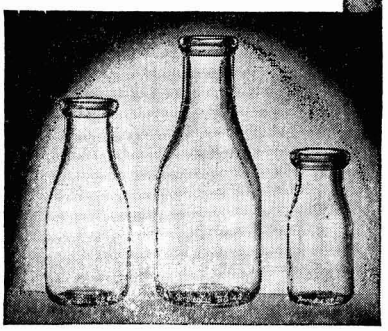
JOURNAL OF DAIRY SCIENCE

Contents

Vol. XXIX, No. 5, May, 194

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATIO



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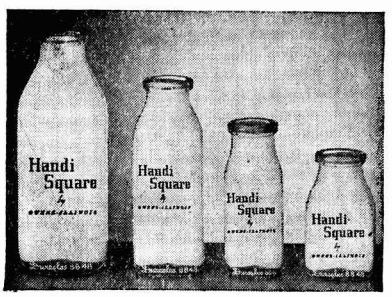

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Published at
NORTH QUEEN ST. AND MCGOVERN AVE., LANCASTER, PA.

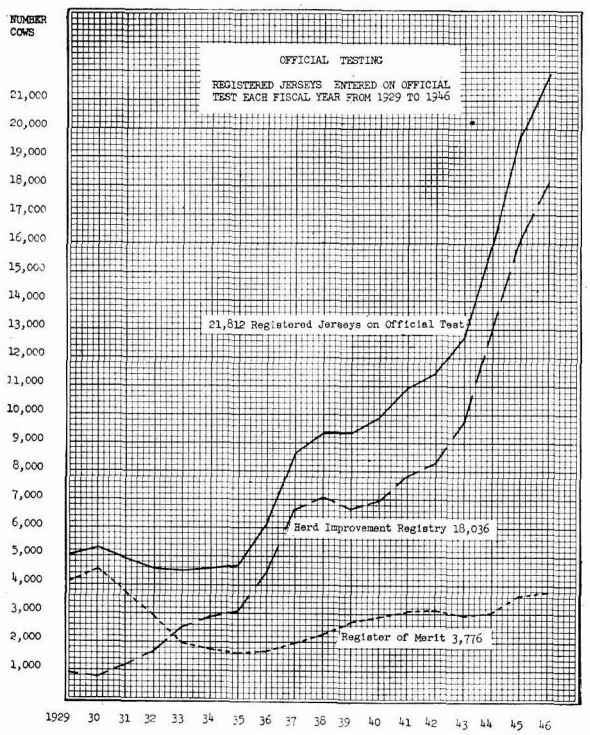
Subscriptions. Price; $6.00 per volume in North and South America; $6.50 in all other countries. Prices are net, postpaid. New subscriptions and renewals are entered to begin with the first issue of the current volume. Renewals should be made promptly to avoid a break in the series. Subscriptions should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Subscriptions for the British Isles and British Empire, except for Canada and Australia, should be ordered through our agents: Messrs. Bailliere, Tindall and Cox, 7 and 8 Henrietta Streets, Convent Garden, London, W. C. 2, England. Subscriptions for Australia should be sent to our agent: John H. Bryant, Herbert St., St. Leonards, N. S. W., Australia.

Advertising should be mailed direct to the Science Press Printing Company, N. Queen St. and McGovern Ave., Lancaster, Pennsylvania.

Post Office Notices of undeliverable copies and changes of address should be sent to R. B. Stoltz, The Ohio State University, Columbus, Ohio.

Entered as second-class matter April 13, 1934 at the postoffice at Lancaster, Pa., under the act of March 3, 1879.



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JOURNAL OF DAIRY SCIENCE

VOLUME XXIX MAY, 1946 NUMBER 5

LIVE WEIGHT VERSUS METABOLIC BODY SIZE IN DAIRY COWS AND GOATS

W. L. GAINES

Illinois Agricultural Experiment Station, Urbana

Received for publication January 5, 1946.

Kleiber and Mead (12) have recently published in this Journal a vigorous[1] critique of the FCM_8/W_1 philosophy. (FCM_8 is milk-energy yield for the first 8 months of lactation, W_1 is live weight within 31 days after parturition of the same lactation, both FCM_8 and W_1 to be measured at each lactation of each cow or goat.) See also their (11) earlier better-tempered critique on the same subject. In regard to the metabolic body size philosophy they state: "This theory, suggested by Kleiber, was tested and confirmed by Brody and his co-workers." The reference here is presumably to Kleiber's (8) masterly paper on body size and metabolism, and his (9) later paper on size of animal and food utilization.

WHAT IS METABOLIC BODY SIZE?

The following two paragraphs are quoted from Kleiber (10):

[1] In some respects their paper shows more vigor than perception. Thus, in their criticism of a short article (3) they dwell on an incidental point in the article and apparently fail to perceive the specifically stated major point, namely that when the equation, $FCM = aW^b$, is used to describe the regression of milk-energy yield on live weight the value of b varies greatly according to the stage of lactation at which live weight is measured (cf. 2, table 1). They present their California data without stating the stage of lactation at which live weight was measured. The FCM_8/W_1 philosophy accepts W_1 as a proper and sufficient measure of size of the cow or goat. It is, of course, their right not to accept that view but failure to designate the stage of lactation at which live weight was measured in their data indicates lack of perception as regards a very important detail. The article (3) in question is based on 11 records and as they rightly contend (and as is recognized in the article) the considerable inherent and environmental variability in milk-energy yield as between cows and lactations renders unreliable (but by no means necessarily false) for general application the fact that in these 11 records FCM_8 is, by the fitted equation, proportional to the 1.07 power of W_1. A similar criticism does not apply to the main comparison for the simple reason that it deals with the same milk-energy yields, only the stage of lactation at which live weight was measured being variable. In general Kleiber and Mead's point concerning variability in milk-energy yield, as between cows, is very important. Of coordinate significance is the variability as between lactation of the same cow, the import of which is not generally and fully appreciated.

. . . An animal may be thought of as a group of equal size-parts. These parts are not thought of as structural elements such as cells or moleculars but as abstract units. By analogy with the engineers' method of measuring motors in number of horse power, biologists can express the size of animals in units of power e.g. as kilocalories of standard metabolism per day which may be abbreviated to "Calsad". . . .

. . . Since the standard metabolism in kilocalories of various animals from rat to steer can be predicted as 72 times the $\frac{3}{4}$ power of body weight in kilograms the number of Calsads in an animal is proportional to the $\frac{3}{4}$ power of its body weight. . . .

From this it appears that metabolic body size is predicted standard metabolism or estimated basal metabolism, and kg.$^{\frac{3}{4}}$ is estimated basal metabolism per day in units of 72 kilocalories. Metabolic body size is not size in the sense of weight.[2] It is size in the sense of metabolism, under the conditions

[2] Brody in his recent invaluable volume uses the term "metabolically effective body weight" (1, p. 383) which he defines as proportional to the 7/10 power of live weight. He states explicitly that ". . . a 1200-lb. cow cannot produce ten-fold the milk energy of a 120-lb. goat at its upper limit, and a 1400-lb. cow cannot produce twice the milk energy of a 700-lb. cow at its upper limit, because large animals have relatively larger supporting structures and, therefore, relatively smaller visceral organs and areas than small animals," (1, p. 856). He explains, by dimensional analysis, that for animals to possess stability the supporting structures must be proportional to the 3/2 power of live weight, or approximately so.

Bone is a typical supporting structure. Mitchell, Card, and Hamilton (13) have published experimental data on live weight and weight of bone in chickens during the course of growth as per the following figures:

Cockrels									
W, gm.	232	449	673	993	1361	1786	2236	2583	3253
Bone, %	16.8	16.0	16.2	18.6	17.9	18.5	18.0	16.2	15.7
Capons									
W, gm.	.....	.....	.....	.....	1375	1702	2285	2684	3188
Bone, %	.....	.....	.....	.....	17.3	18.7	17.1	16.4	15.6
Pullets									
W, gm.	.....	.....	.....	961	1342	1842	2340	.....	.....
Bone, %	.....	.....	.....	16.8	16.7	14.5	14.2	.....	.....

These data indicate no tendency for bone percentage to increase with live weight within sex. But according to Brody's explanation bone percentage must be approximately proportional to $W^{\frac{1}{2}}$. By direct observation bone percentage in a 4-lb. pullet is 15. According to Brody, bone percentage in a 1600-lb. cow would have to be 300 (that is, $15 \times 1600^{\frac{1}{2}}/4^{\frac{1}{2}} = 300$). This absurdity raises a question about the validity of his explanation. His metabolically effective body weight is not weight, but, like Kleiber's metabolic body size, is simply predicted basal metabolism.

When we fit the equation, $Y = aW^b$, to a set of observed Y's and W's we systematically distort W by the exponent b so that W^b is proportional to Y, as near as may be. We must think of W^b not as an actual measurement in units of weight, but as a predicted measurement in units of Y (Y = metabolism in calories, lactating maintenance in lb. DN, milk-energy yield in lb. FCM, or the like). Thus, to say that kg.$^{\frac{3}{4}}$ is the unit of metabolic body size is to say that predicted basal metabolism in kilocalories per 20 minutes is the unit of metabolic body size. If we talk in terms of W^b we must not think in terms of weight. In general, in the fitted equation, $Y = aX^b$, "units" of X^b are units of estimated Y, the unit of estimated Y being a times the original unit of measurement of Y. In the sense of quantitative science, X^b, where b is not unity, has no units except in terms of estimated Y.

prescribed for the measurement of basal metabolism, predicted from live weight by means of an interspecies equation.

Table 1 provides an easy means of finding the numerical value of kg.$^{\frac{3}{4}}$, where live weight is given in lb., no fractions. Table 1 is presented as superior in design and execution as compared with Kleiber and Mead's (12) table 2. As to execution, among the 101 entries of kg.$^{\frac{3}{4}}$ in their table 2, 13 are erroneous, namely at 680, 740, 870, 950, 990, 1330, 1400, 1430, 1460, 1490, 1520, 1550, 1580 lb. (In a later edition of their table, Hilgardia, vol. 16, p. 571, the error at 1400 lb. has been corrected.) In magnitude the errors

TABLE 1

Table for reading metabolic body size, in kg.$^{\frac{3}{4}}$ to closest whole number, where live weight is given in lb., no fractions

	0	1	2	3	4	5	6	7	8	9
1	45	51	58	64	71	78	86	93	101	108
2	116	124	132	141	149	157	166	175	183	·192
3	201	211	220	229	239	248	258	267	277	287
4	297	307	317	327	338	348	359	369	380	390
5	401	412	423	434	445	456	467	478	490	501
6	513	524	536	547	559	571	583	594	606	618
7	630	643	655	667	679	692	704	716	729	742
8	754	767	780	792	805	818	831	844	857	870
9	883	896	909	923	936	949	963	976	990	1003
10	1017	1031	1044	1058	1072	1086	1100	1113	1127	1141
11	1155	1170	1184	1198	1212	1226	1241	1255	1269	1284
12	1298	1313	1327	1342	1356	1371	1386	1400	1415	1430
13	1445	1460	1475	1490	1505	1520	1535	1550	1565	1580
14	1596	1611	1626	1641	1657	1672	1688	1703	1719	1734
15	1750	1765	1781	1797	1812	1828	1844	1860	1876	1892
16	1907	1923	1939	1955	1972	1988	2004	2020	2036	2052
17	2069	2085	2101	2117	2134	2150	2167	2183	2200	2216

To use, find the largest tabular value which is not larger than the given live weight and read metabolic body size from the corresponding marginal figures at side and top of table. An efficient procedure is to arrange the W's in numerical order and divide the array into blocks at the division points shown by the table. Each of the W's in a given block then has the metabolic body size value indicated. For example, W's 1400–1414 inclusive each give metabolic body size 127.

are minor, but inexcusable because the relation 1 kg. = 2.2046 lb. is established and the kg.$^{\frac{3}{4}}$ relation is purely a matter of computation. As to design; their table is formulated by use of uniform intervals of 10 in lb., while the present table is formulated by use of uniform intervals of 1 in the values of kg.$^{\frac{3}{4}}$. The present design provides finer numerical distinctions than the design of their table. However, if live weight is given in 10-lb. units, no fractions, their table is handier to use (first, of course, correcting the 13 errors mentioned).

WHAT IS LIVE WEIGHT?

The FCM_8/W_1 philosophy regards live weight as a directly proportional measure of the amount of protoplasm or work-stuff in the lactating cow or

goat. This view is warranted by the data of Mitchell, Kammlade, and Hamilton (14) showing the weight of protein to be 13.6 per cent of live weight as an average of 6 shorn sheep (average live weight 77 lb.): and by the data of Trowbridge, Moulton, and Haigh (17) showing the weight of protein to be 14.2 per cent of live weight, as an average of 3 cows (average live weight 975 lb.). It seems permissible to say that the amount of protoplasm or work-stuff tends to be proportional to live weight of the two species. Live weight is, accordingly, estimated amount of protoplasm (work-stuff) in lactating cows and goats.[3]

An essential feature of the FCM_8/W_1 philosophy is that live weight at each lactation be measured shortly after parturition, that is, within 31 days (in accommodation to the system of monthly visits by the tester). Morgan and Davis (15) have published valuable detailed data showing the course of live weight with the course of gestation and lactation. (See 1, p. 431 for a graphic abstract of their data.) Clearly, the stage of the reproductive cycle at which live weight is measured should be prescribed: arbitrarily, if necessary; logically and usefully, if possible. W_1 is used in the FCM_8/W_1 philosophy in preference to W at some other stage for several reasons. Live weight may be regarded as a mixed measurement representing "pure size" plus fatness. It is well known that fatness at calving has a considerable influence on milk-energy yield. In a qualitative way that influence is properly recognized by dealing with FCM_8/W_1. Fatness is not properly recognized qualitatively by dealing with W at later stages. In addition to the logic of the case may be cited the experimental observation (5, p. 1041) that FCM_8/W_1 is independent of fatness at calving. It may be too much to say that a pound of fatness has the same influence on milk-energy yield as a pound of "pure size," but from the data available it appears the fatness influence is not less than that of "pure size," pound for pound.

FCM_8/W_1 VERSUS CAL.$_{10}$/METABOLIC BODY SIZE

Both these philosophies recognize milk energy as a proper biological measure of the quantity of milk yield. Both are expressed as an average per day, one in lb. 4 per cent milk for the 8-month partial lactation, the other in kilocalories for the 10-month partial lactation. Practically, calories may

[3] The experimental observations are not as direct or extensive as could be desired but the nature of the data lends confidence in the view that the weight of protein in lactating cows or goats is about 14 per cent of live weight. It is quite clear that the amount of protein does not follow Kleiber's $\frac{3}{4}$-power rule as between cows and goats. If a 975-lb. animal is 14.2 per cent protein, a 77-lb. animal would be 26.8 per cent protein by the $\frac{3}{4}$-power rule. The observations cited would not fail to detect a difference of that magnitude.

A large part of the body protein is represented by the muscle tissues, which are only indirectly involved in the work of lactation. The question arises, is the amount of protoplasm directly involved in the work of lactation proportional to live weight as between cows and goats or as between individuals within species?

be estimated as 1 lb. $FCM = 340$ kcal., hence the difference narrows to the length of partial lactation used.

Official records started off with a 7-day period and later swung to the other extreme of a 365-day period. Popular favor is now with a 305-day period. The 305-day yield, as between individuals is much less affected by the inevitable variability in length of service period (parturition to next conception) than is the 365-day period. In this respect a further advantage is gained by shortening the period to 243 days (8 months) and the FCM_8/W_1 philosophy simply utilizes that biological improvement.[4]

AN IMPORTANT DIFFERENCE

A very important difference in the two philosophies is that one deals with milk-energy yield per unit live weight (live weight measured within 31 days after parturition, without age distinctions), while the other deals with milk-energy yield per unit basal metabolism (basal metabolism estimated as proportional to the $\frac{3}{4}$ power of live weight, presumably average live weight for the 10-month period, with age confined to given lactations, such as first lactations.)[5] The one measures the metabolism of lactation (milk-energy yield) in terms of the amount of work-stuff (protoplasm) in the goat or cow; the other measures the metabolism of lactation in terms of the whole metabolism of the goat or cow under conditions of starvation and the lowest possible level of work. Figure 1 attempts to show graphically, in a diagrammatic way, the nature of the difference in the two philosophies.

EXPERIMENTAL EVIDENCE

Figure 1 may be considered as presenting two alternative postulates. The final test of any postulate is its conformity with the evidence of experimental observations. What experimental evidence is available bearing on the validity of the postulates?

In lactating cows and goats the direct and indirect work of milk secretion precludes the direct determination of basal metabolism because the condition of lowest possible level of work cannot be provided during lactation.

[4] Ordinarily yields are estimated from monthly 1-day determinations and FCM_8 is the average of 8 such determinations while FCM_{10} is the average of 10. Other things equal, an average of 10 is better than an average of 8, but study of individual lactation curves suggests that for biological comparisons, use of the 8-month period gains more by avoiding complications of advanced pregnancy than is lost by disregarding the ninth and tenth monthly tests. Compare figure 17 of Jensen *et al.* (7) and their finding that beyond 36 weeks after calving in the lactation curves of their critical Input-Output investigation, ''. . . pregnancy rather than feed is the dominant factor in controlling milk production.'' For biological comparisons between individuals as to quantity of lactation, a rational procedure seems to be to simply side-step pregnancy complications by dealing with FCM_8. However the difference between FCM_8 and FCM_{10} is a minor rather than a major matter.

[5] Kleiber and Mead do not clearly define their philosophy with regard to these rather important details.

It is feasible to determine lactating maintenance as a function of live weight by straightforward statistical procedure, the principle of which was used by Wood and Capstick (18) many years ago. By such procedure, it has been shown (4) quite conclusively that as between cows and goats lactating maintenance is substantially proportional to metabolic body size, but between

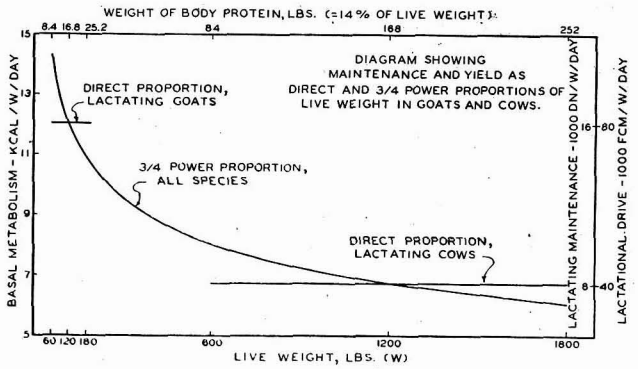

FIG. 1. Diagrammatic comparison of live weight and metabolic body size proportionality theories theories in goat and cow.

The regression lines represent the following equations:

Curved line, all species: basal metabolism in kcal./day = 72 kg.$^{\frac{3}{4}}$

Straight line, goats: lactating maintenance in lb. DN/day = 0.016 W

Straight line, cows: lactating maintenance in lb. DN/day = 0.008 W

Straight line, goats: milk-energy yield in lb. FCM/day = 0.08 W

Straight line, cows: milk-energy yield in lb. FCM/day = 0.04 W

Accepting the weight of body protein (scale at top of diagram) as proportional to live weight, and weight of protoplasm, or amount of work-stuff, as proportional to weight of protein, these relations may be expressed in terms of amount of protoplasm, or work-stuff, by merely changing the proportionality constant.

To make the comparison vivid, basal metabolism, lactating maintenance, milk-energy yield, are presented per unit live weight, plotted against live weight. Inclusion of mice weighing 0.025 lb. to 0.075 lb. makes the comparison still more striking. On the scale of the figure the basal metabolism line (curved line) would be practically vertical and indistinguishable from the ordinate at $W = 0$. At $W = 0.025$ basal metabolism per lb. live weight is 100; at $W = 0.05$ it is 84; at $W = 0.075$ it is 76. The corresponding horizontal line would be microscopic in length.

The impressive fact is the great difference in intensity of metabolism of the protoplasm, as between species, according to the mean size (live weight) of the species. Values of metabolism per unit live weight keep that difference before us; values of metabolism per unit metabolic body size conceal the difference from us.

individuals within either one of the species lactating maintenance is proportional to live weight (4, fig. 3). That is, as concerns lactating maintenance in cows and in goats the two horizontal lines in figure 1 conform to the experimental evidence; the curved line (metabolic-body-size theory) does not conform to the experimental evidence. Unless the observations or

method of analysis can be shown inadequate or invalid it must be concluded that the metabolic-body-size theory with respect to lactating maintenance within cows or within goats is false (but true as between the two species).

It may be legitimate to reason from the known relation between live weight and lactating maintenance in cows or in goats (that is, within species), to the unknown relation between live weight and basal metabolism within species during lactation. There is no need to take that step, however, because it is lactating maintenance and not basal metabolism that is of practical concern (in such matters as feeding standards, efficiency formulas and the like). On the theoretical side it is clear, for lactating cows and goats, that if basal metabolism, within species, is proportional to the $\frac{3}{4}$ power of live weight then lactating maintenance, within species is not proportional to basal metabolism.

The above weight-maintenance relations are based on average live weight, rather than W_1. For reasons above mentioned, it is desirable in considering the weight-yield relation to deal with W_1.

LACTATIONAL DRIVE

This appellation is one of Dr. Brody's apt expressions. The words seem to fit the extraordinary ability of dairy cows and goats to produce milk under the conditions provided on commercial dairy farms. This ability has been developed far beyond the point of maximum good with respect to natural species survival value, until it has become parasitic in nature, beneficial to man rather than the animal. The extraordinary yields require extraordinary energy transformations or levels of metabolism. It seems appropriate to designate the characteristic with a descriptive term.

Lactational drive is quantitatively measured by FCM_8/W_1, which is lactation metabolism per unit time per unit weight of live protoplasm (workstuff), with fatness at calving recognized and discounted. As thus measured lactational drive is confirmed as a real characteristic of dairy cows by the demonstration of pure lines (Nebraska-herd data to be presented from that Station at a later date). It appears that lactational drive is real, objectively measurable as FCM_8/W_1, and highly important in the practical and technical aspects of milk production.

Relative lactation capacity, as used by Kleiber and Mead, is measured by $kcal._{10}/kg.^{\frac{3}{4}}$, which is lactation metabolism per unit time per unit basal metabolism, with fatness not specially recognized as influencing milk yield. It is the ratio of metabolism during lactation to metabolism during starvation and lowest feasible, temporary, level of activity.

The two measures are very different in concept. They are, however, quite similar numerically. In fact Kleiber and Mead (12) generalized that within one herd " . . . one would need the results from more than 500 cows to show a barely significant difference (random probability 5 per cent) between production rate per unit weight and per unit of metabolic body size. . . . "

STATISTICAL SIGNIFICANCE

Davis *et al.* (2) found in 367 lactations of 147 Holstein cows, by the fitted equation, $FCM_8 = aW_1^b$, that FCM_8 is proportional to the 1.01 power of W_1. Kleiber and Mead's generalization, above quoted, would indicate that the numbers are far too small (147 cows whereas more than 500 are required) to give statistical significance to this result as contradicting their metabolic body size theory of milk-energy yield. The result is statistically significant, as shown by the following approach.

