., *45*, No. 1: 51–52. Jan., 1943.

Studies on growth of thermophiles in 4 city milk plants indicated a doubling in number of organisms for every one-and-a-half hours of (continuous) equipment use. Observations indicated that the organisms multiplied and were held for as much as 3 hours in certain parts of the equipment before they were released into the milk. D.P.G.

221. The Maintenance of Viability in *Lactobacillus bulgaricus* Cultures by Growth in Association with Certain Yeasts. V. E. GRAHAM, Univ. Saskatchewan. Jour. Bact., *45*, No. 1: 51. Jan., 1943.

It was found that some film forming yeasts that preserved the viability of *Lactobacillus bulgaricus* in milk were unable to utilize lactic acid as a carbon source in 1% concentration. Some yeasts that did not protect the viability of *L. bulgaricus* were active utilizers of lactic acid. It was concluded that the lactic acid utilization protective theory was in itself inadequate to explain the protective action of the yeasts. D.P.G.

222. Fermentation of Some Simple Carbohydrates by Members of the *Pseudomonas* Genus. LEON STEIN, R. H. WEAVER AND M. SCHERAGO, Univ. Ky. Jour. Bact. *44*, No. 3: 387. Sept., 1942.

The ordinary meat extract sugar broth medium cannot be relied upon to show acid production by members of this genus. Any acid production

at all will be shown by a minimum buffered synthetic medium. Many strains of *Pseudomonas* may utilize sugars without production of evident acid. The best routine method for determination of carbohydrate utilization by this genus is use of a synthetic medium, without indicator, containing the carbohydrate as the only source of energy and of carbon, and with growth as evidence of utilization of the sugar. Glucose was utilized by all of the 24 strains of *Pseudomonas* studied, maltose by the majority and sucrose and lactose were utilized by some. D.P.G.

223. The Problem of Thermoduric Bacteria in Short-Time High-Temperature Pasteurization. V. F. O'DANIEL, Ewing Von-Allmen Dairy Co., Louisville, Ky. Jour. Bact., *44,* No. 3: 387. Sept., 1942.

After the sanitary conditions on the farms of 48 shippers (out of 350) had been improved, the average pasteurization efficiency increased from 89.1% destruction (before cleaning up) to 98.5% (after cleaning up). D.P.G.

224. Dissociation of *Bacillus albolactis*. F. S. ORCUTT, Va. Polytechnic Inst. Jour. Bact. *44,* No. 1: 147. July, 1942.

Bacillus albolactis dissociates into smooth and rough forms which are identical morphologically and physiologically. Both forms appear normally in dairy products in Virginia and could be mistaken for two distinct species. D.P.G.

225. The Effect of Pasteurization on the Nutritive Requirements of Milk Bacteria. J. B. HERSHEY, Health Div. Lab., St. Louis, Mo. Jour. Bact., *43,* No. 1: 117. Jan., 1942.

Counts on raw milk from varied sources using both the old standard medium (beef extract peptone agar) and the new standard medium (trypton-glucose beef extract skim milk agar) showed the same ratio in numbers of bacteria before and after heating in test tubes at the equivalent of usual pasteurization treatment. Cultures of various non-spore forming organisms isolated from milk, counted in the same manner before and after heating, either gave the same counts on both media or failed entirely to grow on the old standard medium. *Escherichia-coli* was not influenced by the type of medium employed following pasteurization when these two media and the dilution method with tryptone glucose beef extract broth were used. The author concluded that when milk was laboratory pasteurized, no change in nutritive requirements of milk organisms before and after pasteurization could be detected by use of the new standard medium as compared with the old. D.P.G.

226. Influence of Growth at Low Temperature on Heat Resistance of *Lactobacillus bulgaricus*. J. G. Voss and W. C. Frazier, Univ. Wis. Jour. Bact., *44*, No. 2: 255–256. Aug., 1942.

Lactobacillus bulgaricus (strain Ga) was carried in sterile reconstituted skim milk at 37.2° and 24.3° C., respectively, and transfers made at 12 and 84 hour intervals, respectively, in order to obtain cultures of equivalent maturity. The 24.3° C. culture was more active at both 37.2° C. and 47° C. after heat-shocking at 63° F. for 30 minutes, in spite of the fact that the 37.2° C. culture cells showed a greater percentage survival of the heat-shocking. This is explained by the fact that the 24.3° cells showed a greater fermentation rate at 37.2° after heat-shocking than did the 37.2° cells. The data are considered of possible significance in preparation of starters for Swiss cheese manufacture. D.P.G.

227. The Effect of Incubation Temperature upon Certain Organisms Cultured in Cream. Elizabeth D. Robinton and Elizabeth F. Genung, Smith Col., Northampton, Mass. Jour. Bact., *43*, No. 6: 778. June, 1943.

The rate of development of *Streptococcus lactis* and *Aerobacter aerogenes* was far greater in sterilized cream than in sterilized skim milk. It was suggested that possibly the protein-lipid membrane around each fat globule offers a greater surface area for available food materials for bacterial growth. Plates incubated at 20° C. showed higher counts than plates incubated at 37° C. D.P.G.

228. Observations on Bacteriological Condition of Creamery Water Supplies. H. F. Long and R. T. Corley, Iowa State Col. Jour. Bact., *44*, No. 2: 255. Aug., 1942.

The water supplies of 70 creameries were examined by special media for *Escherichia-Aerobacter* species, *Pseudomonas putrefaciens*, and total, proteolytic, and lipolytic counts. Approximately half the supplies were unsatisfactory. Contamination was traced to various sources including plant wells, city water supplies, storage tanks and piping. D.P.G.

229. The Heat Resistance of Mixed Cultures of *Streptococcus thermophilus* and Certain Caseolytic Bacteria. H. J. Peppler, Kansas State Col. Jour. Bact., *44*, No. 3: 389. Sept., 1942.

