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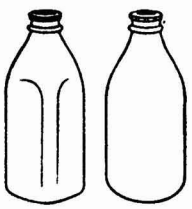

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NOTICE

TO MEMBERS AND SUBSCRIBERS

The December 1950 issue of the JOURNAL OF DAIRY SCIENCE has not yet been completed due to the vast amount of work entailed in getting up the index. Therefore we are mailing the January issue before the December issue in an endeavor to get back on our regular schedule.

The December issue will follow within the next several weeks.

JOURNAL OF DAIRY SCIENCE

VOLUME XXXIV JANUARY, 1951 NUMBER 1

SOME AMINO ACIDS, PEPTIDES AND AMINES IN MILK, CONCENTRATED MILKS AND CHEESE[1]

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1. The Amino Acid Composition of the Proteins of Cow's Milk and of Cheddar Cheese

The importance of milk and cheese proteins as sources for amino acids in the American diet warrants investigation on the effects of preparation and of storage on these food proteins (4). Hodson and Krueger (10) have determined the essential amino acid content of casein and of fresh, dried and evaporated cow's milk. They also studied the changes which these amino acids undergo on prolonged storage of dry skimmilk (11). The first portion of this paper describes the essential amino acid composition of the proteins of cheddar cheese, dried skimmilk and of both freshly prepared and stored evaporated milk.

EXPERIMENTAL

The proteins from commercial samples of spray-dried milk, evaporated milk and cheddar cheese were isolated after precipitation with trichloracetic acid and analyzed for nitrogen, amino nitrogen and amino acids by the methods previously described (3, 5). Whenever two or more proteins were to be compared, the analyses were carried out simultaneously.

RESULTS

The analytical results are given in table 1 together with comparable data from the publications of Hodson and Krueger (10, 11). It appears that in spite of the different kinds of milk used or the length of the curing period, the amino acid patterns of the proteins of raw milk cheddar cheese cured 90 days and pasteurized milk cheddar cheese, fresh or cured, are essentially the same and similar to casein. Likewise, the amino acid content of the proteins of spray-dried milk, evaporated milk and fresh milk do not appear to be significantly different. Storage of the evaporated milk[2] for 6 mo. under the conditions rec-

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1 These investigations were aided in part by grants from the American Dairy Association, Chicago, and from The Borden Co., New York City.

2 Hodson (12) has compared the amounts of seventeen amino acids in evaporated milk, freshly prepared and stored for 5 yr. Of these amino acids, significant losses were observed for only lysine (17 per cent), histidine (17 per cent) and arginine (11 per cent). The customary storage period is less than 15 mos., yet even during this period the losses of the basic amino acids were not greater than 4 per cent in any case.

TABLE 1

Essential amino acids in milk and cheese proteins

(Calculated to 16.0 g. of nitrogen)

Amino acid	Casein		Cheddar cheese			Evaporated milk			Dry skimmilk			Fresh milk	
		(10)[a]	A[b]	B	C	D	E	(10)	Low heat	F (11)	G (11)	(5)	(11)
(*g.*)													
Arginine	3.6*	3.5*	3.5*	3.2*	3.6*	4.1	4.1	3.5*	3.2*	3.2*	3.2*	3.8; 3.2*	3.5*
Histidine	2.9*	2.5*	3.2*	3.3*	3.1*	2.8	2.9	2.0*	2.6*	2.4*	2.4*	2.6; 2.6*	2.4
Lysine	7.1*	7.9*	8.2*	8.4*	8.5*	8.4	8.2	7.6*	8.2*	7.9*	7.9*	7.2*	9.1*
Tyrosine	6.4	5.3*	6.9	6.7	6.9	5.9	6.0	5.0*	6.2	5.4*	5.2*	6.4	5.4*
Tryptophan	1.3	1.1*	1.6	1.6	1.6	1.5	1.6	1.2*	1.7	1.4*	1.4*	1.4	1.4*
Phenylalanine	6.4	4.9*	6.4	6.2	6.5	5.7	5.8	4.3*	6.5	5.8*	5.3*	5.7	4.6*
Cystine	0.4	.....	0.4	0.5	0.5	0.9	0.9	.....	0.8	.....	.....	0.7	.....
Methionine	3.5	2.7*	3.5	3.5	3.6	3.6	3.6	2.3*	3.4	2.6*	2.5*	3.5	1.9*
Threonine	3.6	4.0*	3.7	3.6	4.1	4.0	4.3	5.0*	4.5	4.3*	4.2*	4.1	4.7*
Leucine	9.6*	9.4*	9.0*	8.9*	8.7*	10.8*	9.9*	11.5*	8.7*	10.0*	10.1*	10.6*	11.1*
Isoleucine	6.9*	6.0*	7.1*	7.3*	7.4*	7.2*	7.9*	6.3*	7.0*	6.8*	6.5*	8.5*	6.4*
Valine	7.7*	5.9*	7.8*	7.5*	7.7*	7.6*	8.2*	6.3*	6.5*	6.4*	6.4*	8.4*	6.4*
N.P.N./Total N × 100			16%	23%	8%	7%	7%		9%			5%	

[a] Numbers in parentheses indicate references.

[b] A—raw milk cheddar cheese, cured 90 days.
B—pasteurized milk cheddar cheese, cured 90 days.
C—pasteurized milk cheddar cheese, fresh.
D—total proteins from evaporated milk, prepared when received.
E—total proteins from evaporated milk, prepared six months after number D.
F—fresh sample.
G—old sample.
N.P.N.—nonprotein nitrogen i.e. trichloracetic acid filtrate.
* —Microbiological method.

ommended for storing this product did not result in any demonstrable loss in essential amino acids.

The values in table 1 show general agreement between those found after hydrolysis of the isolated milk proteins and those reported by Hodson and Krueger (10, 11) after hydrolysis of the milk without prior isolation of the proteins. The most notable difference is the higher values for methionine. This is in accord with the literature (3), *i.e.*, "methionine" in casein and milk proteins as found by chemical methods usually is higher than that found by microbiological procedures.

DISCUSSION

After the completion of these experiments, the investigations of Harper and Swanson (9) came to our attention. These investigators found in agreement with the results given in table 1 that the total content of nine amino acids (aspartic acid, glycine, glutamic acid, isoleucine, leucine, lysine, phenylalanine, proline and valine) in cheddar cheese compared favorably with the amino acid composition of casein, with the exception of glycine, which increased with the age of the cheese. The latter amino acid was not analyzed by us.

2. The Amino Acids and Peptides in "Protein-free" Milk and Cheese

Experiments and Results

Preparation of amino acid and peptide fraction from milk. One liter of cold, pasteurized skimmilk (Walker-Gordon) was treated with 100 g. of trichloracetic acid in 100 ml. of ice-water. The precipitate was allowed to form in the refrigerator for 1 hr. and then was removed by centrifugation and thoroughly washed with 1 l. of cold trichloracetic acid. The last traces of precipitate were removed by filtration. The combined filtrate and washings were extracted five times with ether to remove the excess trichloracetic acid. The aqueous layer then was passed through a 200-g. column of the cation exchange resin Duolite C-3 in the H^+ cycle at the rate of 10 ml. per minute. The resin was washed with water until an aliquot of the washings was devoid of lactose, as indicated by a negative Molisch test. The column then was washed with 1 l. more of water as a precautionary measure. The effluent and washings were set aside.

The adsorbed amino acids were eluted from the ion exchange resin with 1 l. of 4 per cent of aqueous NH_3 followed by 1 l. of water. This elutriate was concentrated *in vacuo* to 200 ml., slightly alkalinized with $Ba(OH)_2$ and again concentrated *in vacuo* to remove all the bound ammonia. The ammonia-free solution (negative Nessler's test) was slightly acidified with dilute H_2SO_4, the precipitate of $BaSO_4$ was removed and washed, and the filtrate and washings were evaporated to dryness. The residue was taken up in 10 ml. of 10 per cent of isopropanol. This solution contained 140 mg. or 78 per cent of the nitrogen in the original protein-free milk.

A sample of sweetened condensed milk was stored in an unheated attic for 10 yr. The cans showed no signs of damage but the contents had set to a stiff gel, were dark in color and had a distinctly unpleasant odor. Analyses for amines showed the presence of tyramine only. A sample of freshly purchased sweetened condensed milk did not contain a detectable quantity of tyramine.

The effluent and washings from the first adsorption again were passed through a 200-g. column of Duolite C-3 in the hydrogen cycle as described above. After elution of the adsorbed materials with ammonia and removal of NH_3 from the elutriate, only 15 mg. of nitrogen were obtained. This fraction was added to the first elutriate and the combined solutions were adjusted to contain 11.2 mg. of nitrogen per ml. of solution.

Separation and analysis of amino acid and peptide fractions from milk. An aliquot of the amino acid and peptide-containing solution (AAP) was hydrolyzed with HCl in the usual fashion and two-dimensional paper chromatograms (4) were carried out on equal nitrogen levels of the hydrolyzed and unhydrolyzed material. Unhydrolyzed AAP contained glutamic acid[3] (very strong), glycine (medium), alanine (medium), valine, leucines, aspartic acid and serine (all weak). Hydrolyzed AAP contained aspartic acid (weak), glutamic acid (very strong), serine (medium), glycine (strong), alanine (medium), valine (medium), leucines (medium), threonine (medium), phenylalanine (weak), lysine (weak), arginine (weak), proline (strong). After hydrolysis the quantities of serine, glycine, valine and the leucines apparently were increased in intensity. Threonine, phenylalanine, lysine, arginine and proline were not present in detectable quantities before hydrolysis.

The AAP then was fractionated by placing 35 0.01-ml. aliquots of the solution equidistant from each other and 1 in. from the bottom of an 18 in. × 21 in. sheet of Whatman no. 4 filter paper. Four sheets were used. The chromatograms were developed with water-saturated phenol in an atmosphere of 0.3 per cent of NH_3, HCN and coal gas. The length of the solvent flow was 45.5 cm. The sheets were dried in air, then at 100° C. and viewed under ultraviolet light. Eight distinct bands were seen. The paper was cut into these eight bands at the following points: Band 1, Rf 0.00–0.15; band 2, Rf 0.15–0.26; band 3, Rf 0.26–0.38; band 4, Rf 0.38–0.45; band 5, Rf 0.45–0.53; band 6, Rf 0.53–0.64; band 7, Rf 0.64–0.77; and band 8, Rf 0.77–0.88.[4]

These bands were thoroughly extracted with hot water, the extracts concentrated to dryness and each residue was dissolved in 1 ml. of 10 per cent of aqueous isopropanol. Five-tenth-ml. aliquots of each band were hydrolyzed for 24 hr. with 10 ml. of 6 *N* HCl. The excess acid was removed and the residue was diluted to 1 ml.

An estimation of the amino acids in the free and peptide form in these eight fractions of the AAP was made by means of paper chromatography.

Band 1. Glutamic acid and a trace of aspartic acid were found in the form of the free amino acids. There was no change after hydrolysis.

Band 2. Only free glutamic acid was found.

[3] The method of preparation was such that one would expect the hydrolysis of glutamine to yield glutamic and/or pyrrolidone carboxylic acids.

[4] The symbol Rf, which was introduced by Consden *et al.* (6), is used to express the relation between the distance moved by a substance and by the advancing front of the solvent. Thus

$$\text{Rf} = \frac{\text{movement of substance in cm.}}{\text{movement of advancing front of liquid in cm.}}$$

Band 3. Free glutamic acid (approximately 16 millimols per ml. of original solution) and less than 3 millimols of free glycine per ml. were obtained.

Band 4. Only glycine and a small quantity of serine were seen before hydrolysis. After hydrolysis, there was an increase in glycine and the appearance of glutamic acid, *i.e.*, this band contained a peptide of glycine and glutamic acid.

Band 5. This band did not contain any free amino acids but yielded, on acid hydrolysis, glutamic acid and glycine in equimolar ratios.

Band 6. Only alanine and possibly pyrrolidone carboxylic acid were present in the free state. After hydrolysis alanine (5 millimols), glutamic acid (1 millimol) as well as traces of aspartic acid, serine, glycine and the leucines were found.

Band 7. No amino acids were seen on the chromatograms before hydrolysis. After hydrolysis, glutamic acid (1–2 millimols per ml. of original solution) and small amounts of glycine, valine and the leucines were found.

Band 8. This band also apparently was devoid of free-amino acids, but yielded glutamic acid (5 millimols), proline (5 millimols), glycine (5 millimols), alanine (1 millimol), serine (trace), valine (1–2 millimols) and lucines (3–4 millimols) after acid hydrolysis.

DISCUSSION

The "protein-free" fraction of pasteurized skimmilk was investigated for its content of amino acids in the free- and peptide-form. Glutamic acid was present in the largest quantity of any of the free amino acids.

Evidence is presented for the presence of at least the following peptides in protein-free milk in terms of their acid cleavage products: Glutamic acid-glycine; glutamic acid-glycine-aspartic acid-serine-leucines; glutamic acid-glycine-valine-leucines; glutamic acid-glycine-alanine-serine-valine-leucines-proline.

The order in which the amino acids in the peptides are written is purely arbitrary and does not imply their amounts or structural arrangement in the peptide or peptides. Small quantities of arginine and lysine were observed after hydrolysis of the AAP but these were not identified in any of the above mentioned fractions. They may have been destroyed when the paper chromatograms were dried at 100° C. in order to make the observations under ultraviolet light.

Preparation and analysis of amino acid and peptide fractions from cheese. A 20-g. portion of cheese was ground in a Waring blendor with 100 ml. of ether which contained 1 ml. of glacial acetic acid. The insoluble residue was removed by filtration and the ether extraction was repeated twice to remove the lipid materials. The ether extract gave a negative test with triketohydrindene-hydrate (ninhydrin). Five g. of the air-dried cheese residue were extracted with 25 ml. of 10 per cent aqueous isopropanol for 36 hr. The residue was removed by centrifugation and washed twice with 20 ml. of water each time. The clear filtrates were concentrated to 9 ml., then 1 ml. of isopropanol was added. This solution was used for the analysis of several amino acids after acid hydrolysis.

Table 2 gives the results obtained by microbiological amino acid assay (4)

TABLE 2

Some amino acids obtained after hydrolysis of protein-free milk and cheese
(Calculated as g. of amino acid/16.0 g. of nitrogen)

Amino acid	Protein-free evaporated skimmilk	Protein-free cheddar A[a]	Protein-free cheddar B	Protein-free cheddar C
	(*g.*)			
Lysine	2.5	2.5	lost	2.4
Leucine	2.5	13.8	12.5	9.8
Isoleucine	2.4	5.5	4.9	6.5
Valine	1.5	5.6	4.7	4.8

[a] A—raw milk cheddar cheese cured 90 days.
B—pasteurized milk cheddar cheese cured 30 d.
C—pasteurized milk cheddar cheese cured 210 d.

on three samples of cheddar cheese and on protein-free evaporated skimmilk prepared as described above. The relatively large quantity of leucine in the protein-free filtrates from cheese recalls that leucine first was isolated from cheese filtrates by Proust (15) in 1819. The apparent decrease in the relative quantity of leucine and leucine-containing peptides on curing may be explained by the assumption that leucine and leucine-rich peptides are liberated from the casein at a more rapid rate than some of the other amino acids and leucine-poor peptides or that there is an actual destruction of leucine during ripening.

A number of one- and two-dimensional paper chromatograms (*cf.* 4, 6) were carried out on the aqueous extracts of the cheddar, limburger, and Swiss cheeses. Although the resulting chromatograms were complex because of the presence of amino acids, peptides and amines, the following ninhydrin-reacting substances were definitely identified:[5] aspartic acid, glutamic acid, serine, glycine, threonine, alanine, lysine, arginine, histidine, tyrosine, valine, methionine, leucines, phenylalanine, proline, γ-aminobutyric acid and methionine sulfoxide[6] (table 3). A spot, which increased in color density after the addition of synthetic α-aminobutyric to the cheese extract and was unchanged following acid hydrolysis, also was seen. This is presumed to be α-aminobutyric acid.

The substances listed in table 3 have been given the following numerical values: minus indicates not found, ± possibly present; + trace, 1+ very weak, 5+ strong, 10+ present in relatively high concentration. In assigning these values, account was taken of the fact that some amino acids give considerably more color per mol than others (1, 2).

DISCUSSION

The increasing amount of proteolysis with increasing age of cheddar cheese (table 1) is in agreement with the findings of Harper and Swanson (9) who reported that the total extractable amino acids and some simple peptides, espe-

[5] Tyramine is readily separated from all the other amino acids, peptides, and amines which were investigated, by chromatographing with a 2,6-lutidine-water-ethanol mixture. This observation permits the quantitative determination (2) of tyramine in an aqueous extract of lipid-free cheese.

[6] It is not known with certainty whether methionine sulfoxide is present in the cheese or whether it was formed from methionine on the paper chromatograms.

TABLE 3

Amino acids in the aqueous extracts of various cheeses as found by two-dimensional paper chromatography

	Cheddar A[a]		Cheddar B		Limburger		Swiss A		Swiss B	
	U[b]	H	U	H	U	H	U	H	U	H
Arginine	±	2+	3+	1+	–	1+	–	1+	–	–
Histidine	+	2+	2+	+	±	+	±	+	2+	+
Lysine	±	3+	4+	2+	+	2+	+	2+	4+	5+
Tyrosine	±	1+	+	1+	±	1+	1+	1+	1+	+
Phenylalanine	+	2+	2+	3+	1+	3+	1+	2+	2+	3+
Cystine	–	–	–	+	–	–	–	±	–	±
Methionine	–	+	2+	3+	+	2+	+	2+	1+	3+
Serine	±	3+	4+	5+	+	4+	1+	4+	2+	4+
Threonine	±	2+	3+	3+	±	1+	+	2+	2+	2+
Leucine	2+	3+	4+	5+	2+	4+	1+	3+	3+	6+
Isoleucine	+	2+	3+	5+	2+	4+	1+	3+	3+	6+
Valine	3+	4+	4+	8+	3+	5+	3+	4+	5+	5+
Glycine	±	2+	2+	2+	±	1+	1+	1+	2+	2+
Alanine	2+	3+	4+	3+	3+	3+	3+	3+	4+	5+
Glutamic Acid	3+	6+	10+	10+	5+	10+	5+	10+	10+	10+
Aspartic Acid	+	3+	5+	3+	1+	2+	2+	2+	+	3+
Proline	–	5+	±	3+	–	2+	–	2+	2+	3+
Hydroxyproline	.....	±	.....	±	.....	–	.....	±	.....	±
γ-NH_2 Butyric Acid	–	–	+	1+	5+	5+	2+	2+	1+	1+
α-NH_2 Butyric Acid	–	–	±	±	+	1+	1+	1+	±	±
Ornithine ?	–	.....	–	.....	–	.....	+?	1+?	.....	+?
Methionine Sulfoxide	±	1+	1+	.....	1+	±	1+	1+	1+	1+

[a] Cheddar A—3 wk. old; cheddar B—2 yr. old; Swiss A—1 mo. old; Swiss B—1 yr. old.
[b] U—aqueous extract of cheese; H—hydrolyzed aqueous extract.

cially after removal of any heat coagulable material, showed a definite relationship to the intensity of the flavor of cheese. Virtanen and his collaborators (16, 18) concluded that the characteristic taste of Emmentaler cheese is related to the amino acid composition of the "tear fluids." They believed that the sweetness of the Emmentaler is due to the large quantities of proline liberated and the acidity to the dicarboxylic amino acids, while the presence of arginine would give a bitter taste. In view of the complexity of the composition of the non-protein components of cheddar cheese as given above, it may be somewhat premature to assign the taste and flavor of cheese to a few specific amino acids as reported by Virtanen *et al.* (16, 18) but to concur with the opinion of Harper and Swanson (9).

3. Tyramine and Other Amines in Cheese

Although tyramine was reported in cheese in 1903 by Van Slyke and Hart (17), no quantitative investigations were carried out in this connection until the recent publications of Kosikowsky and Dahlberg (13). These investigators have reviewed the earlier literature and have shown that there is a correlation between the tyramine content of American cheddar cheese and its desirable flavor. They further showed that the flavor was enhanced in cheese made with lactic and *S. faecalis* starters (7, 8) and that these starters produced more flavor and more tyramine than either alone. They also found an increase in titratable acidity with increasing flavor of cheese and in a general way an increase in volatile fatty acids and water-soluble nitrogen.