TABLE 2

Number of lactations required to show a statistically significant difference in the validity of the two postulates, FCM_8/W_1 is independent of W_1 and $FCM_8/kg.^{\frac{3}{4}}$ is independent of W_1, as indicated by selected cases

Number of lactations (n)	367	171	33
Number of cows	147	81	8
Breed	Holstein	Jersey	Holstein*
Mean W_1, lb.	1348	894	1423
Smallest W_1, lb.	934	596	1072
Largest W_1, lb.	1854	1180	1716
$FCM_8 = aW_1^b$, value of b	1.01	0.96	0.73
Mean 1000 FCM_8/W_1, lb./day†	39.1	34.5	42.1
Mean 100 $FCM_8/kg.^{\frac{3}{4}}$, lb. day†	43.0	34.1	46.6
Coefficient of variation in FCM_8	20.0	20.6	12.9
Coefficient of variation in FCM_8/W_1	16.0	16.4	10.2
Coefficient of variation in $FCM_8/kg.^{\frac{3}{4}}$	16.3	16.5	9.8
Regression of 1000 FCM_8/W_1 on W_1 (b)†	+ 0.0001	− 0.0022	− 0.0080
Standard error (SE_b)	± 0.0020	± 0.0039	± 0.0046
Regression of 100 $FCM_8/kg.^{\frac{3}{4}}$ on W_1 (b)†	+ 0.0075	+ 0.0084	− 0.0006
Standard error (SE_b)	± 0.0022	± 0.0039	± 0.0051
Probability for FCM_8/W_1‡	0.96	0.58	0.08
Probability for $FCM_8/kg.^{\frac{3}{4}}$‡	0.0006	0.03	0.90

* Pure line with respect to FCM_8/W_1, being all the daughters of the Holstein bull No. 1H, each daughter with 2 or more lactations in the Nebraska herd. These 33 lactations are included in the preceding 367.

† Note: 1000 $FCM_8/W_1 = 100$ $FCM_8/kg.^{\frac{3}{4}}$ when $W_1 = 933$.

‡ Probability that zero regression might occur by chance, as judged by the particular set of experimental observations. The corresponding number of lactations, N, to give $P = 0.05$ (that is a random probability of 5 per cent) is approximated by the formula $N = 3.8\ (SE_b/b)^2 n$. (For $P = 0.01$ use 6.6 in place of 3.8.)

We have two postulates to be tested against the experimental observations: 1) FCM_8/W_1 is independent of W_1; 2) $FCM_8/kg.^{\frac{3}{4}}$ is independent of W_1. (For present purposes, in the metabolic body size postulate, FCM_8 is used instead of FCM_{10}, and $kg.^{\frac{3}{4}}$ is derived from W_1 by use of table1.) In this form the validity of each postulate can be readily tested. The test for postulate 1 is to determine, by least squares, the values of b and its standard error in the equation, $FCM_8/W_1 = a + bW_1$. If b is not more than 1.95 times as large as its standard error the postulate is valid within the 5 per cent statistical standard. A similar test for postulate 2 is afforded by the equation, $FCM_8/kg.^{\frac{3}{4}} = a + bW_1$. Table 2 presents data of the 367 Holstein lactations, together with data of two other selected cases.

From table 2, as judged by these 367 lactations, the chances are 96 in 100 that postulate 1 is valid, but only 6 in 10,000 that postulate 2 is valid. The statistical distinction between the two postulates is unequivocal.

By the formula given in table 2 the number of lactations that would provide statistical distinction at the 5 per cent level would be $3.8\ (22/75)^2\ 367 = 120$. At the same average number of lactations per cow this would require 48 cows—quite a different matter than 500.

Table 2 presents similar data for 171 lactations of 81 Jersey cows with a similar outcome. At the 5 per cent level the metabolic body size postulate would be invalid with $3.8\ (39/84)^2\ 171 = 140$ lactations (66 cows).

We turn now in table 2 to a special group of 8 cows representing a pure line with respect to lactational drive, and whose 33 lactations correspond closely to the metabolic body size theory of milk-energy yield.[6] The chances are 90 in 100 that postulate 2 is valid and only 8 in 100 that postulate 1 is valid. While postulate 1 is not invalid by the 5 per cent standard, a small increase in numbers would make it so, namely $3.8\ (46/80)^2\ 33 = 41$. Thus, as judged by these 33 lactations, 10 cows with 41 lactations would be sufficient in number to give statistical distinction as to the validity of postulate 2 and invalidity of postulate 1. The low numbers are associated with a low variability in FCM_8/W_1 ($CV = 10.2$).

From the cases of table 2, the indications are that if CV in $FCM_8/W_1 = 16$, CV in $W_1 = 12$, and the regression of FCM_8/W_1 on W_1 is zero the regression of $FCM_8/\text{kg.}^{\frac{3}{4}}$ on W_1 will be statistically significant at the 5 per cent level with 50 cows having an average 2.5 lactations. Reduction of CV in FCM_8/W_1 or increase of CV in W_1 may easily give statistical significance at the 5 per cent level with 10 cows and 40 lactations.

If the regression of $FCM_8/\text{kg.}^{\frac{3}{4}}$ on W_1 is zero, the regression of FCM_8/W_1

[6] This result is not to be construed as a general confirmation of the metabolic body size theory in pure lines, as indicated by the following data on the daughters of each of 5 bulls (the letter indicates the breed):

Bull. No.	*Daughters*	*Lactations*	*Mean* W_1	*Mean 1000* FCM_8/W_1	*Regression of 1000* FCM_8/W_1 *on* W_1
13A	5	19	1055	35.3 ± 0.89	$+0.0064 \pm 0.0085$
15G	8	17	1057	25.0 ± 0.90	-0.0060 ± 0.0082
1H	8	33	1423	42.1 ± 0.76	-0.0080 ± 0.0046
6H	13	35	1324	40.2 ± 1.07	$+0.0016 \pm 0.0061$
9J	9	27	897	35.4 ± 1.24	$+0.0091 \pm 0.0129$

Each of the 5 daughter groups behaves as a pure line with respect to FCM_8/W_1 in that comparison of variance between daughters and variance within daughter gives an F value below the 5 per cent level of significance or intraclass correlation is not significant statistically. The simple average of the 5 regression coefficients is $+0.0006$. That is, these 5 pure lines (3 different levels) on the whole support the FCM_8/W_1 philosophy and contradict the metabolic body size theory of milk-energy yield. The total number of lactations (131) is sufficint to make the result statistically significant.

on W_1 will be statistically significant in a similar way as in the preceding paragraph.

The present direct method of distinguishing statistically between the validity of the two postulates is quite feasible as concerns number of cows and lactations required and not subject to the practically prohibitive requirement of more than 500 cows as deduced by Kleiber and Mead in their generalization previously quoted. Their method is based on the difference in expected yield by the two postulates in comparison to the standard error of the difference. Results by one method do not contradict results by the other method because the two procedures do not measure the same thing. If FCM_8 proportional to W_1 and FCM_8 proportional to kg.$^{\frac{3}{4}}$ (metabolic body size) are regarded as two alternative working postulates as to the relation between live weight and milk-energy yield the present method of testing validity seems preferable to that of Kleiber and Mead.

EXPERIMENTAL EVIDENCE ON GOATS

Data from specific determinations of FCM_8 and W_1 are almost completely lacking for goats. The best data available are those of the London Dairy Show (16) which afford accurate information on live weight and milk-energy yield during a 48-hour period, occurring on the average and most frequently in the seventh month of lactation. For the whole body of 318 records FCM is proportional to the 0.91 power of live weight, by the fitted equation, $FCM = AW^b$. This relation may not correctly represent an unselected population because small goats had to compete on a par with large goats in absolute yield, which introduces a material bias in terms of FCM/W. That is, in terms of the competition a small goat would need a large FCM/W to equal a large goat with a small FCM/W. Bearing that bias in mind these records definitely contradict the metabolic body size theory of milk-energy yield in goats.

To carry out a comparison like that of table 2, the records (20) of 14 goats of the British breed are selected. In these 20 records the regression of 1000 FCM/W on W is $+0.002 \pm 0.075$ (probability of zero regression, 0.98) and the regression of 100 FCM/kg.$^{\frac{3}{4}}$ on W is $+0.064 \pm 0.049$ (probability of zero regression, 0.19). Postulate 1 is valid, but postulate 2 is not invalid by the 5 per cent standard. Judged by these 20 records postulate 2 would be invalid if the number of records was increased to $3.8\ (49/64)^2\ 20 = 45$. This outcome again indicates that statistical distinction of validity as between postulates 1 and 2 does not require anything like 500 cows or goats where one or the other postulate conforms closely to the observations.[7]

[7] In case 3 of table 2 a low number of lactations (41) was indicated as sufficient to distinguish at the 5 per cent level as between the two postulates, and this was associated with a low variability in FCM_8/W_1 ($CV = 10.2$). In these 20 records of goats of the British breed the low number (45) similarly required is associated not with low variability in FCM/W ($CV = 15.0$) but with high variability in live weight ($CV = 17.9$,

WEIGHT–YIELD RELATION BETWEEN AND WITHIN SPECIES

It has been noted above that lactating maintenance per unit live weight is distinctly higher (about 100 per cent higher) for goats than for cows, but within either one of the two species, it is independent of live weight. That is, in the diagram of figure 1 the two horizontal lines correctly represent the observations and these relations may be accepted as experimentally established with respect to lactating maintenance per unit live weight.[8]

With respect to milk-energy yield per unit live weight the case is similar to that with respect to lactating maintenance per unit live weight, provided FCM_8 is used as the measure of yield and W_1 is used as the measure of live weight. However, the weight–yield relation is subject to more inherent and environmental variability than is the weight–maintenance relation. Thus, in footnote 6 we have pure lines ranging in 1000 FCM_8/W_1 from 25.0 to 42.1. It is unlikely that there is any such inherent difference with respect to lactating maintenance per unit live weight among these same cows.

Variability in FCM_8/W_1, as between animals, limits the assignment of very definite values but it appears that, as a species average, FCM_8/W_1 is about twice as high in goats as in cows.[9] Like lactating maintenance per

range = 107–250 lb.). An important advantage of the FCM_8/W_1 philosophy is that FCM_8/W_1 is substantially independent of age. The influence of age on FCM_8, independent of W_1 is practically negligible (cf. 2, fig. 5; 5, fig. 6; and 6, p. 16). In the use of FCM_8/W_1 age may be disregarded, which is not only an advantageous simplification but also provides greater range in live weight. This greater range in live weight, as just pointed out for the above 20 goat records, contributes to solution of the weight-yield relation. Kleiber and Mead (11, 12) limit their California Holstein and Jersey data to first lactations and this limits both the number of lactations and the range of live weight.

[8] This statement is warranted by more than one thousand results from carefully conducted feeding trials which consistently show that lactating maintenance within cows or within goats is not proportional to predicted basal metabolism (metabolic body size, $kg.^{\frac{3}{4}}$), but is rather proportional to a power of live weight in excess of unity. Within cows or within goats the use of $kg.^{\frac{3}{4}}$ (basal metabolism) as a directly proportional measure of lactating maintenance is definitely discredited by the experimental observations. The following words of Benedict are quoted from Brody: "Basal metabolism is one thing. Energy-food requirements for maintenance is quite a different thing . . ." (1, p. 471). Evidently there is occasion to question the use of metabolic body size as a proportional measure of the energy needs for lactating maintenance as within cows and as within goats. By inference the question may be extended to include milk-energy yield. So far as concerns the present paper, the question is raised only as within cows and as within goats, not as between the two species. The weight-maintenance relation as concerns lactating cows and goats is distinctly different within species than it is between the two species (4, fig. 3). A similar statement seems to apply to the weight yield relation, although it is more variable and less definitely established.

[9] If the goat lives twice as fast and half as long as the cow the lifetime milk-energy yield and lactating maintenance needs would be proportional to their live weight by the FCM_8/W_1 philosophy. According to Rubner as cited by Brody (1, p. 679) each would generate 200,000 kcal. per kg. live weight during the life cycle. If $\frac{1}{2}$ of this is milk energy, a 50-kg. goat would have a lifetime yield of 5,000,000 kcal. and a 500-kg. cow,

unit live weight, milk-energy yield per unit live weight (FCM_8/W_1) conforms to the metabolic body size theory as between cows and goats. Does it also so conform within cows and within goats? Is the relation correctly represented by the two discontinuous horizontal lines (FCM_8/W_1 philosophy) of figure 1, or is it correctly represented by the one continuous curved line (metabolic body size philosophy) of figure 1? The answer to the question must be found in the evidence of experimental observations. Adequate data on FCM_8 and W_1, are lacking for goats but there is a considerable amount of such data on cows.

Dealing with those sets of data on FCM_8 and W_1 where the number of observations is large enough to afford reliability in the statistical sense and expressing the relation in the form, $FCM_8 = aW_1^b$, we have the following experimental evidence:

Source	*Breed*	*n*	$\overline{W}_1$	$1000\,\overline{FCM_8/W_1}$	*b*
Neb. Sta.	G	77	960	30.6	0.43
do	A	131	1031	33.3	0.84
do	J	171	894	34.5	0.96
do	H	367	1348	39.1	1.01
Ill. DHIA	J	163	814	36.5	0.98
do	H	255	1203	24.8	1.02

The Nebraska data (2) are all within one herd (at Lincoln). The Illinois data (6) from several herds, are brought within one herd with respect to *b* by statistical treatment.

Of these 6 sets of data, 1164 lactations: 4 sets, 956 lactations, conform very closely to the FCM_8/W_1 philosophy; 1 set, 77 lactations, conforms to neither philosophy although more closely to metabolic body size than to FCM_8/W_1; and 1 set of 131 lactations, falls about half way between the two philosophies. Clearly the experimental evidence supports the FCM_8/W_1 philosophy very well and the metabolic body size philosophy very poorly. It is desirable, of course, to have further experimental observations but the above data will correct the possible impression created by Kleiber and Mead (12) that the FCM_8/W_1 philosophy depends for support entirely on the 11 lactations mentioned above in footnote 1.

If there is a species (cow and goat) difference in FCM_8/W_1, associated with species live weight, as pictured in figure 1, is there a similar breed difference within cows? More specifically, is FCM_8/W_1 greater for Jerseys than for Holsteins? Evidence of the Nebraska data answers, no. Evidence of the Illinois data answers, yes. Clearly more evidence is needed. Is there any Experiment Station (or other) herd of Jerseys to exceed the Nebraska

50,000,000 kcal. If the life of the goat is 10 years, the yield would be 1470 lb. *FCM* per year; and if the life of the cow is 20 years, the yield would be 7350 lb. *FCM* per year. (Note: $1470/50 = 2 \times 7350/500$, or goat $= 2 \times$ cow.) These figures are presented not as being necessarily exact, but rather as representing a point of view.

Station Holstein performance of 1000 $FCM_8/W_1 = 39.1$, on a comparable basis, that is, all lactations of all cows over a term of 20 years? Kleiber and Mead's (11) data indicate such a possibility, even probability, for the California Station Jersey herd, but until the actual data are reported there is no way to be sure.[10]

SUMMARY

This article considers lactating maintenance and milk-energy yield, in cows and goats, as proportional to live weight versus proportional to metabolic body size (kg.$^{\frac{3}{4}}$).

It may be accepted as experimentally established that lactating maintenance per unit live weight as a species average is about twice as high in goats as in cows, or lactating maintenance is proportional to metabolic body size as between the two species. It is equally well established that the same relation does not hold within either one of the two species. Consistently, within species lactating maintenance is proportional to a power of live weight in excess (not significant) of unity.

The weight–yield relation is similar to the weight–maintenance relation, provided live weight is measured as W_1, age is not restricted, and milk-energy yield is measured as FCM_8. Within cows the weight–yield relation is more variable than the weight–maintenance relation, both inherently and environmentally. Validity of the two postulates as indicated by observed values of FCM_8 and W_1 is tested by determining, by least squares the value of b and its standard error in the equation, $FCM_8/W_1 = a + bW_1$ or $FCM_8/\text{kg.}^{\frac{3}{4}} = a + bW_1$. If one of the postulates conforms closely to the observations the other postulate will be invalid by the 5 per cent statistical standard with about 50 cows and 125 lactations in case of CV in $FCM_8/W_1 = 16$ and CV in $W_1 = 12$. These numbers may be easily reduced to 10 cows and 40 lactations if CV in FCM_8/W_1 is reduced or if CV in W_1 is increased.

Six sets of data on FCM_8 and W_1 are available on cows, each set with number of lactations large enough to give reliability in the statistical sense.

[10] Brody (1, p. 857) continues his repeated citations of a "700-lb." Jersey cow with 1000 $FCM_{12}/W = 101.6$. Taken at face value this is a phenomenal performance. (From what we know about feed requirements of lactating cows at such enormous yields this 700-lb. Jersey must have consumed an average of about 35 lb. of grain per day for 365 consecutive days!) He uses the case to show the improbability, for example, that any 2000-lb. Holstein can ever attain 1000 $FCM_{12}/W = 101.6$. That is, dealing with the highest individual Jersey and the highest individual Holstein, the FCM_8/W_1 philosophy is shown to be untenable, as he sees it. Such extremes are of great interest but hardly rule out the need of comparing herd averages with respect to FCM_8/W_1 for the Jersey and Holstein breeds. Years ago Eckles developed a famous herd of Jerseys at the Missouri Station. How does the average FCM_8/W_1 for the Missouri Jerseys compare with the Nebraska Holsteins on the basis of all lactations of all cows? The average life spans of Holsteins and Jerseys are approximately equal and by Rubner's philosophy (footnote 9) it may be anticipated their average FCM_8/W_1's are also approximately equal. Adequate experimental observations must answer the question.

Four sets support the FCM_8/W_1 philosophy almost perfectly; one set supports neither one of the two philosophies, but the metabolic body size philosophy less poorly; one set falls about half way between the two philosophies. The weight of experimental evidence as between cows distinctly supports the philosophy that FCM_8 tends to be proportional to W_1; and distinctly contradicts the metabolic body size theory of milk-energy yield (FCM proportional to kg.$^{\frac{3}{4}}$).

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THE EFFECT OF ROUTE DELIVERY ON THE FLAVOR, RIBOFLAVIN, AND ASCORBIC ACID CONTENT OF MILK

D. V. JOSEPHSON, L. H. BURGWALD, AND R. B. STOLTZ

Department of Dairy Technology, Ohio State University

It has been known for some time that riboflavin and ascorbic acid are readily destroyed by the sun's rays, but only recently has attention been called to this problem in milk (7, 8, 9, 10, 11, 12). The photolability of riboflavin in milk was demonstrated by Williams and Cheldelin (11), who observed as much as 64 per cent loss of this vitamin when milk was boiled in a light room for 45 minutes and only a 5 per cent loss when it was boiled in the dark. More recently Stamberg and Theophilus (9) found as much as 80 per cent loss of riboflavin when quart-size flint glass bottles were exposed to direct sunlight for six hours, while the same milk exposed in brown glass and paper bottles lost less than 10 per cent of its riboflavin. In a more recent paper, Stamberg and Theophilus (10) observed as much as 40 per cent loss after two hours exposure in quart-size bottles, while brown glass and paper containers gave good protection. Holmes and Jones (7) exposed one-half pint bottles of milk to known intensities of sunlight and obtained losses of riboflavin up to 85 per cent in two hours. On a dark rainy day they found about 10 per cent loss in one hour. Their data, in general, show that the loss of riboflavin increases with the intensity of the sunshine. These workers further observed that little ascorbic acid remained after 30 minutes exposure to sunlight.

Naturally, important losses of these vitamins, such as demonstrated by these workers, are significant if milk is permitted to remain on the customer's doorstep for long periods of time. Furthermore, since milk is probably the most important source of riboflavin in the average American's diet, everything within reason should be done to preserve this all-important vitamin. Ascorbic acid is present in rather important quantities in fresh raw milk, but during processing, distribution, and storage this vitamin, too, is frequently depleted to a great extent. Many foods in the normal diet contain rather liberal quantities of vitamin C so its loss is of less importance from the nutritional point of view.

It has been demonstrated that light, particularly direct sunlight, causes a flavor defect in milk which has been variously described as the "sunlight," "activated," or "burnt" flavor (1, 2, 3, 4, 5). This defect has been a matter of considerable concern among distributors who have been confronted with numerous complaints and "returns" of milk having this off flavor. It would seem logical that milk which is subjected to light for a period of time suffi-

Received for publication January 19, 1946.

cient to produce the "sunlight" flavor, would also suffer a considerable loss of riboflavin and ascorbic acid.

It is generally assumed that when the routeman leaves a bottle of milk on the customer's doorstep, the job of the distributor is done. Whether this should or should not be the case, the fact still remains that there exists a very critical period between the time the milk is placed on the doorstep and the time it is taken into the home and placed in the refrigerator. This period of exposure to light, if sufficiently long, could convert an otherwise good quart of milk into one of inferior quality.

In order to ascertain the magnitude of this problem it seemed logical that a study should first be made to determine how long housewives leave milk exposed after it is delivered. Such a study was made during the past summer and fall, together with a rather thorough laboratory investigation of "sunlight" flavor development and vitamin losses during exposure to both sunlight and shade.

METHODS

A total of 13 milk routes in the cities of Columbus, Cleveland, St. Louis, and Boston were carefully studied to determine how soon after delivery the milk was taken in by the housewife. Briefly, the procedure employed in these studies was as follows: The investigator, accompanied by a route supervisor or someone well acquainted with the route, followed the route truck and first recorded how many customers took in their milk within five minutes. Those who did not take in their milk within this period were checked again after 30 minutes and again at 60, 90 and up to 150 minutes if the milk still remained on the doorstep at time of previous re-check. On re-check visits there was naturally some overlapping of time, but this was maintained at a maximum of plus or minus five minutes, which it was felt would cancel out in the overall study. Compact routes in middle-class residential sections were chosen for these studies in order to get a good cross-section picture of the average urban community. In each study routes were selected in different parts of the city so as to get a more composite picture of the particular market.

In laboratory trials when samples of milk were exposed to sunlight or shade, a sufficient number of bottles were exposed so that one bottle could be removed from the group at the end of each of three time intervals. The bottles were placed on a cement surface in the direct sun. Samples exposed in the shade were placed on the opposite side of the building on a similar surface.

Riboflavin analyses were conducted according to the method of Hand (6) with slight variations, and with the aid of a Coleman photofluorometer for measuring fluorescence. Ascorbic acid determinations were made by the conventional direct titration method employing sodium 2,6-dichlorobenzenoneindophenol dye.

