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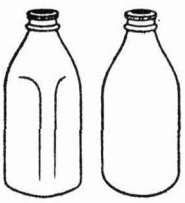

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QUATERNARY AMMONIUM COMPOUNDS AS STERILIZING AGENTS FOR BACTERIAL SPORES

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Since their commercial introduction in this country in 1937, quaternary ammonium compounds have been studied intensively with special reference to their antiseptic and bactericidal properties. The sporocidal activity of these compounds has received much less attention and the published reports reveal a wide divergence in essential findings and conclusions.

The reports of Kayser (14), Dunn (5), Zeissler and Gunther (25), Schubert (24), Scales and Kemp (23), Neufeld and Schütz (20), Hausam *et al.* (8), Dubois and Dibblee (3) and Mueller *et al.* (19) indicate that quaternary ammonium compounds are not particularly effective against bacterial spores. Complete killing of spores in the absence and presence of organic matter was achieved only when the reagents were used in relatively strong concentration (1 to 10 per cent) and the exposures were maintained for considerable periods, frequently at elevated temperatures.

Other investigators using similar source material reported rapid (less than 15 min.) and complete killing of spores in water and broth substrates by low concentrations of quaternaries: 0.5 per cent at room temperature (11); 0.5 per cent at room temperature (9); 0.05 per cent, 37° C. (10); 0.2 per cent, room temperature (16, 17); and 0.250 to 0.0022 per cent, depending on the quaternary and organism used, unstated temperature (12). Green and Birkeland (7) stated that cetyl pyridinium chloride is an effective germicide for bacterial spores. Johns (13) reported that Roccal and Hyamine 1622 in 0.1 per cent concentration at 20° C. killed 99.9 per cent of the spores of *B. panis* in 1 to 3 sec. For the quick sterilization of heavily contaminated instruments, Brekenfeld (2) recommended the use of Zephirol (0.75 to 2 per cent) at boiling temperatures; the efficacy of this procedure was disputed by Zeissler and Gunther (25), a view also supported by the data of Schubert (24).

From this brief review, it is apparent that the efficiency of quaternary ammonium compounds as spore-killing agents is not clearly defined. The present study is an attempt to clarify this subject and to furnish a more satisfactory basis for the evaluation of these compounds as sterilizing agents. The term sterilizing agent is used here in its true sense, *i.e.* complete destruction of the test organisms.

Received for publication July 26, 1949.

In the reports cited, it is noteworthy that all except Hoogerheide (10) and Hucker *et al.* (12) dealt individually with a single quaternary ammonium compound, usually Roccal (Zephiran, Zephirol) and, for the most part, with a single organism. In the present study a representative group of quaternaries was tested against a variety of sporing species. In the study of sporocidal as distinguished from sporostatic activity, emphasis was placed upon those organisms not easily controlled by heat-sterilization methods.

MATERIALS AND METHODS

The following compounds were studied: Roccal[1] (alkyl dimethyl benzyl ammonium chloride), Onyxide[2] (alkenyl dimethyl ethyl ammonium bromide), Ceepryn (cetyl pyridinium chloride), Nopcocide K (dodecylacetamide dimethyl benzyl ammonium chloride), Hyamine 10X (diisobutyl cresoxethoxy ethyl dimethyl benzyl ammonium chloride), Hyamine 1622 (diisobutyl phenoxyethoxy ethyl dimethyl benzyl ammonium chloride), Emcol 888 (alkyl aryl pyridinium chloride), Tetrosan (alkyl dimethyl 3,4-dichloro benzyl ammonium chloride) and Amosol-1 and Amsol-2[3] (chemical components not revealed by manufacturer). These compounds were received in solid (crystalline) form or in concentrated aqueous solutions. The manufacturer's statement of concentration of active ingredient was accepted in calculations of quaternary concentration. The Amosols were mixtures of a quaternary with alkaline detergents; these two compounds were used in the proportions (by weight) indicated without adjustment of quaternary concentration.

The 10 per cent concentrations of the quaternaries were aqueous dilutions of the more concentrated products; lower concentrations were made by dilution of the 10 per cent solutions with suitable proportions of buffers[4] to yield with the inoculum 4 ml. of a final suspension of the desired pH and quaternary concentration.

The tests were made as follows: To a constant volume of buffer or aqueous solutions in tubes was added 0.5 ml. of the spore suspension and the contents mixed by moderate rotation of the tube; the quaternary then was added without touching the walls of the tube with the pipette and the tubes gently rotated and quickly immersed in a glycerine bath thermostatically controlled ($\pm$ 0.5° C.) at the desired temperature. The samples were agitated gently during the first 5 min. and at the end of the exposure period, after which 0.1 ml. quantities of the test suspension were pipetted into flasks containing 40 ml. of the subculture (detoxification) medium. The flasks were shaken gently and stored at the optimum temperature for growth of the organism. Evidence of growth and probable purity was verified in each case by microscopic examination of film preparations.

Detoxification. Several substances of potential value for the detoxification

[1] Also known as Roclina, Rodalon, Zephiran, Zephirol and Benzalkonium chloride.

[2] Also known as Quartol.

[3] A more recently developed product than Amosol-1.

[4] M/15 KH_2PO_4, M/15 $NaHPO_4 \cdot 2H_2O$, M/10 NaOH (stronger solutions of this compound were used when necessary).

of quaternaries were studied; these included agar, starch, lecithin in Tween, crystalline albumin and oxgall. A modification of the Letheen medium (lecithin in Tween 80) of Quisno and Foter (21) was adopted eventually; its composition was: Beef extract (Difco) 3.0 g., Peptone (Difco) 5.0 g., lecithin (soya)[5] 5.6 g., Tween 80[6] 40 g. and distilled water 1,000 ml.

Dubos (4) found that commercial Tweens contain sufficient unesterified fatty acid to inhibit the growth of small inocula of tubercle bacilli which, if removed or inactivated, eliminated the bacteriostatic effect. Since commercial Tween 80 in the required concentration was found to inhibit some of the sporogenic organisms, the purified[7] product was employed. This was without inhibitory activity for minimal inocula. The subculture (Letheen) medium was prepared as needed to minimize the slow hydrolysis which Tween undergoes in aqueous substrates.

The organisms used and their sources were: *Bacillus mycoides* 6462, *B. cereus* 401, *B. sphaericus* 4525, *B. circulans* 7049, *B. polymyxa* 8523, *B. brevis* 8185, *B. pumilus* 7061, *B. aterrimus* 230 (all from N. R. Smith); *B. subtilis* 6, 15 u and *B. stearothermophilus* C_2P_3 (American Can Company); *B. subtilis* LB (L. A. Burkey), *B. metiens* (W. A. Randall); *B. stearothermophilus* 1518, 26 and a mesophilic proteolytic anaerobe 3679 (National Canners Association).

The spores were produced on the surface of plain nutrient agar slopes contained in large flat-sided prescription bottles (those of 3679 in thioglycollate supplement broth). Time of incubation was 3 to 4 wk. at the optimum temperature of the organism. The spore crops were collected in the usual manner, filtered through cotton, washed several times and stored in distilled water at 3° C. The inocula were prepared by dilution of the stock suspensions. The concentration of viable spores during the exposure to the quaternaries, except when otherwise noted, was approximately 5 million per ml., that of the thermophilic flat sour types somewhat less.

RESULTS

The quaternary ammonium compounds first were studied in different concentrations at 30° C. and pH 9.6 with exposure periods up to 30 min. Under these conditions no one of the compounds in concentrations up to 5 per cent consistently killed all the spores in the test suspensions within 30 min. When the temperature of exposure was 60° C., the same result was obtained. At exposure temperatures of 80° C., complete killing of the spores was first observed. The data (table 1) show that sterilization of the samples apparently was achieved by several of the quaternaries, but against only a limited number of cultures. The flat sour thermophilic species (1518 and 26), which were the most susceptible, did not grow in subculture after either 20 or 30 min. contact with three of the quaternaries. Doubling the concentration of the quaternaries at 80° C. and with no adjustment of pH (table 2) slightly increased the over-all sterilizing effectiveness of the compounds but reduced the sporocidal activity in specific instances.

[5] American Lecithin Co., Inc.

[6] Tween 80, Atlas Powder Co.

[7] Approved by R. J. Dubos for use in bacteriological culture medium.

TABLE 1

Sporocidal activity of quaternary ammonium compounds (1–20) at 80° C. (spores exposed in buffer solutions pH 9.6)

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with:									
		Roccal	Onyxide	Ceepryn	Nopcocide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan	Amosol #1	Amosol #2
	(*min.*)										
B. subtilis LB	20	3	3	3	3	5	4	11	3	3	3
	30	5	3	3	3	5	4	30	3	4	3
B. subtilis 6	20	1	1	1	1	1	1	1	1	1	1
	30	1	1	1	1	1	1	1	1	3	3
B. aterrimus 230	20	1	1	1	1	1	1	1	1	0	2
	30	2	1	1	1	1	1	2	1	0	0
B. stearothermophilus 1518	20	0[a]	2	7	1	2	2	0	2	1	1
	30	0	0	2	1	2	2	0	7	2	2
B. stearothermophilus 26	20	0	0	0	1	4	4	0	4	1	1
	30	0	0	0	1	4	4	0	4	1	1
Clostridium sp. 3679	20	3	3	4	3	3	3	3	3	12	0
	30	4	3	3	4	3	3	4	3	0	3

[a] 0 = no growth.

TABLE 2

Sporocidal activity of quaternary ammonium compounds (1–10) at 80° C., no pH adjustment[a]
(spores exposed in aqueous solution)

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with:									
		Roccal	Onyxide	Ceepryn	Nopcocide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan	Amosol 1#	Amosol 2#
	(*min.*)										
B. subtilis LB	20	8	3	3	3	4	3	7	3	3	10
	30	8	3	3	3	11	5	5	3	5	4
B. subtilis 6	20	1	1	2	1	1	1	1	1	2	2
	30	1	1	2	1	1	1	1	1	2	4
B. subtilis 15u	20	4	2	2	2	5	3	14	3	2	2
	30	5	2	2	2	3	3	11	3	4	0
B. stearothermophilis 1518	20	5	9	3	1	2	2	0	3	2	2
	30	0[b]	12	3	1	2	3	0	5	2	2
B. stearothermophilis 26	20	0	0	0	3	14	1	0	0	3	3
	30	0	0	0	3	12	10	0	0	3	3
Clostridium sp. 3679	20	5	5	5	5	5	5	5	5	0	0
	30	5	5	5	5	5	5	5	5	0	0

[a] Amosols approximately pH 12, others ranged between pH 3.6–7.5.
[b] 0 = no growth.

TABLE 3

Sporocidal activity of quaternary ammonium compounds (1–20) at 95° C. with variations in pH (spores exposed in buffer solutions)

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with: Roccal pH				Onyxide pH				Ceepryn pH				Nopcocide K pH				Hyamine 10X pH			
		6.4	8.0	9.6	9.6 M[a]	6.4	8.0	9.6	9.6 M	6.4	8.0	9.6	9.6 M	6.4	8.0	9.6	9.6 M	6.4	8.0	9.6	9.6 M
	(*min.*)																				
B. subtilis LB	20	14	0	0	0	2	1	1	2	2	1	1	2	2	2	5	2	12	14	6	6
	30	35	0	0	0	2	1	1	2	2	1	1	8	2	5	4	5	12	9	5	5
B. subtilis 6	20	3	2	0	0	3	1	1	1	1	3	1	2	10	1	1	4	6	1	1	1
	30	3	3	0	0	3	1	1	1	1	3	2	2	3	2	1	4	5	1	1	2
B. subtilis 15u	20	7	0	0	0	2	2	3	3	5	2	3	4	2	2	3	3	3	9	5	5
	30	9	0	0	0	3	7	3	3	3	2	3	5	2	2	0	0	0	10	4	7
B. aterrimus 230	20	0[b]	1	0	0	0	1	1	3	0	0	2	0	1	0	0	0	1	1	1	3
	30	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	2	2	3
B. stearothermophilus 1518	20	2	3		0	5	3		4	0	4		0	1	3		3	1	3		3
	30	0	0		0	6	3		0	4	4		0	1	0		3	2	3		4
B. stearothermophilus 26	20	0	0	0	2	0	0	0	0	0	0	0	0	1	1	3	0	2	4	3	0
	30	0	0	0	0	0	0	0	0	0	0	0	0	1	4	0	0	3	4	4	7
B. stearothermophilus C_2P_3	20	10	0	0		10	8	0		0	0	0		1	2	0		2	2	2	
	30	0	0	0		0	0	0		0	0	0		1	2	2		2	3	6	
Clostridium sp. 3679	20	3	0	0	0	3	3	3	3	3	3	3	3	3	0	0	0	3	3	3	3
	30	0	0	0	0	3	0	3	3	3	0	3	3	0	0	0	0	3	0	3	3
B. metiens	20				1				0				1				0				3
	30				0				0				1				0				0

TABLE 3—(*Continued*)

Culture	Period of exposure	Day on which growth in subculture was first observed after treatment with: Hyamine 1622 pH				Emcol 888 pH				Tetrosan pH				Amosol #1 pH unadjusted		Amosol #2 pH unadjusted	
		6.4	8.0	9.6	9.6 M	6.4	8.0	9.6	9.6 M	6.4	8.0	9.6	9.6 M		M		M
	(*min.*)																
B. subtilis LB	20	3	3	4	3	10	0	11	0	2	4	4	0	0	0	15	0
	30	3	5	5	12	14	15	0	0	2	4	13	4	0	0	0	0
B. subtilis 6	20	3	1	1	1	3	2		4	3	26	1	1	0	0	0	0
	30	3	1	1	2	3	2	8	2	4	2	0	4	0	0	0	2
B. subtilis 15u	20	2	7	5	4	0	18	0	0	2	5	15	8	0	0	0	0
	30	3	7	4	3	0	0	0	0	2	5	14	0	0	2	0	0
B. aterrimus 230	20	3	2	2	3	0	2	4	0	0	1	0	0	6	0	0	3
	30	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. stearothermophilus 1518	20	1	3		3	0	0		0	4	0		0	3	0	0	0
	30	1	3		3	0	0		0	0	0		0	0	0	0	0
B. stearothermophilus 26	20	2	4	3	0	0	0	0	0	7	4	0	0	0	0	0	3
	30	4	4	3	0	0	0	0	0	0	4	0	0	0	0	0	0
B. stearothermophilus C_2P_3	20	2	4	2		0	0	0		10	0	0		0		0	
	30	2	6	2		0	0	0		0	6	0		0		0	
Clostridium sp. 3679	20	3	0	3	3	0	0	3	0	0	0	0	0	0	14	2	14
	30	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
B. metiens	20				0				1				0		0		4
	30				0				1				0				11

[a] M = buffer contained skim milk (1–500).
[b] 0 = no growth.

TABLE 4

The activity of quaternary ammonium compounds (1–20) at 95° C. against spores dried in milk films on stainless steel strips (spores exposed in buffer solutions pH 9.6)

Culture	Day on which growth in subculture was first observed after treatment with:										
	Check	Roccal	Onyxide	Ceepryn	Nopcocide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan	Amosol #1	Amosol #2
B. subtilis LB	1	7	4	4	4	5	7	10	8	1	1
B. subtilis 6	2	0[a]	2	3	0	2	4	0	0	4	4
B. subtilis 15u	1	8	2	4	3	4	0	0	4	4	1
B. stearothermophilus 1518	2	0	0	3	3	0	0	0	0	1	0
Clostridium sp. 3679	2	0	5	3	0	0	0	0	0	0	0

[a] 0 = no growth.

Tables 3 and 4 show the results obtained when the spores were exposed at 95° C. in buffer substrates of varying pH, with and without traces of skim milk. Even at this near-boiling temperature, most of the compounds in 1–20 (5 per cent) concentration did not sterilize the suspensions. Roccal, the most generally effective sporocidal agent under these conditions, apparently killed all the spores of all the test species in 30 min. at pH 9.6 but not at the less alkaline levels. The diminished sporocidal activity at pH 6.4 and 8.0 was evident, also, with the other compounds. The influence of pH on sporocidal activity was revealed further by the observation that Roccal even in 1–10 concentration at 95° C. did not sterilize the *B. subtilis* 6 culture in 30 min. when distilled water at pH 7.5 was the substrate (unpublished data), although at pH 9.6 and 95° C. Roccal sterilized the suspension within 30 min. at 1–20 concentration. The pH of the Amosols was not adjusted, since the high buffering capacity made this impracticable; these formulations ranged from pH 12 to 13.

Traces of skim milk in the exposure substrate extended the time of sterilization in some cultures, this being most frequent with the thermophilic species. Emcol 888, Tetrosan and the Amosols sterilized the thermophilic and anaerobic cultures but were less effective against the *subtilis* types. The spores of *B. metiens,* unlike those of the other test organisms, are relatively sensitive to heat; in consequence most of the spores are killed by the higher heat treatment *per se.* Survival and growth of these organisms in subculture indicates a high degree of resistance to specific quaternaries on the part of very few relatively heat-resistant spores. Spores that survived contact with the quaternaries usually developed more slowly than those not so treated; however, most samples which finally became positive did so within 10 days. In exceptional instances, evidence of growth by survivors first appeared after 40 or more days of incubation.

The test spores developed readily in the basal (Letheen) medium in minimal numbers (10–12 spores per ml.). Accepted as evidence that the Letheen medium provided satisfactory inhibition of quaternary activity was growth in the Letheen broth by minimal number of spores in quaternary concentration equal to the highest used in the test, with momentary exposure of the spores to the quaternary before their transfer to the inhibitor. Results were recorded as negative when seven to ten samples, all treated alike, failed in each instance to show growth within 60 days; when, during this period, one or more samples in a replicate series revealed growth and microscopic examination of film preparations indicated close correspondence to the test organism, results were recorded as positive.

In the foregoing experiments, the spores were exposed to the quaternaries when suspended in fluids. Under conditions which usually prevail in food processing establishments, the spores may be contained in films of organic matter adhering to the surfaces of equipment. The capacity to reach and kill spores embedded in such films is an essential requirement of a chemical sterilizer. To obtain some information on the activity of quaternaries in these circumstances, spores were dried in skim milk films on small stainless steel strips ($50 \times 8 \times 2$ mm.). The sterile, fat-free strips first were dipped into cold milk heavily inoculated with spores, then suspended flatwise in a level plane on wood supports in

sterile petri plates and held at sub-minimum growth temperatures until the films were thoroughly dry, when the process was repeated two or three times.[8] The strips with adherent layers of dried milk film containing spores then were exposed to the quaternaries in buffer at pH 9.6 and 95° C. After the exposure, the strips were drained quickly and transferred to Letheen broth. One-tenth ml. quantities of the exposure substrate also were subcultured in separate flasks, since these usually contained some flakes of milk film detached from the strips during the heating treatment. Drying of the film is greatly retarded at low temperatures but at higher temperatures some of the mesophilic aerobic spores may germinate before drying of the films is complete.

Differences may be observed in the sporocidal activity of several compounds depending on the method by which they were tested (tables 3 and 4). Although the presence or absence of a protective film may be considered to be the chief variable, the numbers of spores exposed and subcultured were not directly comparable in the two tests. The results shown in table 4 indicate that, when the spores were protected in milk films, none of the quaternaries was able to kill all of the spores in all the test samples. This provides a rigorous test of effectiveness, yet the only safe criterion of the practical value of a sterilizing agent.

Since the germicidal activity of quaternaries may be enhanced by trisodium phosphate (18), some observations were made upon the sporocidal activity of quaternaries in this substrate. Dry milk films containing spores deposited on stainless steel strips as previously described were exposed to the quaternaries (1–20) at 95° C. in aqueous solutions of trisodium phosphate (2.22 per cent). The pH was unadjusted. As with the previously described strip results, the quaternaries did not consistently sterilize all the samples with exposures up to 30 min.

Many investigators have noted the powerful bacteriostatic action of quaternary ammonium compounds (1, 5, 6, 10, 19). Few have reported on the minimum concentration of quaternary required to produce sporostasis.

In tables 5 and 6 are given the approximate limiting concentrations of the compounds in nutrient broth and in skim milk. Since the amount of quaternary necessary to prevent development of the spores may be influenced by the number of spores present, some of the results are given at two levels of spore concentration. Viable vegetative cells were absent from most of the spore crops as harvested; in others, when prolonged incubation of the slopes did not eliminate the vegetative cells, the latter were killed by mild heating (85° C. for 15 min.) before the inoculations were made.

Table 5 shows that all the compounds (except the Amosols) were sporostatic in high dilution in nutrient broth. *B. metiens* was most tolerant, while certain strains of *B. subtilis* and *B. stearothermophilus* were inhibited by less than 1 part quaternary in 5 million.

Increasing the number of spores in broth usually increased the concentration of

[8] Concentration of viable spores in milk used to inoculate strips was approx. 100 million per ml. for *B. subtilis* cultures and 7 million per ml. for cultures 1518 and 3679. Each dip of strip picked up approx. 0.08 g. of fluid milk inoculum.

TABLE 5

Concentration of quaternaries which produced sporostasis in nutrient broth

Culture	No. spores in 10 ml. medium	Roccal	Onyxide	Ceepryn	Nopcocide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan	Amosol #1[a]	Amosol #2[a]
		(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)
B. mycoides 6462	50	1	1	1	1	0.4	1	1	1	40	40
	1,000,000	2	2	1	4	1	1	4	2		
B. metiens	50	2	2	2	2	2	4	4	2	200	1,000
	1,000,000	4	2	4	4	2	10	10	2		
B. cereus 401	50	1	2	1	2	1	1	2	1	40	40
	1,000,000	4	2	4	4	4	4	4	4		
B. sphaericus 4525	50	1	1	1	1	1	1	1	1	40	40
	1,000,000	2	2	2	2	2	4	2	1		
B. circulans 7049	50	1	1	1	1	1	1	1	1	40	40
	1,000,000	4	4	2	2	4	4	4	4		
B. polymyxa 8523	50	2	2	1	1	2	2	2	1	100	100
	1,000,000	4	4	2	2	4	4	4	4		
B. subtilis LB	50	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	20	20
	1,000,000	< 0.4	< 0.4	< 0.4	1	< 0.4	< 0.4	< 0.4	< 0.4		
B. subtilis 6	50	2	1	1	1	1	1	1	1	40	40
	1,000,000	2	4	1	4	1	2	4	2		
B. subtilis 15u	50	< 0.1	0.2	0.4	0.4	0.1	0.4	0.4	1	20	20
	1,000,000	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4		
B. aterrimus 230	50	1	1	1	2	1	1	1	1	40	40
	1,000,000	2	2	2	2	2	2	2	2		
B. pumilus 7061	50	1	1	1	1	1	1	1	1	40	40
	1,000,000	2	1	2	2	2	2	4	1		
B. brevis 8185	50	1	1	1	1	1	1	4	1	40	40
	1,000,000	2	4	2	4	2	4	4	2		
B. stearothermophilus 1518	50	1	0.2	0.2	1	1	1	1	1	> 1	> 1
B. stearothermophilus 26	50	< 0.1	< 0.1	< 0.1	0.2	< 0.1	0.2	0.2	1	> 1	> 1
B. stearothermophilus C_2P_3	50	0.2	< 0.1	< 0.1	0.2	0.2	0.2	0.1	1	0.2	0.2

[a] Data not directly comparable with that of the other compounds.

TABLE 6

Concentration of quaternaries which produced sporostasis in skim milk (50 viable spores /ml.)

Culture	Roccal	Onyxide	Ceepryn	Nopcocide K	Hyamine 10X	Hyamine 1622	Emcol 888	Tetrosan	Amosol #1	Amosol #2
	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)	(*p.p.m.*)
B. mycoides 6462	100	200	200	200	200	200	200	200	2,000	2,000
B. metiens	1,000	1,000	1,000	1,000	2,000	1,000	2,000	1,000	> 2,000	> 2,000
B. cereus 401	200	1,000	1,000	1,000	200	1,000	1,000	1,000	>> 2,000	>> 2,000
B. circulans 7049	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	>> 2,000	>> 2,000
B. polymyxa 8523	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	>> 2,000	>> 2,000
B. subtilis LB	100	200	100	100	< 40	100	100	100	< 1,000	< 1,000
B. subtilis 6	200	200	200	200	200	200	1,000	1,000	2,000	2,000
B. subtilis 15u	100	100	200	200	100	100	100	40	1,000	1,000
B. aterrimus 230	200	1,000	200	1,000	100	200	1,000	200	2,000	2,000
B. brevis 8185	1,000	200	200	1,000	200	200	1,000	1,000	> 2,000	
B. stearothermophilus 1518	< 100	< 100	< 100	< 100	< 100	< 100	< 100	< 100	1,000	1,000
Clostridium sp. 3679	1,000	1,000	200	1,000	1,000	1,000	1,000	1,000	4,000	4,000

quaternary necessary to produce sporostasis; the greatly diminished sporostatic activity of quaternaries in milk is shown in table 6.
In this medium the minimal inhibiting concentration is frequently 1,000 times greater than for nutrient broth with comparable inocula.

DISCUSSION

It generally is recognized that surface film should be removed from equipment by prior detergent action to enable the sterilizing agent to act effectively; however, since this is not always realized, the performance of a germicide in the presence of organic matter is of practical significance.

The results of the foregoing study indicate that quaternary ammonium compounds are not efficient sterilizing agents against bacterial spores. Complete destruction of a spore population rarely can be effected by these compounds within 30 min. and then only at concentration levels and under other conditions of exposure that would greatly limit their commercial usefulness.

The conflicting nature of published reports in this field may be ascribed in small part to the use of different organisms but more particularly, to defects in the evaluation technique. Chief among these is the failure of many investigators to differentiate between sporocidal and sporostatic effects. Although quaternaries are strongly adsorbed on bacterial surfaces (22) and in many substrates may inhibit growth in high dilutions (*loc. cit.*), many have worked with these compounds in the relatively high concentrations necessary to affect spores, yet have made no provision for detoxification of the quaternary transferred with the spores to the subculture medium. In the present study, sporostatis was observed frequently in broth at dilutions so high that it would be impracticable to employ this method to eliminate the inhibiting effect. Of interest in this connection is the demonstration by Kivella *et al.* (15), that it is possible to remove surface-active cations from the surface of bacterial spores by a combination of dilution and vigorous shaking or centrifugation.

SUMMARY

Eight quaternary ammonium compounds and two quaternary-containing detergents were studied in respect to their spore-killing activity in buffer or distilled water solutions.

The sporocidal activity increased with temperatures at 30, 60, 80 and 95° C. The sporocidal activity increased with the degree of alkalinity at pH 6.4, 8.0 and 9.6.

Traces of skim milk and the protection afforded by milk films, in general, decreased the sporocidal activity of the quaternaries.

At the highest observed temperature (95° C.) and most favorable pH (9.6), concentrations of quaternaries up to 1–20 did not kill all the spores of all test samples within 30 min.

Quaternaries were highly sporostatic in nutrient broth and had relatively low sporostatic activity in skim milk.

Discrepancies in the reported sporocidal activity of quaternary ammonium compounds are discussed.

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STUDIES ON THE FEEDING VALUE OF MOW-CURED BALED HAY[1]

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In the search for better methods of preserving high quality roughages much interest has been shown in the mow-curing of hay by both experiment station workers (2, 6, 11, 12, 13, 16, 20) and farmers. Most of the experimental work has centered around the completion of the drying of long or chopped hay and its feeding value. Fewer studies, however, have been made on the mow-curing of baled hay and its feeding qualities (6, 7, 14, 17).

Aitkenhead (1) in 1926 at the Purdue Agricultural Experiment Station seems to have been the first investigator in this country to use supplemental heat to aid in the curing of hay. In more recent years others (3, 6, 16, 17, 18) have applied heat with some success. Since in 1945 practically nothing was known about the mow-curing of baled hay and because field balers were being developed rapidly, a study on the feeding value of mow-cured baled hay was undertaken.