TABLE 4

Amines in various cheeses

Amine	Cheddar				Limburger	Swiss		Rf. in lutidine mixture
	1[a]	2	A	B		A	B	
	(%)	(%)	(%)	(%)	(%)	(%)	(%)	
Phenylethylamine	Not found	Not found	Not found	Not found	±	Not found	Not found	0.90
Tyramine[b]	0.004	0.07	" "	0.015	0.012	0.004	0.020	0.84
Tyramine[c]	0.004	0.08						
Histamine	0.005	0.005	Not found	Not found	Not found	Not found	Not found	0.55
Cadavarine	Not found	0.008	?	+	+	+	+	0.35
Putrecine	" "	Not found	±	+	+	Not found	Not found	0.28

[a] Cheddar 1—pasteurized milk 1 mo. old; cheddar 2—raw milk 3 yr. old; cheddar A—3 wk. old; cheddar B—2 yr. old; Swiss A—1 mo. old; Swiss B—16 mo. old.

[b] Tyramine estimated by paper chromatography.

[c] Tyramine estimated by colorimetry.

However, Dahlberg and Kosikowsky (7, 8) found that tyramine added to cheese curd did not give the cheddar flavor to cheese. It was, therefore, deemed of interest to investigate whether other amines may be formed during the processing of cheese by decarboxylation of amino acids. The non-volatile amines considered most likely in this respect were, besides tyramine, histamine, putrecine, cadavarine, phenylethylamine, agmatine and γ-amino butyric acid.

EXPERIMENTS AND RESULTS

Isolation of amines from cheese. A 5-ml. aliquot of the aqueous extract of the lipid-free cheese, equivalent to 8 g. of cheese, was added to 6 g. of a mixture of K_3PO_4–Na_2SO_4 (1:6 W/W) (14) dissolved in 15 ml. of hot water. The amines were extracted with peroxide-free ether in a continuous liquid-liquid extractor (Scientific Glass Apparatus Co., no. J-1631) for 48 hr. One ml. of 4 per cent aqueous phosphoric acid was placed in the receiving flask.

Control experiments on tyramine, phenylethylamine, histamine, putrecine and cadavarine, alone, in mixtures or added to a casein hydrolysate, indicated complete extraction of all these amines from the aqueous layer. At the end of the extraction period, the ether was removed from the aqueous solution in the receiver flask. The residue was diluted with water, neutralized to pH 7 with NaOH and the solution was saturated with the K_3PO_4–Na_2SO_4 mixture. The amines were extracted with butanol. The butanol extracts were dried with Na_2SO_4, filtered and the amines separated by means of paper chromatography (4, 6) using a mixture of 55 volumes 2,6-lutidine, 25 volumes water and 20 volumes 95 per cent of ethanol.

Some quantitative and qualitative analyses on the amines present in various samples of cheese are given in table 4. In order to check the results found by quantitative paper chromatography,[6] the tyramine in the butanol extract was estimated by means of the Millon-Lugg reaction (4) in several instances. The results are in essential agreement by both procedures.

SUMMARY

The pattern of the essential amino acids in cheddar cheese proteins made from raw or pasteurized milk showed no significant deviations from that of casein.

The amino acid pattern of the proteins of freshly prepared and of stored evaporated milk were similar and equal to the amino acid patterns of the proteins of fresh milk and of dry skimmilk.

An investigation of the amino acids of "protein-free" milk showed the presence of glutamic acid, glycine, alanine, valine, leucines, aspartic acid and serine. The first three are the predominating amino acids in protein-free cow's milk.

Evidence is presented for the presence of several peptides in "protein-free" milk. Glutamic acid and glycine were found in each of the peptides or groups of peptides obtained.

Aqueous extracts of various kinds of cheese were shown to contain all of the amino acids found in casein, as well as γ-amino butyric acid, α-amino butyric acid,

tyramine and, in certain cases, possibly ornithine, histamine, cadavarine and putrecine.

Tyramine may be readily determined in aqueous extracts of lipid-free cheese by one-dimensional quantitative paper chromatography.

I wish to thank A. B. Erikson, Lakeshire-Marty Co., Plymouth, Wis. and H. L. Sipple, Evaporated Milk Association, Chicago, Ill. for the samples of cheese used in the investigation.

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A YEAST-LIKE DEFECT CAUSED BY AN *AEROBACTER* SPECIES IN CREAM[1]

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The typical yeasty, gassy or "boiling" defect that sometimes occurs in raw churning cream during hot weather and is caused by lactose-fermenting yeasts (2, 3, 4) has been somewhat less common in recent years. Although cases of advanced yeast fermentation are less frequent, defects described as yeasty still are noticed often. Another chief cause of gas in dairy products is the development of members of the coliform group of bacteria (2). This group is recognized as being common in raw milk and cream and may produce a variety of flavor and odor defects (1, 2, 6, 7).

In the course of an investigation of the quality of farm-separated cream, a defect was encountered occasionally that suggested the early stages of the yeasty cream defect. A slight gassy condition usually was present, accompanied by a definite "bread dough" odor suggestive of yeast. However, in view of the season of the year and the relatively low atmospheric temperatures often involved, the yeast origin seemed somewhat questionable.

During a later study, involving laboratory samples of milk and cream, the defect was noticed rather frequently over the period of about 1 yr. The "bread dough," yeast-like odor plus the evolution of gas strongly suggested lactose-fermenting yeasts. The defect was similar to that observed under practical conditions in cream stations.

EXPERIMENTAL

The incidence of the "bread dough," yeast-like defect that occurred in laboratory samples from various sources is given in table 1. The defect occurred

TABLE 1

Incidence of a yeast-like defect developing in laboratory samples of milk and cream[a]

	Samples from grade A milk-producing farms Holding temperature		Samples from cream-producing farms Holding temperature	
	10° C.	24° C.	10° C.	24° C.
No. of samples	153	177	68	68
No. developing yeast-like defect	7	26	6	8
Per cent developing yeast-like defect	4.5	14.7	8.8	11.8
Av. no. days for defect to develop	7	3	6	3

[a] 85% of the samples were sterilized milk or cream inoculated with swabs from dairy equipment on farms. The remainder were raw milk and cream samples obtained from the same farms.

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during the winter as well as in warmer seasons. It developed at both 24 and 10° C. although there were fewer instances at the lower temperature. At 24° C. the defect developed in from 1 to 9 days, with an average time of 3 days. At 10° C. the defect developed in from 4 to 10 days, with an average time of slightly under 7 days. Altogether, 47 samples out of 466, or approximately 10 per cent, developed the yeast-like defect.

Bacteriological examinations were made on samples developing the yeast-like condition as well as on samples showing other types of defects. Examinations were made at the time of the first definite change detected organoleptically. Samples were streaked (after suitable dilution) on tryptone glucose extract medium as modified by Jamieson *et al.* (5) by the addition of yeast extract and milk to give a general purpose medium for investigating equipment sanitation. Incubation of plates was at 24° C. for 4 to 5 days. The violet red bile agar method was used for the estimation of coliform bacteria.

The examinations rarely showed any evidence of yeasts. As would be expected from the nature of the samples, the coliform group often accounted for a high proportion of the total flora. Of the 47 samples having the yeast-like defect, 41 were examined for coliform bacteria. Although some of the samples were relatively high in both number and proportion of these organisms, others were low in these respects (table 2). It is recognized that bacteriological exami-

TABLE 2

Incidence of coliform organisms in laboratory samples of milk and cream having a yeast-like defect

Approx. per cent of total flora made up by coliforms	Samples held at:					
	10° C.			24° C.		
	No.	%	Av. no. coliforms per ml. (in thousands)	No.	%	Av. no. coliforms per ml. (in thousands)
< 1	7	54	< 200	11	39	< 200
1–9	1	8	10,000	2	7	10,000
10–49	3	23	25,000	6	22	15,000
50–100	2	15	75,000	9	32	80,000
Totals	13	100		28	100	

nations made at other stages of development might show a different situation. The 24° C. samples tended to have a higher proportion of coliform organisms than did the samples at 10° C. However, the actual numbers (in the same percentage groups) generally were similar at the time of examination. Although not indicated in the table, many samples that did not show the defect were high in the proportions of coliform bacteria.

On picking colonies from agar plates streaked from samples having the yeast-like defect, some cultures were obtained that produced a mild "bread dough" odor at certain stages of development in litmus milk. Frequently, the odor was preceded by slightly "cowy" or slightly aromatic odors characteristic of some organisms in the coliform group. Gas was produced also. As the cultures aged,

coagulation usually occurred and the odor often became more suggestive of yeast. Cultures in tubes of sterilized cream generally gave similar results. After the initial isolations the organism was found frequently in subsequent samples and various cultures were obtained. Preliminary observations indicated that the organism belonged to the coliform group.

Production of the yeast-like defect in cream. Several trials were conducted on the development of the defect in sterilized 20 per cent cream in 2-oz. samples jars at 10, 24 and 30° C. An example of the results is given in table 3. In-

TABLE 3

Odor defects produced in sterilized cream by an Aerobacter species isolated from laboratory samples of milk and cream showing a yeast-like defect

No. of days held	Odor defects developing at holding temperatures of:					
	30° C.		24° C.		10° C.	
	Aerobacter sp.	*Aerobacter* sp. + *S. lactis*	*Aerobacter* sp.	*Aerobacter* sp. + *S. lactis*	*Aerobacter* sp.	*Aerobacter* sp. + *S. lactis*
1	sl. bread dough	sl. bread dough	sl. bread dough	cowy	.....	.....
3	sl. bread dough	bread dough	bread dough	yeast-like	indefinite	indefinite
5	bread dough	yeast-like	indefinite	indefinite	malty, bread dough	malty, sl. bread dough
7	indefinite	definitely yeast-like	dirty	definitely yeast-like	bread dough	malty,
10	.....	.....	.....	.....	bread dough	sl. bread dough

oculations included the coliform organism in pure culture and also in combination with *Streptococcus lactis.*

In the early stages of incubation at 24 and 30° C., the cream usually had a mild "bread dough" odor, although some persons described it as yeasty. The cream surface was gassy and "bubbly" and suggested moist bread dough in appearance. The defect varied somewhat from day to day, being more typical at some stages than at others. During the early stages, *S. lactis* appeared to suppress the defect, but later, the samples that contained both *S. lactis* and the coliform culture were most typical and frequently were described as yeasty. In several cases these samples were so suggestive of yeast that the possibility of yeast contamination during daily examination of the samples was considered. However, examinations on acidified agar failed to demonstrate yeast organisms. Although samples inoculated with the coliform culture alone were often "doughy" in odor, they seldom produced as definite a yeast-like defect as did the samples also containing *S. lactis.* After 7 to 8 days the defect usually was not specific and was described as off, dirty or indefinite. The "bread dough" defect also developed at 10° C. but more slowly and to a lesser degree. The production of the defect at this temperature as well as at the higher temperatures was

characteristic of the original defect in milk and cream samples (table 1). The time element involved also generally was similar.

General characteristics of the organism. Further study of the organism that caused the yeast-like defect indicated that it belonged to the *Aerobacter* genus. Some variations occurred in the changes produced in litmus milk at room temperature. Although acid and gas developed rapidly from freshly picked colonies, reduction required 4 to 5 days. Coagulation sometimes did not occur or was only partial in extent. After several days the cultures usually were ropy. About the same time, proteolysis frequently developed at the surface of the milk but did not become extensive. The "bread dough" odor was not always definite in litmus milk tubes, but varied in intensity with different cultures and their stage of development. All strains isolated were able to grow at pH 4 on potato dextrose agar, but not at pH 3.5.

Tests with other coliform cultures. To determine if the production of the yeast-like defect was a relatively common characteristic of the coliform group, eight pure cultures (four *Escherichia* and four *Aerobacter*) recently isolated from milk were tested in sterilized cream. None of the changes produced by the *Escherichia* cultures resembled the yeast-like defect. Two of the *Aerobacter* cultures caused changes slightly suggestive of the defect at some stage of development. However, they did not produce the condition as definitely and typically as did the cultures isolated from the original defective samples. Presumably, the production of the yeast-like defect is a variable characteristic of *Aerobacter* species and limited to certain varieties.

DISCUSSION

Although the *Aerobacter* species isolated was shown to be the cause of the yeast-like defect, many of the original defective samples were low in coliform organisms at the time of examination (table 2). In cases of bacteriological defects in dairy products, inability to demonstrate large numbers of causative organisms from original samples is not unusual. Such a situation frequently arises from the stage of development at which examinations are made. In the cases involved in table 2 it is possible that the coliform group had passed its peak of development and had become overgrown by other types at the time of examination. Since the coliform count includes both *Escherichia* and *Aerobacter* species, even the samples with high counts do not necessarily indicate corresponding proportions of the causative *Aerobacter* organism. It is possible that a relatively small number of *Aerobacter* species sometimes caused the defect. Since the average time required to develop defects at 10° C. was more than twice as long as at 24° C., the general similarity in coliform counts at the two temperatures (within the same percentage groups) probably is not unusual.

SUMMARY

An *Aerobacter* species of bacteria was isolated as a cause of a yeast-like defect occurring in laboratory samples of milk and cream inoculated from farm dairy utensil sources. The defect produced by the organism in cream, particu-

larly when associated with *S. lactis*, ranged from a mild "bread dough" odor to a definite yeast-like defect. It resembled the condition sometimes observed in farm-separated cream, which suggests early stages of yeast development.

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A COMPARATIVE STUDY OF THREE METHODS OF DETERMINING THE HEAT DESTRUCTION OF LACTOSE IN MILK WITH SPECIAL REFERENCE TO THE PICRIC ACID COLORIMETRIC PROCEDURE[1]

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Renewed interest in methods for determining the lactose content of milk has occurred within recent years as the result of studies which have revealed lactose to be intimately associated with acid production and with the browning reaction in milk heated to high temperatures. In a previous paper in 1945, Gould (4) stated that methods which have been developed for measuring changes in normal milk may not yield comparable results when utilized for milk which has been changed by high-temperature processing. The available data indicate this statement to be applicable to the measurement of lactose.

Several investigators have shown that the heating of milk and lactose solutions to high temperatures for prolonged periods results in a marked decrease in the lactose content as determined polarimetrically (4, 5, 7, 9, 12, 13, 14). When this method was used, the amount of lactose destroyed depended mainly upon the temperature and time of heating, pH and concentration of lactose, and was far greater than was necessary to account for the acid produced. In contrast, when methods other than the polarimetric were used, the lactose changes resulting from heating were much less and, in certain cases, comparatively insignificant (5, 6, 7, 13). For example, in studies in which pronounced polarimetric changes occurred as the result of heat treatment, slight loss in lactose was revealed by the gravimetric copper reduction method (7, 13) and by the chloramine T method (5), somewhat more loss by the iodometric than by the copper reduction method (7) and an actual increase in lactose by the alkaline ferricyanide method (5). The changes revealed by certain of these methods are insufficient to account for the acidity increase which is known to result from lactose destruction in heated milk.

In 1947, in connection with other research on the heat treatment of milk, a study was conducted to compare certain methods for measuring the lactose disappearance in heated milk. Particular attention was given to the adaption and use of the picric acid colorimetric method studied or used by Dehn and Hartman (3), Pacini and Russell (11), Bierman and Doan (2), Knodt and Petersen (8) and recently reported upon by Perry and Doan (10).

Dehn and Hartman (4) developed the picric acid method for the estimation of carbohydrates. The method is based upon the reduction of picric acid to the

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mahogany-red picramic acid in an alkaline medium, and it was demonstrated that the intensity of the color produced is proportional to the concentration of lactose (11). Bierman and Doan (2) used the picric acid colorimetric method for determining the lactose content of milk and milk products. The results were within 0.1 per cent of those obtained by the copper reduction gravimetric method.

Since the picric acid method appeared to be somewhat less tedious and time-consuming than certain other gravimetric and volumetric methods and since it had never been applied to the measurement of lactose in heated milk, it was adopted as one of the methods for this study. At the time this study was made, the picric acid method had not been adapted to photoelectric colorimetry, and one objective of the study was to make this adaptation. However, since the recent paper by Perry and Doan (10) deals with this subject, it obviates the necessity of including repetitious details relative to the use of photometric color measurements for this lactose method.

EXPERIMENTAL PROCEDURE

The milk used was fresh, raw mixed milk obtained from the University herd. The milk was sealed in no. 2 Canco cans and heated. The control samples were heated to 62° C. for 30 min. in a hot water bath; the other samples were heated in an autoclave to 116° C. for 0, 15, 30 and 60 min. The cans were shaken at the end of each heating period. The milk was cooled to about 5° C. immediately following heating and held for examination.

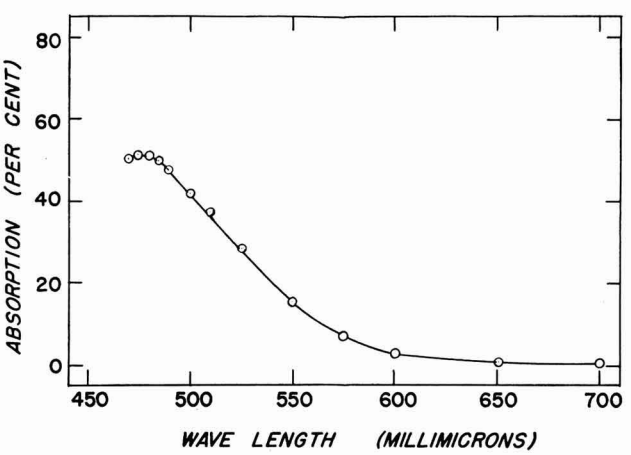

FIG. 1. Absorption curve for the picric acid method for determining lactose using the Cenco-Sheard Spectrophotolometer

The lactose content was determined by the polariscope and copper reduction methods of the A. O. A. C. (1) and by the colorimetric picric acid method as outlined by Bierman and Doan (2), but modified to be used with the Cenco-Sheard Spectrophotolometer.

The procedure adopted for the picric acid method varies from the procedure of Perry and Doan (10) mainly as to quantities of reagents and extent of dilution of the solutions.

Absorption curve data were obtained using a sufficient quantity of a standard lactose solution (1 mg. lactose monohydrate per milliliter of saturated picric acid solution) equivalent to a lactose concentration of 5 per cent lactose on a milk basis. The adsorption curve presented in figure 1 reveals that maximum absorption occurs at a wave length of about 475 mμ. However, because of limitations of light source, it was necessary to use a wave length of 485 mμ for all measurements in this study.

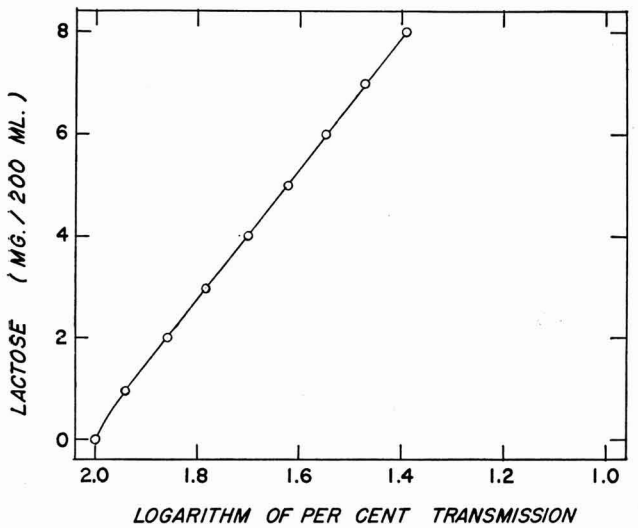

FIG. 2. Standard curve for determining the per cent of lactose by the picric acid method.

The standard curve obtained by using varying concentrations of the standard lactose solution is presented in figure 2. It may be observed that, for the concentrations used, essentially a straight-line relationship exists.

EXPERIMENTAL RESULTS

Preliminary trials revealed that, when applied to milk, the picric acid method exhibited a high degree of reproducibility, with maximum variations of less than 0.05 per cent lactose when several determinations were made upon the same sample.

The recovery ability of the picric acid method was determined by preparing a series of milk samples containing varying concentrations of added lactose. The lactose was added to 60 g. of milk and the mixture diluted to 100 ml. with water. The data presented in table 1 are typical of the several trials run in this experiment and reveal recovery well within 0.1 per cent of the amount of lactose added.