The activity and heat resistance of *Streptococcus thermophilus* was increased when it was grown in association with caseolytic bacteria including *Streptococcus liquefaciens*, *Pseudomonas aeruginosa*, and *Proteus ammoniae*. Milk media enriched with commercial peptones provided heat resistance for pure cultures of *Str. thermophilus* equivalent to that for mixtures of *Str.*

thermophilus and caseolytic bacteria. Similar stimulation and enhanced heat resistance was provided by enrichment of skim milk with heat killed whey cultures of caseolytic bacteria. Heat resistance of *Str. thermophilus* was not enhanced by addition of calcium panthothenate, l-ascorbic acid, riboflavin, thiamin, and niacin, added separately or in combination.

All caseolytic bacteria except *P. aeruginosa* and *Achromobacter lipolyticum* survived 60 minutes at 55° and 58° C. These two were killed in milk in 25 minutes at 55° C. and in 10 minutes at 58° C. D.P.G.

230. A Comparison of Media for the Detection of *Coli-Aerogenes* Organisms in Raw Milk. C. E. SKINNER AND R. M. MARVIN, Univ. Minn. Jour. Bact., *44*, No. 2: 255. Aug., 1942. Abs. Proc. Local Branches.

No significant difference in repression of growth of *coli-aerogenes* organisms was evident with 2% brilliant green bile broth and standard formate ricinoleate broth. False positives occurred in a very considerable number of tubes of the formate ricinoleate broth but only in an insignificant number of cases in the brilliant green bile broth. The false positives were due largely to members of the genus *Proteus*. Members of *Proteus* and *Salmonella* are known to produce gas from formate. The authors thus consider undesirable the inclusion of formates in lactose media for detection of *coli-aerogenes* bacteria. D.P.G.

231. A Study of Certain Factors Which Influence the Apparent Heat Resistance of Bacteria. F. E. NELSON, Kansas Agr. Expt. Sta. Jour. Bact., *44*, No. 3: 389. Sept., 1942. Abs. Proc. Local Branches.

The effect on growth of variations in plating media and temperature and time of incubation of plates was determined for heat-treated bacteria of the following species: *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus durans*, *Streptococcus liquefaciens*, and *Streptococcus zymogenes*. The factors studied had no significant effect on counts of unheated control cultures. For heated bacteria, beef-infusion agar was superior to three other media and for some lactic streptococci the new standard milk agar was superior to ordinary nutrient agar. Incubation at 32° C. usually resulted in higher counts than 21°, 28°, 37° or 42° C. Counts after 96 hours, particularly at the low temperatures, were higher than after 48 hours of incubation. The heated bacteria were more sensitive to the pH of the plating medium than were the unheated bacteria. Considerable increases in plate count of heated bacteria were obtained on addition of small amounts of thioglycollic acid or cysteine to the medium. D.P.G.

232. The Occurrence and Characterization of the Genus Microbacterium. M. L. SPECK, Univ. Md. Jour. Bact., *45,* No. 1: 50–51. Jan., 1943.

In an effort to define more clearly the genus *Microbacterium,* cultures belonging to this genus were isolated from pasteurized and raw milk, Cheddar cheese, dairy farm equipment, and milk stone.

Cultures of the genus *Microbacterium* were isolated from pasteurized and raw milk, Cheddar cheese, dairy farm equipment, and milk stone. Because of their relationship to the genera *Propionibacterium* and *Lactobacillus,* it was suggested that the genus *Microbacterium* be placed closer to these in a system of classification. *Lactobacillus thermophilus* was reported to be a true lactobacillus and entirely distinct from the microbacteria. Because of their high thermal resistance the microbacteria are found in pasteurized milk and milk products. Their metabolism is similar to that of the true lactic acid bacteria. They enter milk primarily from farm milking equipment.

D.P.G.

233. Agar-Agar Manufacture. E. J. FERGUSON WOOD. Food Mfr., *18,* No. 3: 85–86. March, 1943.

Japan was the chief producer of agar-agar before the war. Australia is now producing her own and studies on different types of seeweed have been made there.

Drying is discussed and also several methods and procedures. Natural bleaching by sun gives a desirable product but increases cost of production; because it must be turned every two days and spread thinly it requires space and time.

The manufacturing process is discussed in detail and six steps are given. Yields and the product are listed. J.C.M.

234. Significance and Control of *B. Coli* in Dairy Products. LEWIS SHERE, Diversey Corp., Chicago, Ill. Milk Dealer, *32,* No. 5: 33–34. Feb., 1943.

Data are presented showing the two principal groups of the colon-aerogenes types together with their principal origin. The importance of pasteurization as a control measure is stressed but the question of survival of heat-resistant strains is raised in relationship to complete reliance on standard tests as a measure of determination of healthfulness of dairy products. The author then summarizes the essential control measures necessary in the milk and ice cream plant to insure freedom from contamination from bacteria of the *B. coli* group in dairy products. C.S.T.

BUTTER

235. "Strong," "Rancid," "Bitter," or "Goaty" Flavors in Milk. W. M. ROBERTS, Col. Agr., Univ. Tenn. Report No. 68. Jan. 1, 1943.

A common flavor defect occurring in winter milk and most frequently in the milk from cows in the last stages of lactation is variously described as "strong," "rancid," "bitter," or "goaty."

This flavor defect is rarely encountered in milk from cows on pasture or from those which have recently freshened.

To prevent the development of this flavor in milk it is well to dry off cows near the end of their lactation period.

If a cow is not due to freshen for several months, and her milk goes rancid, the off flavor can be prevented by mixing the milk while still warm and fresh with milk of normal flavor at the rate of one part of milk that will go rancid with five parts of milk which does not.

Another solution to preventing such milk going rancid is to heat the milk while fresh to 143° F. for 30 minutes and then cooling it to below 60° F.

The same treatments for milk can be applied to churning cream to prevent "rancid" or "strong" flavored butter.