PROCEDURE

The feeding value of field-cured and mow-cured baled alfalfa hays was compared in feeding trials conducted during the winter months for a period of 3 successive years. In order to eliminate the factor of different soil conditions, the hays studied were produced on the same field and alternating windrows were used for the field- and the mow-cured hays. All hays except the first cutting in the third year were cut between the one-half and three-quarter bloom stage.

After cutting, the hays for mow-curing were partly dried in the field to a moisture content ranging from 30 to 40 per cent, whereas the field-cured hays were dried to about 20 per cent moisture content. All hays were baled in the field with a pickup baler and hauled immediately to the barn. The hays to be mow-cured were placed in a $20 \times 20 \times 10$ ft. high experimental drying bin with a slatted floor, and air, at the rate of about 25 ft.^{3} per min. per ft.^{2} of floor space, was forced through the hay until cured. In the first 2 yr. unheated air was used, whereas heated air was used in the third year (8.5 and 15° F. rise). The first cutting hays each year contained considerable timothy, while the other cuttings were almost pure alfalfa.

In the first 2 yr. three cuttings of alfalfa hay were studied; two cuttings were used in the third year. The reason for the difference in the third year was that excessive rains in the fore part of June delayed the first cutting until the last week

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of that month. Therefore, only two cuttings were obtained that year. Each of the cuttings of all 3 yr. was fed to dairy cows in test periods of 9 wk.

The groups fed the experimental hays included five to six cows each and consisted of Holsteins, Jerseys, and Guernseys, with the Holsteins predominating. The two groups of cows of each experiment were balanced as evenly as possible at the time of allotment. The hays were fed to the experimental cows as the only roughage at the rate of 2.5 lb. of hay per 100 lb. of live weight. This proved to be about all the hay the cows would consume. A 13.5 per cent total protein grain mixture was fed to the cows on the basis of 1 lb. of grain for each 4 lb. of 4 per cent fat-corrected milk produced by the cows in a preliminary period before each feeding trial. The grain mixture consisted of 400 lb. yellow corn, 200 lb. oats, 100 lb. linseed oil meal, 10 lb. iodized salt and 7 lb. steamed bone meal.

The carotene contents of the hays were determined by a modification of the method of Moore and Ely (19), of the blood plasma by Moore's method (10) and of the butterfat by the method of Koehn and Sherman (8). The butterfat was assayed for vitamin A potency by the usual rat-growth method, using USP reference oil as a standard.

RESULTS AND DISCUSSION

The carotene content of the hays was used as a sensitive index to the state of hay preservation. Table 1 contains the carotene values of the field- and mow-

TABLE 1

Carotene content and grades of the experimental baled alfalfa hays

Yr.	Cutting	Carotene (ppm on dry basis)				Federal grade no.[b]
		As cut	Into barn	Cured	As fed	
1945	1st.—Field	209	67[a]	24	10	
	Mow	209	101	30	13	
	2nd.—Field	226	48[a]	15	9	
	Mow	226	93	56	25	
	3rd.—Field	216	48	31	25	
	Mow	216	52	42	25	
1946	1st.—Field	167	116	26	3	3
	Mow	167	107	27	7	2
	2nd.—Field	303	7[a]	3	2	3, leafy
	Mow	303	85	25	15	1, extra leafy
	3rd.—Field	210	23[a]	.....	12	1
	Mow	210	59	.....	39	1, extra leafy, extra green
1947	1st.—Field	150	9[a]	9	4	2
	Mow	150	16[a]	10	7	1
	2nd.—Field	360	93	15	5	3, leafy
	Mow	360	144	76	48	1, extra leafy, extra green

[a] Rain on the hay in the field.
[b] No Federal grade available in 1945

cured baled hays by each year's cuttings as cut, as going into the barn, as completely cured (after barn drying) and as fed some 5 to 6 mo. later. In table 1, the carotene content also may be compared to official federal gradings of the experimental hays. As a rule, the mow-cured hays graded at least one grade higher than the field cured.

It is very interesting to note the difference in the rate of carotene destruction after the forage was cut and up until the time of storage. This destruction in the field was much more rapid in the case of the second and third cutting hays of the first 2 yr. than the first cutting. One logical explanation for this is the lower temperatures and more hours of cloudiness which would retard the destructive action of the enzyme lipoxidase (9). Any advantage in the lower carotene destruction of the first cutting hays in the field was lost, however, in storage. In all 3 yr. of study, the carotene content of the first cutting hays, regardless of treatment, were relatively low at the time of feeding. This has been observed by others (11). Even heated air (8.5° F. rise) in the third year was of no obvious benefit in curing the first-cutting mow-cured hay. This, however, was due to the facts that the latter hay was past bloom stage and, therefore, relatively low in carotene at the time of cutting and also that most of the carotene was lost in the field before mow-curing.

One of the real advantages of mow-curing of hay is the early removal of the hay from the field without much, if any, rain on it. This is illustrated by the figures in table 1 where rain on the various hays is indicated and the corresponding greater carotene destruction can be noted. In four out of eight sets of experimental hays the mow-curing process allowed the hay to be placed in the barn without rain, whereas, the corresponding field-cured hays were rained on. In only one out of eight cases did rain fall on both the field- and mow-cured hays. Rain does the greatest damage when it falls on hay after the hay is partly dry.

The mow-curing process without supplemental heat showed its greatest advantage in the second cutting hays when the outside air temperature was high and not very humid but showers were rather frequent. Even so, the over-all differences observed in this study between mow- and field-curing are not striking. It appears, then, from the losses in carotene content of the hays that the mow-curing of baled hay with unheated air, using about 25 $ft.^3$ of air per min. per $ft.^2$ of floor space, is difficult and, at best, the results are likely to be quite variable. The application of heat (15° F. rise) in the third year, second cutting, proved to be very successful and appears to offer a much more dependable process for baled hay. From studies by other experiment station workers (5, 12, 13, 20, 21) on the mow-curing of long hay with unheated air, the carotene content of long hay seems to be more easily preserved and, undoubtedly, a higher quality hay usually results than is the case of baled hay dried with unheated air.

In general, the carotene content of the experimental hays is reflected directly in the cows by the carotene content of the cows' blood and butterfat and the vitamin A potency of the butterfat (table 2). The carotene content of the bloods was determined every 3 wk. during the trial. The carotene and the vitamin A potency of composite samples of butterfat were determined also at the end of each 9-wk. feeding trial. The carotene contents of the hays were reflected in the carotene contents of the blood, the milk fat and the vitamin A potency of the milk fat during the successive periods of each trial.

A summary of each 9-wk. feeding trial for the 3 yr. is presented in table 3.

TABLE 2

The carotene content of cows' blood and milk as influenced by feeding baled alfalfa hays

	First cutting						Second cutting						Third cutting			
	1945		1946		1947		1945		1946		1947		1945		1946	
	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow
Carotene in hay (ppm/D.M.)	10	13	3	7	4	7	9	25	2	15	5	48	25	25	12	39
Av. carotene intake (mg./d.)	111	144	33	77	53	92	101	296	24	184	67	620	304	302	152	497
Blood carotene[a] (γ/ml.)																
Initial	7.6	7.5	7.8	8.3	6.6	8.7	2.4	2.7	5.6	5.1	3.4	2.6	3.0	2.4	4.3	1.3
3 wk.	3.4	3.0	6.6	6.4	3.7	5.0	1.8	3.6	2.3	4.8	1.9	3.7	3.3	2.7	4.2	4.7
6 wk.	2.7	3.3	6.0	5.5	2.3	3.2	1.7	3.3	1.4	4.6	1.0	3.3	2.8	2.5	3.2	4.1
9 wk.	2.4	3.0	4.3	4.5	1.8	2.6	1.3	2.8	1.3	4.3	1.6	4.3	2.7	2.4	3.7	4.8
Milk carotene (γ/g. fat)																
Initial	.....	.....	6.6	6.0	5.6	6.0	3.8	3.5	4.1	5.6	1.8	1.8	2.4	3.2	2.8	1.0
Final	2.4	3.2	3.4	3.9	1.9	1.3	2.2	3.5	1.0	2.8	1.0	2.8	3.8	3.5	2.3	3.4
Vitamin A (I.U./g. fat)																
Initial	.....	.....	47	38	28	27	35	37	38	40	16	15	27	28	22	15
Final	27	28	30	35	17	14	25	40	15	22	9	Lost	36	37	22	40

[a] 1945—whole blood; 1946 & 1947—plasma.

TABLE 3

Summary of 9-wk. hay feeding trials (lb.)

	First cutting						Second cutting						Third cutting			
	1945		1946		1947		1945		1946		1947		1945		1946	
	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow	Field	Mow
No. of cows	6	6	6	6	5	5	6	6	6	6	5	5	6	6	6	6
Grain consumed	9.1	9.1	7.8	7.8	9.0	9.3	7.0	7.0	7.7	7.8	6.0	6.1	6.3	6.3	6.5	6.5
Hay consumed	24.6	24.2	26.0	26 0	29.3	28 9	26.4	26 5	28.5	28.7	29.7	30.3	26.3	25.8	30 1	30.1
Av. wt. of cows	1003	1024	1130	1139	1227	1277	1081	1062	1200	1213	1193	1231	1108	1110	1253	1219
Av. gain in wt.	59	57	20	25	24	32	29	46	19	40	34	18	23	35	47	36
Av. daily milk	28.7	27.4	24.2	24.4	26.7	25.4	22.9	23.5	27.1	27.0	22.0	24.7	19.1	18.6	23.2	24.2
Av. daily 4% F.C.M.[a]	29.6	28.6	25.2	25.7	27.2	27.0	23.5	24.6	27.4	28.4	23.5	25.2	20.9	20.0	24.4	25.0
Av. decline 4% F.C.M.	10.7	7.9	3.5	3.9	8.8	14.6	2.9	2.6	3.0	3.9	4.0	0.1	2.3	1.7	4.5	3.6
Av. 4% F.C.M. produced per lb. T.D.N. consumed	1.47	1.44	1.33	1.36	1.27	1.26	1.18	1.22	1.37	1.40	1.16	1.22	1.09	1.05	1.19	1.20

[a]Gaines' formula used (4).

Since the groups of cows from one experiment to another were not necessarily similar, no detailed comparisons should be made from year to year or cutting to cutting. Only the field- and mow-cured hays of the same cutting and year can be compared directly. Although none of the possible comparisons of the value of the hays in table 3 showed significant differences, it is interesting to note two things. In five out of eight trials the cows fed the mow-cured hays produced a small amount more milk than those fed the field-cured. Also, in the case of the second cutting hay, mow-cured in the third year by means of heated air, the cows showed remarkable persistency. Of the five cows in this group, three were producing more milk at the end of the 9-wk. feeding trial than at the beginning, one held her production and one dropped, giving an average decline of only 0.1 lb. of 4 per cent fat-corrected milk for the entire period. The cows in the experiments were fed controlled amounts of hay (2.5 lb. of 100 lb. live weight) and grain. For this reason large differences in the feeding value of the hays would have to exist in order to demonstrate significance.

On the whole, the observations on the feeding value of the experimental hays are not greatly different from those found at other experiment stations. Rollins and Reaves (13) found in a double reversal feeding trial that 4 per cent more milk was produced by the cows fed barn-cured hay as compared to those fed field-cured hay. Morrison and Turk (11), in a summary of 3 yr. of studies, found no significant difference between the barn-cured and field-cured hays as measured by daily milk production and hay consumption. Also, in experiments by Shepherd *et al.* (15, 16) no significant difference was found between the average daily milk production of cows fed barn-cured and field-cured hays.

When equal amounts of hays with similar leaf content and from the same crop are fed, the above results on milk production might well be expected, because from the work by Camburn (2) the digestible protein and total digestible nutrients are about the same for similar hays cured either in the barn or field. Concluding from all observations it would seem that the mow-cured hay probably would have to average at least two grades above that of the field-cured in order to show significant differences between mow- and field-curing processes.

It is only when the mow-curing system consistently produces high quality hay and the field-cured hay has been abused by leaching rains and/or by improper handling for best preservation of hay that significant differences on a pound for pound basis can be expected. In such cases of wide differences between hays cured in the barn or field, palatability also would become a factor in hay feeding experiments. The importance of palatability can be shown more satisfactorily in trials where the hay is fed *ad libitum*.

Another way in which mow-curing systems show up to an advantage is the nutrients preserved per acre. Work by Shepherd *et al.* (5, 15, 16) has shown striking advantages for preservation of hay crops as wilted silage, as compared to field- or mow-cured hay. Again, mow-cured hay showed a distinct advantage over field-cured hay when measured on the basis of the preservation of the original plant nutrients in the field at the time of cutting. However, carotene

was the only nutrient whose losses during the curing processes and storage was studied in our experiments.

SUMMARY

The feeding value of similar baled alfalfa hays cured either in the mow or field was studied over a period of 3 yr. Without the aid of heated air the carotene content of the mow-cured hays was variable, depending on the prevailing weather conditions. When the outside air was cool and humid, as in the cases of the first-cutting hays, the mow drying process was prolonged, with most of the carotene in the hay being destroyed.

The mow-curing of baled hay seems to be a much more difficult process than the curing of long hay because of the difficulty of getting air to pass through the bales, even though loosely packed. The use of supplemental heat is to be recommended as a more dependable processing procedure for baled hay.

When compared in feeding trials in which equal quantities of hays were fed on a cow-weight basis and with concentrates at the rate of 1 lb. for each 4 lb. of 4 per cent fat-corrected milk, no significant differences in feeding value were found between baled hays cured either in the mow or in the field. The mow-cured baled hays seemed to be as palatable to the dairy cows as the field-cured baled hays.

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A SYNTHETIC PABULUM VS. YOLK-CITRATE BUFFER AS A DILUTER OF BULL SEMEN[1]

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In 1939, Phillips (4) developed an egg-yolk buffer for preserving and diluting bull semen. That this buffer was satisfactory for use in artificial breeding was indicated by Phillips and Lardy (5) with limited field trial data. Salisbury, Fuller and Willett (7) reported advantages in substituting sodium citrate for the dibasic sodium phosphate in Phillips' buffer. Use of the yolk-phosphate or the yolk-citrate buffer has become a general practice in artificial breeding.

In 1946, Phillips and Spitzer (6) developed a synthetic pabulum. The essential ingredients were "freshly purified lipids, specific sugars—glucose and galactose—a buffer system, a gum to supply the proper physical consistency and an agent to control bacterial contamination." Limited field trial data indicated that breeding results from the use of such a pabulum might be comparable to those from the use of yolk-buffer.

The object of the investigation reported here was to obtain further information concerning the fertility of semen diluted with this synthetic pabulum[3] as compared with semen diluted with yolk-citrate buffer.

EXPERIMENTAL PROCEDURE

The dry ingredients of the synthetic pabulum were prepared in two compounds by the Department of Biochemistry in accordance with the formula published by Phillips and Spitzer (6). Once each week the compounds were combined in solution for use. Twenty-five g. of compound No. 1 were added to 50 ml. of distilled water at boiling temperature and stirred until the solute was dissolved. Three and one-half g. of compound No. 2 were added and dissolved as completely as possible. The solution then was cooled and stored at a temperature of 40 to 42° F.

The yolk-citrate buffer was prepared twice a week. Thirty-two g. of crystalline $Na_3C_6H_5O_7 \cdot 2H_2O$ were dissolved in 1000 ml. distilled water and brought to boiling. After cooling, this solution was mixed with an equal volume of egg yolk, strained and stored at a temperature of 40 to 42° F.

This field trial employed semen from ten Holstein and nine Guernsey bulls in the University of Wisconsin bull stud. Their 60- to 90-day non-returns for the period of the experiment ranged from 23 to 68 per cent.

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Semen was collected on Monday and Thursday of each week and was used routinely for 3 days. No experimental breedings were made on Sunday. A system of balancing was carried out so that, as far as possible, semen collections were made from all 19 bulls with equal frequency.

Immediately after collection, the semen was examined for motility and diluted 1 : 30 to 1 : 40, depending on the volume collected and the amount of diluted semen needed. Both diluters were warmed in a water bath to 65 to 70° F. prior to being mixed with raw semen. One-half of each semen sample was diluted with the synthetic pabulum and the other half with yolk citrate. The rate of dilution was the same for both treatments.

Inseminations for this experiment were made during the period from May 3 to September 13, 1948. Fertility was measured by pregnancy examinations (8) and was based on both first and second services in herds in Dane County. The two treatments of semen were used alternately during the day so that the same number of inseminations per treatment were made insofar as the number of cows to be bred permitted.

TABLE 1

Breedings with semen from bulls having 60- to 90-day non-returns below 40 per cent for 2 or more mo. during the period of the experiment

Day of use	Treatment	No. of breedings	% Fertile[b]	Chi-square
1[a]	Synthetic pabulum	71	14.1	0.03
	Yolk citrate	71	12.7	
2	Synthetic pabulum	75	9.3	2.08
	Yolk citrate	75	17.3	
3	Synthetic pabulum	64	6.2	4.58[c]
	Yolk citrate	64	18.8	
Over-all 3 d. of use	Synthetic pabulum	210	10.0	3.54
	Yolk citrate	210	16.2	

[a] Day of use 1 is the day of collection.
[b] These fertility percentages are based on pregnancy examinations. The same results based on 60- to 90-d. non-returns would be approximately 6 to 7 % higher. (1)
[c] $P < 0.05$.

TABLE 2

Breedings with semen from bulls having 60- to 90-day non-returns above 40 per cent

Day of use	Treatment	No. of breedings	% Fertile[b]	Chi-square
1[a]	Synthetic pabulum	144	38.2	10.20[c]
	Yolk citrate	144	57.6	
2	Synthetic pabulum	146	31.5	11.88[c]
	Yolk citrate	146	51.4	
3	Synthetic pabulum	142	29.6	14.04[c]
	Yolk citrate	142	51.4	
Over-all 3 d. of use	Synthetic pabulum	432	33.1	36.82[c]
	Yolk citrate	432	53.5	

[a] Day of use 1 is the day of collection.
[b] These fertility percentages are based on pregnancy examinations. The same results based on 60- to 90-d. non-returns would be approximately 6 to 7 % higher. (1)
[c] $P < 0.01$.

Inseminations where the semen was deposited in the cervix instead of the uterus were eliminated. The daily first-service inseminations made by each inseminator and with semen of each bull were balanced so that there were an equal number of breedings for each treatment. The daily second-service breedings were balanced in the same manner. Such elimination of breedings as was found necessary was carried out by the use of random numbers. The data were then tabulated and analyzed by chi-square.

The trial was planned to terminate when a preliminary analysis of the data indicated a real difference in fertility between the treatments of not less than 5 per cent.

RESULTS AND DISCUSSION

The data obtained are presented in tables 1 and 2. The fertility of semen diluted with yolk-citrate averaged 15 per cent above the fertility of semen diluted with the synthetic pabulum. The chi-square test showed this difference to be highly significant. Allowing for the error that one would expect in the average difference found in this trial, the real difference may be considered as lying within the range 7 to 23 per cent.[4] The interaction chi-square (3) between days of use was not significant, indicating that the difference between treatments may be considered the same for all days of use.

Bulls whose 60- to 90-day non-returns were lower than 40 per cent for 2 mo. or more during the experiment were considered as "bulls of relatively low fertility." The results of experimental breedings made with semen from these bulls are shown in table 1. Results of breedings made with bulls of "relatively high fertility" are shown in table 2. The tables appear to show that the difference between diluters was greater for the bulls of higher fertility than for the bulls of lower fertility. However, the statistical analysis did not substantiate this. The analysis also indicated that the results of the trial did not vary significantly between individual bulls, between breeds or between inseminators or services.

In drawing conclusions regarding the level of fertility shown here as resulting from the use of this synthetic pabulum, three considerations should be made: (a) The level of fertility found with this diluter depended to some extent on the fertility level of the bulls used. (b) The fertility percentages shown here are based on pregnancy examinations and would be approximately 6 to 7 per cent higher if 60- to 90-day non-returns had been used (1). (c) The comparison between the two diluters is more important in this trial than is the actual fertility level obtained with the synthetic pabulum.

SUMMARY

In a trial involving 1,284 field inseminations, the fertility was 15 per cent higher with semen of 19 bulls diluted with egg-yolk citrate buffer than with other portions of the same semen diluted with a synthetic pabulum. The real difference may be considered as lying within the range 7 to 23 per cent.

[4] 95% fiducial limits (2).

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THE INFLUENCE OF CRACKED SOYBEANS, SOYBEAN HAY AND VARIOUS KINDS OF CONTAINERS ON THE FLAVOR OF MILK[1]

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The Iowa dairy industry long has been troubled with the development of oxidized and other undesirable flavors in milk. Since soybeans are the chief source of home-grown protein in Iowa, some of these flavors were attributed to soybean feeding. Creamery operators of the state continually reported that they were receiving milk and cream which had what was described as a "soybean" flavor.

Earlier work at this station (4, 5, 6, 12, 13) showed no indications of milk off-flavors when soybeans were fed in the usual amounts. Similar results were obtained by other workers (2, 7, 8, 9, 10). However, since soybeans have been indicated to increase the proportion of unsaturated fatty acids in butterfat, and since a number of farmers occasionally transport their milk in rusty tin cans, it was felt that the exposed surface of the iron might act as a catalyst aiding in the oxidation of the unsaturated fatty acids, thus resulting in oxidized flavors.

An experiment was set up to compare the milk from a group of cows fed cracked soybeans in amounts usually used by Iowa dairymen with that of another group fed similar amounts of linseed oil meal. The milk from both groups was collected in glass, tinned iron and rusty tinned iron containers[3] and scored for flavor.

At the conclusion of this experiment, a second experiment was initiated to answer certain questions raised by the results of the first. Since the first experiment was conducted during the months of April to July, it was felt that work on similar lines should be conducted during the cold months,[4] as there might be a seasonal effect involved. The effect of soybean hay was not studied in the previous work. Therefore, in the second experiment, the effect of soybean hay on the flavor of milk was checked simultaneously with that of cracked soybeans.

EXPERIMENT I

Method of experimentation. Previous work (4) has shown that when selecting cows for flavor studies, selection should involve the flavor scores of their

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[3] For brevity the tinned iron and rusty-tinned iron containers will be referred to subsequently as tin and rusty tin containers, respectively.

[4] The second experiment extended from Nov. through May.

milk. Sixteen Holstein cows were chosen from the college herd and fed a common ration for a preliminary period of 24 days. During this period individual milk samples were collected and scored twice a week for flavor.

On the termination of the preliminary period 10 of the 16 cows were selected and paired as similarly as possible with regard to age, stage of lactation, production of milk and butterfat and the flavor scores of their milk. The cows in each pair then were placed at random in one or the other of two groups.

A double reversal design of feeding was used (table 1) comparing two rations

TABLE 1

Feeding schedule—Experiment I

	Duration	Rations fed to:	
		Group I	Group II
	(*d.*)		
Preliminary Period	24	Standard ration	Standard ration
Transitional period	10	Soybean ration	Linseed oil meal ration
Exp. period I	21		
Transitional period	10	Linseed oil meal ration	Soybean ration
Exp. period II	14		
Transitional period	10	Soybean ration	Linseed oil meal ration
Exp. period III	11		
Transitional period	10	Linseed oil meal ration	Soybean ration
Exp. period IV	13		

which were similar except that the experimental concentrate contained 11.1 per cent cracked soybeans, whereas the control contained an equivalent amount of linseed oil meal substituted for the soybeans. The grain rations contained four parts corn, two parts rolled oats, two parts wheat bran, and one part cracked soybeans or linseed oil meal. This ration was fed at the rate of 1 lb. grain to every 4 lb. of milk produced. Alfalfa hay of fair quality was fed *ad libitum* as the only roughage. A 10-day transitional period was allowed whenever the feeds were reversed.

Milk samples were collected for scoring three times during each feeding period. When sampling, portions of each cow's milk were put in a 0.5-pt. milk bottle, a 1-pt. tin container and a 1-pt. rusty tin container. The rusty tin containers were made as uniformly rusty as possible by scratching one line deeply around the side and two lines across the bottom of the tin plated surface. The untarnished tin containers immediately were replaced by new ones, once any corrosion in them was noticed. All containers were sterilized in an autoclave before being used.

The milk samples were gathered at 3:30 A.M. on collection days and cooled immediately. They were scored about 12 hr. later by experienced judges from the Dairy Industry Department. The score card suggested by the Committee on Score Cards, A.D.S.A. (1, p. 4) was used.

Results and discussion. The flavor score data showing differences between the two rations and differences between the three types of containers are summarized in table 2. The differences in scores of the milk produced by the two

TABLE 2

Average flavor scores of milk held in glass, tin and rusty tin containers for each group during each sampling when the animals were fed either cracked soybeans or linseed oil meal. Experiment I

Feeding periods	Samplings	Soybean ration				Linseed oil meal ration			
		Group	Containers			Group	Containers		
			Glass	Tin	Rusty tin		Glass	Tin	Rusty tin
I	1	I	37.9[a]	37.7	37.5	II	37.9	37.6	37.5
	2		37.2	37.2	37.1		37.3	37.6	37.9
	3		37.2	37.5	37.9		37.4	37.9	37.7
II	1	II	37.6	37.7	37.9	I	37.9	37.6	37.8
	2		37.8	37.9	38.0		37.8	37.8	37.5
	3		37.6	37.7	37.8		37.9	38.0	37.8
III	1	I	38.2	38.1	38.0	II	38.0	37.9	38.1
	2		37.9	38.0	38.0		38.0	38.0	37.8
	3		38.2	38.0	37.9		37.8	38.0	37.8
IV	1	II	37.3	37.2	37.3	I	37.5	37.5	37.3
	2		38.0	37.9	37.9		38.1	38.0	37.9
	3		37.9	37.9	37.9		38.0	38.2	37.8
Container Av.			37.73	37.73	37.77		37.80	37.84	37.74
Ration Av.			37.74				37.79		

[a] Each of these scores is an av. of five scores obtained from the five cows in each group.

rations were so slight that, under the conditions of this trial, soybeans did not produce flavors in milk that were more undesirable than those produced when linseed meal was the concentrate. No oxidized flavors developed in the milk during the experiment, even though no green feed was fed. Little difference in quality occurred in the milk collected and held for a 12-hr. period in glass, in tin and in rusty tin containers.

EXPERIMENT II

Method of experimentation. Sixteen Holstein cows were fed a common ration for a preliminary period of 15 days. At the end of this period 12 cows were selected and placed in three groups as uniformly as possible with regard to flavor of milk, number of previous lactations, stage of lactation, size and production level. A cow from each of the three groups was started on one of the following four experimental rations: ration A—soybean hay as roughage, a basal grain mixture and 11.1 percent linseed meal as the protein supplement; ration B—alfalfa hay as roughage, a basal grain mixture and 11.1 percent cracked soybeans as the protein supplement; ration C—soybean hay as roughage, a basal grain mixture and 11.1 percent cracked soybeans as the protein supplement; and ration D—alfalfa hay as roughage, a basal grain mixture and 11.1 percent linseed meal as the protein supplement. Hay was fed *ad libitum;* grain was fed at the rate of 1 lb. for every 4 lb. of milk produced.

Since four rations could not be tested adequately during the lactation period through the double reversal design, the design suggested by Cochran, *et al.* (3) was used. This design (table 3) reduces variation resulting from cow and ra-

TABLE 3

Feeding schedule—Experiment II

Duration	Rations fed											
	Group I				Group II				Group III			
	Cow no.				Cow no.				Cow no.			
	2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional (*d.*) period ..... 14 Exper. Period I..... 20	A	B	C	D	A	B	C	D	A	B	C	D
	2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional period ..... 14 Exper. Period II.. 17	B	A	D	C	D	C	B	A	C	D	A	B
	2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional period ..... 14 Exper. Period III 18	C	D	A	B	B	A	D	C	D	C	B	A
	2346	2344	2335	2310	2378	2340	2214	2210	2197	2000	1556	1297
Transitional period ..... 14 Exper. Period IV 21	D	C	B	A	C	D	A	B	B	A	D	C

tion differences, progress of lactation and seasonal change. Each group of four cows constitutes an independent experiment designated as a 4×4 Latin square. The important features of the design are: (a) all rations are received by each group during every period, (b) each ration is followed by a different ration from one period to the next and (c) each cow receives a different permutation of rations during the four periods. The cows were kept on a transitional period of 14 days prior to each test period. The schedule of feeding is shown in table 3.