TABLE 1

Recovery of lactose added to milk by the picric acid colorimetric method

Lactose found	Lactose added	Lactose recovered	Variation
(*G./100 g. milk*)	(*G./100 g. milk*)	(*G./100 g. milk*)	(*G./100 g. milk*)
2.71	.....	.....	.....
3.14	0.50	0.43	−0.07
3.64	1.00	0.93	−0.07
4.23	1.50	1.53	+0.03
4.65	2.00	1.94	−0.06
5.20	2.50	2.49	−0.01

A comparison was made of the picric acid, copper reduction and polarimetric methods. The results are presented in table 2. The data reveal good agreement

TABLE 2

Comparison of picric acid, copper reduction and polarimetric methods on heat treated milk

Treatment	Picric acid		Copper reduction		Polarimetric	
	Found	Variation from control	Found	Variation from control	Found	Variation from control
	(%)	(%)	(%)	(%)	(%)	(%)
Control	4.74	.....	4.82	.....	4.76	.....
116° C.— 0 min.	4.69	−0.05	4.86	+0.04	4.79	+0.03
116° C.—15 min.	4.64	−0.10	4.81	−0.01	4.68	−0.08
116° C.—30 min.	4.61	−0.13	4.77	−0.05	4.42	−0.34
116° C.—60 min.	4.59	−0.15	4.72	−0.10	4.02	−0.74

among the three methods when they were applied to the control samples, with values of 4.74, 4.82 and 4.76 per cent lactose for the picric acid, copper reduction and polarimetric methods, respectively. However, when application was made to the heated milk samples, definite differences were noted, with the polarimetric method revealing an appreciably greater decrease in the lactose content than the other two methods for heating periods of 30 and 60 min. at 116° C. For shorter heating periods, agreement was close between the results for all of the methods. In general, the picric acid method reveals slightly greater lactose decreases in the heated milk than the copper reduction method.

DISCUSSION

The reasons for the differences between the results obtained by the three different methods for the heated milk are not completely evident. As has been observed by previous workers, the polarimetric method reveals appreciable losses of lactose as the result of heating, whereas, in general, other methods reveal comparatively slight changes in the amount of lactose present. Perhaps, in so far as optical rotation is concerned, the changes are additive, whereas, from the standpoint of reducing properties, the changes are somewhat compensatory.

The reduction in the rotatory capacity of milk heated to high temperatures may result from destruction of the lactose and the yielding of one or more of the following: (a) substances having non-rotating properties, (b) substances giving rotation values less than lactose and/or (c) substances with distinct levo-

rotatory properties. Hydrolysis of lactose and the subsequent rearrangement of glucose into other hexoses is known to take place under mildly alkaline conditions at 15 to 20° C. and might possibly occur in milk at high temperatures. The formation of fructose with a rotation of –92.25 would result in a marked lowering in the optical rotation of the milk. The presence of ketoses in heated milk has been indicated (7).

SUMMARY

The picric acid method adapted to a photometric procedure for measuring color intensity agrees closely with the copper reduction and polarimetric methods in determining lactose in unheated milk. The method yields results that are reproducible and sufficiently accurate for routine analysis, and it may be performed as quickly as the polarimetric method and much more rapidly than the copper reduction method.

In milk heated to high temperatures for prolonged periods, the lactose losses noted with the picric acid method were slightly greater than those obtained with the copper reduction method but were considerably less than those obtained with the polarimetric method.

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SEDIMENTATION OF SPERMATOZOA AND SETTLING OF DILUTER SOLIDS AND THEIR EFFECTS UPON SURVIVAL OF SPERMATOZOA DURING STORAGE[1]

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It has been a rather general observation that a marked difference exists in the degree of "settling out" of the solids between different samples of semen "extended" with egg yolk diluter. It also has been suggested that rapid or pronounced "settling out" in these diluted semen samples would result in lowered fertility.

Indications that sedimentation may have harmful effects have been found in several investigations where gums or gelatin were added. Weber (6) reported that viscous media were more suitable for semen dilution than fluid ones. Knoop (2) reported improvement in motility maintenance when gelatin was added to semen diluted with an egg yolk-buffer mixture. Among other factors, holding the spermatozoa inactive was given as a possible explanation for the increased survival. Knoop and Krauss (3), using some of the major amino acids of gelatin rather than gelatin in an egg yolk-buffer medium, obtained better storage than that obtained with the egg yolk-buffer medium alone or with gelatin. Numerous investigators have used various gums in spermatozoan diluents. One of the possible desirable attributes of a gum is its capacity for holding the spermatozoa in suspension. Therefore these viscous media could be acting by (a) holding the spermatozoa in suspension and (b) reducing their activity.

It might then be highly desirable to know if a semen specimen can be judged as inferior on the basis of the settling of the solids. This settling is commonly observed in routine shipments of bull semen. The purpose of this investigation is to determine those factors responsible for the settling of the solids, especially in dairy bull semen diluted with egg yolk, and the effects of "settling out" upon livability of the spermatozoa.

MATERIALS AND METHODS

Semen specimens for these experiments were collected weekly or biweekly from bulls in the Station dairy herd during the period of March, 1948, through February, 1949. One hundred fifteen ejaculates from 13 different bulls representing the Holstein, Jersey and Guernsey breeds were used. The semen was collected by the use of an artificial vagina. The techniques of dilution, storage and evaluation are those commonly employed in this and other laboratories.

The motility was scored in thirds of a unit from 0 to 5, 0 representing no motility and 5 the maximum. Per cent survival of the spermatozoa was obtained by the use of a modified staining technique for the differentiation of live

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and dead spermatozoa described by Mayer *et al.* (4). A further discussion of methods can be obtained by referring to the previous paper by the authors (1).

EXPERIMENTAL RESULTS

Factors responsible for the settling of solids in diluted semen. A visual method was used in estimating the settling of egg yolk and semen plasma solids. Observations were made after the storage tubes were placed inside larger, calibrated tubes and held in front of a bright light. By previously selecting uniform storage tubes, readings from the bottom of the clear supernatant and from the top of the entire diluted sample could be converted directly into per cent of the total volume as clear supernatant.

The following were found to be the major factors causing the variation in the amount of settling of yolk and semen plasma solids in a diluted semen specimen:

(a) *Amount of mixing.* In semen samples diluted with one part of egg yolk to one part of citrate solution, the per cent of the total volume present as clear supernatant could be varied, from zero to ninety per cent during a 24-hr. storage period by the amount of shaking or stirring involved in mixing the egg yolk with the citrate or phosphate solutions. In those samples which were thoroughly mixed little or no settling occurred.

(b) *Ratio of egg yolk to citrate solution.* With smaller volume ratios of egg yolk to citrate solution in the diluter, the samples settled at a faster rate. Fifty-four samples, diluted with a medium composed of one part of egg yolk to five parts of citrate solution, had an average of 89 per cent of the total volume as clear supernatant at the end of a 2-day storage period. Thirty-five samples in a mixture containing one part of egg yolk to one part of citrate solution had an average of 31 per cent of the volume as clear supernatant at the end of a corresponding period of time.

(c) *Concentration of electrolytes or Na ions.* The settling of the egg yolk solids could be prevented by replacing all or a part of the buffer solution with non-electrolytes. When mixtures of either citrate or phosphate solutions with sugar solutions were used in egg yolk media, the degree of settling of the egg yolk solids was proportional to the amount of buffer solution used.

(d) *Influence of the individual semen sample.* When the above conditions were standardized, there still was considerable variation in settling, presumably caused by some unrecognizable differences between the individual semen specimens.

Effect of settling of the solids in diluted semen upon the viability of the spermatozoa. Since the chemical or physical characteristics of the diluter constituents might affect livability, attempts were made to keep the type and quantity of the constituents constant, while making comparisons between the rate of settling of the solids and the livability of the spermatozoa. In experiment 1, 9 ml. of egg yolk were stirred with 9 ml. of citrate solution until the egg yolk appeared to be equally distributed throughout. This mixture then was divided into three equal portions which were placed in a refrigerator over night. The first aliquot was used without further treatment, whereas the second aliquot was mixed

thoroughly for 1 min. in a Waring blendor before use as a diluter. The third aliquot was centrifuged to remove the solids prior to its use. An equal volume of semen from the same specimen was added to each of the three diluters. Duration of motility and survival of the spermatozoa during storage were compared in these three mixtures. The average results of 12 such trials are shown in table 1.

TABLE 1

Influence of the amount of settling of the egg yolk solids upon spermatozoan motility and livability during storage[a]

	% of the total volume present as clear supernatant			Motility rating			% survival		
Length of Storage (*hr.*)	60	120	240	60	120	240	60	120	240
Egg yolk one part: one part citrate	46	73	82	2.48	1.35	0.68	82.5	71.9	66.6
Egg yolk one part: one part citrate (blended)	0	0	0	2.48	1.38	0.66	82.5	72.7	67.8
Egg yolk one part: one part citrate (centrifuged)	Solids removed			2.43	1.41	0.83	82.5	71.6	66.9

[a] Averages of 12 trials.

Although there were pronounced differences in the degree of settling of the solids, the average motility rating and per cent survival were very close for the three types of mixing. Table 2 gives the mean squares for the analysis of vari-

TABLE 2

Analysis of variance between samples and treatments using egg yolk media with varying degrees of settling of the solids
(Mean squares only)

	D.F.	Mean squares			
		120 hr. of storage		240 hr. of storage	
		Motility rating	% survival	Motility rating	% survival
Between samples	11	1.593	335.10	0.6145	248.60
Between treatments	2	0.013	3.88[a]	0.0968	4.52
Treatments × sample	22	0.046	1.05	0.0350	8.35

[a] $P < .05$

ance between samples and between treatments at 120 and 240 hr. of storage for these 12 trials. This analysis would indicate that the amount of clear supernatant had no effect upon the motility estimate after storage.

In the second experiment, 35 different samples of semen "extended" with a medium containing equal quantities of egg yolk and citrate solution were divided into five classes according to the per cent of the total volume present as clear supernatant after 60 hr. of storage. There were eight, seven, seven, six and seven samples, respectively, represented in the following classes 0–19, 20–39,

40–59, 60–79 and 80–99 per cent of the total volume as clear supernatant. Figure 1 shows the average motility and per cent survival after 60 hr. of storage for the samples represented in each class.

There was more variation in motility rating and in spermatozoan survival between samples within the classes than between the classes. The individual semen specimen, then, can affect the settling of egg yolk solids, but a large amount of clear supernatant, representing maximum settling of the diluter solids, does not necessarily indicate poor livability of that semen sample.

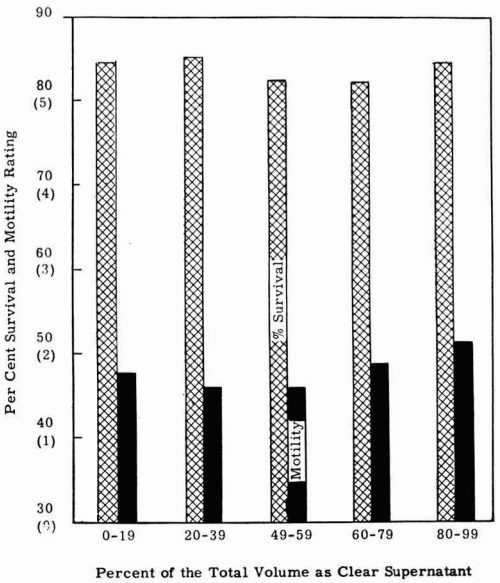

FIG. 1. Comparisons of the degree of settling of egg yolk solids with motility and livability at 60 hours of storage in egg yolk 1 part: 1 part citrate. ( )-Motility rating.

It was shown previously that the rate of settling of egg yolk solids increased as the proportion of egg yolk to citrate solution in the diluting medium was decreased. However, no significant differences were found either in motility rating or per cent survival after storage when 32 semen specimens were compared in media in which the proportions of egg yolk to citrate solution were 1 : 1 and 1 : 5.

Swanson (5) made comparisons between media containing various proportions of egg yolk and citrate solution for the storage of spermatozoa. He used proportions of egg yolk to citrate solution of 1 : 1, 1 : 3 and 1 : 7. Progressive motility after storage was quite similar in these three diluters with the 1 : 7 egg yolk-citrate preparation being slightly inferior.

Sedimentation of the spermatozoa. Some question arose regarding the possible relationship between the settling of diluter solids and the position of the majority of the spermatozoa in the storage tube. During preliminary observations, some of the spermatozoa were found in the clear supernatant in the upper

portion of the storage tube. On the basis of these observations, the development of a more direct method for the estimation of the sedimentation rate of spermatozoa was deemed necessary. Special tubes were made by fusing a small bore stopcock to the bottom of a 12-ml. centrifuge tube. A small portion of the diluted semen could be removed, without disturbing the contents of the tube, at intervals during the storage period for the estimation of the rate of spermatozoan sedimentation. A total of 12 ml. of diluted semen was used in all comparisons with these storage tubes. At the end of a 24-hr. storage period, 0.5 ml. of semen was carefully withdrawn through the stopcock, while a second 0.5 ml. portion was withdrawn at the end of 48 hr. of storage. Removal of these 0.5-ml. portions was accomplished with a minimum of agitation, while the semen samples were at the storage temperature of 4 to 7° C. Spermatozoan concentration counts were made with the aid of a hemacytometer on the original diluted semen specimen at the beginning of the storage period. Concentration counts were repeated after mixing on the remaining portions of the specimen immediately following the withdrawal of the 0.5-ml. portions at 24 and 48 hr. of storage. The degree of sedimentation is expressed as the per cent of the spermatozoa originally present in the entire specimen which remain after the withdrawal of the bottom 0.5-ml. portions at 24 and 48 hr. of storage.

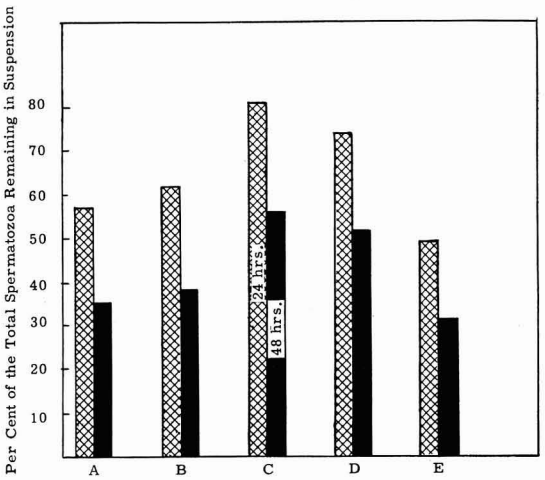

FIG. 2. Effect of various diluting media upon sedimentation of the spermatozoa: A-Egg yolk 1 part: 5 parts citrate. B-Egg yolk 1 part: 5 parts citrate (blended). C-Egg yolk 1 part: 1 part citrate. D-Phillips' synthetic pabulum. E-Egg yolk 1 part: 5 parts of a mixture of (1$NaHCO_3$ + 4 glucose).

Figure 2 demonstrates the relative effects of various types of storage media on the sedimentation of the spermatozoa. Bar A represents the average of 31 samples stored in a diluter composed of one part of egg yolk and five parts of citrate solution. The sedimentation results obtained with all of the other media were compared with those obtained with this medium, using the same semen specimen in the comparative studies.

Media C and D, which contain a higher percentage of solids, held more of the spermatozoa in suspension. Medium E held the fewest spermatozoa in suspension; however, this medium was far superior to the others in maintaining motility and survival of the spermatozoa during storage according to Kampschmidt *et al.* (1).

It seemed possible that the spermatozoan sediment was comprised chiefly of dead spermatozoa or those incapable of movement. To check this point, live-dead cell counts and motility estimates were made upon the suspended spermatozoa and the sedimented layer at 24 and 48 hr. of storage. For the 28 samples studied, an average of 57.04 per cent of the spermatozoa from the top of the tubes were unstained or alive and 53.41 per cent from the sedimented layer were alive at the end of 24 hr. of storage. After 48 hr. of storage the average per cent of live spermatozoa from the top and bottom portions of the storage tube were found to be 55.28 and 55.11 per cent. respectively.

The following experiment was designed to provide a further check on whether the sedimentation rates of the live and dead spermatozoa are approximately equal. Each of the seven samples of semen used was divided into two equal portions, one of which was subjected to heat and cold until death of the spermatozoa was assured. Both portions then were stored in a diluter consisting of one part of egg yolk to five parts of citrate solution. Initially, the untreated control samples contained 56.4 per cent of live spermatozoa and a motility rating of 2.90; whereas the treated samples contained only 1.1 per cent live spermatozoa showing no motility. At the end of a 24-hr. period 57.8 and 57.9 per cent of the spermatozoa remained in suspension in the control and in the treated samples, respectively. Very little difference in the rate of sedimentation of the spermatozoa in the two groups of samples was evident at the end of a 48-hr. period when 34.1 per cent of the total spermatozoa remained in suspension in the control group and 38.6 per cent in the treated group. These results show that at storage temperatures sedimentation of live and dead spermatozoa apparently occurs at almost identical rates.

DISCUSSION

Apparently some characteristic or characteristics of the individual semen specimens affected the settling of the solids from an egg yolk storage medium, since in different specimens diluted with egg yolk media of the same composition and prepared by identical procedures the amount of settling varied considerably. There was, however, no significant correlation between the amount of settling of the solids and the survival of the spermatozoa in these samples. These results suggest that one can not use the presence or absence of settling of the solids nor the amount of settling as a criterion of the storage potentialities of bull semen specimens in egg yolk media.

Viscous media containing gums, gelatine or similar inert viscous materials have been recommended as diluters of semen specimens prior to storage. Reduction of spermatozoan activity and the holding of the spermatozoa in suspension during the storage period have been suggested as beneficial functions of viscous

media. However, the results of this investigation show that spermatozoan viability was maintained at the highest level during a prolonged storage period in media which neither prevented the sedimentation of spermatozoa nor depressed their activity. These results raise some doubt regarding the need for viscous materials as constituents of media used in the storage of bull semen.

SUMMARY

Minor changes in procedure of preparing and mixing egg yolk media were found to have a marked effect on the settling of egg yolk and semen plasma solids.

The visible settling of egg yolk and semen plasma solids during the storage or shipping of a diluted specimen of bull semen apparently has no influence on the duration of motility or survival of the spermatozoa.

The sedimentation rate of the spermatozoa in most of the common semen-diluting media is very rapid under storage conditions. Apparently, at storage temperatures, the minute spermatozoa are behaving as inert particles, since the sedimentation rates of live and of dead cells were almost identical. In view of the rapid sedimentation rate of spermatozoa during storage, a diluted semen specimen should be thoroughly mixed prior to evaluation by the usual techniques or to utilization for artificial insemination.

The evidence presented indicates that the rapid sedimentation of the spermatozoa during storage does not affect spermatozoan survival.

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THE NUTRITION OF THE NEWBORN DAIRY CALF. IV. THE MINIMUM RIBOFLAVIN REQUIREMENT

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There is considerable information indicating that the requirement for the B-complex vitamins may be met by microbiological synthesis in the rumen of the bovine species, once the rumen has reached full functional activity (7, 8, 11, 22). There is growing evidence, however, that prior to the full functional development of the rumen a dietary supply of certain of the B-complex vitamins, including riboflavin, is required for adequate nutrition.

The need for a dietary source of riboflavin by the very young calf has been demonstrated by Wiese *et al.* (23) by feeding a synthetic milk in which riboflavin was lacking and by Warner and Sutton (21) who fed whole milk in which about 96 per cent of the riboflavin had been destroyed by photolysis.

The experiment reported herein was undertaken in order to determine the riboflavin requirement of the dairy calf up to 8 wk. of age.

EXPERIMENTAL PROCEDURE

The deficient basal ration used in this study consisted exclusively of photolyzed whole milk supplemented with vitamin A. The general procedure followed for the preparation of the photolyzed milk was similar to the one described by Warner and Sutton (21). The major modification consisted of the use of a specially designed photolyzing chamber fabricated from a 12×12 in. cylindrical pyrex jar and a 5 × 13.75 in. pyrex tube. This cylindrical chamber was supported by a base that permitted the mercury vapor lamp to be set upright within the center tube of the cylinder. The mercury vapor lamp, a 400 watt lamp emitting rays longer than 3000 Å, was of the same type as previously used in this laboratory (21). Constant agitation of milk during the photolytic process was effected by bubbling nitrogen gas through it at four equidistant outlets. With this equipment, maximum destruction of riboflavin (approximately 97 per cent) was obtained in batches of 33 to 35 lb. of milk within 4.5 hr.