"Rancid" milk or cream is also very difficult to churn. L.H.B.

236. A New Method of Studying the Effect of Bacteria on Butter Flavor. M. C. JAMIESON, Dept. Anim. Path. and Bact., Univ. Manitoba, Winnipeg, Canada. Food Res., *8*, No. 1: 62. Jan.–Feb., 1943.

A simplified, less costly and considerably more convenient method of establishing relationship between bacterial contaminations and flavor spoilage of butter is presented. The method can be used to study the suitability of water supplies for creamery use and for noting the effect of equipment organisms of butter quality.

Specially prepared, clean flavored, low count butter is melted and poured into suitable vials which are refrigerated until required. Inoculations of pure cultures, combinations of cultures, suspected water or preparations from equipment are made by means of pipettes. Then the vials are warmed until the butter medium acquires a mixable consistency. The vials are next vigorously agitated in a mechanical shaker for 20 minutes followed by cooling and solidification. Finally the vials are incubated at the desired temperature for the time required, usually six to eight days at 18° C. (64.4° F.) after which examinations are made. F.J.D.

CHEESE

237. The Bacteria in Brick Cheese During Ripening. E. M. FOSTER, J. C. GAREY AND W. C. FRAZIER, Univ. Wis. Jour. Bact., *43*, No. 1: 45. Jan., 1942.

Brick cheese was made from raw and pasteurized milk by the conventional and washed-curd methods using *Streptococcus lactis* and *Streptococcus thermophilus* as starters, alone and in combination. In more than 1000 cultures from 18 lots of cheese, *Strep. lactis* predominated. It became predominant also in (raw milk) cheese made with *Strep. thermophilus* alone. *Str. thermophilus* was seldom detectable in appreciable numbers after 2 or 3 weeks even in cheese where it was used as the starter. *Lactobacilli* developed in raw milk cheese but were usually absent in pasteurized milk cheese. Of those isolated *Lactobacillus casei* predominated but *Lactobacillus brevis* and *Lactobacillus lactis* were found in several samples. *Streptococcus faecalis, Streptococcus bovis* and *Streptococcus liquefaciens* also were isolated, the first two particularly in pasteurized milk; however, there was no regularity in the ocurrence of these organisms. D.P.G.

238. The Bacteria Involved in the Ripening of Brick Cheese. E. M. Foster, Univ. Texas. Jour. Bact., *43*, No. 1: 123. Jan., 1942.

Abs. Proc. Local Branches. D.P.G.

239. Cheese Defects and Cheese Diseases. W. Dorner, Swiss Dairy Res. Inst., Liebefeld-Bern, Switzerland. Jour. Bact., *43*, No. 1: 46–47. Jan., 1942.

A distinction is made between cheese defects and cheese diseases. A cheese defect is due to faulty operation during manufacture and a cheese disease is due to a specific agent, usually a species of bacteria. These bacteria may be divided into two groups: (1) Obligate agents of disease such as *Clostridium tyrobutricum* van Beynum and Pette and *Bacterium proteolyticum*. These cause the disease even when present in small numbers. (2) Potential agents of disease such as *Clostridium sporogenes*, the cause of "white stinker" in Swiss cheese. Such organisms multiply only if conditions such as too little acidity are provided. In such a cheese they would be favored but in a normal cheese they would remain inactive. D.P.G.

240. Microorganisms Associated with Gassy Swiss Cheese. Harry H. Weiser, Ohio State Univ. Jour. Bact., *43*, No. 1: 46. Jan., 1942.

Bacteriological examination of gassy or split rind Swiss cheese showed anaerobic bacteria and lactose fermenting yeasts. They were classified as *Clostridium perfringens* and *Torula cremoris*. These organisms were absent in normal cheese.

Both types were present in extremely gassy cheese but *T. cremoris* was not isolated from mildly defective cheese. Unsanitary practices on the farm resulted in contamination of fresh milk with these organisms. D.P.G.

CHEMISTRY

241. Food Analysis. Part II. D. W. GROVER, Food Mfr., *18*, No. 3: 87–89. March, 1943.

Determination of volatile fatty acids has been proven to be a useful indication of spoilage. This is discussed. Sugars, starches, gums and titration of reducing sugars and their results are listed. Fermentation products are given and discussed. Flavours, oils, and preservatives, trace elements, arsenic tests and war gases are all discussed in detail. All these are listings of the progress made in each line discussed during 1942. References are listed. J.C.M.

242. Dairy Chemistry. S. J. ROWLAND. Food Mfr., *18*, No. 3: 75–78. March, 1943.

This article is a detailed review of fundamental studies and technological investigations in dairy chemistry. The influence of mastitis on milk composition is discussed. Milk products, cheese, skim milk and their composition are discussed in detail. Pure salt and its uses in the dairy industry are listed.

The defects of milk and its products are explained and also the use of cereal flours as antioxidants. The analysis of milk, determination of lactose-butterfat values and quality of skim milk are listed. Each subject is discussed in detail and contributions in each field by chemists given. A page of references is listed. J.C.M.

DISEASE

243. Intrammary Injection of Homogenized Oil-Sulfanilamide in the Treatment of Bovine Mastitis. J. C. KAKAVAS, Univ. Del., Newark, Delaware. Jour. Bact., *44*, No. 2: 262–263. Aug., 1942.

A preparation of homogenized oil-sulfanilamide was made by mixing one pound of sulfanilamide with 900 ml. of light liquid petrolatum in a mechanical mixer and then homogenizing.

The recommended dosages are: For streptococcic mastitis, 40 ml. of the homogenized mixture daily for 4 days with additional treatment if the infection persists. For staphylococcic mastitis, 80 ml. daily for 4 days with additional treatment if necessary.