The milk samples for scoring collected from each cow twice each week were held in 0.5-pt. milk bottles, 1-pt. tin containers and 1-pt. rusty tin containers. Samples were iced and held about 21 hr. before they were scored. The score card (11, p. 567) was essentially the same as the one used in the first experiment, except that it was more specific in that the intensities of the various flavors were given definite scores.

Results and discussion. Milk samples were collected twice each week from each cow. This procedure yielded six samples (and six scores) for each cow and each container type during each experimental period of the study.

The average flavor scores of the six samples of each cow's milk (for all container types) are shown in table 4 for each of the experimental periods. The scores obtained for the milk held in glass were analyzed statistically for variance;

TABLE 4

Average flavor scores of milk held in glass, tin and rusty tin containers for each cow during each period

Period	Group I						Group II								Group III					
	Cow. no.						Cow. no.								Cow. no.					
	2346		2344		2335		2310		2378		2340		2214		2210		2197		2000		1566		1297	
	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score	Ration	Score
Glass containers																								
I	A	35.8	B	36.5	C	37.5	D	35.7	A	36.6	B	36.1	C	36.4	D	35.8	A	37.2	B	35.1	C	37.4	D	36.1
II	B	36.0	A	36.3	D	36.2	C	36.3	D	36.1	C	36.8	B	35.3	A	36.1	C	36.7	D	35.9	A	36.9	B	36.3
III	C	35.7	D	35.8	A	36.0	B	35.0	B	35.5	A	35.4	D	34.9	C	35.9	D	35.7	C	35.9	B	36.3	A	35.7
IV	D	35.0	C	36.0	B	35.4	A	36.1	C	36.5	D	35.5	A	34.5	B	35.3	B	33.5	A	35.8	D	35.7	C	36.3
Tin containers																								
I	A	35.9	B	36.6	C	36.2	D	36.2	A	36.8	B	36.2	C	36.4	D	35.6	A	35.5	B	35.3	C	37.3	D	35.8
II	B	35.9	A	36.4	D	36.0	C	36.5	D	36.3	C	36.7	B	35.4	A	36.8	C	37.0	D	37.0	A	36.4	B	36.4
III	C	35.6	D	35.7	A	35.8	B	35.5	B	36.2	A	36.4	D	34.9	C	35.9	D	35.6	C	36.2	B	36.3	A	36.5
IV	D	35.3	C	36.5	B	34.9	A	35.7	C	36.8	D	35.3	A	34.9	B	35.7	B	36.0	A	36.0	D	35.9	C	36.3
Rusty tin containers																								
I	A	35.7	B	36.8	C	36.2	D	34.8	A	35.5	B	36.5	C	35.6	D	34.3	A	34.7	B	34.7	C	36.1	D	34.1
II	B	35.8	A	36.1	D	35.9	C	36.3	D	36.1	C	36.7	B	35.7	A	35.7	C	36.8	D	37.0	A	36.1	B	36.0
III	C	36.0	D	35.4	A	35.4	B	35.3	B	35.3	A	35.8	D	34.5	C	35.5	D	35.8	C	36 3	B	36.1	A	35.2
IV	D	35.0	C	36.3	B	35.3	A	35.9	C	36.5	D	35.2	A	34.8	B	35.0	B	33.5	A	36.0	D	35.7	C	35.6

the results are presented in table 5. The scores obtained for milk held in tin

TABLE 5

Analysis of variance of the flavor scores of milk held in glass containers

	Degrees of freedom	Sum of squares	Mean squares
Between groups	2	16.9	8.45
Between cows within groups	9	216.3	24.0 *
Between periods within groups	9	232.5	25.83**
Between rations	3	212.8	70.9 **
Ration X group interactions	6 } 25	45.1 } 175.15	7.5 } 7.0
Error	19 }	130.05 }	6.8 }
Total	48	854.25	

* Significant ($p < 0.05$).
** Highly significant ($p < 0.01$).

and in rusty tin containers were subjected to similar statistical treatment. The results are presented in tables 6 and 7. The "ration X group interactions"

TABLE 6

Analysis of variance of the flavor scores of milk held in tin containers

	Degrees of freedom	Sum of squares	Mean squares
Between groups	2	7.0	3.5
Between cows within groups	9	220.9	24.5*
Between periods within groups	9	162.9	18.1
Between rations	3	156.3	52.1**
Ration X group interactions	6 } 25	56.14 } 201.2	9.3 } 8.04
Error	19 }	145.1 }	7.6 }
Total	48	748.3	

* Significant.
** Highly significant.

TABLE 7

Analysis of variance of the flavor scores of milk held in rusty tin containers

	Degrees of freedom	Sum of squares	Mean squares
Between groups	2	13.3	6.6
Between cows within groups	9	222.3	24.7
Between periods within groups	9	177.7	19.7
Between rations	3	160.0	53.3*
Ration X group interactions	6 } 25	62.1 } 312.4	10.3 } 12.4
Error	19 }	250 3 }	13.1 }
Total	48	885.7	

* Significant.

in all these tables are insignificant and therefore, are included in the experimental error.

The mean square of the ration effects is significantly greater than the error mean square in all three analyses of variance tables, indicating that there are differences between the rations in their effect on milk flavor. The average scores

for each ration in each container (table 8) show that the general trends in the

TABLE 8

Average flavor scores of milk for the different rations when the milk was held in glass, tin and rusty tin containers

Rations	Av. flavor score of milk held in:		
	Glass	Tin	Rusty tin
C. Soybean hay + cracked soybeans	36.4	36.4	36.1
A. Soybean hay + linseed meal	36.0	36.1	35.6
D. Alfalfa hay + linseed meal	35.7	35.8	35.3
B. Alfalfa hay + cracked soybeans	35.5	35.7	35.5

effects of the rations are almost identical for each of the types of containers. Ration C received the highest flavor score in all three containers. Ration A received the second highest score in all three containers. Ration D received the third highest score in the glass and tin containers and the lowest score in the rusty tin container. Ration B received the lowest score in the glass and tin containers and the third highest score in the rusty tin container.

The *t*-test indicated a highly significant difference between ration C (soybean hay and cracked soybeans) and ration A (soybean hay and linseed meal), a significant difference between ration A and ration D (alfalfa hay and linseed meal) and a non-significant difference between ration D and ration B (alfalfa hay and cracked soybeans).

Milk produced on rations B and D, both of which contained alfalfa hay, received the lowest flavor scores in all three kinds of containers, while milk produced on A and C, which contained soybean hay, received the highest scores in all three containers. Probably the differences between rations result from differences between the hays. The protein supplements do not seem to be responsible because the milk which received both the highest and lowest flavor scores was produced on rations containing cracked soybeans, while the rations that contained linseed meal produced milk with scores between those received by milk produced on the cracked-soybean rations.

The data in table 8 indicate that the effect of containers has the same general trend with all rations. The milk in the tin containers scored highest. This was followed in order by the milk in glass and in rusty tin containers. The *t*-test indicated no significant differences between the glass and tin containers but a significant difference between the tin and rusty tin containers. Apparently, tin containers free from rust are as satisfactory as glass containers, but rusty tin containers cause deterioration of milk flavor.

Oxidized flavors occurred 77 times in the 864 samples. These flavors were divided among the rations as follows: 21 occurred in the milk from the cows receiving ration A, 17 in the milk from those receiving ration B, 14 in the milk from those receiving ration C and 25 in the milk from those receiving ration D. Chi-square was computed using a 4×2 table. The differences in occurrence of oxidized flavors could not be attributed to differences in rations.

The oxidized flavors were divided among the containers as follows: 12 occurred in glass, 9 in tin and 56 in rusty tin containers. This difference is highly significant, showing that the rusty tin containers tend to increase the susceptibility of the milk to oxidation. Therefore, results in experiment II are not in agreement with those from experiment I, in which the quality of the milk was not influenced by the type of container. This difference may have resulted from the longer time that the milk was held before scoring in experiment II or to seasonal influences, since experiment II was conducted during the winter months.

As the experiment progressed, unclean flavors became apparent in the milk (table 9). The increase in the occurrence of unclean flavors from the first two

TABLE 9

Occurrence of unclean flavors in milk: per ration, per period

Periods	A Soybean hay + linseed meal	C Soybean hay + cracked soybean	B Alfalfa hay + cracked soybean	D Alfalfa hay + linseed meal	Total
1	3	3	4	5	15
2	1	–	2	1	4
3	6	6	4	7	23
4	16	7	8	9	40
Total	26	16	18	22	

to the last two periods is shown in table 10. The calculated Chi-square showed

TABLE 10

Occurrence of unclean flavors between the first two and the last two periods for soybean and alfalfa hay

Periods	Soybean hay	Alfalfa hay
1 and 2	7	12
3 and 4	35	28
Total	42	40

a highly significant difference in the occurrence of unclean flavors between the first two and the last two periods for soybean hay and a significant difference for alfalfa hay. Since the containers were cleaned thoroughly and sterilized, it is improbable that these unclean flavors came from the containers. At the start of the experiment, the hays seemed similar and of good quality. During period III, when large numbers of unclean flavors began to appear, the hays had a musty odor and appeared to have deteriorated during the experiment. Possibly the change in quality of the hays was responsible for the increased unclean flavors. Caution probably should be exercised when criticizing farmers in regard to the cleanness of equipment, because flavors which seem typical of unclean equipment possibly can be caused by poor quality hay.

Eighty-two samples of milk had unclean flavors. Of these, 26 occurred in the milk from the cows receiving ration A, 18 in the milk from those receiving

ration B, 16 in the milk from those receiving ration C and 22 in the milk from those receiving ration D. Chi-square was computed and the differences in occurrence of unclean flavors could not be attributed to any particular ration.

There seemed to be no influence of container on unclean flavor, for of 82 occurrences of this flavor, 25 occurred in glass, 25 in tin and 32 in rusty tin containers. These differences are not significant.

Rancid flavors occurred only 19 times in the 864 samples and could not be attributed to any particular ration. Every sample of milk was criticized as possessing a feed flavor. The intensity of the feed flavors ranged from slight to distinct. However, these flavors could not be attributed to any particular ration.

Summary. Two experiments were conducted to determine whether soybeans adversely affect the flavor of milk. The first was run during April to July; a grain ration containing cracked soybeans was compared with one containing linseed oil meal. Alfalfa hay was used as the only roughage. The soybean ration yielded milk with flavor scores equivalent to those of milk produced on a linseed meal ration. The type of container used for collection appeared to have no influence on milk flavors and scores during this season of the year.

The second experiment extended from November through May. Alfalfa hay and linseed oil meal were used as control feeds. Neither soybeans nor soybean hay adversely affected the flavor of the milk. A large number of unclean flavors appeared in the milk during the latter part of the experiment. The change in quality of the hay seemed to be responsible for the increase in unclean flavors that appeared in the milk. Contrary to the results of the first experiment, the rusty tin containers increased the susceptibility of milk to oxidation at this season of the year. The failure of the rusty containers to influence oxidative processes in milk during the first experiment may have resulted from the short time the milk samples were held in the rusty containers before scoring or to seasonal effects.

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PARTURIENT PARESIS. IV. THE EFFECT OF UDDER INFLATION UPON BLOOD LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHORUS IN COWS WITH PARTURIENT PARESIS[1, 2]

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Air inflation of the udder was used extensively as a treatment for parturient paresis until the development of calcium therapy. Even today, air inflation is resorted to in cases that do not respond to calcium therapy (23). Seitter (25) reported that inflation of the udders of anesthesized goats and cows produced a marked increase in blood pressure. Later Auger (4), using a more refined technique, concluded that air inflation caused only a slight increase in blood pressure. Maguire (20), Widmark and Carlens (28) and Auger (3) report a marked increase of blood sugar after inflation of the udder with air. Fish (10, 11), Sjollema (26) and Hayden (17) demonstrated that the hyperglycemia occurring after udder inflation resulted from lactose in the blood, presumably absorbed from the mammary gland.

Several workers have shown an increase in blood serum calcium following mammary inflation for milk fever treatment (9, 16, 27). Fish (12) demonstrated an increase in both serum calcium and inorganic phosphates. The mechanism whereby udder inflation produces the above effects has been a source of much conjecture. Peterson and Rigor (19) and Garrison and Turner (12) have reported that milk secretion is practically inhibited when air pressures of 25 to 40 mm. Hg are maintained in the cow's udder. Some (9, 19) attribute the effect of inflation to the cessation of milk secretion, thereby preventing the further uptake of milk precursors from the blood. Others (6, 15) postulate that udder inflation gives rise to afferent stimuli which are responsible for the curative effects.

This work was undertaken to obtain, in more detail, the changes in calcium, magnesium and inorganic phosphorus during recovery from parturient paresis after udder inflation. At the time the work was initiated, no one had reported studies on magnesium levels after inflation. In a recent study of parturient paresis treated by air inflation, Robertson (23, 24) included data on magnesium levels.

EXPERIMENTAL PROCEDURE

The udders of seven Jersey cows with parturient paresis were inflated with air to a pressure of 60 to 70 mm. Hg and the teats taped to prevent the escape of air. The pressure was measured with an aneroid manometer attached to a teat

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[1] Part of these data were taken from a thesis presented by R. P. Niedermeier to the graduate faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Published with approval of the Director of the Wisconsin Agricultural Experiment Station.

TABLE 1

The effect of udder inflation on calcium, phosphorus and magnesium of cows with parturient paresis

Cow no.	12			20			63			494			556			705			732		
Time	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg	Ca	P	Mg
(*hr.*)										(*mg. %*)											
										Preinflation											
	4.6	1.3	2.0	5.2	0.5	3.3	4.6	1.4	4.5	2.8	1.2	2.6	4.4	0.8	2.8	3.1	0.62	3.5	4.5	1.4	4.1
										Postinflation											
0.5	4.8	2.1	1.9	5.0	0.5	3.1	5.1	1.5	4.6	3.4	2.1	2.8	5.1	0.7	2.8	3.7	0.87	3.7	5.1	2.2	3.9
1.5	4.7	1.5	2.2	5.1	0.7	3.3	6.1	2.3	4.6	3.8	3.0	2.6	5.4	0.9	2.8	4.3	1.09	3.8	5.6	2.7	4.0
3.0	5.4	1.7	2.2	5.1	0.7	3.5	6.6[a]	2.5	4.7	4.0	2.2	2.6	4.8[a]	1.0	3.0	4.1	1.06	3.7	5.4	2.9	3.7
5.0	5.9	2.7	2.3	6.0	1.0	3.4	6.3	2.6	4.5	5.3	2.5	2.8	4.9	0.8	3.1	4.3	1.23	3.7	6.0[a]	2.9	3.6
8.0	6.0	3.3	2.4	6.9[a]	1.2	3.5	6.6	2.9	4.3	5.7[a]	3.3	3.1	5.0	1.4	3.2	4.7[a]	1.15	3.7	6.8	2.7	3.4
11.0	6.7	3.8	2.7	7.3	1.4	3.5	6.5	3.0	4.3	5.2	3.6	3.2	5.4	3.7	3.5	4.8	0.87	3.6	7.5	2.1	3.6
14.0	7.1[a]	4.7	2.4	7.6	1.8	3.5	6.8	3.0	4.3	5.1	2.8	2.9	6.0	3.5	3.2	4.6	1.62	3.8	7.4	3.3	3.5
17.0	8.2	6.0	2.6	7.7	1.9	3.0	6.9	2.1	4.3	5.0	1.6	2.8	6.1	3.2	3.3	4.6	2.13	3.8	7.3	4.1	3.3
20.0	8.5	6.4	2.6	7.6	2.8	3.2	7.1	3.3	3.9	5.0	1.8	2.9	6.8	2.8	3.6	4.0	1.70	3.9	7.5	4.4	2.9
36.0							6.1	6.0	3.5										9.8	3.6	2.3
48.0							8.6	6.3	2.6										9.8	3.8	2.0

[a] Time after inflation when cow was found standing.

cannula. The cows were not treated until they were down and unable to rise. A sample of venous blood was drawn before inflation. Post-inflation samples were drawn at 0.5, 1.5, 3, 5, 8, 11, 14, 17, 20, 36 and 48 hr. Samples were analyzed for serum calcium, serum magnesium and inorganic phosphorus, and all analyses were made in duplicate. Blood serum calcium was determined by the method of Clark and Collip (7), serum magnesium according to Simonsen *et al.* (26) and plasma inorganic phosphorus by the Fiske and Subbarow method (13).

RESULTS AND DISCUSSION

Table 1 shows the results of analyses of blood samples for total serum calcium, serum magnesium and plasma inorganic phosphorus. Cow 494 was in a coma at the time of inflation. Cows 20 and 494 were reinflated 3 hr. after the first inflation as a precautionary measure, but no relapses occurred. The time relationships for calving, occurrence of milk fever, treatment and recovery are shown in table 2. Recovery was regarded as the time the cow arose to her feet and stood up of

TABLE 2

Summary of time of treatment and recovery in relation to calving

Cow no.	Parturient paresis		Inflation completed		Recovery	
	Time[a]		Time[a]		Time[a]	
	Hr.	Min.	Hr.	Min.	Hr.	Min.
12	6	00	7	40	9	40
20	7	40	9	00	17	00
63	39	00	46	10	49	10
494	17	40	19	55	27	55
556	15	30	17	00	20	00
705[b]	11	45	14	30	19	30
732	22	00	28	10	33	10

[a] Measured from end of calving.

[b] Suffered relapse 38 hr. after calving and was treated with an intravenous injection of calcium gluconate. A second injection of calcium gluconate was made 76 hr. after calving and the cow died suddenly, after apparent recovery 9 hr. after the second injection.

her own accord. Time of recovery ranged from 3 to 14 hr. after treatment by udder inflation.

The severity of the milk fever cases is reflected in the low total serum calcium and phosphorus in all pre-inflation samples. Inorganic phosphorus values as low as 0.5 mg. per cent also were reported by Allcroft (2) for milk fever cows. With the exception of cows 556 and 705, recovery was noted when the calcium level was near or above 6 mg. per cent. Cow 494 remained on her feet with a serum calcium level of only 5 mg. per cent at 20 hr. after inflation. In the seven cases studied, plasma phosphorus levels increased following udder inflation, but in no case could the phosphorus level be considered within the normal range when the cows got up. Blood serum magnesium levels remained in the normal to high normal range as reported by Allcroft (2) in all cows except 63 and 732, where a hypermagnesemia condition existed. Recovery was uneventful in all cows except 705. In 705 the calcium and phosphorus did not return to normal up to 20 hr. after inflation. Shortly after 20 hr. post-inflation, she had a relapse and

was treated with calcium gluconate. Two days after the first attack 705 died suddenly after apparent recovery.

Robertson (23) found no correlation upon statistical analysis of his data on 19 milk fever cases between blood magnesium levels and the symptomatology classification as proposed by Barker (5). Pribyl (22) suggested the symptoms of milk fever may be due to a magnesium narcosis. Hibbs *et al.* (18) reported that upon intravenous injection of magnesium sulphate into cows, general anesthesia resulted when the serum magnesium reached a level of 7.5 mg. per cent. When the serum calcium then was raised to 17.5 mg. per cent by an injection of calcium gluconate, the cows regained consciousness, even though the serum magnesium remained at the same high level. They suggested that the relationship between serum calcium and serum magnesium may be of great importance in the symptomatology of milk fever. Allcroft (1) found that anesthesia was produced in goats when the serum magnesium was between 13.5 and 14.5 mg. per cent, with the serum calcium around 7 mg. per cent. He further comments (2) on the importance of the calcium and magnesium ratio, as well as the calcium phosphorus ratio in milk fever.

These data show that there is a considerable difference between cows as to the blood levels of calcium, phosphorus and magnesium at the time of recovery. This suggests that possibly the relative levels of the calcium, magnesium and phosphorus are more important in the symptomatology of milk fever than the actual blood level of any one constituent.

SUMMARY

Data are given for the blood levels of calcium, magnesium and phosphorus during the recovery period for seven cases of parturient paresis in Jersey cows which were treated by udder inflation.

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FERTILITY AND LIVABILITY OF BULL SEMEN DILUTED AT VARIOUS LEVELS TO 1:300

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The phenomenal growth of the practice of artificial insemination of dairy cattle in the United States has necessitated the development of means of increasing the number of cows that can be bred to a given sire. This problem is of extreme importance because of the limited number of sires proved to be transmitters of satisfactory levels of milk and fat production. One of the most promising and fruitful solutions has been that of semen dilution.

A series of experiments concerning the fertility of semen diluted at various levels has been conducted by Salisbury and associates. In the first three papers (6, 8, 11) increasingly high dilutions were tried until it was demonstrated that levels as high as 1:100 could be used without lowering breeding efficiency. These studies have been of inestimable value to the dairy industry in that they, along with the development of the egg yolk diluter (5), have made possible the great expansion of the practice of artificial insemination. In later experiments (10) it was shown that, with increase in dilution levels above 1:100, there was a progressive decline in breeding efficiency.

The American Foundation for the Study of Genetics also has been conducting research along this line. During the past 2 yr. a series of controlled experiments has been conducted to determine the fertility of semen diluted above 1:100. It is believed that enough information has been accumulated to enable the establishment of quantitative results. The information obtained from these studies is presented below.

EXPERIMENTAL PROCEDURE

Four experiments were conducted in which each semen collection was split three ways. One-third was diluted 1:100, and the other two portions were diluted at higher levels. Each collection consisted of two or more ejaculates which were mixed together before being added to the diluters. The different dilution levels were rotated among different inseminator groups where the semen was used for breeding. As far as could be predicted when planning each experiment, these groups were equal in regard to number of cows bred and in breeding efficiency. Each experiment, therefore, consisted of two or more Latin squares. In experiments 2 and 4 each Latin square consisted of three collections from a given bull. The semen was from Guernsey and Holstein bulls selected for high fertility when used artificially.

The non-returns in the first three experiments were determined 60 to 90 days after service and were based on first and second services made the day following

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collection. In the fourth trial the non-returns were obtained in a similar manner except that, in an attempt to reduce variation, they were determined 72 to 78 days after service. Figures computed in this way are comparable to the 60- to 90-day values because in both cases 75 days is the average or midpoint.

In experiments 1 and 3 spermatozoan concentration was determined by opacity, using the method of Salisbury *et al.* (9). They observed a correlation coefficient of 0.98 between spermatozoan numbers and opacity. A study has been made by Willett (13) of 110 separate ejaculates from 29 bulls wherein the number of spermatozoa were determined both by the above-mentioned method and by a haemacytometer. With the latter, the semen was diluted 1 : 100 with 0.85 per cent NaCl solution containing a small quantity of chlorazene to kill the spermatozoa. In each case, the cells in all the squares in a ruled area were counted. A correlation coefficient of 0.786 between spermatozoan numbers and 2-(logarithm of light transmission) was obtained when comparing these two methods. Only 62 per cent of the variation in light transmission was, therefore, accounted for by number of spermatozoa. These unsatisfactory results probably were due to the presence of interfering substances in the semen. Perhaps this difficulty would be overcome by adaptation of the technique devised by Emik and Sidwell (4) for ram semen. This technique was published after most of the dilution experiments reported in this paper were completed. In experiments 2 and 4, which were larger in scope than the others, the most accurate spermatozoan counts possible were desired. As a consequence, haemacytometer counts were made as described above.

Experiments 1 and 3 were preliminary in nature and small in scope. In the first, nine collections from five bulls were used, while in the third there were six collections from six bulls. The two large-scale trials, numbers 2 and 4, consisted of 18 collections from 6 bulls and 36 collections from 12 bulls, respectively.

The yolk-citrate diluter (36.0 g. $Na_3C_6H_5O_7 \cdot 2H_2O$ per liter of water in the buffer) was used in the first experiment. In the later ones the same diluter with 6 g. sulfanilamide per liter of buffer was used.

RESULTS

Fertility of semen. The breeding results from the four experiments, along with average figures for spermatozoan numbers, are presented in table 1. The

TABLE 1

Average non-returns from, and number of spermatozoa in, semen diluted at different levels in four experiments

Expt. no.	No. of services	Av. percentage non-returns and sperm numbers by dilution levels				
		1 : 100	1 : 125	1 : 150	1 : 200	1 : 300
1	2,146	60.7 (12.3)[a]	57.5 (9.8)	57.0 (8.2)		
2	3,449	68.3 (12.7)		68.3 (8.5)	65.6 (6.4)	
3	1,441	63.9 (13.0)			63.6 (6.6)	59.9 (4.4)
4	4,338	65.0 (10.1)			61.9 (5.1)	59.5 (3.4)

[a] Figures in parentheses are av. no. (in millions) of spermatozoa per ml. of diluted semen.

number of services for each dilution level is not given, as the figures for each experiment are approximately the same and, for all practical purposes, may be considered equal. It can be seen that in every experiment there is a downward trend with increase in dilution rate or with decrease in number of spermatozoa. None of these differences is significant. Experiments 2 and 4 were designed to enable the statistical measurement of bull and dilution interaction. In neither experiment is this interaction significant, however. If bulls selected at random from the population and not those selected for high fertility had been used in the experiment, it seems logical that a significant interaction would have been obtained. This laboratory has additional limited data suggesting that reduction in breeding efficiency greater than that observed above can result when semen from a bull of questionable fertility is diluted above 1:80.

Salisbury and Bratton (10) reported two experiments wherein citrate-yolk diluter was used in one and citrate-sulfanilamide-yolk was used in the other. With the latter diluter they observed less reduction in non-returns with increase in degree of dilution than with the citrate-yolk. The data presented in table 1 are in line with this observation, for sulfanilamide was used only in the last three experiments. Direct comparisons within one experiment, however, are needed to demonstrate definitely if such differences actually occur.

In the course of the analyses of the data in experiments 2 and 4, the non-return percentages were plotted on graphs against numbers of spermatozoa per ml. of diluted semen. Over-all regression coefficients were calculated. The relationship on the graphs tended to be curvilinear, with the slopes being greater when spermatozoan numbers were less than 6 million. The data from each experiment were, therefore, divided into two groups, and separate regression coefficients were calculated. One group contained samples with 6 million or more spermatozoa per ml. and the other less than 6 million. The over-all range in spermatozoan numbers in each of the two experiments were 4.6 to 17.8 and 1.5 to 17.2 million, respectively. The regression coefficients are presented in table 2. Al-

TABLE 2

Regression of non-return percentages on spermatozoan numbers by individual samples

Expt. No.	All samples		Samples with 6 million or more spermatozoa/ml.		Samples with less than 6 million spermatozoa/ml.	
	No.	b[a]	No.	b	No.	b
2	54	0.77**	47	0.43	7	6.99
4	108	0.78*	44	0.52	64	2.62*

[a] b = regression coefficient—the drop in non-return percentages per million decrease in number of spermatozoa in each ml. of diluted semen.

* Significant. Probability = 0.05 or less.

** Highly significant. Probability < 0.01.

though they suggest curvilinearity, the coefficients representing the two groups in each experiment are not significantly different. More data are needed to establish definitely this relationship.

The two over-all coefficients, 0.77 and 0.78, agree very closely with the corre-

sponding figure of 0.8 given by Salisbury and Bratton (10) for their large-scale experiment. The figures of 0.43 and 0.52 are somewhat higher, however, than that of 0.3 calculated by them from 700 ejaculates used routinely for breeding and with a range of 6,700,000 to 34,600,000 spermatozoa per ml. Apparently these samples included many which were diluted at levels less than 1:100. When such samples are included, the regression coefficient probably is lower than if they were not used. They mentioned that the data from their controlled experiments were not curvilinear even though their ranges in spermatozoan numbers were comparable to those reported in this paper.