Fresh milk was obtained from the University Holstein herd at the time of milking and immediately photolyzed. During photolysis, the milk was allowed to reach a temperature of 60 to 65° C. for at least 30 min. in order to control the growth of microorganisms. The temperature of the milk during treatment was controlled by means of an electric fan. The photolyzed milk was stored at 42° F. and fed within a period of 48 to 60 hr.

Riboflavin was determined in untreated and photolyzed milk and in colostrum by the fluorometric method of Hand (4) with a few minor modifications.

In addition to destroying riboflavin, photolysis may destroy other compounds of importance in the nutrition of the young calf. A survey of literature indicated that vitamin A, thiamine and pyridoxine may be sufficiently reduced by

photolysis that some compensation should be made in order that such milk might be used in evaluating the riboflavin requirements of the calf without the development of other nutritional deficiency complications. Previous experiments (21) had indicated the extent of vitamin A destruction. Consequently, the effects of light treatment on thiamine and pyridoxine content of milk were studied. Thiamine in untreated and photolyzed milk was determined by the method of Hodson (5). Pyridoxine was determined by microbiological methods (1, 19). The results are given in table 1. The destruction of thiamine averaged

TABLE 1

The effects of photolysis on the thiamine and pyridoxine content of milk

Treatment	Thiamine		Pyridoxine	
	γ/ml.	Loss (%)[a]	γ/ml.	Loss (%)[b]
None	0.32	.....	0.64	.....
Photolysis	0.28	12.5	0.24	62.5
None	0.31	.....	0.88	.....
Photolysis	0.27	9.7	0.28	67.1

[a] In samples in which 97.7% of the riboflavin was destroyed.
[b] In samples in which 96.8% of the riboflavin was destroyed.

11.1 per cent in samples of milk in which 97.7 per cent of the riboflavin had been destroyed. Pyridoxine destruction was greater, averaging 64.8 per cent when riboflavin was destroyed to the extent of 96.8 per cent.

The destruction of thiamine was not considered sufficiently great to be a complicating factor in this experiment. In the case of pyridoxine, however, the experimental procedure was modified, as described later in this report, to determine whether the destruction was great enough to produce deficiency symptoms.

Vitamin A in the form of an oil concentrate was fed daily at the rate of 20,000 I.U. per calf. The vitamin A oil was first emulsified with a small amount of milk in a Waring blendor in order to facilitate mixing with the milk fed. The riboflavin supplement was added to the milk at each feeding in the form of a water solution. Fluorescence readings on the stock solution were taken intermittently and fresh riboflavin solutions were prepared when losses in potency were apparent. Riboflavin was determined in every batch of photolyzed milk and the amounts of riboflavin added at each intake level were adjusted accordingly. The milk was not supplemented with iron and copper in view of the work of Knoop *et al.* (9).

The experimental animals consisted of nine male dairy calves, all dropped in the University Dairy Herd. They were taken from their dams at birth and placed in individual pens having raised expanded metal floors. No bedding was used.

The calves were fed their dams' colostrum for the first 48 hr., after which they were fed the experimental diets for a period of 8 wk. The riboflavin intake during the colostrum feeding period was recorded. Sulfathalidine was administered in every case of scours or diarrhea. Water was available at all times.

The nine calves used in the experiment were grouped to receive dietary treatments as indicated in table 2.

TABLE 2

Grouping and dietary treatment of calves

Group	Calf[a]	Treatment of Milk	Approximate daily riboflavin intake (γ/kg. body weight)	Supplemental pyridoxine
I	A-0	Photolyzed	4 (remaining in milk)	None
II	J-I	Untreated	115 (in milk)	None
	J-II	Photolyzed	115 (supplemented)	None
III	J-25	Photolyzed	25 (supplemented)	None
	S-25	Photolyzed	25 (supplemented)	Quantity equal to that in untreated milk.
IV	J-35	Photolyzed	35 (supplemented)	None
	H-35	Photolyzed	35 (supplemented)	Quantity equal to that in untreated milk.
V	J-45	Photolyzed	45 (supplemented)	None
	G-45	Photolyzed	45 (supplemented)	None

[a] Letter indicates breed.

The milk was fed warm (37 to 40° C.) at 12-hr. intervals at the rate of 10 per cent of the body weight per day. The calves were weighed weekly and the quantity of milk fed adjusted after each weighing.

Each week the calves were placed in a 4×1.5 ft. metabolism stall with an expanded metal floor, and a 24-hr. urine specimen was collected for riboflavin assay. Collection was made in an 8-pt. brown glass bottle which contained approximately 25 ml. of concentrated HCl. Contamination with feces was prevented by a water-proof feces bag attached to a blanket put over the calf during the collection period. The total volume of urine collected was measured and representative samples were stored, following the instructions of Slater and Morell (16). In general, riboflavin was determined in the urine within a week by the method of Slater and Morell (17). No major modifications were made except that the photolyzing apparatus sometimes was used instead of sunlight to destroy riboflavin as required in the procedure.

At the end of the experiment, the calves were slaughtered and all except A-0, J-I, J-II and G-45, whose carcasses were used in another experiment, were autopsied in the Veterinary Clinic. In every case, however, the rumen content was collected and incubated at 37° C. for 20 hr. The riboflavin content of the liquid rumen contents before and after incubation was determined. An increase during the incubation period was considered to be indicative of the rate of riboflavin synthesis in the rumen of the calves at the time of slaughtering. The method used for riboflavin determination in the rumen liquid was the same as that used for milk.

RESULTS

The growth rates of the calves are presented graphically in fig. 1. The Ragsdale standard (15) is included for purpose of comparison. The 24-hr. urinary

TABLE 3

The urinary excretion of riboflavin by calves receiving various levels of riboflavin (mg./100 lb. (45.3 kg.) body weight)

Calf no.	Riboflavin intake	24 hr. urinary riboflavin excretion at age of:									Av.[a]
		2 d.	1 wk.	2 wk.	3 wk.	4 wk.	5 wk.	6 wk.	7 wk.	8 wk.	
	(γ/kilo./d.)										
A-0	4	0.86	0.07	0.05	0.07	0.04	0.01	0.04	0.07	0.09	0.06
J-25	25	0.14[b]	0.18	0.07	0.02	0.07	0.10	0.10	0.10	0.16	0.10
S-25	25	0.44	0.14	0.12	0.13	0.10	0.09	0.12	0.07	0.19	0.12
J-35	35	1.01	0.08	0.12	0.11	0.11	0.12	0.08	0.20	0.12	0.12
H-35	35	1.73	0.09	0.09	0.10	0.09	0.16	0.13	0.12	0.07	0.11
J-45	45	0.19[b]	0.18	0.13	0.09	0.32	0.27	0.32	0.24	0.80	0.29
G-45	45	1.00	0.87	0.31	0.70	0.22	0.45	0.38	0.54	0.54	0.50
J-I	115[d]	2.26	3.06	2.04	2.27	1.68	2.06	2.26	2.21	2.72	2.29
J-II	115	0.14[b]	3.42	2.81	2.88	0.05[c]	3.00	3.37	2.87	2.96	2.66

[a] First collection excluded.
[b] First collection made at the age of 4 to 5 d.
[c] Riboflavin supplementation had been discontinued for 3 d. prior to this collection.
[d] Average riboflavin intake in untreated milk.

excretions of riboflavin are given for each calf in table 3. The results of the first collection were not included when the average daily excretion for the entire experiment was calculated. These results were omitted in an attempt to eliminate the influence of the possible difference in riboflavin storage at birth, as well as the differences in intake during the colostrum feeding period.

Control calves. The growth of the J-I and J-II calves was practically identical and similar to the Ragsdale growth standard (15) (fig. 1). Both calves

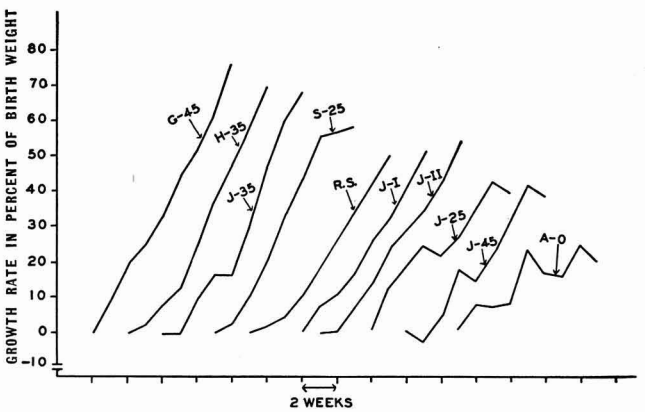

FIG. 1. Curves illustrating the growth rates of calves fed various levels of riboflavin (R. S. refers to Ragsdale growth standard, other letters and numbers refer to calves as given in text).

appeared normal, thrifty and active during the entire experiment. They showed no deficiency symptoms whatsoever. There was no physical evidence of pyridoxine deficiency in calf J-II, even though no pyridoxine supplement was fed. A normal healthy condition of the lungs and intestines was noted in these calves at slaughter. The rumen of J-I was small and contained a few hair balls

but no fluid. It is possible, however, that some liquid, originally present in the rumen, was lost during slaughtering. A few hair balls were found in the rumens of all calves used in this experiment. The rumen of J-II contained approximately 500 ml. of liquid. During incubation, the riboflavin content of this liquid increased about 2.5 times. This is an indication that at the time of slaughtering some riboflavin-synthesizing microorganisms were present in the rumen of this calf.

The growth of the negative control (A-0) was poor and irregular (fig. 1). Excessive lacrimation and salivation appeared when the calf was 2 wk. old. Early during the third week diarrhea occurred which did not respond to sulfathalidine treatment and persisted until the termination of the experiment. When the milk allowances were reduced for a few days, however, the feces became more solid, but diarrhea invariably reappeared when the calf was brought back on full feed. At the end of the experiment, this calf was unthrifty, possessed a rough hair coat and showed definite signs of dry, scaly dermatitis, especially apparent in the area back of the ears. Excessive shedding persisted from the third week of the experiment on; however, severe alopecia as previously reported (21, 23) was not observed. When the animal was sacrificed at the age of 62 days, the rumen contained about 600 ml. of liquid. The riboflavin content of this liquid increased from 0.09 to 0.37 mg. per liter during the incubation period. It is possible, therefore, that some riboflavin was synthesized in the rumen of this calf. This phenomenon may possibly account for the periods of relatively good growth observed during the fourth and sixth week of the experiment. A condition of severe catarrhal enteritis and gangrenous pneumonia was observed when the calf was slaughtered.

25 γ of riboflavin per kilogram of body weight. J-25 grew well during the first 3 wk. of the experiment. During the fourth week, scours appeared, and the animal lost weight (fig. 1). From this time on, an unthrifty condition gradually developed. Excessive shedding accompanied the development of a rough hair coat. Sulfathalidine treatment was without beneficial effects in times of diarrhea. Excessive salivation was observed. The post-mortem examination showed pneumonia, severe catarrhal enteritis and "white spotted" kidneys as the gross pathology. Anemia, characterized by a low erythrocyte and leucocyte count and low hemoglobin content, also was observed.

S-25 grew normally during the first 6 wk., after which growth stopped and body weight remained practically unchanged until the termination of the experiment. At that time excessive loss of hair, a rough hair coat and excessive salivation were the most prominent external symptoms of deficiency. Diarrhea never appeared. The post-mortem examination showed a mild catarrhal enteritis and "white spotted" kidneys. No pneumonia was observed. A normcytic hypochromic type of anemia as observed in J-25 also was noted in S-25.

Some liquid was collected from the rumen of both J-25 and S-25 but incubation effected no change in the riboflavin content of this liquid. There was no indication that pyridoxine supplementation improved the photolyzed milk diet of S-25.

35 γ of riboflavin per kilogram of body weight. These two animals (J-35 and H-35) grew well until the end of the experiment (fig. 1). No external symptoms of deficiency were observed, except for somewhat excessive shedding, especially in calf H-35. Both calves apparently remained thrifty and in good health throughout. Diarrhea, however, appeared in J-35 toward the end of the experiment. Mild catarrhal enteritis and "white spotted" kidneys were revealed by the post-mortem examination. Normocytic hypochromic anemia, as previously noted in calves J-25 and S-25, also was observed in both J-35 and H-35. The rumens of these animals contained some liquid. The riboflavin content of this liquid was not changed during the incubation test.

There were no differences in the performance of these calves which could be attributed to pyridoxine supplementation in the diet of calf H-35.

45 γ of riboflavin per kilogram of body weight. The response of G-45 in growth, thriftiness and general health was excellent until termination of the experiment (fig. 1). No signs of deficiency whatsoever were observed. A normal condition of the viscera, especially the lungs and intestines, was noted at the time of slaughter.

J-45 had diarrhea during the first week of life and lost weight. After recovery, growth was resumed at a normal rate until the fourth week. During that week, for no apparent reason, the calf lost weight, but resumed normal growth during the fifth to seventh week. During the last week of the experiment, diarrhea which responded to the sulfathalidine treatment occurred and the calf again lost weight so that the over-all growth was fairly poor (fig. 1). It may be noted, however, that during the periods of good growth the gain in weight was normal according to the Ragsdale standard. In general, J-45 remained thrifty and very active. No external signs of riboflavin deficiency were observed during the entire course of the experiment. The post-mortem examination showed an entirely normal condition to prevail throughout. No anemia was observed.

The rumen of G-45 contained some liquid which, during the incubation test, showed no change in riboflavin content.

DISCUSSION

Photolyzed milk, as prepared in this experiment and supplemented with vitamin A, was found to be a satisfactory basal ration for the study of riboflavin nutrition in the young calf. When the photolyzed milk diet was fed without riboflavin supplementation (A-0) riboflavin deficiency symptoms developed, which were similar to those previously reported (21, 23). On the other hand, when this basal diet was supplemented adequately with riboflavin, the symptoms did not appear and the performance of the calf fed this ration (J-II) was identical to that of a similar calf (J-I) fed untreated milk. It appears, therefore, that riboflavin is the only limiting factor in photolyzed whole milk supplemented with vitaimn A when fed to calves up to 8 weeks of age.

The destruction of thiamine and pyridoxine by the process of photolysis apparently did not complicate the results. In fact, the destruction of thiamine as

a result of the treatment was small and the destruction of pyridoxine, even though considerable, obviously was without significance for the young calf.

When the photolyzed milk ration was fed without supplementation or when riboflavin was supplemented at the 25 microgram level, gross deficiency symptoms developed. Post-mortem examination showed catarrhal enteritis and "white spotted" kidneys as the gross pathology. These symptoms of riboflavin deficiency were associated with anemia and, in some cases, pneumonia. The latter condition was reported to develop in lambs fed a riboflavin-deficient ration (3). The anemia observed was of the normocytic hypochromic type accompanied by marked decreases in erythrocyte (8.4 to 3.4 millions per ml.) and leucocyte (6,350 to 2,700 per ml.) counts. This type of anemia was found to be similar to the type of anemia reported to be associated with riboflavin deficiency in swine (24) and monkey (2, 20).

When riboflavin was supplemented at the 35 γ level, the calves grew normally and showed no external signs of riboflavin deficiency except, perhaps, for excessive shedding. The post-mortem examination, however, showed a mild catarrhal enteritis and "white spotted" kidneys but no pneumonia. Normocytic hypochromic anemia was observed.

The calves fed riboflavin at the 45 γ level showed no evidence of abnormalities that definitely could be attributed to a deficiency of riboflavin. They showed no external signs of deficiency whatever and post-mortem examination revealed a normal condition to prevail throughout. Anemia was not observed.

The fact that the riboflavin excretions on intakes of 25 and 35 γ per kilogram body weight were essentially the same indicates that the higher of these levels may still be below the minimum daily requirement. On the basis of these observations it appears that the minimum daily riboflavin requirement is between 35 and 45 γ per kilogram of body weight. This requirement is in good agreement with that reported for other mammalian species of comparable age (2, 6, 10, 12, 13, 14, 18).

The question of the significance of microbiological synthesis of riboflavin in the rumen of the milk-fed calf is still unanswered. The failure to observe an increase in riboflavin during the incubation of rumen fluid from a number of the calves in this experiment may be interpreted as indicating that riboflavin-synthesizing organisms were not present. In the case of the two calves where there was evidence of riboflavin synthesis during the incubation of the rumen fluid, evidence of such synthesis *in vivo* could not be detected by urine analysis.

SUMMARY

Nine male calves representing five dairy breeds were used to determine the minimum riboflavin requirement of the dairy calf up to 8 wk. of age.

The basal riboflavin-deficient diet consisted of whole milk in which approximately 97 per cent of the riboflavin had been destroyed by photolysis. The photolyzed product contained an average of 0.04 mg. of riboflavin per liter. This photolyzed milk was supplemented with vitamin A and the various levels of riboflavin studied.

It was found that calves receiving 35 γ or less riboflavin per kilogram of body weight daily developed symptoms of riboflavin deficiency. The deficiency symptoms were more severe at the lower levels. The calves receiving 45 γ or more of riboflavin per kilogram body weight showed no symptoms that could be attributed to a deficiency of the vitamin.

The data obtained indicate that the urinary excretion of riboflavin is a good index of the nutritional status of the calf as far as riboflavin is concerned.

The data obtained indicate that the minimum daily riboflavin requirement of the male dairy calf up to 8 wk. of age is between 35 and 45 γ per kilogram of body weight.

ACKNOWLEDGMENT

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THE EFFECTS OF ESTROGEN AND PROGESTERONE ON THE ARTERIAL SYSTEM OF THE UTERUS OF THE COW

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The uterine arteries of the cow have received less attention than they deserve. Sisson and Grossman (7) state that the uterus is supplied by the small utero-ovarian arteries and the large middle uterine artery. These arteries and their ramifications have been described in detail by Martin and Schauder (5) and Ellenberger and Baum (1).

The arterial structure within the uterus itself is not well known, although it has been described in part by Martin and Schauder (5). Ellenbogen (2) described the coiled nature of the arteries surrounding the uterine horns and pointed out that the caruncular vessels were smaller and less numerous in heifers than in parous cows. The following study was carried out in order to gain a more complete understanding of the arterial system of the uterus in normal animals and to ascertain the effects of the ovarian hormones on this system.

EXPERIMENTAL PROCEDURE

The uteri of six normal and seven ovariectomized cows were collected at the time of slaughter and washed thoroughly in 0.9 per cent saline. Red vinylacetate[1] in acetone then was injected into the middle uterine artery on each side from a syringe. The injection was made as rapidly as possible and continued until no more vinylacetate could be forced into the artery. The injection mass was allowed to harden and the tissue was digested away by immersion in a solution of 2 per cent HCl to which pepsin (20 g. per l.) was added. The digestion was carried out at 37° C. for 48 to 72 hr. The last remnants of tissue were washed away under a fine jet of water. In some cases a portion of one horn of the uterus was removed prior to digestion of the tissue. These portions were used to prepare thick sections for microscopic study.

Data concerning the animals used and the hormones administered are summarized in table 1. The normal animals were of two age groups. Three were unbred heifers between 13 and 16 mo. of age when slaughtered and three were mature cows 5 yr. old or over. One of the mature cows (no. 31) had never been bred; the other two were parous cows.

The total amounts of estrogens and progesterone administered to the ovariectomized cows in the 17-mo. period prior to the time they were slaughtered likewise are shown in table 1. It may be seen that the amounts of estrogens administered covered a wide range, but that the amounts of progesterone given did not vary greatly. The hormones were dissolved in cottonseed oil and injected subcutaneously.

In order to determine if changes produced in the uterine arterial system by

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[1] Ward Scientific Co., Rochester, N. Y.

