JOURNAL OF DAIRY SCIENCE

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RESAZURIN REDUCING TIME AS AN INDICATOR OF BOVINE SEMEN FERTILIZING CAPACITY[1]

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Resazurin is a chemical indicator which, during its reduction, proceeds through a series of color changes. Resazurin is blue in milk or in water solution and reduces to resorufin which is pink in color. Resorufin further reduces to hydroresorufin, a colorless compound. The change to resorufin is irreversible, while the reduction to hydroresorufin is reversible (13). Resazurin has been extensively investigated for use as a rapid indicator of milk quality (2, 15). Its use now has gained considerable popularity because of its rapid reducing time, and it appears more versatile than the older methylene blue test as an indicator of milk quality. Methylene blue also has been used as an indicator of semen quality. This test was developed by Beck and Salisbury (1) and has been found to be quite highly correlated with initial motility and concentration. The basis of the test (1, 14) consists in determining time in minutes for semen diluted at a standard rate with yolk-citrate to reduce a 1 to 40,000 concentration of methylene blue.

The purpose of this paper is to report comparisons of the resazurin reduction time of bull semen to non-return rates, methylene blue reduction time, initial motility, concentration and survival at 3.3 and 45° C.

EXPERIMENTAL

Semen samples were collected with the artificial vagina from eight young bulls at the State College of Washington and from 37 bulls regularly used for artificial breeding at the Northwest and Evergreen Co-op Breeders bull studs. Preliminary studies were started in January, 1948. Resazurin test solutions were prepared in distilled water at the rate of 11 mg. of the dye to 200 ml. The resazurin was procured in tablet form from the National Aniline Division of the Allied Dye Corp. Fresh solutions were made up once monthly and were stored

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in the refrigerator at 3.3° C. in brown glass bottles. No evidence of destruction could be detected up to 1 mo. in storage. The technique of the test was essentially the same as that described by Beck and Salisbury (1) for determination of methylene blue reduction time for semen, with the exception that the semen was undiluted. One-tenth ml. of the test solution was pipetted directly into a small culture tube containing 0.2 ml. of fresh undiluted semen, in a water bath at 45° C. The test sample was rotated in the bath to insure mixing and then layered with mineral oil. The pink endpoint was clear and complete. The white endpoint was adjudged as that time when 85 per cent of the column of semen was white. Methylene blue reduction time was determined in exactly the same manner, using 0.1 ml. of a 1 : 40,000 concentration of methylene blue and 0.2 ml. of undiluted semen.

Initially, resazurin reduction time was compared with such determinations as initial motility, concentration, ascorbic acid content and survival at 3.3 and 45° C. Initial motility in these studies was scored from 0 to 10, with 10 representing the maximum motility. Concentration was determined with the hemocytometer and ascorbic acid by the method of Roe and Keuther (9). In order to compare this test with fertilizing capacity, cooperative experiments were conducted with the two breeding associations located in Washington. Non-return rates (60 to 90 days) for all first and second services were determined on each sample by bulls, by local units and by age of semen at the time of use. Records of initial motility and concentration were kept. Resazurin and methylene blue reduction times were recorded on 507 semen samples unincubated prior to the tests and on 323 semen samples incubated for 30 min. at 45° C. before making the tests. Semen samples used for breeding 20 or more cows (first and second services) were used for statistical analyses, as suggested by Erb *et al.* (4). Non-return rates were expressed in per cent and converted to angles for final analysis. Statistical analyses were according to Snedecor (10).

RESULTS

Comparisons of resazurin reduction time to whole semen ascorbic acid content survival at 3.3 and 45° C. were made on 94 semen samples collected from three bulls between 1 and 2 yr. of age during the first half of 1948. The samples used for comparison ranged from 1 to 10 in initial motility. The results of this phase of the study are shown in table 1. The ascorbic acid content of 94 samples of whole semen average 8.0 mg. per cent as compared with 8.8 mg. per cent for the seminal plasma. For the purposes of this study, it was felt that values for whole semen were more applicable. Thirty semen samples which reduced resazurin to pink in 1 to 5 min. averaged 8.7 $\pm$ 0.51 mg. per cent, as compared with 6.7 $\pm$ 0.68 mg. per cent ascorbic acid for 12 samples requiring 21 to 60 min. for reduction to pink. The five samples which did not completely reduce to pink in 1 hr. averaged slightly higher (7.1 $\pm$ 1.35), but the high standard error reflects the need for more samples in this range. Thirty-six of the 94 samples studied reduced resazurin to the white endpoint in 30 min. or less. The average ascorbic acid content of these samples was 8.5 $\pm$ 0.41 mg. per cent, compared

TABLE 1

Resaurin compared with whole semen ascorbic acid content and survival time at 3.3 and 45° C.

Pink endpoint						White endpoint					
Reduction time interval	No. Samples	Av. time within intervals	Ascorbic Acid	Survival at:		Reduction time interval	No. Samples	Av. time within interval	Ascorbic Acid	Survival at:	
				3.3° C.	45° C.					3.3° C.	45° C.
(*min.*)		(*min.*)	(*mg. %*)	(*d.*)	(*min.*)	(*min.*)		(*min.*)	(*mg. %*)	(*d.*)	(*min.*)
1–5	30	3.6	8.7±0.51	15.2±1.4	81.8± 5.7	0–30	36	18.9	8.5±0.41	14.1±1.0	89.3± 7.7
6–10	21	7.5	7.7±0.50	11.2±1.4	113.8±14.3	31–60	18	40.4	9.0±0.58	12.9±2.0	106.5±14.8
11–15	20	13.2	8.3±0.36	10.5±1.4	113.2±14.6	Partial	..	....	.....	.....	.....
16–20	6	18.0	8.7±1.01	5.6±1.8	84.8±33.8	in 60	23	....	7.4±0.38	10.1±1.9	102.0±13.4
21–60	12	32.5	6.7±0.68	8.8±3.4	46.9±15.5	>60	17	....	6.9±0.68	4.6±1.3	41.9±11.2
>60	5	....	7.1±1.35	3.6±2.4	25.5± 8.2	.....	..	....	.....	.....	.....
Total	94	....	8.0±0.25	11.4±0.8	87.6± 6.1	Total	94	....	8.0±0.25	11.4±0.8	87.6± 6.1

with 6.9 ± 0.68 mg. per cent for 17 samples showing no reduction to white in 60 min. By analysis of variance (93 d.f.) the relationship of ascorbic acid level to resazurin reduction to pink was not significant. The same comparison with the white endpoint approached significance at the 5 per cent level. From these limited data representing all levels of semen quality, it appears the quantity of ascorbic acid in semen does not materially affect the resazurin reduction test.

Survival time under storage (undiluted semen) at 3.3° C. and under incubation at 45° C. varied inversely with reducing times for both the pink and white endpoints. Survival time in each case was measured to zero motility. The variation between the means for the two measures of survival for the respective time intervals for resazurin reduction shown in table 1 were highly significant.

TABLE 2

Comparison of initial motility with resazurin reduction time

Initial motility rating	Pink				White			
	No. samples	Reduced in 1 hr.	Av. reduction time[a]	Range[b]	No. samples	Reduced in 1 hr.	Av. reduction time[a]	Range[b]
		(%)	(*min.*)	(*min.*)		(%)	(*min.*)	(*min.*)
0	6	0.0	—	—	6	0.0	—	—
1	26	76.9	40.3	12–60	27	33.3	40.0	14–54
2	18	77.8	24.6	5–60	19	26.4	27.0	9–59
3	29	79.3	22.3	3–60	27	33.3	39.3	5–60
4	13	84.6	15.5	4–60	13	30.8	25.5	19–60
5	31	96.8	18.3	2–60	31	48.4	34.1	17–60
6	39	100.0	7.2	1–22	39	87.2	32.9	7–60
7	89	100.0	3.9	1–9	89	92.1	22.3	7–60
8	92	100.0	2.9	1–16	92	97.8	20.1	4–60
9	98	100.0	2.5	1–9	98	100.0	16.7	5–30
10	123	100.0	1.2	1–3	123	100.0	9.3	3–30

[a] Av. for samples that reduced in 1 hr. or less.
[b] Range for samples that reduced in 1 hr. or less.

Pink and white reduction endpoints were compared on 564 semen samples with respect to initial motility. The results (table 2) reveal that the six samples rating zero motility also failed to reduce resazurin to purple, which is an intermediate color in the reduction to pink. Some samples failed to reduce to pink in 1 hr. until initial motility exceeded a rating of 5. The average reduction time to pink was more than twice as short with a motility of 6 as compared with a motility of 5. While some samples with motility of 6 to 8 failed to reduce completely to white in 1 hr., the breaking point appears to be between 5 and 6. Only 48.4 per cent of the samples rating 5 reduced to white, as compared with 87.2 per cent for samples rating 6. The 223 samples given initial motility ratings of 9 or 10 all reduced resazurin to white in less than 1 hr. Since it generally is agreed that samples rating below 5 are undesirable for routine use in artificial insemination, correlations were determined for only those samples, used by Northwest Co-op. Breeders, rating above 5. The correlation was – 0.459 for 376 samples for the Guernsey, Jersey and Holstein breeds, which showed

individual breed correlations of – 0.475, – 0.529 and – 0.460, respectively. Similar correlations for white resazurin for all breeds was – 0.232 and was – 0.232, – 0.473 and –0.097 for the Guernsey, Jersey and Holstein breeds, respectively.

Concentration also was reflected in the resazurin reduction times, as shown in table 3. Samples reducing to pink in 5 min. or less and to white in 1 hr. or less, averaged 1 million or more sperm per mm.^{3}. The correlation of pink resazurin and concentration for samples used by Northwest Co-op. Breeders was – 0.399 for all breeds and was – 0.391, – 0.589 and – 0.333 for the Guernsey, Jersey and Holstein breeds, respectively. Similar correlations for white resazurin was – 0.267 for all breeds and – 0.220, – 0.535 and – 0.181 for the Guernsey, Jersey and Holstein breeds, respectively. The correlation of concentration to motility for these same samples also was high, being 0.478 for all breeds and 0.536, 0.554 and 0.288 for the Guernsey, Jersey and Holstein breeds, respectively. The spermatozoa were

TABLE 3

Comparison of concentration of spermatozoa per mm^3 with resazurin reduction time for all semen samples showing an initial motility rating of one or higher

Pink				White			
Reducing time	No. samples	Av. concentration	Range	Reducing time	No. samples	Av. concentration	Range
(min.)		*(thousands /mm^3)*	*(thousands /mm^3)*	*(min.)*		*(thousands /mm^3)*	*(thousands /mm^3)*
1	125	1,850	920–3,540	3 or less	8	2,430	1,920–3,170
2	136	1,340	690–2,280	4–6	49	1,910	690–3,540
3	76	1,100	320–1,760	7–9	66	1,580	630–2,920
4	47	1,050	510–2,200	10–12	76	1,370	820–2,300
5	21	1,090	730–2,240	13–15	72	1,280	500–2,740
6	16	990	590–1,880	16–18	39	1,240	560–2,540
7	7	890	560–1,290	19–21	37	1,070	420–2,070
8	11	990	420–2,040	22–24	19	1,140	510–2,280
9–10	11	900	500–2,370	25–27	10	1,010	650–1,880
11–15	30	910	260–1,780	28–30	21	1,190	590–2,040
16–60	55	740	200–1,760	31–60	57	1,000	500–2,370
>60	23	490	100–940	>60	104	750	200–2,060

centrifuged from 12 samples with high initial motility and high concentration. No resazurin reduction was observed in the seminal plasma of the samples during 1 hr. of incubation. Hence, for detectable reduction of resazurin, motile spermatozoa are required.

Two separate experiments were conducted to test the fertilizing prediction value of the resazurin test. The first involved 34 bulls and 507 semen samples from Northwest and Evergreen Co-op. Breeders. In this trial, resazurin and methylene blue reduction times were determined as outlined previously. The second trial, involving 20 bulls and 304 semen samples from Northwest Co-op. Breeders, varied from the first only in that the undiluted semen was incubated for 30 min. at 45° C. before adding resazurin and methylene blue to the test vials to determine the reduction time. This was undertaken in an effort to speed up reduction of resazurin to white, since preliminary analyses of the data from the first trial indicated no correlation of the white endpoint to non-return rates.

Average reduction time of resazurin to pink was lowered from 2.28 min. per sample to 1.56 min., and average time to reduce to white was lowered from 16.7 to 5.9 min. Methylene blue reduction was slowed by incubating before making the test on undiluted semen. The average reduction time for unincubated semen was 29.4 min., as compared with 84.0 min. for incubated semen. The reason for this is not immediately apparent but possibly indicates that resazurin and methylene blue do not measure the same reducing properties of semen. The correlations between the reduction tests were as follows: (a) Unincubated semen; methylene blue compared with pink and white resazurin was 0.430 and 0.382, respectively, and pink compared with white resazurin was 0.563. (b) Incubated semen; methylene blue compared with pink and white resazurin was 0.499 and 0.596, respectively, and pink compared with white resazurin was 0.741. Even though the average methylene blue reduction time was increased by incubation and the reduction of resazurin to pink and white was decreased, the correlations were higher than for unincubated semen.

Comparisons of resazurin reduction time to non-return rates are shown in tables 4 and 5. Non-return rates are reported by two methods in these tables. The left-hand column in each section of each table shows the non-return rate based on total first and second services on all semen samples studied. The corresponding right-hand column shows the average non-return rate converted from per cent to angles and averaged. These averages then were reconverted to per cent, as shown in the right hand column of each section of each table. This latter procedure was necessary in order to determine correlations and make tests of significance and will be referred to in the discussion. In general, the non-return rates were slightly higher when averaging non-return rates on a per sample basis.

As shown in table 4, 125 unincubated semen samples which were used for breeding 20 or more cows and which reduced resazurin to pink within 1 min. were significantly superior, as shown by the analysis of variance (370 d.f.). The difference between non-return rates for semen reducing resazurin to pink in 1 min. or less, compared with 4 min. or more for all breeds, was 8.0 per cent. This mean difference was highly significant. For incubated semen, 182 samples reduced resazurin to pink in 1 min. or less and were 8.7 per cent higher than incubated semen requiring 3 min. or more. This mean difference also was highly significant (303 d.f.).

Reduction time of resazurin to white on unincubated semen was of no value for estimating fertilizing capacity. When the semen was incubated for 30 min. before making the test (table 5), 43 semen samples reducing resazurin to white in 3 min. showed an average non-return rate of 67.6 per cent, as compared with 66.2 per cent for 40 samples requiring 1 to 2 min. for reduction to white. The latter is 6.0 per cent higher than the average non-return rate of 60.2 per cent for 24 samples requiring more than 10 min. for reduction. The differences between the means for 1, 2 and 3 min., as compared with over 10 min., were highly significant. The variance for between times likewise was highly significant (303 d.f.).

TABLE 4

Comparison of resazurin reduction (pink endpoint) time of undiluted semen and fertilizing capacity

Reduction time (*min.*)	Unincubated					Incubated				
	No. samples	No. services	Non-returns	Samples—available for statistical analysis		No. samples	No. services	Non-returns	Samples—available for statistical analysis	
			(%)	(*no.*)	(% *non-return*)			(%)	(*no.*)	(% *non-return*)
Breed—Guernsey										
1	65	2429	63.8	50	65.2	90	5126	65.6	90	66.4
2	76	2676	59.5	76	61.5	40	2206	63.6	40	64.2
3	42	1118	56.9	28	58.4	4	248	57.4	4	55.6
4	21	794	60.0	19	60.2	2[a]	40	66.7	2	66.7
>4	10	435	53.8	10	54.9					
Total	214	7452	60.2	183	61.6	136	7620	64.8	136	65.4
Breed—Jersey										
1	72	1699	59.2	45	60.4	52	1869	61.4	50	61.6
2	34	867	57.7	25	57.9	32	963	58.3	27	60.6
3	29	653	62.3	18	59.6	4	79	63.3	1	62.1
4	8	160	53.1	4	52.8	7[a]	177	56.5	5	52.0
>4	9	131	62.6	2	64.7					
Total	152	3510	59.3	94	59.7	95	3088	60.2	83	60.8
Breed—Holstein										
1	46	1253	66.3	30	68.4	46	1526	65.5	42	65.6
2	39	970	64.0	35	62.8	33	1224	63.8	31	64.7
3	32	614	64.5	15	65.8	9	377	66.6	9	66.8
4	12	232	58.6	7	60.1	4[a]	110	58.2	3	58.2
>4	12	322	57.4	7	56.0					
Total	141	3391	64.0	94	64.4	92	3237	64.7	85	65.1
All Breeds										
1	188	5381	63.0	125	64.3	188	8521	64.6	182	64.9
2	144	4513	60.1	136	61.2	105	4393	62.5	98	63.4
3	103	2385	60.3	61	59.7	17	705	63.0	14	63.3
4	41	1186	58.8	30	62.1	13[a]	326	58.3	10	56.2
>4	31	888	56.4	19	56.3					
Total	507	14353	60.9	371	61.8	323	13945	63.7	304	64.0

[a] 4 min. or longer

TABLE 5

Comparison of resazurin reduction (white endpoint) time of undiluted semen and fertilizing capacity

Unincubated						Incubated					
Reduction time (*min.*)	No. samples	No. services	Non-returns	Samples available for statistical analysis		Reduction time	No. samples	No. services	Non-returns	Samples available for statistical analysis	
Breed—Guernsey			(%)	(*no.*)	(% *non-returns*)	(*min.*)			(%)	(*no.*)	(% *non-returns*)
1–3	4	91	44.0	2	52.6	1–2	20	1,023	68.4	20	68.2
4–6	25	883	59.7	21	61.2	3	23	1,194	68.7	23	69.7
7–9	32	1,069	60.8	28	62.7	4	21	1,172	63.4	21	64.0
10–12	50	1,616	57.0	40	58.9	5	19	1,147	63.6	19	64.2
13–15	44	1,574	63.1	38	64.4	6	15	834	63.1	15	63.2
16–20	32	1,227	61.9	30	61.2	7	12	691	64.4	12	64.9
21–25	13	459	61.4	10	63.8	8	12	682	62.8	12	63.1
>25	14	533	59.3	14	61.8	9	5	298	63.4	5	62.9
						10	5	318	60.1	5	59.8
						>10	4	261	62.8	4	63.9
Total	214	7,452	60.2	183	61.6		136	7,620	64.8	136	65.4
Breed—Jersey											
1–3	10	252	55.2	6	52.8	1–2	17	600	64.5	16	63.7
4–6	36	911	60.2	26	64.0	3	16	584	62.3	15	63.7
7–9	33	692	58.8	18	57.7	4	13	423	61.7	10	63.1
10–12	25	633	57.4	19	55.8	5	13	395	57.0	11	57.4
13–15	16	406	59.1	11	58.9	6	7	272	54.4	6	58.2
16–20	20	403	62.5	10	60.8	7	7	243	58.0	7	59.2
21–25	3	61	59.0	1	59.4	8	4	129	58.9	3	55.6
>25	9	152	63.2	3	70.7	9	8	201	59.7	7	60.0
						10	2	37	56.8	1	59.9
						>10	8	204	56.4	7	53.8
Total	152	3,510	59.3	94	59.7		95	3,088	60.2	83	60.8

TABLE 5 (*Continued*)

Comparison of resazurin reduction (white endpoint) time of undiluted semen and fertilizing capacity

Unincubated						Incubated					
Reduction time (*min.*)	No. samples	No. services	Non-returns	Samples available for statistical analysis		Reduction time	No. samples	No. services	Non-returns	Samples available for statistical analysis	
Breed—Holstein											
1–3	6	135	63.0	4	64.2	1–2	4	135	65.9	4	66.4
4–6	18	470	63.8	13	65.6	3	6	161	70.0	5	69.9
7–9	17	468	67.3	16	67.8	4	19	658	63.5	18	63.5
10–12	23	576	63.4	15	66.2	5	18	639	64.3	15	64.4
13–15	22	499	62.5	16	59.1	6	8	306	69.9	8	70.9
16–20	13	288	69.4	7	69.6	7	8	328	61.0	7	62.6
21–25	10	231	67.1	6	65.4	8	7	263	66.2	8	65.7
						9	6	181	65.1	6	66.2
>25	32	724	60.4	17	64.8	10	4	129	64.3	1	70.3
						>10	12	437	63.1	13	62.6
Total	141	3,391	64.0	94	64.4		92	3,237	64.7	85	65.1
All breeds											
1–3	20	478	55.2	12	55.2	1–2	41	1,758	67.1	40	66.2
4–6	79	2,264	60.7	60	63.4	3	45	1,939	66.8	43	67.6
7–9	82	2,229	61.6	62	62.6	4	53	2,253	63.1	49	63.7
10–12	98	2,825	58.4	74	59.6	5	50	2,181	62.6	45	62.6
13–15	82	2,479	62.3	65	58.8	6	30	1,412	62.9	29	64.4
16–20	65	1,918	63.1	47	62.4	7	27	1,262	62.3	26	63.2
21–25	26	751	63.0	17	65.4	8	23	1,074	63.1	23	63.3
>25	55	1,409	60.3	34	61.4	9	19	680	62.8	18	62.9
						10	11	484	61.0	7	61.4
						>10	24	902	61.5	24	60.2
Total	507	14,353	60.9	371	61.8		323	13,945	63.7	304	64.0

The correlations for resazurin and methylene blue reduction times for unincubated and incubated semen and non-return rates by breeds are shown in table 6. The correlations for initial motility and concentration to non-return rates also are shown for each trial. Time required to reduce unincubated semen to pink showed a highly significant correlation of −0.141 to fertility. Likewise, in the first trial, resazurin reduction to white, methylene blue reduction time, initial motility and concentration showed no significant correlations with non-return rates. When the semen was incubated, pink resazurin reduced quite rapidly, but did not lose sensitivity, since the correlation of −0.151 to non-return rate was highly significant.

Average time to reduce resazurin to white was approximately three times faster for incubated semen. The correlation for the white endpoint of −0.169 to fertility was highly significant. Likewise, during this trial, initial motility

TABLE 6

Correlation Summary by breeds

Non-return rate compared to:	Guernsey		Jerseys		Holstein		All Breeds	
	No. Samples	r	No. Samples	r	No. Samples	r	No. Samples	r
	Reduction times on unincubated semen							
Pink resazurin	183	−0.291**	94	−0.022	94	−0.210*	371	−0.141**
White resazurin	183	−0.032	94	+0.087	94	−0.186	371	−0.031
Methylene blue	150	−0.073	65	−0.039	73	−0.186	288	−0.046
Initial motility	183	+0.080	94	+0.006	94	+0.006	371	+0.022
Concentration	183	+0.073	94	−0.098	94	−0.194	371	0.000
	Reduction times on incubated semen							
Pink resazurin	136	−0.136	83	−0.217*	85	−0.204	304	−0.151**
White resazurin	316	−0.201*	83	−0.176	85	−0.115	304	−0.169**
Methylene blue	136	+0.011	83	−0.158	85	−0.085	304	−0.090
Initial motility	136	+0.228**	83	+0.061	85	+0.271*	304	+0.207**
Concentration	136	+0.185*	83	+0.060	85	+0.155	304	+0.163**

* = significant
** = highly significant

and concentration were highly-significantly correlated with non-return rate. When the two trials were combined, giving a total of 675 semen samples used on 20 or more cows, correlations of 0.076 and 0.052 for initial motility and concentration, respectively, to non-return rate were observed. This suggests a possible seasonal difference, since semen used for the first trial was collected from July to March and semen for the second trial was collected from March to August. The data also were analyzed by age of semen. Since too few samples were used to breed 20 or more cows during any one 24-hr. period, the correlations were low and erratic. Summarizing on the basis of totals for first and second services revealed that the quality tests herein reported on had no additional predictive value with respect to age of semen at the time of insemination.

DISCUSSION

Resazurin reduction time to pink and white has shown some promise in this experiment as an indicator of semen quality. Ascorbic acid content of the whole

semen did not appear to affect the resazurin reduction time of 94 samples of semen. The average for these 94 samples was 8.0 mg. per cent, which is higher than a range up to 8 mg. per cent reported by Phillips *et al.* (8). VanDemark *et al.* (13) failed to associate methylene blue reduction with ascorbic acid content of semen, although Beck and Salisbury (1) had felt earlier that the two were correlated. These latter authors also reported that the methylene blue test also was largely dependent on concentration and rate of motility. In this respect, resazurin and methylene blue reduction times give relatively similar results. Beck and Salisbury (1) reported correlations of – 0.6532 and – 0.6577 for methylene blue compared with concentration and initial motility, respectively. In this experiment, the correlations of concentration to pink and white resazurin and methylene blue reduction times were 0.399, – 0.267 and – 0.449, respectively, and for initial motility – 0.459, – 0.232 and – 0.493, respectively. The correlation of initial motility to concentration was 0.478.

The time required to reduce resazurin to pink and white was inversely related to survival time at 3.3 and 45° C. Swanson and Herman (11), using grouped data, observed a highly significant correlation between conception rate and survival under storage. Madden *et al.* (7) could demonstrate no significant difference between longevity under cold shock conditions and conception rate. Erb and Shaw (5) could demonstrate no correlation between motility after 30 min. incubation at 45° C. and non-return rate.

Resazurin reduction time to pink gave a highly significant correlation with non-return rate on 371 semen samples. This correlation of – 0.141, plus the close relationship to survival under storage, initial motility and concentration, makes this particular test appear promising. A recent experiment (3) has shown that when concentration of semen was adjusted to approximately 750,000 sperm mm.^{3}, the correlation coefficient between resazurin reduction to pink and non-return rate was – 0.517 on 72 samples. This technique increased the selectivity of the pink endpoint by removing some of the effects of variable concentration. Efforts to set up a series of standards utilizing initial motility, concentration and resazurin reduction failed to improve non-return rates in the sub-classes of superior semen quality over the differentiation observed by using resazurin reduction time to pink as the only criterion.