In the absence of more accurate equipment for the measurement of light intensity, a Weston light meter was used for this purpose, readings being taken by holding the instrument about one foot above the exposed surface of the bottles.

When organoleptic examinations were made for the presence of the "sunlight" flavor, the samples were tasted immediately after exposure and again after being stored for 24 hours in a household refrigerator.

RESULTS

Route Studies

The studies conducted in Columbus, Cleveland, St. Louis, and Boston revealed a very interesting cross-section picture regarding the care of milk after it is delivered to the customer. The data tabulated and presented in table 1 represent a compilation of all data taken during these studies and although they are self-explanatory, some comment should be made regarding them. At the outset, it will be noted that the per cent of deliveries taken in at the various periods appears to vary considerably among the four cities. In St. Louis, for example, 71.9 per cent of the housewives took in the milk within five minutes after delivery, while only 29.6 per cent of the deliveries were taken in within this period in Cleveland. However, when the data are examined further, it will be noted that a total of 48.9 per cent of all deliveries made in Cleveland were placed in delivery boxes or taken into the house, apartment, or refrigerator by the routeman, while only 2.6 per cent of the total were so treated in St. Louis. Columbus and Boston were similar as regards the per cent of deliveries taken in within five minutes after delivery, but Boston routemen placed considerably more milk inside of homes, apartments or in household refrigerators. Since milk placed in delivery boxes or inside homes, apartments or refrigerators is not subjected to light, it was considered "protected" in this study.

Upon examination of the data under the heading of "Total milk protected," it will be observed that all four cities were fairly uniform with respect to the total percentage of customers who actually took in their milk within five minutes or had it protected from light at time of delivery. The weighted averages show that approximately 70 per cent of all deliveries either were taken in by the housewife within five minutes or placed in the house, apartment, delivery box or refrigerator by the routeman. Between the five-minute check and the 30-minute re-check period an additional 10 per cent of deliveries were taken in, and at the 60- and 90-minute re-checks about seven and six per cent, respectively, was removed from the doorstep. During the last hour, between the 90- and 150-minute re-checks, an additional three per cent was taken in by the housewife. The number of customers who left their milk out more than 150 minutes represented 3.3 per cent of all deliveries. It is interesting to note that in all four cities this percentage was

TABLE 1

A summary of route studies in four cities to determine the length of time milk remains exposed to light after delivery

Customer	Number*				%					Total milk protected† %				
	Columbus	St. Louis	Cleveland	Boston	Columbus	St. Louis	Cleveland	Boston	Weighted average	Columbus	St. Louis	Cleveland	Boston	Weighted average
Milk taken in within 5 min.	600	334	163	142	53.9	71.9	29.6	48.8	51.3	63.2	73.8	78.5	70.8	69.6
Milk taken in within 30 min.	748	383	191	174	67.2	82.5	34.7	59.8	61.9	76.5	85.1	83.6	81.8	80.4
Milk taken in within 60 min.	661‡	419	218	202	73.6	90.3	39.6	69.4	68.6	83.9	92.9	88.5	91.4	87.7
Milk taken in within 90 min.	931	431	241	212	83.6	92.9	43.8	72.9	75.1	92.9	95.5	92.7	94.9	93.6
Milk taken in within 150 min.	971	439	263	217	87.2	94.6	47.8	74.6	78.3	96.6	97.2	96.7	96.6	96.7
Milk left in box by routeman	31	6	113	7	2.8	1.3	20.5	2.4	6.4					
Milk placed in house, apt. or refrigerator by routeman	74	6	156	57	6.6	1.3	28.4	19.6	12.0					
Milk left out more than 150 minutes	38	13	18	10	3.4	2.8	3.3	3.4	3.3					

* 2419 customers studied:
Columbus = 1114 customers on five routes of three different companies.
St. Louis = 464 customers on three routes of two different companies.
Cleveland = 550 customers on three routes of three different companies.
Boston = 291 customers on two routes of one company.

† Includes milk taken in by customers, plus that put in box, apartment or house by routeman.

‡ No data taken at 60 minutes on first Columbus study.

very uniform. As a matter of fact all 13 routes in the different cities were very similar in this respect, the extremes being 2.4 to 4.0 per cent.

It is felt that the data shown here represent a fairly accurate cross-section picture of the promptness exhibited by customers in removing their milk from the doorstep after delivery. While it is appreciated that markets vary considerably in their delivery practices, the above tabulation should represent a relatively accurate index for evaluating the importance of this problem from the standpoint of vitamin losses and off-flavor development.

The Effect of Sunlight on Milk

Vitamin losses. Investigators who have published data on the photolysis of riboflavin and ascorbic acid in milk fail to agree on how much riboflavin is lost during a definite period of exposure to sunlight. As has been pointed out by Holmes and Jones (7) some of this is probably due to the varying intensities of sunlight to which the samples are subjected as well as to variations in the temperature of the milk during exposure. Some investigators employed quart-size milk bottles while others used pint or one-half pint sizes. It would seem logical that the contents of the smaller size containers would naturally warm up faster and would also be subjected to a greater surface exposure per unit volume.

In order to determine this relationship, experiments were conducted wherein homogenized milk was filled into two-quart, one-quart and one-half pint square flint glass milk bottles and exposed to strong direct sunlight for periods up to two hours. A duplicate series was exposed simultaneously for the same interval periods on the shaded side of the building.

The results of a complete and representative experiment are recorded in tables 2 and 3. The pronounced difference in vitamin losses of the same milk exposed in different sizes of milk bottles is clearly evident. From this and several other comparisons it was demonstrated that the riboflavin losses in one-half pint bottles were roughly twice as much as those found in two-quart bottles. When the riboflavin losses shown here are compared with those reported by other workers, where the same type of milk was exposed to bright sunshine in the same size bottles and at similar temperatures, there is good agreement. Other experiments, conducted at lower outside temperatures and at lower intensities of sunlight gave results which also agree quite well with most other published work. On the basis of eight trials employing quart bottles of homogenized milk, exposed to sunlight intensities ranging from 160 to 500 Weston degrees and at temperatures ranging from 52 to 88° F., the following percentage ranges for loss of riboflavin were experienced: one-half hour, 10.2–13.9 per cent; one hour, 18.6–28.5 per cent; and two hours, 37.2–45.0 per cent. All sunlight exposures were conducted on clear days between the hours of 10 A.M. and 2 P.M.

The ascorbic acid data in table 2 as well as that obtained in numerous

TABLE 2

*The effect of bottle size on the magnitude of vitamin loss and flavor development in homogenized milk exposed to sunlight**

No.	Size of milk bottle	Hours exposed	Weston light reading	Temperature of milk after exposure	Mg./liter riboflavin	Per cent loss	Mg./liter ascorbic acid	Per cent loss	Degree of sunlight flavor	
									Immediately	24 hours
				°F.						
1	Control milk	...	.....	52	1.37	.....	12.7	.....	.....	.....
2	2 quart	½	300	67	1.23	10.2	5.2	59.0	++	++++
3	1 quart	½	"	70	1.23	10.2	2.7	78.7	++	++++
4	½ pint	½	"	77	1.14	16.8	0.2	98.4	+++	++++
5	2 quart	1	350	74	1.07	21.9	2.3	81.9	++	++++
6	1 quart	1	"	78	0.98	28.5	0.1	99.0	++	++++
7	½ pint	1	"	86	0.79	42.3	0.0	100.0	+++	+++++
8	2 quart	2	"	83	0.86	37.2	0.9	93.0	+++	+++++
9	1 quart	2	"	87	0.76	44.5	0.0	100.0	+++	+++++
10	½ pint	2	"	97	0.42	69.3	0.0	100.0	++++	+++++

* Clear day with unrestricted sunlight. Time 10 A.M. to 12 M. Outside temperature 75–78° F.

TABLE 3

*The effect of bottle size on the magnitude of vitamin loss and flavor development in homogenized milk exposed to shade**

No.	Size of milk bottle	Hours exposed	Weston light reading	Temperature of milk after exposure	Mg./liter riboflavin	Per cent loss	Mg./liter ascorbic acid	Per cent loss	Degree of sunlight flavor	
									Immediately	24 hours
				°F.						
1	Control milk	...	.....	52	1.37	.....	12.7	.....	−	−
2	2 quart	½	25	61	1.37	0.0	11.2	11.8	−	−
3	1 quart	½	"	62	1.38	0.0	10.2	19.7	−	−
4	½ pint	½	"	65	1.35	1.5	7.9	37.8	−	±
5	2 quart	1	"	65	1.31	4.3	9.1	28.3	−	±
6	1 quart	1	"	65	1.30	5.1	7.3	42.5	±	+
7	½ pint	1	"	67	1.23	10.2	5.5	56.7	+	++
8	2 quart	2	"	67	1.26	8.0	6.1	52.0	+	++
9	1 quart	2	"	68	1.21	11.7	5.2	59.0	++	+++
10	½ pint	2	"	71	1.14	16.8	2.4	81.1	++	+++

* Uniform shade. Outside temperature 75–78° F.

other trials clearly demonstrate the extreme photolability of this vitamin and further confirms the conclusion of Holmes and Jones (7) that little if any ascorbic acid remains in one-half pint bottles after exposure to sunlight for 30 minutes. As was the case with riboflavin, the losses of ascorbic acid were less in quart and two-quart bottles than in the one-half pint size.

The results obtained with shaded samples (table 3) demonstrate the great difference in the rate of vitamin destruction between milks exposed to direct sunlight and those held under shaded conditions. In this and other comparative trials the loss of riboflavin in the shade seldom exceeded 25 per cent of that for a comparable sample exposed simultaneously to the direct sunlight. On cloudy or rainy days the losses of riboflavin were usually considerably less than those exposed in the shade on bright days. Twenty trials, in which samples were held in the dark for 24 hours after exposure, confirmed data of Stamberg and Theophilus (10) which demonstrated that the destruction of riboflavin ceases immediately upon removing the samples from the light. On the other hand, ascorbic acid losses continued at a rather rapid rate after exposure.

Ascorbic acid losses in the shade were surprisingly high and only further confirm the extreme sensitivity of this vitamin to light.

Flavor. The development of the so-called "sunlight" flavor was given considerable attention in the course of these studies. Because of the relatively high degree of oxidative stability displayed by most milk produced in summer and fall, very few of the samples exposed to light developed the "oxidized" flavor. However, the development of the characteristic "sunlight" flavor was very pronounced and appeared very quickly. The flavor data shown in tables 2 and 3 are recorded in terms of plus signs, depending upon the intensity of the flavor. These data representing the opinions of three judges, show that the flavor develops rapidly and that it increases in intensity during storage for 24 hours. In some trials the initial intensity of the flavor immediately after exposure or the rate of its development during subsequent storage was much less than in others, but with all freshly pasteurized milks the defect was very evident immediately after exposure.

The development of the sunlight flavor in samples held in the shade is somewhat surprising since the light intensity was relatively low. With one-half pint samples the flavor was frequently evident after one-half hour in the shade. However, with quart bottles of milk, little or no "sunlight" flavor developed until after more than one-half hour's exposure.

Route and Home Care of Milk

In order to have a more complete picture of the problems of vitamin loss and "sunlight" flavor development under actual conditions of milk distribution, a series of studies was conducted.

From the time the milk leaves the milk plant until the routeman has the

load delivered, some six hours frequently elapse. With conventional covered trucks and solid wall (wood) milk cases little direct sunlight actually reaches the bottle surfaces. Wire cases, on the other hand, subject the milk to considerable daylight especially near the front part of the truck. Numerous trials were conducted wherein random quarts of milk were removed from the truck before leaving the plant and every hour during the period of delivery. Some trucks carried both wood and wire cases for the purpose of the experiment. The samples were placed in a dark box and taken to the laboratory for analysis.

The data taken in this study are recorded in table 4. As might be expected, the vitamin losses were somewhat higher in bottles of milk carried in

TABLE 4

The loss of riboflavin and ascorbic acid in route delivery trucks

Trial No.	Weather conditions	Type of milk case	Time on route	Riboflavin loss	Ascorbic acid loss	Degree of sunlight flavor
			hr.	*per cent*	*per cent*	
1	Clear	Wood	1	0.6	26.8	–
	Bright	Wire	1	1.2	27.5	–
	Hot					
	"	Wood	2	4.3	26.1	–
		Wire	2	4.6	34.1	–
	"	Wood	5½	9.1	63.0	–
		Wire	5½	17.7	90.6	–
2	Cloudy	Wood	1	0	0	–
	Warm	Wire	1	0	5.8	–
	"	Wood	3	0	1.9	–
		Wire	3	0.6	11.7	–
	"	Wood	5	1.8	9.8	–
		Wire	5	6.2	40.0	–

wire cases than in those carried in wooden cases. The riboflavin losses were negligible except where milk was on a route for five hours or more. It should also be pointed out that trial 1 was conducted on a very hot, bright summer's day when higher than normal losses could be expected. This particular trial gave the highest percentage losses experienced.

Losses of ascorbic acid again were quite high yet not surprising, in view of the photolability of this vitamin. Although vitamin losses are low in milk carried on retail trucks, there is a potential danger if routemen are careless, especially in exposing wire cases of milk to the direct sunlight for long periods of time.

Since some housewives frequently allow milk to be exposed to light in the kitchen, it was of interest in this connection to determine how such treatment affected riboflavin, ascorbic acid, and the flavor of milk. Two trials were conducted in which quarts of homogenized milk were allowed to stand for two hours on a table in a bright kitchen having an east exposure.

Samples stood for two hours during the forenoon, but were not in direct sunlight. In these two trials riboflavin losses of 3.2 and 4.0 per cent and ascorbic acid losses of 52.0 and 67.4, respectively, were found. In both trials the milk developed a slight but definitely discernible "sunlight" flavor.

DISCUSSION

It is clearly evident that the exposure of milk to sunlight can cause important losses of riboflavin and ascorbic acid as well as impair the flavor of the product. The potential danger periods are those during which the milk is on the truck and after the product is on the customer's doorstep.

Ascorbic acid losses, while large, are not of critical importance because of the liberal quantities of this vitamin acquired from other foods in the average diet. The loss of riboflavin, however, is important because milk constitutes our most important source of this vitamin.

While the losses of riboflavin reported here and by others previously, do appear significant, it is the opinion of the writers that the problem is far less critical than has been assumed by previous workers. From the results of the route studies reported here it is evident that nearly 70 per cent of all milk deliveries are removed from the doorstep within five minutes, and therefore, lose little or no riboflavin.

If we assume that all deliveries in this study were equal as to the number of quarts of milk per customer and that the riboflavin losses during exposure to sunlight were 12 per cent for one-half hour, 20 per cent for one hour, 30 per cent for one and one-half hours, 40 per cent for two and one-half hours, and 60 per cent for that milk left out for more than two and one-half hours, we can calculate, approximately, the percentage of riboflavin that is lost. In so doing we must also consider the facts that, 1) the sun shines only about 55 per cent of the time (average of U. S. Weather Bureau records for areas studied), and 2) at least one-half of all milk that is left out is on the shady side of the street or in other shaded places. Since losses under shaded conditions do not exceed 25 per cent of that experienced in direct sunlight, this factor must be considered in any calculation. On the basis of the above facts and assumptions it has been calculated that about 3.25 per cent of the riboflavin is lost after milk is delivered to the customer's doorstep. This figure is, undoubtedly, too high because of the fact that, in general, milk is delivered in the morning when the light intensity is lower than during the hours when laboratory trials were run. Support for the view was found in analyses of samples picked up during the route studies. Three quarts of milk (each from different days) which were known to have been exposed to the direct morning sun for two and one-half hours, showed riboflavin losses of 26.2, 28.6 and 33.1 per cent in contrast to the 40.0 per cent average assumed above. Another intangible factor, which is not considered in the above calculation, is the question of the temperature during the period of

exposure, which is known to affect the rate of riboflavin loss. During winter months, when milk is exposed at lower temperatures, losses would be expected to be less. Furthermore, the route studies reported here were conducted in typical residential sections of cities and it might be expected that the percentage of apartment deliveries ("protected milk") is higher, for the nation as a whole, than is shown here.

It is the feeling of the writers that the problem of the "sunlight" flavor is of greater significance than the loss of part of the riboflavin. Most milk that is subjected to sunlight for as little as one-half hour develops the very unpleasant "sunlight" flavor.

It is appreciated that the small average loss of riboflavin demonstrated here is somewhat misleading when it is considered that in many cases, where housewives are careless in the matter of caring for milk, the resulting loss might be an important factor in the nutrition of the families in question. However, it is felt that the correction of this situation will not require any revolutionary changes in milk containers or in conventional delivery practices, but can be largely rectified by a program of consumer education. Such a program initiated through the combined efforts of distributors and dairy promotional agencies can do much to disseminate such information to consumers and thereby make milk a more nutritious and more palatable food.

SUMMARY

Approximately 70 per cent of all retail milk deliveries are removed from the doorstep or protected from light within five minutes after delivery while 3.3 per cent of customers leave milk exposed for more than two and one-half hours.

Under experimental conditions of exposing milk to sunlight, the loss of ascorbic acid is extremely rapid with only insignificant quantities remaining after 30 minutes. Losses of this vitamin in the shade are also quite rapid.

Riboflavin losses vary depending upon the intensity of sunlight, the temperature of the milk, and the size of bottles in which the milk is exposed. Under conditions of direct sunlight exposure for extended periods of time, milk loses significant quantities of this vitamin. On the other hand, the loss of riboflavin from milk exposed to shade does not exceed 25 per cent of that experienced in the direct sunlight.

The photolysis of riboflavin stops when milk is removed from light while losses of ascorbic acid continue at a somewhat slower rate.

The "sunlight" flavor, which develops in milk during exposure, appears to be a more critical problem than the loss of some of the riboflavin. On the basis of route studies and certain basic assumptions, it is believed that the average loss of riboflavin in all milk delivered does not exceed 3.25 per cent.

It is the feeling of the writers that a program of consumer education in the care of milk can be of great value in maintaining the palatability and nutritional qualities of this important food.

Further practical and fundamental studies are being conducted on the matters of "sunlight" flavor development and vitamin losses, the results of which will be reported in the near future.

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THE EFFECT OF SULFANILAMIDE UPON THE LIVABILITY AND METABOLISM OF BOVINE SPERMATOZOA

C. B. KNODT AND G. W. SALISBURY*

Department of Animal Husbandry, Cornell University, Ithaca, New York

The importance of bacteriological control of semen produced for use in artificial breeding of dairy cattle has been presented in reports by Gunsalus *et al.* (3, 4). These workers, while able to keep bacteria of semen at a minimum by aseptic methods of collection and handling, were never able to eliminate bacterial contamination entirely. Some bacterial types were normal inhabitants of the male genital tract, others were invaders of the tract, and still others were introduced into the semen at collection or later. *Pseudomonas aeruginosa* organisms, apparently harbored deep in the reproductive tract of some bulls, were shown to be deleterious to conception in artificial breeding. No one has yet been able to determine the effect of bacteria on the results of metabolic studies of spermatozoan physiology, though most workers have dismissed the problem as being of little or no consequence. Finally, the possible transmission of certain infections to cows through use of semen containing the responsible bacterium has not been thoroughly investigated.

These facts suggested the desirability of studying the effects of certain bacteriocidal or bacteriostatic agents on the livability and metabolism of bull spermatozoa. Shettles (10) in 1940 reported that survival and activity of human spermatozoa were not adversely affected by the addition of up to 160 mg. of sulfanilamide, or of sulfapyridine per 100 ml. of Baker's fluid used for diluting human semen. Thus, it was logical that the present investigation proceed with the study of one of these chemotherapeutic compounds and its effect on bull spermatozoa. The present report deals with the effect of sulfanilamide.

EXPERIMENTAL

The problems which required solution in these investigations were as follows:

1. What levels of sulfanilamide would be required to prevent growth of the bacterial types found in semen routinely collected from bulls;

2. What effect would sulfanilamide have on the livability and metabolic activity of spermatozoa and, finally;

3. What effect would the required levels of sulfanilamide have on the semen as treated when used for routine artificial insemination? This latter

Received for publication January 21, 1946.

* The authors are indebted to Merck and Co., Inc., Rahway, N. J., for the supplies of sulfanilamide and to Professor I. C. Gunsalus, Laboratory of Bacteriology, Cornell University, for many helpful suggestions.

problem is being investigated in a separate experiment and will be reported later.

Effect of sulfanilamide on bacterial growth. The first experiments were conducted using turbidimetric methods (6) for determining bacterial growth. Fresh bull semen was diluted at the rate of 1 part of semen to 10 parts of an isotonic sodium citrate solution. This solution was 3.6 grams $Na_3C_6H_5O_7.2H_2O$ per 100 ml. of water distilled over glass. To certain batches of citrate solution sulfanilamide was added at varying concentrations and all solutions were simultaneously autoclaved for 20 minutes at 15 lb. pressure.

The technique for determining the effect of various levels of sulfanilamide on bacterial growth consisted of measuring the change in density of each diluted semen sample during incubation for 72 hours at 37.5° C. The original density was due not only to spermatozoa, but to materials in the seminal plasma and bacteria. The change in nephelometer readings, made at 24-hour intervals, was considered as due to bacterial growth. Motility observations were made on each incubated sample to determine the effect of sulfanilamide on the livability of the spermatozoa.

In the first experiment 4 ejaculates were used to each of which sulfanilamide was added at levels of 0, 10, 20, 50, 100, and 200 mg. per 100 ml. In this experiment all spermatozoa with sulfanilamide added lived better than did the spermatozoa in samples containing no sulfanilamide. However, as was to be expected, bacterial growth was enormous in the samples containing no sulfanilamide, the optical density doubling in 72 hours. As bacteria made up a relatively small portion of the oriignal density, this indicates that bacterial numbers increased manyfold. However, at the 200 mg. per 100 ml. level there was only a 3.6 per cent increase in density.

In two subsequent experiments using 5 and 8 separate ejaculates and sulfanilamide levels of 0, 10, 20, 50, 100, 200, 500, and 1000 mg. per 100 ml. no increase in density was observed for the levels of 200 mg. per 100 ml. or above. This fact established 200 mg. per 100 ml. as the minimum level consistent with bacteriological control. Also, in these two experiments all levels of sulfanilamide aided in maintenance of motility as compared to the controls. The maximum increase in livability was with the 200 mg. per 100 ml. concentration, the duration of motility being depressed slightly by the 500 and 1000 mg. per 100 ml. amounts.

Level of sulfanilamide for optimum livability of spermatozoa in yolk-citrate. To determine the effect of sulfanilamide on spermatozoa under conditions comparable to those in routine artificial insemination it was necessary that the semen be diluted with the yolk-citrate commonly used here. The primary object was to determine the effect of the different amounts of sulfanilamide on spermatozoan activity. Because of the presence of the yolk no studies of the change in density could be made. How-

ever, routine microscopic examinations afforded a ready means of determining within limits the effect of sulfanilamide on bacterial growth.