The preparation when used as described resulted in no ill effects. Of 103 cows, 100 infected with *Streptococcus agalactiae* and 3 with *Streptococcus uberis*, cures were effected in 92 animals for 89.3%, and partial or no improvement in 11 cows for 10.7%. Of the 265 quarters treated, 251 or 94.7% were cured and 14 or 5.3% were not cured. D.P.G.

244. Rheological Properties of Bovine Cervical Mucus. G. W. Scott Blair, A. T. Cowie, and S. J. Folley, Univ. Reading, England. Amer. Jour. Physiol., *101*, No. 3: 11P–12P. Nov., 1942.

Earlier work had shown that the viscous and elastic properties of bovine cervical secretions vary regularly during the estrous cycle. Plasticity predominates in pregnancy and elasticity in the non-pregnant, and especially estrous, condition.

Accuracy of diagnosis obtained with pregnant cows (80 in all) is comparable with that obtainable by rectal palpation. The present test is purely empirical; improvement in theoretical knowledge of the rheological conditions involved should lead to increased accuracy. D.E.

245. Mechanism of Action of Certain Sulfonamide Compounds. Albert Dorfman and Stewart A. Koser, Univ. Chicago. Jour. Infect. Dis., *71*, No. 3: 241–252. Nov.–Dec., 1942.

In respiration experiments by the Warburg method the oxygen uptake by washed cells of dysentery bacilli in a medium slightly deficient in nicotinamide was markedly inhibited by sulfathiazole. Similar results reported in an earlier paper had been obtained with the structurally similar sulfapyridine. Sulfanilamide did not inhibit nicotinamide stimulated respiration. Acetylation of the amino group of sulfapyridine did not lessen its inhibiting property, indicating that the inhibition was associated with the pyridine ring and not with the sulfanilamide part of the molecule. P-aminobenzoic acid did not reverse the inhibition of nicotinamide stimulated respiration by sulfapyridine and sulfathiazole. Quantitative evidence is presented to indicate that these drugs compete directly with nicotinamide and related compounds in the respiratory processes. Sulfapyridine, sulfathiazole, and sulfanilamide all inhibit the growth of dysentery bacilli, but sulfanilamide is less effective than the other two. This inhibition of growth can be reversed by p-aminobenzoic acid used in amounts proportional to the concentration of the drugs. The authors suggest that compounds of the sulfapyridine and sulfathiazole type interfere with certain essential reactions in addition to those inhibited by sulfanilamide. J.F.C.

246. Asphyxiated Bacteria as a Vaccine in Tuberculosis. Truman Squire Potter, Univ. Chicago. Jour. Infect. Dis., *71*, No. 3: 232–236. Nov.–Dec., 1942.

Avian tubercle bacilli grown on glycerine agar for one month were deprived of oxygen and incubated for 2 additional months under moist conditions at 38° C. These cultures when injected in massive doses into pigeons failed to produce any lesions of tuberculosis. The cultures were then employed in an attempt to immunize rabbits, after which the treated

animals and susceptible controls were injected with living cultures of avian tubercle bacilli. The treated rabbits were not completely protected from the infection, but they were markedly more resistant than were the control animals. J.F.C.

247. The Death of Tubercle Bacilli Subjected to Oxygen Deprivation in the Presence of Moisture and of Warmth. TRUMAN SQUIRE POTTER, Univ. Chicago. Jour. Infect. Dis., *71*, No. 3: 220–224. Nov.–Dec., 1942.

In one experiment 6 cultures of bovine tubercle bacilli were grown on glycerine agar for 1 month and then sealed in glass tubes that had been freed of oxygen by evacuation, washing with hydrogen gas, and chemical reduction with heated palladium sponge. The anaerobic tube contained water to provide moist conditions. Four control cultures were held aerobically under like conditions of moisture and temperature. After 2 months of additional incubation the cultures were examined by subculture and by guinea pig inoculation. All cultures held anaerobically failed to grow in subculture and to induce any lesions of tuberculosis in guinea pigs. All cultures held aerobically gave positive results.

In a second experiment the growth of human tubercle bacilli was removed from the medium upon which it was grown and sealed in anaerobic tubes in the manner described above. Examinations of cultures were made after 20, 25, 30, 36, and 42 days. A control culture was positive after 42 days. All of 5 anaerobic cultures examined after 20 days and 1 of 2 examined after 25 days were positive. The remaining 14 cultures held anaerobically 25 to 42 days were negative in subculture and guinea pig inoculation. J.F.C.

248. Physiological Studies of Brucella. I. Quantitative Accessory Growth Factor Requirement of Certain Strains of Brucella. N. B. MCCULLOUGH AND LEO A. DICK, Univ. Texas, Austin. Jour. Infect. Dis., *71*, No. 3: 193–197. Nov.–Dec., 1942.

Accessory growth factor requirements were studied for one strain each of *Brucella suis*, *Br. abortus*, and *Br. melitensis*, all laboratory strains that grew aerobically. The basal medium was the synthetic medium described by Koser et al. (cf. JOUR. DAIRY SCI., *25*, No. 1: A6, 1942). *Br. suis* and *Br. melitensis* required thiamin and nicotinic acid for growth. The addition of calcium pantothenate aided in the initiation of growth of *Br. suis*. *Br. abortus* required thiamin and biotin. In the medium used in these experiments the quantities of the accessory growth factors recommended for maximum growth of Brucella organisms are thiamin 0.2 μg. per ml., nicotinic acid 0.2 μg. per ml., calcium pantothenate 0.04 μg. per ml., and biotin

0.001 μg. per ml. No difference was found when the accessory growth factors were sterilized in the medium and when they were sterilized separately by filtration and added to the sterilized medium. J.F.C.

249. Physiological Studies of Brucella. II. Accessory Growth Factor Requirement of Recently Isolated Strains of *Brucella abortus*. N. B. McCULLOUGH AND LEO A. DICK, Univ. Texas, Austin. Jour. Infect. Dis., *71*, No. 3: 198–200. Nov.–Dec., 1942.