SUMMARY

Resazurin was tested as a possible indicator of fertilizing capacity. The test for reduction time was made by using 11 mg. of resazurin dye in 200 ml. of distilled water. One-tenth ml. of this solution was added to 0.2 ml. of undiluted semen and incubated at 45° C. Time required for reduction to pink, the first endpoint, and reduction further to white was recorded for 924 semen samples from 45 bulls representing the Guernsey, Jersey and Holstein breeds. The relationship of the pink and white endpoints to survival at 3.3 and 45° C. (94 semen samples), initial motility (564 semen samples), concentration (558 semen samples) and methylene blue reduction time (376 semen samples) was high. The time required to reduce to pink in unincubated semen showed a highly sig-

nificant correlation of −0.141 to non-return rate on 371 semen samples which were used for 20 or more first and second services. The white endpoint showed a slightly higher correlation of −0.169, as compared with −0.151 for the pink endpoint when the semen was incubated for 30 min. at 45° C. before making the test.

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THE DETERMINATION OF LINOLEIC ACID IN MILK FAT

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Until recently, when the photoelectric spectrophotometer came into general use as an aid in fat analysis, it has been practically impossible to obtain accurate data on the amounts of the unsaturated acids in fats. Most of the naturally occurring fats do not possess chromophores but possess structures which may be altered by chemical means to produce groups which absorb radiant energy. For example, linoleic acid possesses a diene grouping and when treated with alkali forms an isomer containing a conjugated double bond, which structure causes an absorption of light in a region of the ultraviolet spectrum. The intensity of the absorption may be used as a basis for measuring the amount of linoleic acid present in a fat.

Mitchell *et al.* (1) described a procedure for the quantitative estimation of linoleic and linolenic acid content of various fats and oils. By using pure linoleic and linolenic acid, they obtained reference standards at the points of maximum absorption, namely 234 mμ and 268 mμ, respectively, which may be used in the determination of these acids in mixtures of other fat acids. Beadle and Kraybill (2) later published reference values for linoleic acid and linolenic acid which they obtained with a Beckman spectrophotometer. Riemenschneider *et al.* (3), using an adsorption fractionation technique, were able to isolate methyl linoleate which gave a higher spectrophotometric absorption coefficient than previously reported. Brice and Swain (4), by using alkaline glycerol as an isomerizing medium, described a method for simultaneous spectrophotometric determination of non-conjugated and conjugated diene, triene and tetraene fat acid constituents of vegetable oils, animal fats, their soaps and purified fat acid preparations. Stainsby (5) describes a method for the determination of linoleic acid by oxidation of the fat in acetone, followed by titration of the acidic glycerides after removal of the steam-volatile acid products.

The literature dealing with the determination of linoleic acid in milk fat is limited. Eckstein (6), by using the lead-salt method of separating the saturated from the unsaturated fat acids in milk fat, was able to obtain only about 0.2 per cent linoleic acid and about 0.1 per cent linolenic acid. Hilditch *et al.* (7), in an examination of the glycerides of milk fat which had been separated by low temperature crystallization from acetone, reported the linoleic acid content to be about 5.5 per cent. Later, Hilditch and Jasperson (8), with the aid of a quartz spectrograph and using a concentrate of the more unsaturated acids of cow milk fat, prepared by lithium salt separation, reported the presence of a total of 2 per cent non-conjugated and 2 per cent conjugated octadecadienoic acid. They also reported traces of conjugated and non-conjugated octadecatrienoic acids.

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White and Brown (9), in a study of the tetrabromide method of estimating linoleic acid in fat acid mixtures, were able to definitely identify linoleic acid in butterfat by actual isolation of the tetrabromide.

With the aid of a Beckman spectrophotometer and the adoption of some of the existing procedures, the amount of linoleic acid present in milk fat has been determined.

EXPERIMENTAL

Preparation of isomerizing reagent. The method used for the preparation of the ethylene glycol-KOH isomerizing reagent was essentially as described in specific detail by O'Connor *et al.* (10). The reagent was prepared in an atmosphere of nitrogen, was colorless and permitted the use of a larger sample of the milk fat acids in the procedure. The reagent consists essentially of a solution containing 7.5 g. of 85 per cent. KOH per 100 ml. of ethylene glycol.

Preparation of milk fat acids. Samples of butter were converted to butteroil by heating the butter at 60° C. until melted. Prolonged heating was avoided in order to eliminate oxidation. The melted fat was separated from the curd and water by centrifuging in glass bottles. Approximately 100 g. of the butteroil then were saponified as prescribed by Jamieson (11). The potassium soaps were converted to the free fat acids by means of HCl and the free fat acids were isolated by extracting with peroxide-free ethyl ether. The last traces of ether were removed under vacuum.

Isomerization of milk fat and milk fat acids. Samples containing approximately 0.1 g. fat or fat acids were weighed out in small glass vials and were added to 10 ml. of the ethylene glycol-KOH solution in pyrex test tubes that were being held in a constant temperature bath at 180° C. as prescribed by O'Connor (10). After 25 min. the tubes were removed from the bath and cooled quickly in a cold water bath. The contents of the tube then were transferred quantitatively to a 100-ml. volumetric flask, using 95 per cent alcohol purified by distillation over Zn and KOH, to wash out the tubes. The solutions usually require further dilution before they can be used in the spectrophotometer. A sample of the isomerizing reagent was treated similarly and was used as the reference material in the spectrophotometer.

Spectrophotometric measurements. A Beckman DU photoelectric spectrophotometer employing a hydrogen lamp was used to measure the optical densities. The solutions, after being filtered through sintered glass funnels just before being used, were placed in 1-cm. cells for reading of the optical densities. Density readings were made in the range 224–270 mμ. The specific absorption coefficient (a) was calculated for the various wavelengths, using the equation Specific $a = \frac{E}{cl}$, where a = specific absorption coefficient, E is the optical density (obtained as a direct reading on the spectrophotometer), c is the concentration of solute in grams per 1000 ml. and l is the length in centimeters of solution through which the radiation passes.

RESULTS

With milk fat acids. Figure 1 shows a typical absorption curve for isomerized milk fat acids. At 234 mμ, the intensity of absorption is due to diene and triene conjugation which results from the isomerization of the linoleic and apparent linolenic acids present. The absorption at 268 mμ is due to a triene conjugation. This absorption is a measure of the apparent linolenic acid present in the milk fat acids. It has been shown by others (10, 12) that small but interfering absorption takes place at 268 mμ even though the presence of linolenic

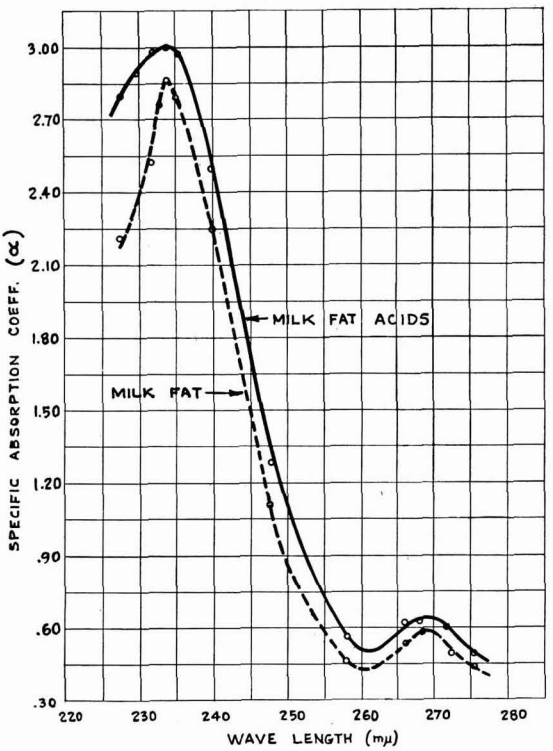

FIG. 1.—The specific absorption coefficients of isomerized milk fat and milk fat acids in alcohol solutions at different wavelengths of light.

acid can not be proved by actual isolation of a hexabromostearic acid. However, in the case of the milk fat acids, although no attempt was made to actually prove the presence of linolenic acid by the isolation of hexabromostearic acid, the absorption at 268 mμ is greater than is warranted by an interfering substance due to isomerization of the linoleic acid. Therefore, it has been called the apparent linolenic acid present in the fat. Since both diene and triene conjugation absorb radiant energy at 234 mμ, it is necessary to make a correction at 234 mμ, in cal-

culating the amount of linoleic present, for the absorption due to diene conjugation resulting from the apparent linolenic acid present in the fat. The equations for calculating the amount of linolenic and linoleic acids are as follows:

$$\text{per cent linolenic acid} = Y = \frac{a\ (268\ \text{m}\mu) \times 100}{53.2}$$

$$\text{per cent linoleic acid} = \frac{a\ (234\ \text{m}\mu) - \left(\frac{Y}{100} \times 60.9\right)}{86.0} \times 100$$

where 53.2 is the specific absorption coefficient of pure linolenic acid at 268 mμ, 60.9 is the specific absorption coefficient of linolenic acid at 234 mμ and 86.0 is the specific absorption coefficient of pure linoleic acid at 234 mμ.

Absorption values for unisomerized milk fat and milk fat acids dissolved in iso-octane, using iso-octane as a blank, (4) increased very slightly only in the region of wavelengths of approximately 269 mμ, indicating that practically no conjugated systems existed in the original fat.

Table 1 shows the amount of linoleic acid and apparent linolenic acid calculated to be present in some of the samples analyzed.

TABLE 1

Milk fat source	Linoleic acid after correction for triene conjugation	Octadecatrienoic acid calculated as linolenic acid
	(%)	(%)
1. Winter butter (past. cream)	2.11	1.29
2. Spring butter (unpast. cream)	2.17	1.05
3. Same (past. cream)	2.11	1.20
4. Whey cream butter (Swiss)	2.31	1.11
5. Summer butter (past. cream)	2.42	1.09

In order to test the accuracy with which the spectrophotometric observations may be made, pure linoleic acid was used to fortify some of the butter fat acid samples, using up to a maximum of 3 per cent linoleic acid based on the weight of the milk fat acid sample. An identical sample of milk fat acids was used in the blank. The maximum deviation from complete recovery of the linoleic acid at 234 mμ was $\pm$ 0.8 per cent for a solution containing 1 per cent added linoleic acid and $\pm$ 0.5 per cent of the amount present for a solution containing 3 per cent added linoleic acid.

With milk fat. Figure 1 also shows the absorption data obtained with isomerized milk fat. Table 2 gives the values obtained for the percentage of linoleic acid in milk fats, when the milk fat or its fat acids are used in the determination. Sample 1 was a fresh butterfat, while sample 2 had been prepared and stored at a temperature of 40° C. for 1 yr. before isomerization and optical density readings were made.

SUMMARY

A spectrophotometric method is described for the determination of linoleic acid in milk fat. Values are given for the linoleic acid and apparent linolenic

acid content of samples of milk fat obtained from whey and from summer and winter milk.

TABLE 2

Milk fat source	Linoleic acid	Octadecatrienoic acid calculated as linolenic acid
	(%)	(%)
1. Summer 1949 milk fat	2.62	1.02
Summer 1949 milk fat acids	2.63	1.17
2. Summer 1948 milk fat	2.71	0.77
Summer 1948 milk fat acids	2.64	0.81

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AN ALL-ROUGHAGE RATION FOR BULLS[1, 2]

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The rapid development of large scale artificial breeding programs in areas devoted largely to the raising of dairy cattle has resulted in the establishment of breeding rings dependent upon the maintenance of large bull studs. It is not surprising, therefore, that the feeding of bulls has received increased attention during the last few years.

The cost of feed is an important contributory factor to the over-all cost of maintaining such large bull studs, and any means whereby these costs can be reduced should prove of considerable advantage. The relatively high cost of grain-concentrate mixtures suggests a more economic utilization of such mixtures as an important step in this direction. One measure advocated by Reid, *et al.* (8, 9) is the use of simple, rather than complex, concentrate mixtures.

Although the inclusion of grain in the ration generally has been accepted as necessary in the feeding of dairy bulls used for breeding purposes, there is little experimental evidence either to support or refute this practice. Since the bull is a ruminant, it should be able to make the best use of roughage feeds, and an investigation into the possibility of feeding a ration devoid of any concentrate mixture appeared justifiable.

The roughages used to make up such a ration necessarily must be of a high quality and the over-all digestible crude protein and TDN content of the ration should be maintained at the levels known to give satisfactory results. It was realized that in order to develop such a ration it would be necessary to utilize large amounts of silage. It generally is believed that too much silage results in "excess middle" and a consequent falling off in the libido of bulls. There are again no pertinent data in support of this idea. It was hoped, therefore, that the use of a ration consisting of good hay and a high level of good quality silage would provide information on the latter subject.

EXPERIMENTAL

Selection of bulls. Twenty bulls, 10 Holsteins and 10 Guernseys, were selected on the basis of their age, general thrift, vigor and condition, heart and paunch girth, semen quality and, where available, the past year's conception rate, in such

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a way that they could be divided into two equalized groups. Group I was designated the "control" group, while group II was the "experimental" group. The above data for the individual bulls, as well as the distribution of the paired bulls between the two groups, are presented in table 2.

Unfortunately, 4 mo. after the experiment was started three of the bulls (one experimental Holstein and two control Guernseys), due to circumstances beyond the control of the experiment, were sold for slaughter. Therefore, data pertaining to these bulls were discarded.

Rations and management of bulls. A ration consisting of 5 lb. of a concentrate mixture (ground corn and oats, wheat bran, linseed oil meal, soybean oil meal, bone meal, blood meal, tankage and mineral salt mix), 15 lb. of grass-legume silage and good quality mixed hay fed *ad libitum* per bull per day, had

TABLE 1

Rations fed and calculated intake of T.D.N. and D.C.P. per bull per day, based on the intake of a 2000-lb. bull

	lb. feed/100 lb. live weight	Total intake	T.D.N.[a]	D.C.P[a]	N.R.
		(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Control ration:					
Grain mixture	0.25	5.0	3.9	0.7	
Silage (grass-legume)	0.75	15.0	3.0	0.45	
Hay (grass-legume)[b]	1.25	25.0	11.2	1.25	
Total			18.1	2.40	1:6.5
Experimental ration:					
Silage (grass-legume)	2.25	45.0	9.0	1.35	
Hay (grass-legume)[a]	1.25	25.0	11.2	1.25	
Total (grass-legume)[a]			20.2	2.60	1:6.7

[a] Calculated from Morrison's tables on the composition of feeds.
[b] Hay was fed *ad libitum*, the intake used in the above calculations being based on an estimate.

been used with excellent results since the inception of the stud in which the experiment was set up. This ration was used, therefore, as the control (lot I) against which an all-roughage ration (lot II) could be tested.

The hay consisted of alfalfa and brome grass, together with a small amount of red clover. The silage was made from these same three forage crops, 20 lb. of molasses being added per ton of silage. The two rations, together with data calculated from Morrison's tables (5), on their TDN, digestible crude protein content and nutritive ratios are presented in table 1.

The bulls used in the experiment were treated in the same way as the rest of the bulls in the stud, *i.e.*, exercise, time of feeding, semen collection (5-day intervals) etc. were continued in the same manner as had been done prior to the setting up of the experiment.

Observations such as volume, initial motility and density by microscope estimation of ejaculates were made routinely with each collection. The duration of motility in storage was determined from time to time. The semen was used in

TABLE 2

Representative data on various characteristics of typically paired bulls and the basis of pairing the bulls

Bull	Group allocated	Age at start of expt.	Heart girth		Paunch girth		Vigor, thrift and condition		Semen rating		Conception rate	
			Initial	Final	Initial	Final	Initial	Final	Pre-exptl. period	Exptl. period	Pre-exptl. period	Exptl. period
		(*yr. mo.*)	(*in.*)	(*in.*)	(*in.*)	(*in.*)					(%)	(%)
Holstein												
H 20	I	10 – 0	90.5	89	101.5	101	Ex	Fair	4	4	63.3	71.4
" 34	II	11 – 4	98	95.5	110.5	111	Good	Good	4	4	64.7	63.3
" 26	I	5 – 0	94.5	95.5	105	107.5	Ex	Ex	4	4	61.4	68.8
" 35	II	5 – 8	94	90	105	106.5	Ex	Good	4	4	61.8	65.1
" 37	I	1 – 8	75	88	87.5	101	Ex	Ex	4	4	66.5	70.9
" 38	II	1 – 9	77	85	93.5	104	Ex	Ex	4	4	69.8	71.4
Guernsey												
G 51	II	8 – 5	83	82.5	99	100	Ex	Ex	4	4	26.9	60.4
" 47	I	6 – 8	84.5	88.5	99.5	105	Ex	Ex	4	4	66.6	68.8
" 36	II	6 – 7	87	85	101	102	Ex	Ex	4	4	56.0	62.9
" 57	I	1 – 2	63.5	78	76.5	93.5	Ex	Ex	3–4	4	No rec	67.5
" 58	II	1 – 2	63.5	76	73	90	Ex	Ex	4	4	No rec	69.3

the field for artificial insemination and all pertinent records were kept by the breeding ring.

Chemical analyses were conducted on semen from all the bulls at the start and end of the experiment. At 4-wk. intervals during the course of the experiment, semen for chemical analysis was taken from one-fourth of the bulls in such a way that semen collection schedules of the breeding ring were undisturbed. The analyses carried out included ascorbic acid, total nitrogen, non-protein nitrogen and acid and alkaline phosphatase activity.

The ascorbic acid content of the semen was determined by means of a modification of the method of Mindlin and Butler (4), using a Fisher electrophotometer. These determinations were made immediately following collection of the semen.

Total nitrogen was determined by means of a semi-micro Kjeldahl method, $CuSO_4$ being used as catalyst for the digestion.

Since it was desired to determine the level of non-protein nitrogen in whole semen, it was necessary to develop a method in which the non-protein nitrogen of the sperm cells would be liberated. Zittle and O'Dell (12) reported that the cell wall of the spermatozöon can be dissolved in the presence of an alkali and Na_2S, and this observation was utilized in developing the following method: Two ml. of semen were transferred to a 25-ml. volumetric flask, 2 ml. of a 0.2*M* Na_2S solution in 2 *N* NaOH were added and the mixture was allowed to stand for 5 min. A clear, straw-colored viscous solution resulted. Two ml. 2*N* HCl were added while shaking the flask vigorously and a white precipitate started to form; 10 ml. of a 10 per cent trichloracetic acid solution were added to complete the precipitation of the proteins. The flask was shaken vigorously and then allowed to stand for 15 to 30 min., the contents made up to volume with distilled water and the precipitate filtered off by means of a fluted Whatman no. 42 filter paper. A 5-ml. aliquot of the clear filtrate was used for the determination of nitrogen by the semi-micro Kjeldahl method. (After the filtration had stood for a short time, a fine white precipitate settled out. This was due to the liberation of free S from the Na_2S reagent.)

The estimation of phosphatase in semen was obtained with a modified method developed by Johnson (3). In the latter method, a 2-ml. aliquot is taken from the reaction mixture for testing purposes. The advantage of the small aliquot lies in the fact that it avoids the formation of a precipitate. A 0.1*M* ethylene diamine-citrate solution of the desired pH (5.0 for the acid- and 9.3 for the alkaline-phosphatase) was used as buffer.

The time of survival of the spermatozoa was determined in the semen samples that were taken for chemical analysis. A portion of the semen was diluted immediately after collection with egg yolk-phosphate diluent (6) to which penicillin had been added at a level of 100,000 units per 100 ml. diluent. A dilution rate of 1:20 was used and the diluted semen was stored in a refrigerator at 4° C. Motility was estimated every second day with the aid of a warm-stage microscope.

In order to obtain a measure of the fertility of the two groups of bulls, non-return data were assembled in the following manner: (a) 90-day non-return data from cows receiving first service or first service after calving, were utilized. A

cow was recorded as a 90-day non-return if she was not reported for a second insemination within 90 days from the date of the first service. (b) Only paired data were used, *i.e.*, where a pair of inseminations, using semen from a control and an experimental bull, was conducted on the same farm and within the same month. (c) All possible pairs of inseminations on a given farm were recorded, with the reservation that data from an insemination involving a given cow were not used to make up more than one pair of inseminations.

By taking the above steps it was possible to minimize differences due to variations in farm management as practiced by individual farmers and to the varying ability of individual inseminators, since in most cases a given farm was served by a single inseminator. The utilization of pairs of inseminations made in the same month helped to reduce the effects of seasonal influences.

RESULTS

Chemical analyses. The results of the analyses of the semen for ascorbic acid, total nitrogen and non-protein nitrogen are presented in table 3. Only the average values for the two groups are given, but the values for the different 12-wk. periods are included to serve as an indication of the variations from one period to the next. Apparently, the type of ration had no effect on the total and non-protein nitrogen levels. Variations in the level of these constituents in different ejaculates from the same bull indicate that the small differences shown in the table are not significant. Although the level of ascorbic acid in the semen shows a noticeable decrease over the experimental period, this is reflected in both groups and probably is not attributable to the rations fed.

The acid and alkaline phosphatase levels of the semen are given in table 4. Apparently, the levels of both of these semen constituents were slightly elevated in the case of the bulls on the all-roughage ration. Unfortunately, these determinations were started relatively late in the experiment, so that there were no data giving a comparison of the phosphatase levels of the semen of the two groups of bulls prior to the experiment. Furthermore, the phosphatase levels obtained on different ejaculates, even from the same bull, vary over a wide range, while one of the experimental Guernseys had such a low level of alkaline phosphatase that no appreciable activity could be obtained with three different ejaculates.

Examination of the data on volume of the first and second ejaculates indicated that there was no breed difference between the Guernsey and Holstein bulls in this respect. Comparisons of volumes before and during the experiment indicated that high volume bulls continued to produce large volumes despite the ration used. It was apparent that the experimental ration was without effect on ejaculate volume. The ejaculates averaged approximately 5.5 to 6.5 ml. per ejaculate with little or no difference between the first and second ejaculates.

Detailed data on the initial motility and density of ejaculates are not presented. However, the semen rating for each bull, as estimated on the basis of both initial motility and density of ejaculates taken during the last few months of the experiment, together with a similar rating for the pre-experimental period,

are given in table 2. From these data it appears that the all-roughage ration had no effect on these semen characteristics.

The average values for the duration of a motility rating greater than "1+" for semen from the two groups of bulls gave a difference between them of less than 12 hr. of storage time, which was not considered significant. The experimental ration apparently had no detrimental effect.

During the early part of the experiment, the young Guernsey bulls on the experimental ration showed considerable roughing of the hair coat, but this condition cleared up as the experiment progressed. One of the veterinarians employed by the breeding ring judged the bulls on the basis of "general thrift, vigor and condition" at the start and the close of the experimental period. A comparison of bulls on the basis of the above showed that there was no difference between the bulls fed the two rations.

It generally is believed that too much silage may result in "paunchiness" in bulls. The paunch- and heart-girths of each of the bulls were measured at the start and close of the experiment and these measurements are listed in table 2. In no case among the mature bulls was there any marked increase in paunch girth after 12 mo. on the all-roughage ration. Increases in the paunch girth of the younger bulls were the result of growth as evidenced by the simultaneous increase in heart girth, and by similar increases observed in the control animals.

The average fertility of the bulls in the two groups is summarized in table 2. The per cent non-returns for the Holsteins were 71.5 and 70.0 per cent for the control and the experimental animals, respectively, while the corresponding values for the Guernseys were 67.8 and 65.1 per cent. When considered from a practical aspect, this difference in the fertility of the two groups appears unimportant. This conclusion further was borne out by statistical analysis, the differences not being significant when tested by means of the "Chi-square" test (11).

DISCUSSION

The data concerning the phosphatase activity of the semen were insufficient to demonstrate any significant trend, but it is of interest to compare these results with the findings of Reid *et al.* (7) who reported that the levels of both acid and alkaline phosphatase in the semen of bulls receiving a complex concentrate mixture were "markedly elevated" above that of the semen from bulls receiving a simple concentrate mixture. A further discrepancy with our data lies in the relative amounts of acid and alkaline phosphatase. Reid and his co-workers reported that the mean level of alkaline phosphatase was considerably higher than that of acid phosphatase, while in the present study the opposite was found to be the case.

To date, no controlled experiments have been conducted to investigate to what extent silage can be used in the rations of bulls. Reid and his co-workers (8, 9) were able to show that the concentrate mixture used in bull rations can be made considerably less complex without any detrimental effect upon the composition of the blood or the semen quality.

Branton *et al.* concluded that 1 lb. of hay together with 0.4 to 0.5 lb. concentrate mixture daily per 100 lb. body weight was sufficient to meet the needs of bulls used for artificial insemination purposes.

Contrary to general beliefs, measurements of the paunch girth of the animals indicated that an all-roughage ration could be fed with silage at a level three times higher than that normally recommended without the development of "excessive middle."

Although not set up with this in mind, the above study serves to confirm the work of Branton *et al.* (2) who were able to show that, for the nutrition of bulls, "animal protein was not superior to the plant proteins" under the conditions of their experiment. When the results presented in this paper are studied in conjunction with those obtained by Reid *et al.* (9) and Branton *et al.* (2), it seems justifiable to conclude that, provided sufficient energy is supplied in the ration and the level of protein is adequate, the source of this protein is not of major importance.

Whereas the present study was conducted with bulls ranging from 14 mo. to 11 yr. of age, the question as to whether such an all-roughage ration can be fed to fast-growing bulls less than 1 yr. of age, was not answered by this experiment. Furthermore it is not known what the effects of this ration will be if it was fed over a period longer than 12 mo. The present study is being continued for another 12 mo. in order to obtain further information on this aspect of the problem.

In conclusion, it may be stated that the feeding of bulls, used for artificial breeding purposes, on a ration consisting solely of roughages seems to hold considerable promise. The adoption of such a feeding practice by artificial breeding rings should prove of considerable economic importance not only to the rings, but also to the dairy industry in general.

SUMMARY

A study has been made of the effects of an all-roughage ration including a high level of silage upon dairy bulls in a controlled experiment for a 12-mo. period.

Measurement of the ascorbic acid, total nitrogen and non-protein nitrogen content of semen indicated that there was no observable difference in the levels of these constituents in semen from bulls fed the all-roughage or the control ration. Although the levels of acid and alkaline phosphatases appeared slightly elevated in the semen of the bulls on the all-roughage ration, it was not possible to arrive at a definite conclusion as to the significance of the differences reported.