For these studies fresh bull semen was diluted with the yolk-citrate diluent composed of one part of fresh egg yolk to one part of the isotonic sodium citrate solution, previously mentioned. The diluted semen was gradually cooled to the temperature of storage, 5° C., and stored in stoppered test tubes. The test tubes were opened at two-day intervals to subsample for microscopic examination of spermatozoan activity. The aliquots were obtained by use of sterile pipettes.

A series of preliminary experiments indicated that every level of sulfanilamide improved the livability of the spermatozoa, as had been the case when the yolk was not included. Additions of 1000 mg. per 100 ml., however, depressed livability as compared with the certain lower levels of added sulfanilamide. These experiments established the desired levels at from 100 to 500 mg. per 100 ml., which were used for the next experiment.

Fourteen separate ejaculates were diluted at 1:9 and 1:49 with the yolk-citrate and stored for 20 days at 5° C. These two dilution rates were chosen as the range of dilution then employed by the New York Artificial Breeders' Cooperative, Inc., for routine artificial breeding. Also, it was desired to determine if sulfanilamide additions would eliminate the dilution effect on livability previously reported from this laboratory (7).

The 14 ejaculates had a mean concentration of 967 thousand spermatozoa per $mm.^3$, mean methylene blue reduction time of 7.0 minutes, and a mean motility of 69 per cent motile spermatozoa moving at a rate of 3.3, where 4.0 is considered optimum. The samples were observed every 2 days for percentage and rate of motile spermatozoa.

The motility data of the experiment after 10 and 20 days' storage at 5° C. are shown in table 1. The observations made at each 2-day interval were used for a statistical analysis of variance. Highly significant differences were shown for sulfanilamide levels with 300 mg. per 100 ml. being optimum. In addition, the statistical interactions of ejaculate × treatment, and dilution × treatment were highly significant, aso. Sulfanilamide had no apparent influence in preventing the dilution effect, for the difference between dilution rates was highly significant statistically. The mean motility of the entire experiment for the 1:9 rate was 43 per cent, and for 1:49, 37 per cent.

While objective bacterial counts were not made on these diluted semen samples, microscopic observations at the time of motility examinations indicated great bacterial growth after 10 to 12 days of storage in those samples without added sulfanilamide. On the other hand, no bacteria were observed after storage at the 200 mg. per 100 ml. or higher concentrations of sulfanilamide.

Effect of sulfanilamide on glucose utilization and lactic acid production.

TABLE 1

The effect of sulfanilamide additions to yolk-citrate upon spermatozoan livability. Mean of 14 ejaculates

Days of storage	1:9*						1:49*					
	mg.% sulfanilamide						mg.% sulfanilamide					
	0	100	200	300	400	500	0	100	200	300	400	500
	% motile spermatozoa						% motile spermatozoa					
10	35	44	54	58	46	40	24	39	46	48	40	34
20	7	16	20	26	16	9	0	6	10	15	8	1
	Rate of motility						Rate of motility					
10	1.1	1.3	1.6	1.6	1.4	1.2	0.8	1.1	1.3	1.3	1.1	1.0
20	0.5	0.8	0.9	0.9	0.8	0.5	0.0	0.5	0.5	0.7	0.5	0.4

* Parts of semen to yolk-citrate.

Earlier reports from this laboratory (8, 13) indicated that glucose loss in semen samples diluted with yolk-citrate and stored for 10 days at 5° C. was not related to the livability of the spermatozoa. However, lactic acid accumulation in such samples was highly correlated with livability.

Later, Salisbury and VanDemark (9), in studying the dilution effect, found that semen ejaculates when diluted to a greater extent with yolk-citrate produced somewhat more lactic acid per 10^8 cells than did the same semen which was diluted less. On the other hand, the proportion of the glucose which disappeared on storage, and which was recovered as lactic acid, was much lower for the more dilute samples. It was felt that this unrecovered glucose might have been oxidized at a greater rate by the less concentrated spermatozoa or that bacteria might have oxidized it.

Thus, in the present investigation it was of interest to know not only how sulfanilamide might effect glycolysis of spermatozoa, but what effect it would have on the recovery of glucose as lactic acid. Therefore, 10 ejaculates of the semen diluted with yolk-citrate and stored for 6, 12, and 18 days were analyzed for glucose and lactic acid. Glucose analyses were

TABLE 2

The effect of sulfanilamide upon glucose and lactic acid metabolism of ejaculated bull semen stored at 5° C. in yolk-citrate for 18 days. Mean of 10 determinations

	Rate of dilution	mg.% sulfanilamide					
		0	100	200	300	400	500
mg.% glucose loss	1:9	105	86	80	66	63	60
	1:49	82	46	19	10	8	5
mg.% lactic acid gain	1:9	82	83	89	96	92	76
	1:49	24	27	26	22	20	20
% glucose utilized recovered as lactic acid	1:9	78	97	111	145	146	127
	1:49	29	59	137	220	250	400

determined by the method of Horvath and Knehr (5) and lactic acid by the method of Barker and Summerson (1). Determinations were made on Somogyi filtrates (11).

The data on glycolysis during 5° C. storage for 18 days are shown in table 2. The breakdown of glucose to lactic acid, glycolysis, was actually stimulated by sulfanilamide in some concentrations. At the 1:9 dilution maximum stimulation was at the 300 mg. per 100 ml. concentration. For the higher dilution rate maximum lactic acid production was obtained with the 100 and 200 mg. additions.

What appears to be of equal interest in table 2 is the data concerning the percentage of glucose loss recovered as lactic acid. In those tubes without sulfanilamide the percentage of glucose loss recovered as lactic acid was relatively small, but increased with each addition of sulfanilamide. Thus it is possible that bacteria utilized a part of the glucose not recovered as lactic acid in this and the previous investigations. However, as will be shown in the next section, sulfanilamide depresses oxygen consumption of spermatozoa and may function not only to suppress bacterial growth, but to suppress oxidative systems in diluted semen, including the possible oxidation of lactic acid or oxidation of glucose by the spermatozoa themselves.

The recovery of lactic acid in excess of glucose utilized probably is due to the metabolism of glycogen or other carbohydrate materials which the authors have found to be present in both semen and egg yolk.

Effect of sulfanilamide on oxygen consumption by washed spermatozoa. For this study 10 fresh bull-semen ejacualtes were used. The semen was cooled and then centrifuged. The seminal plasma was removed by aspiration and the spermatozoa were washed twice with 0.9 per cent NaCl, which was added at the same temperature as the spermatozoa. The washed spermatozoa were then suspended in Krebs calcium-free Ringer phosphate fluid (12). The concentration of spermatozoa was determined turbidimetrically and the sample diluted to a final concentration of 1 billion spermatozoa per ml. Three-quarters of a ml. of this spermatozoan preparation was placed in the Krebs calcium-free fluid in the Warburg flasks. The final concentrations of 0, 100, 200, 300, and 500 mg. per 100 ml. of sulfanilamide in the flasks was obtained by using Krebs fluids with appropriate sulfanilamide concentrations. The final concentration of spermatozoa was 250 million per ml.

The oxygen consumption was measured by the direct method of Warburg under air at 37.5° C. (2). The CO_2 was absorbed by 0.2 ml. of 20 per cent KOH in the center cup containing a small piece of filter paper. The manometers were shaken at the rate of 120 oscillations per minute. After one-half hour of endogenous oxygen consumption 0.25 ml. of a 1.2 per cent glucose solution was tipped from the sidearm into the reacting flask.

The data are presented in table 3. They show a marked effect of increasing sulfanilamide concentration on the utilization of oxygen by washed spermatozoa. The effect was more marked in the absence of glucose than in its presence, for glucose itself apparently reduced oxygen consumption.

TABLE 3

The effect of sulfanilamide upon the oxygen utilization of washed spermatozoa. Mean of 10 ejaculates*

	mg.% sulfanilamide				
	0	50	100	300	500
Endogenous for ½ hour	12.3	10.5	9.4	7.1	6.3
100 mg.% glucose for 1 hour	5.7	5.5	5.5	4.7	4.2

* Expressed as $mm.^3$ oxygen utilized/10^8 spermatozoa/hour (ZO_2).

DISCUSSION

The experiments presented in this paper demonstrate that sulfanilamide may be added to ejaculated bull semen diluted with yolk-citrate without decreasing the livability of the spermatozoa. A level of 300 mg. of sulfanilamide per 100 ml. of diluter gave, on an analysis of variance, a highly significant improvement in spermatozoan livability, it prevented bacterial growth, and appears to be optimal on the basis of these experiments.

Sulfanilamide markedly affected the glucose and lactic acid metabolism of diluted bull semen. The addition of 300 mg. of sulfanilamide per 100 ml. of diluter gave an increased accumulation of lactic acid at the 1 : 9 dilution whereas the greatest accumulation was at the 100 mg. level with the 1 : 49 dilution. Glucose utilization was greatest in the conrols; sulfanilamide at all levels, depressed the metabolism of this substance. Also, sulfanilamide depressed the oxygen utilization by washed spermatozoa under basal conditions, as well as in the presence of added glucose.

For certain of these experiments 3 different batches of sulfanilamide have been simultaneously tested. The results were similar for all batches. In the routine handling of the citrate buffer with added sulfanilamide precautions should be taken to keep it away from the direct rays of the sun.

The effect of sulfanilamide on fertility of diluted bull semen is under investigation. Preliminary data indicate that it has no deleterious influence on conception rate. However, at the present writing definite conclusions on this problem cannot be drawn.

A method of collection of bull semen free from bacteria is not now available. However, it would appear from these studies that in sulfanilamide or other sulfonamides a key to the control of bacteria in semen and control of possible spread of certain infections by artificial breeding is available. The action of sulfanilamide is selective for it is either bacteriocidal or bacteriostatic, but permits bull spermatozoa to live longer.

SUMMARY

1. The addition of 300 mg. of sulfanilamide per 100 ml. of yolk-citrate diluent, gave a significant improvement in the livability of ejculated bull spermatozoa over a 20-days-storage period and prevented bacterial growth.

2. Sulfanilamide depressed glucose and oxygen utilization at all concentrations studied.

3. The accumulation of lactic acid was increased both absolutely and in relation to the glucose utilized in the presence of sulfanilamide.

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VITAMIN C, HYDROGEN PEROXIDE, COPPER AND THE TALLOWY FLAVOR IN MILK

VLADIMIR N. KRUKOVSKY AND E. S. GUTHRIE
Department of Dairy Industry, Cornell University, Ithaca, N. Y.

In a preceding paper it has been disclosed that the reaction which produces the tallowy flavor can be inhibited by quick and complete oxidation of ascorbic acid in milk and that the reaction can be induced again by the addition of ascorbic acid (5).

This phenomenon together with the results obtained to date tends to indicate that the reaction responsible for the breakdown of the lipid fraction of the milk is a coupled reaction, possibly initiated under favorable conditions by a catalyst. In this connection it is of importance to note that ascorbic acid is the only form of vitamin C present in milk at the time of its removal from the mammary gland, or immediately after pasteurization (4), whereas the tallowy flavor is ordinarily detected in milk containing considerable amounts of dehydroascorbic acid as well. Equally important is the stimulative effect of the partial photochemical or chemical oxidation of the ascorbic acid in the milk to dehydroascorbic acid upon the development of the tallowy flavor during its subsequent storage in the dark (5). It suggests that the oxidation of the lipids of the milk is coupled to that of ascorbic acid when a certain equilibrium between ascorbic and dehydroascorbic acids has been established. Accordingly, copper as an accelerator of ascorbic acid oxidation by atmospheric oxygen to dehydroascorbic acid might help to bring the system more quickly to its critical point, or, in other words, to a condition under which the reaction is permitted either to deviate from its original course or to be replaced by a new one.

Furthermore, there is a good reason to believe that the promoter (an enzyme) of ascorbic acid oxidation with added H_2O_2 is largely responsible for its quick conversion in the milk to dehydroascorbic acid, and that if H_2O_2 were formed in the photochemical reaction (3) it might oxidize the residual ascorbic acid, as in the case of milk to which it has been added. Consequently, it was assumed that if a trace of H_2O_2 were present in the milk at the point when all of the ascorbic acid was oxidized, it might initiate in the presence of a suitable catalyst the breakdown of the lipid fraction of the milk (5). As a matter of fact, the tallowy flavor was sometimes induced by copper, added to milk at the end of the first reaction, even though less effectively as compared with that in milk containing ascorbic acid. Thus, the reason for the depletion of milk of its total vitamin C content prior to storage was to effect the destruction of the products of the first reaction by the following heat treatment that takes place in pasteurization. In such

Received for publication January 25, 1946.

a milk the tallowy flavor was not induced in the presence of copper (0.1 p.p.m.).

It was apparent therefore that at the end of the first reaction the residual H_2O_2, together with the catalyst, could constitute an additional factor for the promotion of the tallowy flavor in milk.

The following experiment was carried on to test further the theory that the reaction which produces the tallowy flavor is initiated more rapidly when a certain pressure between ascorbic and dehydroascorbic acids has been established. A sample of fresh mixed milk was pasteurized at 61.6° C. (143° F.) for half an hour and then quickly cooled to room temperature, at which point the ascorbic acid content of the milk was increased to 50 mg. per liter. Part of the milk was then treated with a carefully calculated quantity of 30 per cent H_2O_2 solution to produce milk containing dehydroascorbic acid only. Immediately after, by dilution of the portion of milk containing 49.43 mg. of ascorbic acid with the one containing 45.69 mg. of dehydroascorbic acid per liter, respectively, a series of samples were prepared which had progressively decreasing ascorbic acid contents but retaining their total vitamin C content at approximately the same level. The samples were stored at 0 to 5° C. (32° to 41° F.) in the absence of light. The ascorbic acid content of the milk was followed by direct titration with 2,6-dichlorophenol-indophenol in acid solution, but the total vitamin C content was determined by the Gunsalus and Hand method (2).

The data presented in figure 1 are rather conclusive in showing that there is a relationship between the pressures of ascorbic and dehydroascorbic acids on one hand and the production of the tallowy flavor on the other; and that the breakdown of the lipid fraction of the milk is initiated more readily when ascorbic and dehydroascorbic ratio is less than one or approaching one. Since dehydroascorbic acid in aqueous solutions is less stable than the reduced form of vitamin C, it would appear that unless the rate of ascorbic acid oxidation to dehydroascorbic acid surpasses that of dehydroascorbic acid to non-reducible substances, the necessary amount of dehydroascorbic acid could not be accumulated in the milk.

Accordingly the protective influence of ascorbic acid when added in large quantities to milk or to any other fat-containing food product can be attributed to the exhaustion of dissolved oxygen prior to the establishment of a favorable equilibrium between these two forms of vitamin C. If atmospheric oxygen is not made available at the point of the depletion of the occluded oxygen, the chances are that dehydroascorbic acid would be decomposed before the arrival of the new supply of oxygen. As a consequence, the oxidative process must start over again. It does not imply, however, that, because of the limited supply of oxygen made available at each successive point, the oxidative deterioration of the fat could be postponed indefinitely. It is necessary to consider as well the effect of the time of storage

upon the susceptibility of fat to oxidation (induction period), and that with the progress of time the conditions favoring the reaction would be correspondingly augmented.

Thus the differences between the samples of milk fortified with large amounts of ascorbic acid, in their abilities to resist the reaction which produces the tallowy flavor, can be attributed to the ability of milk to promote

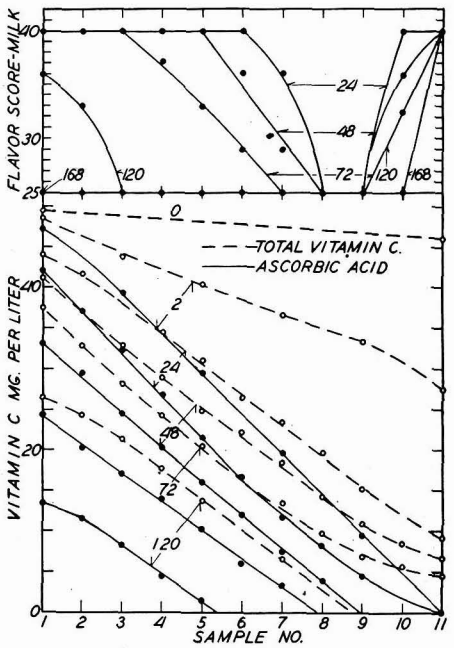

FIG. 1. The relationship between ascorbic and dehydroascorbic acids pressures in the milk (samples 1 to 11) at the end of 2, 24, 48, 72 and 120 hours holding period at 0° to 5° C. and the development of the tallowy flavor. The flavor score (the upper part of the figure) indicates: 40, no criticism; 35–40, acceptable; and 25, unsuitable for consumption.

ascorbic acid oxidation in the presence of atmospheric oxygen and the continued availability of the latter. This statement is further supported by our observations which indicated that under normal conditions the rate of ascorbic acid oxidation in so-called "susceptible milk" is apparently greater than that of less susceptible or "non-susceptible milks."

The data of figure 1 also show that with the passage of time irrespective of the initial ascorbic and dehydroascorbic acids ratios, the oxidized form of vitamin C tends to approach the level parallel to that for the ascorbic acid content of the samples.

It has already been remarked that a catalyst (an enzyme) might be responsible for the quick oxidation of ascorbic acid in milk by added H_2O_2. The evidence for this belief was the following: In a previous paper it has has been shown that, depending upon the ascorbic acid content of the fresh milk, from 0.021 to 0.03 ml. of 30 per cent H_2O_2 were needed to oxidize ascorbic acid quickly and completely. At that time it was observed that with further increase in the volume of H_2O_2 added to milk the rate of ascorbic acid oxidation was noticeably retarded. The results of this experiment, since repeated several times, are graphically presented in figure 2.

In this case the samples were prepared by dilution in sequence of milk containing 0.816 ml. of added H_2O_2 with normal milk, to produce a series of samples containing 0.816, 0.408, 0.204, 0.102, 0.051, 0.0382, 0.0286, 0.0215, 0.016, 0.012, 0.009, 0.0068 ml. of 30 per cent H_2O_2 per liter of milk, respectively.

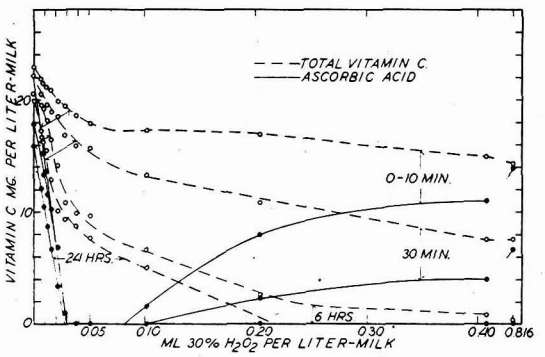

FIG. 2. The inverse relationship between the volumes of 30 per cent H_2O_2 solution added to milk and the time required to oxidize ascorbic acid either partially or completely.

The data show that the rate of ascorbic acid oxidation varied inversely with the volume of hydrogen peroxide added to milk. In the presence of 0.068 to approximately 0.08 ml. of the agent, ascorbic acid was oxidized either partially or completely within 0 to 10 minutes of time, respectively, whereas more than 30 minutes were required to complete its oxidation in the samples treated with 0.204 to 0.816 ml. of H_2O_2. This progressive retardation of ascorbic acid oxidation suggests that the reaction may be catalyzed by an enzyme, which in turn is slowly inactivated by H_2O_2. The data show also that, in the presence of as much as 0.1 ml. of H_2O_2, the oxidation of ascorbic acid to dehydroascorbic acid was promoted at a considerably faster rate than that of dehydroascorbic acid to non-reducible substances. Further increase in the volume of H_2O_2 added to milk resulted in the progressive retardation of ascorbic acid oxidation and more rapid destruction of dehydroascorbic acid. The break in the curve for the latter apparently occurred at the point coresponding to 0.2 ml. of H_2O_2; but at 0.816 ml.,

TABLE 1

The relationship between the amounts of 30 per cent hydrogen peroxide added to milk prior to pasteurization, vitamin C content, added copper, added ascorbic acid (Table 1–A) and the development of the tallowy flavor in milk subsequently held at 0 to 5° C.

Held at 0 to 5° C.	Untreated control sample		Ml. of 30% hydrogen peroxide per liter of milk prior to pasteurization at 143° F. for 30 minutes																					
			0.0068		0.009		0.012		0.016		0.021		0.028		0.038		0.051		0.102		0.204		0.408		0.816	
	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.
days	*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*	
After H_2O_2 added	18.1	18.8	14.2	18.1	12.0	17.3	9.6	16.6	7.1	16.3	4.3	14.9	0.0	13.8	0.0	13.8	0.0	12.7	1.7	12.6	6.0	12.4	9.6	12.2	12.0	12.0
and pasteurized	12.0	12.7	8.1	8.5	7.1	7.8	5.3	5.7	2.8	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	8.9	12.0	5.7	7.9	4.9	6.3	3.9	4.9	0.7	2.1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4	0.0	5.9	0.0	2.9	0.0	2.1	0.0	1.6	0.0	0.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Flavor score																									
1	40		40		40		40		40		40		40		40		40		40?		40		40		40	
2	40?		40?		40?		34–T.		40		40		40		40		40		40?		40		40		40	
4	25–T.		25–T.		25–T.		25–T.		40		40		40		40		40		40		40		40		40	
8	.....		.....		.....		.....		40		43*		43		40		40		40		40		40		40	
	Copper added 0.1 p.p.m.																									
1	4.2	10.9	2.1	6.3	1.4	5.0	0.3	5.0	0.0	1.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4	0.0	2.7	0.0	1.6	0.0	1.0	0.0	1.0	0.0	0.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	Flavor score																									
1	25–T.		25–T.		25–T.		25–T.		40?		40		40		40		40		40?		40		40		40	
2	.....		.....		.....		.....		40		40		40		40		40		40		40		40		40	
4	.....		.....		.....		.....		40		40		40		40		40		36–T.?		40		40		40	
8	.....		.....		.....		.....		40		40*		40		40		36–T.		36–T.?		40		40		40	

dehydroascorbic acid was destroyed as fast as formed. The last sample, as the data in table 1–A indicate, was an exceptional one in many respects. The addition of ascorbic acid either alone or with copper to milk depleted of its total vitamin C content not only failed to induce the tallowy flavor, but also the sample lost its vitamin C content at a much faster rate than the nearest one in the same series.

The differences between ascorbic and dehydroascorbic acids in their rates of oxidation with H_2O_2 indicate, therefore, that the second reaction, involving dehydroascorbic acid oxidation with H_2O_2 to non-reducible substances, was not effected by the promoter of ascorbic acid oxidation.

These results are supported by the observations of Steinman and Dawson (12) who concluded that the rate of dehydroascorbic acid decomposition is increased by the addition of H_2O_2, apparently because of the bimolecular reaction between dehydroascorbic acid and H_2O_2. They also stated that the initial stage of decomposition is pseudomolecular in character and is not due to an oxidation which is catalyzed by a metallic ion.