TABLE 1

Summary of data on animals used and hormones administered
Normal cows

Open heifers				Open cows			
No.	Breed	Age at slaughter	Stage of estrous cycle when slaughtered	No.	Breed	Age at slaughter	Stage of estrous cycle when slaughtered
53	Grade Hol.	16 mo.	8 d. postestrum	31	Grade Hol.	5 yr.	16 d. postestrum
55	Grade Guer.	16 mo.	1 d. postestrum	C–4	Grade Hol.	Approx. 8 yr.	21 d. postestrum
67	Hol.	13 mo.	20 d. postestrum	C–5	Hol.	14 yr.	5 d. postestrum

Ovariectomized-hormone treated cows

No.	Age at slaughter	Breed	Total hormones given in 17 mo. prior to slaughter				Hormones given in 26 day period prior to slaughter			
			Stilbestrol	Estradiol Benz.	Dienestrol	Progesterone	Days	Stilb.	Days	Progest.
	(*yr.*)		(*mg.*)	(*I.U.*)	(*mg.*)	(*Rb.U.*)		(*mg./d.*)		(*Rb.U./d.*)
11	8	Gr. Guern.	34.18	0	0	2162	16 4	1 2	6	35
36	5	Gr. Guern.	1685.89	23,000	0	1960	16 4	1 2	6	35
26	8	Gr. Guern.	1679.43	0	0	1225	16 10	1 2	0	0
35	5	Gr. Guern.	1610.40	0	0	1540	16 10	1 2	0	0
45	8	Gr. Hol.	8.78	0	0	1357	.....	0	.....	0
47	5	Gr. Guern.	26.49	0	12.25	997	.....	0	.....	0
39	5	Gr. Hol.	4.50	28,000	0	1015	.....	0	.....	0

short-term treatments with the ovarian hormones could be detected, two of the ovariectomized cows were given stilbestrol for 26 days prior to the time they were slaughtered, two were given stilbestrol followed by progesterone and three were given no treatment during the same period. The details of these treatments are shown in table 1.

RESULTS AND DISCUSSION

Several points concerning the general anatomical relationships of the uterine

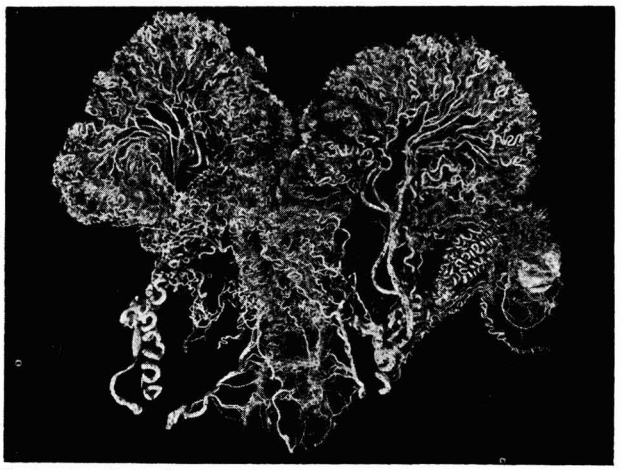

FIG. 1. Vinylacetate injection-corrosion preparation of the entire uterus and both ovaries of a 5-yr.old cow slaughtered at 16 d. postestrum. The active corpus luteum was in the right ovary. Cow no. 31.

arteries are worthy of note. The middle uterine artery gives rise to numerous primary branches in the broad ligament (fig. 1). The two primary branches

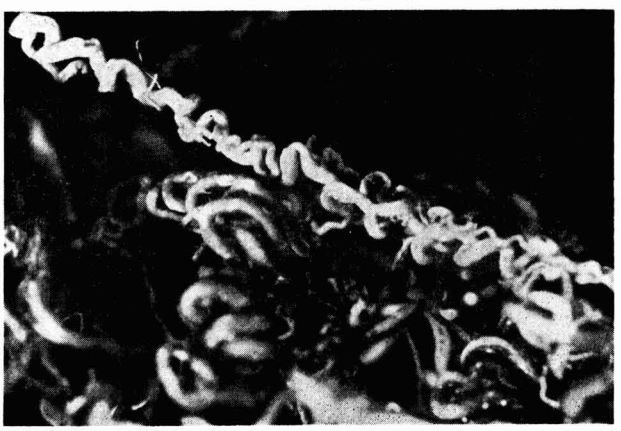

FIG. 2. "T"-shaped arteriole in the longitudinal muscle layer of the uterus. An arcuate artery runs vertically through the center of the photograph. Cow. no. C-4. Injection-corrosion preparation.

nearest the cervical end of the uterus are particularly well developed and run caudal beyond the *os uteri*. Each primary artery gives rise to a varying number of arcuate arteries which encircle the horn of the uterus and anastomose opposite the mesometrial border. These arcuate arteries are thrown into coils of decreasing diameter and increasing complexity and are developed to a much greater degree in mature cows than in heifers.

The outer muscular layer is supplied by small "T"-shaped arterioles arising from the arcuate arteries (fig. 2). These arterioles are quite convoluted, and the combined length of the two arms may vary from 0.5 to 1.0 cm. They are remarkably adapted to the tissue which they serve.

The endometrium is supplied by complex branches and subbranches of the arcuate arteries. These endometrial arterioles differ markedly in heifers and mature cows. In the uteri of the 13- to 16-mo.-old heifers the endometrial arterioles are nearly straight (fig. 3). In mature cows the endometrial arterioles are

FIG. 3. Endometrial arterioles in the uterus of a 16-mo.-old heifer at 8 d. postestrum. Note that the arterioles are essentially straight. Cow no. 53. Plastic-corrosion preparation.

more numerous and are coiled on their central ends to a much greater degree (fig. 4).

FIG. 4. Endometrial arterioles in the uterus of a 5-yr-old cow slaughtered at 16 d. post-estrum. The high degree of coiling of many of the arterioles is evident. Contrast with fig. 3 showing the endometrial arterioles of a 16-mo.-old heifer. Injection-corrosion preparation.

The smaller number and lesser degree of coiling and development of the arterioles supplying the endometrium of the uterus of heifers may well be contributing factors to the lower conception rate found in heifers than in mature cows.

The phenomenon of metestrous bleeding occurs much more frequently in heifers than in mature cows. An average of the figures given by Krupski (4), Trimberger (8) and the authors (3) indicates that heifers under 2 yr. of age bleed during 86 per cent of their estrous cycles, while cows over 2 yr. of age bleed in only 52 per cent. Ruptured endometrial capillaries are the principal source of this blood, as Weber *et al.* (9) recently have shown. The higher incidence of metestrous bleeding in heifers and its lower incidence in mature cows possibly may be attributed to the differences in the endometrial arterioles. The spiral arterioles in the uterus of the mature cow reduce the blood pressure acting on the endometrial capillaries and, at the same time, do it in such a way as to maintain an axial rather than a turbulent flow of blood. This pressure-reducing system is developed to a much lesser degree in heifers and metestrous bleeding

in such animals possibly is more frequent for this reason. The reasons for stating that a system of coiled arterioles reduces blood pressure have been presented by Reynolds (6) and are briefly as follows: (a) coiling increases the length of the vessel that must be traversed by the blood per unit of distance in the tissue in question (endometrium); (b) the diameter of the coiled vessels decreases rapidly and (c) engineers studying the flow of liquids in coiled tubes have found that there is a greater pressure loss due to friction in coiled tubes than in straight tubes. This pressure loss due to friction would be further accentuated by the elasticity of the arterioles and by the fact that they carry a pulsating flow.

In general, the caruncular arterioles are more tightly coiled than the other endometrial arterioles, and possess unusually thick walls containing many elastic fibrils (fig. 5). They appear to be somewhat more developed in the uteri of mature cows than heifers.

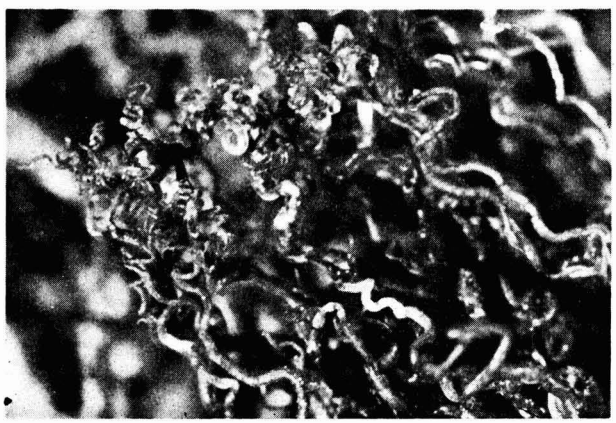

FIG. 5. Coiled caruncular arterioles in the uterus of a mature cow slaughtered during late proestrum. These arterioles are from 10 to 50 mμ in diameter. Plastic injection-corrosion preparation.

It is not possible to pick out clear-cut and consistent differences in the endometrial arterioles ascribable to definite stages of the estrous cycle. Most of the sections made from the injected material were unsatisfactory because the hardened plastic tended to break up when cut, so that small changes in the endometrial arterioles could not be studied accurately.

In the ovariectomized cows the number and the degree of coiling of the endometrial arterioles were correlated with the total amounts of the ovarian hormones given over a 17-mo. period. Most of the endometrial branches of the arcuate arteries disappeared in the uteri of those cows which received only small amounts of estrogens. On the other hand, those which received large amounts of estrogens during the 17-mo. period had endometrial arterioles which compared favorably in number and in degree of coiling to similar arterioles in the uteri of normal cows of the same age (fig. 7). Those cows which received the largest amounts

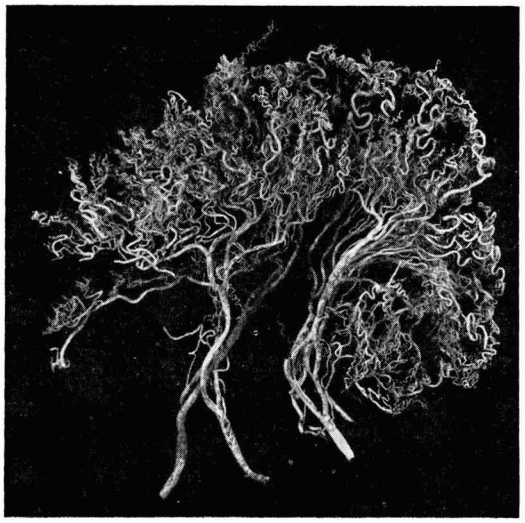

FIG. 6. Plastic injection-corrosion preparation of the right horn of the uterus of a 5-yr.-old cow ovariectomized 17 mo. prior to the time of slaughter. The amounts of ovarian hormones given after ovariectomy were small (table 1.) Most of the endometrial arterioles have disappeared. Contrast with fig. 7. Cow no. 39.

of estrogens over the 17-mo. period also received slightly more progesterone. However, the differences in the amounts of progesterone given are so small that it appears that the differences observed may be attributed for the most part to the estrogenic hormones.

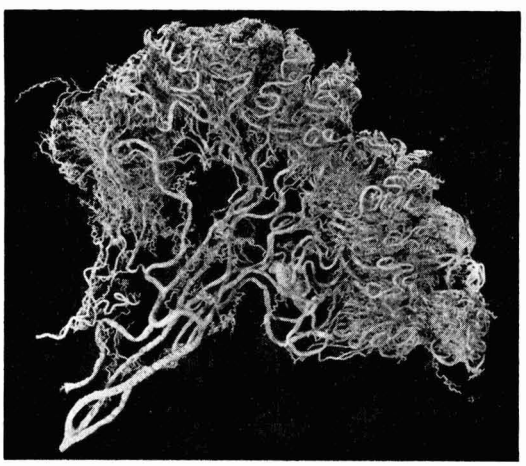

FIG. 7. Plastic injection-corrosion preparation of a portion of the right horn of the uterus of a 5-yr.-old cow ovariectomized 17 mo. prior to the time of slaughter. A large amount of stilbestrol was given after ovariectomy (table 1). The endometrial arterioles are numerous and coiled. Cow no. 35.

No differences were found in the endometrial arterioles attributable to the different treatments given in the 26-day period prior to slaughter. Here, as in the case of the normal cycle, small changes may have occurred which could not be assessed accurately.

SUMMARY

A study of the uterine arteries of six normal and seven ovariectomized cows by the plastic injection-corrosion technique revealed the following points:

(a) The caruncular arterioles of normal cows are extremely coiled.

(b) The endometrial arterioles of 13- to 16-mo.-old heifers are essentially straight, while similar arterioles in the uteri of mature cows are more numerous and more highly coiled. These facts appear significant in relation to the lower conception rate encountered in heifers and in relation to the more frequent occurrence of metestrous bleeding in heifers.

(c) The disappearance of the endometrial arterioles that normally occurs following ovariectomy can be prevented by administration of the ovarian hormones. Estrogenic hormones appear to be the most effective in this respect.

(d) It was not possible by this method to detect differences in the arterial development of the uteri of normal cows which might be associated with definite stages of the estrous cycle.

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VIABILITY OF BULL SPERMATOZOA AS INFLUENCED BY ELECTROLYTE CONCENTRATION, BUFFER EFFICIENCY AND ADDED GLUCOSE IN STORAGE MEDIA[1]

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Observations in this laboratory suggested that a reduction in the quantity of sodium-containing buffers and an increase in glucose in diluting media would result in improved storage of stallion and bovine spermatozoa. Previously, Lardy and Phillips (6) and Salisbury and VanDemark (14) presented evidence that the addition of small amounts of glucose to an egg yolk-buffer diluting mixture increases and prolongs active motility of the spermatozoa. Further, Dubincik (3), Milovanov (10) and Bogart and Mayer (2) reported that a high concentration of electrolytes in a diluting medium has a deleterious effect on spermatozoa. On the basis of these observations, comparisons were made between several diluters now in use and diluters from which a part or all of the sodium-containing buffer salts had been replaced with non-electrolytes, usually one of the various sugars.

However, the maintenance of the pH during prolonged storage in diluters with reduced buffer salt concentrations presented a major problem and initiated a search for more efficient buffers. Sodium bicarbonate proved beneficial in media used for metabolic studies with spermatozoa, according to Redenz (13), Henle and Zittle (5), Lardy, *et al.* (7) and Moore and Mayer (11). Since sodium bicarbonate is not a highly ionizable salt and also has proved to be an efficient buffer, it was selected for study in this investigation.

MATERIALS AND METHODS

Collection and dilution. The semen was collected with an artificial vagina and cooled immediately to 15 to 20° C. and held there until the experimental period. The time between collection and dilution was from 1 to 3 hr. in all experiments. Collections were made weekly or biweekly from 13 dairy bulls representing the Holstein, Jersey and Guernsey breeds.

The volume ratio of semen to diluter in all experiments was 1 : 10. The ratio of egg yolk to the mixture of buffer and sugar solutions in the diluter was 1 : 5. All of the ingredients were dissolved separately in glass-distilled H_2O in concentrations giving a solution isosmotic with semen plasma. These solutions then were pipetted into flasks containing egg yolk to give the desired quantities in the various storage media.

The vials of diluted semen were placed in a beaker of water and cooled slowly in the refrigerator to a storage temperature between 4 and 7° C.

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Evaluation. Motility estimates were made at the time of dilution and every 24 hr. thereafter until the sample had been stored for a period of 10 days. The motility was scored in thirds of a unit from 0 to 5; 0 representing no motility and 5 the maximum.

The proportion of the spermatozoa surviving a storage interval was determined by the staining technique developed by Lasley *et al.* (8) and as modified by Easley *et al.* (4) and Mayer *et al.* (9). The staining mixture was composed of 2 per cent fast green (FCF) and 0.8 per cent erythrosin B in 100 ml. of *M*/8 phosphate buffer. The slides were made immediately after dilution and after 60-, 120- and 240-hr. storage intervals. The survival percentage was obtained by dividing the fraction of spermatozoa unstained at the end of a given storage period by the fraction found unstained immediately after dilution.

The pH values of the samples were determined at 120- and 240-hr. storage intervals with a Beckman glass electrode pH meter.

RESULTS

Comparisons were made between portions of the same semen specimen diluted with media composed either of: (a) one part of egg yolk mixed with five parts of isosmotic sodium citrate solution, (b) one part of egg yolk with five parts of 5 per cent glucose solution or (c) one part of egg yolk plus five parts of a solution containing various proportions of glucose and sodium citrate. In each successive comparison, an additional one-tenth of the citrate solution was replaced with glucose solution. At least twelve trials were run in each comparison, using the same semen specimen in each trial. As the proportion of glucose to citrate solution was increased, there was, in every case, a corresponding increase in the survival of the spermatozoa during storage. The improved survival between each successive increase in glucose also was large enough to be of importance. The motility of the spermatozoa was maintained at increasingly higher levels with each successive increase in glucose until one-half or more of the citrate had been replaced with glucose. Replacing more than one-half of the citrate lowered the buffering capacity of the diluting medium to such an extent that the pH was not maintained during the entire 10-day storage period.

Figure 1 shows the average spermatozoan motility and survival in 32 semen samples stored in a medium composed of one part egg yolk and five parts of citrate solution (*curve B*) and in one composed of one part egg yolk and five parts of a mixture of one citrate plus four glucose solution (*curve A*). It is quite apparent that spermatozoan survival was maintained at a higher level in the medium containing glucose. This medium also maintained the spermatozoan motility at a higher level during the first 5 days of storage. However, motility declined at a rapid rate after the fifth day of storage in the glucose-containing medium. It was noticed that this decline in motility was paralleled by a decline in pH whereas pH was maintained in the non-sugar containing citrate medium. A regression analysis was designed to test whether the increasing differences in the pH of these two media could account for the changes in motility rating of the spermatozoa after the fifth day of storage. When the difference in motility rating be-

tween the two media on successive days was adjusted to a constant pH difference, based on regression of motility on pH among samples within the same diluter and storage interval classes, it remained practically constant during the last 5 days. The rapid drop in motility after the fifth day of storage probably could be accounted for by the rapid lowering of pH in the poorly buffered solution. It was observed in these studies and has been shown previously that when the pH drops below a certain minimum level (approximately 5.3 to 5.5) the spermatozoa become immobile. In media with a higher concentration of glucose and a correspondingly lower concentration of buffer salts than those just reported, a more rapid drop in motility than that shown in figure 1 was noticed. However, even

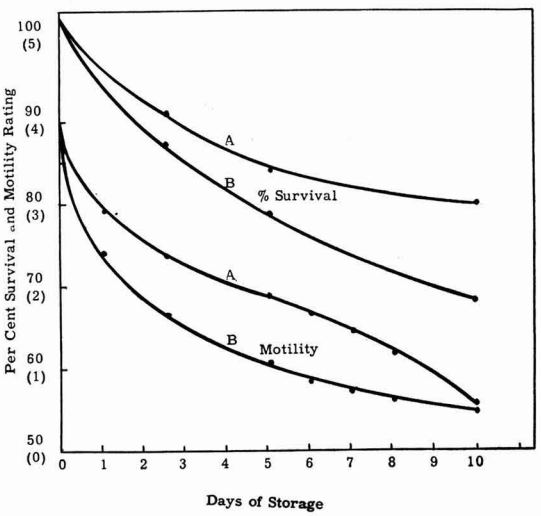

FIG. 1. Effect of replacing 4/5 of the citrate solution with glucose solution upon spermatozoa motility and livability during storage: A-Egg yolk 1 part: 5 parts of a mixture of (1 citrate + 4 glucose). B-Egg yolk 1 part: 5 parts of citrate. ( )-Motility rating.

with the decreased buffering capacity, an increased maintenance of survival during a 5-day storage period was observed in the media with decreased sodium ion concentrations, suggesting a more detrimental effect of sodium ions than low pH values on spermatozoan survival.

It was necessary to find a more efficient buffer in order to maintain a high level both of motility and survival during a prolonged storage period in glucose-containing media with a reduced concentration of buffer salts. Sodium bicarbonate was selected for reasons previously stated.

Figure 2 shows a comparison between spermatozoa stored in media containing one part egg yolk plus five parts of sodium citrate solution (*curve B*) and one part egg yolk plus five parts of a mixture of one part of 1.3 per cent $NaHCO_3$ solution and four parts of 5 per cent glucose solution (*curve A*). The curves represent the average of 25 trials, dividing the same specimen of semen for dilution with the two different storage media in each trial. The superiority of medium A

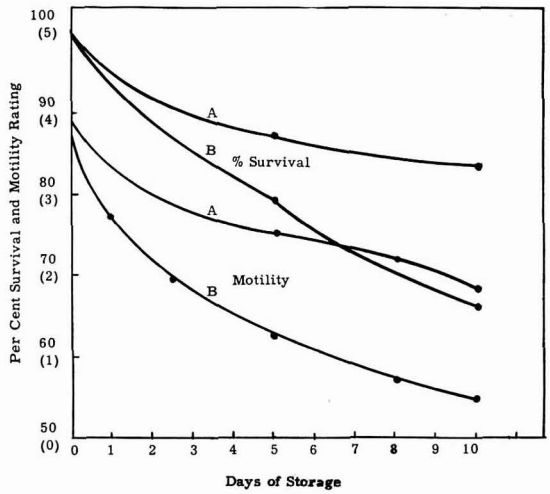

FIG. 2. Comparison between a glucose-sodium bicarbonate mixture and sodium citrate in an egg yolk medium: A-Egg yolk 1 part: 5 parts of a mixture of (1 $NaHCO_3$ + 4 glucose). B-Egg yolk 1 part: 5 parts of citrate. ( )-Motility rating.

is marked and unquestionably real. The pH was maintained at the same level by both media throughout the storage period, despite the lower concentration of buffer salt in medium A, attesting the superior buffering characteristics of $NaHCO_3$.