Continuing their earlier studies and employing the same synthetic medium, the authors studied the growth factor requirements of 41 recently isolated strains of *Brucella abortus*. Strains requiring increased CO_2 atmosphere failed to grow on the synthetic medium even when thiamin, nicotinic acid, calcium pantothenate, and biotin were supplied in various concentrations. After acclimatization to aerobic conditions, 30 of the 41 strains grew when all 4 accessory factors were present. Twenty-three strains had an absolute requirement of only thiamin and biotin. Both nicotinic acid and calcium pantothenate enhanced the growth of some but not of all of these 23 strains. J.F.C.

250. The Necessity for the Standardization of Tuberculins for Cattle-Testing. JANET R. McCARTER AND E. G. HASTINGS, Univ. Wis. Jour. Bact., *45*, No. 1: 69. Jan., 1943.

More careful standardization of tuberculins of cattle would obviate some of the non-specific tuberculin reactions in cattle. Studies on humans indicated that the differences observed in the skin activities of tuberculin proteins of varying antigenicities seems to be purely quantitative; the number and size of reactions to a non-antigenic P.P.D. can be increased by increasing the dose. D.P.G.

251. *Streptococcus agalactiae* Agglutinins in Bovine Blood Serum and Whey. W. N. PLASTRIDGE AND LORNA CUNNINGHAM, Storrs Agr. Expt. Sta., Storrs, Conn. Jour. Bact., *43*, No. 6: 779. June, 1942.

The macroscopic slide test was employed to test bovine blood serum and whey for presence of *Streptococcus agalactiae* agglutinins. Titers of 1 to 3 or higher were considered indicative of infection. Examination of whey indicated insufficiently high titers to consider it successful for this form of diagnosis.

When applied to blood serum the test detected about 80% of the infected cows. Antigens of the 11 types of *Streptococcus agalactiae* are considered necessary in examination of cow's blood for agglutinins of this organism. D.P.G.

252. History of Brucellosis. ARTHUR PARKER HITCHENS, Dept. Pub. Health and Preventive Med., Univ. Pa. Med. Ann. Dist. Columbia, *12*, No. 2: 55–59. 1943.

A lecture. R.E.L.B.

FOOD VALUE OF DAIRY PRODUCTS

253. Experiments on Milk Anemia in Rats with Special Consideration of Sheep Milk Anemia. HUBERT VOLLMER AND GERDA-LIESE MELDE, Institut für Pharmakologie und experimentelle Therapie der Universität Breslau. Klin. Wochschr., *20*, No. 1: 17–19. 1941.

Rats were fed sheep, cow or goat milk exclusively for a long period. All 3 types of milk caused a hypochromic anemia. The anemia produced by goat or sheep milk was inhibited (hemoglobin in the sheep milk experiments), transitorily improved (erythrocytes in the sheep milk experiments) or extensively removed (goat milk experiments) if the diet of the milch sheep or milch goats was supplemented with carrots. When the rats with the sheep milk anemia were placed on a mixed diet, the recovery from the anemia could be hastened by supplementary feeding of carrot juice. The course of the sheep milk anemia was essentially more favorable than that of the goat milk anemia. The better general condition of the rats fed sheep milk is believed to be due to the higher content of fat and protein in sheep milk. R.E.L.B.

254. Influence of Cooking Utensils on Destruction of Vitamin C (Zeitschrift für Vitaminforschung, Berne, No. 1–2, 1942.) Arch. Ped., *59*, No. 586. Sept., 1942.

"Fleisch determined by titration the loss of vitamin C in milk, potatoes, kohlrabi, cauliflower and apricots caused by cooking in different types of kitchen utensils. A noticeable influence is already exerted by the length of time required until the boiling point is reached. The longer the time, the greater the destruction of vitamin C. Since pyrex has a poor heat conduction capacity, its destruction of vitamin C is greater than is the case in aluminum utensils. The destruction of oxydases by the cooking process does not prevent or reduce the destruction of vitamin C after the cooking process. This was ascertained over a period of 24 hours. When milk is heated in utensils of various materials for the same length of time, vitamin C destruction is least in pyrex; then follow aluminum, well tinned copper utensils, enamel ware, enameled cast steel and badly tinned copper. However if the utensils of various materials are heated with the same degree of doneness, poorly tinned copper, pyrex, enameled cast steel, double boiler and particularly the fireless cooker all destroy more vitamin C than does aluminum. This applied to all the aforementioned foods." (Jour. A.M.A.) H.P.

255. Antirachitic Action of Irradiated Milk (Kinderarztliehe Praxis, Leipsig, April, 1940). Arch. Ped., *58*, No. 10: 679. Oct., 1941.

"Sheer states that the antirachitic effects of irradiated milk have been partly forgotten and that confused ideas exist regarding irradiated milk. Many are of the opinion that irradiated milk has prophylactic but no therapeutic action. The author demonstrates the great antirachitic power of irradiated milk on the basis of new investigations. He reviews twelve cases of rickets occurring in different seasons of the year. Roentgenograms of five cases show that even severe forms of rickets are cured within a few weeks. The cure is not as rapid as when large doses of irradiated ergosterol are given. Feeding with irradiated milk has the therapeutic effect of the daily administration of about 6,000 international units. The children received the quantity of milk that corresponded to their age and their state of nutrition. They were given no other antirachitic treatment. That irradiated milk exerts no harmful effect has been proved by observations on 500 children. This milk is unchanged in odor and taste. Although the therapeutic action of irradiated milk has been demonstrated, its chief value is in the prophylaxis of rickets, because it can be proved for every child and the expense involved is small." (Journal A.M.A.) H.P.

MILK

256. A Simple Basis for the Evaluation of Fluid Milk of Different Fat Contents. D. R. CARPENTER, Salem, Va. A Mimeographed Report of Roanoke Milk Board.