As can be seen from the data in table 1, when semen is diluted at 1:100 it contains on the average about 12 million spermatozoa per ml., and at 1:200, 6 million. On the basis of the data presented above, therefore, there is a decline of about 3 per cent in non-return rate when the dilution level is extended from 1:100 to 1:200. This figure determined by means of the regression coefficients agrees closely with the information obtained directly by comparing in table 1 the non-returns for these two dilution levels.

In Salisbury and Bratton's (10) experiment using citrate-sulfanilamide-yolk diluter and in the experiments reported in this paper, it has not been possible to demonstrate significant differences in non-returns between dilution levels of 1:100 and 1:200. Their difference is 2.5 per cent. Apparently the experiments have not been sensitive enough or on a large enough scale to establish significance. The differences have been quite consistent, however, and, therefore, probably can be assumed not to be due to chance.

It must be kept in mind that, in the four experiments reported in this paper, the data are based on inseminations made the day following collection of semen. The maximum storage time was approximately 36 hr. Thus the results may not be applicable to longer storage periods. Non-return rates calculated from the few services made the second day following collection indicate that there was an over-all downward trend in non-return rate with increase in age of semen but that the relative difference between the dilution levels remained about the same.

By the extension of dilution levels from 1:100 to 1:200 the number of cows that could be bred to a given bull would be almost doubled. It seems reasonable that a sacrifice of 3 per cent in non-return percentage could sometimes be made in order to utilize outstanding sires to the maximum, especially if breeding efficiency is otherwise at a high level. The question of whether or not to make such a sacrifice is one of economics and must be decided by the stud manager in accordance with his individual situation.

Livability of spermatozoa. While the four experiments were being conducted, observations were made of spermatozoan motility in all samples at 2-day intervals until the semen had been stored in a refrigerator at 4° C. for 12 days. Simultaneously with the fourth experiment another study which included duration of motility at the dilution level of 1:50, was being made of the semen from most of the collections. Since motility observations might be of interest because of the wide range in dilutions, the data from the collections studied in both experiments were combined and are presented in table 3. The semen was graded

TABLE 3

Average motility (in per cent) during storage of spermatozoa in semen diluted at four levels, and number of samples containing no motile spermatozoa at 12 days. (Observations per datum: 32. Total observations: 896.)

Time	Av. % motility at dilutions of:				L.S.D.[a]
	1:50	1:100	1:200	1:300	
Fresh	73	73	73	73	
2 d.	68	65	59	56	3
4 d.	59	57	48	44	5
6 d.	51	43	35	29	5
8 d.	43	34	24	18	5
10 d.	33	21	13	12	4
12 d.	23	12	8	4	4
Samples with no motile spermatozoa at 12 d.	0	6	11	15	

[a] Least difference required for probability of 0.01.

on the basis of the per cent of spermatozoa showing any degree of motility. Estimates were made to the nearest 10 per cent. In the table, for each storage period the least significant difference required for a probability of 1 per cent is given. The numbers of samples containing no motile spermatozoa on the twelfth day of storage also are given because the determination of the presence or absence of motile spermatozoa is considered to be more objective than determination of motility percentages. By means of the chi-square test it was determined that the probability, according to Crow's chi-square chart (3), of the differences in number of dead samples being due to chance is 0.0001. Whichever criterion is used, it can be seen that the livability of spermatozoa during storage decreases with increase in dilution rate. These livability studies are in line with the fertility studies reported above. Salisbury *et al.* (8, 12) observed a similar decline in livability with increase in rate of dilution with dilution levels up to 1:100.

These livability studies indicate that there is much room for improvement of the diluters now available. Salisbury (7) attributed the effect of high dilutions to the harmful action of oxygen. In later work, Van Demark *et al.* (12) presented data which indicate that oxygenation is mainly but not solely responsible for lowered livability of spermatozoa stored for a number of days in semen diluted at high levels. Studying the immediate effect of dilution upon motility, Cheng *et al.* (2), on the other hand, present evidence indicating that oxygen is not a factor and suggest that the "leaching" of one or more necessary substances from the spermatozoan may be the contributing cause of the harmful effect of high dilutions. This problem merits additional study.

Correlation studies. The data from experiment 4 were used to determine the correlation between non-return rates, spermatozoan numbers and motility of spermatozoa after either 2 or 8 days of storage. Calculations were made with all samples together, with samples containing 6 million or more spermatozoa per ml. and with samples containing less than 6 million spermatozoa.

The coefficients significant only at the 5 per cent level of probability were for non-returns and spermatozoan numbers with all samples (0.23) and with samples with less than 6 million spermatozoa (0.24), and also for non-returns and spermatozoan numbers with 2-day motility held constant by partial correlation using all samples (0.23). The only correlation significant at the 1 per cent level of probability was between sperm numbers and 8-day motility using all samples (0.29).

The correlation analyses indicate that decrease in spermatozoan numbers was a more important factor causing reduction in breeding efficiency with increase in dilution rates than the depression of spermatozoan motility by the high dilutions. The correlations between spermatozoan numbers and nonreturn rates were changed only slightly when the effect of motility was held constant.

These results are somewhat contrary to those obtained by Cheng and Casida (1) with rabbits in a study to determine the number of spermatozoa required for maximum and partial fertility. Their correlation coefficients indicated that the effect of dilution upon motility was of much greater importance than the actual reduction in numbers of spermatozoa. In their work a much wider range in dilution levels was used than in the experiments reported in this paper. With comparable dilution levels comparable results might have been obtained. Cheng *et al.* (2) have studied the effect of high dilutions upon motility of unstored bull spermatozoa in yolk-citrate. Their data suggest that the immediate effect upon motility of the dilution levels compared in experiment 4 would be slight.

The significant correlation of 0.23 between non-returns and sperm numbers obtained in experiment 4 with all samples is almost identical to that of 0.24 obtained by Salisbury and Bratton (10).

SUMMARY AND CONCLUSIONS

1. Four controlled experiments were conducted to study dilution levels above 1:100. A total of 11,372 services from 69 collections from bulls selected for high fertility were involved. In every experiment there was a downward trend with increase in dilution level, but none of the differences was significant.

2. In two of these experiments with a total of 7,787 services from 54 collections, accurate spermatozoan counts were made and regression coefficients were calculated. The citrate-sulfanilamide-yolk diluter was used. The relationship between non-return percentages and spermatozoan numbers at dilutions above 1:100 appears to be curvilinear.

3. As the number of spermatozoa decreased from approximately 12 million to 6 million, there was a decrease of approximately 0.5 in non-return percentage per million decrease in number of spermatozoa per insemination. Within this range in spermatozoan numbers, which corresponds roughly to dilution levels of 1:100 and 1:200, respectively, when 1 ml. of semen is used per insemination, there is, therefore, an over-all decline in non-returns of approximately 3 per cent.

4. When the diluted semen contained less than 6 million spermatozoa per ml., there was a drop of over 2.6 per cent in non-returns per million decrease in number of spermatozoa per insemination.

5. Motility observations are reported on a total of 128 samples from 32 collections studied at 2-day intervals during a storage period of 12 days. Dilution levels of one to 50, 100, 200 and 300 were compared. There was a marked decrease in livability of spermatozoa with increase in dilution rate.

6. Correlation analyses indicate that decrease in spermatozoan numbers was more important in causing the decrease in non-return rates with the dilution levels studied in this experiment than the direct depression of motility by high dilutions.

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STALE-FLAVOR COMPONENTS IN DRIED WHOLE MILK. II. THE EXTRACTION OF STALE BUTTER OIL FROM STALE DRIED WHOLE MILK BY ORGANIC SOLVENTS

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In a previous paper (4), it was reported that the stale-flavor components which develop in spray-dried whole milk during storage were found to be concentrated in the butter oil when prepared according to the usual method. However, due to unavoidable homogenization in the spray-drying process, the recovery of butter oil from the dried whole milk, and hence the removal of the stale-flavor components was very inefficient (approximately 35 per cent). Consequently, the possibility of obtaining stale butter oil directly from the whole milk powder by extraction with organic solvents was investigated.

For such a method to be applicable, the recovery of the butter oil must be reasonably high, the stale-flavor components must be extracted with the butter oil and the solvent must not interfere in any way with the flavor judgments of the products.

THE EFFICIENCY OF THE EXTRACTION PROCEDURE

Preliminary work indicated that the stale-flavor component could be extracted with the butter oil from stale, spray-dried whole milk by organic solvents. However, suitable techniques would be needed to improve the efficiency of the extraction procedure and to prevent the interference of solvent flavors with the judgment of the product. In order to improve the efficiency of extraction of the butter oil from the spray-dried whole milk, several modifications involving the type of whole milk powder extracted, the pretreatment of the powder before extraction and the extracting solvents were investigated.

Manufacture and storage of dried whole milk. Two types of dried whole milk were prepared in a pilot-size experimental spray drier. Powder no. 104 was manufactured from condensed whole milk without previous homogenization, while powder no. 105a was made from uncondensed, unhomogenized milk. The conditions of manufacture and storage are indicated in table 1.

General experimental methods. In all experiments, a weighed sample of the milk powder of known fat content, as measured by the Mojonnier method, was pretreated in the manner specified and extracted in a Soxhlet apparatus with a measured volume of solvent. The solvent was maintained at the boiling point by immersing the flask in a constant-temperature water bath. After the specified number of trips of the syphon, the solution was removed from the Soxhlet apparatus and the solvent evaporated from the butter oil under reduced pressure. The extracted butter oil was weighed to determine the efficiency of the method.

The anhydrous ethyl ether used was prepared by redistilling U.S.P. grade

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ethyl ether over metallic sodium. The petroleum ether was distilled over solid KOH and the fraction boiling between 30 and 41° C. collected.

Experimental results. While a considerable number of extractions were performed, only typical examples of the various procedures are reported in table 2. In experiments 1 and 2, powder no. 104, was extracted with anhydrous ethyl ether and with petroleum ether, respectively, without pretreatment. Poor recoveries were obtained with both solvents.

TABLE 1

Manufacturing data for dried whole milk

	Batch no.	
	104	105a
Raw milk:		
Source	University herd	University herd
Fat content (%)	4.3	3.9
Preheat treatment:		
Temp. (° *C.*)	77–79	71
Time (*min.*)	21	30
Condensing:		
Temp. (° *C.*)	46–71	none
Vacuum (*mm. of Hg*)	49–64	.....
Time (*hr.*)	1.2	.....
Total solids (%)	35.24	.....
Fat content (%)	11.37	.....
Cooling & storage:		
Final temp. (° *C.*)	12–15	.....
Method	surface cooler	.....
Storage time (*hr.*)	22.5	.....
Storage temp. (° *C.*)	~ 7	.....
Drying:		
Spraying temp. (° *C.*)	54–61	55–61
Spraying pressure (*lb./in.*^{2})	450–550	450–600
Spray nozzle size	63–17	69–20
Inlet temp. (° *C.*)	135–154	121–149
Outlet temp. (°*C.*)	85–97	82–107
Fat content (%)	31.60	29.56
Moisture content (%)	2.07	2.33
Solubility index	0.6	< 0.1
Powder storage:		
Time of storage (*mo.*)	> 8	< 1
Temp. of storage (° *C.*)	~ 7	~ 7

Since Holm *et al.* (2) had reported that the fat is more readily extracted by carbon tetrachloride from a powder prepared from uncondensed, unhomogenized milk, powder no. 105a was extracted without pretreatment in experiments 3 and 4. Satisfactory recoveries were obtained with both solvents. However, this powder developed a tallowy flavor quickly, which made it unsatisfactory for the investigation of the stale flavor.

Lampitt and Bushill (3) had observed that the amount of fat that could be extracted by organic solvents from dried whole milk prepared by the usual spray-drying process was increased by hydrating the powder to approximately 8 per

cent moisture. In experiment 5, dried whole milk no. 104 was stored in evaporating dishes and placed in desiccators over water at room temperature. After storage for 44.25 hr. under these conditions, the moisture content of the powder, as determined by the toluene-distillation method, was 7.87 per cent. Extraction of this powder with anhydrous ethyl ether yielded satisfactory recoveries. However, the time required to hydrate the powder by this method was longer than desirable.

The dynamic method suggested by Wilson (5) was employed in experiment 6. Air was drawn by means of a high-vacuum pump through the following succes-

TABLE 2

Typical results of various methods for the solvent extraction of butter oil from dried whole milk

	Expt. no.						
	1	2	3	4	5	6	7
Powder no.	104	104	105a	105a	104	104	104
Moisture content (%)	2.07	2.07	2.33	2.33	2.07	2.07	2.07
Special pretreatment	none	none	none	none	static hydration	dynamic hydration	alcohol treatment
Moisture content after pretreatment (%)	.....	.....	.....	.....	7.87	9.20	.....
Wt. of powder (*g.*)	40.00	40.03	40.00	40.00	176.8	40.01	402.1
Fat content of powder (%)	31.60	31.60	29.56	29.56	29.73	29.30	31.60
Solvent	anhydrous ethyl ether	petroleum ether b. p. < 41° C.	anhydrous ethyl ether	petroleum ether b. p. < 41° C.	anhydrous ethyl ether	petroleum ether b. p. < 41° C.	petroleum ether b. p. < 41° C.
Vol. of solvent (*ml.*)	400	300	400	500	750	340	2620
Temp. of extraction (°*C.*)	43	49	43	49	40	43–49	47–48
Time of extraction (*hr.*)	3.5	6	3.25	3	4.25	5.5	4.5[a] 11.0 4.25
No. of trips of syphon	.....	18	.....	.....	19	19	18[a] 19 20
Wt. of extracted butter oil (*g.*)	1.00	4.31	11.50	9.30	51.2	11.11	115.5
Recovery of butter oil (%)	7.9	34.1	97.5	78.5	97.5	94.6	90.9

[a] The powder was divided equally between extraction thimbles. The variations in time of extraction are due to different periods for tripping of the syphon caused by differences in construction of the extraction apparatus used.

sive stages: a charcoal adsorption tube, a washing bottle filled with water maintained at room temperature by immersion in a water bath, a modified Regnault dew point hygrometer, a tube containing 600 g. of dried whole milk no. 104 and a second hygrometer. At 1-hr. intervals, the absolute humidity of the air on both the inlet and outlet sides of the powder was determined. Throughout the hydration period, the air in the inlet side was maintained at as near saturation as possible by regulating the flow of air with a stopcock at the pump. The absolute

humidity was 22.8 to 24.3 mm. of mercury on the inlet side and 2.4 to 9.5 mm. of mercury on the outlet side, while the air temperature was 24.0 to 25.8° C. After 25 hr. of treatment, the moisture content of the dried whole milk was determined by the toluene-distillation method and a weighed sample extracted with petroleum ether in the usual manner. Satisfactory recoveries of butter oil were obtained.

To further reduce the time required for pretreatment of the dried whole milk, the possible application of an observation of Lampitt and Bushill (3) was studied. These investigators had noticed that, in freeing the fat for extraction by hydration of the milk powder to a moisture content of approximately 8 per cent, the lactose was crystallized. They also found that the lactose crystallized when the powder was dispersed in 95 per cent ethyl alcohol. To test whether pretreatment of the powder with alcohol also would free the fat for extraction and to develop the most satisfactory technique, a variety of experiments were performed. Experiment 7 illustrates the most satisfactory procedure used. Approximately 400 g. of dried whole milk no. 104 was agitated with 3,400 ml. of 95 per cent ethyl alcohol and 25.8 ml. of distilled water for 1 hr. and filtered. The alcoholic filtrate was divided into three portions, each of which was concentrated to approximately 850 ml. by evaporation under vacuum at room temperature. Eight hundred and fifty ml. each of petroleum ether and distilled water then were added to each portion and the mixture agitated for 2 min. in a separatory funnel. After the alcohol-water layer had been discarded, the petroleum-ether layer was used to extract the treated powder. Satisfactory recoveries of fat were obtained.

Discussion. In interpreting the results of this study, it must be kept in mind that the purpose was not to explore completely the effect of various factors upon the extraction procedures, but rather to develop efficient and usable methods for this step in the isolation of the stale flavor component. Therefore, no conclusions are justified with respect to small differences in recovery of butter oil, but certain general observations are possible.

While there were several differences in the methods of manufacture and storage of the two dried whole milks extracted, the chief difference was the drying of a condensed (powder no. 104) and an uncondensed (powder no. 105a) milk. The results of the first four experiments, may be considered as confirming the observation of Holm *et al.* (2) that fat is extracted more completely by organic solvents from a powder prepared from an uncondensed than from a condensed product. Unfortunately, the powder prepared from the uncondensed whole milk was so unstable that it was unsuitable for the study of stale flavor. A reasonable hypothesis for the instability of this product is that the physical condition of the powder which makes the fat more available for solvent extraction also makes it more susceptable to oxidation.

The results of the experiments in which the dried whole milk was hydrated to approximately 8 per cent moisture before extraction confirm the observations of Lampitt and Bushill (3) that the fat was readily extracted by organic solvents from dried whole milk when pretreated in this manner. As was expected, the rate of hydration was more rapid when the dynamic method of Wilson (5) was used.

The experiments employing the alcohol pretreatment indicate that satisfactory recoveries of butter oil can be obtained by agitation for 1 hr. of the dried whole milk with 95 per cent ethyl alcohol to which sufficient water has been added to hydrate the powder to 8 per cent moisture followed by extraction with petroleum ether in the usual manner, provided the fat dissolved in the alcohol used in the pretreatment is recovered. The reaction involved in the freeing of the fat for extraction by the alcohol pretreatment apparently approaches completion in 1 hr. However, shorter periods of treatment were not investigated. Some slight improvement in recovery was indicated by experiments in this series when water was added to the 95% alcohol. However, additional information is needed before any conclusions concerning the optimum amount to be used can be made. Several hypotheses may be suggested for the mechanism of the process whereby the fat is freed by the alcohol pretreatment. The alcohol may serve as a medium by which the water may be brought into more intimate and continuous contact with the lactose in the milk powder, thus speeding the crystallization process. This, in turn, would free the fat for extraction by destroying the continuity of the lactose glass which may entrap the fat. In this case there should exist between the alcohol and the lactose a competition for the water and additional water in the alcohol should shift the equilibrium toward the formation of more crystalline lactose and the consequent freeing of more fat for extraction. However, the evidence is only suggestive and the other possibilities that the alcohol simply serves as a medium in which the lactose crystals may grow or that the alcohol may effect the other components of the powder so as to make it more porous cannot be eliminated.

A METHOD FOR THE REMOVAL OF SOLVENT FLAVORS FROM BUTTER OIL

When the solvents were removed from the butter oils in the previous study by evaporation under vacuum at approximately 40° C., the butter oil, reconstituted with fresh skim milk to the approximate composition of the original milk (3.8 per cent fat), possessed sufficient solvent flavor to interfere with the establishment of its stale-flavor threshold value. Investigation of other common methods of solvent removal did not yield satisfactory results. Hence, a method specifically designed for this purpose was developed.

Experimental Method. Briggs (1) had observed that, by the adsorption of gases on activated cocoanut charcoal at liquid-air temperatures from low-pressure systems, higher vacuums could be obtained than by use of high-vacuum pumps alone. Also it is known that organic solvents are adsorbed strongly by charcoal at low temperatures. Therefore, the special tube shown in fig. 1 was constructed. Butter oil and the solvent to be investigated were added to the pyrex flask A. Cocoanut charcoal (~31 g.) was placed in the pyrex tube B, which was connected to the flask by means of a ground-glass joint. The flask A and its contents were held at a constant temperature of 40° C. in water bath, while the entire apparatus was agitated continuously by a Boerner shaker. The bulk of the solvent was removed with an aspirator through stopcock C, and then a vacuum pump was attached and the pressure reduced to 0.1 mm. of mercury or less. After the sys-

tem had been evacuated for a sufficient length of time, the charcoal in the tube B was degassed by being heated with a Bunsen burner while the pump was still in operation. The heating period (usually 15–20 min.) was complete when no change in the pressure of the system could be noted after the charcoal had been heated for a 5-min. period in a closed system. With the stopcock still closed, the charcoal bulb was allowed to cool to room temperature, and then the pressure of the system was further reduced to 10^{-5} mm. of mercury or less by immersion of the bulb in liquid air contained in a 1 l. Dewar flask. After a sufficient time, the bulb was removed from the liquid air, the stopcock C was opened and the

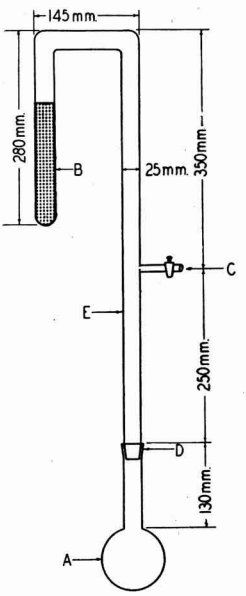

FIG. 1. Apparatus for removal of solvents from butter oil: A—500 ml. pyrex flask for butter oil and ether. B—Cocoanut charcoal (31 g.). C—Stopcock leading to vacuum pump. D—Ground glass fitting. E—25 mm. pyrex tube.

flask containing the butter oil was disconnected from the bulb. The pressure in the system during the process was determined by means of a high-vacuum arc tester which gives a pale-blue arc at less than 0.1 mm. of mercury pressure and no discharge at 10^{-5} mm. of mercury pressure or less. While this method for measuring the pressuure may seem somewhat qualitative, it was satisfactory for the purpose of this study.

Time study. To determine the minimum time required for evacuation with the high-vacuum pump and for the charcoal adsorption for the solvents used in this study, the duration of these treatments was varied and the butter oils obtained, blended with fresh skim milk to 3.8 per cent fat and scored for solvent flavor by

a panel of experienced judges. Their observations are recorded in table 3.

Discussion. In the development of this procedure no attempt was made to determine the effect of changes in such variables as weight and type of charcoal, design of apparatus, or amount of butter oil, since a satisfactory method was developed by controlling the time of evacuation and the time of adsorption on cocoanut charcoal at liquid-air temperature. Although there are some inconsistencies

TABLE 3

Effect of time of evacuation and time of charcoal adsorption upon the completeness of solvent removal from butter oil

Sample no.	Solvent	Wt. of butter oil	Vol. of solvent	Time of evacuation	Time of adsorption on charcoal	Judging		
						No. of judges	Control[a]	Treated samples[a]
		(*g.*)	(*ml.*)	(*hr.*)	(*hr.*)			
1	ethyl ether	40	400	21.8	.....	4	2+ 2−	4+
2	ethyl ether	40	400	21.8	1.0	4	2+ 2−	4+
3	ethyl ether	40	400	21.8	3.0	4	2+ 2−	4−
4	ethyl ether	40	400	21.8	24.0	4	2+ 2−	4−
5	ethyl ether	40	400	.....	3.0	4	1+ 3−	4+
6	ethyl ether	40	400	4.1	3.0	4	1+ 3−	4+
7	ethyl ether	40	400	1.0	3.0	4	1+ 3−	4+
8	ethyl ether	40	400	24.0	3.0	4	1+ 3−	2+ 1 (?) 1−
9	ethyl ether	40	400	16.0	5.0	2	2−	1+ 1 (?)
10	ethyl ether	40	400	8.25	10.6	3	3−	1 (?) 2−
11	ethyl ether	40	400	8.0	3.0	3	3−	3−
12	ethyl ether	40	400	4.0	3.0	2	2−	2−
13	ethyl ether	40	400	4.0	2.0	3	3−	3−
14	ethyl ether	150	250	4.0	2.0	3	3−	3−
15	methyl alcohol & pet. ether (1/1)	150	500	4.0	2.0	4	4−	4+
16	methyl alcohol & pet. ether (1/1)	150	500	4.0	4.0	2	2−	2−
17	methyl alcohol & pet. ether (1/1)	150	500	4.0	3.0	3	3−	1+ 2−

[a] (+) indicates presence of solvent flavor. Apparently, in the initial experiments some of the judges had difficulty in recognizing the solvent flavor since solvent flavor was sometimes reported in the fresh control. Results are included only to add weight to the results of the later experiments.

in the results reported in table 3, subsequent use has confirmed the conclusions reached in the time study that satisfactory removal of ethyl ether from at least 150 g. of butter oil was accomplished by 4-hr. evacuation with the pump at pressures of 0.1 mm. of mercury or less and 2-hr. adsorption on cocoanut charcoal at liquid-air temperatures, while the mixture of petroleum ether and methyl alcohol required an additional 2-hr. adsorption on the cocoanut charcoal.

The results in table 3 indicate that evacuation alone, even for 21.8 hr., is insufficient to reduce the solvent concentration in the butter oil below the threshold value. Apparently, pressures of solvent vapor of the order of 0.1 mm. of mercury are in equilibrium with a concentration of solvent in the butter oil which is above the threshold value. However, as also indicated by the data, some evacuation with the pump is necessary to reduce the total amount of solvent in the system below a certain value before the adsorption on the charcoal can reduce the concentration in the butter oil below the threshold value. The time of adsorption on charcoal required is apparently determined by the rates of diffusion of solvent in the various states and the dimensions of the apparatus.

THE REMOVAL OF STALE-FLAVOR COMPONENT WITH THE BUTTER OIL FROM THE STALE DRIED WHOLE MILK BY SOLVENT EXTRACTION

With the development of a special procedure for eliminating the interferences of solvent flavors with the judgments of the butter oil, a series of experiments was performed to determine whether the stale-flavor component was removed efficiently from the dried whole milk with the butter oil by solvent extraction.

Experimental methods. Three different procedures for pretreatment of the dried whole milk were employed, all of which had been found to yield satisfactory recoveries of butter oil on subsequent extraction with organic solvents. The conditions of extraction and solvent removal are supplied in table 4. After the solvent was removed from the extracted butter oils, they were blended with fresh skim milk to the composition of the original whole milk (4.3 per cent fat) and their stale-flavor threshold values were determined by the procedure described in our previous publication (4). Samples of the dried whole milk before pretreatment were reconstituted with distilled water to the same composition and their threshold values determined. These results also are recorded in table 4.

Discussion. Consideration of these results indicates that by all methods investigated the threshold value of the stale extracted butter oil is approximately the same as the percentage of fat from the stale reconstituted whole milk present at its threshold value.[1] Thus, it is indicated that the amount of the stale-flavor component per unit weight of fat is approximately the same for both the extracted butter oil and the dried whole milk from which it was extracted. Therefore, it can be concluded that, within the limits of experimental error, the efficiency of extraction of the stale-flavor component is proportional to the efficiency of extraction of the butter oil and that 90 per cent or more of the stale-flavor component is removed along with the butter-oil by the procedures used. In the

[1] Calculated by the following formula: The per cent fat is reconstituted whole milk times the threshold value of stale reconstituted whole milk.

first experiment, the presence of an off-flavor which was considered to be different from that of ethyl ether and which could not be removed by the solvent-removal technique indicated the possible formation of flavored reaction products

TABLE 4

Effect of pretreatment and solvent extraction upon the efficiency of removal of stale-flavor component from dried whole milk

	Expt. no.		
	1	2	3
Powder no.	104	104	104
Pretreatment	static hydration as in table 1, expt. 5	dynamic hydration as in table 1, expt. 6	alcohol treatment as in table 1, expt. 7, except time of treatment was 7 hr.
Moisture content after pretreatment (%)	7.87	9.20	.....
Wt. of powder (*g.*)	176.8	198.5	205
Fat content of powder (%)	29.73	29.30	31.60
Solvent	anhydrous ethyl ether	pet. ether b.p. < 41 °C.	pet. ether b.p. < 41 °C.
Vol. of solvent (*ml.*)	750	850	850
Temp. of extraction (°*C.*)	40	43–49	47
Time of extraction (*hr.*)	4.25	23.75	9.17[a] 4.17
No. of trips of syphon	19	18	18
Temp. of solvent removal (°*C.*)	40	40	47
Pressure during evacuation (*mm. of Hg*)	> 0.1 for 4 hr. < 0.1 for 4 hr.	< 0.1	> 0.1 for 1 hr. < 0.1 for 4 hr.
Time of evacuation (*hr.*)	8	20.2	5
Time of adsorption on charcoal (*hr.*)	2.25	4	4
Wt. of extracted butter oil (*g.*)	51.2	56.0	61.8
Recovery of butter oil (%)	97.5	96.2	95.4
Threshold value of stale reconstituted whole milk (%)[b]	(6) 40 ± 9	(6) 50 ± 9	(8) 50 ± 8
Fat from stale reconstituted whole milk at threshold value (%)[c]	1.7 ± 0.4	2.2 ± 0.4	2.2 ± 0.4
Threshold value of stale extracted butter oil (%)[b]	(4) 2.8 ± 0.9	(8) 1.8 ± 0.5	(6) 1.0 ± 0.5
Comments	Medicinal, Solvent Pyrolysis Product		

[a] The alcohol-treated powder was divided into 2 equal fractions and extracted in 2 different Soxhlet extractors. The difference in time of extraction is due to the design of the apparatus.