The buffer salts of Phillips' synthetic pabulum (Phillips and Spitzer, 12) were replaced with glucose and $NaHCO_3$ to see if the beneficial effects just described could be secured by a reduction in electrolytes in other types of media. The modified pabulum contained 3.2 per cent glucose, 0.45 per cent $NaHCO_3$, 1.5 per cent Lipositol, 3.0 per cent gum acacia and distilled H_2O to volume.

Phillips' synthetic pabulum and egg-yolk citrate buffer were compared in field inseminations by Bayley *et al.* (1). The fertility was 15 per cent higher using portions of semen specimens from 19 bulls diluted with egg yolk-citrate buffer than that obtained with other portions of the same semen specimens diluted with the synthetic pabulum. In this laboratory, comparisons between the original pabulum and egg yolk-citrate buffer showed the pabulum to be inferior in maintaining motility and survival during storage.

Comparisons between semen specimens diluted with the original pabulum and with the pabulum as modified showed a marked increase in both duration of spermatozoan motility and survival during a 10-day storage period in the modified pabulum over that in the original pabulum. In 12 trials with the original pabulum, the average spermatozoan motility rating at 240 hr. of storage was 0.51 and the per cent survival was 57.2. The modified pabulum maintained an average motility rating of 2.33 and a survival percentage of 78.1 with portions of the same semen specimens. The modified pabulum also was superior to egg yolk-citrate buffer in 12 within-sample comparisons. The modified pabu-

lum did not, however, yield storage results equal to those obtained with the medium composed of egg yolk and $NaHCO_3$ and glucose solutions.

Sugars other than glucose were used in diluting media in order to compare the results obtained with those secured with the glucose-containing media. Table 1 shows the results obtained when isosmotic solutions of various sugars were

TABLE 1

Effects of various sugar solutions in combination with citrate solution upon motility and livability of spermatozoa during storage in an egg yolk medium

Diluter	Motility rating after			% survival after		
	60 hr.	120 hr.	240 hr.	60 hr.	120 hr.	240 hr.
Egg Yolk 1: 5 citrate	1.67	0.97	0.59	85.9	75.6	66.1
Egg Yolk 1: 5 mixture of (1 citrate + 4 glucose)	2.26	1.82	0.63	90.2	85.8	82.9
Egg Yolk 1: 5 mixture of (1 citrate + 4 fructose)	2.20	1.86	0.58	90.1	83.4	82.4
Egg Yolk 1: 5 mixture of (1 citrate + 4 galactose)	1.97	1.53	0.56	88.7	82.6	78.4
Egg Yolk 1: 5 mixture of (1 citrate + 4 lactose)	1.62	0.86	0.41	90.0	81.4	71.4
Egg Yolk 1: 5 mixture of (1 citrate + 4 sucrose)	1.43	0.62	0.00	89.1	81.3	68.2

used in media containing one part of egg yolk to five parts of a mixture of one part citrate solution plus four parts sugar solution. The results shown in table 1 are averages of seven trials using portions of the same specimen of semen with all of the different media. Those media which contained sugars metabolizable by spermatozoa (glucose and fructose) maintained higher spermatozoan survival during storage than any of the others. However, the diluters containing non-metabolizable sugars (lactose, galactose and sucrose) maintained a higher spermatozoan survival percentage than did the egg yolk-citrate medium. The motility in the glucose- and fructose-containing media could be maintained at a higher level during prolonged storage if the pH drop was averted by using the $NaHCO_3$ buffer.

DISCUSSION

In this investigation, the effects of replacing varying proportions of the buffer solution in egg yolk media with solutions of glucose and other sugars were studied. In general, the relative proportion of sugar solution in an egg yolk-citrate-sugar medium markedly influenced the survival of spermatozoa during storage. Other proportions of glucose to citrate solution than those shown by the graphs were used in comparative experiments and the results followed the same general trend as previously described.

The type of sugar added is an important factor in determining the livability of the spermatozoa in that medium. It was shown (table 1) that the sugars which have been reported to be metabolized by spermatozoa give better storage results. One might surmise that the added glucose was aiding spermatozoan survival only because of its utilization in metabolic activities. However, the results of Bogart and Mayer (2), using stallion spermatozoa, and the results

obtained in this investigation, using bull spermatozoa in media containing non-metabolizable sugars, suggest that some factor in addition to the metabolic utilization of the sugar is responsible for the increased spermatozoan survival. Evidence for additional factors is presented in this paper, as it was shown that each successive increase in glucose solution or decrease in citrate solution promoted increased spermatozoan livability during storage. The evidence suggests that the decreased concentration of the sodium ion aids spermatozoan survival.

When combinations of phosphate buffer and glucose solution in egg yolk media were used, results similar to those with the citrate-glucose solution were obtained. Decreasing the proportion of phosphate solution to glucose solution increased the survival of spermatozoa during storage. Bogart and Mayer (2), working with stallion spermatozoa, showed that sodium ions were definitely detrimental to these cells during storage. The results reported in this investigation on the beneficial effects of replacing most of the sodium citrate or sodium phosphate solution with a glucose solution would indicate that bovine spermatozoa also might be adversely affected by sodium ions.

Most of the differences in the storage results shown in figure 2 probably could be explained by the added metabolizable sugar and a reduction of the electrolytes. There may, however, be additional stimulating effects due to the carbonate ions. The two media maintained about the same pH throughout the storage period. The increased buffering capacity imparted by $NaHCO_3$ is illustrated in figure 2. It will be noted that undiluted citrate solution was mixed with the egg yolk, while the $NaHCO_3$ solution was diluted with four volumes of a 5 per cent glucose solution; yet, as previously stated, the pH was maintained at approximately the same level in these two media during the 10-day storage period.

In storage media with insufficient buffering capacity, the pH decreases rapidly during the later stages of the storage period. A decrease or a complete cessation of motility accompanies the pH changes. However, the decrease in the percentage of surviving or viable spermatozoa lags behind the pH and motility changes. Determination of the percentage of viable spermatozoa by the differential staining technique indicates that a large proportion of the non-motile cells may be alive. This finding would raise some question concerning the reliability of a motility rating as a measure of spermatozoan survival during a prolonged storage period, since a low pH will immediately decrease or even eliminate spermatozoan motility, but fails to kill the spermatozoa until considerable time has elapsed.

CONCLUSIONS

A reduction in the quantity of sodium-containing buffer salts in a diluting medium promoted increased survival of bull spermatozoa during storage. Sugar solutions, especially solutions of metabolizable sugars, were most satisfactory for replacing solutions of buffer salts in the medium. However, reducing the proportion of buffer salts in the medium was beneficial only if a sufficient quantity remained to maintain an optimum pH level during the entire storage period.

A diluting medium composed of one part of egg yolk and five parts of an isosmotic mixture of one part $NaHCO_3$ solution (1.3 per cent) plus four parts glucose solution (5 per cent) gave better results than any diluter studied as a storage medium for bull spermatozoa.

It has been suggested that a motility rating may not be an accurate criterion of spermatozoan survival during the late stages of a prolonged storage period in the absence of adequate amounts of buffer substances.

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THE EFFECT OF INTERRUPTION OF MILKING ON THE PROTEIN FRACTIONS OF MILK[1]

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Various workers (3, 4, 5, 7, 10, 11, 12, 13, 14, 17) have found that interrupting milking causes a marked increase in the per cent total protein of milk. Porcher (12) and Porcher and Muffet (13, 14) report that the percentages of albumin and globulin in milk increased, while the percentage of casein decreased. However, Laxa (7) found that the milk from cows not milked for 3 days contains no albumin and globulin, increased amounts of albumoses, peptones and amino acids and a normal amount of casein. The object of this study was to determine the effect of interrupting milking for 10 days on the casein, globulin, albumin and non-protein nitrogen of milk.

EXPERIMENTAL

Animals. During October 1949, twice-daily milking of four Holsteins and one Jersey was discontinued for 10 days. The average number of lactations for these cows was 2.6 ± 0.5, and the average day of lactation at the beginning of the experimental period was 237.4 ± 54.0. The two Holstein cows in the control group were milked twice daily during their experimental period which was in April and May, 1950. These cows were in their fourth and seventh lactations and had been lactating 215 and 52 days at the beginning of the experimental period. The feeding, management and treatment of all cows were essentially those reported earlier by Mercer *et al.* (8).

Samples. Representative samples of the six milkings immediately prior to interruption and of the first six milkings after milking was resumed were obtained from the five treated cows. Similar samples were obtained from the two control cows in which case milking was not interrupted. All milk samples were immediately quick-frozen and held at $-18°$ C. until the analyses were made in May and June, 1950.

Analyses. Casein was determined by Moir's method (9), but larger amounts of acetic acid and sodium acetate were needed to reach a pH of 4.6 in the precipitation of casein from the milk obtained after the interruption period. Globulin was precipitated from the casein-free filtrate with one-half saturated Na_2SO_4 according to the method of Howe (6). Albumin was determined in the casein- and globulin-free filtrate by Rowland's trichloroacetic acid method (15). The nitrogen content of the precipitates and the protein-free filtrate was determined by the Macro Kjeldahl method (2). Protein concentrations of casein, globulin

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and albumin were obtained by multiplying their respective percentages of nitrogen by 6.38. Standard statistical procedures (16) for the analysis of variance were used to test for differences.

RESULTS

When the amounts of milk obtained from the cows (table 1) were compared,

TABLE 1

The effect of a 10-d. period of interrupted milking on the pounds of milk secreted by dairy cows

	Milkings before interruption					
	−6	−5	−4	−3	−2	−1
Controls	17.2 ± 4.4[a]	13.6 ± 3.9	16.7 ± 5.2	13.3 ± 3.9	17.9 ± 4.4	13.3 ± 3.8
Treated	16.7 ± 6.3	12.0 ± 4.9	16.3 ± 5.4	12.5 ± 5.1	15.0 ± 6.7	11.2 ± 4.2
	Milkings after interruption					
	+1	+2	+3	+4	+5	+6
Controls	18.5 ± 5.0	14.5 ± 2.5	17.2 ± 3.9	14.2 ± 2.8	17.5 ± 4.0	13.4 ± 3.6
Treated	5.6 ± 2.4	2.0 ± 0.9	2.7 ± 3.1	2.3 ± 1.4	3.4 ± 2.3	2.1 ± 1.8

[a] Mean ± standard deviation.

the amount after interruption of milking was found to be less ($P < 0.01$) than that obtained from the same animals before the 10-day interruption period and less ($P < 0.01$) than that from the control cows.

The per cent total nitrogen was higher after the interruption period in the milk obtained from the treated cows. The differences between the per cent nitrogen before interruption of milking and that after interruption were significant ($P < 0.01$) within the treated group, but no significant differences were found between the control group and the treated group. The total amount of nitrogen in the postinterruption milk was less ($P < 0.01$) than that of the milk drawn before interruption and also less ($P < 0.001$) than that of the milk obtained from the control cows.

The per cent casein (fig. 1) of the milk was not markedly changed by interrupting milking. The total amount of casein in the milk after interruption was much less ($P < 0.001$) than that before interruption and also less than that in the milk of the control cows. The per cent of the total nitrogen as casein (fig. 2) found in the milk after interruption was less ($P < 0.01$) than that in the milk obtained from the control cows and measurably less ($P < 0.001$) than that in milk drawn before interruption of milking.

The per cent albumin (fig. 1) after interruption was greater than that prior to interruption of milking within the treated group ($P < 0.001$) and also greater than that for the control group ($P < 0.05$). The total amount of albumin in the milk after interruption was less ($P < 0.05$) than that before interruption and less ($P < 0.01$) than that in the control cows' milk. A larger percentage of the total nitrogen in the postinterruption milk was albumin (fig. 2). When the per cent of the total nitrogen as albumin in postinterruption milk was compared

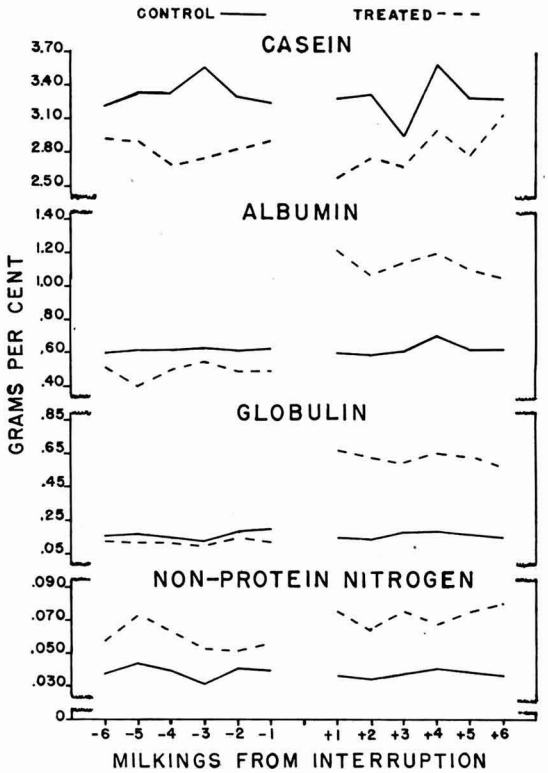

FIG. 1. The effect of a 10-d. period of interrupted milking on the per cent of casein, albumin and globulin proteins and the per cent non-protein nitrogen in milk.

to that in preinterruption milk of the treated group, the difference was significant ($P < 0.001$); also, it was less than that in the control cows' milk ($P < 0.01$).

Postinterruption milk was found to contain larger ($P < 0.001$) percentages of globulin (fig. 1) than the preinterruption milk contained. No statistical differences were found between the total globulin in the preinterruption milk, in the postinterruption milk and in the control cows' milk. Of interest is that the the first milking after interruption of the treated group contained, on the average, one and one-half times as much globulin as the control group; however, this difference was not statistically significant. The per cent of the total nitrogen as globulin (fig. 2) was higher in the post- than in the preinterruption milk ($P < 0.001$) and than in the milk from the control cows ($P < 0.01$).

The per cent non-protein nitrogen (fig. 1) in postinterruption milk was greater ($P < 0.05$) than in the preinterruption milk and also greater ($P < 0.001$) than in the milk from the control cows. The treated cows' milk contained more ($P < 0.01$) non-protein nitrogen after interruption of milking for 10 days than before. When the per cent of the total nitrogen as non-protein nitrogen (fig. 2)

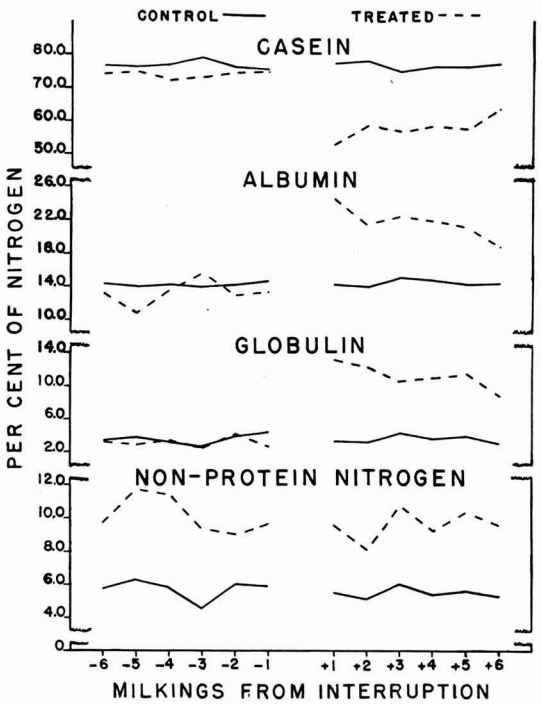

FIG. 2. The effect of a 10-d. period of interrupted milking on the per cent nitrogen distribution as casein, albumin and globulin and non-protein nitrogen in milk.

was calculated, no statistically significant differences between preinterruption, postinterruption and control cows' milk were found.

DISCUSSION

After a 10-day interruption of milking, determination of the protein fractions of milk indicates that proteins are resorbed from the udder and that there are changes in the protein components. The data are essentially in agreement with those of Porcher (12, 13, 14). Whether degradation of casein results in the formation of albumin- and globulin-like substances as suggested by Porcher or whether these substances pass into the udder during interruption of milking awaits further experimentation with more precise measurements. Further, the increase in leucocytes in suspended milk (1, 5, 12, 13, 14) complicates the data, since leucocytes contain nitrogenous substances. Which protein fraction(s) upon precipitation contained the leucocytes has not been determined.

The lack of agreement between our data and Laxa's (7) and between Porcher's (12, 13, 14) and Laxa's might be explained by the difference in treatment of the cows. Our data and Porcher's were from cows handled normally, except that milking was interrupted, whereas, Laxa's interrupted milking period was while the cows were being transported from Russia to Prague over a 3-day period and, in addition, no preinterruption milking samples were taken.

It would seem advantageous in future studies on interrupted milking to determine whether the globulin fraction contains antibodies. If so, it should be possible to provide a product with increased antibody content that could be fed to newly born calves when colostrum is not available.

SUMMARY

The effect of interruption of milking for a period of 10 days on the casein, globulin, albumin and non-protein nitrogen content of milk has been studied in seven cows. The per cent casein was found not to change, while the per cents of globulin, albumin and non-protein nitrogen of milk were significantly increased by interrupting milking. Total globulin was not altered, but the total amounts of casein, albumin and non-protein nitrogen in the postinterruption milk were significantly less than those in the milk obtained before interruption. The per cent of the total nitrogen as casein was decreased, the per cent of the total nitrogen as albumin and as globulin was increased and the per cent of the total nitrogen as non-protein nitrogen was unchanged by interruption of milking.

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USE AND INTERPRETATION OF MILK FLOW CURVES IN MEASURING VARIATIONS IN THE RESPONSE OF COWS TO MACHINE MILKING[1, 2]

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During recent years, increasing experimental attention has been directed toward problems involved in mechanical milking. In studying the response of cows to various machine milking conditions, the total yield, the speed of milking and the possible effects on the health of the udder have been factors of primary interest.

In general, the method used in measuring milk yields and speed of milking has been to suspend the milker unit from a spring balance and record the accumulated weights at various intervals throughout the milking operation. This method was used by Matthews *et al.* (5) and by Foot (3) to study the normal response of cows to machine milking. It was used by Petersen (7), Smith and Petersen (9, 10) and by Dodd and Foot (1) in studying milking rates under various experimental situations. Elting and LaMaster (2) used essentially the same technique in recording milk weights by 30-sec. intervals from a combine-type milker. Matthews *et al.* (6) investigated the milking rates of individual quarters by using a specially designed machine that directed the milk from each quarter into a separate container fitted with a glass gauge and a graduated scale calibrated in pounds of milk of average specific gravity.

In 1927, Gaines (4) reported the development of an instrument which produced a continuous graphic record of the weights of milk as it accumulated in the milking machine. No description was given nor have there been any subsequent reports observed in the literature pertaining to the use of this device. An ingenious electrical recording apparatus recently developed by Whittleston (11) gives a continuous graphic record of the entire milking operation. This method has the advantage, according to the author, of being almost entirely automatic. The recorder can be operated by ordinary milkers without the presence of a technician in the barn. Petersen (8) has used a continuous-feed kymograph to obtain graphic measurements of the milk flow during machine milking.

One of the difficulties in obtaining an accurate appraisal of milking speed is that of determining the end point of each milking. Foot (3) established the end point as the point at which the increment of milk yield fell below 0.3 lb. during two consecutive 20-sec. periods. A similar method was reported by Smith and

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[2] The data contained in this publication are a part of the data in a thesis presented by the senior author to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] Now at Mississippi State College.

Petersen (9). However, the latter investigators stated that such an end point does not represent the extent to which the udder has been evacuated but rather a decline in flow to a point where possible injury may result. In Whittleston's studies (12) the end point was determined by a sight glass arrangement. Machine stripping was started when the rate of flow dropped to 0.5 lb. per minute, and the teat cups were removed when the rate of flow once again dropped to this level.

Since the milk flow curve is being used as a measurement of the response of cows to various machine milking practices, the normal stability of this measurement is important. Matthews *et al.* (5) showed that the milking rate is influenced some by total milk yield, but that individual cows differ markedly regardless of yield. After plotting the cumulative milk weights for individual milkings, Foot (3) observed that each cow had a characteristic rate-of-flow curve, which, under normal conditions, did not vary greatly from day to day.