Regardless of the ration fed, the initial motility, density and volume of ejaculates, as well as the longevity of the spermatozoa in storage, were similar. The "over-all condition" and health of the bulls was maintained on the all-roughage ration. "Excessive middle" did not develop despite the feeding of high silage levels.

On the basis of fertility data it appears that the two rations were equally efficient in maintaining the reproductive ability of the bulls.

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PARTITION OF ORALLY ADMINISTERED RADIOACTIVE PHOSPHORUS IN THE BLOOD AND MILK OF THE DAIRY COW[1]

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The present knowledge of the blood precursors of phosphates in milk is based mainly on data obtained by a comparison of the phosphorus content of arterial and mammary venous blood. These data (3, 4, 9, 10, 11, 12, 13, 17, 18) seem to show that the mammary gland removes only plasma inorganic phosphate from blood. Consequently, this has been considered the sole precursor of all phosphorus occurring in the various compounds in milk. Since exchange of phosphates between blood plasma and tissues is very rapid and the equilibrium between inorganic phosphates and organic esters also is labile, the slightest excitation of test animals may affect the results obtainable with the arterio-venous difference technique. Therefore, additional information regarding phosphorus metabolism of the mammary gland was considered desirable.

Aten and Hevesy (1), working with goats, were the first to use labeled (radioactive) phophorus in milk formation studies. The data presented by these workers appear to be in good agreement with the results obtained with A-V difference technique. However, at one time interval in these experiments, the specific activity of the main phosphorus fractions in milk reached a higher level than the simultaneous value of plasma inorganic phosphorus, the fraction which showed the highest specific activity in blood. A direct comparison of these values was difficult, because the effect of the subcutaneously administered radioactive phosphate lasted a relatively short time, and no definite conclusion could be drawn by comparing the simultaneous values of the specific activity of plasma inorganic phosphates and milk phosphates in different fractions. To explain the differences in the simultaneous specific activity values in blood and milk, it was assumed that 3 to 4 hr. are required before blood plasma phosphates are excreted in the milk.

Very few additional data (2, 7) regarding the partition of radioactive phosphorus in blood and milk have been published since the above work. It appeared desirable to repeat this work in such a way as to reduce the rate of change in specific activity values and obtain these values over a longer period of time. This was accomplished by oral administration of radioactive phosphorus instead of subcutaneous or intravenous injection.

EXPERIMENTAL METHODS

A Jersey cow (no. 982UF) from the Florida Agricultural Experiment Station dairy herd, weighing 857 lb. and producing about 20 to 22 lb. of milk per day

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was chosen for the experiment. During the entire experiment, the cow was fed regularly twice a day according to the usual practices in the herd. The mixed concentrates (17 per cent total crude protein) contained the usual mineral additions of 1 per cent each of common salt, marble dust ($CaCO_3$) and steamed bonemeal. She grazed on a fertilized pasture. On December 11, 1947, the cow was milked at the usual time, *i.e.*, 3 : 30 to 3 : 40 p.m. On December 12, at 4 : 45 a.m., before the cow had consumed any feed, labeled disodium phosphate containing 247.5 γ of phosphorus with an activity of approximately 3.1 millicuries[4] was administered orally in about 200 ml. of water. The cow was milked the first time at 5 : 30 to 5 : 38 a.m. Because of the unavailability of pituitrin or oxytocin, the amount of milk secreted during the 59 min. after administration of the isotope was considered to be in proportion to the interval between milkings. After the first milking, the cow was milked three times at 4-hr. intervals. On the following days, the cow was milked twice a day at the usual milking times. The first blood sample was drawn on December 12, at 5 : 44 a.m. and subsequently, immediately after every milking. The samples were taken from the coccygeal artery using Saarinen's (16) technique.

Radioactivity was measured in solution with a dipping-type Geiger counter, using aliquots from the solution administered to the animal as standards according to usual procedures. Activity values are expressed in terms of micrograms of labeled phosphorus, whereas specific activity values are expressed as micrograms of labeled phosphorus per gram of total phosphorus. Counts were made from 15-ml. aliquots either directly or after proper dilution of the sample. Only the first milk sample was concentrated before the reading was taken.

The total amount of phosphorus in each fraction was determined colorimetrically using the method of Kuttner, *et al.* as modified by Saarinen (15) and the blood plasma acid soluble phosphates were extracted by the procedure therein described. Blood plasma phospholipids were extracted according to Bloor's (5) procedure and purified by redissolving in dry ether. Casein was precipitated from skimmilk after the method of Brereton and Sharp (6). Milk serum phosphorus was determined in an aliquot of the casein filtrate.

RESULTS AND DISCUSSION

The values for total phosphorus and labeled phosphorus in blood and milk samples are presented in tables 1 and 2. It will be noted in table 1 that the blood plasma acid soluble phosphorus was distinctly radioactive in the first blood sample, taken 59 min. after the oral administration of the P_{32} phosphate. In this sample and in the second blood sample, taken about 4 hr. later, the blood plasma phospholipids did not show any activity, *i.e.*, the orally administered radioactive phosphorus was at first absorbed into the blood stream in an acid soluble form. When these values are compared with the results presented in table 2, it will be observed that in the milk secreted during the 4-hr. period preceding the drawing of the second blood sample, the milk serum acid soluble phosphorus was consider-

[4] The radioactive phosphorus was obtained from the Oak Ridge National Laboratory on authorization by the U. S. Atomic Energy Commission.

ably radioactive, and, likewise, the casein phosphorus. This indicates that the casein phosphorus had originated from the acid soluble phosphate fraction of the blood and not from the phospholipid fraction, but does not necessarily support the common view that the inactive phosphorus of phospholipids is not utilized simultaneously by the mammary gland.

The other results presented in table 1 show that the actual amounts of P_{32} in blood and plasma are at about the same level. The activity in milk is from 10 to 20 times higher than that of the blood and plasma. This is due mainly to the larger amount of total phosphorus in milk.

The values for blood and plasma labeled phosphorus in table 1 show two maxima. The first occurs in plasma 5 to 6 hr. after administration of the phosphate and the second in sample 6 at about 30 hr. later. The first maximum is due entirely to the activity of the acid soluble phosphates, but the second is due

TABLE 1

Labeled and total phosphorus in blood

Sample no.	Date and sampling time	Whole blood labeled P	Blood plasma labeled P	Plasma acid soluble P		Plasma phospholipid P	
				Labeled P	Total P	Labeled P	Total P
		(γ/100 ml.)	(γ/100 ml.)	(γ/100 ml.)	(mg./100 ml.)	(γ/100 ml.)	(mg./100 ml.)
1	Dec. 12, 5:44 a.m.	0.00023	0.00027	0.00012	9.81	0.00000	7.48
2	Dec. 12, 9:40 a.m.	0.00550	0.00667	0.00703	10.10	0.00000	8.42
3	Dec. 12, 1:36 p.m.	0.00562	0.00581	0.00533	11.22	0.00048	8.04
4	Dec. 12, 5:50 p.m.	0.00381	0.00303	0.00273	11.50	0.00056	7.67
5	Dec. 13, 5:45 a.m.	0.00733	0.00733	0.00517	11.87	0.00301	7.67
6	Dec. 13, 3:45 p.m.	0.00815	0.00803	0.00517	11.59	0.00296	6.84
7b	Dec. 14, 3:45 p.m.	0.00775	0.00724	0.00397	12.16	0.00552	6.99
8b	Dec. 15, 3:45 p.m.	0.00601	0.00567	0.00188	12.16	0.00487	7.48

to activity of both acid soluble and phospholipid fractions, although the most marked increase in the phospholipid fraction occurred still later (samples 7b and 8b).

On the basis of the form of the blood labeled phosphorus, it may be assumed that the first increase was due to absorption of a portion of the P_{32} that passed directly to the omasum or abomasum or was absorbed from the rumen. The second increase occurred after the feed given with the tracer should have been largely absorbed (8, 14) and probably followed normal absorption, although interaction with bone and other tissues may have been involved. Although nearly simultaneous, the fluctuations were wider in the plasma than in the blood suggesting a diffusion equilibrium of soluble phosphorus compounds between plasma and erythrocytes.

As shown in table 2, the activity of the skimmilk was considerably higher than that of whole milk during the first 58 hr. of the experiment. While the diluting effect of the milk fat would explain part of this difference, the initial

TABLE 2

Labeled and total phosphorus in milk

Sample no.	Date and milking time	Milk	Whole milk labeled P	Skimmilk labeled P	Acid-soluble serum P in skimmilk		Casein P in skimmilk	
					Labeled P	Total P	Labeled P	Total P
		(*g.*)	(γ/*100 ml.*)	(γ/*100 ml.*)	(γ/*100 ml.*)	(*mg./100 ml.*)	(γ/*100 ml.*)	(*mg./100 ml.*)
1	Dec. 12, 5:30–5:38 a.m.	4,495	0.0000585	.....	.....	92.2	.....	24.3
2	Dec. 12, 9:30–9:40 a.m.	1,317	0.0223	0.0341	0.0285	102.9	0.0036	23.9
3	Dec. 12, 1:30–1:36 p.m.	1,589	0.0704	0.1041	0.0819	104.7	0.0168	25.2
4	Dec. 12, 5:30–5:40 p.m.	1,861	0.0957	0.1219	0.0995	101.0	0.0242	25.2
5	Dec. 13, 5:30–5:40 a.m.	4,585	0.1122	0.1219	0.0875[a]	110.3	0.0242	23.9
6	Dec. 13, 3:30–3:40 p.m.	4,086	0.1050	0.1242	0.0928	98.2	0.0210[a]	21.5
7a	Dec. 14, 5:30–5:40 a.m.	6,265	0.0909	0.1018	0.0773	107.2	0.0196	25.1
7b	Dec. 14, 3:30–3:40 p.m.	2,479	0.0869	0.0933	0.0686	99.7	0.0175	23.6
8a	Dec. 15, 5:30–5:40 a.m.	6,538	0.0632[a]	0.0522[a]	0.0480[a]	101.5	0.0137	22.9
8b	Dec. 15, 3:30–3:40 p.m.	3,541	0.0497	0.0458	0.0464	107.2	0.0121	23.7
9a	Dec. 16, 5:30–5:40 a.m.	5,085	0.0352	0.0406	0.0383	95.9	0.0104	24.8
9b	Dec. 16, 3:30–3:40 p.m.	3,541	0.0380	.....	.....	.....	.....	.....
10a	Dec. 17, 5:30–5:40 a.m.	5,403	0.0294	.....	.....	.....	.....	.....
10b	Dec. 17, 3:30–3:40 p.m.	3,723	0.0249	.....	.....	.....	.....	.....
11a	Dec. 18, 5:30–5:40 a.m.	5,085	0.0204	.....	.....	.....	.....	.....

[a] Variable readings.

differences probably are due to the slow increase in the activity of milk phospholipid phosphorus as noted previously in this laboratory. During the period from 60 to 80 hr. after the beginning of the experiment, samples 8a and 8b, the whole milk showed higher activity than the skimmilk. While some of the duplicates were somewhat variable, the variations were not so great as to invalidate the conclusions.

It was during this period that the blood plasma phospholipids showed a very high activity (table 1, samples 7b and 8b), and it was suspected that, contrary to general belief, the blood plasma phospholipids had passed into the milk.

The activity of the milk phospholipid phosphorus fraction was determined on the samples following 8b, but the relative activity of whole milk and skimmilk were nearly the same and the blood plasma phospholipid phosphorus had decreased to a point where the results could not be used to check the above observation. To evaluate the transfer of blood plasma phospholipids in the mammary gland, it probably will be necessary to follow the path of intravenously administered P_{32} labeled bovine blood phospholipids.

When considered on the basis of micrograms of labeled phosphorus per gram of total phosphorus, the data in tables 1 and 2 demonstrate that the phosphorus in both the casein and milk serum fractions must have originated in the acid soluble phosphorus fraction of blood plasma rather than in the phospholipid fraction. Aten and Hevesy (1) noted that 1 to 3 hr. were required for casein formation. This and the fact that during the first 34 hr. of the experiment the specific activity of casein phosphorus expressed as micrograms of labeled casein phosphorus per gram of total casein phosphorus increased smoothly to a maximum, while the specific activity of milk serum phosphorus showed marked variations, indicates that part of the milk serum phosphorus may originate from a different source than does casein phosphorus.

While the esterified phosphates of blood were not determined separately, the specific activity of phosphorus in the milk serum and casein reached and maintained a level so much higher than the blood plasma acid soluble phosphate fraction that the difference in milk serum and casein phosphate levels could hardly have been affected by the ester phosphates even if they were inactive.

These results are in general agreement with those of Aten and Hevesy (1) who reported that the specific activity of the milk inorganic and casein phosphorus was about 1.7 times higher than that of the blood plasma inorganic phosphorus at 4.25 hr. after subcutaneous administration. From the results in tables 1 and 2, it can be seen that after about 5 hr. the specific activity of the milk serum phosphorus and of the casein phosphorus was always higher than that of the plasma acid soluble phosphorus, although the general trend of the three values was similar. It was only after 59 hr. that the specific activity of the milk serum phosphorus fell to the highest value obtained for plasma acid soluble phosphorus.

Since it is unlikely that this could be due to a differential behavior of the isotopes P_{32} and P_{31}, a more probable explanation would be that plasma inorganic phosphorus is comprised of two or more forms with differing specific ac-

tivities and that there exists a preferential absorption by the mammary gland of the higher specific activity fraction.

The phosphorus of certain labile phosphate esters, such as the acye phosphates, is included in the inorganic phosphorus as usually determined. Consequently, it probably will be necessary to study the true inorganic phosphates and the labile organic phosphates before definite conclusions can be drawn concerning the blood precursors of the acid soluble and casein phosphorus of milk.

SUMMARY

When phosphorus isotope P_{32} was given orally to a cow in mid-lactation, the blood showed a marked activity after 59 min., mainly due to the activity of blood plasma acid-soluble phosphorus fraction. Later, two activity maximums were noted in both whole blood and blood plasma; the first appeared about 5 to 6 hr. after the beginning of the test and the second one about 30 hr. later. During the first of these periods, only the acid-soluble phosphorus fraction in plasma was labeled. The blood plasma phospholipid phosphorus fraction did not show any activity until several hours later. The increase in the specific activity of phospholipid phosphorus fraction also was much slower than in the plasma acid-soluble phosphorus fraction. The comparison of the specific activity of phosphorus in different blood and milk fractions at different periods following administration of P_{32} shows clearly that both the acid-soluble phosphorus in the milk serum and the casein phosphorus originate from the blood plasma acid-soluble phosphorus fraction and not from the phospholipid phosphorus fraction. On the basis of the proportionally high activity of both the casein phosphorus and the acid-soluble phosphorus in milk serum, it is considered that possibly only one fraction of the phosphates usually determined as blood plasma inorganic phosphates serves as the main precursor of the phosphorus in milk. There was some evidence to indicate that blood plasma phospholipids also may be removed from the blood by the mammary gland.

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THE EFFECT OF STERILE COPULATION ON TIME OF OVULATION IN DAIRY HEIFERS[1]

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The females of most species of mammals ovulate spontaneously, whereas some species ovulate only after copulation or some other form of sexual excitement. Cattle ovulate spontaneously but differ from most other species in that ovulation does not occur regularly until postestrum. There has been considerable speculation as to the effect of copulation on the time of ovulation in dairy cattle, especially since the extensive adoption of artificial breeding and the possible lack of sexual stimulation by this process of breeding. There are no published data concerning the effect of copulation on time of ovulation in the bovine. Marshall (8) was of the opinion that coitus was necessary for ovulation in sheep towards the end of the normal breeding season. Comprehensive studies by McKenzie, *et al.* (7) showed that sterile copulation had no effect on time of ovulation in ewes, but did shorten the estrual period.

This study was undertaken to determine the effect of sterile copulation on time of ovulation in the bovine and on other phenomena related to estrus.

The time interval between the end of estrus and the release of the ovum in dairy animals has been determined by a number of investigators. Brewster and Cole (3) found this interval to be 14.5 hr. for cows and 11.5 hr. for heifers. Nalbandov and Casida (9) recorded data on 72 estrual periods of grade cows and found that the time of ovulation from the end of heat normally varied from 10 to 18 hr., while Asdell (1) reported a range of 13.5 to 15.5 hr. After a comprehensive study, Trimberger (11) reported that the average ovulation time of cows was 10.7 hr. and of heifers 10.2 hr. after the end of estrus.

Hammond (6) observed the sexual cycles of three heifers and noted that the length of the cycle was decreased following estrus in which the heifers were serviced by a vasectomized bull. Two of the three heifers went out of heat more quickly following copulation than if service was not permitted. Chapman and Casida (5) stated that clinically normal cows which did not conceive to service of fertile bulls had longer subsequent estrual cycles than those which were not serviced.

EXPERIMENTAL METHODS

Thirty heifers from the University of Wisconsin herd, consisting of 21 Holsteins, 5 Guernseys, 3 Jerseys and 1 Brown Swiss, were used for this study. The heifers varied in age from 12 to 18 mo. and were confined to pasture lots during the experimental period, which was from June 16 to October 4. The heifers were

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paired as evenly as possible according to age and breed, and the heifers of each pair were assigned arbitrarily to different groups.

The first estrus of the animals in group A was decided arbitrarily to be a control, and that of the B group and experimental period, so that contemporary information might be obtained during the course of the experiment. At the subsequent estrus, the treatments were reversed for both groups. Consequently, of the four estrual periods observed for each heifer, there were two experimental and two control periods. An experimental period differed from a control period only in that a heifer was mated with a vasectomized bull. An effort was made to mate the heifers during that phase of the estrual period when sexual receptivity was most intense. Four of the heifers were dropped from the experiment because of estrual abnormalities and another was sold.

The animals were observed for the onset of estrus twice daily at 6 a.m. and 6 p.m. The only acceptable criterion of estrus was willingness of the heifer to stand while being mounted by a bull or by another heifer. The many other external manifestations of estrus, such as the flow of mucus from the vulva, highly vascular, swollen vulval lips, general restlessness, bellowing, attempting to ride other females and ruffled hair coat over the tail head, were not considered conclusive evidence that a heifer was in heat. However, these signs were helpful in detecting approaching estrual periods. As soon as a heifer was noted in heat, she was confined to the barn.

The time of ovulation was determined by the rectal palpation method, which was utilized by Schmid (10). Later work has shown a close agreement between the findings by rectal palpation and post-mortem data (Brewster *et al.*, 4). The heifers were examined per rectum shortly after being noticed in heat. The size, position and tone of any follicles in either ovary were determined and recorded. If the follicle was turgid, the next examination was made after the animal went out of heat, from which time palpations were made at 2-hr. intervals until ovulation occurred. In cases where the follicle was found to be soft, rectal palpations at 2-hr. intervals were begun immediately. Time of ovulation was established as the midpoint between the last examination when the follicle was intact and the subsequent examination 2 hr. later when the follicle had collapsed. In one case the follicle ruptured during palpation, and since the follicle was noted to be flabby and apparently ready to rupture, the time of that ovulation was established as the time of palpation. Rectal palpations of all heifers were performed by two or more workers, each recording his observations independently of the other.

The end of estrus was determined by checking the heifers every 2 hr. with other females or with a yearling Holstein bull, or both. The heifers were aproned to prevent copulation when a bull was used. The midpoint between the last check when the heifer stood quietly for mounting and the subsequent check when mounting was not permitted was taken as the time when the heifer went out of estrus. Usually the heifers were checked again 2 hr. later to confirm the previous observation.

The switchback technique was utilized, so that a simple group comparison could be employed in the analysis.

RESULTS AND DISCUSSION

The data showing the effect of sterile copulation on the time of ovulation are presented in table 1. The average time intervals between the end of estrus and

TABLE 1

The effect of sterile copulation on time of ovulation

GROUP A

	Time from end of heat to ovulation			
	Control	*Exptl.*	*Control*	*Exptl.*
No. of estrual period—	1	2	3	4
Heifer no.	(*hr.*)	(*hr.*)	(*hr.*)	(*hr.*)
22	10.50	7.25	5.00	11.25
25	8.50	5.50	9.25	3.75
26	2.25	2.75	14.50	4.25
28	12.00	10.00	14.25	4.00
31	11.75	10.75	10.75	7.00[b]
34	13.25	12.50	9.00	5.00
35	14.25	11.00	12.50	16.00
39	8.00	5.00	14.00	8.00
42	9.75	2.00	10.00	12.25
43	10.00	1.75	6.25	8.00
45	4.75	5.25	6.25	7.50
47	6.75	4.00	8.25	13.00
48	9.25	6.50	10.00	2.50
Mean =	9.31	6.48	10.00	7.88

GROUP B

Heifer no.	*Exptl.*	*Control*	*Exptl.*	*Control*
15	10.00	12.75	11.75[a]	8.75[b]
16	10.00	8.75	13.50	9.00
18	7.75	8.75	12.00	10.50
20	8.25	6.75	2.00	12.00
21	8.00	8.50	11.00	12.75
23	4.75	15.50	4.50	8.00
27	4.75	5.50	6.75	8.25
29	5.25	14.50	10.00	16.00[b]
30	2.00	6.00	8.25	6.25[b]
40	6.75[c]	7.00	11.00	10.50
44	14.00	13.00	6.50	12.50
46	12.00	9.50	7.75	12.75
Mean =	7.79	9.71	8.75	10.60

[a] 4th consecutive estrual period.
[b] 5th consecutive estrual period.
[c] Mean of a double ovulation.

Summary of statistical analyses

	ΣX	ΣX^2	T
Group A =	+155.75	1788.94	3.220**
Group B =	− 68.75	2522.06	

T_{01} for 23 d.f. = 2.807. (P = < .01)

the rupture of the follicle were 7.73 hr. and 9.91 hr. for the experimental and control groups, respectively. The interval for the control period was similar in length to that obtained by Trimberger (10). The difference of 2.18 hr. was

highly significant. The method of analysis used was that of Brandt (2). The values O_1, O_2, O_3 and O_4 were assigned to estrus periods 1, 2, 3 and 4, respectively, for both groups, and the formula $O_1 - 3O_2 + 3O_3 - O_4$ was utilized to determine the individual variates. The results of the tabulations are shown at the foot of table 1.

The individual ovulation intervals varied greatly for any particular heifer, and there was a wide range in the time of ovulation for both the control and the experimental periods. For the control periods the range was from 2.25 to 16.00 hr., while for experimental periods it was from 1.75 to 16.00 hr. However, when the average ovulation intervals for the control and experimental periods were compared, the variation was small. Although no special effort was made, metestrum bleeding was observed to be associated with 60 of the 100 estrual periods. A bloody discharge was observed to occur from one heifer before ovulation during a control estrual period.

Table 2 presents the data showing the effect of sterile copulation on the length

TABLE 2

Effect of sterile copulation on length of estrual period

GROUP A				
Treatment—	*Control*	*Exptl.*	*Control*	*Exptl.*
No. of estrual period —	*1*	*2*	*3*	*4*
Mean (hr.)	21.92	19.62	20.48	17.79
GROUP B				
Treatment —	*Exptl.*	*Control*	*Exptl.*	*Control*
No. of estrual period —	*1*	*2*	*3*	*4*
Mean (hr.)	18.79	19.94	16.67	22.10
	Experimental		*Control*	
Over-all mean (hr.)	18.22		21.11	

of time the heifers remained in heat. Since only two examinations were made daily for the detection of heat, the error in the time of the onset of heat may be quite large. However, since both experimental and control groups were handled similarly, this error should be balanced. It was noted that heifers in heat often stood quietly to be mounted by a bull after they no longer would stand for another female. Therefore, a bull was always used to check the termination of heat so that it could be determined as accurately as possible. The average length of estrus of all experimental periods was 18.22 hr., while the average for the control groups was 21.11 hr. The range in length of the experimental periods was 5.00 to 33.25 hr., and for the control periods, 6.00 to 41.00 hr. When the figures which were recorded as the length of each individual estrual period were treated statistically, using the same technique as before, there was no significant difference between the length of the experimental estrual periods and the length of the control periods.

Coitus was found to have no effect on the length of the succeeding cycle. The average length of the 50 estrual cycles following the estrual period during which copulation was permitted was 21.8 days, while the 50 cycles following non-copulatory estrual periods averaged 21.7 days in length. This observation is not in agreement with that of Hammond (6) or of Chapman and Casida (5).

SUMMARY

The effect of sterile copulation on the time of ovulation was observed on 25 heifers representing four dairy breeds.

The heifers ovulated, on an average, at 7.7 hr. following the end of estrus when serviced by a vasectomized bull, as compared to 9.9 hr. when not serviced. The difference was highly significant.

The average length of non-serviced estrual periods was 21.1 hr., compared with 18.2 hr. for estrual periods during which copulation occurred. Statistical analysis showed the difference to be insignificant.

Sterile copulation had no effect on the length of the subsequent estrual cycle.

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THE DETERMINATION OF PROTEIN SULFHYDRYL GROUPS WITH IODINE AND O-IODOSOBENZOATE BY AN AMPEROMETRIC TITRATION.[1]

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Iodimetric titrations have been used extensively to determine reducing matter in many biological systems. Iodine itself in acid media reacts not only with such low molecular weight reductants as ascorbic acid and glutathione but also with some proteins. Hess and Sullivan (6) found that the amount of iodine reduced by native proteins in acid solution corresponded to their cysteine contents as determined by colorimetric analysis of acid hydrolysates. It is fairly well established that there are several degrees of reactivity or availability of the sulfhydryl groups of proteins. Anson (1) found that iodine will react with all of the sulfhydryl groups of native egg albumin and iodoacetamide with about half of them but that the reagents nitroprusside and acid ferricyanide show a negative test.

o-Iodosobenzoate was first proposed by Hellerman *et al.* (8) as an oxidant for the quantitative determination of protein sulfhydryl groups. Hellerman *et al.* (7) determined by inhibition tests that o-iodosobenzoate oxidizes only part of the sulfhydryl groups of the enzyme urease, but they also showed that it oxidizes cysteine, glutathione and apparently the sulfhydryl groups of guanidine-denatured proteins quantitatively to the respective disulfide compounds.