[b] The numbers in parenthesis represent the no. of judgments. Rejected judgments are not included.

[c] Calculated by the following formula: % Fat in reconstituted whole milk × threshold value of stale reconstituted whole milk.

from the ethyl ether and some component of the butter oil. Since this off-flavor was not present in the other experiments in which petroleum ether was used as the extracting solvent, it appears to be the more suitable solvent for this study.

SUMMARY

In this study of the stale flavor which develops in dried whole milk on storage, it was necessary to develop a more efficient method for obtaining stale butter oil from the dried whole milk.

An investigation of various Soxhlet-type extraction procedures with organic solvents resulted in two suitable procedures which yielded better than 90 per cent recovery of stale butter oil.

Difficulties encountered in the removal of solvent from the extracted butter oil necessitated the development of a special technique to reduce the solvent concentration in the butter oil to the point where it did not interfere with the organoleptic judgment of the product.

The stale-flavor component was extracted with the butter oil by these procedures in approximately the same ratio to the fat as existed in the original dried whole milk and therefore may be considered to be better than 90 per cent extracted from the dried whole milk.

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A NEW INDICATOR METHOD FOR THE DETERMINATION OF DIGESTIBILITY AND CONSUMPTION OF FORAGES BY RUMINANTS

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The search for an indirect method for measuring the digestibility and consumption of feedstuffs by animals has been in progress for many years. A satisfactory procedure conceivably could eliminate the necessity of long, tedious and expensive digestion trials. In addition to determining the digestibility of dry and green feeds, an adequate method also would allow the indirect measurement of the quantity of pasture herbage consumed by grazing animals. Many other obvious applications of such a method are possible.

Most of the proposed methods require that certain reference substances used as "indicators" occur naturally in or be added to the feedstuff being examined. For the purpose of estimating consumption, it is essential that the reference substance be a normal constituent of the feed. Based upon the difficulties encountered in the use of various indicator methods it would seem that the ideal method should possess the following features: (a) It should employ a reference material which occurs naturally and in a measurable quantity in the feedstuff; which is indigestible and, therefore, completely recoverable in the feces; and, for which the chemical analysis is simple, accurate and rapid. (b) The recovery of the reference substance from the feces must not be influenced by treatment of the feed (curing methods, heat, etc.), by stage of maturity or by irregular passage of the "indicator" through the gut. (c) The equilibrium of the reference substance in the feces with that in the feed must be established soon after feeding is begun in order that short time trials may be used.

The authors are not aware of any reports giving previous attempts to employ natural plant pigments or chromogens[1] as reference substances. A summary of the voluminous literature dealing with the use of silica, iron oxide, chromic oxide and lignin as indicators will not be attempted in this report.

It was the object of this study to determine whether forages contain natural chromogenic substances which are indigestible, completely recoverable in the feces and, therefore, adaptable to use as a reference material for the indirect measurement of digestibility and consumption of forages by ruminants.

EXPERIMENTAL PROCEDURE

In the first part of the investigation conventional digestion trials were con-

[1] For lack of better terms, "chromogen(s)" and "chromogenic substances" are employed throughout this report to refer to substances in solution absorbing light. Whether or not the substance(s) absorbing light at 406 mμ is a natural plant pigment or is chromogenic is not known.

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ducted with a mixed forage of which aliquots had been cured by ensiling and by drying in the field, barn and oven. All hays were fed to four wethers weighing 70 to 90 lb. in one replication of a 4×4 Latin square, whereas silage and barn-dried and field-cured hays were fed to three bull calves weighing 350 to 375 lb. in one replication of a 3×3 Latin square. No feed other than these forages was fed. Ten-day preliminary and fecal collection periods were employed. The forages fed and the resultant feces produced in these 25 trials provided the materials used in the first part of the study.

The ratio of the dry matter consumed in forage to that excreted in feces was established for each animal and forage in the conventional trial as a prerequisite to the pursuit of the problem. In order to ascertain whether any naturally occurring chromogenic substances present in the forages were completely recoverable in the feces, absorption measurements were made of acetone extracts of the forages and of their fecal products in the visible spectral range. Samples of the forages as fed and the resultant fresh feces were weighed in quantities proportional to the dry matter consumption-excretion ratio for a given animal. It is apparent that this procedure would allow the demonstration of indigestible substances should they exist. The samples were extracted in the same volume of 85 per cent acetone and the absorption spectrum was determined for each extract, using a Beckman DU model spectrophotometer. According to well established laws and assuming a minimum of interference, solutions of similar source absorbing the same quantity of light at a given wavelength theoretically contain equal quantities of the same chromogenic substance. Therefore, it follows that at wavelengths where equal quantities of light were absorbed by the forage and corresponding fecal extracts (disregarding the possible presence of interfering substances), some chromogenic substance(s) was present in both extracts in the same quantity. Furthermore, the presence of such isosbestic points would indicate that the chromogen(s) of forages responsible for the absorption of light at the corresponding wavelengths is indigestible and may be recovered completely from the feces. Upon determining the absorption spectra of the extracts of the different forages and corresponding feces samples it was found that an isosbestic point consistently existed near 406 mμ, as shown in figure 1. Although isosbestic points occurred at other wavelengths, the amount of light absorbed was undesirably low and in some cases appeared to be characteristic of a certain kind of forage. Since a high degree of absorption occurred at 406 mμ, indicating that a relatively large quantity of the chromogen(s) responsible for this absorption was present in the extracts, attention was largely given to this wavelength.

In order to employ these findings in an indicator technique for the measurement of forage digestibility and consumption, it was necessary, first of all, to quantitate the absorption measurements of the extracts in terms of a known chromogen at 406 mμ. Since the chromogen(s) responsible for absorption at 406 mμ was unknown and since the absorption maximum of Na_2CrO_4 in aqueous solution is reasonably close (370–375 mμ) to 406 mμ, Na_2CrO_4 was employed for this purpose. Calibration of the spectrophotometer at 406 mμ was effected by

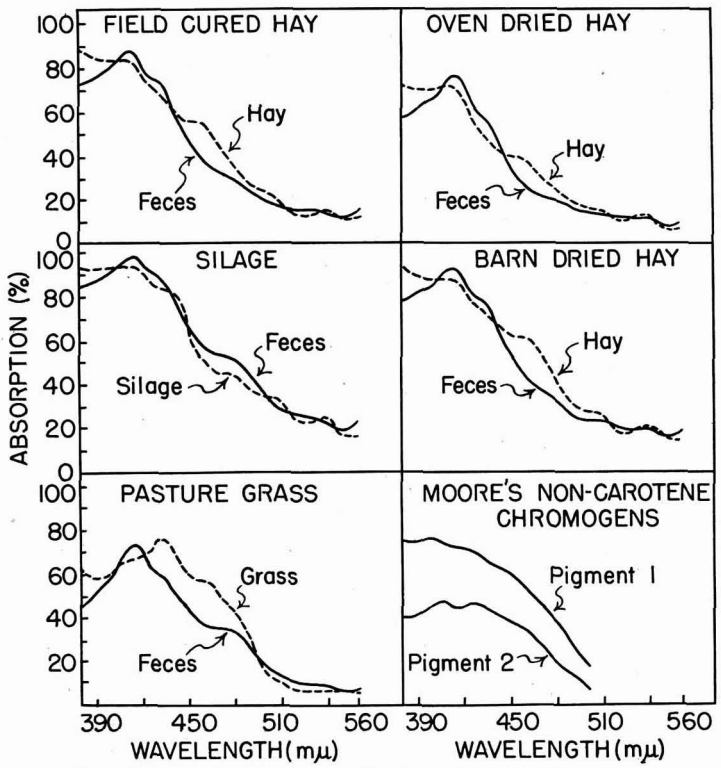

FIG. 1. Absorption spectra of acetone extracts of forages and of corresponding feces showing the formation of the isosbestic point at 406 mμ. The spectra of the extracts of hays and silage and corresponding feces were obtained from the studies conducted with wethers, whereas those of pasture grass and its resultant feces were obtained using steers.

making absorption measurements on solutions of Na_2CrO_4 ranging in concentration from 0 to 20 mg. per cent. The amount of light absorbed by a solution containing 1 mg. per cent Na_2CrO_4 was termed equivalent to 1 unit of "chromogen" per 100 ml. of extract. For the particular instrument used in this study, the relationship of the concentration of chromogen in extracts to the quantity of light absorbed is expressed by the equation $Y = 49.2379 - 23.8010\ X$, where Y = units of chromogen per 100 ml. of extract and X = log of the per cent of transmitted light.

Following these preliminary studies and as a second part of the investigation, the method finally adopted was tested further with hay containing a large proportion of Ladino clover. This was an attempt to determine whether the chromogen(s) under scrutiny were common to more than one plant species. Although the hays and silage used in the first part of the study were mixed, one plant possibly could have been the source of the pigment.

In addition to these trials, a study was made of the method's applicability to pasture grass. A known quantity of freshly clipped timothy-mixed grass forage was fed four times daily at 4-hr. intervals to three steers (two Holsteins and one Hereford weighing 375 to 500 lb.) confined in a barn and fitted with fecal collection bags. The pasture grass was studied at three timothy growth stages characterized as vegetative, boot to early head and full bloom, giving a total of nine trials. Simultaneously, total fecal collections were made from three other steers allowed to graze similar grass. Grass for analysis was sampled beginning 2 days prior to and during the first 2 days of fecal collection. The collection of feces was made over a period of 4 consecutive days. During the first day of the third trial the harness on one Holstein steer was damaged irreparably, and consequently, data were obtained on only two steers during this trial.

For all forages examined in these studies the analyses for chromogen(s) were conducted on feces as voided (undried) and on forage material in the same state as that fed to the animals.

All moisture measurements were made by the toluene distillation method. Care was taken to protect all samples and their extracts from light insofar as this was possible. All samples not in immediate process of analysis were kept in a refrigerator at 1 to 5° C.

Details of Adopted Method. Although the size of forage and feces samples convenient for extraction and the degree of dilution of the original extract will vary with the chromogen(s) content of the forage being studied, table 1 summarizes the weights and volumes found to be practicable in this study.

TABLE 1

Convenient sample weights, extract volumes and dilution rates

Material	Approximate sample wt.	Vol. of original extract	Dilution of original extract
	(*g.*)	(*ml.*)	(*diln. factor*)
Hay, mixed grass	10–12	2000	2–3
Hay, Ladino	5–6	2000	2–3
Silage, mixed grass	20–25	2000	2–4
Pasture grass (largely timothy)			
vegetative stage	20	2000	2–3
boot to early head	20	2000	2–3
full bloom stage	20	2000	2
Feces of sheep fed:			
Hay, mixed grass	5–10	1000	3–5
Hay, Ladino	3–5	1000	5–8
Silage, mixed grass	4–6	1000	4–5
Feces of bulls and steers fed:			
Hay, mixed grass	6–10	1000	2
Silage, mixed grass	5–7	1000	2–3
Pasture grass			
vegetative stage	5–7	1000	5
boot to early head	5–7	1000	4
full bloom stage	5–7	1000	2–3

Samples were weighed on filter paper of a diameter commensurate with the bulkiness of the sample and transferred with the paper to a 500-ml. boro-silicate

Waring blendor cup equipped with a large rubber stopper covered with aluminum foil. A glass tube extending from the base of and through the stopper approximately 6 to 8 in. above the cup was found to prevent leakage from around the stopper caused by pressure due to increased temperature accompanying blending. Two hundred fifty to 400 ml. of 85 per cent (by volume) acetone (depending upon the final volume of extract desired) were added to the weighed sample and the blending was begun. The blendor was allowed to run approximately 3 to 7 min. during which the cup was removed at intervals from the motor unit and placed in an ice-water bath. The number of times cooled during an extraction was determined by the degree of heating. The contents of the cup were transferred quantitatively to a Buchner funnel containing Whatman no. 42 paper, filtered by suction, and the macerate was washed with 85 per cent acetone. The residue then was returned to the blendor cup and extracted in the same manner two or more times, depending on the degree of pigmentation of the successive extracts, toughness of the material being extracted and the fineness of maceration of the residue. Results were found to be readily reproducible when the final washings were clear and when stems were well macerated. Green timothy grass in the full bloom stage, clipped into short pieces with shears, was considerably more difficult to extract completely than the same grass at an earlier growth stage. More extensive treatment was required for this kind of grass than for any of the other materials extracted. Feces from pasture grass-fed steers was the most easily extracted material studied.

The extracts were made to a known volume and a portion of this sufficiently large to prepare a final dilution was filtered by gravity through Whatman no. 40 or 42 filter paper.

An absorption measurement then was made on the properly diluted extract at 406 mμ, using a Beckman spectrophotometer. The units of chromogen(s) per gram of dry matter were calculated according to the equation shown above. Substitution of the chromogen(s) values in the following equation allows the derivation of the apparent digestion coefficient for any nutrient or the dry matter without a knowledge of the total quantity of feces produced or of the forage consumed. Apparent digestibility $= 100 - \left(100\frac{a \cdot x \text{ in feces}}{b \cdot x \text{ in forages}}\right)$, where a = units of chromogen per g. forage, b = units of chromogen per g. feces and x = per cent of specific nutrient.

When the total yield of feces is known, the daily dry matter intake may be determined according to the following equation: Dry matter consumption (g./day) = $\frac{(\text{units of chromogen(s) per g. dry feces}) \times (\text{g. of dry matter in feces per day})}{\text{units of chromogen(s) per g. dry matter in forage}}$

RESULTS

The average rates of recovery from the feces of chromogen(s) absorbing light at 406 mμ for the forages studied are summarized in table 2. Table 3 sum-

TABLE 2

Recovery at 406 mμ of chromogen(s) from feces of animals receiving various forages

Forage	Experimental animals		Recovered in feces (%)	
			Av.	Range
Field-cured hay	Wethers	(4)[a]	102.0[b]	99.9–106.2[b]
	Bull calves	(3)	100.8	100.2–101.7
	All animals	(7)	101.5[b]	99.9–106.2[b]
Barn-cured hay	Wethers	(4)	99.0	96.2–100.3
	Bull calves	(3)	101.1	98.4–104.2
	All animals	(7)	99.9	96.2–104.2
Oven-dried hay	Wethers	(4)	99.9	97.4–102.3
Silage, hay crop	Wethers	(4)	98.6	96.4–100.7
	Bull calves	(3)	100.8	99.0–102.6
	All animals	(7)	99.5	96.4–102.6
Ladino clover hay	Wethers	(2)	100.9	100.6–101.3
Pasture grass:				
vegetative stage	Steers	(3)	100.9	97.2–102.4
boot to early head stage	Steers	(3)	102.7	98.8–105.8
full bloom stage	Steers	(3)	100.3	94.4–103.3

[a] Figures in parentheses represent the no. of animals used.

[b] When the datum on one wether for which only one fecal analysis was made is disregarded, the av. and range of recovery become 100.7 (99.9–101.2) and 100.7 (99.9–101.7) % for wethers and all animals, respectively, on field-cured hay.

TABLE 3

Av. dry matter digestion coefficients of forages estimated by the chromogen method as compared to those derived from conventional digestion trials

Forages	Experimental animals		Dry matter digestibility (%)	
			Conventional trial	Chromogen method
Field-cured hay	Wethers	(4)[a]	53.2[b]	54.8[b]
	Bull calves	(3)	53.8	54.1
	All animals	(7)	53.4[b]	54.5[b]
Barn-cured hay	Wethers	(4)	53.3	52.7
	Bull calves	(3)	55.2	56.0
	All animals	(7)	54.1	54.1
Oven-dried hay	Wethers	(4)	55.4	55.4
Silage, hay crop	Wethers	(4)	48.2	47.4
	Bull calves	(3)	52.6	53.0
	All animals	(7)	50.1	49.8
Ladino clover hay	Wethers	(2)	68.0	68.3
Pasture grass:				
vegetative stage	Steers	(3)	72.9	73.3
boot to early head stage	Steers	(3)	66.3	67.2
full bloom stage	Steers	(3)	58.0	58.2

[a] Figures in parentheses represent the no. of animals used.

[b] When the datum on one wether for which only one fecal analysis was made is disregarded, the average dry matter digestibility as determined by the conventional and chromogen(s) methods, respectively, becomes 53.3 and 53.6% for wethers and 53.5 and 53.9% for all animals on field-cured hay.

marizes the data for the digestion coefficients derived from conventional trials and from the chromogen(s) method, while figures for the actual intakes and those calculated from the chromogen(s) data are shown in table 4. These data

TABLE 4

Av. daily dry matter intakes of forages estimated by the chromogen method as compared with the actual daily dry matter consumption

Forage	Experimental animals		Dry matter intake (g./day)	
			Actual	Chromogen method
Field-cured hay	Wethers	(4)[a]	454[b]	461[b]
	Bull calves	(3)	3142	3166
	All animals	(7)	1606[b]	1620[b]
Barn-cured hay	Wethers	(4)	474	467
	Bull calves	(3)	3402	3467
	All animals	(7)	1729	1753
Oven-dried hay	Wethers	(4)	443	445
Silage, hay crop	Wethers	(4)	356	352
	Bull calves	(3)	2627	2654
	All animals	(7)	1330	1338
Ladino clover hay	Wethers	(2)	712	719
Pasture grass:				
vegetative stage	Steers	(3)	4077	4114
boot to early head stage	Steers	(3)	4949	5095
full bloom stage	Steers	(3)	4096	4112

[a] Figures in parentheses represent the no. of animals used.

[b] When the datum on one wether for which only one fecal analysis was made is disregarded, the av. quantity of dry matter consumed daily as determined by actual measurement and the chromogen(s) method, respectively, becomes 507 and 510 g. for wethers and 1825 and 1838 g. for all animals.

are not, in all cases, derived from duplicate measurements on feces and most of those showing the greatest deviation from perfect recovery were the result of a single determination. This was necessitated by the time required in working out certain details of the method, especially during the early stages. Therefore, it would seem that for forages similar to those examined in this investigation, absorption measurements on acetone extracts of forage and the resultant feces of animals consuming the respective forage will allow the estimation of digestion coefficients and dry matter consumption in close agreement with the actual (that derived from a conventional digestion trial), assuming that the usual care is taken in the conduct of the trial.

Equally good results were obtained with the proposed method when applied to a study of clipped pasture grass during three growth stages as when employed for the study of hays and hay-crop silage. The rates of recovery of the chromogen(s) from the feces of wethers, bull calves and steers were similar.

The mixed forage cured by four different methods ranked in descending order of chromogen concentration as follows: silage, oven-dried hay, barn-cured hay and field-cured hay. Although no extensive investigation has been made of the stability of the chromogen(s) studied, it was found to be light labile.

However, chlorophyll and carotene were lost more readily upon exposure to sunlight than the chromogen(s) responsible for the absorption at 406 mμ. No great loss of chromogen(s) was observed from extracts allowed to sit at room temperature in the dark from several days. Chromogen analyses made at intervals of a month on the same feces stored at 1 to 5° C. were in very close agreement. It is indicated that chromogen analyses do not have to be made on strictly fresh material. However, more extensive studies dealing with stability are in progress and may alter this general picture.

In a few cases where animals refused a small quantity of the forage offered, the chromogen content of the orts was much less than that of the forage offered. Even in the case of chopped hays, a marked degree of selective feeding was observed. This was indicated not only by the low chromogen level, but also by the low protein and high fiber content of the refused feed.

The digestibility and consumption of pasture grass dry matter by grazing steers, as estimated by the proposed method, are shown in table 5. These intake

TABLE 5

Comparison of dry matter digestibility and consumption of pasture grazed at three growth stages as determined by the dry matter consumption-excretion ratio method and the chromogen(s) method

Steer no.	Dry matter digestibility (%)		Estimated dry matter intake (g./day)	
	D.M.C.–E.R. method[a]	Chromogen method	D.M.C.–E.R. method[a]	Chromogen method
Vegetative stage				
0		79.1	3292	4267
36		76.8	5443	6350
39		75.8	4672	5227
Av.	72.9[b]	77.2	4469	5281
Boot to early head stage				
0		74.8	3579	4787
36		65.0	5202	5003
39		68.2	3879	4103
Av.	66.3[b]	69.3	4220	4631
Full bloom stage[c]				
0		67.4	3135	4017
39		64.4	4041	4744
Av.	58.1[b]	65.9	3588	4381

[a] Abbreviation for "dry matter consumption-excretion ratio" method.

[b] Dry matter digestion coefficients for clipped grass fed to "inside" steers as shown in table 3 are assumed to be the same for grass consumed by grazing steers when the dry matter consumption-excretion ratio method is employed. Therefore, these figures are recorded in this table in order that they may be readily compared to those obtained by the chromogen(s) method.

[c] Three grazing steers were begun in the study of grass at full bloom stage, but one steer was eliminated because of damage incurred to fecal collection bag harness.

estimates are somewhat higher than those determined by the simultaneous dry matter consumption-excretion ratio method. The digestibility of dry matter of the grazed grass was appreciably higher than that of the barn-fed clipped grass (table 3), indicating that grazing animals tend to select the more nutritive portions of the grass.

DISCUSSION

The results indicate that it is feasible to use the chromogen(s) of forages absorbing light at 406 mμ as an "indicator" for digestibility and consumption measurements. The method as proposed is simple, rapid and accurate. It appears to be readily applicable to many kinds of ruminant nutrition experiments involving feeding trials, lactation trials and, especially, studies dealing with the evaluation of pastures in which a measure of consumption rate as well as digestibility is highly essential.

One weakness common to all methods employing naturally occurring "indicators" as applied to pasture studies is the inability to analyze grass truly representative of that selected by the grazing animal. If the substance used as the reference material was of the same concentration throughout the plant, the seriousness of the problem would be greatly minimized. However, the problem exists when either the lignin or chromogen ratio technique is applied. The lignin content of the leafy portions of the plant is much lower than that of the stems, whereas for the chromogen(s) measured at 406 mμ the reverse relationship was found. The data obtained in this study show that the intake of grazing steers estimated by the chromogen method is higher than that calculated on the basis of the dry matter consumption-excretion ratio which was determined for steers fed known quantities of similar grass. The quantity of dry matter consumed (estimated by chromogen method) by grazing steers was higher than that of similar steers fed a maximum level of clipped grass in the barn. However, it is doubtful that a difference as great as that observed actually existed. Since the level of chromogen(s) was higher in leaves than in stems and since the steers tended to select the leaves, probably because of greater palatability, the grass actually consumed by the steers probably contained a higher concentration of the chromogenic substance(s) than the analysis of clipped grass indicated. Therefore, a higher-than-actual intake was most probably the resultant estimate. This hypothesis would appear to be substantiated further by the fact that the chromogen(s) content of the grass refused by the barn-fed steers was much lower than that of the grass offered.

Data recently published by Forbes and Garrigus (1) show that the intake of steers estimated by the lignin ratio method generally is lower than that estimated by the dry matter consumption-excretion ratio technique. An explanation for this seems to be that the portion of the plant actually consumed by the animal is lower in lignin than the representative grass upon which the lignin analyses were made.

Regardless of the accuracy which may be demonstrated for the proposed method (chromogen), the lignin ratio or any other indirect method for evaluating forages when the material analyzed is the same as that fed, precise consumption data under grazing conditions is precluded at the present time by the inability to obtain samples of grass which are representative of that consumed by grazing animals.

An attempt was made in this study to confine grazing steers to an area which within 4 days would be grazed down to a level approximating the height of the stubble from which the grass was clipped for the barn-fed steers. Since the forage received by the barn-fed steers was sampled on an aliquot basis at each clipping (four times daily), plucked samples were taken at corresponding times from the area being grazed. These 16 samples, each weighing 165 g., were used to form a 4-day composite sample upon which moisture and chromogen analyses were conducted. The observed grazing habits of the steers pointed to a fallacy in this method of sampling. During the first 2 days, the steers grazed the leaves and, thereafter, the remaining stems. Judging from the degree of fill, the intake of these steers was greatest during the first day of grazing and was progressively less each successive day. However, the procedure used for obtaining the plucked samples did not allow for the supposedly reduced intake on successive days, *i.e.*, the samples taken were not proportional to the consumption of the steers. Since the chromogen level of plucked samples was considerably lower than that of clipped samples, a greater stem to leaf ratio existed in the plucked sample than in either the clipped sample or the grass actually consumed by the steers. Accordingly, the quantity of chromogenic material in the grass consumed by the barn-fed steers was used in calculating the data for the grazing steers.

The dry matter consumption-excretion ratio method originally suggested by Garrigus and Rusk (2) for use in pasture evaluation generally is considered to be the best method available for critical studies. However, the major recognized weakness of this procedure as applied to grazing studies is the assumption that the digestibility of the forage selected by grazing animals is the same as that of clipped grass fed to "inside" steers. The data derived by the chromogen method (table 5) for the pasture grass used in this study indicate that grazing animals select grass of a higher digestibility than that of grass from the same source but clipped and fed. Although the data are too few to allow definite conclusions, an examination of the body weight gains of the grazing steers indicates that the dry matter intake calculated by the chromogen method approaches more closely the probable intake based upon daily digestible nutrient requirements than do the estimates derived from the dry matter consumption-excretion ratio method. Therefore, these data suggest that the consumption estimates effected by the proposed method are more nearly comparable with production response than are the estimates made by the method suggested by Garrigus and Rusk (2). However, the chromogen(s) content of the grass consumed by grazing steers probably was higher than that resulting from the analysis of clipped grass, so the dry matter intake and digestibility estimates probably are somewhat higher than the actual figures.

Although the identity of the chromogen(s) employed in the proposed method is not essential to the execution of the method, it may be that a knowledge of its nature would lead to the improvement of the procedure. In addition, information in this connection would be of interest from a purely scientific standpoint. One possibility as to its identity is suggested by the spectral studies made of various non-carotene chromogens (fig. 1) extracted from dehydrated alfalfa meal

by Moore (3). The absorption spectra for the crude acetone extracts employed in the method proposed here resemble the spectra found by Moore for pigments 1 and 2 (in Skellysolve B) (fig. 1) which he had classified among a group of non-carotene chromogens. Moore (3) detected a very low vitamin A activity for these pigments used as a group in biological assays with rats. Whether or not pigments 1 and 2 were responsible for any part of the vitamin A activity found is not known.

Since the recovery of the pigment in the feces was so consistent for all forages studied, and since it would seem improbable that several chromogens consistently would exhibit an absorption relationship resulting in the formation of isosbestic points near 406 mμ, one chromogenic substance apparently is responsible for the absorption observed. However, since acetone is a polar solvent, combination of the solvent with some substance to form a chromogen capable of absorption at 406 mμ is possible. Further study employing non-polar solvents to examine this possibility is planned.