The objects of this investigation were threefold: first, to establish a satisfactory method of measuring the rate of milk flow throughout the milking operation; second, to determine the repeatability of the milking response when cows are milked under standard conditions; and third, to study some methods

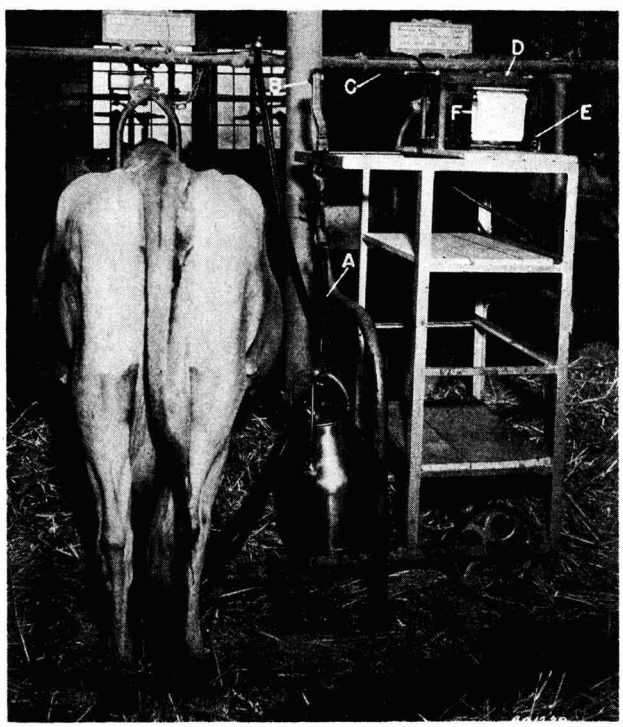

FIG. 1. Photograph of the milk flow recorder. *A*—Extension coil spring; *B*—Ball-bearing pulley; *C*—Copper wire connecting spring and recording assembly; *D*—Pen assembly; *E*—Counter weight; *F*—Continuous-feed kymograph.

of interpreting differences in the response of cows to machine milking as shown by milk flow curves.

DESCRIPTION OF MEASURING APPARATUS

A continuous-feed kymograph[4] was adapted in a manner somewhat similar to that employed by Petersen (8), to obtain graphic measurements of the rate of milk flow during the entire machine milking operation. The kymograph (*F*, fig. 1) was mounted on a cart at about the level of a cow's back. An extension coil spring[5] (*A*, fig. 1) with a stretch coefficient of 5.5 lb. per inch of elongation and an elastic limit of 10 in. was suspended from the edge of the platform on which the kymograph was mounted. The lower end of the spring was attached by means of a small copper wire (*C*, fig. 1) passing over a pulley (*B*, fig. 1) to a pen assembly (*D*, fig. 1) mounted over the kymograph. The pen assembly consisted of a sliding bar to which was attached a capillary pen.

During the milking operation, the milker pail was suspended from a hook in the lower end of the coil spring. The weight of the milk accumulating in the pail caused the spring to stretch, thus exerting a downward pull on the copper wire, which in turn pulled the pen assembly across the kymograph paper. Simultaneously, the kymograph, operating in low gear, rotated the kymograph paper at a rate of 2.12 cm. per minute. This produced a graphic record of the amount of milk per unit of time. After removal of the pail from the coil spring, the pen assembly was returned to its original position by the force from a counter weight (*E*, fig. 1).

Examples of the milk flow curves obtained by this apparatus are shown in

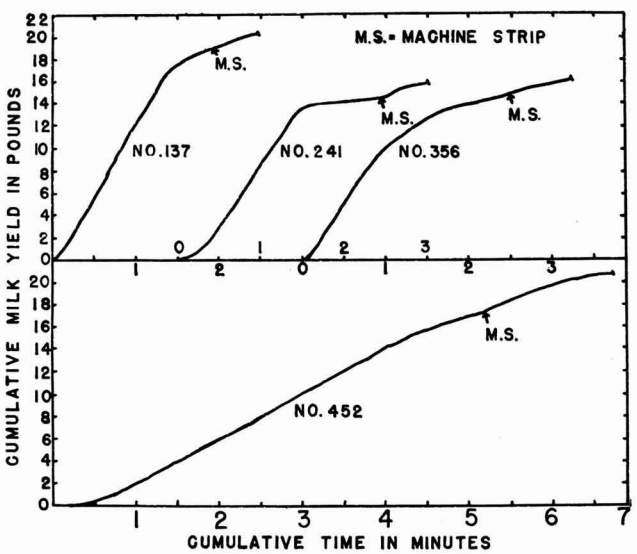

FIG. 2. Examples of milk flow curves obtained during the process of machine milking.

[4] Phipps and Bird, Richmond, Va.

[5] American Spring and Wire Specialty Company, Chicago, Ill.

figure 2. In order to obtain a reading conveniently and quickly at any point along the curve, a transparent scale that could be placed directly over the graphs was devised. For this purpose a piece of celluloid was marked with seconds on the horizontal axis and pounds on the vertical axis.

EXPERIMENTAL PROCEDURE

Repeatability of the milking response was studied in three different ways: (a) Day to day, for 3 consecutive days; (b) week to week, for 6 consecutive wk.; (c) lactation to lactation during corresponding stages of two successive lactation periods. Graphic milk flow recordings were obtained from 28 cows in the first study, 12 in the second and 25 in the third. These cows were selected at random from the College milking herd. Each group included cows of Ayrshire, Guernsey, Holstein and Jersey breeds, cows of different ages and cows in different stages of lactation.

Measurements were restricted to the evening milkings throughout this study. A standard procedure was followed in milking the cows. Milk let-down was stimulated by wiping the teats and floor of the udder for about 20 sec. with a heavy flannel cloth wrung out of chlorinated water at a temperature of 110 to 130° F. This was followed by stripping two or three full hand squeezes of milk from each quarter into a strip cup. A time interval of approximately 1 min. elapsed between stimulation and application of the teat cups. In order to reduce the possible disturbing influence that the presence of the measuring apparatus might have on the cow, milk let-down always was stimulated before rolling the cart alongside the cow.

A standard, upright pail-type milking machine was used. This machine operated with 15 in. of vacuum in the pipeline and 48 pulsations per minute.

The end point of milking was determined by the slope of the curve. Machine stripping was started when a definite decrease in the rate of flow was indicated by the curve flattening out into a plateau. The teat cups were removed when the milk flow curve once again formed a plateau. Hand stripping was practiced in all cases as a check on the completeness of machine milking.

Over a 3-yr. period from 1946 to 1949, routine graphic milk flow recordings were taken at three different stages of lactation from most cows in the College herd. In addition, various experiments dealing with methods of stimulating milk let-down were conducted, in which kymograph measurements were taken. Data have been drawn from these sources in studying methods of interpreting differences in the response of cows to machine milking.

RESULTS

In studying the milk flow characteristics of cows milked by machine, it is important to employ a method of measuring milk flow that is sensitive enough to reflect basic differences in the response of cows and yet simple enough that it does not upset the cow during milking. With the kymograph, as herein adapted, a continuous cumulative milk flow curve could be obtained which showed the rate of flow at any point during the milking operation. The majority

of cows were not visibly disturbed by the presence of this apparatus during milking. In a few instances some cows appeared nervous, and milk let-down was affected by the presence of the measuring apparatus. This condition was alleviated by stimulating milk let-down before placing the measuring apparatus next to the cow. After two or three milkings, most cows did not pay any attention to the measuring apparatus.

Differences in the response of cows to machine milking were reflected in the shape and length of the milk flow curve. A cursory examination indicated that each cow had a rather characteristic milk flow curve that changed little in appearance from day to day. To illustrate some of the different types of milk flow curves encountered, four examples are reproduced in figure 2. Three of these cows, 137, 241 and 356, were selected at random from the records of a group of relatively fast-milking cows. Despite the fact that the milking time was somewhat similar, the shape of the curve was quite different for each of these cows. The fourth curve, from cow 452, was selected at random from the records of a group of slow-milking cows. In the case of cow 452 the milk yield was about the same as that obtained from cow 137, but the time required to obtain that yield was nearly three times as long.

While such differences are easily seen when graphically presented, no tests of significance were known which could be applied to the shape of the curve. Thus, the problem existed of reducing the curve to numerical measurements so that statistical treatment might be applied. Numerical measurements selected should be representative of the important features of the milk flow curve. Furthermore, before using such measurements in testing differences between experimental situations, something should be known about the normal repeatability of such measurements observed under standard machine-milking conditions.

After a number of different segments of the curve were considered, five numerical measurements were selected as the most probable indices of differences in the response of cows to machine milking. A description of these measurements follows:

(a) Milk yield (*MY*). Obviously milk yield should be considered, but the question arose whether or not it should include hand strippings. In studying the response of cows to machine milking, it appeared logical to make comparisons on the basis of the milk yield obtained by machine as one item and hand strippings as another.

(b) Machine time (*MT*). It was recognized that measurements of machine time based on the judgment of the machine operator may be erratic. It is difficult, if not impossible, to determine with any degree of consistency, a precise end point of milking when relying entirely on the appearance and feel of the udder. Therefore, machine time, as determined in these investigations, represents a measured distance between two points on the milk flow curve. The first point marked the beginning of milking, or the exact time when the fourth teat cup was applied. The second point, marking the completion of milking, was that point on the curve where there was no further increment of milk. For the majority of cows this end point was clear cut, but in some cases, particularly among the slower

milking cows, there was a tendency to continue giving milk at a very slow rate as long as the machine was left on the udder. In these cases the end point of milking was marked at that point where the increment amounted to less than 0.4 lb. per minute.

(c) Per cent of the milk yield obtained during the first 2 min. of milking (*%2M*). This measurement was adopted because it is a simple objective measure of the speed of milk withdrawal that can be obtained in any barn without the use of a complicated measuring apparatus. Its usefulness depends, of course, on how representative it is of accurately determined machine time. This will be brought out later.

(d) Average rate of flow (*AR*). Average rate, expressed in pounds of milk per minute, was obtained by dividing machine yield by machine time. It is an expression of the speed of milk withdrawal and has been used rather commonly by other investigators. Because it is partially a product of machine time, its accuracy is dependent upon the accuracy of the machine time determination.

(e) Maximum rate of flow (*MR*). This measurement was obtained by measuring the angle formed between the horizontal axis and an intersecting line drawn parallel to the steepest slope of the curve. It is of interest because it is an important feature in determining the pitch of the milk flow curve.

The repeatability of the milk flow response by cows when milked under carefully controlled standardized milking conditions was remarkable. Coefficients of intraclass correlation were computed for *MT, %2M, AR* and *MR* from data obtained from 28 cows during three consecutive evening milkings. The correlations were *MT,* 0.893; *%2M,* 0.928; *AR,* 0.883; *MR,* 0.956. This shows that the milkings "within a cow" were quite similar, whereas cow-to-cow variations with respect to these measures differed greatly. A similar study with similar results was made with 12 cows, each of which was milked at weekly intervals during the 6-wk. period purposely beginning with the fifth week of lactation. The correlations were *MT,* 0.873; *%2M,* 0.895; *AR,* 0.800; *MR,* 0.890. Thus, it is clear that *MT, %2M, AR* and *MR,* as herein measured, were each highly repeatable during selected parts of the lactation period.

To study the repeatability of the milking response from one lactation to another, graphic milk flow records were obtained from 25 cows at corresponding stages of lactation during two successive lactation periods. The average yield per milking amounted to 15.6 and 17.2 lb., respectively, during the two lactation periods. The shapes of the milk flow curves from one lactation to the next were remarkably similar. The average machine time in the first lactation studied was 3.6 min., compared with 3.7 min. for the next lactation. The average rate of flow was slightly greater during the second lactation studied, amounting to 4.7 lb. per minute compared with 4.3 in the earlier lactation. Maximum rate of flow was the same for both lactations but was maintained for a longer period of time during the second lactation. Correlation coefficients were computed between the numerical measurements obtained from the same cow during the two lactation periods. The correlations were *MT,* 0.871; *%2M,* 0.893; *AR,* 0.895; *MR,* 0.945. Thus, it is apparent that the milking process was highly repeatable

from one lactation to the next among the animals studied. Furthermore, the repeatability of these measures shows that the milking response is a characteristic feature of the individual cow.

Since the numerical measurements under consideration were shown to be highly repeatable from day to day, from week to week during part of the lactation period, and even between successive lactation periods when the same stage of lactation was considered, it seemed of interest to determine the interrelationship of these measures. Because machine time is of greatest practical interest, it was decided to determine the relationship of the other measurements to machine time. For this purpose 61 milk flow curves were selected wherein the end point of milking was clearly defined, assuring a fairly accurate determination of machine time. No records were used where there had been more than 0.2 lb. of hand strippings. This group of records included 16 from the Ayrshire breed, 10 from Guernseys, 19 from Holsteins and 16 from Jerseys; measurements were taken at various stages of lactation, from the same cows in two different lactations and from cows that were subjected to various experimental treatments wherein the shape of the milk flow curve had been shown to be affected.

Four additional measurements, which may be found useful under certain experimental conditions, were included in this study. These were: 1. Starting time (*ST*). This term was used to designate the time interval between attachment of the machine and the beginning of the maximum rate of flow. Such a measurement is of interest in indicating whether milk let-down had occurred at the time the machine was applied. 2. Duration of maximum rate (*DMR*). The time during which milk flowed at the maximum rate. 3. Machine stripping yield (*MSY*). The yield of machine strippings in pounds. 4. Machine stripping time (*MST*). The time in seconds devoted to machine stripping.

TABLE 1

Simple linear correlation coefficients between machine time and other numerical measurements taken from the milk flow curves

Measurement correlated with machine time (*MT*)	Correlation coefficient 59 D/F[a]	Significance
Milk yield (*MY*)	0.132	n.s.[b]
Per cent of yield obtained in two min. (%2*M*)	0.922	***
Average rate of flow (*AR*)	0.598	***
Maximum rate of flow (*MR*)	0.566	***
Duration of maximum rate of flow (*DMR*)	0.444	***
Starting time (*ST*)	0.438	***
Machine strippings, yield (*MSY*)	0.240	n.s.
Machine strippings, time (*MST*)	0.376	***

[a] D/F—degrees of freedom.
[b] n.s.—non-significant.
*** —significant at the 0.1 per cent level.

As shown by results in table 1, all of the selected measurements were correlated significantly with machine time except the milk yield and the yield of machine strippings. The outstanding correlation was found between %2*M* and *MT*, the coefficient being 0.922. Because of this high correlation, the %2*M* is use-

ful as an objective measure of the speed of milk withdrawal. This measure might be especially useful in farm studies, where it is not practical to use special equipment in measuring the rate of flow. Such a measurement can be obtained simply by weighing the milk at 2 min. and again at the end of milking and then doing the necessary arithmetical computations.

It might be expected that the machine time would depend, to a large extent, on the average rate of milk flow throughout the milking process. The correlation coefficient of 0.598 obtained between *MT* and *AR* indicates, however, that only about 36 per cent of the variation encountered in machine time can be accounted for by differences in the average rate of flow. Thus, it is apparent that the average rate of flow is too gross a measure to be used in explaining variations in machine time.

An even smaller percentage of the variation in *MT* could be accounted for by the three maximum rate variables, *MR, ST* and *DMR,* when these measures were considered individually. When the three maximum rate variables were considered collectively, however, a much closer relationship to *MT* was found. The multiple correlation between *MT* and the three maximum rate variables, *MR, ST,* and *DMR,* was 0.936, which accounts for about 87 per cent of the variation in machine time. However, this correlation is little better than the simple correlation 0.922 between *MT* and *%2M*. Moreover, the multiple correlation between *%2M* and *MR, ST* and *DMR* was 0.956. This implies that the single variable, *%2M,* is virtually equivalent to the three maximum rate variables, as regards the relations of *MT*. It does not imply that there are no features of the milk flow curve which are not best measured by one or more of the maximum rate variables.

DISCUSSION

With proper adaptations, as herein described, the continuous feed kymograph can be used to obtain graphic measurements of the rate of milk flow throughout the milking process. Such measurements reflect basic differences in the response of cows to machine milking. Hence, this is a useful method in studying differences in the milking response that might be inherent in the cow or that might be created by experimental variations in methods of milking. In order to interpret statistically any differences found, numerical measurements which are representative of the important features of the milk flow curves are necessary. Several such measures are discussed in this paper and their interrelationship is shown. As an objective measurement of the time required to milk a cow, the per cent of the milk yield obtained during the first 2 min. of milking was found to be the most reliable single measure. It is suggested that this measure might be especially useful in farm studies, where it is not practical to use special equipment in measuring the rate of flow.

In agreement with the observations reported by Foot (3), it was found that each cow had a rather characteristic milk flow curve. Of particular interest is the fact that each cow not only responded in a characteristic fashion to machine milking, but that this response was highly repeatable, even to the extent of being quite similar between corresponding stages of two lactation periods, when stand-

ard milking methods were followed. Since the milking response of the cow seems to be a characteristic feature which is highly repeatable, the question arises as to the extent that the variations are caused by environmental conditions, and to what extent the variations represent inherited characteristics. Furthermore, since there was so little variation from day to day when standard milking methods were followed, the question arises as to what might happen if the milking methods were changed. These questions will be considered further in future publications from this station.

SUMMARY

A method is described for adapting the use of a continuous-feed kymograph to obtain graphic measurements of the rate of milk flow during the machine milking process.

Various numerical measures taken from the milk flow curves were highly repeatable, not only from day to day, but from week to week over a 6-wk. period, and from lactation to lactation where similar stages of lactation were compared.

Cows differ in their response to standardized methods of machine milking in a characteristic individual fashion, as shown by milk flow curves.

Numerical measurements of average rate of flow, maximum rate of flow, when maximum flow starts, how long maximum flow is sustained, per cent of the milk yield obtained during the first 2 min. of milking and time required to machine strip, selected from the milk flow curves for the purposes of statistically interpreting differences in the milking responses of cows, were found to be significantly correlated with machine time.

Time required to milk any cow depends primarily on these three variables—(a) maximum rate of flow, (b) when maximum flow starts and (c) how long maximum flow is sustained. The interrelationship of these three factors to the total machine time was shown by the highly significant multiple correlation coefficient of 0.936.

As a simple objective measurement of the time required to milk a cow, the per cent of the milk yield obtained during the first 2 min. of milking is highly reliable, having a simple correlation of 0.922 with machine time and a multiple correlation of 0.956 with the three maximum rate variables.

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THE EFFECT OF CERVICAL, UTERINE AND CORNUAL INSEMINATION ON FERTILITY OF THE DAIRY COW

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Since artificial insemination was first introduced in this country as a routine dairy cattle breeding technique, much research has been devoted to semen and to the male. Fewer planned and controlled investigations have been undertaken involving the insemination technique in the female.

The history of the development of the present most commonly employed technique of rectal fixation of the cervix and insemination into the cervix or beyond it into the body or the horns of the uterus is obscure. So far as the authors know, the technique was first demonstrated in this country by K. A. Larsen, a Danish veterinarian, who aided the staff at the New Jersey College of Agriculture at Rutgers University in the development of the first commercial artificial insemination venture with dairy cattle in the United States in 1938.

Prior to the use of that method, the vaginal speculum commonly was used to fix the *os cervix* and shallow insemination was made into that opening. Though Russian studies had shown earlier that 0.2 ml. of semen introduced into the cervix was as effective as 4.0 ml. injected into the vagina of the cows (8), no experimental evidence was available at that time on which to estimate the relative efficiencies of the two methods, but the Danish technique rapidly was accepted as being superior. Later observations supported this acceptance.

With a limited number of range cows, Lasley and Bogart (9) observed a higher efficiency of conception for those cows inseminated by the uterine method as compared to those inseminated into the cervix with the aid of a speculum. Raps (10) showed a weighted average difference of 6.7 per cent advantage in favor of deep cervical or intra-uterine insemination as compared to the speculum technique for first-service cows. A negligible difference was observed for second-service cows.

Holt (7) reported a 56.1 per cent conception for inseminations into the uterus as compared to 33.4 per cent for cervical inseminations with the aid of a speculum. However, one technician made the intra-uterine inseminations, while another made the intra-cervical inseminations, thus confounding the issue under test.