The plan is based on the fact that both the fat and non-fat content of milk have value, and that the ratio of the value of the fat to the value of the skimmilk in equal amounts by weight, is constant at any given time in any given area, and that it varies only slightly even over long periods.

The values are based in terms of 4% milk and are applicable at the moment when the ownership passes from the producer to the distributor.

Conversion tables of value factors for different percentages of butterfat from 0% to 100% are given.

Examples for using same are given.

To show the validity of this method of valuation a "table of costs" is given showing the distributor's costs f.o.b. at his receiving platform for skim, 2% milk, 20% cream, 35%, and of butterfat, when 4% is purchased at prices varying from $1.60 to $4.40 per 100 pounds, and the producers are paid according to the "value-factors" given in the conversion table mentioned above.

The advantages cited for this system of evaluation are: (1) It evaluates the entire content of whole milk; the fat and the non-fat, or skim. (2) It is equitable for all producers whether they are producing milk of high or low

fat tests. (3) It is equitable for all distributors in a given area regardless of the quantity of certain milk products sold, because it tends to make the percentage of profit to the distributor uniform on all products handled. (4) It removes the need for a price differential to cover milk of different contents of fat. (5) It removes the necessity for having more than two classes of Grade A milk in a distributing plant. (6) It affords a scientific basis for the making of retail price schedules. (7) It is useful to producers, dairy herd improvement associations, and others, in judging the efficiency of dairy cows producing milk of different fat contents. (8) It is so simple in its operation that every producer and every distributor can understand it.

The second part of the report deals with evaluating fat and solids-not-fat. It takes into consideration that skim in the hands of the distributor is blended skim having an average concentration of solids-not-fat which would correspond to milk of the average fat test for the plant. So methods for evaluating solids-not-fat for both distributor and producer are given together with examples for their use.

For reference and comparison a "conversion table" of value factors having a distributor's differential of 0.017 is given together with a "table of costs" showing the cost of fat, skim, 2% milk, 20% cream and 35% cream when 100 lbs. of 4% milk costs range from $1.60 to $4.40. L.H.B.

257. Bacteriological and Practical Aspects of Paper Containers for Milk. M. J. PRUCHA AND P. H. TRACY, Univ. Ill. Agr. Expt. Sta., Bul. 495. 1943.

Three types of paper milk containers, Pure-Pak, Canco, and Sealright were studied. The study included the sanitary, physical and practical aspects of these containers.

Paper for use in making containers can be obtained almost free of bacteria; of 170 samples of paperboard studied, almost 60% had counts of less than 100 bacteria per gram and the average was 120. It was also found that paper undergoes self purification when stored in rooms where it dries.

A bacteriological examination made of 2,607 finished quart containers showed about 33% of them to be sterile, while only about 1% gave a count of more than one organism per milliliter of capacity. The highest count found was 5,300 for the container. The test for coliform bacteria was negative on all containers tested.

Pure-Pak containers were found not to be contaminated to any extent during the processes of finishing their fabrication at the milk plant.

The bactericidal action of paraffining was tested by contaminating containers and then examining them for bacteria after paraffining. Even when containers were heavily contaminated, only an occasional container showed the presence of the inoculating organism after paraffining.

In one test of 1200 containers handled in the usual manner except that the operators' hands were heavily contaminated by dipping them into a bacterial suspension, and the containers being paraffined for about 24 seconds at temperatures of 170°, 180° and 190° F., all containers were found to be free from the contaminating organism except one. Apparently the paraffin imprisons the bacteria and keeps them from getting into the milk.

The temperature of the paraffin regulates the amount that will adhere to the container; the higher the temperature of application the less retained.

That bacteria do not penetrate the walls or seams of paper containers was proven by submerging filled containers in a bacterial suspension for as long as 48 hours without any of the bacteria of the suspension being found in the milk.

The amount of moisture absorbed by the container when filled with milk varies with the time and temperature of storage. Heavy paper containers absorbed more moisture than light ones.

The temperature of milk changes more slowly in paper bottles than in glass.

Paper bottles stood as much as 80 pounds pressure before they leaked.

Paper containers offer some protection to the ascorbic acid content of milk and to the development of "burnt" or "sunshine" flavor when exposed to sunlight; the changes taking place less rapidly than when exposed in glass bottles.

Opinions of 300 customers concerning the relative merits of Pure-Pak and glass bottles are given as are those for 136 customers concerning the relative merits of Canco and glass bottles. The opinions are in favor of the paper bottle over the glass.

"In the opinion of the consumers, as well as by actual plant tests, milk containers made from paper are practical. After four years of using glass and paper bottles at intervals, 95% of the consumers who returned questionnaires stated that they preferred the paper bottle." L.H.B.

258. The Bacterial Flora of Virginia Milk and Its Relationship to Standards in this State. F. S. ORCUTT, Va. Polytechnic Inst. Jour. Bact., *44*, No. 1: 145. July, 1942.

Abs. Proc. Local Branches. D.P.G.

259. Comparison of Methods for the Detection of Coliform Bacteria in Milk. L. A. SANDHOLZER, A. WALKER AND M. STRONG, U. S. Pub. Health Service, Norfolk, Va. Jour. Bact., *44*, No. 1: 147. July, 1942.

Abs. Proc. Local Branches. D.P.G.

260. The Effect of Flash Pasteurization and Subsequent Treatment on the Phosphatase Value of Cream. W. H. BROWN AND P. R. ELLIKER, Purdue Univ. Jour. Bact., *43*, No. 1: 118. Jan., 1942.

Comparable results on phosphatase value of cream were obtained with the laboratory tests of Scharer and with Neave's modification of the Kay and Graham method when vat or holder pasteurized cream was examined. However, when the flash systems of pasteurizing were used, considerable variation in phosphatase value of the cream occurred. In many cases the value changed from negative to positive following storage of the cream at a low temperature. Phosphatase value for salted and the respective unsalted cream were similar when the Scharer method was used, but with the modified Kay and Graham method addition of NaCl and subsequent storage resulted in a change from negative to positive. This difference was more apparent in samples stored at higher temperatures. D.P.G.