Sources of Error. The errors inherent in the conventional digestion trial appear to be common to this method also. In addition, analytical errors are possible; the major one of these experienced during the investigation was incomplete extraction of the pigments from certain kinds of samples. Mature, tough timothy-mixed grass was considerably more difficult to extract completely than the same kind of grass at an early growth stage, or than finely ground hay samples. Feces, probably because of maceration by the animal, were extracted more easily than forage materials. However, sheep feces, especially those containing in excess of 60 per cent dry matter, were more difficult to extract than those of bull calves or steers. Sampling errors were believed to be of the greatest magnitude with silage and with the green fresh grass clipped to approximately 0.5 in. lengths before weighing. Samples weighing approximately 20 g. were employed to aid in counteracting this error. In an attempt to arrive at a representative chromogen figure on green grass, six or more analyses were made. This problem appeared to be minimized with regard to finely ground hays and feces as excellent agreement was found in most cases in the analysis of duplicate samples. The blending of a sample in acetone to a temperature where acetone is being lost (reducing the ratio of acetone to water) poses a problem which was not explored. This could be an error in that it may influence the absorption of light by a given extract. However, this was controlled, as far as possible, by cooling the blended samples at intervals in an ice-water bath. The Na_2CrO_4 solutions used as standards may respond to the Beer-Lambert law at 406 mμ in a manner different from that of the chromogen(s) responsible for the absorption measured at this wavelength and thus cause errors. However, the rates of recovery observed in this study would justify the use of Na_2CrO_4 in this way. It is the desire of the authors to attempt the isolation of the chromogen(s) in at least a crude form and examine the feasibility of its use in the calibration of the method.

SUMMARY

Mixed forage of the same source cured by barn, oven and field drying and by ensiling, hay consisting largely of Ladino clover, and pasture grass (largely timothy) at three different growth stages were fed to wethers and/or bull calves and/or steers in 36 conventional digestion trials. The dry matter digestibility of these forages ranged from 48.2 to 72.9 per cent.

Spectral examinations of acetone extracts of the forages studied and their corresponding fecal products revealed that some chromogen(s) absorbing light at 406 mμ was completely recoverable in the feces. The average rate of recovery of the chromogenic substance(s) in the feces of animals fed the forages used in these studies was 100.5 per cent for the 36 trials.

As a result of these studies, a simple, accurate method employing the chromogen(s) absorbing light at 406 mμ as a reference substance was devised for the estimation of digestibility and consumption of forages by ruminants. This method apparently has a wide range of applicability in nutrition studies with ruminant animals, especially in the study of pastures where a direct measure of consumption is impossible.

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THE ACCURACY OF LINEAR BODY MEASUREMENTS OF DAIRY CATTLE[1]

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Measurements such as weight, milk production, egg production, speed and linear body measurements are often used in animal husbandry for determining progress of breeding methods or the effects of different rations. Such measurements can have a high degree of objectivity and are adaptable to statistical analyses. The validity of the conclusions drawn from the statistical analyses depends partly on the accuracy of the original measurements. The present study is concerned with the amount of random error in linear body measurements of dairy cattle.

Phillips and Dawson (7) concluded that measurements taken directly from hogs were more accurate and required less time than obtaining measurements from photographs or by a livestock scaling stick. Phillips and Stoehr (8) found that measurements taken directly from sheared sheep generally were more accurate than those obtained from photographs. By 11 repetitions of 25 measurements on each of nine Jersey cows and ten Jersey heifers, Lush and Copeland (5) found that there was little or no correlation between the average size of the measurement and the random error in taking that measurement. Single observations of measurements were, in most cases, accurate enough to obtain significant differences between animals, but averages based on two or more repetitions of the measurements were more accurate. On comparable measurements the absolute sizes of the errors they found were approximately of the same order as those in the present study.

Lush, *et al.* (4), in studying weights of cattle, concluded that the average of 3-day weights was not absolutely accurate, but the random error was only 57 per cent of that of 1-day weights. Bean (2) and Baker *et al.* (1) found that single-day weights were more reliable than 3-day weights, but this contradictory finding can be attributed to the grouping of their data. They grouped their data into subgroups on the basis of the weights on the first day. The range within these groups on the first day was thus fixed within bounds, but these same groups could, on the second and third days, contain weights above or below the limits set for the group. This automatic effect of the method of grouping was mainly responsible for the contradictory conclusions that single-day weights are more accurate than the average of 3-day weights. Concerning weights of steers, Patterson (6) found that by two extra weights the mean square between animals could be reduced by only 0.65, 2.44 and 0.95 per cent in three groups of data. From this, he concluded that using 11 animals with single-day weights was more

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efficient than using 10 animals with 3-day weights, as judged by the standard error for animals.

The general conclusion from such studies is that extra weighings and measurements result in more accurate data, but there is a question from case to case as to whether the gain in precision is enough to warrant the extra trouble, time and expense of taking the extra data.

SOURCE AND DESCRIPTION OF DATA

The data for this study were from the Iowa State College Holstein herd from 1931 to 1946. Five characteristics, *i.e.*, wither height, chest depth, body length, heart girth and paunch girth, were measured three times at each of the ages, 6 mo. and 1, 2, 3, 4, 5 and 7 yr. The measurements were recorded to the nearest millimeter. The paunch and heart girth measurements were taken with a steel tape in a plane perpendicular to the body axis at the largest and smallest circumferences of the barrel, respectively. Chest depth, wither height, and body length were measured with calipers. Wither height was the vertical distance from the highest point over the withers to the ground. Chest depth was the vertical distance from the back to the floor of the chest at the shallowest part of the chest. Body length was the horizontal distance from the point of the shoulder to the end of the pin bones.

One measurement of each characteristic was made and recorded as the "first order" measurement. The animal then was moved to a new position and each characteristic was measured again; this was recorded as the "second order" measurement. The "third order" measurements were taken after the animal had been moved again to a new position. This procedure was followed at each of the seven ages at which measurements were taken. Care was taken each time to have the animal in a natural position standing rather squarely on all four legs. At the beginning of the project one man took most of the measurements, but in later years three men took the measurements so that one man would make only one measurement of a characteristic on each animal at each age. Beginning in 1943, the name of the man who took each measurement was recorded so that the effect of possible differences in the way various men used the measuring instruments could be measured.

ANALYSIS OF DATA

Estimating the variance components. Suppose P cows were each measured three times for each characteristic studied. Now, if the order in which each of the three measurements was taken is known, the data for each characteristic can be classified according to two criteria, namely, by cow and by order. The mathematical model for the data would be of the form

$$Y_{ijk} = \mu + A_i + B_j + X_{ijk} \qquad (1)$$

where Y_{ijk} is the observed value of a characteristic on the i-th cow for the j-th order: μ is the mean of all observed values, A_i is the amount by which the i-th cow is above or below the general mean. B_j is the amount the j-th order differs from the general mean, and X_{ijk} is the error associated with the Y_{ijk}. The X_{ijk}

are caused by such things as mistakes in reading the instruments, differences in the pressure with which the instruments were applied, genuine changes in the animal such as taking in and letting out breath, interactions between orders and cows and many other things which are not controlled easily.

If now *3P* equations of type (1) are written in place of the observed values, the measurements are expressed in terms of the components on which interest is centered. The sizes of the cow, order and error components of variance were estimated by an analysis of variance an example of which is shown as table 1. The sums of squares for total, for cows and for orders were calculated in the usual way as given in Snedecor (11). The error sum of squares was estimated by subtracting the sums of squares for cows and for orders from the total.

The differences between cows were significant far beyond the 0.01 level of probability for each characteristic and at each of the seven ages. This was expected because of the wide variation among the animals. The differences

TABLE 1

Analysis of variance for paunch girth of 3-yr.-old cows

Source	Sum of squares	Degrees of freedom	Mean square	Expected mean squares	Estimated variance component
Total	97,181.38	731			
Cows	96,416.83	243	396.78**	$\sigma^2E + 3\sigma^2C$	$\sigma^2C = 131.74$
Orders	3.76	2	1.88	$\sigma^2E + 244\sigma^2D$	$\sigma^2D = 0.002$
Error	760.79	486	1.57	σ^2E	$\sigma^2E = 1.57$

** Significant at the 0.01 level of probability.

between orders were significant at the 0.01 level of probability in 19 of the possible 35 cases and significant at the 0.05 level in two more cases, although the components for orders were too small to be important practically. Sixteen of the 19 highly significant cases were at the four youngest ages. Perhaps nervousness of the younger animals contributed to the order differences among them. Order differences also are partially confounded with differences between the men taking the measurements. Differences between the mean measurements by different men on the same animal were too small to have been important practically although, apparently, they were statistically significant in some cases. The details of this analysis are not shown since the man differences were confounded completely with the order differences until the last few years and were confounded partially with them then. Table 2 shows the cow, order and error components of variance for each characteristic at each age. The order and error components are very small when compared with the cow components.

Relative value of one, two, and three measurements of a characteristic. So far as the sizes of the variance components σ^2c and σ^2e in table 2 show correctly the variance from these causes, the relative value of measuring a characteristic one, two, three or k times can be estimated by the formula:

$$\text{Relative Value} = \frac{\sigma^2c}{\sigma^2c + \frac{\sigma^2e}{k}}.$$

This formula is used with the assumption that the cows are a random sample, as they would be in most breeding experiments. As k becomes larger the relative value of the estimate approaches 1.00. When $k = 1$, the relative value of the estimate is at its lowest point and the relative value is the same as the intraclass correlation. Table 3 gives the relative values of one, two and three measurements of each characteristic at each age. One measurement of each characteristic seems accurate enough for most purposes. A possible exception is body length, for which estimates based on single measurements have relative values of 0.834 to 0.907, but estimates based on three measurements range from 0.936 to 0.967.

The relative values of the measurements are approximately the same for the same characteristic at the different ages. If the relative values for the various

TABLE 2

The components of variance for each characteristic at each age

Age and no. of cows	Component[a]	Characteristic				
		Wither ht.	Chest depth	Body length	Heart girth	Paunch girth
	C	14.98	4.85	24.86	33.26	78.19
6 mo.	D	0.03	−trace	0.03	0.06	0.09
367	E	0.57	0.21	2.59	0.93	1.43
	C	13.73	5.80	29.06	49.80	85.33
1 yr.	D	0.05	−trace	0.04	0.06	0.08
348	E	0.76	0.45	3.00	1.18	1.66
	C	11.39	5.27	21.40	54.52	136.03
2 yr.	D	0.02	0.01	0.20	0.08	0.03
329	E	0.71	0.40	3.92	1.81	1.37
	C	13.26	6.26	22.09	54.58	131.74
3 yr.	D	0.01	−trace	0.10	0.08	trace
244	E	0.77	0.38	4.10	2.09	1.57
	C	15.06	4.99	21.22	51.47	143.83
4 yr.	D	0.02	0.01	−0.02	0.04	0.01
161	E	0.73	0.34	3.54	1.17	1.83
	C	16.52	6.43	25.67	53.08	108.07
5 yr.	D	0.03	trace	−0.01	0.06	−0.01
108	E	0.67	0.30	3.49	2.04	1.77
	C	10.91	3.66	25.66	64.09	165.36
7 yr.	D	0.01	0.01	−0.13	0.01	−trace
38	E	0.57	0.41	5.11	1.71	2.16

[a] C = Cow component of variance.
D = Order component of variance.
E = Error component of variance.

ages of one characteristic are ranked in numerical order of their size as 1, 2, 3, . . . 7, a rectangular distribution is obtained. If there is no difference between the relative values at the different ages, the sums of the ranks at the seven ages should be approximately equal, and a chi square test of the mean ranks of the seven ages would detect any age difference in the relative values. Table 4 gives the ranks; the number of ranks is seven, corresponding with the number of ages, and the number of sets of ranks is five, corresponding to the five characteristics. In cases of ties, the relative values were assigned the average value of the ranks for which they were tied. A discussion of this method is given by Friedman (3).

TABLE 3

Relative values of estimates based on 1, 2, and 3 measurements of each characteristic at each age

Age	No. of repetitions	Characteristic				
		Chest depth	Wither ht.	Body length	Heart girth	Paunch girth
6 mo.	1	0.958	0.963	0.906	0.973	0.982
	2	0.978	0.981	0.951	0.986	0.991
	3	0.986	0.987	0.966	0.991	0.994
1 yr.	1	0.929	0.948	0.907	0.977	0.981
	2	0.963	0.973	0.951	0.988	0.990
	3	0.975	0.982	0.967	0.992	0.994
2 yr.	1	0.929	0.941	0.845	0.968	0.990
	2	0.963	0.970	0.916	0.984	0.995
	3	0.975	0.980	0.942	0.989	0.997
3 yr.	1	0.943	0.945	0.844	0.963	0.988
	2	0.971	0.972	0.915	0.981	0.994
	3	0.980	0.981	0.942	0.987	0.996
4 yr.	1	0.937	0.954	0.857	0.978	0.987
	2	0.963	0.976	0.923	0.989	0.994
	3	0.978	0.984	0.947	0.992	0.996
5 yr.	1	0.956	0.961	0.880	0.963	0.988
	2	0.977	0.980	0.936	0.981	0.994
	3	0.985	0.987	0.957	0.987	0.996
7 yr.	1	0.900	0.951	0.834	0.974	0.987
	2	0.947	0.975	0.900	0.987	0.994
	3	0.964	0.983	0.936	0.991	0.996

Using a modification of Friedman's formula, a chi square value of 5.35 with six degrees of freedom was found for testing the significance of differences in the ranks. Since this has a probability of 0.5, one can say that there is no indication of a difference in the relative accuracy of the measurements of the characteristics at the different ages. There also was no difference in the cow components of variance at the different ages. This method of ranking would not pick out an interaction between age and characteristic. Friedman's formula was modified only to the extent necessary to allow for having used fractional values where there were ties. The use of fractional values changes the sum of the squares of the first p integers.

TABLE 4

Ranks of the relative values for each of the characteristics

Characteristic	Age						
	6 mo.	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.	7 yr.
Wither ht.	7	2.5	2.5	5	4	6	1
Chest depth	7	3	1	2	5	6	4
Body length	6	7	3	2	4	5	1
Heart girth	4	6	3	1.5	7	1.5	5
Paunch girth	2	1	7	5.5	3.5	5.5	3.5
Sum of ranks	26	19.5	16.5	16.0	23.5	24	14.5
Mean rank	5.2	3.9	3.3	3.2	4.7	4.8	2.9
Deviation from mean rank	1.2	−0.1	−0.7	−0.8	+0.7	+0.8	−1.1

It was not necessary to make a chi square test of the ranks of the characteristics within each age to show that there was a highly significant difference between the relative accuracy value for the five characteristics. Paunch girth was the most accurate and was followed by heart girth, wither height, chest depth, and body length in that order. The ranks were in the same order at each of the ages.

The error standard deviations and coefficients of variation. Unless otherwise specified, the standard deviations referred to in this section are those due to errors of measuring. The coefficients of variation referred to are those found by expressing the error standard deviation as a percentage of the mean. Table 5

TABLE 5

The mean, error standard deviation and coefficient of variation for each characteristic at each age

Characteristic	Age						
	6 mo.	1 yr.	2 yr.	3 yr.	4 yr.	5 yr.	7 yr.
Wither ht. S.D.[a]	0.76	0.87	0.84	0.88	0.86	0.82	0.75
$\bar{X}$	98.9	114.9	129.3	133.6	135.3	136.1	138.4
C.V.	0.77	0.76	0.65	0.66	0.63	0.60	0.54
Chest depth S.D.	0.46	0.67	0.63	0.62	0.58	0.55	0.64
$\bar{X}$	46.1	57.0	68.5	71.3	73.3	74.5	71.9
C.V.	1.01	1.17	0.92	0.86	0.79	0.73	0.88
Body length S.D.	1.61	1.73	1.98	2.02	1.86	1.87	2.26
$\bar{X}$	105.9	128.7	151.0	158.9	163.3	165.4	167.6
C.V.	1.52	1.35	1.31	1.27	1.15	1.13	1.35
Heart girth S.D.	0.97	1.08	1.34	1.45	1.08	1.43	1.31
$\bar{X}$	119.5	148.2	181.1	186.3	191.3	194.2	202.2
C.V.	0.81	0.73	0.74	0.78	0.57	0.73	0.65
Paunch girth S.D.	1.20	1.29	1.17	1.25	1.35	1.33	1.47
$\bar{X}$	147.0	178.5	221.5	230.3	237.1	240.2	248.6
C.V.	0.82	0.72	0.53	0.54	0.57	0.55	0.59

[a] S.D. and $\bar{X}$ are expressed in cm.

gives the standard deviation, the mean and the coefficient of variation for each characteristic at each of the seven ages. The standard deviations at 6 mo. seem to be a little smaller than those at the other ages. On ranking the standard deviations within each row in table 5 and then comparing the columns, a chi square of 10.56 was found. With six degrees of freedom this chi square is significant at the 0.11 level of probability. Most of the small age difference seems only to reflect the fact that the standard deviations at 6 mo. of age are smaller than those at other ages.

The coefficients of variation are largest at 6 mo. and 1 yr. A chi square test of the mean ranks at the various ages gave a value of 18.15. With six degrees of freedom this is significant at the 0.01 level. This difference comes mainly from the large coefficients of variation at the first two ages. The coefficients of variation were smallest for paunch girth. Next in order came wither height, heart girth, chest depth and body length.

How random errors and rounding affect correlations. In animal breeding it is often necessary to correlate one characteristic with another. If σ^2X_o and σ^2Y_o are the observed variances of two characteristics X and Y, σ^2X_e and σ^2Y_e the independent error variances involved in measuring the two characteristics and σ^2X and σ^2Y the true variances of the two characteristics, then $\sigma^2X_o = \sigma^2X + \sigma^2X_e$ and $\sigma^2Y_o = \sigma^2Y + \sigma^2Y_e$. Shewhart (10) has shown that the true correlation between X and Y is $\frac{\sigma X_o \ \sigma Y_o}{\sigma X \ \sigma Y}$ times as large as their observed correlation. In studying body measurements, if error variance, σ^2E, and the variance due to differences between cows, σ^2C, are known, the amount the random errors of measuring affect correlations can be shown. For example, if one were studying the correlation between chest depth, X, and wither height, Y, of 3-yr. old cows with the present data, the correlation between the observed average measurements would be $\frac{\sigma X \ \sigma Y}{\sigma X_o \ \sigma Y_o} = \frac{(2.502)\ (3.641)}{(2.527)\ (3.676)} = 0.9807$ as large as the true correlation.

In working with any continuously distributed variable, some rounding must be done. Sheppard (9) long ago showed that the variance component for rounding is 1/12 where unity is the width of the grouping class; this is widely known as "Sheppard's correction". In this study the measurements were rounded to the nearest millimeter, and this error of rounding was included in the error of measuring. If the average measurements of the characteristics are rounded to the nearest centimeter, the variances of these averages would be increased by the amount 1/12. The observed variances would now include the cow component, the error component and a component, 1/12, due to rounding. The observed correlation between the rounded averages of the chest depth and wither height measurements of 3-yr.-old cows would be $\frac{(2.502)\ (3.641)}{(2.543)\ (3.687)} = 0.9716$ as large as the true correlation. In these data random errors of measuring and errors of rounding to the nearest centimeter would have little effect on correlations between the body measurements.

DISCUSSION

The error components of variance did not change significantly with age and they would be affected very little by the heterogeneity of the group studied. In studying body measurements one would expect to find the following error standard deviations (in cm.): wither ht., 0.75 to 0.88; chest depth, 0.46 to 0.67; body length, 1.61 to 2.26; heart girth, 0.97 to 1.45; and paunch girth, 1.17 to 1.47. The cow components of variance in this study are for random samples at specific ages and for one breed and are smaller than cow components from random samples that would include all ages and different breeds. The cow component is thus dependent on the heterogeneity of the group studied, but in general, one would expect cow components of intra-breed variance to be of the order of those found in this study. Consequently, one measurement of each characteristic except body length is accurate enough for practical purposes, as

the cow components are so much larger than the error components. Two or three measurements of body length give approximately the same accuracy as single measurements of the other four characteristics. Even though single measurements are accurate enough for practical purposes, they do not provide a check on gross errors such as misreading a measurement by 10 or more cm. Duplicate and triplicate measurements provide a check on such gross errors.

In working with a more homogeneous group as found in some feeding experiments, the cow components of variance would be much smaller than those in this study. Consequently, the errors of measuring would be a relatively greater source of trouble as they presumably wouldn't change in absolute size. Under such conditions one might be justified in taking two or three measurements of each characteristic to obtain more accuracy as well as to check for gross errors.

Regarding conclusions drawn from measurements subject to random errors of measuring, Tryon (12) says, "When a difference between groups is empirically found, that difference is more significant in proportion as the measuring device is less reliable." Woods (13) says, "The worse we think the material the more certain we may be of our conclusions, provided there is no bias in favor of the results." Tryon's statement implies that the difference necessary for significance increases as the random error of measuring increases and, as Woods intimates, if there is a significant difference even when a large random error is involved, conclusions are more certain. Woods' statement applies only to the case where the hypothesis that the samples involved are from the same population is rejected; there would be less certainty in accepting the hypothesis.

SUMMARY AND CONCLUSIONS

The cow, order and error components of variance for the five body measurements, *i.e.*, wither height, chest depth, body length, heart girth and paunch girth, were observed at each of the seven ages, 6 mo., 1, 2, 3, 4, 5 and 7 yr. There were 367 animals measured at 6 mo., 348 at 1 yr., 329 at 2 yr., 244 at 3 yr., 161 at 4 yr., 108 at 5 yr., and 38 at 7 yr. Measurements were in centimeters.

In all cases, the order and error components of variance were very small as compared to the cow components. The largest error components were found in measuring body length. There they ranged from 2.59 to 5.11, while the cow components for body length ranged from 21.22 to 29.06. The smallest errors were for chest depth and these ranged from 0.21 to 0.45, while the cow components ranged from 3.66 to 6.43. Although significant in some cases, the order components were too small to be of practical importance.

The relative accuracies of the measurements of the characteristics were high. Paunch girth was the most accurate, for single measurements had a mean relative accuracy of approximately 0.986, while the corresponding figure for heart girth was 0.971. Single measurements of wither height and chest depth had relative accuracies of 0.952 and 0.936, respectively. Body length was least accurate, for single measurements had a relative accuracy of 0.866, but the average of three measurements brought the relative accuracy up to 0.951. There was no signifi-

cant difference between the relative accuracies at the seven different ages. The relative accuracies of the five characteristics did differ among each other with high statistical significance.

The error standard deviations did not increase significantly with the age of the animal, but the coefficients of variation were larger at the two youngest ages than at the older ages. The five characteristics differed significantly from each other in error standard deviations and in their coefficients of variation.

The random errors of measuring and also the errors of rounding make the observed correlations between body measurements smaller than the true ones, although this effect was negligible in the present study. Using the average of three measurements and rounding each average to the nearest centimeter reduced the correlation between chest depth and wither height of 3-yr.-old cows to 0.972 of what it would have been if there had been no errors of rounding or measuring. If the averages had been taken to the nearest millimeter the correlation would have been 0.981 as large as the true correlation.

It is concluded that single measurements are accurate enough for most practical purposes provided one can be sure that no gross errors such as reading a measurement 10 cm. too large or too small go undetected.

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PARTURIENT PARESIS. V. BLOOD SERUM LEVELS OF CITRIC ACID AND CALCIUM IN NORMAL PARTURIENT COWS AND COWS WITH PARTURIENT PARESIS[1]

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In recent years evidence has been accumulating that citric acid plays an important role in calcium metabolism in the body. Dickens (3) found that the hard substance of bone contained a large store of citric acid. Nicolaysen and Nordb (8) have reported that lack of vitamin D results in a decrease in bone citric acid content. Shohl (10) has demonstrated the favorable effect of oral administration of citrate buffer in the prevention and cure of rickets in rats on a rachitogenic diet. The effect is not due solely to the alteration of the pH of the intestinal tract.

The relation of citric acid to calcium excretion and urinary calculus formation has been studied by Shorr *et al.* (11, 12) and by Kissin and Locks (6). These investigators have demonstrated a decreased citric acid excretion with urinary calculi, and suggest an increased intra-renal citrate oxidation in this disease. Gomori and Gulyas (4) were able to increase urinary excretion of calcium in puppies by injecting 8 to 30 ml. per kg. of body weight of a 4 per cent solution of sodium citrate. The blood calcium remained essentially unchanged. Marek *et al.* (7) administered sodium citrate intravenously to cows and produced an 8 per cent increase in calcium excretion, chiefly via the kidneys.

That certain hormones may bear some relation to citric acid metabolism has been pointed out by several investigators. Alwall (1) injected parathormone intramuscularly into dogs and produced an increase in blood serum calcium from a normal level of 11.1 mg. per cent to 15.6 mg. per cent 13 hr. after injection. The corresponding rise in blood serum citric acid over the same time interval was from 76.5 γ per ml. to 94.7 γ per ml. Both calcium and citric acid had returned to normal 36 hr. post injection. Gomori and Gulyas (4) histologically observed the bones of puppies into which sodium citrate was injected and commented on the similarity of the lesions to those produced by injection of parathyroid extracts.

Shorr *et al.* (11, 12) have established a relation between certain of the steroidal reproductive hormones and citric acid excretion. This work demonstrated for the first time a very interesting relationship between estrogen and androgen production, citric acid metabolism and urinary calculi.

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[1] These data were taken from a thesis presented by the senior author to the faculty of the Graduate School, University of Wisconsin, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

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Inasmuch as levels of the steroidal reproductive hormones fluctuate markedly around the time of parturition Grollman (5) and since relationships between calcium and citric acid and between estrogens and citric acid have been demonstrated, a study was undertaken to determine the relationship between blood citric acid levels with parturient paresis and normal parturitions.

EXPERIMENTAL PROCEDURE

This experiment was conducted in three herds and covered a period of time extending from February 1947 to December 1948.

The initial phase of the study was conducted in the Jersey herd of Biltmore Farms at Biltmore, North Carolina. This was done in conjunction with the study on the effect of prepartum milking on the incidence of parturient paresis or milk fever reported by Smith and Blosser (13). All cows in this initial phase were prepartum milked. A total of 18 Jersey cows was studied in this phase, of which two developed milk fever.

The second phase of the study was done in the dairy herd of the University of Wisconsin. Six Guernsey, four Holstein and nine Jersey cows were studied. Five of the nine Jersey cows developed parturient paresis and one was prepartum milked.

The third phase of the study was made in the dairy herd of the State College of Washington. Four Jersey cows were used in this phase and one of them developed milk fever.

Cows in the second and third phases were handled in accordance with accepted management and feeding practices at the time of calving. Calves were permitted to nurse and cows were not milked completely dry for the first 48 hr. postpartum.

Blood samples were drawn daily, generally 3 to 5 days prior to parturition and from 3 to 5 days subsequent thereto; a few cows samples were obtained as early as 10 days prepartum and as late as 10 days postpartum. Ordinarily, samples for a given cow were drawn at the same time each day. In the studies made at Biltmore Farms, blood serum was sent in refrigerated cartons to Madison, Wisconsin, three times weekly for analysis.

Blood serum citric acid was determined by the method of Perlman *et al.* (9) in the first two phases, and by the method of Taussky and Shorr[3] (14) in the third phase. The latter method was somewhat more sensitive and reproducible with the small amount of blood serum used, but over-all results of the two methods were essentially the same. Blood serum calcium was determined by the method of Clark and Collip (2).

Cows were considered as calving normally if they did not exhibit gross clinical symptoms of any disease at calving. A few of the so-called normally calving cows were on the verge of parturient paresis, as is evident from their blood picture.

RESULTS AND DISCUSSION

The data obtained in this experiment are summarized in table 1, which presents mean levels of blood serum calcium and citric acid at different times pre-

[3] The *n*-heptane used in citric acid analyses by the method of Taussky and Shorr was kindly furnished by the Phillips Petroleum Co., Bartlesville, Okla.