In preliminary experiments with egg albumin and β-lactoglobulin, using the o-iodosobenzoate procedure (not in guanidine), essentially the same titration value was obtained for the native as for the guanidine-denatured protein. The fact that iodine apparently reacts with all of the sulfhydryl groups of native proteins and o-iodosobenzoate with only the more reactive or accessible ones, such as are present in guanidine denatured egg albumin (8), suggested that in titrating proteins by the o-iodosobenzoate method part of the oxidation may be due to iodine, since the excess o-iodosobenzoate is determined by liberation of iodine from iodide ion in an acid medium. Apparently the stoichiometry of the oxidation, whether produced by iodine or o-iodosobenzoate, is similar.

The presence of proteins may obscure the iodine end point, whether determined by the blue starch-iodine color or by the yellow color of iodine itself. Since this difficulty is encountered not only in direct iodine titrations but also in the o-iodosobenzoate method, a more precise method of determining the end point

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was sought. This paper reports the use of an amperometric adaptation of the "dead stop" titration of Foulk and Bawden (4) for this purpose. This method depends on the depolarizing action of iodine on a polarized platinum cathode and is especially applicable for the determination of small amounts of iodine in opaque sols such as milk.

METHOD

The apparatus employed was similar to that usually used for the "dead stop" titration (4, 13). A potential of 10 to 20 mv. (usually 10 mv.) was maintained across a pair of 5-cm. bright platinum electrodes immersed in the solution. A galvanometer having a sensitivity of 0.02 microamperes per mm. was included in the circuit to measure the current which flows while the cathode is being depolarized by the iodine. Iodide ion keeps the anode depolarized throughout the titration. Stirring was accomplished by means of a magnetic stirrer.

The detailed procedure for the titration is as follows: Two to 20 ml. of solution at a pH of 6.6 to 7.0 (protein sols containing 0.25 to 3.0 g. per 100 ml. may be used) are introduced into a 100-ml. beaker, followed by 4.0 ml. of approximately 0.005 *N* sodium o-iodosobenzoate from a 5-ml. burette graduated to 0.01 ml. The mixture is gently stirred for 2 to 3 min. and during this time a flask containing 5 ml. of 1*N* HCl (or enough to give a final pH of 1.5 to 2.0), 5 ml. of freshly diluted 3 per cent KI and 10 ml. of standardized freshly diluted 0.002 *N* $Na_2S_2O_3$ is prepared. The contents of this flask then are poured and rinsed into the beaker and the volume made to approximately 100 ml. with distilled water.[3] With constant stirring the mixture is titrated with more of the 0.005 *N* o-iodosobenzoate until free iodine is present as indicated by a slight permanent deflection of the galvanometer. More of the solution is added in increments and the volumes added (including the original 4.0 ml.) are plotted against the galvanometer readings. Extrapolation of the plot to zero current flow gives the end point. A blank is run on the solvent (*i.e.*, water, buffer, etc.) in exactly the same manner and this constitutes a standardization of the o-iodosobenzoate against the standard thiosulfate. When iodine is used directly as the oxidant, the sol is first acidified, KI added and iodine or iodate titrated into the solution. Calculations of cysteine percentages were made on the assumption that sulfhydryl groups are oxidized to the disulfide (6, 8).

RESULTS AND DISCUSSION

In figure 1 is shown the titration of casein sols of two concentrations in phosphate buffer. Extrapolation of the curves to zero current flow shows that no o-iodosobenzoate or iodine was reduced. Identical results are obtained using a direct iodine titration. The apparent reducing capacity of casein if the starch-iodine end point had been used is illustrated clearly in figure 1. Iodine does not form a visible complex with starch until the iodine normality is $1\text{–}10 \times 10^{-6}$ *N*,

[3] This large volume was found necessary with casein-containing sols as milk. If the casein sol is not first diluted up with 30 or 40 ml. of water before the acid and iodide are added, a precipitate forms which clogs up to the electrodes and interferes with the readings.

depending somewhat on the concentration of iodide ion and the type of starch used (10, 11). In the present investigation, titration of a purified amylose starch fraction produced a blue color at a concentration of 2.5×10^{-6} N free iodine (galvanometer reading = 50 mm.). However, casein apparently adsorbs part of the iodine from the aqueous phase and, since the blue color with starch will not appear until the concentration of free iodine has reached 2.5×10^{-6} N, the casein would appear to have a considerable reducing capacity (in this case amounting to 0.15 per cent cysteine) if starch were used to detect the end point. All proteins tested except gelatin exhibited the ability to decrease the slope of the plot by this adsorptive process. The iodine adsorbed apparently is held reversibly, since it can be removed readily by a back titration with thiosulfate.

The slope of the plot depends on the final volume of the solution which

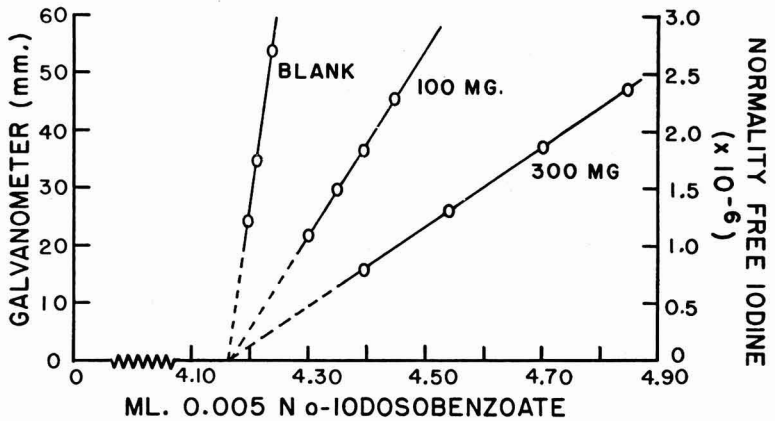

FIG. 1. Titration of casein with o-iodosobenzoate, showing the effect of protein concentration on the amount of iodine required to yield a given galvanometer reading. The normality of free iodine corresponding to galvanometer readings was computed from the increments of o-iodosobenzoate added in the blank titration.

determines the normality of iodine and also on the rate of stirring which affects the diffusion of iodine to the electrodes. The magnetic stirrer employed produced a constant rate of stirring for a given titration but some variability occurred between titrations. It has been observed repeatedly that duplicate titrations extrapolate to the identical end point even though the slopes differ. Consequently, while it is necessary to maintain a constant stirring rate during a given titration, it is not essential to do so from one titration to another.

In order to determine the specificity of the method for protein groups, several amino acids and proteins were titrated by the o-iodosobenzoate method and by the direct iodine titration. The plots for casein and gelatin, which do not contain sulfhydryl groups, extrapolate back to the same point as the blank; those for egg albumin and β-lactoglobulin extrapolate to values characteristic of the protein and proportional to the quantity present. An example of the titration as applied

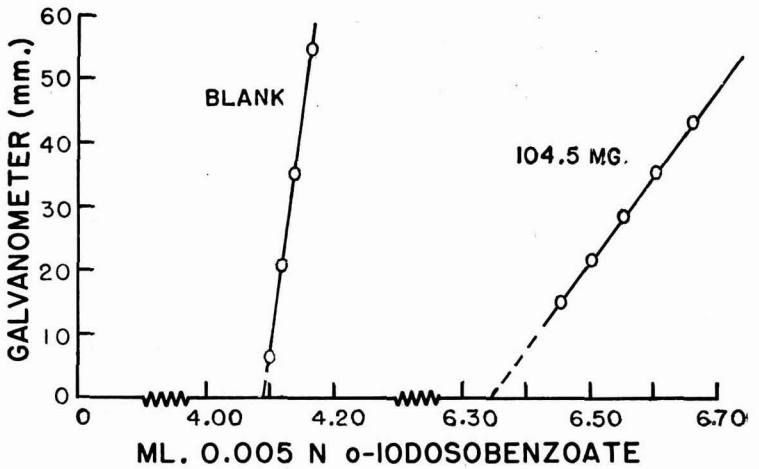

FIG. 2. Titration of 104.5 mg. of crystalline β-lactoglobulin. The calculated normality of the o-iodosobenzoate is 0.004988 since 10 ml. of 0.002040 *N* sodium thiosulfate was used in the blank or standardization titration. The difference in titer between the blank and the β-lactoglobulin sol shows that the β-lactoglobulin has reduced 0.0112 m.eq. of oxidant which is equivalent to 1.30% cysteine.

to β-lactoglobulin is given in figure 2. The reducing capacities of the proteins and amino acids, calculated as cysteine percentage, are presented in table 1. In agreement with Hellerman *et al.* (7, 8) these results indicate a stoichiometric

TABLE 1

Reducing capacity of various proteins and amino acids

Protein or amino acid[a]	Reducing power	
	Direct iodine titration	o-Iodosobenzoate titration
	(*as % cysteine*)	
Casein[b]	0.00	0.00
Gelatin[c]	0.00	0.00
Egg albumin[d]	1.51	1.12
β-Lactoglobulin[e]	1.30	1.30
	(m.eq./m.eq. cysteine)	
Cysteine[f]	3.42	1.00
Glutathione[g]	1.03	0.94
Amino acid mixture[h]	0.00	0.00
Cysteine[f] + amino acids[h]	3.45	0.99

[a] All in phosphate buffer, pH 6.6, μ=0.1.
[b] Prepared according to the method of Van Slyke and Baker (14).
[c] Difco Bacto brand.
[d] Recrystallized four times according to the method of Kekwick and Cannan (9).
[e] Crystallized according to the method of Bull (3).
[f] Pfanstiehl reagent grade (fresh supply).
[g] Eimer and Amend C.P. grade (old supply).
[h] 5 mg. each of cystine, methionine, histidine, phenylalanine, tryptophan and tyrosine.

oxidation of cysteine and glutathione to the disulfides. This is a direct oxidation by the o-iodosobenzoate since the nitroprusside test of these two materials is abolished by o-iodosobenzoate alone. Direct iodine titration of cysteine carries the oxidation to further stages (12), but this effect does not occur with glutathione (15). Direct iodine titration of egg albumin evidently causes some over-oxidation which does not occur if o-iodosobenzoate first is allowed to react with the protein, but both methods give identical results for β-lactoglobulin. The sulfhydryl groups appear to be the only protein groups oxidized by these reagents.

The cysteine contents of egg albumin and β-lactoglobulin calculated from the results of the titration (o-iodosobenzoate method) on the basis of the assumption that the stoichiometry of the oxidation corresponds to the formation of the disulfide are in reasonable accord with values in the literature obtained by other

TABLE 2

The effect of pretreatment with o-iodosobenzoate on the reducing power of egg albumin and β-lactoglobulin

Protein	Treatment	Reducing power	
		o-Iodosobenzoate and iodine reduced	o-Iodosobenzoate reduced (actual)
		(as % cysteine)	
β-Lactoglobulin	Water + dialysis	1.28	
β-Lactoglobulin	o-Iodosobenzoate + dialysis[a]	1.26	0.02
Egg albumin	Water + dialysis	1.10	
Egg albumin	o-Iodosobenzoate + dialysis[a]	0.98	0.12

[a] 0.40 m.eq. of o-iodosobenzoate added per gram of protein and the excess removed by exhaustive dialysis in Visking sausage casings against phosphate buffer pH 6.6, μ=0.1.

methods (2, 5). This fact furnishes some justification for using this method of calculation. The results seem to represent the total sulfhydryl content of these proteins.

To determine accurately what proportion of the oxidation is caused by o-iodosobenzoate itself, egg albumin and β-lactoglobulin were treated with o-iodosobenzoate, exhaustively dialyzed against buffer and finally titrated. The results, given in table 2, show that o-iodosobenzoate reacts with few if any of the reducing groups of native β-lactoglobulin but with about 10 per cent of those of native egg albumin. Thus, the o-iodosobenzoate titration as applied to native proteins actually involves principally oxidation by the iodine formed upon acidifying the sol and adding iodide. The advantage of using the o-iodosobenzoate treatment on protein systems is that any very reactive sulfhydryl groups such as seem to be present in egg albumin will not be over-oxidized by iodine. Fresh milk proteins apparently do not contain such reactive groups, but there is evidence that they are formed by heat treatment of the milk serum proteins.

SUMMARY

An amperometric adaptation of the "dead stop" titration technique has been applied to determine the sulfhydryl groups of proteins with o-iodosobenzoate and iodine. As applied to native proteins, the oxidation is largely produced by the iodine liberated in the course of determining the excess o-iodosobenzoate.

The method appears to be specific and quantitative for sulfhydryl groups, since any very reactive groups which might be overoxidized by iodine react first with the o-iodosobenzoate.

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THE REDUCING CAPACITY OF MILK AS MEASURED BY AN IODIMETRIC TITRATION[1]

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In recent years the reducing components of milk have been studied extensively in relation to various processing procedures. The capacity of acidified milk or of a deproteinized acid filtrate of milk to produce 2,6-dichlorophenolindophenol has been widely used as a measure of the ascorbic acid content. In addition to ascorbic acid, the sulfhydryl groups of the milk proteins must be considered in attempting to elucidate the reducing system. Neither nitroprusside (15) nor thiamine disulfide (5, 6) is reduced by fresh milk, but capacity to reduce these reagents is produced by heat treatment. Ferricyanide at pH 6.6 is reduced by milk at 50° C., the capacity being largely accounted for by the ascorbic acid and the proteins present (1). The capacity to reduce ferricyanide is augmented by heat treatment, mainly as a result of the production of reductants by sugar-protein interactions (1, 6).

Larsen *et al.* (12), using a modification of the *o*-iodosobenzoate method of Hellerman *et al.* (7, 8), found that the reducing power of sols of the serum proteins decreased upon heat treatment, particularly in the presence of air. These decreases tended to parallel the improvement produced by such heat treatment in the baking quality of serum protein preparations and of skimmilk itself. More recently, Larson and Jenness (13) modified the *o*-iodosobenzoate method by use of an amperometric detection of the end point. They demonstrated that when applied to native egg albumin and β-lactoglobulin the method involves chiefly oxidation of sulfhydryl groups by iodine liberated at pH 1.5 to 2.0 in the course of determining the excess *o*-iodosobenzoate, rather than by the *o*-iodosobenzoate itself at a pH of 6.6 to 7.0. This paper reports the application of the method of Larson and Jenness (13) to milk. The constituents of milk which exhibit reducing power in this method and some of the effects of heat treatment have been studied.

Gould (4), using a method adapted from that of Woodward and Fry (21) for determining the glutathione content of blood serum, reported that sulfosalicylic acid filtrates of milk had much higher reducing capacities in an iodate-iodine titration than could be accounted for by the ascorbic acid present but reached no definite conclusions as to the identity of other reductants. This finding may be interpreted in view of the results of the present investigation.

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METHODS

The *o*-iodosobenzoate titrations were made on 10-ml. samples by the method of Larson and Jenness (13). Also, some titrations were made on sulfosalicylic acid filtrates with iodate according to the method of Gould (4). Ascorbic acid was determined by titrations with 2,6-dichlorophenolindophenol of metaphosphoric-trichloracetic acid filtrates prepared according to Doan and Josephson (3). The dye was standardized against ferrous ammonium sulfate as recommended by Stewart and Sharp (19). Analyses for nitrogen distribution were performed by the method of Rowland (17), using a micro-Kjeldahl method that combines digestion with selenium oxychloride as suggested by Pepkowitz and Shive (16) (except that the perchloric acid was omitted) with the distillation and titration technique of Ma and Zuazaga (14).

EXPERIMENTAL

Constituents responsible for the reducing power. The following experiments were made to determine the respective contributions of the fat phase, the colloidal phase and the materials in true solution to the reducing power as determined by the *o*-iodosobenzoate iodimetric procedure.

(a) *The fat phase.* The contribution of the fat phase was determined by comparison of the reducing capacities of whole milk, skimmilk and cream from a single original lot of fresh mixed milk collected from the separator at the University creamery. The data given in table 1 show definitely that the fat phase

TABLE 1

Reducing capacities of whole milk, skimmilk, cream and an emulsion of milk fat

Material	Fat	Reducing capacity		
		Iodimetric	Dye[a]	"Non-ascorbic"[b]
	(%)		(*m. eq./l.*)	
Whole milk	3.95	0.495	0.187	0.308
Skimmilk	0.01	0.477	0.178[c]	0.299
Cream	50.0	0.533	0.083[c]	0.450
Milk fat in gelatin	16.7	0.00	.....	.....

[a] 2,6-dichlorophenolindophenol.
[b] By difference.
[c] Ascorbic acid contents of the skimmilk and cream are lower than would be expected, probably due to oxidation during and following separation.

as well as the plasma contributes to the non-ascorbic reducing capacity. On a volumetric basis the non-ascorbic reducing capacity of the fat phase is somewhat greater than that of the plasma, since the cream had the highest titration value. Since no reducing capacity was exhibited by an emulsion of milk fat in gelatin (16.7 per cent fat), it is logical to conclude that the reducing power of the fat phase involves the materials adsorbed on the fat globules. The contribution of the fat phase to the reducing power of whole milk is very small, however, because of its low concentration therein.

(b) *The proteins and dissolved constituents.* Fresh whole milk representa-

tive of an entire milking of a single cow[3] of the University herd was obtained at milking time. Fractionations were made by dialysis to determine the relative contributions of the dialyzable and non-dialyzable constituents. Furthermore, dialysis experiments were set up in which portions of milk serum were dialyzed against milk and buffer, respectively. Dialysis was performed by placing the materials in Visking sausage casings which then were equilibrated in the desired medium on an inclined rotating turntable in a room at 5° C. Volumes were measured carefully before and after dialysis, and all of the titrations were made about 26 hr. after milking. Details of the fractionations and treatments as well as the results are given in table 2. The titration results show that the reducing

TABLE 2

Reducing capacity of milk fractions

Fraction no.	Material dialyzed	Treatment		Milk constituents in fraction	Reducing capacity[a]	
		Dialysis medium	Type of dialysis		Iodimetric	Dye
					(*m. eq./l.*)	(*m. eq./l.*)
1	Whole milk	None	None	All	0.577	0.244
2	50 ml. whole milk	6 l. buffer[b]	Exhaustive[c]	Non-dialyzable	0.362	0
3	60 ml. water	1950 ml. milk	Equilibrium[d]	Dialyzable	0.227	0.247[e]
4	30 ml. serum[f]	1950 ml. milk	Equilibrium	Serum protein and dialyzable	0.577	0.244
5	60 ml. serum	6 l. buffer	Exhaustive	Serum protein	0.360	.....

[a] Calculated to basis of original milk.
[b] Phosphate buffer pH 6.6, $\mu = 0.1$.
[c] Three 2-l. portions of buffer over 24 hr.
[d] Equilibrated for 24 hr.
[e] Titrations with dye made in presence of metaphosphoric-trichloracetic acid coagulant.
[f] Serum prepared by precipitating casein from 100 ml. milk with 10 ml. 10% acetic acid and 10 ml. 1 *M* sodium acetate.

power of milk as determined by this method is the summation of the effects of certain dialyzable constituents and the serum proteins. The combined contributions of the fat and caseinate, represented by the difference between fractions 2 and 5 or between 1 and 4, are negligible. While the previous experiment demonstrated that the fat phase does have reducing power, it is present in too small an amount in whole milk to contribute significantly. Purified casein was shown in a previous paper (13) to have no reducing power. This has been further verified by titration of a caseinate sol prepared by centrifuging skimmilk in the Sharples supercentrifuge and dispersing the caseinate gel in a milk dialysate prepared by dialyzing 300 ml. of distilled water against 10 gal. of raw skimmilk. Such a preparation had a reducing power identical to that of the dialysate itself. The values for the reducing capacity of the milk serum protein in this sample

[3] The general picture obtained with this milk was confirmed with another lot of milk from a second cow.

are equivalent to about 0.053 m.eq. per gram or 0.64 per cent cysteine whether determined directly (fraction 5) or by difference (fraction 4 minus fraction 3). This figure is in close agreement with data published previously (12) on other preparations of the serum protein mixture.

Ascorbic acid undoubtedly predominates among the dialyzable reducing materials. Titration of milk dialysate with *o*-iodosobenzoate gives approximately the same value as is obtained by 2,6-dichlorophenolindophenol titration of the dialysate or of deproteinized milk.

Variations in reducing capacity among samples. Reducing titration values for a number of fresh and commercial samples of whole and skimmilk are presented in table 3. These results exhibit considerable variability among samples,

TABLE 3

Reducing capacity of various samples of milk

Sample[a]	Iodimetric	2,6-dichlorophenol indophenol	Non-ascorbic	As cysteine[b]
	(*m. eq./l.*)	(*m. eq./l.*)	(*m. eq./l.*)	(%)
Whole milk:				
1— 1 hr.[c]	0.572	0.226	0.346	0.76
1—26 hr.	0.512	0.143	0.369	.....
2— 1 hr.	0.642	0.265	0.377	.....
2—26 hr.	0.574	0.202	0.372	.....
3—26 hr.	0.577	0.244	0.333	0.60
Skimmilk:				
4—Fresh	0.640	0.280	0.360	.....
5—Commercial	0.357	0.085	0.272	0.52
6—Commercial	0.388	0.073	0.315	0.54
7—Commercial	0.288	0.053	0.235	0.48
8—Commercial	0.341	0.067	0.274	0.66
9—Commercial	0.325	0.087	0.238	0.50
10—Commercial	0.335	0.085	0.250	0.55
11—Commercial	0.324	0.077	0.247	0.55

[a] Samples 1, 2, 3, and 4 were from single milkings of individual cows.
[b] Calculated as per cent cysteine in the serum protein.
[c] Time of titration after milking.

not only in the total iodimetric reducing capacity but also in the "non-ascorbic" category which represents the serum proteins. In general, the commercial raw skimmilks, which contained very little ascorbic acid, tended to be lower in non-ascorbic reducing capacity, not only on the basis of milliequivalents per liter, but also when calculated as cysteine percentages of the serum proteins. The data for whole milk samples 1 and 2 were confirmed in an independent analysis by H. A. Harland of the Division of Dairy Husbandry, who also has observed a range of 0.219 to 0.334 m.eq. per liter in non-ascorbic reducing capacities on 20 samples of commercial raw whole milks comparable to that obtained by us for commercial raw skimmilks. Gould's (4) data also are of the same order of magnitude, his non-ascorbic values ranging from 0.214 to 0.387 m.eq. per liter for 14 samples. Since Gould's method of titration is similar to ours, and the results correspond, it appeared likely that his treatment with sulfosalicylic acid did not entirely precipitate the serum proteins. The validity of this explanation

TABLE 4

Reducing capacity and efficiency of sulfosalicylic acid as a protein precipitant

Sample	Reducing capacity			Nitrogen		
	Iodimetric		2,6-dichloro-phenol-indophenol	Non-casein[a]	In sulfo-salicylic filtrates[b]	Non-protein[a]
	o-Iodoso-benzoate	Gould Method				
	(*m. eq./l.*)			(*mgm./100 ml.*)		
Commercial skim[c]						
12—No heat	0.324	0.297	0.077	112.	70.0	27.
—155° F.	0.246	0.216	0.057	.....	57.8	.....
13—No heat	0.335	0.312	0.085	112.	70.0	26.
—165° F.	0.236	0.111	0.060	.....	44.0	.....
14—No heat	0.341	0.260	0.067	106.	71.0	27.
—185° F.	0.206	0.025	0.047	.....	33.4	.....
15—No heat	0.325	0.297	0.087	121.	71.0	30.
—195° F.	0.227	0.069	0.073	.....	32.7	.....
Reconstituted Dry skim[d]						
16—Freeze-dried	0.290	0.295	0.025	126.6	76.6	31.5
17—Spray dried						
145° F.	0.258	0.203	0.023	128.5	74.6	33.3
190° F.	0.100	0.044	0.066	63.0	42.6	34.3

[a] According to method of Rowland (17).
[b] Filtrate prepared according to method of Gould (4).
[c] All heat treatments were of 30 min. duration.
[d] Reconstituted in the amount of 10 g./100 ml.

is attested by the data in table 4. Sulfosalicylic acid as used by Gould does not precipitate completely the proteins of unheated milk, but it does more nearly do so in the case of heated milks.

Effect of heat treatments. The change in reducing capacity produced by

TABLE 5

Effect of heat treatment on the o-iodosobenzoate reducing capacity of milk, milk serum proteins, and crystalline β-lactoglobulin

Temperature for 30 min.	Reducing capacity as cysteine		
	Reconstituted skimmilk[a]	Serum proteins[b]	β-lactoglobulin[b]
(° C.)	(%)	(%)	(%)
	Heated in nitrogen, titrated immediately on cooling		
Control	0.58	0.69	1.30
78	.....	0.65	1.03–1.10[c]
	Heated in air, titrated shortly after cooling		
64	.....	0.59	1.15
69	0.37	0.50	.....
73	.....	0.32	0.72
78	0.20	0.28	0.63
83	.....	0.17	.....
97	0.22	0.20	0.43

[a] Freeze-dried unheated milk reconstituted in the amount of 10 g./100 ml. The ascorbic acid content of this milk was negligible. Cysteine percentages calculated on basis of serum protein in unheated control.
[b] In phosphate buffer at pH 6.9, $\mu = 0.1$.
[c] Greater precautions to exclude air were taken in the case of the milk serum protein sol than for the β-lactoglobulin sol.

subjecting skimmilk (reconstituted freeze-dried nonfat dry milk solids), milk serum protein sol, and β-lactoglobulin sol to 30-min. heat treatments at temperatures of 64 to 97° C. is shown in table 5. These data confirm the previous report (12) that heat treatment decreases the sulfhydryl reducing capacity of milk serum proteins. Probably this change is due to oxidation, since it is largely prevented by excluding air from the sample during heating.

DISCUSSION

This study shows that the *o*-iodosobenzoate titration method as modified by Larson and Jenness (13) is applicable to milk and other dairy products. Indeed, it was partly with the object of applying the method to opaque solutions such as milk that the method was devised. The fact that the fat phase (but not the fat itself) reduces *o*-iodosobenzoate and/or iodine in this determination is interesting in view of the reports of Josephson and Doan (10) and of Townley and Gould (20) that the materials adsorbed on the fat globule are a source of heat-labile sulfides in milk.