261. Irradiation of Skimmed Milk (Zeitschrift fur Kinderheilkunde, Berlin, Aug. 9, 1940). Arch. Ped., *58*, No. 9: 577. Sept., 1941.

"Sheer points out that a considerable portion of the total cholesterol content of milk is combined with the proteins, particularly lactalbumin. He deduces that the antirachitic power of irradiated milk is not limited to its fat content. Thus it seems of interest to determine whether skimmed milk can be rendered antirachitic by irradiation. From comparative experiments with irradiated whole milk and irradiated skimmed milk, he learned that irradiated skimmed milk has an antirachitic action which will cure a severe florid rickets, although time required for such cure is somewhat longer than with the use of irradiated whole milk." (Jour. A.M.A.) H.P.

262. Effective Public Relations in a Local Market. T. KLINE HAMILTON, Diamond Milk Products, Columbus, Ohio. Internal. Assoc. Milk Dealers Assoc. Bul., *35*, No. 4: 51–61. Jan., 1943.

A proper balance between producer prices, consumer prices, employee rates and dealers margin makes for good public relations. The relations among these groups, viz., within the industry, also are vital to good public relations. Laying plans with the current trend, which is a greater participation by various groups in the determination of milk business policy, is wise. It is clear that we must tell and retell the story of milk to the public. Keep all groups fully informed of changes in policy so as to build public confidence and prevent public relations problems from developing.

E.F.G.

263. Wartime Sugar Replacement Problems. J. F. ERB, Ohio State Univ., Columbus, Ohio. Internatl. Assoc. Milk Dealers Assoc. Bul., *35*, No. 3: 39–47. Jan., 1943.

It is suggested that the balance between sugar and chocolate is more important than the actual percentage of the individual constituents. That 1.5% of a certain cocoa with 6% sugar gave about the same final flavor as .75% cocoa and 4.75% sugar. The high solids content of ice cream tends to mask flavors so that nearly three times as much sugar and cocoa are needed as in chocolate milk to give the same intensity of flavor. Consumer preference tests by the author indicate chocolate milk cannot be reduced below 5% sugar and still be sweet enough. In substituting other sweeteners for sucrose 50% replacement is the safe maximum if unfavorable results are to be avoided.

The following table shows the percentage of each sweetener to use in replacement to get the same degree of sweetness in the chocolate milk.

Equivalent values of various sweeteners in chocolate milk drink

Sweetener	No. of lbs. of sweetener equal to 1 replaceable lb. of sucrose	Percentage to use with ½ sucrose replaced		Percentage to use with ¼ sucrose replaced	
		% sucrose	% other sweetener	% sucrose	% other sweetener
Sucrose	1.0	5.0	0.0	5.0	0.0
Dextrose	1.4	3.8	1.7	2.5	3.5
Standard corn syrup	2.6	3.8	3.1	2.5	6.5
Corn syrup solids	2.2	3.8	2.6	2.5	5.5
High conversion corn syrup	2.2	3.8	2.6	2.5	5.5
Malt syrup	1.3	3.8	1.5	2.5	3.2
Honey	1.2	3.8	1.5	2.5	3.0

Dextrose is the only sweetener giving good results in 100% replacement. Its cost exceeds sucrose so that it likely would not be used when sucrose is plentiful. E.F.G.

264. Transportation. J. B. EASTMAN, Director of Defense Transportation, Washington, D. C. Internatl. Assoc. Milk Dealers Assoc. Bul., *35*, No. 2: 27–35. Dec., 1942.

Pooled or cooperative deliveries can produce maximum conservation. Collective action taken for the sole purpose of conserving motor vehicles does not constitute a violation of the laws. Where uniform conditions of delivery hove not been set up in a particular market then attention should be given to that at once. It is suggested that county transportation plans be worked out through producers, motor vehicle operators, processors and other groups. When such a plan is submitted to O.D.T. it will be submitted to the Department of Justice to examine for possible anti-trust violations. If approval is given to the plan a local administrator will be appointed and all details with respect to its operation will be handled locally.

To deal rapidly with more serious shortage conditions, General Order No. 21 with its Certificates of Necessity has been put into effect. This specifies to each operator maximum mileage and minimum load. The object is to reduce mileage and at the same time maintain essential services. E.F.G.

265. Address of President at the 35th Annual Convention. A. G. MARCUS. Internal. Assoc. Milk Dealers, Chicago, Ill. Assoc. Bul., *35*, No. 1: 3–21. Dec., 1942.

A report is given of the many ways in which the association has been actively cooperating with the federal agencies such as O.D.T., O.P.A., etc., during the past year in furthering the war effort concurrently with the prosecution of the normal activities of the association. Attention is called to the fact that milk is being recognized as the leading food in war food economy by the federal government and as such its production and distribution is a matter of great concern. Members of the association have been kept informed of developments touching the industry through the medium of the News letter.

Progress is reported on the revised Laboratory Manual, and the new Plant Manual now being prepared.

More knowledge of the very serious widespread deficiency of calcium in the diet of the American people and the serious results therefrom, suggest that the food value of milk should be emphasized from this angle. The calcium deficiency in from 50 to 70% of the population is serious. This deficiency results in nervous disorders since calcium is the dominant nerve center controller. Emphasis upon the large calcium content of milk would be of especial value at a time of more restricted diets due to war conditions.

In promoting the use of milk by those who do not now use it in sufficient quantities for health it should be kept in mind that as revealed by a recent Chicago survey that from 43 to 47% of the people definitely do not like milk. Many others were found who do not understand the importance of milk to health and mental well being. To reach the above groups is a matter for serious consideration. E.F.G.