TABLE 1

Mean levels of calcium and citric acid in the blood serum of normally calving and parturient paresis cows previous and subsequent to parturition

		10	5	4	3	2	1	D. of parturition	1	2	3	4	5	10
Normally calving cows	No. of Analyses	4	13	11	15	22	27	31	31	29	28	9	8	2
	Calcium (*mg.* %)	11.0	10.3	10.3	10.2	10.0	10.3	8.7	8.4	9.6	10.1	10.5	10.7	11.0
	Citric Acid (*mg.* %)	6.62	5.46	7.17	7.20	6.92	6.24	4.70	3.73	4.93	5.64	5.66	5.98	4.78
Parturient paresis cows	No. of Analyses	3	2	4	3	5	7	9	9	9	7	3	5	3
	Calcium (*mg.* %)	11.1	10.7	10.6	10.5	11.4	10.6	6.9	6.8	6.7	9.2	9.9	10.4	10.9
	Citric Acid (*mg.* %)	5.05	5.86	5.04	7.57	9.48	8.63	3.86	2.20	2.29	2.66	7.08	4.82	5.80

and postpartum. In computing the means, only those days were used where figures for both calcium and citric acid levels of the blood serum were available.

In analyzing the data, the question arose whether the normally calving cows of all three breeds and both prepartum- and non-prepartum-milked cows could be used to compare to the milk fever cows, all of which were Jerseys. Considerable data were available on the blood serum citric acid levels on the day before, the day of and the day following parturition. When these data were submitted to an analysis of variance, there was no significant variation in blood serum citric acid levels between breeds or between prepartum and non-prepartum milked cows.

It is evident from table 1 that there is some relation between calcium and citric acid levels of the blood serum in both normal and milk fever cows. Increases or decreases in serum calcium are accompanied by concurrent increases or decreases in serum citric acid. A statistical analysis of the data based on 231 comparisons in cows calving normally showed a correlation of + 0.40 between calcium and citric acid levels of the blood serum. In milk fever cows the correlation based on 76 comparisons was + 0.46. In both normal and milk fever cows these figures represent highly significant correlations between calcium and citric acid levels of the blood serum.

Data were available on blood serum citric acid levels for 17 cows both 3 and 1 days prepartum. An analysis of variance of these data did not indicate any significant differences in serum citric acid levels between 3 and 1 days prepartum in either the normally calving or the milk fever cows.

Table 1 indicates a drop in mean level of serum citric acid in both normally calving and milk fever cows at the time of parturition. An analysis of variance involving blood serum citric acid levels on 33 cows 1 day prepartum, the day of parturition, and 1 day postpartum was made. For convenience of discussion, $+1$ will be used to indicate one day prepartum; 0, the day of parturition; and -1, one day postpartum. The apparent drop in serum citric acid between $+1$ and -1 was found to be highly significant for both milk fever and normally calving cows. There was a greater drop in blood serum citric acid in milk fever than in normal cows. The difference between normally calving and milk fever cows in this respect was highly significant.

Based on 3-day totals ($+1$, 0, -1) there were no significant differences in citric acid levels of the blood serum between milk fever and normally calving cows. This fact, plus the greater drop (highly significant) in blood serum citric acid levels in milk fever cows over the same period of time, indicates indirectly that serum citric acid levels were significantly higher in milk fever cows than in normally calving cows 1 day prepartum. This difference between normally calving and milk fever cows is borne out by the data presented in table 1. On day $+1$ the cows which subsequently developed milk fever had a mean level of 8.63 mg. per cent citric acid in their blood serum as compared to 6.24 mg. per cent for the normally calving cows. By day -1 blood serum citric acid in milk fever cows had declined to 2.20 mg. per cent as compared to 3.73 mg. per cent in normally calving cows.

Data on the citric acid levels of the blood serum for the day of parturition and the first 3 days subsequent to parturition (*0, – 1, – 2, – 3*) were available on 42 cows (264 analyses). An analysis of variance was made on these data. The following facts became evident as a result of this analysis: (a) Citric acid values increased more slowly following parturition in cows developing milk fever than in normally calving cows (highly significant). (b) Citric acid levels of the blood serum were lower for the period studied (*0, – 1, – 2, –3*) in milk fever cows than in normally calving cows (highly significant). (c) The citric acid values for the day of parturition as compared to 3 days postpartum were significantly different for all cows; in the normally calving cows, citric acid values were higher 3 days postpartum than at parturition, but in the milk fever cows the reverse situation was true.

The length of time during which serum citric acid values persisted at a low level following calving in the milk fever group can be at least partially explained by relapses which occurred in several of the milk fever cows as late as 3 days postpartum. The relapses which occurred were consistently associated with low serum citric acid values. If more data were available, it would be interesting to study the speed with which serum citric acid levels recover following the last attack of milk fever.

Both normal and milk fever cows exhibit somewhat higher citric acid levels on the third day prepartum than on the third day postpartum. The difference is much more marked in milk fever than in normal cows.

Considering the blood serum citric acid picture in its entirety, a drop in serum citric acid has been demonstrated to occur at parturition in both normal and milk fever cows. This drop closely parallels the drop in blood serum calcium which has been demonstrated many times by numerous workers. The prepartum levels of serum citric acid are greater in milk fever than in normally calving cows, and the drop is also of greater magnitude and to lower levels in the former. Low levels of serum citric acid persist for a longer time in milk fever than in normal cows.

The correlation between citric acid and calcium levels of the blood serum at the time of parturition having been established, it is difficult to say which of the two changes that occur in milk fever is the more basic. No likely explanation is advanced herewith. The demonstration by Shorr *et al.* (11, 12) that both estrogens and androgens play a role in calcium and citric acid metabolism makes possible the speculation that they are responsible in some way for the changes occurring in citric acid and calcium levels of the blood at the time of parturition, since levels of the steroidal hormones are undergoing marked changes during this period.

SUMMARY

Blood serum citric acid and calcium analyses were run on twenty-two Jersey, six Guernsey and four Holstein cows from 10 days prepartum to 10 days postpartum. There were no significant differences in blood serum citric acid between breeds or between prepartum- and non-prepartum-milked cows. There was a

highly significant correlation between blood serum citric acid and calcium over this period of time. Serum citric acid levels did not show any appreciable change between 3 and 1 days prepartum, but there was a definite drop in both milk fever and normally calving cows between 1 day prepartum and 1 day postpartum. This drop was of greater magnitude in milk fever than in normal cows.

Following parturition, serum citric acid levels increased in normally calving cows and decreased in cows which developed milk fever. Part of this difference in behavior can be attributed to relapses which occurred in several of the cows with milk fever.

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It is our pleasure, after sixteen years, to welcome you back this summer to the campus of Cornell University in the heart of our great dairy state of New York. Our grounds, our buildings, our staff are being readied for you as you filter into our midst from all parts of our country, Canada and many foreign lands. The days of June 20, 21 and 22 with you as our guests and we as your host, we anticipate with joyously warm feelings and with pride denoting no small amount of satisfaction that you have chosen Cornell as the place for your 1950 annual meeting.

The priceless tradition of our University founded by Ezra Cornell is that of freedom and public service which has been spelled out in our colleges from its very opening. The love of truth and the disinterested pursuit of truth wherever it leads, is what we seek. Your American Dairy Science Association has similar objectives as you approach the multitude of problems in the fields of dairy production, manufacturing, marketing and in your resident and extension teaching. These fundamental freedoms which the living spirit of Cornell exemplifies, you definitely promulgate and adhere to. No more fitting place could have been located, we hope, for your annual meeting. Our traditions are mutual.

Cornell is ready to receive you when you arrive. May your stay with us be socially delightful, your meetings scientifically productive and educationally valuable. We most cordially invite you and welcome you, our friends in the dairy industry, to our campus "far above Cayuga's waters", this coming summer.

Sincerely yours,
C. W. DEKIEWIET, *Acting President,*
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PAPERS FOR THE 1950 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association will be held June 20 to 22, 1950, at Cornell University, Ithaca, N. Y. All members who are planning to present papers should submit the title of their paper accompanied by *two* copies of an abstract of not more than 200 words not later than March 15 to the chairman of the program committee of their respective section. The committee chairmen are as follows:

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The general program committee feels that in order to make it possible for more members to participate in the program no member should present more than two papers. No title will be considered that is not accompanied by a suitable abstract. It also is hoped that more contributions will be received from senior staff members and from the laboratories of industry.

Because of the difficulties encountered in showing slides, it is recommended that each speaker distribute mimeographed copies of his data, together with a brief summary of his paper.

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ABSTRACTS OF LITERATURE

F. E. NELSON, Editor
Ames, Iowa

MILK AND MILK PRODUCTS

MILK PRODUCTION

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CONTENTS

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ABSTRACTS OF LITERATURE

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

BOOK REVIEW

1. Judging Dairy Products, 2nd ed. J. A. NELSON AND G. M. TROUT. Olsen Publishing Co., Milwaukee, Wis. 494 pp. 1948.

This edition not only includes the judging of more dairy products than the original book, but the information is much more detailed. The score cards for milk, ice cream, butter and cheese have been standardized to a flavor score of 45 points. The flavor scoring guides given for each product are very helpful in instructional work and are improved over those given in the old book.

The judging of chocolate milk, skimmilk, goat's milk, fermented milk, evaporated milk, condensed milk, dry milk solids and cream are handled very well, also.

Different types of cheese with respect to process of manufacture, characteristics and judging or grading are discussed. The Cheddar or swiss, brick, limburger, blue-veined, cottage and cream cheeses are included.

Ice cream also is considered in more detail, with special flavors and sherbets receiving attention.

This is an excellent manual for the plant operator as well as the judge of dairy products, as the cause of defects in dairy products and the remedies given should find practical application in most dairy plants. W. S. Rosenberger

ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

2. Brucellosis therapy: studies on the effect of streptomycin and sulfadiazine in experimental brucellosis in guinea pigs. L. S. HOLM AND S. H. MCNUTT, Univ. of Wis., Madison. Am. J. Vet. Research, **10,** 37: 336–340. Oct., 1949.

Male guinea pigs inoculated with 1 million *Brucella* organisms were used as the test animals. Para-amino-benzoic acid did not alter the course of the infection. Streptomycin or sulfadiazine had a slight effect when given singly and a definite curative effect when given together. When treatments were initiated on the day of infection practically 100% bacteriologic cures developed. Treatment started at 2, 5 or 10 d. after infection was also highly effective. Doses of 10,000 units streptomycin plus 120 mg. sulfadiazine given once daily were just as effective as 5,000 units streptomycin plus 120 mg. sulfadiazine divided into 2 or 5 doses/d. No toxicity was observed as a result of the treatments. E. W. Swanson

3. Epizoötiology of mastitis: the relative importance of extended exposure and of age in the spread of Streptococcus agalactiae infection. R. ORMSBEE AND O. W. SCHALM, Univ. of Cal. Berkeley. Am. J. Vet. Research, **10,** 37: 306–313. Oct., 1949.

Data from a large commercial dairy herd collected over more than 3 yr. time were used to check the validity of the "age factor" hypothesis of mastitis susceptibility. Two outbreaks of *S. agalactiae* infection occurred after the herd had been freed of such infection, which exposed a total of 629 clean cows of various ages over a period of 4 mo. The incidence of infection from 1st to 5th or higher lactation was respectively 9.5, 19.0, 21.1, 20.3 and 23.7%. Under extended exposure the same herd prior to eliminating the infection had had age incidence of 16.7, 23.7, 44.8, 71.5 and 76.6% for 1st to 5th or higher lactation. When the clean cows were exposed, 205 had been infected previously and cured by chemotherapy and 236 in a comparable group had not been infected previously. The resulting incidence of infection in these groups was 21.95 and 19.92%, respectively, while according to the "age factor" hypothesis nearly all of the cows in the former group should have been reinfected. These data indicate that degree of exposure is of primary importance in establishing *S. agalactiae* infections and that clean cows of all ages or previous mastitis history react similarly to the same exposure. E. W. Swanson

4. Over de behandeling van streptococcenmastitis met penicilline en de bestrijding van streptococcenmastitis met penicilline en autovaccin. (The penicillin treatment, a reliable therapy against streptococcal mastitis.) (English summary.) O. BOSGRA, R. POST AND D. REMPT.

Netherlands Milk & Dairy J., **3**, 3: 155–161. July–Sept., 1949.

Experiments performed with penicillin and vaccine treatments are described. Five seriously infected herds of cattle with a total of 100 cows were investigated, in which 20% of the quarters of the udder were found positive. These quarters were treated by intramammary injection with 25,000 to 50,000 units of penicillin, depending on the amount of milk produced. This dosage was given in 2 lots with a 24-hr. interval, using distilled water as a solvent. Investigation of the quarters 1 wk. later showed that the mastitis-positive percentage had dropped to 4%. Cattle free from mastitis were treated at the same time with a mixed vaccine prepared from the mastitis strains involved in the experiment. Six mo. later, 13% of the penicillin-treated cows were found positive, giving a 9% increase. The vaccinated cows now had a mastitis percentage of 9%, also a 9% increase.

The following conclusions were drawn: penicillin treatment is a reliable therapy against streptococcal mastitis. No noticeable immunity is produced, either in animals which have been cured of infection or in those inoculated with a specific vaccine. Cattle, suffering at a given time from a streptococcal infection of the udder, are not more sensitive to an infection by the streptococcus concerned than cattle which are at that time free from this infection. A. F. Tamsma

5. Het onderzoek op tuberculose bij het rundvee in Nederland en endele beschouwingen over de huidige stand ervan. (Testing cattle for bovine tuberculosis in the Netherlands and some views of the present situation.) (English summary.) C. F. Van Oijen and G. B. R. Willems, State University of Utrecht, Holland. Netherlands Milk & Dairy J., **3**, 2: 91–112. April–June, 1949.

After a short historical review of the campaign against bovine tuberculosis in the Netherlands, the present situation is described. In the past, farmers volunteered in local organizations, which later were united into Provincial Unions. A few months after the end of this war the eradication of tuberculosis in cattle was regulated by law. The state and local authorities combine with farmers' organizations and organizations of the dairy industry to meet the costs and losses for individual farmers. In many districts milk from tuberculosis-free herds warrants a premium.

The measures consist of the following: (1) a preliminary clinical examination and elimination from the herd of obviously sick animals, (2) intradermal (or conjunctival) tuberculination of the whole stock, (3) segregation of reactors from non-reactors, marking of reactors, (4) clinical examination and microscopical investigation of secreta, to detect open cases of tuberculosis (in special cases the microscopical examination is confirmed by the culture of tubercle bacilli and/or inoculation of guinea pigs), (5) immediate slaughtering of all open cases, (6) raising of calves so called tuberculosis-free, *i.e.*, feeding with milk from cows known to be free from tuberculosis, protection against any possibility of infection, (7) compulsory pasteurization of skim milk and whey destined for feeding of live stock, (8) re-examination of non-reactors at least every year; there is a growing tendency to repeat the tuberculination and the clinical examination twice a year and (9) progressive elimination of reactors and their replacement by tuberculosis-free animals.

During the last years of the war and the first years thereafter, unfavorable conditions caused some positive reactors in some herds which were thought to be free from tuberculosis. At present, a very large number of herds, especially in breeding centers, are free from tuberculosis. The authors expect that tuberculosis in cattle will be reduced to a minimum in a few years.

A. F. Tamsma

6. The response of cattle to penicillin preparations following intramuscular injection. E. V. Morse, Cornell Univ., Ithaca, N. Y. Am. J. Vet. Research, **10**, 37: 314–317. Oct., 1949.

The effectiveness of various penicillin preparations was measured by assaying blood plasma and urine at intervals following intramuscular injection of 1 and 1.5 million units. Crystalline penicillin G in aqueous solution was absorbed and excreted rapidly, only traces being found in plasma at 7 hr. Penicillin in oil and beeswax maintained more than trace levels of penicillin in plasma for 48 hr. Procaine penicillin G in oil gave practically the same results. Procaine penicillin in peanut oil and 2% aluminum monostearate maintained good blood and urine concentration for 72 hr. and traces were found up to 120 hr. Single injection sites were more efficacious than double sites. E. W. Swanson

7. The relative importance of antibodies and Vitamin A in preventing disease in young calves. (Abs.) F. Blakemore *et al.*, Inst. of Animal Path. and Dunn Nutritional Lab., Cambridge. Biochem. J., **42**, 2: xxx. 1948.

When colostrum, known to be rich in vitamin A as well as containing antibodies, is not given to young calves, "white scour" often results. Attempts to prevent this abnormality by giving vitamin A were not successful, but when calves were

inoculated with precolostrum, demonstrated to contain antibodies in concentrated form, they were protected. The protective value of colostrum is assumed to be associated with its globulin content. A. O. Call

Also see abs. no. 50.

BUTTER

O. F. HUNZIKER, SECTION EDITOR

8. Konsistenz der Butter. Teil II. (The body characteristics of butter. Part II.) English summary. W. MOHR AND F. SCHULZ. Die Milchwissenschaft, **3,** 12: 362–366. Dec., 1948.

Butter cutting trials with Alfa butter at temperatures of from 15 to 22° C. indicated that the resistance to cutting varied inversely to the temperature of the butter; the cutting velocity being constant at 0.1 cm./cc. By increasing the cutting velocity from 0.0001 to 0.25 cm./sec. a measurable increase in the force required to cut the butter was observed. The slope of curves obtained was similar in the case of normal and of "layer type" Alfa winter butter. The slope for crumbly, ripened cream Alfa winter butter was slightly more steep than for normal Alfa winter butter. Best results were obtained when the butter samples were cut at 10° C. at a velocity of 0.01 cm./sec. At greater velocities the response of tht balance was too slow, whereas at lower velocities the time required was rather long and the butter warmed up to room temperature.

The body of normal and of crumbly butter, tempered to 10° C., was compared by determining the "break point" of a stick of butter 10 × 2× 2 cm. with the force applied in the center of the stick at a velocity of 0.1 cm./sec. Crumbly butter had a much lower "break point", *i.e.,* it required less force to break the stick, than did normal butter. Normal butter containing air pockets gave values similar to those of crumbly butter. The defect "layer type" Alfa butter could not be detected by the above method.

The "break point" method can be used to advantage with crumbly winter butter only.

Measurements of the firmness of butter by means of comparison of the cutting resistance with the cone flow point and with the "break point" can serve as a guide for detecting crumbliness in butter. For greasy, soft summer butter comparisons of cutting resistance to cone flow point are of value. I. Peters

9. Aktuelle kvalitetsproblemer ved produksjon av smor. (Quality problems in the production of butter.) Meieriposten, **38,** 27: 473–475. July, 1949.

In the annual report from Norske Meieriers Salgscentral for 1948, Olav Benterud summarizes the work done on quality problems. Results from 4610 churnings at 37 creameries were studied for 2.5 yr. The fresh butter was judged at 2.5 to 3, 5 to 6, 7.5 to 8 and 9 to 10 mo. The temperature at judging was 13 to 14° C. Six judges officiated. A comparison was made between the quality of butter from low and high acid cream. The high acidity was preferable when the pH was regulated. For fresh butter, a pH of 5 to 6 gave the best results and at this pH the average score was 10.7 for non-coagulated cream and 11.1 for coagulated cream. With a pH over 6, the scores were 10.7 and 11.0. The lowest average score was that for sweet cream butter, namely 10.4.

With storing under refrigeration, the butter from neutralized cream was just as high in quality as butter from low acid cream. Sweet cream butter, at 10 mo. storage, had decreased only 0.3 point, indicating very good keeping quality. In butter from non-coagulated cream and a pH over 6 the decrease in score was 0.6 point, and at a pH of 5 to 6, the decrease was 1.3 points. For coagulated cream the decrease was 0.8 point with pH over 6, 1.8 with a pH content of 5 to 6 and 2.4 points with the pH content under 5. This was for butter held in cold storage for 10 mo.

There was a greater decrease in score for ripened, coagulated, cream butter, than in unripened, non-coagulated, cream butter but since the score of the fresh butter made from ripened coagulated cream was larger than that from uncoagulated cream, the final scores were approximately the same when the pH was over 6 and between 5 and 6.

The results for butter from high acid cream with a pH under 5 would indicate that this butter is unsuitable for storage purposes. Earlier experiments have shown a direct relation between the pH value in butter and its keeping quality. The most suitable pH seems to be between 5.5 and 6, where butter is to be stored for 6 mo. The best butter had a pH from 5.51 to 6.21.

The butter was scored immediately after taking it out of storage and again after it had been stored at 13 to 14° C. for 2 wk. There usually was a decrease in score between that of the butter scored immediately after removing it from storage and that of butter scored after it had been left out of storage for 2 wk. There seemed to be no greater decrease in score during this 2-wk. period of the 10-mo. storage butter than in the fresh butter. If an oxidized defect is not present when the butter is removed from storage, it is not likely to develop before the butter is consumed.

There seemed to be no difference in the quality of washed and unwashed butter. Experiments were made using sodium bicarbonate or hydrate.

This was used without mixing it with the butter salt.

Comparisons were made by 78 churnings being done with a roller-type churn and 81 churnings with a roller-less churn, the "O.H.K." churn. The butter was judged after 3 to 4 d. at 13° C. and again after 17 to 18 d. at 13° C. It was found that when the roller-less churn was used, better results were obtained in quality, moisture distribution and yield. Working the butter in a roller-less churn resulted in a closer textured butter with less air content. With a low pH content, the greater air content aided the oxidation defects. A high pH and poor moisture distribution helped to further bacteriological defects.

G. H. Wilster

Also see abs. no. 19.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

10. Formula for determining the value of skim. L. C. THOMSEN, Dept. Dairy Ind., Univ. of Wis. Nat. Butter Cheese J., **40,** 11: 24–26, 51. Nov., 1949.

The value to be placed on any skim milk that is transferred to by-products is a difficult problem in cost accounting. Although a free and competetive market may be lacking for skim milk, it usually will exist for cream. With this in mind, the following formula is presented to be used in determining the value of skim milk:

$$\frac{[(A+B)-C\times F\times (D+E)]\times 100}{H}=I$$

In which

A = Amt. paid/cwt. for whole milk, f.o.b. plant.
B = Handling costs (receiving and separating) /cwt. of milk.
C = Fat in each 100 lb. of milk purchased.
D = Price paid for butterfat, f.o.b. plant (of equal quality to that received in the whole milk) in cream which may be bought.
E = Handling charge (receiving)/lb. of butterfat in cream which may be bought.
F = A constant.
H = Lb. of skim milk obtained from each 100 lb. of milk purchased.
I = Value of skim milk/cwt.

The constant "F" will be characteristic of the factory. It is the ratio of the amount of butterfat that must be purchased in the form of cream to that purchased in whole milk to produce an equivalent amount of end product, normal losses of handling both products being considered.

H. E. Calbert

11. Body of cultured cream. E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y. Milk Plant Monthly, **38,** 10: 70–73. Oct., 1949.

The body of cultured cream is largely dependent on the pasteurization temperature and homogenization pressures used in its preparation. Therefore, 18% cream should be pasteurized at 165° F. for 30 min. and homogenized at 3000 lb. pressure if a firm dry body is desired. Following homogenization, the product should be cooled to 72° F. and inoculated with a 2% starter transfer, allowing 15 hr. for ripening. The final acidity in terms of lactic acid should be 0.65% at the termination of the ripening period. The product may then be cooled to 40° F. without agitation. The final evaluation of the body of cultured cream is facilitated by the use of a plummet designed by L. D. Hilker. Plummet readings of 6 to 8 appear to be the most satisfactory from the consumer standpoint. J. A. Meiser

12. The manufacture of quality buttermilk. N. C. ANGEVINE, Meyer Blanke Co., St., Louis. Mo. Milk Plant Monthly, **38,** 10: 26–30, 32, 35. Oct., 1949.

Only fresh skim milk of excellent quality should be used in the manufacture of cultured buttermilk. If non-fat dry milk solids are used, 8.5 lb. of a spray-processed product should be dissolved in 10 gal. of water at a temp. of 80 to 90° F. Pasteurization is then accomplished at 185° F. for 30 min. or 200° F. for 45 to 60 min. followed by cooling to 70 to 72° F. A 1% inoculation requires a ripening period of 12 to 15 hr. without agitation. This should produce a resulting acidity of 0.80 to 0.85% and signal the addition of butterfat and salt, the latter at the rate of 1 lb./100 gal. of buttermilk. The addition of butterfat in the form of granules may be accomplished by churning the vat of buttermilk at the ripening temp. with the addition of 2% fat or by adding chilled granules directly to the buttermilk when agitating. Also, dropping of melted butterfat into the chilled product is permissible. Following the ripening period, the buttermilk should be cooled to 60° F. or below, accompanied by agitation to break the curd. Storage of the bottled product should be at 45° F. for several hr. J. A. Meiser

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

13. Professor, Dr. phil. et scient. Sigurd Orla-Jensen in memoriam. (Professor Sigurd Orla-Jensen) M. T. SODE MOGENSEN. Nordisk Mejeri-Tidsskrift, **15,** 6: 67–69. 1949.

Prof. Sigurd Orla-Jensen and his wife had returned from a trip June 11 to their home in Copenhagen, Denmark trusting that some relief might come for the threatening heart condition which caused the sudden death of Prof. Orla-Jensen on Fri., June 24, at the age of 78. He was born in Copenhagen, Nov. 28, 1870. He held a position as mechanical engineer at the Carlsberg Brewery for a few years but he became interested in dairy manufacturing and spent 2 yr. studying with Prof. Segelcke at the Royal Agricultural College. He sought further training with Weigmann in Kiel, with Duclaux at the Pasteur Institute in Paris and with von Freudenreich in Bern. He was 29 yr. of age when he was placed in charge of the dairy research experiment station at Bern, Switzerland. In 1906 he became professor of biological chemistry at Denmark's Polytechnic High School. He held this position until 1946 although from 1941 he served as a substitute for Prof. Henrik Dam who was in the U. S. at that time.

In 1919, Prof. Orla-Jensen's famous monograph, "The Lactic Acid Bacteria", was published and this brought him world renown. A supplement to it was published in 1942. His text book on dairy bacteriology was translated into English, German, Dutch, Finnish and Russian. His interest in bacteriology, microbiology and chemistry was international and he was present regularly at international dairy conventions. On one occasion he was a guest of the Argentine government and when he visited the U. S. he was popular for his articles and interviews.

At the age of 76, Prof. Orla-Jensen became interested in finding out how to maintain optimum health during the later years of life. He worked in cooperation with the Danish bacteriologist, E. Olsen, and the medical director, T. Geill, of Copenhagen. They achieved some worthwhile results through the study of diet and the part that the factors of diet played in producing certain bacteria in the intestinal flora which, in turn, influenced general health.

The final problem to which Prof. Orla-Jensen gave much attention was the curing of cheese by a quick method and in a way to bring about a characteristic desirable flavor, which would create a demand for cheese for export.

He will be missed by all who knew him. Those who saw him in the setting of his beautiful garden and summer cottage at Karlslunde know how he loved beauty. Denmark lost one of its great sons with the passing of Prof. Sigurd Orla-Jensen.

G. H. Wilster

14. Is the methylene blue reduction test of any value? C. K. JOHNS, Central Exptl. Farm, Ottawa, Can. J. Milk and Food Technol., **12,** 5: 267–269, 278. Sept.–Oct., 1949.

The modified inversion technique should outweigh any disadvantages formerly associated with the methylene blue reduction test because the reduction time is more comparable to the standard plate count, the dye is decolorized uniformly throughout the tube and the reduction time is shortened, especially with low count milks.

H. H. Weiser

15. Lysogenic strains of lactic streptococci. B. REITER. Nature, **164,** 4172: 667. 1949.

A good many strains of lactic streptococci, including some commercial lactic acid cultures, were found to be lysogenic. Starters composed of strains which are lysed by the phages of lysogenic strains are unsuitable for cheese making and should be avoided. R. Whitaker

16. Effect of calcium on the development of streptococcal bacteriophage. D. I. SHREW. Nature, **164,** 4168: 492. 1949.

Ca stimulated phage development in 8 strains of *S. cremoris* when present to the extent of 0.001M with 0.02 to 0.007M as the optimum, depending on the media used. The Ca may be in the form of milk serum or as chloride. The addition of citrate reverses the stimulatory effect of Ca. Phage development, as measured by lysis, was not stimulated by Mg ions. R. Whitaker

17. The lactic acido-proteolytic bacteria and the genotypicity of the bacterial enzymes. C. GORINI. Univ. of Milan. Enzymologia, **12,** 2: 82–87. 1947.