Since, at the end of the first reaction, H_2O_2 can be a variable component of the new system in which the catalyst plays a part, it was of interest to learn if there is a condition under which the breakdown of the lipid fraction of the milk could proceed rapidly and to completion. Consequently, there were two problems to solve. First, to determine the effect of H_2O_2 when added to milk in excess of that required to oxidize its ascorbic acid content prior to the heat treatment upon the susceptibility of milk to tallowy flavor in the presence of subsequently added copper. Second, the ability of copper to promote the breakdown of the lipid fraction of the milk by the residual H_2O_2. For these reasons milk was treated with different amounts of 30 per cent H_2O_2 both prior to and after the heat treatment. It was thought that dehydroascorbic acid and the residual H_2O_2 would be destroyed in one case and retained in the other, at least for a period of time. Thus, two series of samples were prepared by following the same dilution technic as previously described, with the exception that the experiments were performed by using two batches of milk from the same cows on two different days.

The data presented in table 1 show that, with the exception of one or possibly two samples of milk, the tallowy flavor was not promoted by copper in the milk completely depleted of its total vitamin C content by the procedures just described. Even then the flavor developed at a comparatively slow rate, its intensity varied from day to day, and in some cases it was hard to ascertain if the flavor was present at all. It is possible that in this particular case the reaction was started during ascorbic acid oxidation with H_2O_2, which coincidently was appreciably retarded at this point (fig. 1), and, although it was not allowed to continue because the necessary components of the system were destroyed by the following heat treatment, the lipid sensitivity to copper-induced oxidation was somewhat affected.

TABLE 1–A

Ascorbic acid readded after hydrogen peroxide treatment and pasteurization

Held at 0 to 5° C.	Untreated control sample		Ml. of 30% hydrogen peroxide per liter of milk prior to pasteurization at 143° F. for 30 minutes																					
			0.0068		0.009		0.012		0.016		0.021		0.028		0.038		0.051		0.102		0.204		0.408		0.816	
	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.
days	*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*	
After pasteurized and A.A. added	12.0	12.7	8.1	8.5	7.1	7.8	5.3	5.7	2.8	3.2	0.0	0.0	0.0	0.0	0.0	.....	0.0	.....	0.0	.....	0.0	.....	0.0	.....	0.0	.....
	32.6	33.3	29.0	29.0	27.6	28.7	26.6	26.9	23.0	23.7	20.9	21.2	20.2	19.8	20.2	19.1	19.8	20.2	20.9	19.5	20.2	19.1	20.9	19.8	20.9	19.5
0 min.	32.6	33.3	27.9	29.0	27.6	28.3	26.2	25.8	22.3	24.0	20.2	20.9	19.5	20.2	19.1	19.8	19.5	19.8	18.4	19.5	17.7	18.4	14.5	17.0	10.6	11.0
1	27.9	32.2	24.8	29.0	24.0	27.9	21.9	25.8	19.8	22.6	16.6	19.8	15.9	19.1	15.9	18.4	15.9	18.4	15.6	19.1	11.3	16.3	2.5	9.6	0.0	1.4
4	14.9	24.5	12.3	20.3	10.7	19.2	8.5	17.3	6.7	14.4	4.3	11.7	3.2	10.7	1.6	9.0	1.0	9.0	3.7	10.4	1.9	7.2	0.0	2.1	0.0	0.0
	Flavor score																									
1	40		40		40		40		40		40		40		40		40		25–T.		25–T.		30–T.		40–Ca.	
2	40		40		40		40		40		40		40*		40		25–T.		.....		.....		.....		40	
4	25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		.....		.....		.....		.....		40	
	Copper added 0.1 p.p.m.																									
130 min.	31.2	32.6	26.6	27.9	26.2	27.6	23.7	25.8	20.9	22.3	18.0	20.2	17.7	19.1	17.7	18.4	17.7	18.8	17.2	18.4	13.8	17.0	6.0	13.1	1.8	4.2
1	20.2	28.7	17.7	24.8	17.0	23.7	16.6	22.7	13.1	19.8	10.3	17.2	9.2	16.3	7.8	15.6	6.7	15.6	6.0	14.9	1.4	11.3	0.0	4.6	0.0	0.0
4	0.0	11.2	0.0	9.1	0.0	8.5	0.0	6.9	0.0	6.4	0.0	5.9	0.0	5.0	0.0	4.3	0.0	4.5	0.0	4.5	0.0	3.2	.....	1.3	.....	.....
	Flavor score																									
1	40		40		40*		39–T.		40		40		25–T.		25–T.		25–T.		25–T.		25–T.		39–T.		40–Ca.	
2	25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		25–T.		.....		.....		.....		.....		36–T.		40	
4	.....		.....		.....		.....		.....		.....		.....		.....		.....		.....		.....		25–T.		40	

A.A. = Ascorbic acid; T.C. = Total vitamin C.

Remarks: Flavor score indicates: 40, no criticism; 35–40 acceptable to most consumers; 25, unsuitable for consumption.

Symbols indicate: T., tallowy, and Ca., cabbage flavor and odor, respectively. Underlined numbers indicate samples of milk which developed pleasant almond-like flavor. *, milk tasted fresh at the end of 2 weeks of storage.

On the other hand the data presented in table 1–A indicate that the tallowy flavor could be quickly promoted by the addition of ascorbic acid to milk which was completely depleted of its total vitamin C content, providing it was treated with less than 0.8 ml. of H_2O_2 per liter shortly before pasteurization. It was obvious, therefore, that ascorbic acid either alone or with copper catalyzes the reaction which produces the tallowy flavor much more effectively than the copper alone. Conversely, when H_2O_2 treatment was applied to pasteurized milk, the products of ascorbic acid oxidation, such as dehydroascorbic acid and the residual H_2O_2, were retained in the samples,

The data on the effect of the addition of progressively increasing amounts of H_2O_2 to pasteurized milk upon the development of the tallowy flavor during its storage at low temperatures are presented in table 2. They show that in the case in which the volume of 30 per cent H_2O_2 added to milk was not in excess of that required to oxidize completely ascorbic acid to dehydroascorbic acid (at 0.021 ml.), the tallowy flavor was not induced by added copper. It also indicates that dehydroascorbic acid when present alone is not involved in the reaction responsible for the development of the tallowy flavor. However, it should be noted that from this point on with an increase in the volume of H_2O_2 added to milk up to 0.1 ml. per liter, inclusively, the catalysis of the tallowy flavor by copper followed an ecliptic path, with its high point at 0.051 ml. and the low ones at 0.028 and 0.102 ml., respectively, and that copper failed to promote it in the milk originally containing 0.204, 0.408 and 0.816 ml. of H_2O_2.

These results definitely indicate the existence of a critical region within which the oxidation of the lipid fraction of the milk with H_2O_2 is catalyzed by copper. Furthermore, the development of the tallowy flavor in the control portion of the milk treated with 0.051 ml. of H_2O_2 suggests the possibility that the favorable conditions for the copper catalysis of the tallowy flavor were established prior to its addition. In contrast to the milk described in table 1, the reaction was allowed to continue because of the presence of dehydroascorbic acid and of the residual H_2O_2. Undoubtedly, the development of the tallowy flavor in both the control and the portion of milk with copper and treated with 0.051 ml. of H_2O_2 was not a mere coincidence. It indicates that, under these conditions, dehydroascorbic acid may take part in the reaction resulting in the breakdown of the lipid fraction of the milk.

For the foregoing reason it was necessary to corroborate the results by an experiment in which dehydroascorbic acid was not a factor. This was done by the addition of 0.03, 0.06, 0.09 and 0.12 ml. of 30 per cent H_2O_2 per liter to portions of milk which was depleted of its total vitamin C content in the presence of 0.03 ml. of added H_2O_2 and the following heat treatment that takes place in pasteurization. The reaction which produces the tallowy flavor was not stimulated by the addition of H_2O_2 to milk completely depleted of its total vitamin C content. Whereas the development of the tallowy

TABLE 2

The relationship between the amounts of 30 per cent hydrogen peroxide added to milk after pasteurization, vitamin C content, added copper and the development of the tallowy flavor in milk subsequently held at 0 to 5° C.

Held at 0 to 5° C.	Unheated control sample		Ml. of 30% hydrogen peroxide per liter of pasteurized milk																			
			0.0068		0.009		0.012		0.016		0.021		0.028		0.038		0.051		0.102		0.204		0.408		0.816	
	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.	A.A.	T.C.
days	*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*		*mg./l.*	
After pasteurized and H_2O_2 added	16.1	17.4	13.4	17.4	10.0	16.8	8.7	16.4	5.4	15.7	0.0	14.4	0.0	15.1	0.0	13.4	0.0	14.1	5.4	13.7	8.7	13.0	11.0	12.0	13.4	13.4
1	12.7	16.7	8.7	13.7	6.0	12.0	3.7	9.7	1.3	7.3	.....	6.0	.....	4.7	.....	4.0	.....	3.7	.....	0.7	.....	0.0	.....	0.0	.....	.....
3	7.3	12.0	3.0	8.4	1.7	6.3	0.7	5.3	0.0	3.0	.....	3.0	.....	0.0	.....	2.3	.....	2.0	.....	0.0	.....	0.0	.....	.....	.....	.....
	Flavor score																									
1	40		40		40		40		40		40		42		40		40?		40		40		40		40	
3	37–T.		25–T.		25–T.		25–T.		38–T.		40		42		40		36–T.		40		40		40		40	
5	25–T.		.....		.....		.....		39–T.?		42		42		40?		38–T.		40		40		40		40	
7	.....		.....		.....		.....		39–T.?		42		42		40		25–T.		39–T.?		40		40		40	
	Copper added 0.1 p.p.m.																									
1	7.3	14.7	3.7	10.7	2.0	9.0	1.0	8.7	0.0	8.0	0.0	5.7	0.0	4.3	.....	4.0	.....	3.7	.....	1.3	.....	1.0	.....	.....	.....	.....
3	0.0	7.3	0.0	5.0	0.0	4.0	0.0	4.7	.....	3.0	.....	3.0	.....	0.0	.....	2.0	.....	1.3	.....	0.0	.....	0.0	.....	.....	.....	.....
	Flavor score																									
1	29–T.		25–T.		25–T.		25–T.		29–T.		40		40		40		36–T.		40		40		40		40	
3	25–T.		.....		.....		.....		29–T.		42		40		36–T.		25–T.		40		40		40		40	
5	.....		.....		.....		.....		36–T.		42		40		32–T.		.....		32–T.		40		40		40	
7	.....		.....		.....		.....		32–T.		42		38–T.		25–T.		.....		25–T.		40		40		40	

Flavor scores and symbols—see footnote of Table 1–A.

flavor induced by copper followed an ecliptic path again, thus confirming the existence of a critical region for the reaction involving H_2O_2, copper catalyst, and the lipids of milk. The limits of the critical region are probably determined by the availability of the residual H_2O_2 at the end of the first reaction, on one side, and, on the other, by the inhibitory chemical changes produced in the milk by the addition of excessive amounts of H_2O_2. Of course a relationship might exist between the amounts of H_2O_2 added to milk and the catalytic properties of the copper.

Furthermore, in order to ascertain whether or not the sensitivity of milk to copper-induced tallowy flavor was affected by H_2O_2 treatment, portions of milk used in the preceding experiment were depleted of their total vitamin C content by heat in the presence of 0.03, 0.06, 0.09, 0.12 and 0.15 ml. of H_2O_2. Each sample was pasteurized immediately after the H_2O_2 was added. Through out the duration of the experiment (9 days) these samples behaved approximately the same way as the similar ones described in table 1. The promotion of the tallowy flavor was again found to be slow and erratic. Only one sample which was treated with 0.09 ml. of H_2O_2 prior to pasteurization developed the tallowy flavor, and then not until the seventh day of the holding-period.

In conclusion it should be noted that in the case in which the amounts of H_2O_2 added to milk were in excess of those required to oxidize the ascorbic acid of the milk, especially after pasteurization, a rather pleasant almond-like flavor developed in the samples. Its presence was restricted to the area within which the tallowy flavor was induced by copper ecliptically (table 2), and its intensity followed essentially the same path.

Barron, DeMeio, and Klemperer (1) postulated that during ascorbic acid oxidation by atmospheric oxygen with the cupric ion as a catalyst, the metallic ion is reduced to cuprous form, and that the H_2O_2 is formed during the subsequent reoxidation of the catalyst. This by-product of the reaction is then readily split into water and oxygen. Their results show an uptake of one atom of oxygen per molecule of substrate at different pH values from 4.17 to 6.60. Hand and Chase (3) reported, however, an uptake of 1.19 and 1.85 atoms of oxygen per molecule of substrate at pH value of 6.85 (slightly higher than that of average milk) with copper and light as catalysts, respectively. They thought that the differences between copper and light in their effects on the oxygen combining power of vitamin C can be accounted for by the assumption that copper catalyzes the oxidation of ascorbic acid by H_2O_2. At the same time, Steinman and Dawson (12), using, pyrophosphate buffer, observed that, at a pH value of 6.3, the oxygen-combining power of vitamin C decreased when oxidized aerobically by ionic copper in the presence of catalase. These authors concluded that their results offer convincing evidence of H_2O_2 formation in the cupric oxidation of ascorbic acid.

Consequently Olson and Brown (10) attributed the development of the

oxidized flavor in washed cream containing both ascorbic acid and copper (added) to the reaction involving phospholipid oxidation by H_2O_2 formed according to the scheme proposed by Barron and his associates. It should be born in mind, however, that by the numerous dilutions and reseparations these authors changed completely not only the multi-system of milk plasma, but also the physical properties of the cream, whereas the amounts of ascorbic acid which were added to the washed cream were those which some investigators think prevents oxidized flavor. Furthermore, their results show that at the end of the experimental trial (72 hours' duration period) the oxidized flavor developed in washed cream containing ascorbic acid, but no added copper. Obviously, the development of the oxidized flavor in the sample of cream just described could not be explained by the assumption that the free H_2O_2 was formed as well in the sample without copper, especially if consideration is given to the fact that the tallowy flavor was not induced by H_2O_2 when added alone to washed cream or to milk depleted of its total vitamin C content by the rapid oxidative method, or under certain conditions, to milk containing ascorbic acid. Neither could the promotion of the tallowy flavor in milk by partial oxidation of ascorbic acid to dehydroascorbic acid in the presence of added H_2O_2 be attributed to direct reaction between the agent and the lipids of milk.

Although the evidence presented in the preceding paragraphs definitely indicates that the oxidation of the lipid fraction of the milk by H_2O_2 could be catalyzed by copper, nevertheless it was evident that the reaction might take place only after all of the ascorbic acid is oxidized. Thus, if the H_2O_2 was formed during ascorbic acid oxidation by atmospheric oxygen with copper as a catalyst, it would be undoubtedly used to promote ascorbic acid oxidation first. As a matter of fact, we observed that the addition of H_2O_2, in amounts required to oxidize ascorbic acid completely, to milk previously treated with 0.1 p.p.m. of added copper, did not result in a faster promotion of the tallowy flavor, when compared with milk to which copper was added after all of the ascorbic acid were oxidized. In both milks the reaction was largely dependent upon the presence of the residual H_2O_2 at the point when all of the ascorbic acid was oxidized. Because of this, and in spite of the indirect evidence showing that H_2O_2 is formed during ionic copper oxidation from cuprous to cupric forms (aerobic) (3, 12), it would be reasonable to assume that free H_2O_2 is not formed in the milk during ascorbic acid oxidation by atmospheric oxygen with copper catalyst.[1]

These observations, together with the knowledge that the addition of 0.1 p.p.m. of copper to fresh pasteurized milk invariably results in the stimulation of the tallowy flavor, suggest that the reaction resulting in the development of tallowy flavor is a coupled reaction which is initiated when a certain

[1] In a recent paper, L. W. Mapson noted that although the chloride ions accelerate the rate of reduction of Cu^{++} by ascorbic acid, they inhibit the oxidation of Cu^{+} by O_2 (13).

equilibrium between the two forms of vitamin C has been established. Copper as an accelerator of ascorbic acid oxidation to dehydroascorbic acid might thus help to bring the system more rapidly to its critical point.

It has already been remarked that the physical state of washed cream differs from that of normal cream. With each successive washing and reseparation, the fat emulsion becomes less and less stable and easily breaks down, resulting in a partial reversal of the phases. Because of this a continious fat phase film might form at the surface of the cream. It would affect the rate of diffusion of atmospheric oxygen into the sample, as well as the rate of ascorbic acid oxidation. Consequently, the protective influence extended by the addition of 610 mg. of ascorbic acid to washed cream, as the data of Olson and Brown show (10), could be attributed to the exhaustion of the oxygen in the form of air which was incorporated into the cream by mechanical manipulations prior to the establishment of a critical equilibrium between the two forms of vitamin C. Thus the three-day holding period was not long enough to warrant a definite conclusion, since during that time the oxidized form of vitamin C was undoubtedly destroyed faster than it could be renewed by the oxidation of ascorbic acid.

It is of importance to note that ascorbic acid oxidation is responsible for the inactivation of several biologically active compounds (9, 11, 12), and that depending upon the environmental conditions the oxidation or inactivation of the other components of the system is either stimulated or prevented. Moreover, not all of the secondary reactions can be attributed to the activity of H_2O_2 formed during ascorbic acid oxidation. In their study of the ascorbic acid, ascorbic acid-oxidase, and oxygen system, Steinman and Dawson (12) and Powers and Dawson (11) have observed that the inactivation of the oxidase during the course of the reaction is largely prevented by the addition of catalase and peroxidase. The latter authors presented convincing evidence to show that the reaction which causes the inactivation of the oxidase is not due to the H_2O_2 formed in the reaction, but to some other factor, despite the fact that both catalase and peroxidase when added to the system markedly protect the enzyme against inactivation. McCarty (9), in his study of the inactivating action of ascorbic acid on the substance which induces transformation of pneumococcal types, came to the conclusion that this action of ascorbic acid (0.010 M) results from autoxidation, because the cupric ion markedly enhances the inactivating action of ascorbic acid (0.001 M) which when present alone in smaller concentration causes only partial inactivation of the substance. The cupric ion by itself did not alter the activity, whereas the effect of preformed H_2O_2 was found to be low. It was necessary to add a considerably higher concentration of H_2O_2 than is liberated in the course of the minimally effective amounts of ascorbic acid in order to bring about an inactivating effect comparable to that achieved by ascorbic acid.

Milk is a multi-component system containing biologically active materials. The activity of one of them, namely milk lipase, was shown to be dependent on the environmental conditions (temperature changes, Cu, oxygen) (6, 7, 8). It is possible, therefore, that however small the changes in the environmental conditions produced by chemical or physical means (exposure to light, addition of H_2O_2 and Cu, oxygen tension, etc.) might result in the stimulation of the activity of some of the biological agents present in the milk, and in the inactivation of the others. The environmental conditions would also determine the trend, extent and the magnitude of the reactions involved.

SUMMARY

1. It has been demonstrated that ascorbic acid oxidation is an essential link in the chain of the reactions resulting in the development of the tallowy flavor in milk, and that apparently the oxidation of the lipid fraction of the milk is coupled to that of ascorbic acid when a certain equilibrium between ascorbic and dehydroascorbic acids has been established.

2. The evidence is presented to show that a promoter (an enzyme) of ascorbic acid oxidation by H_2O_2 might be responsible for its quick conversion to dehydroascorbic acid, and that it is not the free H_2O_2 formed in the course of ascorbic acid oxidation which causes the breakdown of the lipid fraction of the milk, resulting in the development of the tallowy flavor. This view is supported indirectly by the observations indicating that H_2O_2 formed in the reaction would be used to oxidize the residual ascorbic acid as in the milk to which it was added; and that in the absence of a suitable catalyst the addition of H_2O_2 to milk completely depleted of its total vitamin C content does not result in the development of the tallowy flavor.

3. The reaction which produces the tallowy flavor is catalyzed by added copper in the presence of H_2O_2, providing the agent was added to milk completely depleted of its total vitamin C content by rapid oxidative method, or the amount of the agent added was in excess of that required to oxidize its ascorbic acid content. However, under the experimental conditions the catalysis of the tallowy flavor by copper were confined in a critical region, the limits of which were apparently determined by H_2O_2 concentration.

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A SIMPLIFIED EXTRACTION–DISTILLATION METHOD FOR THE DETERMINATION OF THE VOLATILE FATTY ACIDS OF CHEESE*

K. L. SMILEY,[1] F. V. KOSIKOWSKY, AND A. C. DAHLBERG
Department of Dairy Industry, Cornell University, Ithaca, New York

A simple method for determining the volatile fatty acid content of cheese would be a valuable tool for the dairy chemist concerned with the ripening of cheese. Hiscox, Harrison, and Wolf (4, 5, 6) discussed this problem and proposed a method based on water extraction of the fatty acids from the cheese, and ether extraction of the fat and fat-soluble fatty acids with subsequent removal by washing the ether with dilute alkali. The acids in the extractions were then liberated by acidifying, and distilling with steam. The objections to this method are the great amount of time necessary to make a determination and the fact that a special glass-joined steam still of empirical dimensions is required.

All the volatile fatty acids are very soluble in ether and all except the short-chain acids are relatively insoluble in water. It seemed probable, therefore, that if cheese could be put into an acid solution consisting of dissolved, emulsified, and suspended materials, the fatty acids could be readily extracted by the use of ether. The volatile fatty acids can also be distilled quantitatively from an aqueous solution in an ordinary flask by use of a soluble salt, such as magnesium sulphate, to raise the boiling point, as shown by Friedemann (2). In determining the free volatile acids of butter, Ferris, Redfield and North (1) used a regular Kjeldahl apparatus in their distillation procedure. It was felt that if these principles could be successfully applied to cheese, a shorter, more rapid method requiring no special apparatus could be developed to determine the volatile acidity.

The object of this work was to attempt to develop a more rapid method and to observe more critically the retentive effect of nonvolatile fatty acids upon distillation of volatile fatty acids.

EXPERIMENTAL RESULTS

Simplified Extraction-Distillation Method

Preparation and extraction of cheese. In the method as finally adopted, a 10-gram representative sample of cheese weighed on a Torsion balance (sensitivity 4 mg.) was ground in a mortar with warm 10 per cent phos-

Received for publication January 28, 1946.

* The authors are indebted to the National Cheese Institute for assistance in financing this study through a research grant to Cornell University, and to Mrs. Jean Beaver for making some of the analyses.