While it is probable that the principal dialyzable reductant is ascorbic acid, no claim can be made that it is the only one. The *o*-iodosobenzoate titration of milk dialysate is approximately the same as the titration with 2,6-dichlorophenolindophenol but it must be recognized that neither method is specific for ascorbic acid.

Considerable variability in the sulfhydryl content of the serum protein fraction of various samples of milk was observed. β-Lactoglobulin undoubtedly is the principal contributor to the reducing power of this fraction, since crystalline preparations reduce 0.104 to 0.110 m.eq. of iodine per gram (13). Thus, if β-lactoglobulin represented 50 per cent of the milk serum proteins, it alone would account for titration values in the range obtained for the latter. Electrophoretic analyses indicate that components having the mobility of β-lactoglobulin comprise at least 50 per cent of the proteins of milk serum (2, 18). β-Lactoglobulin constitutes the major portion of the classical "lactalbumin" fraction. It is not known definitely whether the variations in sulfhydryl content of the various serum protein samples reflect the relative amount of β-lactoglobulin present, although this is strongly suspected of being the case.

The results show definitely that Gould's iodate titration of a sulfosalicylic acid filtrate actually involved some protein sulfhydryl groups because the deproteinization was incomplete. These proteins are responsible for the reducing power which he observed over and above that of the ascorbic acid present. Undoubtedly, the "destruction" of a reducing system by heat to which he referred involved both decrease of the protein sulfhydryl groups and increased precipitability of the serum proteins by sulfosalicylic acid. Evidently, that fraction of the serum protein which is precipitated by sulfosalicylic acid from unheated milk contributes little to the reducing power since titrations by Gould's method yield results comparable to those obtained by the *o*-iodosobenzoate procedure.

The fact that the decreases in reducing capacity produced by heating are similar for milk serum protein sols, β-lactoglobulin and skimmilk again indicates that β-lactoglobulin probably is the principal constituent involved. Apparently,

heating activates the sulfhydryl groups so that they become susceptible to oxidation by molecular oxygen. This oxidation, however, does not necessarily mean formation of disulfides from the sulfhydryl groups, since Larsen *et al.* (12) could find no increase in the cystine content of heated milk serum proteins as determined on the hydrolysate by the method of Kassel and Brand (11), even though the apparent titer had decreased. An alternate explanation of the loss of titratable sulfhydryl groups on heat treatment is that the protein micelle unfolds upon heating and assumes upon cooling a new configuration such that the sulfhydryl groups are shielded from the action of the *o*-iodosobenzoate or iodine. In the light of recent evidence (13) indicating that iodine and not *o*-iodosobenzoate is the principal oxidant and that iodine oxidizes all the sulfhydryl groups of native or denatured proteins (9, 13), steric hindrance does not seem adequately to explain the loss of sulfhydryl groups, although it may be a minor factor. Even though the loss of sulfhydryl groups appears to be due to oxidation by molecular oxygen, neither the kinetics of these processes nor the conditions affecting them have been studied thoroughly, and thus the data of table 5 should be regarded as preliminary results which indicate the similarity of the changes occurring in β-lactoglobulin, the serum protein mixture and milk itself. These results may not represent the maximum oxidation, since indications have been obtained that further decreases occur upon holding the cooled sample for periods up to 48 hr. Presumably such factors as diffusion of oxygen into the sample and the temperature of holding influence the rate of oxidation. At present, any attempt to use non-ascorbic reducing capacity as an index of the extent of heat treatment to which a sample of market milk may have been subjected is premature. The observed variability in the non-ascorbic reducing capacity of samples of fresh milk and the fact that oxidation may continue at slow and variable rates after heating complicate the relationship.

SUMMARY

The reducing systems of milk have been studied by an iodimetric titration employing *o*-iodosobenzoate. The fat phase, the serum proteins and the dialyzable portion all exhibit reducing capacity in this method. Since milk fat emulsified in gelatin has no reducing capacity, the materials constituting the natural "fat globule membrane" must be responsible for reduction by the fat phase. Titrations of purified crystalline β-lactoglobulin indicate that it probably is the principal reducing constituent of the serum proteins. Undoubtedly, ascorbic acid is the chief dialyzable reductant.

Considerable variability was found in the reducing capacity of the serum proteins in various samples of milk; commercial raw milks tended to give lower values than fresh milks.

Sulfosalicylic acid as used by Gould does not precipitate quantitatively the serum proteins from raw milk, but the efficiency of precipitation is greater in heated milk. Thus, the decrease produced by heat treatment of milk in the iodimetric titration values of sulfosalicylic acid filtrates is due to both decreased reactivity of protein sulfhydryl groups and increased precipitability of the serum proteins. The similarity in decreases of the reducing capacity for skim-

milk, purified serum proteins and crystalline β-lactoglobulin again suggests that β-lactoglobulin is the principal reducing component of the milk proteins. The decrease in reducing titer upon heat treatment probably is due to oxidation by molecular oxygen, since heat treatment of deaerated samples in the presence of nitrogen produces little or no decrease.

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STUDIES OF HEATED MILK
III. MODE OF FORMATION OF CERTAIN FURAN COMPOUNDS[1]

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The need exists for more fundamental information relating to chemical changes induced in milk by high temperature treatment. To augment present knowledge, research has been progressing with the objective of identifying the compounds produced in milk by high temperature treatment and to elaborate the mechanism of their formation.

The presence of furfuryl alcohol in skimmilk heated to high temperature has been reported previously. It was suggested that lactose or ascorbic acid might serve as the origin of the compound (6). More recent work has precluded the possible action of ascorbic acid in this connection and reduced the essential components of the reaction to lactose and casein. Some additional observations were that no furfuryl alcohol is produced by heating aqueous systems of lactose and glycine, glucose and casein or galactose and casein, the principal end product being 5-hydroxymethyl-2-furfural in these instances. Small quantities of the latter compound were shown to be present also in heated skimmilk (5).

Excepting the isolation of furfuryl alcohol from coffee brew (8), there appears to be no information in the literature concerning the presence or mode of formation of this compound by heating food stuffs. Thus, the potential significance of furfuryl alcohol in the heat degradation of milk, its possible relationship to hydroxymethylfurfural and the decomposition of sugars warranted further study.

EXPERIMENTAL

The experimental procedure involved a uniform heat treatment of milk samples and simplified systems by autoclaving for 2.5 hr. at 127° C., unless otherwise indicated. The pH values of these samples before and after autoclaving were determined with a Beckman model M instrument employing a glass electrode. Preparation and ethyl ether extraction of the autoclaved samples have been described (5). The components of the ether extract, following removal of the solvent on a warm water bath, were separated by vacuum distillation at pressures below 1 mm. Hg. The distillation apparatus consisted essentially of one 10-ml. distilling flask delivering into a second of like capacity. The latter was immersed in either a dry ice or cold water bath as needed. The course of the distillation was followed by measurement of refractive index with an Abbe refractometer. After considerable experience with the use of this apparatus and the type of material being distilled, it was found possible to obtain relatively pure yields of furfuryl alcohol and hydroxymethylfurfural. Where these two compounds were

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encountered in this study, their identification was accomplished according to procedures previously reported (5, 6).

Simplified systems employing lactose. The formation of furfuryl alcohol in lactose-casein systems, but not in those of lactose and glycine (5), suggested that some native property of casein might be modifying the lactose degradation. Of such properties, buffering capacity, the association of copper ions and the presence of basic groupings in the protein seemed worthy of consideration.

Accordingly, 1-kg. samples of 15 per cent lactose solution were prepared in combination with each of the following: glycine (30 g.), glycine (30 g.) plus copper sulfate (2 ppm. cupric ion), lysine hydrochloride (8 g.), lysine (6 g.), sodium bicarbonate (20 g.), 3 *N* HCl (sufficient to adjust pH to 2.5). The quantities of furfuryl alcohol and hydroxymethylfurfural recovered from the autoclaved samples are presented in table 1.

TABLE 1

The amounts of certain furan compounds isolated from various heated[a] lactose systems

Systems[b] studied	Before heating	After heating	Neutral ether-extractable matter	Furfuryl alcohol	Hydroxy-methyl-furfural
	(*pH*)	(*pH*)	(*g.*)	(*g.*)	(*g.*)
Lactose + Glycine (30 g.)	7.0	4.1	0.41	None	0.38
Lactose + Glycine (30 g.) + Cu^{++} (2 ppm.)	7.0	4.1	0.40	None	0.35
Lactose + Lysine HCl (8 g.)	4.6	3.6	0.45	None	0.40
Lactose + Lysine (6 g.)	8.9	4.2	0.91	0.25	0.24
Lactose + $NaHCO_3$ (20 g.)	8.3	4.8	2.80	0.87	Trace
Lactose + HCl	2.5	2.4	0.20	None	0.20

[a] 127°C. – 2.5 hr.

[b] 1-kg. quantities of 15% lactose solutions with the indicated material added.

The effect of pH in heated skimmilk. The data of table 1 indicate that pH is a vital factor affecting the formation of furfuryl alcohol and hydroxymethylfurfural from lactose. Those systems having basic initial pH produced significant quantities of furfuryl alcohol, whereas the acidic or neutral systems produced only hydroxymethylfurfural. Thus, it might be presumed that increasing the acidity of milk would favor the production of hydroxymethylfurfural and reduce the amount of furfuryl alcohol formed during heat treatment. This was observed to be the case. In demonstrating this point, 2-kg. samples of condensed skimmilk (30 per cent total solids) were used. One sample was adjusted to pH 4.8 with 3 *N* HCl; a second sample was retained unaltered (pH 6.4). Following autoclaving, 0.60 g. of hydroxymethylfurfural was recovered from the acidified sample. Furfuryl alcohol could not be isolated from this sample, although qualitative tests suggested that trace quantities of the compound might be present. The milk sample with pH unadjusted yielded 0.47 g. of furfuryl alcohol but no measurable quantity of hydroxymethylfurfural.

Lactose-$NaHCO_3$ systems. The relatively high yield of furfuryl alcohol obtained from the lactose-$NaHCO_3$ sample, as shown in table 1, appeared to be a significant finding and was investigated further. One and one-half kg. samples of 10 per cent lactose solution combined with varying amounts of $NaHCO_3$ were autoclaved for 6 hr. at 127° C. One sample employing a NaH_2PO_4–NaOH buffer (pH 6.5) was included also in the trial. Data were taken relative to changes in pH and the amounts of furfuryl alcohol formed in the samples during heating (table 2). These data demonstrate that buffer capacity of the lactose solution affects the yield of furfuryl alcohol. A change in pH to acidic conditions is beneficial to the yield; however, too rapid a shift to acidic conditions is apparently detrimental to the yield. This mechanism is considered in some detail under the discussion section.

TABLE 2

The amounts of furfuryl alcohol formed and the changes in pH of heated[a] lactose solutions[b] containing varied amounts of $NaHCO_3$

Sample no.	$NaHCO_3$ added	Before heating	After heating	Furfuryl alcohol
	(*g.*)	(*pH*)	(*pH*)	(*g.*)
1	5	8.1	4.5	0.15
2	10	8.2	4.6	0.46
3	20	8.3	4.8	1.07
4	40	8.3	5.1	0.25
5	60	8.3	5.6	0.15
6	80	8.3	6.8	trace
7	*	6.5	5.2	0.22

[a] 127° C.–2.5 hr.
[b] 10% by weight.
* 20 g. $NaH_2PO_4 \cdot H_2O$ added and pH adjusted to 6.5 with 3 *N* NaOH.

Furan compounds as intermediates. With respect to the mechanism of furfuryl alcohol formation, the possibility existed that some other furan compound might serve as a "precursor". Of such compounds, consideration was given to furfural and hydroxymethylfurfural. Although furfural should be readily recovered by the ether extraction technique employed, it was conspicuous by its absence in this and previous investigations (4, 5, 6). Conceivably, it could be reduced to furfuryl alcohol under the conditions of the reaction.

The addition of 3-g. quantities of furfural or hydroxymethylfurfural to 2-l. samples of skimmilk (9 per cent total solids) prior to heating did not increase the amounts of furfuryl alcohol produced during autoclaving. Ether extraction of these samples recovered 0.9 g. of furfural and 1.65 g. of hydroxymethylfurfural, respectively. The quantity of furfuryl alcohol recovered from both samples containing the added furan compounds, as well as from a control sample, was 0.1–0.2 g. It is evident, therefore, that the compounds considered do not serve as "precursors" of furfuryl alcohol in heated milk. They appear to undergo partial destruction during the heating treatment.

Various sugars as sources of furfuryl alcohol. It was noted previously that

glucose or galactose when heated in a casein solution gave rise to hydroxymethylfurfural but not furfuryl alcohol (5). Maltose, sucrose and methyl-α-D-glucopyranoside were studied in similar experiments. One-kg. samples containing 10 per cent of the sugars and 20 g. of Na_2CO_3 were autoclaved for the 2.5-hr. period. It was noted that maltose produces furfuryl alcohol, but that sucrose and methyl-α-D-glucopyranoside do not.

Control experiments. Small quantities (3 g.) of furfuryl alcohol could be recovered to the extent of 95 per cent from aqueous solution (2 l.) with the ether extraction procedure and apparatus[2] used in these experiments. Recovery of hydroxymethylfurfural under these conditions was 93 per cent. No allowance is made in these recoveries for manipulative losses in weighing and drying, thus extraction of the compounds was approximately quantitative. The stability of pure lactose solutions to the heat treatment used in this study has been demonstrated previously (5).

DISCUSSION

The results of this study indicate that the formation of furfuryl alcohol from lactose is fundamentally a consideration in carbohydrate chemistry. Although furfuryl alcohol is one of the principal heat-generated compounds of milk or lactose-casein systems, findings herein show that the compound may be produced in pure lactose solutions having the required pH and buffer capacity (tables 1 and 2). With reference to milk, it appears that various protein groups and soluble salts create such conditions.

The data in table 1 clearly show a relationship between furfuryl alcohol and hydroxymethylfurfural. In both acidified skimmilk and lactose systems, initial pH values below 6.0 appeared to favor the formation of hydroxymethylfurfural at the expense of furfuryl alcohol. This relationship was reversed under more alkaline conditions. Pigman and Goepp (7) state that sugars exhibit their maximum stability at acid conditions rather than at pH 7. Thus, it might be expected that heat degradation of lactose in milk (pH 6.6) would resemble lactose degradation under weakly alkaline conditions.

The formation of furfuryl alcohol from lactose or maltose but not from glucose, galactose or sucrose suggests that the disaccharide molecule with a 1,4 linkage between the hexose components is necessary in the parent compound. It seems logical, also, to assume that the glucose portion of lactose, having the hemiacetal configuration, would undergo degradation most readily and would, therefore, provide the carbon skeleton for furfuryl alcohol. This contention is supported by the general susceptibility of hemiacetals to chemical reaction and the observed stability of methyl-α-D-glucopyranoside under the experimental conditions employed.

The synthesis of furfuryl alcohol, a 5-carbon compound, from a glucose component containing 6 carbons raises a question as to how the extra carbon is eliminated. Evans *et al.* (1, 2) have theorized that the amount of formic acid produced by alkaline degradation of glucose or galactose is a partial indication of

[2] Ace Glass Co., Inc., Vineland, N. J.

cleavage at the 1,2 position in the hexose. They state further that a pentose residue should be the other resultant of the reaction. Insofar as is known, the demonstration of furfuryl alcohol as a degradation product of lactose constitutes the only direct evidence that such a pentose is formed. The work of Gould (3) has established the fact that formic acid is the principal volatile acid of heated milk. Whittier and Benton (9) have shown that the origin of such acids in heated milk is lactose. It is proposed that formic acid and furfuryl alcohol are related in the same mechanism of lactose degradation. This mechanism may be as follows (fig. 1):

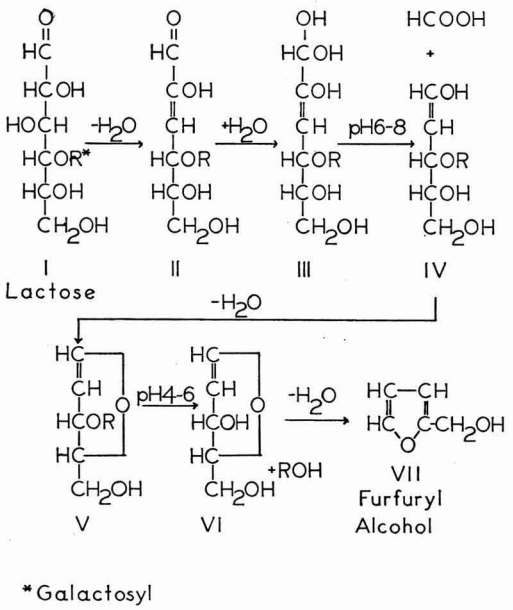

FIG. 1. A proposed mechanism for the chemical conversion of lactose to furfuryl alcohol.

In figure 1, the removal of a molecule of water between the 2,3 positions of lactose (I) is suggested by data from Wolfrom, *et al.* (10) on the degradation of glucose. The resulting enol II or its hydrate III decomposes at pH values above approximately 6 to yield formic acid and the structure IV. The definition of such pH requirements is indicated by the fact that at more acid reactions hydroxymethylfurfural is formed, which compound maintains the 6-carbon chain intact. Ring closure of IV is accomplished by removal of water. At pH values below approximately 6, galactose is hydrolyzed from V. The necessity of this pH condition is indicated by the data in table 2 which shows that a shift of pH from 8.3 to 6.8 accomplished little or no conversion of lactose to furfuryl alcohol. The removal of a final molecule of water from structure VI results in furfuryl alcohol VII.

If the enols II or III in figure 1 did not eliminate formic acid, as might be the case at pH values below 6, the reaction would be somewhat modified. Under these conditions the aldehyde group would remain intact and the end product of the reaction would be hydroxymethylfurfural. This latter reaction scheme is essentially a variation of that presented by Wolfrom *et al.* (10) for the conversion of glucose to hydroxymethylfurfural. An additional alternative may involve hydrolysis of lactose as the first step.

SUMMARY

The mechanism by which furfuryl alcohol and hydroxymethylfurfural are heat-generated in condensed skimmilk and certain lactose systems has been studied. The importance of pH and buffer capacity in the reactions concerned has been demonstrated and discussed. Condensed skimmilk and weakly alkaline lactose systems produced both furfuryl alcohol and hydroxymethylfurfural. Acidified condensed skimmilk and neutral or acidic lactose systems yielded significant quantities of hydroxymethylfurfural but no furfuryl alcohol.

The structure of the lactose molecule or that of a similar sugar, maltose, was shown to be rather specifically required in furfuryl alcohol formation.

A proposed mechanism for the conversion of lactose to furfuryl alcohol has been presented schematically. In this mechanism the production of furfuryl alcohol is related to that of formic acid. It is suggested that a variation of the mechanism accounts for the conversion of lactose to hydroxymethylfurfural.

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CHANGES IN WEIGHT OF THE REPRODUCTIVE ORGANS OF THE DAIRY COW AND THEIR RELATION TO LONG-TIME FEEDING INVESTIGATIONS

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Changes in body weights of dairy cows on long feeding trials involve more than changes in body fat. These weight changes may entail growth, gain or loss of fat, fetal development and alimentary contents. "Pasture Investigations Technique" (1) prepared in 1943, recommended (p. 358) that 3.53 lb. of total digestible nutrients be allowed in computations for each pound of gain and 2.73 lb. per pound of loss in body weight. When, and to what extent these weight changes occur, requires more exact consideration than given previously (1, 5, 11, 12). The Subcommittee on Animal Nutrition of the National Research Council (12) and Morrison (15) recommend requirements for body maintenance, milk production and cows advanced in gestation. The nutrient requirements recommended for cows in advanced gestation are liberal in allowing for body maintenance, milk production, if any, storage of reserve fat and mineral matter for the next lactation and the relatively low requirements for fetal development.

When attempts are made to regulate body weights of cows on long-time investigations by adjusting feed offerings, consideration should be given to that part of total body weight attributable to gestation, namely: changes in the uterus, placenta, embryo, accompanying fluids and slight ovarian changes.

LITERATURE CITED

Eckles (4) attempted to measure nutrients required to develop the bovine fetus and found them too small to determine on a per day basis. He reported 3.9 lb. of dry matter in the amniotic fluid and placenta, while a 75 lb. Jersey calf contained 20.2 lb. of dry matter. He stated: "Four Jersey calves analysed at birth contained an average of 73.09 per cent of water. Data available indicate that breed is not a factor influencing the composition of new-born calves. The amniotic fluid weighs about thirty pounds and contains approximately 95 per cent water. The placenta weighs about 18 pounds, of which approximately 85 per cent is water.

"A Jersey cow produces a total of only fifteen or twenty, and a Holstein twenty or twenty-five pounds of dry matter in the fetus and its accompanying fluid and membranes. . . . The actual energy in the fetus and its accompanying fluid and membranes calculated from the weights and composition was 56.4 therms, a figure surprisingly close to the calculated requirement of 47.4 therms."

Yapp (19) found a maximum of 2.17 per cent of dry matter in the amniotic fluids from nine bovine fetuses and stated: "The highest total solids found for fetus and placenta (combined) was just over 24 per cent and the minimum, 2.56 per cent. Per cent ash varies from 0.82 in the youngest fetuses (41 days) to

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4.14 in the oldest (277 days); ether extract from only a trace to almost 4.0 per cent."

Non-pregnant uteruses analysed 21.7 per cent dry matter and 19.2 per cent protein, as compared with 16.8 and 13.5 per cent, respectively, in pregnant uteruses.

Haecker (6) and Hills (9) determined maintenance requirements of dry barren cows, from which the recommended requirements were liberalized conservatively by the Committee on Animal Nutrition (12) and Morrison (15). It is logical to assume, with Eckles (4) and Yapp (19), that nutrients required for combined development and maintenance of the highly moist bovine reproductive tissues may approach a level with those of the cow's more dense body.

Hayden (8) observed an average increase in body weights of 164 lb. with 426 Jersey cows before calving and a loss of 102 lb. at parturition, not allowing for involution of the uterus. It is logical to assume that a large part of the 62 lb. difference represented storage of body fat which may be available during the subsequent lactation period.

Putnam and Henderson (17) observed with 56 Ayrshires that the gains in body weight were not great before the fifth month of pregnancy. Under their conditions, "from 75 per cent to 85 per cent of the gain came in the last four months of pregnancy." They ascribed part of the weight increases to growth and calculated that this amounted to 27 per cent of the gains during the second gestation, and 20 per cent in the third pregnancy. These animals averaged 59 mo. of age at the third parturition.

With Holstein-Friesians, Moseley *et al.* (16) noted an average change from 1,435 lb. before calving, to 1,280 lb. on the next day, attributing 97 lb. to the calf and 58 lb. to placenta and fluids. This did not account for subsequent involution of the uterus.

Bartlett (2) analysed body weights of 59 milking Shorthorns and observed a 13.75 per cent increase in body weight attributable to pregnancy, based on the farrow weight after parturition. He estimated the loss of weight upon calving to be a 90-lb. calf and 80 lb. of placenta, amniotic fluids, etc.

Morgan and Davis (14) tabulated weight changes due to calving, growth and condition for 656 pregnancies in cows of four dairy breeds. The average decrease of Jersey cows from loss of fetus, membranes and fluids at calving amounted to 7.4 to 9.5 per cent of the cow's weight. Ayrshires, Guernseys and Holstein-Friesians did not vary widely beyond these percentages.

Swett *et al.* (18) analysed data on contents of 113 gravid bovine uteruses (55 from Beltsville and 58 from 13 cooperating state experiment stations). He found average weights of uteruses and contents (including vaginas) to range from 2.3 lb. at 14 days, to 148.5 lb. at 276 days in gestation. "After allowing for the weight of the nonpregnant uterus (3.57 pounds—average for combined breeds) these figures represent the portion of a cow's gains in live weight during pregnancy that are not attributable to changes in her condition (fatness)."

EXPERIMENTAL

As pregnant cows were discarded from the Florida Agricultural Experiment

Station dairy herd, weights were taken of the uteruses, fluids, placentae and fetuses. Non-gravid uteruses were weighed, as a base to compute increases due to pregnancy. Most of these cows were slaughtered and reproductive organs severed from the vagina posterior to the os uterus. The gravid uterus and ovaries were separated and weighed. Age of each fetus was computed from the date of service, even though Miller and associates (13) pointed out that conception may occur 1 to 3 days after service. Relation of length of gestation period to birth weights of Jersey calves in the station herd was determined.

Weights of 37 Jerseys, one Guernsey-Jersey, and one Guernsey embryo, together with weights of the placentae, fluids and uteruses, supply data applicable in interpreting weight changes of Jerseys. The combined weight of the ovaries and corpus luteum was between 10.7 and 22.5 g. and did not exceed weights of ovaries from non-pregnant cows. They are included in weights of "total uterus and contents."

The average weight of eight non-gravid uteruses was 1.4 lb. (see table 1). Gravid uteruses weighed up to 12.9 lb. at 7.5 to 8 mo. in gestation. About a 10 per cent increase in weight was observed beyond this time by Hammond (7), Bergmann (3) and one Florida observation.

TABLE 1

Weights of non-gravid uteruses from dairy cows

Cow	Breed	Age	No. of gestations	Interval since calving	Net uterus[b]
		(*yr. mo. d.*)		(*d.*)	(*lb.*)
123-F	Jersey	4–7–19	barren	0	0.8
695-UF	Jersey	5–8–22	barren[a]	0	1.8
954-UF	Jersey	7–5–21	4	2	13.3[c]
950-UF	Jersey	6–10–0	4	29	1.25[d]
806-UF	Jersey	8–11–21	6	82	1.8[e]
95-F	Jersey	3–8–7	2	98	0.95
601-UF	Jersey	12–0–11	9	104	1.85
107-F	Guernsey	4–2–28	2	289	1.6
906-UF	Jersey	7–6–16	5	505	1.17

[a] Used previously, as an uncertain breeder, in stilbestrol investigation.
[b] Uterus severed at the os uterus, and including the latter.
[c] Died within 54–58 hr. of milk fever and uterine complications after calving. This weight was not used in computations.
[d] In 950-UF, locations of 80 cotyledons were visible on inner uterine wall.
[e] Estrus occurred on day previous to slaughter; uterus was congested. Eight non-gravid uteruses average 1.4 lb. The separated vagina of 695-UF weighed 1.45 lb.; vulva 0.55 lb.