This group of lactic acid bacteria appears to be differentiated on the basis of their reactions on proteins rather than on carbohydrates. These organisms demonstrate considerable variation in their enzymatic activities in general. This variation and dissociation may be readily reversible and the author concludes that the classification of this group will not be possible until the basic enzyme patterns of the various species are recognized. J. J. Jezeski

18. The ester-hydrolyzing enzyme systems of Aspergillus niger and of Penicillium roqueforti. P. J. FODOR AND A. CHARI, Hebrew Univ., Jerusalem. Enzymologia, **13,** 5: 258–267. 1949.

Monobutyrin, methyl butyrate and olive oil were used as substrates in the presence of M/5 phosphate buffer with incubation at 30–38° C. for 48 hr. Glycerol extracts of mycelia and media in which the molds had grown were used as

sources of the enzymes. Free fatty acids were estimated by titration. On the basis of pH optimum studies, evidence was obtained which suggested that 2 types of esterases were produced by each of the molds. An intracellular enzyme extracted from the mycelia had an optimum at pH 6.5 (or below), while the enzyme present in the culture medium had an optimum about pH 8.0 Sodium taurocholate inhibited both types of enzymes. J. J. Jezeski

19. Die Bedeutung der Wasserstoffionenkonzentration bei der Konservierung von Nahrungsmitteln und Viehfutter. (The significance of hydrogen ion concentration in the preservation of foods and feeds.) English summary. A. I. VIRTANEN. Die Milchwissenschaft, **3,** 12: 353–361. Dec., 1948.

Microbial growth is limited, in general, to the pH range of 1.0 to 13.0 Proteins are not readily attacked below pH 4.0. Thus, if silage is adjusted to between pH 3.0 to 4.0 it readily can be kept from spoilage.

Butter is stored best at pH 6.0 to 7.0. At this pH level the formation of fishy and oily flavors is prevented. Bacterial spoilage at this pH must, however, be prevented by proper pasteurization of cream and sanitary butter manufacture.

Animal products, such as fish meal, meat meal, etc. can be preserved by the addition of slaked lime to pH 12.0 and by storage in air-tight containers. The oxidation-reduction potential, similar to the pH, plays an important part in the preservation of all types of nutrients.

I. Peters

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

20. Lipolytic flavors of milk. N. P. TARASSUK AND E. L. JACK, Univ. of Cal., Davis. Milk Plant Monthly, **38,** 10: 48. Oct., 1949.

Lipolytic flavors produced by the action of lipase on milk fat is accelerated by disruption or distortion of the absorbed fat globule layer through homogenization, shaking or heat shock of raw milk. Also, certain milks are naturally lipolytically active and will develop spontaneously a rancid flavor. In the latter case, it is necessary that the milk be cooled before lipolysis begins which suggests that lipase is present in the milk serum prior to cooling.

J. A. Meiser

21. The "sunlight" flavor in milk. D. G. KEENEY, Penn. State College. Milk Plant Monthly, **38,** 10: 54. Oct., 1949.

A sunlight flavor may be produced artificially by the addition of 25 to 75 p.p.m. of formaldehyde to milk flashed to 167° F. or above. Colorimetric determination of the amount of formaldehyde reacting in the milk was less than the sensitivity of the method used. Although the dialyzable portion of the skim milk appears to promote this flavor, exposure of the diffusate to sunlight did not result in a flavor development.

J. A. Meiser

22. Separation and estimation of saturated C_2–C_8 fatty acids by buffered partition columns. VIVIEN MOYLE, E. BALDWIN AND R. SCARISBRICK, Univ. of Cambridge. Biochem. J., **43,** 2: 308–317. 1948.

A chromatographic method of separating the lower molecular wt. naturally occurring fatty acids is given. Phosphate-buffered silica gels are used as the solid phase and mixtures of chloroform and n-butanol as the solvents. Reported recoveries of fatty acids in various mixtures range from 97 to 103%. A. O. Call

23. The mechanism of fatty acid oxidation. L. F. LELOIR, Fundac. Campomar, Buenos Aires. Enzymologia, **12,** 4: 263–276. 1949.

Critical papers are reviewed dealing with fatty acid oxidation in animal tissues and oxidation by microorgansms. The results are interpreted on the basis that a reactive two-carbon compound is formed as the result of beta-oxidation of the fatty acids, as well as from pyruvate oxidation. Thus, the final stages of both fatty acid and carbohydrate oxidation may have a common pathway. J. J. Jezeski

24. Liberation of amino acids from raw and heated casein by acid and enzyme hydrolysis. L. V. HANKES, W. H. RIESEN, L. M. HENDERSON AND C. A. ELVEHJEM, Univ. of Wis., Madison. J. Biol. Chem., **176,** 2: 467–476. Nov., 1948.

Previous work showed soy bean protein was affected adversely by heat. This investigation shows that autoclaving casein at 15 lb. pressure for 4 min. did not affect the ease with which the amino acids were liberated and made microbiologically available. The only ill effects from a 20 hr. period was a reduction in the cystine availability. A table shows the percentage of the various amino acids liberated from the raw and heat-treated casein as measured after acid hydrolysis (alkaline for tryptophan and tyrosine) and enzymatic digestion. A. O. Call

25. Amino acid composition of α-casein and β-casein. W. G. GORDON, W. F. SEMMETT, R.

S. CABLE AND M. MORRIS, Eastern Regional Research Lab., Philadelphia, Pa. J. Am. Chem. Soc., **71,** 10: 3293–3297. Oct., 1949.

The amino acid composition of whole casein and its 2 major components, α-casein and β-casein, was determined. Essentially all of the nitrogen of each protein was accounted for in terms of known amino acid residues and amide nitrogen. Most striking differences in α-casein and β-casein were observed in proline, tryptophane, cystine and tryosine content. Histidine, glutamic acid, threonine and amide nitrogen were considered to be present in equal concentration. The small differences in glycine, isoleucine and serine content are believed to be significant.

β-casein exhibited a greater solubility in ethanol-water mixtures, probably due to the larger proportion of non-polar groups. The amino acid composition also may explain the differences in electrophoretic mobility of α-casein and β-casein. In solutions both acid and alkaline to the isoelectric points of the proteins, α-casein had a higher mobility. The higher proportions of cationic and anionic groups in α-casein would explain this observation.

H. J. Peppler

26. The chemical composition of acropeptides from casein and their behaviour towards enzymes. A. FODOR, P. J. FODOR AND S. KUK-MEIRI, Hebrew Univ., Jerusalem. Enzymologia, **12,** 2: 101–106. 1947.

Acropeptides, nonhydrolytic protein derivatives which do not represent open chains of amino acids and therefore do not possess terminal amino or carboxyl groups, were prepared by dissolving casein in water-free glycerol at 135–145° C. and then precipitating in alcohol. The smallest unit obtained appeared to be an octapeptide and a larger one was studied which contained 9 such units. From data obtained on amino acid composition, elementary analysis and presence of NH_2 and free COOH groups (side chains) it has been concluded that the casein molecule is composed of these amino acid complexes held together by associative linkages. J. J. Jezeski

27. A comparative study of the behavior of proteins and acropeptides towards proteinases. P. J. FODOR, S. KUK-MEIRI AND A. FODOR, Hebrew Univ., Jerusalem. Enzymologia, **12,** 2: 107–113. 1947.

The relative action of pepsin-HCl and a pancreatic proteinase toward casein and an acropeptide presumably containing 72 amino acid units was studied. The amount of cleavage caused by the action of pepsin on the acropeptide was proportional to the number of free carboxyl groups and that caused by the pancreatic enzyme was proportional to the number of free NH_2 groups. This acropeptide was similar to casein in its behavior toward the hydrolytic enzymes studied. J. J. Jezeski

28. De formoltitratie; een practijkmethode voor de bepaling van het gehalte van koemelk aan "totaal" eiwit en aan caseïne. (The formol titration; a practical method for the determination of total protein and casein in cow's milk.) (English summary.) H. J. BANNENBERG AND W. VAN DEN HOEK, College of Agriculture, Wageningen, Holland. Netherlands Milk & Dairy J., **3,** 3: 162–177. July–Sept., 1949.

The formol number w as determined in 21 milk samples without using potassium oxalate and total protein and casein content also were determined. A conversion factor was calculated by dividing the determined formol titre by the percentage protein (or casein). This conversion factor was 1.011 ± 0.038 $(r = +0.55)$ for total protein and 0.807 ± 0.034 $(r = +0.44)$ for casein; the greatest possible deviations from the true values were 10 and 12%, respectively. Another experiment was run the same way with 28 samples of milk using the Pyne modification with potassium oxalate. Here the conversion factor for total protein was 0.350 ± 0.005 $(r = +0.94)$, and for casein, 0.278 ± 0.004 $(r = +0.93)$; the greatest possible deviations from the true values being 4.3% in both cases. Composite samples were used in the previous experiments. The influence of the stage of lactation was checked by examining 15 milk samples of single cows in different stages of lactation using the Pyne method. These conversion factors agreed with the composite milk values, except for the last 2 mo. of lactation which gave a somewhat higher total protein value (0.361 ± 0.011), while the first 4 d. of lactation gave unreliable results. The milk acidity changed the formol titre only when the milk no longer was acceptable for manufacturing purposes. The conclusion was that the Pyne method can be used for determining the protein and casein content with sufficient accuracy. A. F. Tamsma

Also see abs. no. 43.

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

29. Flow diversion valve. E. C. HARTMAN (assignor to Taylor Instrument Co.). U. S. Patent 2,484,622. 4 claims. Oct. 11, 1949. Official Gaz. U. S. Pat. Office, **627,** 2: 528. 1949.

A flow diversion valve for high temperature, short time pasteurizers is described. The forward flow outlet valve assembly is equipped with a leak-detecting opening to prevent the passage of under pasteurized milk while the valve is in the diverted position. R. Whitaker

30. Het vraagstuk van de afvalwateren der zuivelindustrie. (Waste liquids in the dairy industry.) (English summary.) J. H. A. SCHAAFSMA, State Dairy Consultant. Netherlands Milk & Dairy J., **3,** 2: 142–154. April–June, 1949.

Methods of treatment and disposal of dairy waste waters, the present state of affairs and prospects and possibilities in the Netherlands are discussed. Since 1920 the Government Institute for Purification of Waste Waters was mainly in charge of this work for the dairy industry.

The varying character and composition of dairy waste and consideration of local circumstances make it impossible to give a general procedure for purification. First of all, disposal of organic material caused by losses in the manufacturing process and loss of by-products should be reduced to a minimum. The remaining waste liquid should be purified by some oxidative method which is cheap and efficient. Most methods, differing technically, are based on the same microbiological oxidation process of organic material by oxygen of the air. Difficulties can be caused by too high concentration or too low pH caused by lactic acid bacteria. The waste liquid must be kept fresh; sometimes lime or chlorine can help here. In the Netherlands most dairy factories are satisfactory, while some isolated cases still are unsatisfactory. Due to a lack of proper legislation these cannot be forced to change procedures. Without too much cost there is an opportunity for improvement through proper investigation by a sanitary adviser and using the facilities available. Further research to establish more economical purification systems and international interchange of results and ideas should be strongly encouraged and organized. A. F. Tamsma

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

31. Cost—Each individual product in the ice cream plant. E. J. MATHER, National Dairy Products Corp. Ice Cream Trade J., **45:** 10, 62. Oct., 1949.

Key personnel should know the cost of each flavor in bulk ice cream and the cost of each product, such as: bulk, pt. package, qt. package, 0.5 gal. and 1 gal. packages, slices, molds and other products, including the cost of each novelty. To arrive at the price to dealers, manufacturing, selling, administration and other cost factors should be known.

To arrive at a correct delivery cost per product, the space occupied by the unit of sale of each product can be related in terms of points to a gal. of bulk ice cream as a base unit with a point value of 1. A table can be established for all products with point values related to bulk ice cream according to the space occupied.

One of the best ways to reduce cost in the plant is to employ better men, or give those already employed a better education so they will be in a position to find and use the methods that will produce the best products at the lowest possible price. W. H. Martin

Also see abs. no. 10.

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

32. Availability of the magnesium of grass to the ruminant. R. J. GARNER.. Nature, **164,** 4167: 458. 1949.

Mg is not made available by the action of the gastric juice of ruminants, but is liberated from vegetable cells by the ruminal organisms. Free Mg does not exist in the alkaline rumen but becomes available by the action of the abomasal HCl. R. Whitaker

33. The fermentation of cellulose in vitro by organisms from the rumen of sheep. H. R. MARSTON, Univ. of Adelaide, S. Australia. Biochem. J., **42,** 4: 564–574. 1948.

An apparatus intended to similate the conditions found in the rumen is described. Water suspensions of cellulose, from birch bark in 2 cases and from filter paper in 2 cases, to which inorganic salts were added and then inoculated with a "community of microorganisms" from the rumen contents of sheep, were fermented. The predominate products of dissimilation were acetic and propionic acids, CO_2 and methane. Smaller quantities of formic, butyric, pyruvic and lactic acids as well as acetaldehyde were reported. The energy metabolism of the ruminant is discussed in view of these findings.

A. O. Call

34. The nutritive value for the calf of colostrum and its fractions. (Abs.) R. ASCHAFFENBURG *et al.,* Natl. Inst. for Research in Dairying, Univ. of Reading and Research Inst. of Animal Path., Royal Veterinary College. Biochem. J., **42,** 2: xxx-xxxl. 1948.

Both Ayrshire and Shorthorn colostrum were separated with a super-centrifuge into fatty and non-fatty fractions. Various combinations of these 2 fractions were made with dried skim milk and margarine and then fed to a total of 66 calves. Best results were obtained with the untreated colostrum group, followed by the group fed the non-fatty fraction. The essential factor in the aqueous phase of colostrum is concluded to be active in small concentrations. The vitamin A reserves at 5 wk. were not related to the initial intake. A. O. Call

Also see abs. no. 7, 46.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

35. Superovulation and ovum transfer in cattle. R. E. UMBAUGH, Foundation of Applied Research, San Antonio, Texas. Am. J. Vet. Research, **10,** 37: 395–305. Oct., 1949.

Superovulation was induced in cows by implanting a pellet containing 1500 to 3000 r. u. of pituitary gonadotrophin followed in 4 d. by intravenous injection of 500 to 1500 r. u. of pituitary gonadotrophin or by daily subcutaneous injection of 50 to 500 r. u. pituitary gonadotrophin for 5 d. followed by intravenous injection of 25 to 1000 r. u. pituitary gonadotrophin. Pregnant mare serum gonadotrophin and chorionic gonadotrophin were not effective for superovulation. Ova were collected at the time of spontaneous ovulation, 26 hr. following the intravenous injection, by aspirating the follicles with capillary pipettes via a flank incision. Percentage recovery of ova and percentage fertilization were reduced in the high-level hormone treatments. Treatment with stilbestrol or progesterone did not change the fertilization ratio. Following superovulation an average of 23.4 ovulation points/cow were counted and 10.4 ova/cow were collected from the oviducts, but only 5.8/cow were fertilized. Sixteen fertilized ova were transferred to the fallopian tube of 1 cow the day after estrus, and 17 ova were transferred to another. Multiple embryos developed but were aborted prematurely. E. W. Swanson

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

36. Supporting arrangement for milkers. H. B. BABSON (assignor to Babson Bros. Co.). U. S. Patent 2,483,516. 8 claims. Oct. 4, 1949. Official Gaz. U. S. Pat. Office, **627,** 1: 134 1949.

A frame surrounding a milking stall is described having a moveable arm, counterbalanced by a weight, to support a milking machine vessel. R. Whitaker

37. Teat cup. L. DINESEN. U. S. Patent 2,484,696. 1 claim. Oct. 11, 1949. Official Gaz. U. S. Pat. Office, **627,** 2: 548. 1949.

This teat cup for milking machines has a rigid outer shell and a flexible inner tube. The space thus formed is connected to a source of pulsating vacuum. R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

38. Double seal milk can. J. A. HOPWOOD. U. S. Patent 2,484,624. 5 claims. Oct. 11, 1949. Official Gaz. U. S. Pat. Office, **627,** 2: 528. 1949.

To provide additional protection, the cover of this milk can seals in 2 places. The cover not only fits snugly into the throat of the can, but the outside rim of the flared-can-top engages a skirt attached to the inside of the can lid. R. Whitaker

39. Gladsaxe Mejeri, et af Storkobenhavns mest moderne Mejerier. (Gladsaxe Mejeri—One of Greater Copenhagen's Most Modern Dairies.) Nordisk Mejeri-Tidsskrift, **13,** 6–7: 57, 58. 1947.

The Gladsaxe dairy plant presents an exterior which is typical of a large, remodeled milk plant. Upon coming into the building it is apparent at once that the interior is equipped so as to be technically and hygienically up to date and efficient. Working conditions are good, with ample room for all of the necessary operations but with a working plan that eliminates the waste of time and effort.

A large bottle washing machine is in use and when the bottles have been washed, rinsed and sterilized they travel in cases, through an opening in the wall, directly to the bottle-filling machine. From there, the filled bottles travel on a track, on which the cases of filled bottles roll into the cooling room.

The milk as it is received and weighed into tanks is handled in the same efficient, time- and labor-saving manner. It goes through the separator and into a plate pasteurizer. From there it goes into aluminum tanks on a balcony and it is agitated by the use of air under pressure.

The large vacuum filling machine has 16 filling outlets. In the bottom of each milk tank are pipes which are connected with the vacuum filling machine. There is no way of making errors and having buttermilk flow into the bottles intended for fresh milk or vice versa. Everything is done accurately and hygienically. The cases of bottled, cooled milk travel on rollers into a large milk storage room that has a capacity of 20,000 l. This room is cooled by an Atlas cool-

ing apparatus. The cases then travel out to the milk delivery platform where the milk trucks wait to receive them.

Large modern storage rooms are provided for dairy products and supplies. A large basement is equipped with a fresh-water tank and a brine tank, as well as with a lunch room, dressing room and wash room for workers in this modern plant. A floor plan accompanies the article.

G. H. Wilster

Also see abs. no. 14, 20, 21, 29.

MILK SECRETION

V. R. SMITH, SECTION EDITOR

40. Synthesis of the short-chain fatty acids of milk fat from acetate. G. POPJÁK, S. J. FOLLEY AND T. H. FRENCH, Univ. Reading, England. Arch. Biochem., **23,** 3: 508–510. Oct., 1949.

The probability that the short-chain acids of milk fat (C_4-C_{14}) originate from acetate has been indicated by results obtained *in vitro* with mammary gland slices from ruminants as well as non-ruminants. Pregnant rabbits injected with $CH_3C^{14}OONa$ exhibited a high rate of incorporation of C^{14} into glyceride fatty acids extracted from the mammae. Fractionation of these fatty acids revealed that the volatile acids contained 7–18 times more isotope than the non-volatile acids. The highest C^{14} content was found in the water-soluble fraction comprised chiefly of butyric and caproic acids. The low C^{14}-content of unfractionated fatty acids of the liver indicates that this organ is not the source of the highly active fatty acids in the mammae. H. J. Peppler

Also see abs. no. 41, 42.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

41. The effect of thyroxine on the metabolism of lactating cows. 1. General results and nitrogen metabolism. E. C. OWEN, The Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **43,** 2: 235–243. 1948.

Eight Ayrshire cows were used in an investigation to determine the effect of subcutaneous injection of 10 mg. of thyroxine/d./cow. The cows were subjected to 3 different levels of feed intake. Thyroxine increased catabolism, as shown by an increase in pulse rate. This resulted in an increase in milk yield, a loss in body wt., an increase in the urine excretion and a negative nitrogen balance. The negative nitrogen balances were inhibited somewhat by an increase in feed. The composition of the milk during thyroxine administrations showed an increase in fat and solids as well as an increase in nitrogen in the fat-free portion, as compared with that of the controls.

A. O. Call

42. The effect of thyroxine on the metabolism of lactating cows. 2. Calcium and phosphorous metabolism. E. C. OWEN, The Hannah Dairy Research Inst., Kirkhill, Ayr. Biochem. J., **43,** 2: 243–247. 1948.

In connection with thyroxine studies (see previous abstract) Ca balances were made on 8 Ayrshire cows. In all but 1 case the Ca balances were negative before, during and following the administration of thyroxine; however, in 2 cases where the cows were fed more liberally, the output of Ca was increased by giving thyroxine.

P balances were made on only 2 cows. They both showed positive balances throughout the experiment and giving thyroxine tended to increase the amount retained. The P content of the milk was increased significantly by thyroxine but the Ca content was unaffected. A. O. Call

43. Preparation of radioactive iodocasein. C. F. HAMILTON, MARSCHELLE H. POWER AND A. ALBERT, Mayo Clinic and Mayo Foundation, Rochester, Minn. J. Biol. Chem., **178,** 1: 213–216. March, 1949.

A procedure, following in general the method of Reineke and Turner, is given for the preparation of iodocasein containing radioactive iodine (I^{131}). It is intended for human metabolism studies. A. O. Call

44. The effects of large doses of various sulfonamides injected intravenously in dairy cattle. L. M. JONES, H. A. SMITH AND M. H. ROEPKE, Iowa State College, Ames. Am. J. Vet. Research, **10,** 37: 318–326. Oct., 1949.

The sulfonamides sulfathiazole, sulfapyridine, sulfadiazine, sulfamerazine, sulfamethazine and sulfaquinoxaline were each injected into separate groups of cows as the sodium salt dissolved in 500 cc. of water. One series of cows received 1 injection of 60 g. of each sulfonamide. Another series received 2 60-g. injections 7 to 10 d. apart, and a 3rd series of cows was given the same plus 2 90-g. injections. Blood concentrations were maintained best by sulfamethazine, followed in order by sulfapyridine, sulfamerazine, sulfadiazine, sulfaquinoxaline and sulfathiazole. Sulfaquinoxaline was very toxic, resulting in the death of 1 cow following the first injection, and was not used for higher level studies. The other sulfonamides also caused weakness and collapse of some of the cows but recovery was rapid. One cow given the highest dosage of sulfapyridine died from hemorrhage. Livers and kidneys of

all cows showed damage. Extensive myelin degeneration of sections of the spinal cord and of the sciatic and median nerves was found in paralyzed cows following sulfaquinoxaline injection. Body temperature and blood urea were not varied from normal. E. W. Swanson

45. Studies on the gross anatomy of the bovine liver. I. The distribution of the blood vessels and bile ducts as revealed by the vinylite-corrosion technique. L. M. JULIAN AND K. B. DEOME, Univ. of Cal., Berkeley. Am. J. Vet. Research, **10,** 37: 331–335. Oct., 1949.

Different colored vinylite solutions were injected into the vascular systems and bile ducts of the liver and a study made of the subgross anatomy. Portal circuits clearly were demonstrated. Numerous arterial plexuses were found around the bile ducts. The article is well illustrated.
E. W. Swanson

46. The absorption of ammonia from the rumen of the sheep. I. W. MCDONALD, Biochem. Lab., Univ. of Cambridge. Biochem. J., **42,** 4: 584–587. 1948.

The general circulation blood of sheep contains very little, if any, ammonia, while about 1.5 mg. ammonia N/100 ml. is present in the venous blood which comes from the rumen. A nitrogen cycle in the digestive tract is postulated, wherein ammonia from the rumen is converted in the liver to urea, which may be secreted in the saliva and returned to the rumen where it is again converted to ammonia. A. O. Call

47. Amino acid composition of β-lactoglobulin and bovine serum albumin. W. H. STEIN AND S. MOORE, Rockefeller Inst. for Medical Research, N. Y. J. Biol. Chem., **178,** 1: 79–91. March, 1949.

Chromatographic fractionations of the amino acids of β-lactoglobulin and bovine serum albumin hydrolysates have been made using starch columns and a mixture of n-butyl alcohol, n-propyl alcohol and HCl as the solvent. Recoveries of about 98% of the amino acids on a wt. basis are reported. The results are in good agreement with previously reported values.
A. O. Call

Also see abs. no. 6, 23, 35.

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

48. Some new quaternary ammonium compounds and their properties. J. C. L. RESUGGAN, F.R.I.C. Director and Chief Chemist, The British Hydrological Corp. Dairy Inds., **14,** 8: 819–822. Aug., 1949.

Some new twin-chain quaternary ammonium compounds are described with regard to solubility, bactericidal properties and compatability with certain anionic and non-ionic compounds. The results show that with the twin-chain compounds a greater degree of solubility could be attained without sacrificing much bactericidal activity. Didecyldimethylammonium bromide, having a total of 20 carbon atoms in its 2 chains, is more soluble than with a single long chain compound containing 20 carbon atoms. With greater degree of solubility there is less tendency for precipitates to be formed with other compounds.

In testing quarternary ammonium compounds for bactericidal properties, each quaternary compound must be recognized as a special case and the proper inhibitor selected to prevent bacteriostatic effect. G. H. Watrous, Jr.

49. Laboratory evaluation of cleaner-sanitizers for use on dairy farms. F. W. BARBER, National Dairy Research Lab., Inc., Oakdale, L. I., N. Y. J. Milk and Food Technol., **12,** 5: 257–266. Sept.–Oct., 1949.

A heat resistant culture of *E. coli,* known to be resistant to the action of quaternary ammonium compounds, was added to a cleaner-sanitizer test solution. A known concentration of ice cream mix was added to supply the organic matter comparable to farm conditions in cleaning dairy utensils. The study included variable factors such as temperature changes, degree of water hardness and organic matter as they may effect the bactericidal action of quaternary ammonium compounds. The technique approximates the claims made by manufacturers of sanitizing compounds used in actual practice. H. H. Weiser

50. Effectiveness of hypochlorite and quaternary ammonium compounds in a mastitis sanitation procedure. K. R. SPURGEON, W. J. HARPER AND P. R. ELLIKER, Purdue Agr. Expt. Sta., W. Lafayette, Ind. Milk Plant Monthly, **38,** 10: 42–46. Oct., 1949.

To determine the effectiveness of hypochlorites and quaternary ammonium compounds in destroying mastitic organisms, milking machine teat cups were inoculated with *S. agalactiae* by swabbing with a milk suspension of the organism. After inoculation, the cups were rinsed a predetermined number of times in varying concentrations of the germicides under study and allowed to drain for periods ranging from 30 sec. to 5 min. Actual counts of the surviving organisms were obtained by inoculating cold, sterile skim

milk which in turn was plated on veal infusion blood agar. Of the hypochlorites and quaternary ammonium compounds selected for the final study, none destroyed all of the *S. agalactiae* but the reduction in numbers suggested the application of these compounds in milking procedures where teat cups are dipped between cows. Factors affecting the efficiency of these compounds in destroying the test organism were the use of prolonged exposures, the use of two successive rinses and the use of increasing concentrations of the germicide. The high numbers of surviving organisms on rubber teat cup inflations that possess cracks or checks emphasized the inadequacy of germicidal treatment when worn out or improper equipment is used on the farm. J. A. Meiser

51. Tiermedizinische Milchhygiene im Rahmen des Reichsmilchgesetzes. (Veterinary milk hygiene in the scope of the government milk law.) English summary. E. PAARMANN. Die Milchwissenschaft, **3,** 12: 371–372. Dec., 1948.

Considering the financial losses in cattle and in beef as a result of bovine tuberculosis and also the health hazards to the milk-consuming public, the author strongly suggests that a systematic obligatory irradication program of tuberculin infected cattle should be started in Germany.

I. Peters

PUBLICATIONS OF THE XII INTERNATIONAL DAIRY CONGRESS.

In conjunction with the XII International Dairy Congress in Stockholm, August 1949, the dairy specialists of 40 countries submitted more than 400 reports containing much data of scientific and practical value. These papers were printed in 5 volumes. A sixth volume contains General Reports, prepared by eminent specialists who have summarized each of the 33 different subjects taken up for discussion at the Congress. A further volume, no. 7, will comprise a report on the actual Congress and a record of the oral debates. This volume is now being edited and will be released in 1950. Further particulars are given below.

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