266. Your Days May be Numbered in the Milk Business by the Service Left in Your Present Trucks and Tires. A. E. FRIEDGEN. Milk Dealer, *32*, No. 5: 26–27, 64–66. Feb., 1943.

The conservation of trucks, tires and gasoline is essential to preservation of business life in the milk industry. Waste in any or all parts of delivery equipment means possible curtailment of jobs, and of continuation in business aside from monetary losses since many parts are irreplaceable. Directions for maintaining operating efficiency in every phase of truck operation are listed and delivery Do's and Don't's listed together wth mileage and

gasoline, savings as effected by various speeds and methods of truck operation are given which, if observed, may help a milk plant "live to see our victory" and to continue its business of milk delivery to consumers.

C.S.T.

267. Machinery Maintenance in War Time. E. H. FORSTER, Cherry-Burrell Corp. Milk Dealer, *32*, No. 4: 22–23, 56–57. Jan., 1943.

"Machinery maintenance is actually a battle against time" and means daily care and a planned program for both large and small plants. Basic requirements for all plants are (1) definite assignment of responsibility for maintenance of each piece of equipment for daily peak efficiency; (2) regular and thorough inspection under a follow-up filing system for each machine; (3) organization of all data by plant superintendent in order to replace all parts and to train new operators when either are needed; (4) list all local repair facilities in case of emergency breakdown for special parts, particularly electrical equipment and (5) enlist the aid of trained dairy machinery specialists from the dairy machinery manufacturers. C.S.T.

268. Improper Layout and Installation of Piping Invites Trouble. C. T. BAKER, Atlanta, Ga. Milk Dealer, *32*, No. 3: 74. Dec., 1942.

The importance of proper plant layout in pipe installation is stressed together with relationship of pipe size to pump size, the directness of lines, parallel and series flow, suction and discharge lines and location of valves. Concrete examples of economies effected through changes in pipe layout and installations whereby losses in power, capacity and revenue in plant operation are cited. C.S.T.

269. Selling Now to Insure Business Tomorrow. C. W. ESMOND, G. P. Gundlach and Co., Cincinnati, Ohio. Milk Dealer, *32*, No. 3: 80. Dec., 1942.

Instances of a number of continued advertisements by large concerns with no merchandise to sell are cited. Demand for milk may well exceed the supply but the need for keeping a milk concern's name, prestige and integrity before the public is stressed if that concern is to survive. C.S.T.

MISCELLANEOUS

270. New Swedish Yeast. ANON. Food Mfr., *18*, No. 3: 84. March, 1943.

A new substitute for extract of meat will appear on the market soon. It is a yeast produced from sulphite lye. Trials carried on over six months have been entirely successful. A general description of the process is given.

J.C.M.

271. Evaporative Drying Systems. FRANK H. SLADE. Food Mfr., *18*, No. 3: 70–74. March, 1943.

Drying of perishable foodstuffs is discussed. The theory of drying is also explained. Temperature, humidity and air motion for different foods are listed and tables are given. Methods of drying and the driers are discussed and explained by the use of illustrations and tables. The general design for drying is given. The article is too detailed to abstract completely. Principles given apply to dairy foods. J.C.M.

272. Packaging for Army Rations. ANON. Food Mfr., *18*, No. 3: 90–91. March, 1943.

Captain Melson Q.M.C. presents the ideas in this article. The problems of packaging are discussed concerning Army rations which must withstand temperatures from 20° below zero to 130° above or higher. Rains, high humidity, etc., all add to the problems. The food supply is very important and even a small percentage of spoilage may be a hazard.

Capt. Melson wants a fibre shipping case as strong and resistant to water and humidity as wood. Several ideas on this need are listed, along with some on protection from vapor. Cellophane and other films are discussed.

A search for the protection of flour, cereals, etc. from insects is in progress. Dried products also present a problem. They need protection from infestation.

Watertight and water resistant papers are of interest at the moment; also fibre bodied cans. It is also desirable to employ shipping cases which are proof against rats. Much information and suggestions are given in this article by Capt. Melson that should constitute a useful guide for those concerned with the technical side of packaging research. J.C.M.

273. Food Research of Tomorrow. ANON. Food Mfr., *18*, No. 3: 83–84. March, 1943.

This article is an abstract of another article. Nutrition is the topic and its relation to health. Supply, sanitation and palatability are the aims of the researchers. Each change in research must be measured by nutrition. Studies will concern themselves with practical problems. Four agencies of research are listed and each one has a special job to do. Each one is explained in detail. J.C.M.

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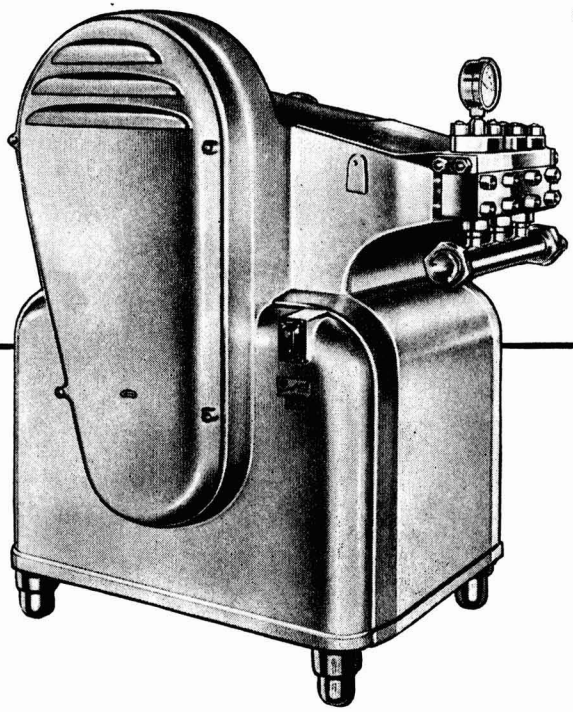

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