The chorion and amnion were not separated in most instances, consequently only total moist weights of fetal membranes (placenta) are given. Their increase during gestation was up to 7.6 lb. at 278 days. Eckles (4) observed weights of 10.5 to 18.5 (average of 15.8) lb. for three placentae from Jerseys at full term.

The amounts of fluids contained in the fetal membranes varied widely with individuals, being approximately 1.2 lb. or less prior to 2.5 mo. in gestation. A rapid gain occurred in the next 2 mo., with only a slight further increase until the last month of gestation. In a single instance, Eckles determined a total embryonic fluid weight of 32.7 lb. at nearly full term upon slaughter of the cow.

One Florida Jersey had 56 lb. of fetal fluids at 278 days. Hammond (7) commented concerning embryonic fluids as follows: "Whether the cessation of the increase in foetal fluids at the 5th month of pregnancy is connected in any way with the definite changes which occur in the udder at this time (in Shorthorn heifers) it is not possible to say without experimental evidence; but . . . an actual absorption of foetal fluids into the maternal circulation might supply a cause."

Individual Jersey fetuses varied in weight as birth weights of full-term calves also vary. These records (table 2) showed that a Jersey fetus attained a weight of about 1.0 lb. at about 3.5 mo. in gestation, that it weighed about 12 lb. at 6 mo. and that growth increases were rapid thereafter. Fetuses weighed over 40 lb. at 8 mo. in gestation. Average birth weights of 759 Jersey calves in the

TABLE 2

Changes in weights of uterus and contents during gestation

Age of fetus	Age of dam	Uterus and contents	Fetus	Sex	Fetal fluids	Fetal membranes	Empty uterus
(*d.*)	(*yr.*)	(*lb.*)					(*lb.*)
31	14	4.3[a]	0.27 g.	?	3.32 g.	2.30 g.	4.3[a]
31	5	1.11	0.28	?	20.48	3.14	1.05
34	3	2.3[a]	0.47	?	.....	6.42	2.0[a]
35	6	1.47	0.51	?	62.97	5.72	1.31
36	3	2.2[a]	0.60	?	28.8	3.11	1.48[a]
39	4	1.5	1.01	?	.....	15.3	.....
44	6	1.09	1.70	M	63.67	17.71	0.85
44	5	1.27	1.72	M	87.50	10.20	1.06
56	7	6.1[a]	10.64	M	453.0	70.0	4.92[a]
59	3	2.61	11.94	M	419.28	125.0	1.23
72	9	3.8	43.74	M	720.5	142.0	1.75
74	6	3.1	44.0	F	546.0	101.0	1.5
77	5	2.6	56.66	M	707.0	100.0	0.69
89	6	4.7	172.5	M	1023.0	202.85	1.6
93	6	6.4	0.5 lb.	M	3.13 lb.	0.64 lb.	2.13
100	3	7.0	0.62	F	3.2	0.84	2.4
109	7	10.0	1.08	M	4.86	0.76	3.3
113[b]	5	12.3	1.06	F	5.2	1.05	2.0
121	2	14.95	2.9	M	8.2	1.05	2.85
127	6	16.8	1.96	F	9.59	1.4	3.85
127	7	18.8	2.18	M	11.07	1.65	3.9
131	3	21.0[a]	2.45	F	11.2	1.65	4.9[a]
138	4	20.6	3.7	M	11.0	1.7	4.1
148	5	26.5	4.0	F	15.0	2.25	5.0
151	8	23.85	4.4	F	12.15	1.9	5.55
155	4	19.6	6.2	F	7.1	1.8	4.5
158	4	32.0[a]	7.4	M	14.0	2.3	8.3[a]
166	4	26.7	8.9	M	7.75	4.05	6.0
177	4	31.4	10.1	F	11.1	3.2	7.1
180	5	36.0[a]	12.0	M	10.15	4.3	9.55[a]
186	9	26.9	15.0	M	6.1	2.13	5.6
204	6	41.6	19.1	F	9.05	3.9	9.3
228	4	62.5	30.5	F	13.0	5.5	13.5
231	4	64.85	36.0	M	12.15	5.35	11.35
235	7	58.6	28.0	M	14.3	5.0	11.3
236	3	71.5	42.0	F	10.9	6.0	12.6
236	3	75.45	45.0	M	14.5	..... (15.95)	.....
245	3	77.6	41.0	M	17.1	6.6	12.9
278	3	140.0	61.45	M	56.35	7.6	14.6

[a] Combined weight, including the vagina.
[b] Guernsey.

Florida station herd over an 18-yr. period were determined. The 392 males ranged between 27 and 80 lb., averaging 55 lb., whereas 367 heifer calves ranged from 27 to 76 lb., the average being 52 lb. They tended to weigh less following short gestation periods.

Total weights of 39 uteruses and contents (all but seven weights excluded the vagina) ranged between that of non-gravid uteruses (1.4 lb.) and 140 lb. at 278 days in gestation. The latter contained a 61-lb. male fetus.

Increases of body weights due to gestation are calculated in table 3. Naturally, there would be no allowance or gestation in the case of an unbred cow. Also, the increase in weight of the uterus and contents during the first 60 days of gestation is negligible. The weight increase is estimated to amount to 5 lb. at 90 days in gestation, and increases to about 122 lb. at full term. This includes weight changes due to the fetus, placenta, fluids and involution of the uterus within 2 wk. after calving.

At parturition the uterus of the full-term cow weighed 14.6 lb., whereas involution reduced the non-gravid uteruses to an average of 1.4 lb. Applying Yapp's percentages for dry matter and protein to these weights leaves an unaccounted reduction of uterine tissue by 2.15 lb. of dry matter (1.59 lb. of protein).

The period immediately following calving is one of physiological underfeeding, ordinarily. It is presumed that this small nutrient difference may leave the cow's body during involution over a period of several days, whereas the fetus, placenta and fluid leave the cow's body shortly.

The 280-day gestation-weight estimate was combined from (a) average birth weight of 392 male Jersey calves in Florida, (b) three placentae reported by Eckles (4), (c) the difference between the combined weights of placentae and fluids (14) and the three placentae (4), and (d) the net empty uterus weight with the 278-day Jersey male fetus.

Morrison's maintenance standard (15) is regarded as sufficiently liberal to

TABLE 3

Weight estimates of uteruses and contents and suggested allowances in body weight for stage of gestation with Jerseys

Age of fetus	Reproductive organs and contents	Fetus	Membranes	Fluids	Net empty uterus	Weight increase due to gestation
(*d.*)	(*lb.*)				(*lb.*)	(*lb.*)
0	1.4	0	0	0	1.4	0
30	2.5	0.25 g.	3.1 g.	11.2 g.	2.4	negligible
60	2.6	12.0 g.	125.0 g.	0.9 lb.	1.4	1
90	6.4	0.5 lb.	0.6 lb.	3.1 lb.	2.2	5
120	14.0	1.7 lb.	1.0 lb.	8.1 lb.	3.2	12
150	23.0	4.3 lb.	1.9 lb.	11.5 lb.	5.3	22
180	32.0	11.1 lb.	3.2 lb.	10.6 lb.	7.1	31
210	44.5	19.5 lb.	4.4 lb.	11.1 lb.	9.5	43
240	76.0	40.0 lb.	6.3 lb.	16.7 lb.	13.0	75
270	111.7	51.3 lb.	13.5 lb.	32.6 lb.	14.2	110
280 ±	123.6	55.0 lb.	15.8 lb.	38.2 lb.	14.6[a]	122[a]

[a] The immediate drop in body weight at calving in this instance would be 109 lb., with an expected further reduction of 13.2 lb. (14.6–1.4) within 2 wk. due to involution of the uterus.

provide for actual maintenance of the cow's net body, as well as for reproduction. Greater increases than indicated in table 3 for gestation would be considered gains in actual body weight. Less than those amounts would be regarded as weight losses. They would be computed at 3.53 and 2.73 lb. of total digestible nutrients, respectively, and either credited or debited to the nutrients involved in respective total digestible nutrients computations.

Two methods of computing nutrients involved in analyses of feeding results were compared. Since no appreciable change occurred due to pregnancy during the first 60 days of gestation, the period of weight corrections would be the remaining 220 days of the gestation period. An 800-lb. cow will be used to illustrate application of weight corrections for gestation.

If maintenance requirements were computed daily by the Morrison standard on the basis of gross body weights (800 to 922 lb.), the accumulative total would amount to 1,551.97 lb. of total digestible nutrients. This does not evaluate the 122 lb. at the rate of 3.53 lb. of total digestible nutrients per pound of gain.

On the other hand, if maintenance needs had been computed in the same manner, and gains credited at 3.53 lb. of total digestible nutrients per pound for the 122-lb. gain due to pregnancy, the computation would have made it appear that an additional 430.66 lb. of total digestible nutrients had been received from the feed. This entails above a 27 per cent error from an over-evaluation. Converted to practical terms, 430 lb. of total digestible nutrients in 220 days would equal about 860 lb. of hay, or 3,071 lb. of fresh Napier grass leaves (10) or similar pasture grass. This error is appreciable either in technical terms or in practical interpretation of research results.

In view of the above, it is suggested that corrections for stage of gestation listed in table 3 be allowed before applying the factors of 3.53 and 2.73 to the gains or losses, respectively, in gross body weights of pregnant Jersey cows in long feeding trials. Corresponding factors need to be developed for other dairy breeds. Any attempts to regulate body weights by limiting feed allowances, should make due allowance for advancing stage of gestation during extended feeding trials.

SUMMARY AND CONCLUSIONS

Weights of nine non-gravid and 39 gravid uteruses and contents are presented, based on dairy cows slaughtered in the station herd. Significant changes in gross body weight occurred between the 61st day of gestation and full term. Division of these weight changes was approximated for Jersey cows at monthly intervals until ready to calve.

Using an 800-lb. cow as an example, computation of total digestible nutrient requirements by Morrison's standard, with 122-lb. gains evaluated at 3.53 lb. of total digestible nutrients per pound of gain, would amount to 1,983 lb. of total digestible nutrients. On the other hand, if growth and maintenance of the more highly moist fetus and associated tissues were attained within the liberal Morrison standard, an excess of over 430 lb. of total digestible nutrients would have been computed—a difference of over 27 per cent of maintenance would have been incurred over the last 220 days of gestation.

It is suggested that gross body weights be computed to net weights (less gains due to gestation) when trying to control body weights by regulating the level of concentrates fed during long feeding trials.

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A COMPARISON OF THE ALLEN VOLUMETRIC BLOOD FAT PROCEDURE WITH AN EXTRACTION PROCEDURE[1, 2]

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The method proposed by Allen (1, 2) for the determination of fat in blood plasma is easily and quickly carried out and permits the rapid determination of large numbers of samples. This method has been used to advantage in several studies (2, 3, 9, 10), but unfortunately the method has not been subjected to a critical study. Allen (2) made a comparison between Bloor's method for total fat and his own method on three samples of plasma and obtained values by his method, which were 36.8, 68.9 and 70.9 per cent of the total lipids obtained by Bloor's procedure. Based on the work of Petersen and Herreid (4) who developed the reagent for the determination of fat in buttermilk, it was concluded that the difference was due to the absence of phospholipids in the fat separated from plasma by the reagent.

No actual fractionations of this lipid column or comparisons with different plasma lipid fractions have been reported. Such comparisons are presented in this report.

EXPERIMENTAL PROCEDURE

Four cows, two Guernseys and two Holsteins, were used for this study. Blood samples were taken from the jugular vein at weekly intervals. Potassium oxalate was used for an anti-coagulant and NaF was used as a preservative. The extractions were made immediately after centrifuging and the same samples were analyzed by the modified Allen procedure the following day.

Three different approaches were used in this investigation. First, the composition of the Allen fat column was determined. Secondly, the fractionations made on the Allen fat column were compared to the values obtained by the direct extraction of the same plasma. Lastly, a statistical study was made of the relationships between the Allen lipid values and the total plasma lipids and lipid fractions. Twenty-seven samples were analyzed for this part of the study.

A modification of the Allen volumetric procedure used for routine analysis in this laboratory was used in this study. For greater accuracy, the length of the fat column was measured through a glass window in a constant temperature bath by means of a reading microscope mounted on a micrometer slide as reported by Shaw (8). In addition, precision-bore capillary tubes with a diameter of 1.016 mm. were used in making the fat tubes so that the calibration factor was

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practically identical for all tubes. An additional small, but very helpful, improvement was made in the course of this study. Perhaps the most troublesome part of this procedure has been the necessity of inserting a small wooden plug in the top of the capillary tube before placing the tube in the water bath. This frequently resulted in the moving and breaking of the fat column so that this reading became less accurate. This was solved by not flaring the top of the capillary tube and placing a small piece of adhesive tape over the end of the tube instead of inserting wood plugs.

For the extraction and fractionation of the plasma lipids, Saarinen's method was used (5, 6, 7). Of the two alternatives proposed in the latter paper for the extraction of the total lipids, the more accurate extraction with alcohol-ether (2:1) was used in this study. The total lipids were saponified with a saturated aqueous solution of NaOH using reflux condensers. The phospholipid fatty acids values determined by the difference between the total lipids and the lipids other than phospholipids were converted to total phospholipid values, using the factor 1.44.

TABLE 1

The percentage composition of the Allen fat column

Sample no.[a]	Total cholesterol	Ester cholesterol	Free cholesterol	Fatty acids in cholesterol esters	Neutral fat	Phospholipids
	(%)	(%)	(%)	(%)	(%)	(%)
1	54.6	47.0	7.5	30.5	11.5	3.4
2	59.5	51.4	8.1	31.8	4.0	4.6
5	55.6	46.0	15.6	30.6	12.8	1.0
9	56.1	46.8	10.7	31.0	12.4	0.4
10	55.8	44.3	11.5	29.4	14.6	0.2
Av.	56.3	47.1	10.8	30.7	11.1	1.9

[a] No. 1 and 2 represent mixed plasma samples, and no. 5, 9, and 10, single cows.

RESULTS

The percentage composition of the fat column obtained by the volumetric method was determined on two mixed plasma samples and on individual samples from three other cows. The results are presented in table 1. In this case, the phospholipids were calculated on the basis of the total phosphorus in the purified fat. These data show that the fat column contained small but variable amounts of phospholipids. The main part of the lipids consisted of cholesterol esters, free cholesterol and neutral fat. The proportions of these fractions varied within the normal range, which indicated that most of the lipids were liberated by the volumetric procedure. The uniform composition of the different fat samples undoubtedly is due to the fact that the cows were fed similarly.

For purposes of comparison, four of the above plasma samples also were subjected to the complete fractionation procedure of Saarinen. The values obtained in the analysis of the lipids separated from these same samples by the Allen procedure were calculated back to the actual level in the blood plasma on the basis of the Allen total lipid value. The results of this comparison are shown in table 2. The data show that the lipid fraction consisting of cholesterol and cholesterol

TABLE 2

A comparison of lipid fractions (Mg./100 ml. plasma) separated from plasma by the Allen and Saarinen procedures

	Sample 1		Sample 5		Sample 9		Sample 10		Average	
	A[a]	S[b]	A	S	A	S	A	S	A	S
Total cholesterol (*mg./100 ml.*)	116.9	152.7	94.0	99.4	119.4	128.2	130.3	129.1	115.2	127.4
Ester cholesterol (*mg./100 ml.*)	100.6	118.3	77.7	72.9	99.6	105.1	103.4	110.2	95.4	101.6
Free cholesterol (*mg./100 ml.*)	16.3	34.4	26.3	16.5	19.8	23.1	26.9	18.9	19.8	23.2
Fatty acids in cholesterol esters (*mg./100 ml.*)	65.3	65.6	51.7	48.4	66.0	69.8	68.6	73.2	62.9	64.3
Neutral fat (*mg./100 ml.*)	24.6	0.8	21.6	43.3	26.5	28.5	34.1	29.3	26.7	27.3
Phospholipids (*mg./100 ml.*)	7.3	.....	16.9	.....	12.8	.....	0.5	.....	10.5	.....

[a] A = Allen procedure
[b] S = Saarinen procedure

and glycerol esters was removed almost as completely by the Allen volumetric procedure as by the Saarinen extraction procedure. In addition to the above lipid fractions, the Allen procedure included small amounts of phospholipids.

A summary of the data on 27 samples which were subjected to the Allen procedure and simultaneously fractionated more completely by the Saarinen procedure is presented in table 3. The mean value obtained by the Allen procedure was 63.3 $\pm$ 1.17 per cent of the total lipids determined by the extraction procedure. The Allen lipid value was very similar to the value obtained for the lipids other than the phospholipids. The latter fraction averaged 83.1 $\pm$ 1.41 per cent of the value obtained by Allen's procedure. This indicates that some lipids of phospholipid origin were included in the Allen fat column.

It will be noticed that rather high correlations were obtained between the Allen value and total lipids, lipids other than phospholipids and total cholesterol. Since only small amounts of phospholipids are separated by the Allen procedure,

TABLE 3

The average (M) of different lipid fractions with standard errors (m_M) and the correlations of other fractions to Allen's lipid values (n=27)

Lipid fraction	Plasma lipids $M \pm m_M$	Correlation to Allen's lipid value $r \pm m_r$
	(*mg./100 ml.*)	
Allen's lipid value	258.1 $\pm$ 10.3	.....
Total lipids	530.9 $\pm$ 17.4	0.866 $\pm$ 0.048
Phospholipids	301.7 $\pm$ 14.6	0.667 $\pm$ 0.107
Total cholesterol	127.8 $\pm$ 5.3	0.846 $\pm$ 0.055
Ester cholesterol	110.9 $\pm$ 5.1	0.803 $\pm$ 0.068
Free cholesterol	16.9 $\pm$ 1.6	0.346 $\pm$ 0.169
Total lipids minus p-lipids	229.2 $\pm$ 7.0	0.868 $\pm$ 0.047
Fatty acids in cholesterol and glycerol ester fraction	101.4 $\pm$ 3.7	0.403 $\pm$ 0.161

it is probable that the high correlation between the Allen value and the total lipids is accidental due to the limited data. Additional data are needed to establish these relationships more precisely.

CONCLUSIONS

The fractionation of the Allen lipid column showed that only small amounts of phospholipids were present. A comparison of these fractions with fractions obtained by extraction of the same plasma gave similar values, indicating that the lipids other than phospholipids are separated rather completely by the Allen procedure. The lipids other than phospholipids were 83.1 $\pm$ 1.41 per cent of the Allen lipid value. The latter was 63.3 $\pm$ 1.17 per cent of the total plasma lipids. The correlation between the Allen lipid value and the value for plasma lipids other than phospholipids was high ($r = 0.866 \pm 0.047$).

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THE VALIDITY OF THE ALLEN VOLUMETRIC PROCEDURE FOR THE DETERMINATION OF BLOOD LIPIDS OF COWS ON DIFFERENT FEEDING REGIMES[1, 2]

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In a previous paper by Chung *et al.* (1), a comparison was made between a modified Allen blood fat procedure and a fractionation procedure based on the extraction of blood lipids. This study indicated that the Allen procedure was satisfactory under normal conditions for the determination of the plasma lipid fraction composed of the lipids other than phospholipids. However, it was deemed advisable to obtain additional data to establish the relationships more specifically and especially to determine the value of the procedure under varying feeding regimes. This report deals with the validity of a modification of the Allen volumetric method for the determination of blood lipids of cows on different levels of energy and fat intake.

EXPERIMENTAL PROCEDURE

For this study, blood samples were drawn from cows which had been fed on different levels of fat and energy intake in connection with another project which was under way simultaneously. These rations resulted in marked variations in the plasma lipids. A group of 12 cows, seven Holsteins, two Guernseys and three Ayrshires, was used for this study. Two to three blood samples were drawn from each cow 14 to 18 days after each change in feeding. The blood was drawn from either the coccygeal artery by a method developed by Saarinen (2) or from the jugular vein. A total of 155 blood samples was analyzed.

All blood samples for this experiment were drawn between 8 and 10 a. m. during the last part of each period. Potassium oxalate was used as an anticoagulant and about 0.1 per cent of NaF was used as a preservative. The plasma was analyzed immediately after centrifuging, except in one case when the blood samples were stored in a refrigerator 1 day.

The analytical methods were the same as those used in the previous paper (1), except that the total lipids were not saponified in the first set of samples. Consequently, the phospholipids were determined directly as true phospholipids.

RESULTS

During the various test periods the plasma total lipids varied from 213.3 to 623.3 mg. per cent. The plasma phospholipids paralleled the total lipids closely

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TABLE 1

The relationships between the Allen volumetric plasma lipid value and different plasma lipid fractions obtained by Saarinen's procedure

Lipid fraction	$M \pm m_M$	Correlations to Allen lipid value $r \pm m_r$
	(mg./100 ml.)	
Allen lipid value	220.4 ± 4.3	
Total lipids	375.4 ± 6.7	0.663 ± 0.045
Phospholipids	152.2 ± 5.0	0.109 ± 0.079
Total cholesterol	151.5 ± 2.7	0.766 ± 0.033
Ester cholesterol	103.9 ± 2.1	0.750 ± 0.035
Free cholesterol	47.6 ± 1.4	0.382 ± 0.069
Total lipids minus phospholipids	223.2 ± 3.5	0.836 ± 0.024
Fatty acids in the cholesterol glycerol ester fraction	71.7 ± 1.7	0.550 ± 0.056

as has been observed previously by Saarinen (3). The other lipid fractions, including the volumetric value, varied more independently of the total lipid values. The mean volumetric value was 58.6 per cent of the mean total lipid value and 98.5 per cent of the value for lipids other than phospholipids.

The correlations to the volumetric lipid value were calculated separately for each plasma lipid fraction. The coefficients of correlations along with the means and standard errors are shown in table 1. Not only was the mean value obtained by the volumetric procedure (219.81 ± 4.29) very similar to the mean value for the lipid fraction other than phospholipids (223.19 ± 3.53), but the coefficient of correlation between these two values was relatively high (0.836 ± 0.024). The coefficient of correlation between the volumetric value and the plasma phospholipids was very low (0.109 ± 0.079). This was to be expected due to the large variations in plasma lipids produced by variations in feeding. The other correlations are about what would be expected on the basis of the percentage representation of these fractions in the lipids other than phospholipids.

These data establish the fact that the volumetric procedure is fairly well adapted to the determination of lipids other than phospholipids under markedly different feeding conditions.

TABLE 2

Correlation between true total lipids minus the Allen lipid value and the Saarinen phospholipid fraction

	Present data (n – 155)		Chung's data (n – 27)	
	$M \pm m_M$	Correlation to p-lipids obtained by Saarinen method $r \pm m_r$	$M \pm m_M$	Correlation to p-lipids obtained by Saarinen method $r \pm m_r$
	(mg./100 ml.)		*(mg./100 ml.)*	
Phospholipids obtained by the Saarinen method	152.2 ± 5.0	.....	301.7 ± 14.6	.....
Total lipids minus the Allen lipid value	155.0 ± 5.6	0.905 ± 0.015	272.8 ± 10.9	0.780 ± 0.181

The above fact indicated that it would be possible to determine the plasma phospholipids by the difference between the total lipids and the Allen volumetric lipid value. To test this hypothesis, coefficients of correlations between the true phospholipid fraction and the total lipids minus the volumetric lipid value were calculated from both the present data and from the data by Chung *et al.* (1). These results, along with the means and standard errors, are presented in table 2. The coefficients of correlation were 0.905 ± 0.015 and 0.817 ± 0.065, respectively, which suggests that this procedure may be followed in estimating plasma phospholipid values.

SUMMARY AND CONCLUSIONS

Determinations of blood plasma lipids were made on 155 blood samples from 12 cows on markedly different feeding regimes. All of the samples were subjected to analysis by an extraction procedure and by a modification of the Allen volumetric procedure.

A relatively high coefficient of correlation (0.836 ± 0.024) was found between the mean volumetric value and the lipid fraction consisting of the total lipids other than phospholipids, which shows that the former method is fairly reliable for the determination of this lipid fraction.

On 155 samples a high coefficient of correlation (0.905 ± 0.015) between the true phospholipid value and the difference between the total lipids and the Allen volumetric lipid value suggests that phospholipids can be estimated fairly well merely by determining the volumetric lipid value and the total lipid value.

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USE OF PROPYL GALLATE TO DEFER DEVELOPMENT OF OXIDIZED FLAVOR IN MARKET MILK[1]

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One of the most difficult off-flavors to control in market milk is the oxidized flavor which may develop after a few days storage. This off-flavor is especially troublesome during the winter period.

Chilson *et al.* (2) and others (1, 4) have shown that the addition of ascorbic acid to market milk will defer the development of oxidized flavor for several days, but when the ascorbic acid is depleted, the off-flavor develops. Ascorbic acid is depleted quickly in the presence of added copper, and a more pronounced oxidized flavor develops than would have developed had not ascorbic acid been added (2). Such uncertain control measures may be somewhat unsatisfactory in many commercial milk plants. If a simple and practical treatment could be devised that would eliminate oxidized flavor for at least 1 wk. of storage, such a treatment would be accepted enthusiastically by milk processors.

Propyl gallate has been recommended as an antioxidant to be used in butter for candy manufacture (5). It was effective in controlling oxidized flavor in dried whole milk and dried ice cream mix (6). Propyl gallate is an approved ingredient in stabilized lard and lard and vegetable fat mixtures, the maximum content allowed being 0.01 per cent (5).

The research herein reported was undertaken to determine the effectiveness of propyl gallate in controlling the development of oxidized flavor in market milk.