[1] Present address, Hiram Walker & Sons, Inc., Peoria, Illinois.

phoric acid (50–55° C.). The ground cheese was washed quantitatively from the mortar into a 250-ml. centrifuge bottle until a total of 50 ml. of phosphoric acid solution was used. Ten ml. of ethyl alcohol and about 35 grams of $MgSO_4.7H_2O$ were added to the bottle and the mixture shaken. Fifty ml. of ethyl ether were then added to the bottle and the contents shaken vigorously for about 20 seconds. The pressure should be released carefully to avoid the stopper blowing out and spilling some of the contents. This bottle was then centrifuged one to two minutes at 2000 r.p.m. The ether layer was drawn off into a suction flask by means of a water-suction pump and the suction line was rinsed with 10 ml. of fresh ether. Two more extractions were carried out in the same manner except that no additional alcohol or $MgSO_4$ was added and that 35-ml. portions of ether were sufficient for each of the last two extractions. The ether was then transferred from the suction flask to a separatory funnel. Twenty-five ml. of N/10 NaOH were used to rinse out the suction flask and were then added to the contents of the funnel. The funnel was gently agitated with a rotary motion. Too vigorous shaking results in an emulsion which makes it difficult to separate the layers. The aqueous layer was drawn off into a 300-ml. Erlenmeyer flask. The ether layer was washed five additional times with 25 ml. of N/10 NaOH until 150 ml. of alkali had been used. By carefully heating on an electric hot plate or steam bath, the ether was driven from the combined alkaline washings in less than one hour.

Distillation and titration of the residue. While the ether was being driven off the alkaline washings, the residual material in the centrifuge bottle was transferred to an 800-ml. Kjeldahl flask. A total of exactly 230 ml. of distilled water was then added, part of it being used to rinse the centrifuge bottle. The solution, already acid due to the presence of phosphoric acid, was refluxed over a low flame on a wire gauze square three to five minutes to drive off the carbon dioxide. After slight cooling exactly 20 ml. of freshly boiled distilled water were used to rinse down the refluxer. The flask was placed on an asbestos board with a three-inch opening on an ordinary Kjeldahl distillation apparatus except that the traps were replaced by glass tubes of 10 mm. inside diameter to reduce refluxing to a minimum. A couple of glass beads were found essential to prevent bumping toward the end of distillation. The distillate was collected through Whatman No. 2 paper until 280 ml. had been obtained. It is imperative that not more than 280 ml. be collected because burning of the remaining contents will produce erratic results. The distillate was titrated with N/10 NaOH, using phenolphthalein as indicator. The funnel containing the filter paper was left in position for the second distillation involving the alkali washings.

Distillation and titration of the alkali washings of ether. The ether-free alkali washings were transferred to an 800-ml. Kjeldahl flask using three 15-ml. portions of distilled water to rinse the flask. About 35 grams of

$MgSO_4.7H_2O$ were added and enough 50 per cent H_2SO_4 to adjust to pH 2, using pHydrion paper as indicator. Refluxing and distillation procedures were followed similar to that used with the residual material except that the distillation was continued until the contents of the Kjeldahl flask started to crystallize. This can be readily detected when the material in the flask becomes murky. It is important not to heat the flask after crystallization because the H_2SO_4 will then decompose and give erroneous results. The distillate was passed through the same condenser tube and was filtered through the same paper used for the first distillation and collected in an Erlenmeyer flask. It was titrated directly.

Neutral alcohol was used to rinse the insoluble acids from the condenser. The rinsings were caught in the funnel with the filter paper which had retained the remainder of the insoluble acids during the two distillations. These alcohol rinsings were titrated.

The sum of the titrations of the first and second water distillates and of the alcohol rinse was considered the volatile acidity of the cheese. Results were expressed as ml. N/10 acid per 100 grams of cheese.

A COMPARISON OF METHODS

In order to critically evaluate the reliability of this improved technique, more aptly called the simplified extraction-distillation method, the results from a number of cheeses were compared to the standard Hiscox method and in some cases to the direct steam distillation method. The direct steam distillation was made by distilling 500 ml. from 20 gm. of cheese made up to 100 ml. with CO_2-free water. Table 1 gives the results from eight Cheddar cheeses covering a wide range of volatile acids. The simplified extraction-distillation method compared very favorably with the Hiscox method. The direct steam distillation method gave low results for all cheeses, this being characteristic of the method as shown previously by Hiscox, Harrison, and Wolf (5).

Further analyses were extended to cover various other types of cheese. Table 2 shows the results from seven different types of cheese. In each instance the results from the simplified extraction-distillation method were in very good agreement with those of the Hiscox method.

The Retention of Volatile Fatty Acids by Nonvolatile Fatty Acids

In his work on the determination of volatile alcohols and acids Friedemann (2) observed that short chain fatty acids, such as acetic, butyric, caproic, and caprylic could be quantitatively recovered by direct distillation in the presence of $MgSO_4$. An experiment was conducted to observe the effect of $MgSO_4$ upon the percentage recovery of fatty acids during direct distillation, using all acids in the series from butyric through myristic.

A quantity of pure fatty-acid solution was added to a clean 800-ml.

TABLE 1

*A comparison of three different methods in the determination of volatile fatty acids in Cheddar cheeses**

Cheese	I Direct steam			II Hiscox							III Simplified extraction-distillation				
	Combined		Total	Water phase		Fat phase		Residue		Total	Ether extract		Residue		Total
	Sol.	Insol.		Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		Sol.	Insol.	Sol.	Insol.	
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
1	5.2	0.5	5.7	4.9	0.6	2.0	1.5	2.9	2.7	14.6	9.6	2.9	2.0	0.7	15.2
2	74.5	1.3	75.8	72.5	1.7	4.6	5.5	4.3	5.3	93.9	72.0	8.2	12.5	1.1	93.8
3	35.8	1.4	37.2	37.6	1.6	2.7	4.4	2.2	3.1	51.6	33.2	6.5	7.5	0.4	47.6
4	11.5	4.0	15.5	13.9	0.9	2.3	1.6	1.4	2.5	22.6	12.1	3.9	6.1	2.7	24.8
5	27.4	0.5	27.9	28.4	0.6	3.2	1.1	2.2	1.7	37.1	25.9	3.2	5.6	1.6	36.3
6	35.5	0.8	36.3	39.7	0.4	1.3	1.5	2.6	1.6	47.1	34.8	4.9	7.9	1.7	49.3
7	44.0	0.9	44.9	46.9	1.1	2.1	5.1	4.0	2.0	60.6	38.2	6.3	15.0	2.3	61.8
8	28.9	0.8	29.7	29.8	1.1	3.3	3.0	2.9	1.0	41.5	28.9	5.6	5.1	0.8	40.4
Average	.....	.....	34.1	.....	.....	.....	.....	.....	.....	46.1	.....	.....	.....	.....	46.2

* All values expressed as ml. N/10 acid per 100 grams cheese.

TABLE 2

*A comparison of the Hiscox method and the simplified extraction-distillation method in the determination of volatile fatty acids in various types of cheese**

Type of cheese	Hiscox							Simplified extraction-distillation			
	Water phase		Fat phase		Residue		Total	Ether extract	Residue	Combined	Total
	Sol.	Insol.	Sol.	Insol.	Sol.	Insol.		Sol.	Sol.	Insol.	
	ml.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
1. Blue	13.0	6.5	2.5	9.0	2.0	4.5	37.5	19.7	8.5	12.2	40.4
2. Switzer	39.6	1.3	2.0	1.8	1.4	1.0	47.1	38.0	7.7	3.8	49.5
3. Chantelle	22.9	0.7	1.4	1.0	2.4	1.5	29.9	22.2	6.4	2.8	31.4
4. Camembert	23.0	5.7	2.2	8.1	†	†	39.0	27.0	4.5	8.4	39.9
5. Snappy cheese food	14.9	1.1	3.2	4.5	4.7	1.2	29.6	19.9	5.3	5.6	30.8
6. D'Oka (Trappist)	37.0	2.8	4.8	6.8	†	†	51.4	36.9	4.6	7.7	49.2
7. Leiderkrantz	89.0	2.1	4.7	4.2	2.2	1.5	103.7	84.3	13.7	4.8	102.8
Average	.....	.....	.....	.....	.....	.....	48.3	.....	.....	.....	49.1

* All values expressed as ml. N/10 acid per 100 grams cheese.
† Residue washings combined with washings from fat and both distilled over at same time.

Kjeldahl flask and titrated with N/10 NaOH to get the exact amount of acid present. About 300 ml. of H_2O were added and enough H_2SO_4 to lower the pH to 2.0. Approximately 35 grams of $MgSO_4.7H_2O$ were added and the solution distilled to the point of crystallization. The distillate was titrated and the recovery calculated.

Table 3 shows the percentage recovery of the fatty acids under the stated conditions. The results confirm the conclusions of Friedemann that distillation with $MgSO_4$ will quantitatively recover acetic, butyric, caproic, and caprylic acids. In addition, these data (table 3) show that caproic and lauric acids can also be quantitatively recovered under similar conditions. Myristic acid was not volatile enough even under the elevated temperature provided by $MgSO_4$ to be completely distilled, but was about 70 per cent recovered.

TABLE 3

The percentage recovery of fatty acids by direct distillation with $MgSO_4$ present in the distillation mixture

Fatty acid	Percentage recovery
Acetic	100
Butyric	100
Caproic	100
Caprylic	100
Capric	100
Lauric	100
Myristic	71

The known retentive rôle played by fat during distillation does not concern itself in the simplified extraction-distillation and Hiscox methods as there is no fat present in the distillation mixtures. However, such nonvolatile acids as palmitic and oleic, if present, are carried over into the distillation mixture. If these nonvolatile acids exert a retentive effect upon the volatile fatty acids it would be important to establish this fact. The following experiment was designed to furnish information on this subject.

Quantities of tenth normal volatile fatty acids, ranging from 2.0 to 6.0 ml. amounts, were added to a specific quantity of palmitic or oleic acid (0.25 and 0.50 gram, respectively) in 300 ml. of distilled water. Approximately 35 grams of $MgSO_4.7H_2O$ were added to the mixture followed by the addition of 50 per cent H_2SO_4 until a pH of 2 was obtained. After distilling the acid mixture to the point of crystallization the distillate was titrated and percentage recovery of the fatty acids calculated.

TABLE 4

The effect of nonvolatile fatty acids upon the recovery of individual volatile fatty acids during direct distillation with $MgSO_4$.

Volatile acids	0.25 gram palmitic acid in distillation mixture	0.5 gram oleic acid in distillation mixture
	Percentage recovery	Percentage recovery
Butyric (C_4)	100.	100.
Caproic (C_6)	100.	100.
Caprylic (C_8)	93.2	92.8
Capric (C_{10})	84.4	79.1
Lauric (C_{12})	71.1	53.6
Myristic (C_{14})	36.5*	20.6*

* Approximately 70.0 per cent myristric acid is recovered during direct distillation with $MgSO_4$ and no nonvolatile fatty acid.

The nonvolatile acids, palmitic and oleic, exerted a retentive effect on some of the higher chain fatty acids during distillation. However, for those fatty acids which are mostly concerned with the volatile acidity of cheese the effect of nonvolatile acids was negligible. Table 4 shows that fatty acids up through caproic are unaffected during distillation by nonvolatile acids, while from caprylic to myristic there is a varying effect as the percentage recovery ranges from 93.2 to 20.6 per cent. The percentage recovery is indirectly related to the number of carbon atoms present in the volatile fatty acids, a relationship which is clearly shown in table 4.

DISCUSSION

The advantages of the simplified extraction-distillation procedure become very apparent when such factors as economy of time and utilization of regular apparatus are considered. It takes approximately four hours to complete an analysis on one cheese sample using the new procedure, whereas the

Hiscox method requires well over forty hours. The use of the Kjeldahl distillation apparatus eliminates any great delay which might occur in case of breakage with special glass stills, and, because of its efficient design, enables the laboratory technician to greatly increase the number of samples that can be handled at one time.

In the actual method, phosphoric acid was chosen because it is a good cheese solvent. The purpose of the alcohol was to afford better contact between the ether and water when the extraction was carried out. In the early portion of the method $MgSO_4.7H_2O$ was added because it allowed the cheese proteins to float on the aqueous layer as a crust after centrifuging, greatly facilitating the separation of the ether from the water. It also increased the efficiency of the extraction, probably because it lowered the solubility of the ether in the water phase. In the latter portion of the method $MgSO_4$ was used to raise the boiling point of the solution.

During the distillation of the residue the turbidity of the solution, as a result of the presence of proteins, prevented easy observation of crystallization. Hence, this distillation was conducted on a volume basis. In the case of the alkali washings of the ether extract there was no turbidity and consequently the distillation could be carried to crystallization.

Washing of free fatty acids from an ether fat solution was tried in accordance with the directions of Hiscox, Harrison and Wolf (5) except that six 25-ml. portions of N/10 NaOH were used instead of six 50-ml. portions as these authors suggested. Known amounts of volatile fatty acids were dissolved in pure fresh butter oil and the solution added to ether in a separatory funnel. The ether solution was then washed with the six portions of dilute alkali. When the washings were acidified and distilled in the presence of $MgSO_4$ it was found that all the volatile acids tried from butyric up to and including lauric were recovered completely. This also confirms the work of Gould and Johnson (3) who found that dilute alkali will remove practically all the fatty acids from a rancid fat. The results obtained on fat blanks alone were the same as the direct titration of the fat showing little or no saponification due to the alkali.

The retentive effect of the nonvolatile acids is probably not as great as the effect of butter fat. Hiscox and Harrison (4) recovered 95, 54, and 19 per cent of caprylic, capric and lauric acids, respectively, in the presence of butter fat. Our results show 93, 84, and 70 per cent recovery for these same acids in the presence of palmitic acid. These results are not entirely comparable due to the great differences in the distillation techniques used. They do, however, show that in either the method presented here or in the technique of Hiscox, Harrison, and Wolf (5) there is still a retentive factor present. However, since most of the volatile acidity is due to short-chain acids, the retentive effect of the nonvolatile acids during the distillation is of minor importance.

Hiscox and Harrison (4) distilled over 90 to 100 per cent of the volatile fatty acids in the presence of cheese proteins so the retention of fatty acids by proteins was much less marked than by the fat. It appears obvious in our work that the retention of volatile acids by the proteins in the residue did not present any appreciable error as only a relatively small amount of fatty acids was carried in the residue and practically all of this was recovered during direct distillation.

Recovery experiments were performed to see if the ether extraction of the acid cheese mush was efficient. Known amounts of the volatile fatty acids were added to the acid cheese mush and the regular procedure, previously described, was followed. Results indicated that about 95 per cent of the butyric acid added was recovered by three extractions, while about 85 per cent of added lauric was recovered. Further extractions failed to recover significant amounts of volatile acids. However, in actual analyses on a number of cheeses it was noted that ether extraction alone gave significantly lower results than those obtained with the Hiscox method. An increase in the number of ether extractions with increased amounts of ether did not remedy this situation, although using four 50-ml. portions of ether instead of the customary three did recover more of the volatile acids. It was not until the residue was distilled and the titration value added to that of the ether extraction that a very close agreement with the Hiscox method was obtained.

SUMMARY

A method for determining the volatile fatty acids of cheese is described. The method, referred to as the simplified extraction-distillation method, is based on two distillations of acid cheese solution, one associated with the fat phase and the other with the residual material. After ether extraction of the acid-cheese mixture, the volatile fatty acids were removed from the ether with dilute alkali. The alkali rinses were heated to drive off the ether, then acidified, refluxed to remove carbon dioxide, and distilled in the presence of $MgSO_4$ until crystallization occurred. In the meantime, 250 ml. of distilled water were added to the residual material and the solution distilled until 280 ml. of distillate were collected. The sum of the titrations from the two distillations plus that of the alcohol rinse represented the total volatile acids of the cheese.

This new method and the method of Hiscox, Harrison, and Wolf (5) gave nearly identical results for a wide variety of cheeses. It has advantages over the latter method in that no special apparatus is necessary, more distillations can be accomplished in the course of a day, and the time required to analyze a sample of cheese is very greatly reduced.

The findings of Friedemann (2) that acetic, butyric, caproic, and caprylic acids can be quantitatively recovered by distilling in the presence of $MgSO_4$ were confirmed. In addition, capric and lauric acids were quantitatively

recovered under similar conditions, while myristic acid was incompletely recovered.

Nonvolatile fatty acids, such as palmitic and oleic, exerted a retentive effect on caprylic, capric, lauric, and myristic acids during direct distillation in the presence of $MgSO_4$ with the degree of retention being in direct relationship to the number of carbon atoms present in the volatile acids. When butyric and caproic acids were distilled, the nonvolatile acids exerted no retentive effect.

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DEFLUORINATED ROCK PHOSPHATE AS A PHOSPHORUS SUPPLEMENT TO THE RATIONS OF DAIRY CATTLE[1]

T. W. GULLICKSON AND FLOYD C. OLSON[2]
Minnesota Agricultural Experiment Station, St. Paul, Minnesota

The scarcity of steamed bone meal during the war period made it necessary to find a satisfactory substitute that could be used to supplement dairy herd rations that are deficient in phosphorus. Raw rock phosphate, the natural choice for such use, has been proven unsatisfactory because of its high fluorine content which renders it toxic. Within the last few years several methods have been perfected for removing the fluorine from natural phosphates to a level approximating that in bone meal, making the resulting products safe for feeding to cattle. Recently, however, Barrentine, Maynard, and Loosli (1), working with rats, observed considerable difference in the availability of the calcium and phosphorus in defluorinated rock phosphates produced by these methods. The Florida station (2) reported rather undecisive results regarding the comparative palatability of defluorinated superphosphate and bone meal for cattle, but no comparisons were indicated in regard to their values as sources of phosphorus.

The following report presents briefly the results obtained in trials in which a defluorinated rock phosphate[3] was compared with steamed bone meal in its ability to serve as a supplement to phosphorus-deficient rations of dairy cattle. The process followed in its manufacture was described as "the calcination of phosphate rock in addition to the use of acids all of which maintains the original ortho form of phosphate in the product." The product as fed compared favorably in composition with that of steamed bone meal. It contained approximately 29.8 per cent calcium and 14.0 per cent phosphorus. The fluorine content was under 0.10 per cent, which according to Mitchell (3) makes it safe, under all reasonable conditions for feeding to cattle.

EXPERIMENTAL

Four grade Holstein heifers, E416, E418, E426 and E441, all about 21 months old when started, were used in the trials. The experiment lasted 17 months, the first six months of which was a preparatory period during which all animals were fed similar phosphorus-deficient rations. During the last eleven months, the experimental period, the same plan of feeding as before was followed except that bone meal was added as a supplement to the

Received for publication January 30, 1946.

[1] Published with the approval of the Director as Paper No. 2271, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] Resigned July 1, 1945.

[3] Supplied by International Minerals and Chemical Corporation, Chicago, Illinois.

rations of E418 and E441, respectively, and E416 and E426 received equivalent additions of phosphorus to their rations in the defluorinated rock phosphate. Each ration with supplements added was designed to provide adequate protein but slightly less than the required amount of phosphorus. The mineral supplements were added directly to the grain ration at each feeding.

All heifers were fed twice daily in mangers designed for cattle on experiment and records were kept of amounts of feedstuffs and supplements fed and consumed. Chemical analysis, including calcium and phosphorus determinations, was made of all feeds and supplements fed. The inorganic phos-

TABLE 1

Phosphorus intakes in ration and supplement, and blood plasma inorganic phosphorus concentration of each heifer during the preparatory and experimental periods

Animal No.	Kind of supplement	Phosphorus intake daily			Blood plasma inorganic phosphorus
		In ration	In supplement	Total	
Preparatory period					
		grams	*grams*	*grams*	*mg.%*
E416	None	7.35	.....	7.35	3.06
E426	"	7.44	.....	7.44	3.38
Average	.....	7.40	.....	7.40	3.32
E418	None	7.23	.....	7.23	2.82
E441	"	8.00	.....	8.00	3.31
Average	.....	7.62	.....	7.62	3.07
Experimental period					
		grams	*grams*	*grams*	*mg.%*
E416	Defluor. phosphate	7.40	2.79	10.19	4.40
E426	"	7.85	2.87	10.72	4.79
Average	.....	7.63	2.83	10.46	4.60
E418	Bone meal	7.39	2.70	10.09	4.42
E441	"	8.61	2.80	11.41	5.52
Average	.....	8.00	2.75	10.75	4.97

phate content of blood plasma and the weight of each animal was determined regularly every 30 days. Observations relating to their physical condition, general appearance, evidence of pica and anorexia, were made daily. Heifers, when in the barn, were stanchioned in comfortable stalls equipped with drinking cups. When weather permitted, they spent from two to six hours daily outdoors in a grass-free yard. Results were measured in terms of general appearance of animals, appetite shown for good-quality low-phosphorus content prairie hay, rate of gain in weight, and by the indicated concentration of inorganic phosphate of the blood plasma.

RESULTS AND DISCUSSION

Some of the more significant data relating to phosphorus intakes, weights

and inorganic phosphate content of the blood plasma of all animals during the periods of the experiment are presented in tables 1 and 2.

The data presented in table 1 clearly shows that the basal rations fed were in all cases deficient in phosphorus. This is indicated by the subnormal concentration of inorganic phosphorus in the blood plasma of all animals during the preparatory period. During this period all heifers also exhibited marked pica, poor general appearance and indifferent appetite for hay as well as other characteristic symptoms typical in cattle suffering from a deficiency of phosphorus in their rations.

TABLE 2

Weights and average daily gains of heifers at various intervals during the preparatory and experimental periods

Animal No.	Weight at			Average daily gain from		
	Start	6th month	End	Start to 6th month	6th month to end	Start to end
			Preparatory period			
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
E416	746	825	825	0.44	.....	0.44
E426	795	880	880	0.47	.....	0.47
Average	770	852	852	0.46	.....	0.46
E418	765	832	832	0.37	.....	0.37
E441	848	949	949	0.56	.....	0.56
Average	806	890	890	0.47	.....	0.47
			Experimental period			
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
E416	825	857	1006	0.18	0.99	0.55
E426	880	959	1082	0.44	0.82	0.61
Average	852	908	1044	0.31	0.91	0.58
E418	832	885	1082	0.29	1.31	0.76
E441	949	1044	1199	0.53	1.03	0.76
Average	890	964	1140	0.41	1.17	0.76

As is indicated in table 1 the average blood plasma phosphate concentration in each animal was considerably higher during the experimental than for the preparatory period. This rise or increase in phosphate concentration occurred simultaneously and to approximately the same extent in all animals in both groups. The rise became apparent within 30 days after feeding of supplements was started, and although the concentrations fluctuated quite widely from month to month, the trend throughout was upward for all animals. All the heifers also showed marked improvement in their physical appearance and appetite for prairie hay, and pica which was exhibited by all animals at the start, gradually disappeared as the experimental period progressed. From these results and observations it must be concluded that the phosphorus supplied in the supplements was being utilized by the heifers.