METHODS

Six trials were conducted, each consisting of split samples with the following treatment: 1. Control milk; 2. control milk plus 20 mg. of propyl gallate per liter of milk; 3. control milk plus 20 mg. propyl gallate per liter with 0.5 ppm. of added copper; 4. control milk plus 30 mg. of ascorbic acid per liter and 0.5 ppm. of added copper; 5. control milk plus 0.5 ppm. of added copper; 6. control milk plus 20 mg. of propyl gallate per liter plus 30 mg. of ascorbic acid per liter plus 0.5 ppm. of added copper. The 500-ml. samples used in each trial were taken from freshly pasteurized milk (holder process) from the college herd. Treatments were begun immediately after the samples were obtained. The propyl gallate was dissolved in glycerine before being added to the milk. The copper was added as an aqueous solution of $CuSO_4$. The ascorbic acid was added as a powder and thoroughly mixed with the milk by shaking. The samples were placed in quart milk bottles and then stored in a cabinet refrigerator at approximately 35° F. All samples were examined for the presence of oxidized flavor when fresh and after 1, 2, 3, 5, 7 and 14 days of storage. Samples were scored 40

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points if no oxidized flavor was evident. A score of 30 was used to denote a very pronounced oxidized flavor. Thus, scores from 40 to 30 denote the intensity of the off-flavor. Selected samples were analyzed chemically for ascorbic acid content and the oxidation-reduction potential determined.

RESULTS

Since the flavor scores on corresponding samples of the six trials were nearly identical, varying only slightly in the intensity of the oxidized flavor on a particular day, the results are shown as average scores in table 1. After one day of

TABLE 1

The effect of propyl gallate upon the development of oxidized flavor in market milk (average of 6 trials)

Treatment	Days stored						
	Fresh	1	2	3	5	7	14
	Average flavor scores[a]						
Control—none	40	39.0	37.6	36.0	33.5	32.3	30
Control plus 20 mg. propyl gallate/l.	40	40	40	40	40	40	40
Control plus 20 mg. propyl gallate/l. plus 0.5 ppm. of Cu.	40	40	40	40	40	40	40
Control plus 30 mg. ascorbic acid/l. plus 0.5 ppm. of Cu.	40	35	33	30	30	30	30
Control plus 0.5 ppm. of Cu.	40	36	33.3	33	31.7	31.3	30
Control plus 0.5 ppm. of Cu. plus 30 mg. ascorbic acid plus 20 mg. propyl gallate/l.	40	40	40	40	40	40	40

[a] The milk used usually had a slight cooked or feed flavor, but a score of 40 was used to designate the absence of an oxidized flavor at the beginning of the trial. Scores from 40 to 30 indicate the degree of oxidized flavor.

storage, two of the six control samples were slightly oxidized, giving an average score of 39 for the six control samples. After 2 days in storage four of the control samples were oxidized, and by the fifth day all were distinctly oxidized, having an average score of 33.5.

The samples that contained added ascorbic acid and copper usually developed the most pronounced oxidized flavor in the shortest storage time, while those containing only copper were second. The control samples developed the least intense oxidized flavor of the samples not protected with propyl gallate.

All 18 samples that did not contain propyl gallate developed a definite oxidized flavor. Those containing propyl gallate with or without added copper developed no oxidized flavor within 14 days. The oxidized flavor was scored according to its intensity and objectionableness, regardless of the type. The control samples seemed to have more of a cardboardy or papery flavor, while those containing added copper were usually fishy, or tallowy and fishy, and those samples containing added ascorbic acid and copper usually were tallowy, or cardboardy and tallowy.

In four of the six trials, addition of propyl gallate to the control milk lowered the oxidation reduction potential an average of 0.059 volts. The initial potentials were from + 0.165 to + 0.280 volts. The greater reductions usually occurred on

samples having the higher initial potential. This is in agreement with the results of other investigations on oxidized flavor, which have demonstrated that a substance or process which controls or retards oxidized flavor also reduces the oxidation-reduction potential, at least temporarily (4).

Analyses of six fresh and four stored samples for ascorbic acid content showed that the addition of propyl gallate had practically no effect on the oxidation of ascorbic acid. The amount of ascorbic acid in five fresh samples to which propyl gallate was added was the same as the amount in the control samples. One fresh sample containing propyl gallate had 3 mg. or 17 per cent less ascorbic acid than the control milk. Analyses of three stored samples containing propyl gallate showed that they contained the same amount of ascorbic acid as the corresponding controls, and one stored sample containing propyl gallate showed a loss of 1.3 mg. or 33 per cent, as compared to the control.

DISCUSSION

Greenbank (3) and others (4) have shown a relationship between the oxidation-reduction potential and the development of oxidized flavor in milk. At least a part of the flavor-protective action of added ascorbic acid has been attributed to the fact that the oxidation-reduction potential is lowered. Since propyl gallate does not stabilize ascorbic acid, yet prevents the development of the oxidized flavor in milk even in the presence of added copper, it would seem that there is a difference in the nature of the protective actions afforded by these two substances. More information regarding the mechanisms involved in the protective actions of these substances would be interesting, but is beyond the scope of this paper.

It was intended that this study should include work to ascertain the minimum amount of propyl gallate that would protect milk from oxidation for a storage period of 14 days. However, by the time this phase was started, the college herd had been on spring pasture for several weeks and the milk no longer developed an oxidized flavor spontaneously, and even with added copper only a mild oxidized flavor developed after several days' storage.

Twenty milligrams of propyl gallate per liter was selected as the amount to use for this experiment, based on the amount used in dry whole milk by research workers of the Quartermaster Food Institute (6). A few limited trials indicated that 10 mg. of propyl gallate per liter of milk were effective in protecting against oxidized flavor development for a period of 7 days, and as little as 1.25 mg. per liter gave some protection. It seems reasonable to believe that substantially less than 20 mg. per liter should give ample protection to milk stored and distributed under normal commercial conditions.

It should be emphasized that propyl gallate may be classed as a drug. It is not a food product. Therefore, the addition of this product to milk or milk products for other than experimental purposes might bring prosecution, if not approved by enforcement officials.

CONCLUSION

The addition of propyl gallate to freshly pasteurized milk at the rate of 20

mg. per liter was found to prevent the development of oxidized flavor effectively for a 14-day storage period at 35° F. The propyl gallate treatment was equally effective with or without 0.5 ppm. of added copper.

The ascorbic acid, natural or added, was not stabilized by the propyl gallate.

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WHITE MUTANTS OF PENICILLIUM ROQUEFORTI[1]

S. G. KNIGHT, W. H. MOHR AND W. C. FRAZIER

Department of Agricultural Bacteriology, University of Wisconsin, Madison

Induced mutation of microorganisms has become a useful tool in microbiological research. Lederberg (3) cites many instances where studies of mutants have contributed to our knowledge of microbial genetics and physiology. From a practical point of view, Backus *et al.* (1) have produced by ultraviolet irradiation mutants of *Penicillium chrysogenum* that yield more penicillin than the parent. This is a preliminary report of a series of studies on normal and mutant strains of *Penicillium roqueforti.* These studies were undertaken for the purpose of obtaining information about the genetics and physiology of *P. roqueforti* in the hope that such information might be of value in the manufacture of mold-ripened cheese.

METHODS

Two molds designated as *P. roqueforti,* strains 1 and 2, were obtained from the Division of Dairy Husbandry of the University of Minnesota. These strains, as well as the mutants therefrom, were grown on a medium made by mixing equal parts of sterile "V8" vegetable juice and sterile 6 per cent agar.

Mutants were obtained by ultraviolet irradiation of spores that had been spread on the surface of vegetable juice agar in a petri dish. The petri dishes were placed 18 cm. from a Westinghouse "Sterilamp," and the inoculated surface of the medium was irradiated for about 20 min., a treatment which killed almost all of the spores on the medium. After irradiation the plates were incubated at 25° C. Many of the molds that grew after incubation of the plates were mutants, but only the mutants that produced white spores were picked for further study. Spontaneous mutation of the green molds to white molds never was observed.

To study the lipolytic and proteolytic activity, the parent and mutant strains were grown on sterile milk that had been adjusted to 8 per cent butterfat. Lipolytic activity was estimated by the amount of volatile, water-soluble acid produced from 100 ml. of milk fortified with fat. Proteolytic activity was estimated from the amino nitrogen produced, as assayed by the Van Slyke procedure.

RESULTS

After ultraviolet irradiation many of the surviving spores formed colonies of molds that unquestionably were mutants. The mutants most frequently found were slow-growing types. Mutants that were nonsporulating or had oddly formed hyphae were observed occasionally. Mutants that formed white spores

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[1] This research was supported in part by funds supplied under the Federal Research and Marketing Act of 1946, and is published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

but otherwise were normal were uncommon. Nevertheless, ten stable white-spore mutants were obtained and four of them were used in this study. White mutants 1–5 and 1–10 were obtained from *P. roqueforti,* strain 1, and white mutants

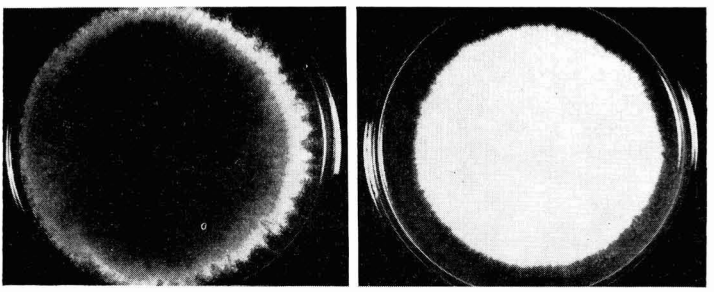

FIG. 1. Normal *P. roqueforti* (strain 1) and a typical white mutant (strain 1–10).

2–1 and 2–10 were obtained from strain 2. Figure 1 is a photograph of a normal *P. roqueforti* and a typical white mutant. The white mutants apparently are stable, since all attempts to induce reversion to green spores have been unsuccessful. Furthermore, when mixtures of various combinations and proportions of spores from ten white mutants were mixed and plated in an attempt to produce colored heterocaryons, only colonies with white spores were formed.

TABLE 1

The ml. of N/0.05 KOH necessary to titrate the soluble volatile acid produced by the lipolytic activity of the normal and white P. roqueforti on 100 ml. milk plus 8% butterfat at 25° C

Strain	ml. of N/0.05 KOH (*days of incubation*)		
	5	9	15
green (1)	6.1	6.7	8.5
white (1–10)	6.3	7.3	17.2
white (1–5)	5.3	6.0	8.2
green (2)	6.5	7.4	11.7
white (2–10)	7.8	11.5	17.5
white (2–1)	6.8	8.0	10.3

TABLE 2

The amino-N produced by the proteolytic activity of normal and white P. roqueforti on milk plus 8% butterfat at 25° C

Strain	mg. of amino-N/ml. (*days of incubation*)		
	5	9	15
green (1)	0.57	1.05	1.22
white (1–10)	0.46	1.05	1.28
white (1–5)	0.45	1.01	1.25
green (2)	0.45	0.86	1.08
white (2–10)	0.34	0.96	1.48
white (2–1)	0.46	1.07	1.08

A comparison of the lipolytic activity of the normal molds and the white mutants is shown in table 1. Apparently lipolytic activity is not physiologically associated with green color and the color characteristics can be lost without modifying lipolysis. Whether or not the increased lipolytic activity of mutants 1–10 and 2–10 is the result of an ultraviolet induced change has yet to be deter-

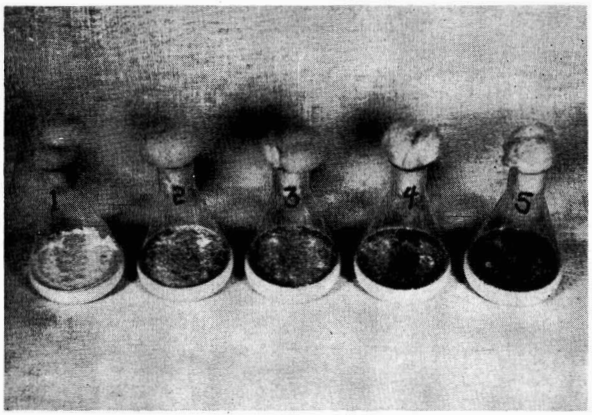

FIG. 2. *P. roqueforti* strain 2 grown on milk with and without added iron.
Flask 1.....0.0 mg. $FeCl_3$ added
" 2.....0.5 " " "
" 3.....1.0 " " "
" 4.....2.0 " " "
" 5.....4.0 " " "

mined. Table 2 shows that proteolysis, like lipolysis, is not physiologically associated with green coloration.

During an investigation of the metals associated with the green coloration of normal *P. roqueforti,* it became evident that the iron requirements of the normal molds and the white mutants were quite different. Figure 2 shows the effect of iron ($FeCl_3$) added to whole milk on sporulation of strain 2. Table 3 gives the

TABLE 3

The influence of iron on the weight of mycelium produced by P. roqueforti 2 and 2–10 on whole milk at 25° C

$FeCl_3$ added	Dry weight of mycelium	
	Strain	
	green (2)[a]	white (2–10)
(mg./100 ml.)		
none	0.74	1.01
0.5	0.76	1.11
1.0	0.85	0.95
2.0	1.04	1.02
4.0	1.13	1.07

[a] Mycelium from flasks shown in fig. 2.

dry weight of the mycelium of strain 2 in each of the flasks in figure 2, as well as the weight of strain 2–10 that was grown at the same time. Similar results were obtained with strains 1 and 1–10. From these data it is evident that the white mutants will grow well in milk without additional iron, whereas the green parents grow and sporulate poorly unless iron is added. Finely divided metallic iron and the iron in ferric citrate, ferric lactate, ferric chloride and ferric sulfate was available.

DISCUSSION

The results of these experiments indicate that the green color of *P. roqueforti* can be lost permanently by induced mutation without markedly changing the lipolytic and proteolytic activity or the growth of the mold. Hence, roquefort-type cheese made with the white molds would be lacking the green venation usually associated with such cheese, but probably would be normal in other respects. Mold-ripened cheese made from the white mutants would be desirable for the manufacture of cheese spreads and blends and should appeal to consumers who are prejudiced against eating the conventional roquefort-type cheese which to them appears "moldy." That at least one of the white mutants can be used for the manufacture of roquefort-type cheese has been shown by Jezeski *et al.* (2).

It should be possible to produce mold-ripened cheese with new flavors and textures by use of *P. roqueforti* or other molds, the lipolytic or proteolytic activity of which has been modified by mutation. It could be that the increased lipolytic activity of mutant 2–10 (table 1) is due to such a mutation, or that a more lipolytic strain has been selected by isolation of single spores from the parent culture.

The high iron requirements for good growth and sporulation of *P. roqueforti* might be very significant in the manufacture of mold-ripened cheese. The fact that milk might not contain enough iron for good growth of the green molds was noted first on pasteurized milk obtained from the University Dairy. However, it was found that not all samples of milk from the Dairy were deficient in iron; apparently, contamination from utensils provided enough of the essential metal in some instances. All samples of milk that were obtained directly from the cow and never were in metal utensils were iron-deficient for the green molds but not for the white mutants. It is probable that lack of available iron in the milk sometimes may result in poor growth of the mold in roquefort-type cheese. Because of the low iron requirements of the white mold, milk probably contains enough iron for the manufacture of "white mold" cheese. It is interesting that the absence of color in the spores of the mutants should decrease the amount of iron needed for growth and that the green mold at low levels of iron both grew and sporulated poorly. The relationship between pigmentation of the spores and growth of the mold is being studied.

There is evidence that the color in *P. roqueforti* is determined by a single gene since only white heterocaryons have been obtained from various combinations of ten colorless mutants. If color were determined by more than one gene it would be unlikely that the same gene would have been hit in each of the ten

mutants. That heterocaryosis does occur has been proved by producing stable pale green heterocaryons as a result of anastymosis between a colorless mutant and a normal green parent. Anastymosis of hyphae has been observed frequently by microscopic observation. Nevertheless, since all of the factors involved in the synthesis of heterocaryons are not known, definite conclusions in regard to the genetics of the green color of *P. roqueforti* spores should not be made at this time.

The practical applicability of these findings is being developed through the Wisconsin Alumni Research Foundation in cooperation with the Division of Dairy Husbandry, University of Minnesota.

SUMMARY AND CONCLUSIONS

Strains of *Penicillium roqueforti* with white spores have been obtained by ultraviolet-induced mutation of the green mold.

The white mutants were produced without marked changes in the lipolytic, proteolytic or growth rates of the mold.

Evidence is presented to show that normal *Penicillium roqueforti* probably requires more iron for good growth than may be present in mixed samples of milk. Milk that was produced without contact with a metal container was a poor medium for the green mold unless iron was added.

The white mutants required less iron for growth than the green parents and grew normally in milk to which no iron had been added.

Apparently one gene is responsible for the green pigmentation of the spores of *Penicillium roqueforti.*

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COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

Atlantic City, N. J.—Oct. 16, 1950

Teams from 26 State Agricultural Colleges participated in this, the sixteenth annual contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

ALL PRODUCTS

Individuals

*1. Edward Schuch, Iowa State College
*2. Thomas E. Gilmore, Mississippi State College
3. Willis E. Parkin, University of Connecticut
4. Duane Osam, Iowa State College
5. James Stanton, Ohio State University
6. William E. Sandine, Iowa State College
7. Graham E. Hall, University of Connecticut
8. James C. Sutherland, Michigan State College
9. Richard H. Andrews, Mississippi State College
10. Jack Davis, Mississippi State College

Teams

1. Iowa State College
2. Mississippi State College
3. University of Connecticut
4. Michigan State College
5. Ohio State University
6. University of Georgia
7. Cornell University
8. Oklahoma A. & M.
9. Purdue University
10. North Carolina State College

* Tied for first place; tie broken in favor of Edward Schuch on flavor score of 60.67 compared with flavor score of 67.84 for Mr. Gilmore.

BUTTER

Individuals

1.	Farris E. Ashe, University of Tennessee	11.25
2.	Richard H. Andrews, Mississippi State College	11.67
3.	Edward Schuch, Iowa State College	12.50
4.	Kenneth Van Patten, Michigan State College	13.17
5.	Richard J. Stucky, University of Minnesota	13.67
5.	Edward B. Hanna, West Virginia University	13.67
7.	Thomas E. Gilmore, Mississippi State College	14.17
8.	William E. Sandine, Iowa State College	14.34
9.	Donald G. Sickafoose, Ohio State University	14.67
10.	George Farrell, Purdue University	15.17

Teams

1.	Iowa State College	42.18
2.	Mississippi State College	44.01

3.	Purdue University	46.92
4.	University of Minnesota	48.17
5.	University of Tennessee	50.42
6.	Ohio State University	50.67
7.	University of Maryland	53.34
8.	Michigan State College	53.35
9.	Cornell University	56.35
10.	University of Connecticut	57.51

CHEESE

Individuals

1.	Thomas E. Gilmore, Mississippi State College	23.55
2.	Edward Schuch, Iowa State College	24.00
3.	Kenneth Van Patten, Michigan State College	25.58
4.	Duane Osam, Iowa State College	25.58
5.	James C. Sutherland, Michigan State College	26.33
6.	James Stanton, Ohio State University	26.67
7.	Earl M. Harvey, University of Nebraska	26.76
8.	Ervin C. Hamme, Pennsylvania State College	27.01
9.	Willis E. Parkin, University of Connecticut	28.14
10.	Graham E. Hall, University of Connecticut	28.33

Teams

1.	Iowa State College	78.92
2.	Michigan State College	85.49
3.	Purdue University	88.52
4.	University of Connecticut	91.64
5.	Mississippi State College	94.33
6.	Ohio State University	95.18
7.	North Carolina State College	100.05
8.	Cornell University	101.68
9.	University of Georgia	102.58
10.	University of Nebraska	103.68

ICE CREAM

Individuals

1.	Richard H. Andrews, Mississippi State College	25.00
2.	Jack Davis, Mississippi State College	26.00
3.	Hilmer H. Schuelke, Texas A. & M.	28.33
4.	Joe Otto Brown, North Carolina State College	29.00
5.	Stanley L. Ruxton, University of Connecticut	31.00
5.	Willis E. Parkin, University of Connecticut	31.00
7.	Robert W. Skinner, University of Tennessee	31.25
8.	Farris E. Ashe, University of Tennessee	31.50
9.	Graham E. Hall, University of Connecticut	32.00
9.	James A. Paul, Cornell University	32.00
9.	James Stanton, Ohio State University	32.00
9.	Aaron B. Karas, Cornell University	32.00

Teams

1.	Mississippi State College	86.67
2.	University of Connecticut	94.00

3.	Texas A. & M.	98.58
4.	University of Tennessee	98.75
5.	Cornell University	104.00
6.	University of Georgia	104.17
7.	Iowa State College	104.50
8.	Michigan State College	104.67
9.	North Carolina State College	107.50
10.	Ohio State University	108.50

MILK

Individuals

1.	Willis E. Parkin, University of Connecticut	15.32
2.	Gale G. Ripma, Michigan State College	18.72
3.	Edward Schuch, Iowa State College	23.42
4.	James C. Otto, University of Minnesota	23.84
5.	Thomas E. Gilmore, Mississippi State College	24.60
6.	H. Douglas Cope, Ohio State University	24.75
7.	Harold Windlam, University of Georgia	25.06
8.	Jack E. Conrad, University of Maryland	26.00
9.	James Stanton, Ohio State University	26.05
10.	Roger B. Thompson, University of Massachusetts	27.45

Teams

1.	University of Connecticut	73.17
2.	Michigan State College	81.40
3.	Iowa State College	81.91
4.	University of Georgia	85.97
5.	Ohio State University	87.40
6.	Mississippi State College	87.54
7.	Oklahoma A. & M.	88.66
8.	University of Maryland	91.20
9.	Texas Technological	93.72
10.	University of Nebraska	95.89

NATIONAL INTERCOLLEGIATE DAIRY CATTLE JUDGING CONTEST
NATIONAL DAIRY CATTLE CONGRESS—1950

Waterloo, Iowa

TEAM RANK—ALL BREEDS

1.	Ohio	2081
2.	Iowa	2026
3.	Pennsylvania	2022
4.	Kentucky	2000
5.	Calif. State Polytechnic	1980
6.	Maryland	1978
7.	Missouri	1966
8.	Ontario Agr. College	1947
9.	Cornell	1924
10.	Texas Tech.	1922

HIGH INDIVIDUALS IN JUDGING ALL BREEDS

1.	Herman Rickard, Ohio State	702
2.	Carl Young, Ohio State	700
3.	G. J. Lyon, Iowa State	695
4.	James Fish, Pennsylvania State	690
5.	W. Earle Roger, Ontario Agr. College	689
6.	Cecil Burnette, Kentucky	685
7.	James Moxley, Maryland	683
8.	Ben Broesma, Calif. Polytechnic	681
9.	T. A. Burgeson, Missouri	680
10.	William E. Davis, Jr., Ohio State	679

AYRSHIRE

	Teams			*Individuals*	
1.	Calif. Polytechnic	425	1.	Lawrence Barba, Calif. Poly.	146
2.	N. Carolina State	423	2.	(W. Earle Roger, Ontario Agr.	144
3.	Kentucky	415	3.	(Engimar Sveinsson, Wash. State	144
4.	Pennsylvania	405	4.	(Max Sink, N. Carolina	143
5.	Maryland	402	5.	(Robert Johnson, Calif. Poly.	143
6.	Ohio State	398	6.	(James Martin, Kentucky	143
7.	Univ. of Missouri	396	7.	(Tommie McPherson, N. Carolina	142
8.	Washington State	394	8.	(Richard Riggs, Purdue	142
9.	Ontario Agr. College	392	9.	(M. D. Rinner, Iowa State	142
10.	(Purdue	391	10.	Arthur Korte, Missouri	141
11.	(Kansas	391			

BROWN SWISS

1.	Iowa State	431	1.	Herman Rickard, Ohio State	148
2.	Ohio State	426	2.	(Engimar Sveinsson, Wash. State	147
3.	Univ. of Ill.	404	3.	(William Shenton, Iowa State	147
4.	Ontario Agr. College	391	4.	(M. D. Rinner, Iowa State	146
5.	Washington State	390	5.	(Ben Broesma, Calif. Poly.	146
6.	Kentucky	389	6.	(Carl Young, Ohio State	146
7.	Univ. of Wis.	388	7.	Cecil Burnette, Kentucky	142

8.	(Maryland	387	8.	(James Fish, Penn. State	141
9.	(Calif. Poly.	387	9.	(Ray Briggs, Cornell	141
10.	Connecticut	380	10.	Earl Spurrier, Maryland	140

GUERNSEY

1.	Pennsylvania State	433	1.	(Herman Rickard, Ohio State	148
2.	Ohio State	431	2.	(James Fish, Penn. State	148
3.	Iowa State	422	3.	(M. D. Rinner, Iowa State	148
4.	Ont. Agr. College	419	4.	(Carl Young, Ohio State	147
5.	Texas Tech.	418	5.	(James Moxley, Maryland	147
6.	Kentucky	417	6.	Charles Harding, Penn. State	146
7.	Purdue	413	7.	Vestal Shipman, Texas Tech.	145
8.	Cornell	410	8.	(W. Earle Roger, Ont. Agr. College	144
9.	Maryland	404	9.	(T. A. Burgeson, Missouri	144
10.	Okla. A & M	398	10.	Robert Peterson, Purdue	143

JERSEY

1.	Ohio State	418	1.	E. B. Morgan, Louisiana State	147
2.	(Texas Tech.	417	2.	Robert Strickler, Kansas	144
3.	(Cornell	417	3.	(Tommie Hewlett, Texas Tech.	143
4.	Calif. Poly.	416	4.	(Don House, Cornell	143
5.	(Penn. State	405	5.	(G. J. Lyon, Iowa State	143
6.	(Louisiana State Univ.	405	6.	(Charles Harding, Penn. State	142
7.	Missouri	401	7.	(Ben Broesma, Calif. Poly.	142
8.	Iowa State	399	8.	(C. B. Smith, Texas A & M	142
9.	Okla. A & M	394	9.	(T. A. Burgeson, Missouri	142
10.	Kansas	393	10.	(W. Earle Roger, Ont. Agr. College	141
			11.	(W. E. Davis, Jr., Ohio State	141
			12.	(Herman Rickard, Ohio State	141

HOLSTEIN

1.	Maryland	415	1.	William Shenton, Iowa State	146
2.	Kentucky	413	2.	James Fish, Penn. State	144
3.	Ohio State	408	3.	(William Curry, Maryland	142
4.	Penn. State	407	4.	(Ward Ricter, Wisconsin	142
5.	Iowa State	402	5.	(G. J. Lyon, Iowa State	141
6.	Missouri	399	6.	(Don House, Cornell	141
7.	Illinois	397	7.	(W. E. Davis, Jr., Ohio State	140
8.	Texas A & M	394	8.	(Carl Young, Ohio State	140
9.	Univ. of Wis.	388	9.	(Ed Thomason, Okla. A & M	139
10.	Cornell	385	10.	(Cecil Burnette, Kentucky	139
			11.	(T. A. Burgeson, Missouri	139
			12.	(Robert Hertzog, Missouri	139

NEW MEMBERS FOR 1950

ARKANSAS

HORTON, OTIS H., Dept. of Animal Industry, Univ. of Arkansas, Fayetteville

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BOYLE, KENNETH D., Calif. State Polytechnic College, San Luis Obispo
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ABSTRACTS OF LITERATURE

Prepared in cooperation with the
International Association of Ice Cream Manufacturers
and the Milk Industry Foundation

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

824 Medicator for cows' teats. M. H. NEWELL. U. S. Patent 2,523,478. 8 claims. Sept. 26, 1950. Official Gaz. U. S. Pat. Office, **638,** 4: 1092. 1950.

A hand-operated device flushes cows' teats with liquid medication. R. Whitaker

825. An important problem facing dairymen is ketosis and the dairy cow. C. B. KNODT. Can. Dairy Ice Cream J., **29,** 9: 84. Sept., 1950.

See abs. no. 690, Oct., 1950.

826. Brucella ring test antigen prepared by reduction of a tetrazolium salt. R. M. WOOD, Johns Hopkins Univ., Baltimore, Md. Science, **112:** 86. 1950.

Details are given for carrying out the brucella ring test using a tetrazolium salt (4, 4′-bis) 3, 5-diphenyl-2-tetrazolinium)-biphenyl dichloride) instead of hematoxylin to stain the brucella antigen. The tetrazolium salt is reduced by living cells to an intensely colored violet-blue formazan. Apparently the reduction takes place inside the cell, and hence the antigenic specificity of the cell surface is not altered. Lots of antigen prepared over the last 2 yr. using the tetrazolium method all have been of uniform color intensity, specificity and sensitivity and have remained stable over prolonged periods under normal conditions of use and storage.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

827. De controle op de kwaliteit van te exporteren kaas. (Quality control for export cheese). F. KEESTRA, Zuivel-Kwaliteits controle-Bureau-Z. K. B., Amsterdam, Holland. Neth. Milk and Dairy J., **4,** 2: 148–155. 1950.

The Z. K. B. (quality control bureau for dairy products) has handled in Holland the quality control for butter since 1937, dried milk since 1946, cheese since 1948. The Z. K. B. is an organization of the dairy industry under government supervision. Cheese may be exported only if the "Holland" brand of the Dutch cheese control is on it. This means that it has been checked for composition and purity. Neither can it pass the customs without an export certificate of the Z. K. B. for quality control. Quality requirements are of the negative type, several regulations mentioning what is not allowed. Thus, the cheese may not look bad from the outside or be out of shape. The rind may not have serious faults or cracks or have a wrong color. The inside may not have serious faults. Odor and taste may not be abnormal. A minimum age is required, 6 wk. in winter, 4 wk. during the summer and 2 wk. more if the cheese is sealed in paraffin or similar material.

There are no positive requirements because different countries and even parts of countries want different properties. The manufacturer places a brand on the cheese dealing with the composition and purity. The exporter has to place his number on every packing unit. From this the origin of the cheese always can be found out later on. A. F. Tamsma

Also see abs. no. 837.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

828. A use of ascorbic acid in frozen homogenized milk. R. B. ANDERSON, C. W. BETZOLD and W. J. CARR, Sixth Army Area Food Lab.,

Seattle, Wash. Food Technol., 4, 7: 297–300. 1950.

Milk was processed after ascorbic acid had been added at the rate of 0, 1.5, 3.0, 6.0 and 12.0 g./100 lb. of milk. The milk was pasteurized at 75° C. for 15.8 sec., homogenized at 58–60° C. under 1700 lb. pressure, cooled to 3° C., placed in commercial qt. paper cartons and stored at −17.8 to −16.7° C. for 30, 60 or 90 d. At the end of the storage period the samples were thawed 8 hr. at 19–20° C. then held 15–17 hr. at 4.4° C. Samples fortified with 0, 1.5 and 3 g. of ascorbic acid/100 lb. of milk had a strong, definite or slight oxidized flavor, while the samples fortified with 6 or 12 g. of ascorbic acid/100 lb. of milk were free from off-flavors. Approximately 1.25 g. added ascorbic acid were expended in protecting the flavor of the milk during processing and 30-d. storage. Milk fortified to the 6-g. level contained approximately 128 mg. of vitamin C./qt. after 30 d. of storage and 108 mg./qt. after 90 d. storage.

E. R. Garrison

829. Meringues and method of making the same. J. A. Snelling (assignor to Proctor and Gamble Co.). U. S. Patent 2,524,333. 12 claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, **639,** 1: 153. 1950.

Nonfat dry milk solids are mixed with not over 9% by weight with a mixture of alkali and alkaline earth sulphates and chlorides and an edible acid in such proportions that when 3–10 parts by weight of water is added to each part of milk powder in the meringue powder, the pH will be between 5 and 7. R. Whitaker

830. Process for the production of artificial bristles and the like from protein. T. L. McMeekin, T. S. Ried, R. C. Warner and R. W. Jackson (assignors to U. S. A., as represented by Secy. of Agr.). U. S. Patent 2,521,738. 5 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638,** 2: 415. 1950.

A fibre having a tensile strength of not less than 0.8 g./denier, is made by kneading iso-electric casein with water at 80–100° C. until plastic, extruding into air at 95–110° C., stretching the filament, treating with an anti-sticking agent, followed by hardening in a bath, stretching again and rehardening before drying under tension.

R. Whitaker

831. Recovery of lactalbumin. G. Josh and M. E. Hull (assignors to Armour and Co.). U. S. Patent 2,521,853. 6 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638,** 2: 444. 1950.

A coagulable protein is added to whey and the pH adjusted to 4–5. The mixture then is heated and the liquid drained off the 2 coagulated proteins. R. Whitaker

832. Process for the manufacture of foam producing albuminous products and their application in foodstuffs and luxuries. J. Lenderink. U. S. Patent 2,522,050. 10 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638,** 2: 494. 1950.

Casein or other protein is hydrolyzed at about pH 10 with $Ca(OH)_2$ and $Mg(OH)_2$ at a temperature below boiling for at least 2 d. until the mixture contains 5–40% polypeptides and has strong foaming properties. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

833. Isolements de ferments lactiques particuliers au lait de brebis et au fromage de roquefort. (Isolation of lactic acid fermentors characteristic of sheep's milk and roquefort cheese.) C. Alais. Lait, **30,** 297: 349–359. July–Aug., 1950.

Lactic acid cultures isolated from roquefort cheese and from sheep's milk exhibited distinctly different characteristics when cultured in sheep's milk as compared with cow's milk. Strength of the cultures when carried in sheep's milk remained high for protracted periods. In cow's milk, the organisms rapidly lost capacities to produce acid and to inhibit contaminants. These cultures, carried in sheep's milk, were observed to yield excellent results when used in the manufacture of roquefort cheese; however, they were entirely unsatisfactory when employed in production of blue cheese (made from cow's milk). Reasons for preferential growth of cultures in sheep's milk are discussed. S. Patton

834. Recherche, dans le lait en nature de certaines bacteries pathogenes pour l'homme. (Examination of raw milk for certain bacteria pathogenic to man.) G. Guillot, A. Nevot and G. Thieulin. Lait, **30,** 297; 337–349. July–Aug., 1950.

Methods for detecting the principal bacteria, pathogenic to man and incident to milk, are presented and discussed. S. Patton

835. The effect of hypochlorite and quaternary ammonium compounds, used in udder washes, on the chemical composition and bacterial flora on the milk produced. E. M. Kesler, C. B.

KNODT and J. J. REID, Penn. State College. J. Milk & Food Technol., **13:** 288–291. Sept.–Oct., 1950.

One quaternary ammonium compound (200 ppm.) and 200 and 400 ppm. concentrations of chlorine were compared with clean water in this study. Both sanitizers were considered equally ineffective when used under comparable conditions in checking the spread of organisms usually associated with mastitis. Although a general reduction of the udder microflora of the cows was noted, no apparent differences were observed between treatments on the chloride content or pH values of the milk produced.

H. H. Weiser

836. Antibiotics in milk and discussion of problems encountered. F. J. DOAN. Can. Dairy Ice Cream J., **29,** 9: 35–36. Sept., 1950.

Antibiotics such as penicillin, aureomycin, sulphamethazine and streptomycin, used for the treatment of mastitis infections in the udders of producing dairy cows, have been reported in the milk from such cows for several milkings after treatment. In many cases arrested acid development has resulted when such milk is used in the manufacture of various types of cheese and buttermilk. At present, the only satisfactory control of the problem of antibiotics in milk is to try to get the producer to keep the milk from treated udders out of the milk shipped to the dairy. It probably is best to discard no less than 3 milkings following the treatment. H. Pyenson

837. Preliminary report of effect of mastitis curatives on cheese making. A. BRADFIELD. Can. Dairy Ice Cream J. **29,** 9: 37–38. Sept., 1950.

The results, so far, indicate that problems may be expected in cheese making if the newer methods of mastitis treatment which depend upon the use of antibiotics become common.

H. Pyenson

838. The site of action of penicillin. 1. Uptake of penicillin on bacteria. D. ROWLEY, P. D. COOPER and P. W. ROBERTS, St. Mary's Hospital, Paddington, and E. L. SMITH, Glaxo Laboratories, Ltd., Greenford, Middlesex. Biochem. J., **46,** 2: 157–161. 1950.

The preparation of radioactive penicillin, using ^{35}S in the medium, is described. By tracing this radioactive penicillin, the amount of penicillin attached to the bacterial cells can be estimated. The action seems to be due to a direct chemical reaction. The penicillin concentration attained inside "sensitive" or growing bacterial cells was much greater than in the medium, but for resistant or resting cells it was much less. Attempts were made to block the uptake of penicillin, as well as to remove the attached penicillin from bacterial cells. A. O. Call

839. The microbiological determination of pyrimidines with lactobacilli. R. B. MERRIFIELD and M. S. DUNN, Univ. of Cal., Los Angeles. J. Biol. Chem., **186,** 1: 331–341. Sept., 1950.

An assay procedure for free and combined pyrimidines has been developed employing *Lactobacillus brevis* (ATCC 8287) and *L. helveticus* (ATCC 335). Only uracil and thymine were found active toward *L. helveticus,* which revealed a strict requirement for free pyrimidines. *L. brevis,* however, utilized both free and combined pyrimidines and exhibited essentially the same activity toward uracil. cytosine, orotic acid, uridine, cytidine, diammonium uridylate and cytidylic acid. Pyrimidine concentrations employed during assay were 0.3–5.0 γ uracil/ml. medium with *L. brevis,* 0.3–1.0 γ uracil/ml. medium with *L. helveticus* and 0.27–1.07 γ thymine/ml. medium with *L. helveticus.*

H. J. Peppler

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

840. Alanine, glycine and proline contents of casein and its components. W. G. GORDON, W. F. SEMMETT and M. BENDER. E. Reg. Research Lab., Philadelphia, Pa. J. Am. Chem. Soc., **72,** 9: 4282. Sept., 1950.

By means of the radioisotope derivative technique whole casein and its 3 components, α-, β- and γ-casein, were analyzed for alanine, proline and glycine. Results corrected for moisture and true ash reveal that whole casein contains 3.2% alanine, 2.0% glycine and 10.6% proline. These values are in close agreement with those reported in the literature. H. J. Peppler

841. Rancidity in milk and cream and discussion of milk-lipase. E. G. HOOD. Can. Dairy Ice Cream J., **29,** 8: 58–62. Aug., 1950.

The defect produced by milk-lipase action commonly is called rancidity. Conditions must be suitable for lipase activity, otherwise it cannot break down the fat.

The enzyme usually shows the most activity at a temperature of 37–40° F. and a pH of 8.4–8.6. Traces of heavy metals have an inhibiting action on milk lipase. Rancidity is most likely to occur when the cows are in advanced stage of lactation and have been milking for a year or more without freshening. Cows at the

end of lactation period also may produce rancid milk. Green feed will reduce the incidence of rancid flavors in milk. Rancidity can be induced by homogenization at a temperature under 130° F., violent agitation and by a temperature treatment—precool to 40° F., reheat to 80° F. and recool to 50° F. Milk produced in winter months is more subject to rancidity than milk produced in late spring and summer.

H. Pyenson

842. Le colostrum et le lait dans leur rapports avec l'immunite du jeune. (Colostrum and milk in connection with immunity of the young.) E. LEMETAYER, L. NICOL, O. GIRARD, R. CORVAZIER et M. CHEYROUD. **Lait, 30,** 297: 359–373. July–Aug., 1950.

The work concerning placental vs. colostral transmission of immunity to the newborn of a number of mammals is reviewed at length. The study deals with levels of antibodies in the blood and colostrum of mares vaccinated or hyperimmunized against tetanus or diptheria. In the case of the mare, the prepartal colostrum invariably carries a higher level of antibodies than does the blood. The colostrum level drops very rapidly at the time of birth. The authors propose that hormone-induced changes in the gland and dilution effect, due to increase in the quantity of secretion are responsible for reduced antibody titre in colostrum at birth. S. Patton

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

843. A study on the performance of a side-opening milk cooler. G. H. WATROUS, JR. Can. Dairy Ice Cream J., **29,** 9: 44–46. Sept., 1950.

No significant differences were noted in bacterial levels obtained on milk cooled in the side-opening spray-type cooler, as compared to the conventional immersion-type cooler, either with or without water agitation in the latter. The side-opening cooler cooled milk below 50° F. in less than 45 min. compared to 8.5 hr. without agitation and 2.25 hr. with agitation in the immersion-type cooler. The final temperature of the milk in the side-opening cooler varied between 42 and 45° F. In the immersion type cooler the final temperature ranged between 34.4 and 39.5° F. No significant difference in electricity consumption with either cooler was noted.

H. Pyenson

844. Apparatus for the production of ice cream. D. WESTMORELAND. U. S. Patent 2,524,616. 5 claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, **639,** 1: 224. 1950.

A continuous ice cream freezer consisting of 3 horizontal cylinders one above the other is described. Mix enters the top cylinder, where air is incorporated by a rotating hollow dasher and some cooling takes place. From the top cylinder the ice cream flows to the middle and then to the lowest cylinder, both of which are equipped with rotating blades which scrape the cylinder walls. The jacket of the bottom cylinder is flooded with boiling refrigerant, which expands to a gas in the jacket of the middle cylinder and then flows through the jacket and hollow dasher of the top cylinder. R. Whitaker

845. Automatic control for the freezing of ice cream. A. J. TACCHELLA (assignor to Steady Flow Freezer Co.). U. S. Patent 2,522,648. 17 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638,** 3: 773. 1950.

As ice cream is withdrawn for serving from this freezer, additional mix and air are automatically admitted to maintain a constant overrun. R. Whitaker

846. Frozen custard machine. B. H. WOODRUFF. U. S. Patent 2,523,853. 16 claims. Sept. 26, 1950. Official Gaz. U. S. Pat. Office, **638,** 4: 1191. 1950.

A freezer for making soft ice cream, frozen custard, etc. consists of a horizontal refrigerated cylinder with a rotating dasher and scraper blades. Mix is metered from a supply tank into an inlet in the freezer in proportion to the amount of soft frozen product withdrawn for serving.

R. Whitaker

847. Scraper for freezing apparatus. C. ERICKSON and E. SPELLMAN. U. S. Patent Reissue 23,267. 15 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638,** 2: 397. 1950.

A scraper blade, pivoted on arms attached to the dasher of an ice cream freezer, is so designed that it may be easily removed for cleaning.

R. Whitaker

848. Pasteurizing system. R. E. OLSON and G. E. HELLER (assignors to Taylor Instrument Co.). U. S. Patent 2,522,796. 7 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638,** 3: 810. 1950.

An electrical system of controlling the temp. in a high-temp., short-time milk pasteurizing system, including milk-to-milk regeneration, a final milk to water heater and a flow diversion valve is described. The flow diversion valve is actuated

by either a decrease in temp below that desired or by an increase in the desired velocity of the milk flow. R. Whitaker

849. Can washer. A. W. SMITH (assignor to Rice and Adams Corp.). U. S. Patent 2,522,310. 11 claims. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638,** 2: 561. 1950.

A straight-line milk can washer of the rising jet type is described. R. Whitaker

850. Label holder for milk cans. S. PETERSEN. U. S. Patent 2,522,398. 1 claim. Sept. 12, 1950. Official Gaz. U. S. Pat. Office, **638,** 2: 585. 1950.

A slide is provided on a cross arm of milk can lids for holding a removable label for identifying the can and contents. R. Whitaker

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

851. The utilization of non-protein nitrogen in the bovine rumen. 5. The isolation and nutritive value of a preparation of dried rumen bacteria. M. L. MCNAUGHT and J. A. B. SMITH, Hannah Dairy Research Inst., Kirkhill, Ayr, and K. M. HENRY and S. K. KON, Univ. of Reading. Biochem. J., **46,** 1: 32–36. 1950.

Batches consisting of 2–3 l. of bovine rumen liquid were taken from a fistula. They were incubated with added maltose and urea and the rumen bacteria then separated by a Sharples super-centrifuge. This procedure was repeated until a total of 130 l. were processed. The yield was about 3.5 g. bacteria/l. The conversion of non-protein N to protein is demonstrated. In composition the dried rumen bacteria are similar to dried yeast. In biological value the material compares favorably with "dried-milk protein." A. O. Call

852. The utilization of non-protein nitrogen in the bovine rumen. 6. The effect of metals on the activity of the rumen bacteria. M. L. MCNAUGHT, E. C. OWEN and J. A. B. SMITH, Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **46,** 1: 36–43. 1950.

The effects of various concentrations of Cu, Co, Mo and Fe on the development of rumen bacteria in rumen liquid were studied using *in vitro* techniques. The tolerated and toxic levels (in ppm.) were Fe, 100 and 1000; Cu, 10 and 25; Co, < 10 and 1000; Mo, 100 to 1000 and 2000. The effects of several organic chelating agents also were studied. A. O. Call

853. Deposit and residue of recent insecticides resulting from various control practices in California. W. M. HOSKINS, Univ. of Cal., Berkeley. J. Econ. Entomol., **42,** 6: 966–973. Dec., 1949.

DDT was used on alfalfa for insect control. The hay was fed to dairy cows. DDT in the milk measured 15–23% of the DDT intake on the feed. Benzene hexachloride appeared in cow's milk within 24 hr. after being sprayed on the cow. Other data of DDT, DDD and parathion on alfalfa are included. E. H. Fisher

Also see abs. no. 842, 857, 858.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

854. Milking machine pulsator. S. P. WALL (assignor to Rite-Way Prod. Co.). U. S. Patent 2,523,795. 9 claims. Sept. 26, 1950. Official Gaz. U. S. Pat. Office, **638,** 4: 1175. 1950.

A device for causing pulsations in the vacuum line of a milking machine is described. R. Whitaker

855. Teat cup claw. W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,524,193. 5 claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, **639,** 1: 117. 1950.

A four-outlet manifold for connecting the teat cup tubes of a milker to an intermittent vacuum supply is described. R. Whitaker

856. Milker timer. W. H. HARSTICK (assignor to International Harvester Co.). U. S. Patent 2,524,194. 5 claims. Oct. 3, 1950. Official Gaz. U. S. Pat. Office, **639,** 1: 118. 1950.

A device causing pulsations in a vacuum supply for milking machines is desribed. R. Whitaker

857. Calf feeder. F. J. HABERKORN. U. S. Patent 2,522,820. 3 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638,** 3: 816. 1950.

A calf feeder consisting of a lid containing an outlet terminating in a nipple, which fits on the top of a cylindrical vessel holding liquid calf food is described. The device is placed in operation by inserting it in a holder attached to a wall, which holds it so the nipple is on the bottom. R. Whitaker

858. Calf feeder. H. J. LARSON. U. S. Patent 2,522,757. 8 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638,** 3: 801. 1950.

A tube, mounted in a vertical wall, extends to the bottom of a pail of liquid calf food, the upper end terminating in a nipple. R. Whitaker

859. Weaning basket. L. E. COX. U. S. Patent 2,523,820. 1 claim. Sept. 26, 1950.

Official Gaz. U. S. Pat. Office, **638,** 4: 1182. 1950.

A basket-shaped device for covering a cow's udder is held in place by straps over the cow's rump and back. R. Whitaker

860. Device for assisting parturition of animals. B. N. FRANK. U. S. Patent 2,522,508. 4 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638,** 3: 738. 1950.

An obstretical device for assisting calving in cattle is described. R. Whitaker

861. Handcart for milk cans and the like. R. E. PUTMAN. U. S. Patent 2,522,894. 4 claims. Sept. 19, 1950. Official Gaz. U. S. Pat. Office, **638,** 3: 834. 1950.

A 2-wheeled cart for easily transporting a can of milk is described. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

862. Chocolate ice cream and discussion of formula. C. W. DECKER. Can. Dairy Ice Cream J., **29,** 8: 78–82. Aug., 1950.

Chocolate ice cream represents approximately 15% of the total ice cream sales. Dutch process cocoa containing 20–22% cocoa fat produces a chocolate ice cream without bitterness or harshness. 1.5% chocolate liquor or blend, 3% coca and 18% sugar makes a good chocolate ice cream. A portion of the liquor may be replaced by cocoa at the rate of 0.25% cocoa for each 0.5% chocolate liquor. Any changes in the chocolate ice cream formula should be made gradually and preferably in the slack season of the year. H. Pyenson

Also see abs. no. 844, 845, 846, 847.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

863. Packaging whipping cream in pressurized containers. E. GRAHAM. Crown Can Co., Philadelphia, Pa. Food Technol., **4,** 6: 225–229. 1950.

The development of single-trip metal containers for pressurized whipped cream and the principle of whipping by effervescence are outlined. To properly pasteurized cream containing approximately 30% butterfat are added 5–10% of sugar, vanilla flavoring and stabilizer (dehydrated egg albumen, sodium caseinate, gelatin, skimmilk powder). Usually 7 fluid oz. of the mix are placed in the 12-oz. pressure container on regular dairy fillers, the metal gasketed cap with valve assembled is clinched to the top, then the cans pass to the gasser. The gas (N_2O or 85% N_2O and 15% CO_2) is added through the container valve to yield an equilibrium pressure of 75–90 p.s.i.g. The gassed container then is shaken vigorously for 10–30 sec. to hasten equilibrium between gas and mix and to partially clump the butterfat. An average overrun of about 250% is obtained or a yield of 25 fluid oz. of whipped cream from the original 7 fluid oz. Since the internal pressure tends to drop as the contents are dispensed from the container, the overrun of the whipped cream decreases accordingly. A "dry" firm whip is desired. A "wet" whip is associated with a low equilibrium pressure and insufficient agitation. Drainage varies inversely with the butterfat content and the gas pressure but can be decreased by the addition of stabilizer. Homogenized cream whips equally as well as regular cream by the aeration process but requires more agitation after gassing and therefore, generally is not used. Homogenized cream, however, shows less tendency to creaming and plugging and requires less shaking by the housewife before using. N_2O is regarded as being non-toxic and is widely used as an anesthetic. The gas (85% N_2O and 15% CO_2) exerts a bacteriostatic effect upon the cream but the product should be stored in a refrigerator until dispensed. E. R. Garrison

864. Cream separator. B. F. DOSCHER. U. S. Patent 2,523,561. 4 claims. Sept. 26, 1950. Official Gaz. U. S. Patent Office, **638,** 4: 1114, 1950.

A device for removing the cream from the top of a cream-top type of glass milk bottle, without mixing with the skim layer is described. R. Whitaker

865. Bottle crate. D. T. TICHENOR (assignor to United Steel and Wire Co.). U. S. Patent 2,519,800. 4 claims. Aug. 22, 1950. Official Gaz. U. S. Pat. Office, **637,** 4: 1173. 1950.

A wire milk bottle crate is described. R. Whitaker

Also see abs. no. 848.

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

866. Production of milk substitutes. L. NICHOLLS. Food Manufacture, **25,** 3: 95. 1950.

This reviews the potential application of nutritional substitutes (particularly in areas such as topics where milk generally is unavailable), the protein and vitamin dietary requirements and the use of soya "milk". K. G. Weckel

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

867. Quaternary ammonium compounds as sanitizers and cleaner sanitizers. P. R. ELLIKER. Can. Dairy Ice Cream J., **29**, 8: 64–66, 76, 84. Aug., 1950.

Some quaternary ammonium compounds are combined with non-ionic wetting agents and certain alkaline cleaning compounds to provide a combination cleaner or detergent and sanitizer. Quaternary ammonium compounds sometimes termed cationic, surface active agents, form a deposit on the surface of equipment, producing a germicidal or bacteriostatic film. They are characterized by a high degree of stability. Whether these compounds are toxic to humans has not been settled. In general, quaternaries are effective in destruction of Gram-positive bacteria but usually are slower than hypochlorites in destruction of Gram-negative bacteria. Quaternaries appear to be less effective in destruction of bacterial spores than are the hypochlorites but seem to be able to prevent germination of spores and growth of spore-forming types. As little as 10 ppm. quaternary in milk may seriously retard growth of lactic acid starter bacteria in starters, cultured milk or cheese milk. Organic matter definitely interferes with germicidal activity of quaternary ammonium compounds. Hard water salts, such as those containing Ca, Mg and Fe tend to inactivate quaternaries. The eosin titration method may be used to determine concentration of quaternary ammonium compounds.

H. Pyenson

Also see abs. no. 834.

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