It will be observed that heifers made only slightly greater average daily gain in weight while receiving the supplements than during the preparatory period. This is especially true of E416 and E426, the heifers that were fed defluorinated product. In this connection it should be explained that as appetite for prairie hay improved, somewhat less grain was fed in order to maintain the phosphorus content of the basal ration at the desired low level. Consequently, digestible nutrients intakes did not increase directly with volume of feed consumed. There was in fact a slight decline in nutrient intake during the first two or three months which accounts for the slower rate of gain indicated (table 2) for the first six months of the experimental period. During the last five or six months of this period, however, hay consumption increased greatly with consequent rise in energy intake and marked increase in rate of daily gain in weight. The higher average daily gains made by the heifers fed bone meal as compared to those fed defluorinated rock phosphate during a similar period of time is only slightly (if at all) significant. No difference in palatability of the two supplements was discerned during the progress of the trials.

SUMMARY AND CONCLUSIONS

A defluorinated rock phosphate was compared with steamed bone meal in its ability to serve as a satisfactory supplement to phosphorus-deficient rations of dairy cattle. Four grade Holstein heifers about 21 months of age were used. They were fed similar phosphorus-deficient basal rations during the 17-months period of the experiment. During the last eleven months two of them were fed steamed bone meal as a supplement to their rations; the other two received equivalent additions of phosphorus from a defluorinated rock phosphate product. Total phosphorus intakes were kept slightly below the requirements of each animal.

Under the conditions of the experiment no significant difference was observed between defluorinated rock phosphate and steamed bone meal in availability of their phosphorus to dairy cattle. Animals fed the bone meal made slightly greater gains in weight. No difference was noted in the palatability of the two supplements as fed.

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PROGRAM

FORTY-FIRST ANNUAL MEETING

OF THE

AMERICAN DAIRY SCIENCE ASSOCIATION

IOWA STATE COLLEGE

AMES, IOWA

JUNE 18–20, 1946

PROGRAM COMMITTEE

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University of Missouri

G. W. SALISBURY,
Cornell University

FLOYD ARNOLD,
University of Maryland

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Cornell University

DWIGHT ESPE,
Iowa State College

DWIGHT SEATH,
Louisiana State University

REGISTRATION

MEMORIAL UNION

IOWA STATE COLLEGE

Meetings will be held in buildings on the campus of Iowa State College and headquarters will be in the Memorial Union.

SCHEDULE OF PROGRAMS

Date and Time	General	Production	Extension	Manufacturing
Tuesday				
June 18, 1946				
8:00	Registration			
9:30–12:00	Opening Session			
1:00– 4:00		Section A Section B	Section	Section
4:00– 5:00	Committees	Committees	Committees	Committees
7:30	Reception (Informal)			
Wednesday				
June 19, 1946				
9:00–12:00		Joint Business and Symposium		Symposium
1:00– 3:00		Section A Section B	Section	Section
3:00– 4:00		Business	Business	Business
4:00– 5:00	Committees Campus Tours	Committees Campus Tours	Committees Campus Tours	Committees Campus Tours
7:30	Mixer			
Thursday				
June 20, 1946				
7:00	Southern Section Breakfast			
9:00–11:00		Joint Symposium		Section
11:00–12:00		Business	Business	Business
1:00– 3:00		Section A Section B	Section	Section
3:00– 5:00	Business			
6:30	Banquet Installation of Officers, Borden Awards			

COMMITTEE MEETINGS

Suitable rooms will be available for all committees and for other groups which may desire to meet. Elmer Hansen will have charge of room assignments for the Extension and Production Sections and F. E. Nelson for the Manufacturing Section.

PROJECTION EQUIPMENT

Lanterns will be available, upon request, for projection of standard and $2'' \times 2''$ slides. Projectors for 8 and 16 mm. movies also will be available by arrangement.

DAIRY FARM—HERD—MILKING PARLOR

All persons attending the meetings are invited to visit the Dairy Husbandry Farm which is located approximately three-fourths of a mile south of the campus. The milking parlor will be in operation from 4:00–6:00 both morning and afternoon.

GENERAL PROGRAM

Tuesday, June 18, 1946

8:00 REGISTRATION, Memorial Union.

9:30–12:00 OPENING SESSIONS, *Great Hall, Memorial Union.*

C. A. IVERSON, *Head, Department of Dairy Industries,* Presiding.

Introduction of Officers and Guests.

Address of Welcome—DR. CHARLES E. FRILEY, *President, Iowa State College.*

Presidential Address—J. A. NELSON, *President, American Dairy Science Association.*

Guest Speaker—E. S. ESTEL, *Editor, Creamery Journal; Manager, Dairy Cattle Congress; Secretary, Iowa State Dairy Association.*

Announcements.

1:00– 4:00 SECTIONAL MEETINGS.

Production Section A, Vitamins, *Room 19, Agricultural Hall.*

Production Section B, Hormones, Blood, and Disease, *Agricultural Assembly Room, Agricultural Hall.*

Manufacturing Section, Dry Milk Products, *Room 118, Dairy Industries Building.*

Extension Section, D.H.I.A. Work, *Room 117, Agricultural Hall.*

4:00– 5:00 COMMITTEE MEETINGS.

7:30 RECEPTION, *Women's Gymnasium.*

Wednesday, June 19, 1946

9:00–12:00 SECTIONAL MEETINGS.

Manufacturing Section, Quality Symposium, *Room 118, Dairy Industries Building.*

Joint Business and Symposium Production and Extension Sections, Committee Reports—Breeds Relations, *Agricultural Assembly Room, Agricultural Hall.*

1:00– 3:00 SECTIONAL MEETINGS.

Manufacturing Section, Cheese and Evaporated Milk, *Room 118, Dairy Industries Building.*

Production Section A, Thyroprotein, Protein, Minerals, *Room 19, Agricultural Hall.*

Production Section B, Feeding and Management, *Agricultural Assembly Room, Agricultural Hall.*

Extension Section, Artificial Breeding—Exhibits, *Room 117, Agricultural Hall.*

3:00– 4:00 BUSINESS MEETINGS OF SECTIONS.
Extension Section, *Room 117, Agricultural Hall.*
Manufacturing Section, *Room 118, Dairy Industries Building.*
Production Section, *Agricultural Assembly Room, Agricultural Hall.*

4:00– 5:00 COMMITTEE MEETINGS.
CAMPUS TOURS.

7:30 MIXER, *Great Hall, Memorial Union.*

Thursday, June 20, 1946

7:00 BREAKFAST-SOUTHERN SECTION, *Pine Room, Memorial Union.*

9:00–11:00 SECTIONAL MEETINGS.
Manufacturing Section, Bacteriology and Chemistry, *Room 118, Dairy Industries Building.*
Joint Symposium Production and Extension Sections, Herd Health and Sanitation, *Agricultural Assembly Room, Agricultural Hall.*

11:00–12:00 BUSINESS MEETINGS OF SECTIONS.
Extension Section, *Room 117, Agricultural Hall.*
Manufacturing Section, *Room 118, Dairy Industries Building.*
Production Section, *Agricultural Assembly Room, Agricultural Hall.*

1:00– 3:00 SECTIONAL MEETINGS.
Extension Section, 4-H Club Work, Quality Programs, *Room 117, Agricultural Hall.*
Manufacturing Section, Chemistry, Sanitation, Teaching, *Room 118, Dairy Industries Building.*
Production Section A, Hay, *Room 19, Agricultural Hall.*
Production Section B, Breeding, *Agricultural Assembly Room, Agricultural Hall.*

3:00– 5:00 GENERAL BUSINESS SESSION, *Agricultural Assembly Room, Agricultural Hall.*

6:30 ANNUAL BANQUET—INSTALLATION OF OFFICERS AND PRESENTATION OF BORDEN AWARDS.

SECTIONAL PROGRAMS

Tuesday, June 18

Afternoon Session, *Room 117 Agr. Hall*

FLOYD J. ARNOLD—*Chairman*

1:00– 1:15 **Opening Business Session.**

1:15– 4:00 **D.H.I.A. Work.**

E1. Considerations in the Development of Dairy Herd Improvement Association Work—a panel discussion led by J. F. KENDRICK, *Bureau of Dairy Industry, U.S.D.A.*

E2. More Accurate Records on More Cows Through County Testing Laboratories—J. E. STALLARD, *University of Wisconsin.*

E3. Practices Used in Dairy Herd—Improvement Associations to Evaluate Pasture—R. B. BECKER, *University of Florida.*

E4. Report of Testing Committee—W. T. CRANDALL, *Cornell University.*

E5. Effective Use of Herd Records—R. E. HORWOOD, *Michigan State College.*

4:00– 5:00 **Committee Meetings.**

Wednesday, June 19

Morning Session, *Agr. Assembly Room, Agr. Hall*

FLOYD J. ARNOLD AND GLENN SALISBURY—*Co-Chairmen*

9:00–12:00 **Joint Symposium Production and Extension Sections.**

Breeds Relations—Committee Reports.

A. Pasture and Roughage Committee, R. B. BECKER, *Chairman, University of Florida.*

B. Program of the Purebred Dairy Cattle Association, G. A. BOWLING, *Secretary.*

C. Breed Relations Committee, H. A. HERMAN, *Chairman, University of Missouri.*

D. Uniform Rules for Official Testing, FLOYD JOHNSTON, *Iowa State College.*

E. Dairy Cattle Breeding Committee, C. L. BLACKMAN, *Chairman, Ohio State University.*

F. Program Suggestions for Inter-Breed Groups, E. E. SCHEIDENHELM, *Rutgers University.*

G. Round Table Discussion, BREED ASSOCIATION REPRESENTATIVES.

Afternoon Session, *Room 117, Agr. Hall*

FLOYD J. ARNOLD—*Chairman*

1:00– 3:00 **Artificial Breeding, Exhibits.**

E6. Inspection of Exhibits, each exhibit will be explained by the person in charge.

E7. Report of Exhibit Committee, ARTHUR R. PORTER, *Iowa State College.*

E8. Organization and Operation of Artificial Breeding Associations, C. A. HUTTON, *University of Tennessee.*

E9. Some New Management Methods for Artificial Breeding Units, E. J. PERRY, *Rutgers University.*

E10. Schools and Program for Training Inseminators, RAYMOND ALBRECTSON, *Cornell University.*

3:00– 4:00 **Business Session.**

4:00– 5:00 **Committee Meetings.**
Campus Tours.

Thursday, June 20

Morning Session, *Agr. Assembly Room, Agr. Hall*

FLOYD J. ARNOLD AND GLENN SALISBURY—*Co-Chairmen*

9:00–11:00 **Joint Symposium Production and Extension Sections.** Herd Health and Sanitation—a panel discussion led by W. E. PETERSEN, *University of Minnesota.*

11:00–12:00 **Business Session**—*Room 117, Agr. Hall.*

Afternoon Session, *Room 117, Agr. Hall*

FLOYD J. ARNOLD—*Chairman*

1:00– 3:00 **4-H Club Work, Quality Programs.**

E11. New Developments in 4-H Club Work, J. C. NAGEOTTE, *Pennsylvania State College.*
a. Discussion of 4-H Club Projects.

E12. Extension Work in Quality Improvement of Dairy Products, A. B. NYSTROM, *Bureau of Dairy Industry.*

E13. Minnesota's Dairy Quality Improvement Program, H. R. SEARLES, *University of Minnesota.*

3:00– 5:00 **General Business Session,** *Agr. Assembly Room, Agr. Hall.*

Evening

6:30 **Annual Banquet—Installation of Officers and Presentation of Borden Awards.**

MANUFACTURING PROGRAM

Tuesday, June 18

Afternoon Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

1:00– 4:00 **Dry Milk Products.**

M1. A New Quantitative Method for Determining the Solubility of Milk Powders, J. FRANK CONE, U. S. ASHWORTH, *State College of Washington.*

M2. Drying Milk by the Use of Cold, OTTO F. HUNZIKER, *Dairy Consultant, La Grange, Illinois.*

M3. Biochemical Changes in Whole Milk and Ice Cream Mix Powders During Storage, N. P. TARASSUK, E. L. JACK, *University of California.*

M4. The Role of Moisture and Oxygen Levels on the Keeping Quality of Dry Ice Cream Mix, R. W. KUNKEL, S. T. COULTER, AND W. B. COMBS, *University of Minnesota.*

M5. Factors Related to the Brown Discoloration of Powdered Whole Milk, WALTER KRIENKE AND P. H. TRACY, *University of Illinois.*

M6. Observations on the Relative Keeping Quality of Powdered and Frozen Whole Milk, P. H. TRACY, JOHN HETRICK, AND WALTER KRIENKE, *University of Illinois.*

M7. Physico-chemical Factors Affecting the Reconstitutability of Dry Whole Milk, G. H. WILSTER, OTTO SHREITER, AND P. H. TRACY, *University of Illinois.*

M8. Changes in the Fluorescence of Dry Whole Milk on Storage, R. JENNESS, S. T. COULTER, AND R. LARSON, *University of Minnesota.*

M9. Changes in Reducing Materials in Dry Whole Milk During Storage, L. K. CROWE, S. T. COULTER, AND R. JENNESS, *University of Minnesota.*

M10. Drying Milk in Nitrogen or Carbon Dioxide, S. T COULTER, *University of Minnesota.*

M11. Relation of the Temperature of Separation and Heat Treatment Given the Serum Solids to the Keeping Quality of Spray Dried Ice Cream Mix, HARRY PYENSON AND P. H. TRACY, *University of Illinois.*

4:00– 5:00 **Committee Meetings.**

Wednesday, June 19

Morning Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

9:00–12:00 **Quality Symposium**—H. F. JUDKINS, *National Dairies, New York, Chairman.*

A. Milk.—G. M. TROUT, *Michigan State College;* C. V. CHRISTIANSEN, *Bowman Dairy Co., Chicago, Ill.*

B. Ice Cream.—A. C. DAHLBERG, *Cornell University;* E. C. SCOTT, *Swift & Company, Chicago, Ill.*

C. Butter.—R. V. HUSSONG, *University of Illinois;* G. W. SHADWICK, *Beatrice Creamery Co., Chicago.*

D. Cheese.—W. V. PRICE, *University of Wisconsin;* H. L. WILSON, *Kraft Cheese, Chicago, Ill.*

E. Manufactured Products.—P. H. TRACY, *University of Illinois;* O. F. GARRETT, *M & R Dietetic Laboratories, Columbus, Ohio.*

Afternoon Session, *Room 118, Dairy Industries Building*

G. C. NORTH, *Chairman*

1:00– 3:00 **Cheese and Evaporated Milk.**

M12. Gas Packing of American Cheddar Cheese, N. S. GOLDING, J. N. REYNOLDS, AND C. C. PROUTY, *State College of Washington.*

M13. The Action of the Associate Starter Organisms in American Cheddar Cheese, C. C. PROUTY AND N. S. GOLDING, *State College of Washington.*

M14. Studies on the Resistance of Various Cheese Cultures to the Action of Bacteriophage, F. J. BABEL, *Iowa State College.*

M15. The Influence of the Quality of Milk and Curing Temperatures on the Ripening of Pasteurized-Milk Cheddar Cheese, GEORGE P. SANDERS, HOMER E. WALTER, AND RALPH P. TITTSLER, *Bureau Dairy Industry, U.S.D.A.*

M16. The Manufacture of Grating Type Cheese, GEORGE H. WATROUS, *Pennsylvania State College.*

M17. The Influence of Age and Degree of Ripeness on the Activity of Cheese Cultures, H. C. OLSON AND RUSSELL BEACHBOARD, *Oklahoma A & M College.*

M18. A Study of the Physical Changes which Effect the Storage Life of Evaporated Milk, B. H. WEBB, E. F. DEYSHER, AND C. F. HUGNAGEL, *Bureau of Dairy Industry, U.S.D.A.*

M19. Observations on the Judgment of Flavor Defects in Dairy Products, D. V. JOSEPHSON, *Ohio State University.*

3:00– 4:00 **Business Session.**

4:00– 5:00 **Committee Meetings.**

Campus Tours.

Thursday, June 20

Morning Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

9:00–11:00 **Bacteriology and Chemistry.**

M20. A Water Soluble Antioxident for Milk and Milk Products, N. P. TARASSUK AND J. L. HENDERSON, *University of California.*

M21. Isolation of Whey Proteins and their Conversion into Food Products, L. A. BURKEY AND H. E. WALTER, *Bureau of Dairy Industry, U.S.D.A.*

M22. Variations in the Acid Degree of Milk Fat as Affected by Churning and Extraction Procedures, B. C. JOHNSON AND I. A. GOULD, *University of Maryland.*

M23. Concentration of the Free Fatty Acid Portion of Milk Fat by Alcohol Extraction, I. A. GOULD, *University of Maryland.*

M24. The Direct Microscopic Count as a Method for Counting Bacteria in Pasteurized Milk, M. P. BAKER, *Iowa State College.*

M25. Non-lactose Fermenting Yeasts and Yeast-like Fungi from Cream and Butter, STANLEY H. F. CHINN AND F. E. NELSON, *Iowa State College.*

M26. Improvements in the Phosphatase Test for Milk and for Cheese, GEORGE P. SANDERS AND OSCAR S. EAGER, *Bureau of Dairy Industry, U.S.D.A.*

M27. The Relationship of the Growth of all Bacteria and Coliform Bacteria in Pasteurized Milk Held at Refrigeration Temperatures, A. C. DAHLBERG, *Cornell University.*

11:00–12:00 **Business Session**—*Room 118, Dairy Industries Building.*

Afternoon Session, *Room 118, Dairy Industries Building*

L. K. CROWE—*Chairman*

1:00– 3:00 **Chemistry, Sanitation, Teaching.**

M28. Some Observations Regarding the Effect of Various Wave Lengths of Light on the Riboflavin Content and Flavor of Milk, D. V. JOSEPHSON, *Ohio State University.*

M29. Sanitization of Creamery Water Supplies with Various Cationic Bactericides, C. JENSEN, *North Dakota Agricultural College.*

M30. Manufacture of Powdered Cream Mix for Whipping by Aeration, HARRY PYENSON AND P. H. TRACY, *University of Illinois.*

M31. Influence of Nonfat Dry Milk Solids on the Nutritive Value of Bread, LLOYD K. RIGGS, ANABEL BEATY, AND ARNOLD H. JOHNSON, *Kraft Cheese Co., Chicago, Illinois.*

M32. A Device to Aid in Determining the Effectiveness of Dairy Detergents, E. I. FOUTS AND T. R. FREEMAN, *University of Florida.*

M33. Studies of a "Self Washing" Cream Separator, E. O. HERRIED, RALPH HUSSON, AND P. H. TRACY, *University of Illinois.*

M34. An "In Training" Course for Workers in the Dairy Manufacturing Industry, P. H. TRACY AND E. O. HERRIED, *University of Illinois.*

3:00– 5:00 **General Business Session,** *Agr. Assembly Room, Agr. Hall.*

Evening

6:30 **Annual Banquet—Installation of Officers and Presentation of Borden Awards.**

PRODUCTION PROGRAM

Tuesday, June 18

Afternoon Session

1:00– 4:00 SECTION A—**Vitamins,** *Room 19, Agr. Hall,* G. W. SALISBURY, *Chairman.*

P1. The Effect of Massive Doses of Vitamin D on the Blood Picture of Dairy Cows at Parturition, J. W. HIBBS, *Ohio Agricultural Experiment Station.*

P2. Carotene and Vitamin A in the Blood and the Liver of Newborn and Colostral Fed Calves, G. H. WISE, M. J. CALDWELL, F. W. ATKESON, AND J. S. HUGHES, *Kansas Agricultural Experiment Station.*

P3. The Placental Transmission of Vitamin A and Carotene in the Bovine, A. A. SPIELMAN, J. W. THOMAS, AND J. K. LOOSLI, *Cornell University.*

P4. Utilizing All Colostrum in Calf Feeding, H. E. KAESER AND T. S. SUTTON, *Ohio State University and the Ohio Agricultural Experiment Station.*

P5. Effect of Vitamin Supplements on Survival of Newborn Calves, R. G. HANSEN, P. H. PHILLIPS, AND I. W. RUPEL, *University of Wisconsin.*

P6. The Effect of Feeding Vitamin A on the Blood Picture and on Liver Storage in Calves, J. W. HIBBS AND W. E. KRAUSS, *Ohio Agricultural Experiment Station.*

P7. The Relationship of Prepartum Diet to the Carotene and Vitamin A Content of Colostrum, J. W. THOMAS, A. A. SPIELMAN, AND K. L. TURK, *Cornell University.*

P8. Vitamin A and Globulin Content of Colostrum Milk, R. G. HANSEN, P. H. PHILLIPS, J. W. WILLIAMS, AND V. R. SMITH, *University of Wisconsin.*

P9. The Effect of Vitamin A Deficiency on Reproduction in Dairy Bulls, R. E. HODGSON, S. R. HALL, W. J. SWEETMAN, H. G. WISEMAN, AND H. T. CONVERSE, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P10. Relation of Carotene Levels to Fertility in Dairy Bulls, I. R. JONES, J. O. SCHNAUTZ AND J. R. HAAG, *Oregon State College.*

SECTION B—**Hormones, Blood, and Disease,** *Agr. Assembly Room, Agr. Hall,* D. M. SEATH, *Chairman.*

P11. The Adrenal Cortex in Relation to Ketosis in Dairy Cows, J. C. SHAW, *University of Maryland.*

P12. Effect of Stilbestrol on Lactation and Reproduction, SIDNEY MARSHALL, R. B. BECKER, AND P. T. DIX ARNOLD, *Florida Agricultural Experiment Station.*

P13. Elimination of Male Hormone by Lactating Dairy Cows, C. W. TURNER, *University of Missouri.*

P14. A Technique for Intravenous Feeding of Ruminants with Some Observations on the Effect of the Administration of Various Substances Upon the Secretion of Milk Fat, A. I. MANN, *University of Connecticut,* AND J. C. SHAW, *University of Maryland.*

P15. Blood Picture of Louisiana Dairy Cattle, L. L. RUSOFF AND P. L. PIERCY, *Louisiana State University.*

P16. Studies of the Growth and Blood Composition of Dairy Calves Fed Remade Skimmed Milk after Three Days of Age, P. M. REAVES AND L. R. ARRINGTON, *Virginia Polytechnic Institute.*

P17. The Effect of Complete Milking on Blood-Serum Calcium of Cows at Parturition, VEARL R. SMITH, *University of Wisconsin.*

P18. The Occurrence of Various Bacterial Infections and Their Significance in Bovine Mastitis, L. A. BURKEY, W. W. SWETT, AND CECELIA R. BUCKNER, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P19. Some Results in Treating Mastitis by Means of Sulfanilamide, Tyrothricin, and Penicillin, H. A. HERMAN, J. E. EDMONDSON, A. C. RAGSDALE, AND O. S. CRISLER, *University of Missouri.*

P20. The Effect of Ingestion of D.D.T. upon Dairy Cows, N. N. ALLEN, H. A. LARDY, AND H. F. WILSON, *University of Wisconsin.*

4:00– 5:00 **Committee Meetings.**

Wednesday, June 19

Morning Session, *Agr. Assembly Room, Agr. Hall*

FLOYD J. ARNOLD AND G. W. SALISBURY—*Co-Chairmen*

9:00–12:00 **Joint Symposium Production and Extension Sections.** Breeds Relations—Committee Reports.

A. Pasture and Roughage Committee, R. B. BECKER, *University of Florida, Chairman.*

B. Program of the Purebred Dairy Cattle Association, G. A. BOWLING, *Secretary.*

C. Breed Relations Committee, H. A. HERMAN, *University of Missouri, Chairman.*

D. Uniform Rules for Official Testing, FLOYD JOHNSTON, *Iowa State College.*

E. Dairy Cattle Breeding Committee, C. L. BLACKMAN, *Ohio State University, Chairman.*

F. Program Suggestions for Inter-Breed Groups, E. C. SCHEIDENHELM, *Rutgers University.*

G. Round Table Discussion, BREED ASSOCIATION REPRESENTATIVES.

Afternoon Session

1:00– 3:00 SECTION A—**Thyroprotein, Protein, and Minerals,** *Room 19, Agricultural Hall,* G. W. SALISBURY, *Chairman.*

P21. Thyroid Secretion Rate of Growing, Pregnant, and Lactating Albino Rats, R. A. MONROE AND C. W. TURNER, *University of Missouri.*

P22. Some Physiological Effects of Feeding Thyroprotein to Dairy Cows, L. A. MOORE, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P23. The Influence of a Synthetic Thyroprotein When Fed to Dairy Cows Over an Extended Period of Time, RALPH P. REECE, *New Jersey Agricultural Experiment Station.*

P24. Effect of Feeding Thyroprotein to Dairy Cows on the Protein Composition and Content of Milk, A. H. VANLANDINGHAM AND GEORGE HYATT, JR., *West Virginia Agricultural Experiment Station.*

P25. The Influence of Feeding Synthetic Thyroprotein on Fertility of Bulls, A. B. SCHULTZE AND H. P. DAVIS, *University of Nebraska.*

P26. Utilization of Non-Protein Nitrogen by Dairy Heifers, I. R. JONES AND J. R. HAAG, *Oregon State College.*

P27. Shark Meal as a Protein Supplement in Dairy Calf Rations, SIDNEY P. MARSHALL, *Clemson Agricultural College.*

P28. Ground Mungbeans as a Protein Supplement in Rations for Dairy Cows, A. H. KUHLMAN AND H. W. CAVE, *Oklahoma A & M College.*

P29. A Preliminary Report of a Study of the Effects of Manganese on Calcium Metabolism in Lactating Cows, J. T. REID, K. O. PFAU, C. B. BENDER, AND R. L. SALISBURY, *New Jersey Agricultural Experiment Station.*

SECTION B—**Feeding and Management,** *Agricultural Assembly Room, Agricultural Hall,* D. M. SEATH, *Chairman.*

P30. Needed Dairy Feed Ingredient Research, R. T. PARKHURST, *Flory Milling Company, Inc., Bangor, Pennsylvania.*

P31. Ground Ear Corn as Compared to Ground Shelled Corn in a Simple Grain Mixture for Milk Production, C. F. MONROE AND W. E. KRAUSS, *Ohio Agricultural Experiment Station.*

P32. Ten Years Evaluation of Bluegrass Pastures, H. B. MORRISON AND FORDYCE ELY, *Kentucky Agricultural Experiment Station.*

P33. Silage from Normal and from Drouth Damaged Corn, C. F. MONROE, A. E. PERKINS, C. E. KNOOP,

AND LOUISE SKINNER, *Ohio Agricultural Experiment Station.*

P34. The Unknown Lactation Factors in Corn Silage, C. F. HUFFMAN, S. T. DEXTER, AND C. W. DUNCAN, *Michigan State College.*

P35. A Preliminary Report on the Study of Factors Influencing Rumen Microflora, A. L. BORTREE, K. M. DUNN, R. E. ELY, AND C. F. HUFFMAN, *Michigan State College.*

P36. The Physiological Role of the Rumen of the Young Bovine as Indicated by the Growth and Blood Composition of a Rumen-ectomized Calf, G. H. WISE, R. R. LINK, W. W. THOMPSON, AND M. J. CALDWELL, *Kansas Agricultural Experiment Station.*

P37. Effect of High Humidity and High Temperature on Dairy Cows, D. M. SEATH AND G. D. MILLER, *Louisiana Agricultural Experiment Station.*

P38. The Causes and Extent of Variations in Weight of Dairy Cows Weighed at Half-hour Intervals over Ten-day Periods, N. N. ALLEN, *University of Wisconsin.*

3:00– 4:00 **Business Session,** *Agricultural Assembly Room, Agricultural Hall.*

4:00– 5:00 **Committee Meetings.**
Campus Tours.

Thursday, June 20

Morning Session, *Agr. Assembly, Agr. Hall*

FLOYD J. ARNOLD AND G. W. SALISBURY—*Co-Chairmen*

9:00–11:00 **Joint Symposium Production and Extension Sections.** Herd Health and Sanitation—a panel discussion led by W. E. PETERSEN, *University of Minnesota.*

11:00–12:00 **Business Session**—*Agricultural Assembly Room, Agricultural Hall.*

Afternoon Session

1:00– 3:00 SECTION A—**Hay,** *Room 19, Agricultural Hall,* G. W. SALISBURY, *Chairman.*

P39. Standardizing Hay Palatability Trials with Dairy Calves, H. S. WILLARD, *University of Wyoming.*

P40. Comparative Value of Alfalfa, Mixed Grasses and Legume, and Timothy Hays for Young Dairy Calves, C. L. NORTON, *Cornell University.*

P41. Feeding Barn Dried Hay to Dairy Heifers, C. E. WYLIE, JOHN EWING, AND S. A. HINTON, *University of Tennessee.*

P42. Progress Report on Comparing the Efficiency of Three Methods of Harvesting and Preserving Forage Crops, R. E. HODGSON, J. B. SHEPHERD, L. G. SCHOENLEBER, H. M. TYSDAL, AND W. H. HOSTERMAN, *Bureau of Dairy Industry, Bureau of Plant Industry, Soils and Agricultural Engineering, and Production and Marketing Administration, Agricultural Research Administration, U.S.D.A., cooperating.*

P43. Nutritive Value of Barn-cured Hay, K. L. TURK AND C. L. NORTON, *Cornell University.*

P44. A Still Unidentified Nutrient in Roughages and in Milk, A. M. HARTMAN AND C. A. CARY, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P45. Fan Ventilated Long Hay: A Chemical Study, A. E. PERKINS AND LOUISE SKINNER, *Ohio Agricultural Experiment Station.*

P46. Comparative Feeding Value of Wilted Alfalfa Silage and Alfalfa Hay for Dairy Cows, J. B. SHEPHERD AND W. J. SWEETMAN, *Bureau of Dairy Industry, Agricultural Research Administration, U.S.D.A.*

P47. Early vs. Late Cut Lespedeza Hay for Dairy Cows, JOHN EWING AND C. E. WYLIE, *University of Tennessee.*

SECTION B—**Breeding,** *Agricultural Assembly Room, Agricultural Hall,* G. W. SALISBURY, *Chairman.*

P48. Crossing Zebu with Northern Breeds of Cattle in Jamaica, JOHN W. HOWE, *Iowa State College.*

P49. Some Experiences with Central Artificial Breeding Organizations, H. A. HERMAN, W. K. DINWIDDIE, AND M. J. REGAN, *University of Missouri.*

P50. The Use of Pregnancy Diagnosis with Artificial Breeding, L. E. CASIDA, G. R. BARRETT, AND C. A. LLOYD, *University of Wisconsin.*

P51. The Effect of Certain Coal-Tar Dyes Used for Semen Identification on the Livability and Fertility of Bull Spermatozoa, J. O. ALMQUIST, *Pennsylvania State College.*

P52. The Effect of Feeding Potassium Iodide and Skim Milk Powder on Spermatogenesis, C. E. KNOOP, *Ohio Agricultural Experiment Station.*

P53. Seasonal Effect on Spermatogenic Activity in the Bull, ERNEST MERCIER, *Cornell University and Ministry of Agriculture of Quebec Province, Canada.*

P54. Time of Insemination and Conception Rate in Artificial Breeding, G. R. BARRETT AND L. E. CASIDA, *University of Wisconsin.*

P55. Artificial Insemination as a Means of Genetic Improvement, J. L. LUSH, *Iowa State College.*

P56. Deleterious Recessive Genes in Dairy Bulls Selected at Random, S. W. MEAD, P. W. GREGORY, AND W. M. REGAN, *University of California.*

3:00– 5:00 **General Business Session,** *Agricultural Assembly Room, Agricultural Hall.*

Evening

6:30 **Annual Banquet—Installation of Officers and Presentation of Borden Awards.**

VOLUME XXIX MAY, 1946 NUMBER 5

JOURNAL OF DAIRY SCIENCE

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION
R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with
INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec, Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
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JOURNALS

American Butter Review
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American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics
Australian Journal of the Council for Scientific and Industrial Research

Biochemical Journal

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

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National Butter and Cheese Journal
New Zealand Journal of Science and Technology

Oil and Soap

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SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

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

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BOOK REVIEW

134. Food Industries Manual.—An Encyclopaedia of Food Manufacture. Fourteenth Edition. T. CROSBIE-WALSH, General Editor. Leonard Hill, Limited, 17 Stratford Place, W.I., London, 1945.

This revised edition of the Food Industries Manual is divided into a number of sections, each of which is compiled by one or more recognized British authorities in the particular field. Of 1062 pages, 294 are devoted to the Dairy Industry. This section is compiled by the well-known dairy scientist, J. G. Davis, of the National Institute for Research in Dairying, Reading, England. Other fields covered in this Manual are Cereals, Canning and Preserving, Meat Products, Pickles and Sauces, Food Dehydration, Storage and Refrigeration, Packing and several others.

The book is unique in that under each section the material is presented alphabetically in encyclopedic form so that the reader may refer to a particular subject directly without the use of the index. In general, subjects are treated briefly.

The Dairy Industry sections is covered quite generally with by far the greatest emphasis being placed on the bacteriological aspects of dairy technology. Methods, media, equipment and procedures for most bacteriological techniques are outlined in detail. Problems with starters, ropy milk, sweet curdling, etc., are examples of commercial problems, which are well covered. Very little detail is given on the commercial manufacture of butter and practically nothing is presented on the subject of ice cream. Cheese, particularly cheddar and the British varieties are covered in some detail and methods of manufacture are given. D.V.J.

BACTERIOLOGY

135. The Bacteria "Count"—An Estimate Capable of Accurate Interpretation. JAMES D. BREW, University of Tennessee, Knoxville, Tenn., and Robert S. Breed, N. Y. State Experiment Station, Geneva, N. Y. Amer. Jour. Public Health, *35*, No. 7: 683. 1945.

In view of uncontrollable variabilities, agar plate and microscopic estimates should be used conservatively. Estimates may be used safely in judging whether a milk is in "acceptable," "satisfactory" or "unsatisfactory" condition but fine distinctions are not jusified. Wherever the agar plate method is used, it should be remembered that many of the high and low temperature organisms that occur so frequently in pasteurized milk and cream may not grow at 37° C. and that some fastidious organisms will not

grow well on even the new standard agar. While some dead bacteria may appear in stainable condition in making microscopic estimates, it should be remembered that dead bacteria are just as significant as the living in interpreting the past history of a given sample of milk. No high quality market milk or cream contains an excessive number of bacteria, either living or dead. M.W.Y.

BUTTER

136. Flavor Reversion in Edible Fats. A. E. Bailey. Southern Reg. Res. Lab., New Orleans, La. Oil and Soap, *23*, 55–58. Feb., 1946.

Bailey defines flavor reversion in fats as "the appearance of objectionable flavor from less oxidation than is required to produce true rancidity" (oxidative). Reversion occurs after the absorption of very small amounts of oxygen. Marine oils and vegetable oils such as soybean, linseed and rapeseed oils that contain fatty acids with more than two double bonds are more susceptible to this defect. In unhydrogenated oils a second type of flavor reversion results in fishiness. This is apparently due to a reaction between phospholipides (probably lecithin) and highly unsaturated fatty acids (or their products).

Flavor reversion in hydrogenated oils is only destroyed by prolonged treatment. Evidence recently obtained shows that isolinoleic acid, a product obtained by hydrogenating linolenic acid in the middle or 12:13 double bond, is probably the source of the flavor and odor defect. Present knowledge, however, does not permit a hypothesis being advanced regarding the mechanism of the reaction between oxygen and isolinoleic acid or other materials actually responsible for the flavor and odor. The solution of the problem may be found in improving hydrogenation processes that will avoid the presence of isolinoleic acid. J.L.H.

CHEESE

137. Clarifying Milk for Cheesemaking. E. G. Hood and I. Hlynka, Dept. of Agriculture, Ottawa, Canada. Nat. Butter & Cheese Jour., *37*, No. 1: 38. Jan., 1946; *37*, No. 2: 39. Feb., 1946.

At one of the larger cheese factories in eastern Ontario, cheese was made from clarified and unclarified milk. It was demonstrated that clarification removed sediment. Cheese quality was improved in flavor and texture. Of the cheese made from clarified milk, a total of 98.7% were first grade as compared with 89.3% of cheese made from unclarified milk. Fat losses in the whey were reduced by clarification from 3.67 to 3.01 pounds of fat per 1000 pounds of whey in one series and from 2.60 to 2.47 pounds in another series. The clarified milk developed acid at a faster rate. No adverse effects of clarification were noted. W.V.P.

138. Manufacture of Parmesan Cheese from Raw and Pasteurized Milk. E. T. BERTONASCO, Stella Cheese Co., Cumberland, Wis. Natl. Butter and Cheese Jour., *37*, No. 2: 34. Feb., 1946.

The Parmesan cheese made in the United States is the type known in Italy as Reggiano Emiliano or Parmigiano and as Reggiano Lodigiano. The Lodigiano has a lower fat content. Milk is standardized to produce a cured cheese with 36% fat in the dry matter and 32% moisture. Pasteurized whey or milk is prepared for starter by inoculating with equal amounts of *Bacterium lactis acidi* Leichmann, and *Streptococcus thermophilus;* incubation is at 122° F. for 16 hours to develop 0.7 to 0.8% acidity. Starter is added at the rate of 2 to 4%. Liquid rennet or powder is used to give coagulation in 15 minutes at 90 to 95° F. The curd is first cut into pieces the size of a hazel nut and finally reduced to the size of rice kernels. When the particles are "the proper size and firmness," the temperature is raised to 122° to 133° F. in 1½ to 2 hours. From cutting to the end of cooking, the acidity increases from 0.02 to 0.03%. When experience indicates the correct matting point, the curd is dipped by slipping a linen curd cloth under the curd mass and hoisting it out of the kettle with block and tackle. The curd is drained briefly, divided, placed in metal hoops, pressed and later held 2 or 3 days in round wooden hoops called "fascere." The cheese, which at 3 days should contain 40 to 42% moisture, is brine salted for 15 to 30 days, and cured at 58 to 60°. It is turned frequently, rubbed with oil and finally vegetable black. The cheese is cured for 16 to 24 months and averages 6.5% yield.

Defects of Parmesan may be acid texture, acid flavor, lack of flavor, weak, lack of granular structure, or gassy texture. Usually these defects are caused by faulty acid development, improper starter management, lack of moisture control, or inferior milk. Pasteurized milk can be used for Parmesan by increasing the amount of starter and the temperatures of setting, cooking, and curing. The cheese is used chiefly in the grated form or may be dried in 6 to 18 hours at 60 to 80° F. Temperatures of 120° F. or more have been frequently adopted. The dehydrated cheese is sometimes blended with other cheese or dry skim milk or both to alter its flavor or keeping qualities. W.V.P.

CHEMISTRY

139. Oxygen Absorption of Methyl Esters of Fat Acids and the Effect of Antioxidants. A. J. STIRTON, J. TURER, and R. W. RIEMENSCHNEIDER. Eastern Regional Res. Lab., Philadelphia, Pa. Oil and Soap, *22*, 81–83. April, 1945.

The Barcroft-Warburg apparatus was used to measure the oxygen absorption of methyl esters of fat acids at 100° C. The methyl esters of linolenic,

linoleic, oleic and stearic acids and the distilled methyl esters of lard as well as mixtures of the four individual esters were studied. The mixtures absorbed oxygen at a rate that could be approximately predicted from the rate of oxygen absorption of each component acid and the percentage of each present.

The influence of certain antioxidants on the rate of oxygen absorption was also determined. The most effective antioxidants studied were combinations of citric acid with nordihydroguaiaretic acid and with propyl gallate. Citric acid also showed synergistic action with the other antioxidants studied. J.L.H.

140. Purification of Lactic Acid. Production of Methyl Lactate from Aqueous Solutions of Crude Acid. E. W. FILACHIONE and C. H. FISHER. Eastern Regional Res. Lab., U. S. Dept. Agr., Philadelphia 18, Pa. Jour. Ind. Eng. Chem., Ind. Ed., *38*, No. 2: 228–232. Feb., 1946.

A new method for purifying lactic acid or preparing methyl lactate from crude aqueous lactic acid is described. Methanol vapor is passed through aqueous lactic acid, the effluent vapors are condensed and either methyl lactate is recovered by distillation or purified lactic acid is obtained by hydrolysis of the condensate. Other esters of lactic acid can be prepared similarly with appropriate alcohols. The process can be used with crude unfiltered fermentation liquors. The effect of variables on the volatization of lactic acid with methanol vapor was studied. B.H.W.

DISEASE

141. Differential Bactericidal Activity of Bovine Serum Toward Strains of Brucella Abortus of High and Low Virulence. M. R. IRWIN and B. A. BEACH. Jour. Agr. Res., *72*, No. 2: 83. Jan., 1946.

The bacterial action of the serum from both normal and vaccinated cattle has been shown to depend on the combined activity of antibody and complement. The serum of normal animals usually has an appreciable bactericidal activity at dilutions of 1:40 or 1:80, some individuals at 1:160, rarely higher. The serum of animals vaccinated as adults, or nearly so, showed a definite antibacterial activity to *Brucella Abortus* at dilutions of 1:1280 and even at 1:10, 240 in some individuals. The serum of these individuals showed partial agglutinating reactions no higher than 1:100, but with that from one cow at 1:200. In the majority of the tests, undiluted or diluted serum destroyed more organisms of strain 19 than the more virulent strains. These tests show that even in the almost complete absence of agglutinating antibodies, the serum of a vaccinated animal may exert antibacterial activity against *Brucella Abortus* greater than that of serum of normal animals.

These results substantiate a previous report on a parallel finding in the serum of animals once infected, but with a titer of agglutinating antibodies no higher than in normal cattle. H.P.

142. **Outbreak of Infectious Hepatitis, Apparently Milk-borne.** W. J. MURPHY and L. M. PETRIE, Georgia Department of Public Health, Atlanta, Ga., and Samuel D. Work, Jr., Forsyth, Ga. Amer. Jour. Public Health, *36*, No. 2: 169. 1946.

The purpose of this report is to present epidemiological evidence that the icterogenic agent of infectious hepatitis may be transmitted by milk. In 1945, a small outbreak of 10 cases occurred in Forsyth, Georgia. All of the patients drank milk supplied by a single dairy. Circumstantial evidence points definitely toward a single milk supply as the source of the outbreak. M.W.Y.

ICE CREAM

143. **Commercial Ice Cream Making.** CHESTER D. DAHLE, Pennsylvania State College. Circular 277. 30 pages. 1945.

This circular presents a brief but rather complete description of practical commercial ice cream making. The calculation of mixes is covered thoroughly with many type formulas presented as examples.

All phases of ice cream processing are described and definite recommendations and suggestions are made regarding temperatures of pasteurization and storage, pressures for homogenization, etc. Acidity and its standardization is covered in detail with recommendations made regarding the use of various neutralizers.

The freezing process together with the practical problems of overrun control are discussed. The question of hardening and distribution procedures are considered and helpful suggestions made regarding proper handling.

The matter of ingredients for ice cream making is briefly, but well covered. Sugars, stabilizers, flavors and egg products are discussed and recommendations made regarding amounts to use. Suggested formulas for sherbets, ices, frosted malteds and custards are also given. D.V.J.

PHYSIOLOGY

144. **Effect of Ambient Air Temperature and of Hand Temperature on Blood Flow in Hands.** C. R. SPEALMAN, Naval Medical Research Institute, Bethesda, Maryland. Amer. Jour. Physiol., *145*, 2: 128–222. Dec., 1945.

At any given hand temperature, blood flow was greater the warmer the body. This effect was most pronounced in moderately cold hands (ca. 15° C.) where blood flow in the case of the uncomfortably warm environment was about twenty times that in the uncomfortably cold environment, and

least pronounced in very cold hands (ca. 10° C.) where the difference was only about threefold. (The reviewer raises the question whether there is a similar relationship between the blood flow in the cow's body and the udder.) D.E.

145. **Consideration of the Mechanism of Neutralization of Endogenous Gonadotrophic Hormone of the Rat by Antigonadotrophic Serum.** HERBERT S. KUPPERMAN and ROLAND K. MEYER, Dept. of Zoology, Univ. of Wisconsin, Madison. Amer. Jour. Physiol., *145*, 2: 181–185. Dec., 1945.

The authors point out that the physiological action of antigonadotrophic sera upon the hypophysis and gonads of normal animals produces simultaneously in a single animal both the effects of castration and hypophysectomy. By injection of parabiotic triplet rate they conclude that antigonadotrophic sera can inhibit the endogenous gonad-stimulating hormone in the blood stream after its release from the pitiutary gland. D.E.

146. **The Influence of Sodium Lauryl Sulfate on the Biologic Response to the Gonadotrophins and to Insulin.** FRITZ BISCHOFF, Santa Barbara Cottage Hospital Research Institute, Santa Barbara, Calif. Amer. Jour. Physiol., *145*, 2: 123–129. Dec., 1945.

The subcutaneous injection of sodium lauryl sulfate produces an edematous area which may be effectively used in delaying resorption of the gonadotropins, thus augmenting the effect of the sheep pituitary and chorionic gonadotropins. D.E.

MISCELLANEOUS

147. **Soybean or Vegetable Milk.** A Resume and Bibliography. A. K. SMITH, and A. C. BECKEL. Oil and Protein Division, Northern Regional Res. Lab., Peoria, Ill. Chem. and Eng. News, *24*, No. 1: 54–56. Jan., 1946.

A short discussion of soybean milk, its preparation, characteristics and composition as compared with cow's milk is presented. The bibliography includes 124 references. B.H.W.

148. **Standards for Measuring Creamery Efficiency.** PROF. L. C. THOMSEN, University of Wisconsin, Madison. Natl. Butter & Cheese Jour., *37*, No. 2: 36. Feb., 1946.

Efficiency in a creamery is the effective operation of that business to produce the desired results with a minimum waste of effort. This efficiency cannot be measured simply by butter "overruns." It can only be attained by study of efficient plants, labor costs, composition control, manufacturing losses, utilization of by-products, equipment, cost accounts and market outlets. W.V.P.

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