In the enthusiasm for the technique involving manipulation of the genital tract through the rectal wall, there has been a tendency to advocate deep insemination. Apparently this practice has developed as a result of the earlier observations which indicated that several hours were required by the spermatozoa to traverse the reproductive tract of the cow (3). Thus, the supposition was made that deposition of the semen as close as possible to the site of fertilization would conserve the energy of the spermatozoa and result in a higher proportion of

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conception. There seems to be no published experimental evidence to support this view; on the contrary, recent investigations by VanDemark and Moeller (14) have shown that spermatozoa may reach the infundibulum of the cow in heat 4 min. or less after insemination in the cervix.

An earlier criticism of intra-uterine insemination had to do with the possible production of intra-uterine trauma and with the breaking of the natural cervical barrier to entry of harmful bacteria into the estral uterus. This reasoning may yet prove valid in light of the recent researches of Tanabe and Casida (13) and the well-known fact that semen, even with careful handling, contains numerous bacteria (6), and when appropriate chemotherapeutic agents and antibiotics are added to diluted semen, improved conception results (1, 4, 12).

One objectionable feature of the speculum technique, *i.e.*, the frequent ballooning of the vagina (10), is avoided when the inseminating instrument is guided into and through the cervix by manipulation of the genital tract from the rectum. It is the purpose of this report to give the results of a series of experiments designed to determine whether or not intra-uterine inseminations result in higher conception rates than does insemination into the cervix, using in both cases the per rectum procedure.

EXPERIMENTAL

Preliminary experiments. To get experimental evidence on the proper point of deposition of semen when using the technique of manual fixation of the cervix through the wall of the rectum, two preliminary studies were made under the direction of the senior author in cooperation with the New York Artificial Breeders' Cooperative. The first, supervised by F. I. Elliott (5), involved two technicians each of whom made approximately the same number of inseminations at each of three sites of semen deposition, using 1.0 ml. of yolk-citrate diluted semen. In reaching the first site, a glass catheter of approximately 5 mm. diameter was passed through the cervix and then returned to the middle of the cervix for deposition. In the second procedure, 1.0 ml. of the diluted semen was deposited just inside the uterus, and in the third, approximately 0.5 ml. was deposited deep in each horn. The results were confounded by differing numbers of inseminations at each site and by the use of semen from different bulls.

TABLE 1

Site of insemination study by F. I. Elliott, fall, 1942 (5)

	Site of insemination		
	Cervix	Uterus	Horns
Services	103	150	104
5 mo. non-returns to service	64	97	65
% 5 mo. non-returns	62	65	62

The second study was designed to eliminate bias and confounding by using semen from the same bull on a single day for each of the three sites of insemination and by randomizing the order of sites from day to day of the experiment.

This study was supervised by G. W. Brandt, in cooperation with G. W. Trimberger and R. Albrectsen, all then of the Dairy Extension Staff at Cornell University. It involved 11 different technicians who made equal numbers of inseminations at each of three sites. The same three sites of insemination were used as in Elliott's study with the exception that some diluted semen was deposited in the cervix at each insemination. In the cervical site, the entire 1.0 ml. of yolk-citrate diluted semen was deposited in the approximate middle of the cervix. For the intra-uterine technique, about two-thirds of a milliliter, as indicated on the syringe, was deposited in the body of the uterus and one-third of a milliliter was deposited in the cervix as the catheter was being withdrawn. When inseminations were made into the horns of the uterus, about one-third of a milliliter was deposited in each horn and one-third was left in the cervix.

The procedures followed made this experiment laborious and consequently limited the number of inseminations. The results are summarized in table 2.

TABLE 2

Site of insemination study by G. W. Brandt, spring, 1946

	Site of insemination		
	Cervix	Uterus plus cervix	Horns plus cervix
Services	193	193	193
5 mo. non-returns to service	123	121	111
% 5 mo. non-returns	64	63	58

The differences noted were not statistically significant. However, the results of both of these studies did raise the question of the necessity for insemination beyond the cervix if results equally as effective could be obtained by the simplest of the three insemination techniques. Moreover, insemination into the cervix might prevent accidental abortions which are possible when deep uterine deposition is used on pregnant cows. Sometimes cows do show estrus in early pregnancy (2).

The Illinois experiment. To obtain more evidence on the question of insemination techniques, a cooperative study was undertaken with the Northern Illinois Dairy Cattle Breeding Co-op at Dundee. Eighteen different technicians cooperated in the study. The experiment was designed so that during 1 mo. each technician inseminated all cows in a particular site. The following month he inseminated all cows in a different site, and during the final month of the experiment, he inseminated in the third site. The order of insemination sites for the three single-month periods varied from one inseminator to another so that all possible combinations were obtained, and equal numbers of technicians were using each of the three different sites during each period.

The 18 technicians were divided into six groups of three each according to their experience and previous record as inseminators. Each group of three involved a separate sub-experiment designed as a Latin square. As each of the bulls in the stud was used nearly the same number of times in each of the three

periods and as each technician used semen from each bull for a proportionate number of breedings in each period, the possible confounding effect of the source of semen used was eliminated as an influence in the experiment.

The experiment was started on December 1, 1949, and extended through February 28, 1950. The semen was routinely diluted with the 2.9 citrate-sulfanilamide-yolk extender. The number of spermatozoa introduced with each 1.0-ml. insemination was 15 million or more, which is higher than the minimum levels established by Salisbury and Bratton (11). Even if spermatozoan numbers had been reduced considerably through use of the extender, this would not have been a confounding factor, since all semen samples were used for insemination in each of the three sites called for in the design. The three deposition sites were: (a) into the cervix just past the first cervical fold; (b) into the body of the uterus just posterior to the point of bifurcation; and (c) into each uterine horn midway between the bifurcation and the utero-tubal juncture. In this experiment all of the 1.0 ml. of diluted semen was deposited in the cervix or the uterus and approximately 0.5 ml. in each horn when that technique was used.

The experiment was limited to cows inseminated for the first time in the particular service period and to the use of semen from Holstein-Friesian bulls. The number of first-service cows inseminated by a single technician at a particular experimental site during 1 mo. ranged from 45 to 333. The average was approximately 122 first-service cows per month per technician. These inseminations, together with observations of the number of returns, constitute the data upon which statistical analyses were made. An analysis of variance of the percentage of the total number of first-service cows inseminated per technician per month which did not require rebreeding within a 60- to 90-day interval was used to test the statistical significance of the minor deviations from the mean results observed.

RESULTS

A total of 6,600 first-service cows were inseminated with semen from 16 Holstein-Friesian bulls by 18 technician-inseminators during the experimental period. The numbers of cows inseminated at each of the three designated sites of insemination were not widely different. More cows were bred in December and January than in February, reflecting the smaller number of days in the latter month as well as the slight trend in seasonal breeding. This fact, however, was not a confounding influence on the results of the experiment which are summarized in table 3.

An analysis of variance of these data further subdivided by technician-inseminators reveals that none of the differences shown in table 3 were of significance. In fact, the only significant deviation from the mean of 64.6 per cent 60- to 90-day non-returns was that associated with the several groups of three technicians each, which was part of the experimental design. The variances associated with the three sites of insemination and with the interaction of sites by groups of technician-inseminators were of the same magnitude, both being smaller than the error variance, indicating that one group of inseminators exhibited the

TABLE 3

Effect of site of semen depostion on the fertility of first-service cows

Month	Site of insemination											
	Cervix			Body of uterus			Horns of uterus			Total, all sites		
	Services	Non returns	% Non returns	Services	Non returns	% Non returns	Services	Non returns	% Non returns	Services	Non returns	% Non returns
Dec.	838	504	60.1	737	474	64.3	808	526	65.1	2,383	1,504	63.1
Jan.	684	443	64.8	851	577	67.8	761	485	63.7	2,296	1,505	65.5
Feb.	805	542	67.3	534	331	62.0	582	383	65.8	1,921	1,256	65.4
Total, all months	2,327	1,489	64.0	2,122	1,382	65.1	2,151	1,394	64.8	6,600	4,265	64.6

same general level of skill in getting cows with calf at one site of semen deposition as at another. The design did not permit a statistical test of whether or not a single technician consistently obtained superior conception results from deposition of the semen at one than at another site, although careful examination of the basic data of the investigation failed to reveal evidence of such a trend.

DISCUSSION

The data obtained in the three different experiments follow the same trend, indicating that no advantage in the average results was obtained for insemination beyond the mid-point of the cervix. These results are consistent with the important observations recently made by VanDemark and Moeller (14) that in the cow during estrus either motile or non-motile spermatozoa inseminated in the cervix reach the infundibulum in a few minutes.

The 60- to 90-day non-return results of these experiments do not indicate that inferior results on the average will be obtained from uterine or cornual insemination arising from uterine trauma or by introduction of the diluent or contaminants into the uterus. On the other hand, the experiments as conducted did not permit a critical examination of such possibilities as pregnancy progressed. However, the evidence presented here strongly suggests that no benefit is to be expected by insemination beyond the cervix.

Especially convincing supporting evidence for recommending cervical insemination only is the fact that slaughter experiments now in progress in this laboratory have produced visible uterine trauma by accepted techniques of uterine insemination. Furthermore, abortions or resorptions have occurred in pregnant cows deliberately inseminated in the uterus, while pregnancy was not disturbed in cows inseminated in the middle of the cervix.

SUMMARY

The results of three different experiments, one involving the insemination of 6,600 cows with semen from 16 Holstein-Friesian bulls for the first time during the service period showed that, on a non-return to heat basis of assaying conception, equally satisfactory fertility was obtained from deposition of the diluted semen into the cervix, the body of the uterus or the uterine horns, when the inseminating tube was guided into the reproductive tract by the rectal technique. These results are in accord with recent observations on the rate and mode of spermatozoa travel in the cow and argue against the advisability of intra-uterine insemination when equally effective results may be obtained by the simpler and safer technique of intra-cervical insemination.

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DIURNAL VARIATIONS IN CALCIUM, MAGNESIUM AND CITRIC ACID LEVELS OF THE BLOOD SERUM[1, 2]

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Before extensive work is attempted to study the variations of a specific blood constituent as related to disease, it is almost imperative that the normal daily variation and the diurnal variation of that constituent be established. This enables the investigator to determine whether variations observed during the course of an experiment reflect normal variations in that constituent or whether the changes have some pathological significance. In previous papers on parturient paresis (1, 2, 4, 5, 9, 10), the blood serum calcium, magnesium, and citric acid and blood plasma phosphorus levels have been studied at the time of parturition in normal cows and in cows subsequently developing parturient paresis.

Palmer and Eckles (7) have shown that significant daily fluctuations occur in the total blood serum calcium. The extreme variability in blood inorganic phosphorus is well recognized. Palmer *et al.* (6) found that the inorganic phosphorus in the blood varied markedly from day to day and from hour to hour. Their work also demonstrated an effect of exercise on blood phosphorus.

No information is available on the diurnal variation of calcium or on the daily and diurnal variation of magnesium and citric acid in the blood of dairy cattle. Studies are presented herewith which deal with these subjects.

EXPERIMENTAL PROCEDURE

In the trial set up to determine diurnal blood serum calcium variation, six Jersey cows in various stages of lactation were used, and samples of venous blood were taken from the jugular vein at 4-hr. intervals for three consecutive 24-hr. periods. All analyses were run in duplicate and averaged.

Four mature Jersey cows at varying stages of lactation were used to determine the daily and diurnal variation in blood serum magnesium and citric acid. In order to study normal daily variation in the serum magnesium and citric acid, the cows were bled daily for 9 days at 9:00 p.m. In order to study diurnal variations, cows were bled four times daily at 6-hr. intervals from the fourth to the seventh days inclusive (with the exception of one 3:00 a.m. bleeding). Times chosen for bleeding were 3:00 a.m., 9:00 a.m., 3:00 p.m. and 9:00 p.m.

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The 3:00 a.m. bleeding was made immediately preceding the morning milking, and the 9:00 p.m. bleeding immediately following the evening milking. The 9:00 a.m. and 3:00 p.m. bleedings were both in excess of 3 hr. following the morning and noon milkings, respectively.

The cows were bled in the stanchion and were not subjected to any undue exercise immediately before the samples were drawn. However, from 2 hr. to 0.5 hr. before the 9:00 a.m. bleeding, cows were getting some exercise in a dry lot. All blood samples were drawn from the external jugular vein.

Blood serum calcium was determined by the method of Clark and Collip (3), blood serum magnesium by the method of Simonsen *et al.* (8), and blood serum citric acid by the method of Taussky and Shorr[3] (12).

RESULTS AND DISCUSSION

Statistical analyses were made essentially as outlined by Snedecor (11). Results from the study on the diurnal variation in blood serum calcium are presented in table 1. An analysis of variance showed that there was no significant

TABLE 1

Total serum calcium for individual cows bled at 4-hr. intervals over three 24-hr. periods

Cow	24-hr. period	Total blood serum calcium (Mg. %) at:					
		6 p.m.	10 p.m.	2 a.m.	6 a.m.	10 a.m.	2 p.m.
705	1	9.9	9.7	9.6	9.4	9.3	9.2
	2	9.3	10.0	10.3	10.1	10.5	10.6
	3	10.8	10.7	10.2	9.5	10.7	10.7
720	1	10.5	10.4	10.6	10.6	10.1	10.2
	2	10.7	10.4	11.0	10.5	10.8	10.6
	3	10.8	10.4	11.0	10.5	10.2	10.8
696	1	9.7	10.6	10.3	10.1	9.9	10.4
	2	9.9	10.3	10.8	10.6	10.2	10.3
	3	10.5	10.1	10.3	9.4	9.4	9.4
732	1	10.6	11.0	10.6	10.5	10.8	10.9
	2	10.8	10.9	11.1	10.8	11.1	10.8
	3	10.5	10.8	10.1	10.5	10.7	10.9
B57	1	9.8	10.4	10.5	10.3	10.0	10.1
	2	10.4	10.6	11.0	10.5	10.4	10.5
	3	10.5	10.0	9.8	9.9	9.5	10.2
7-J	1	10.5	10.8	10.7	9.9	10.0	10.5
	2	10.3	10.7	10.7	9.9	10.6	10.4
	3	11.0	10.6	10.4	9.8	10.2	10.1
Av. for all cows		10.4	10.5	10.5	10.2	10.2	10.4

difference in the total blood serum calcium levels at different times of the day based on 4-hr. interval samples, nor was the difference between days significant. As one might expect, the difference in total serum calcium level between animals was highly significant. The interaction between animals and times indicates that all animals responded about the same at different times of bleeding. The

[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.

high significance of the interaction between animals and periods shows that all animals do not vary the same from day to day. Thus, while for one cow the lowest average blood serum calcium level may have occurred during the first 24-hr. period, for another cow the low point may have occurred on the second or third 24-hr. period. This analysis is based upon data taken over all animals.

From these results it is apparent that the time of day at which a blood sample is taken is not critical as regards the total calcium level of the blood serum, and, therefore, it is not necessary to bleed at the same time each day in order to obtain an accurate comparison of the total serum calcium level from one day to another.

Data obtained from the study on the daily and diurnal variation of magnesium in the blood serum are presented in table 2. An analysis of the data

TABLE 2

Daily and diurnal variations in citric acid and magnesium levels of the blood serum

Day	Time	Blood serum citric acid (*mg.* %) of cow no.				Blood serum magnesium (*mg.* %) of cow no.			
		2106	2116	2117	2118	2106	2116	2117	2118
3–25	9: 00 p.m.	3.55	5.50	4.34	5.02	2.23	2.43	2.60	2.41
3–26	9: 00 p.m.	4.13	5.46	4.38	5.29	2.15	2.30	2.33	2.30
3–27	9: 00 p.m.	3.95	5.26	4.38	5.02	2.39	2.23	2.47	2.30
3–28	9: 00 a.m.	1.92	4.09	3.42	4.45	1.83	2.27	2.39	2.46
	3: 00 p.m.	2.66	5.42	4.23	6.34	2.46	2.47	2.30	2.53
	9: 00 p.m.	3.34	4.99	4.55	5.22	2.39	2.42	(a)	(a)
3–29	3: 00 a.m.	3.16	5.46	4.75	5.30	2.47	2.54	2.48	2.45
	9: 00 a.m.	3.10	4.69	4.80	5.82	2.31	2.46	2.36	2.36
	3: 00 p.m.	3.21	4.97	4.79	4.46	2.41	2.19	2.30	(a)
	9: 00 p.m.	3.59	5.78	5.21	5.49	2.39	2.31	2.42	2.30
3–30	3: 00 a.m.	3.72	2.89	4.02	4.37	2.47	2.41	2.41	2.47
	9: 00 a.m.	3.85	5.29	4.59	5.49	2.36	2.22	2.30	2.30
	3: 00 p.m.	4.41	5.41	4.56	5.78	2.33	2.35	2.46	2.41
	9: 00 p.m.	3.68	4.82	3.91	5.09	2.23	2.31	2.48	2.38
3–31	9: 00 a.m.	4.39	3.36	4.02	5.09	2.10	2.30	2.23	2.28
	3. 00 p.m.	3.71	4.39	3.84	(a)	2.73	2.42	2.56	2.63
9	9: 00 p.m.	3.39	4.25	3.98	4.82	2.42	2.36	2.53	2.41
4–1	3: 00 a.m.	3.45	5.01	4.73	5.30	2.42	2.30	2.53	2.33
	9: 00 a.m.	4.12	5.01	4.73	5.05	1.90	2.21	2.38	2.41
	9: 00 p.m.	4.20	5.38	4.42	6.85	2.41	2.42	2.36	2.30
4–2	9: 00 p.m.	5.01	6.03	4.89	5.18	2.39	2.55	2.48	2.30
Av. for entire period		3.64	4.93	4.40	5.27	2.32	2.36	2.42	2.38

[a] Blood sample lost or broken in centrifuge.

shows that in 57 magnesium determinations made on 4 consecutive days at 3: 00 a.m., 9: 00 a.m., 3: 00 p.m. and 9: 00 p.m., the average was 2.38 mg. per cent. The average for sixteen 9: 00 a.m. bleedings was 2.28 mg. per cent as compared with 2.43, 2.38 and 2.44 for fifteen 3: 00 p.m., fourteen 9: 00 p.m. and twelve 3: 00 a.m. bleedings, respectively. The mean difference of the 9: 00 a.m. bleeding as compared with the total number of bleedings when checked by the method of Tukey (13) was significant. Thus, these studies show that while the variation is not great, a diurnal variation in serum magnesium does exist, with the lowest point at 9: 00 a.m.

The analysis of variance further showed that the variation in serum mag-

nesium levels between cows in this experiment was not statistically significant. A total of 81 samples analyzed for the various cows at the times indicated in table 1 averaged 2.37 mg. per cent (table 2). There was no significant daily variation over the 9-day period. The means for the 9:00 p.m. bleedings over this period were 2.42, 2.27, 2.35, 2.40, 2.36, 2.35, 2.43, 2.37 and 2.43 mg. per cent for days one through nine, respectively.

Table 2 shows the individual variation in citric acid levels of the blood serum during the 9-day period covered by this trial. Based on 83 analyses, the mean citric acid level for this trial was 4.55 mg. per cent. There was a highly significant variation between cows. The average citric acid levels at the 9:00 p.m. bleedings varied significantly for days one through nine: these averages were 4.60, 4.82, 4.65, 4.62, 5.02, 4.38, 4.11, 5.21 and 5.28 mg. per cent, respectively. There was no significant variation in blood serum levels of citric acid at 3:00 a.m., 9:00 a.m., 3:00 p.m. and 9:00 p.m.

As a result of this experiment, one can say that, while there was a definite variation between cows and a daily variation, there was no significant diurnal variation in blood serum citric acid.

SUMMARY

Six Jersey cows were bled six times daily at 4-hr. intervals to determine the diurnal variation in blood serum calcium. The variation between cows in serum calcium levels was significant, but daily and diurnal variations were not significant.

Four Jersey cows were bled daily for 9 days and four times daily at 6-hr. intervals for 4 days during days four, five, six and seven of the 9-day period and blood serum citric acid and magnesium were determined. No significant difference existed between cows or between days in serum magnesium levels. However, a small but significant diurnal variation existed, with the low level being observed at the 9:00 a.m. bleeding.

Serum citric acid levels showed a significant variation between days and a highly significant variation between cows, but no significant diurnal variation. Therefore, under the conditions of the experiment, the time of day at which samples were taken had no significant effect upon serum calcium and citric acid levels. The blood serum magnesium was lowered at one bleeding.

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A STUDY OF THE USE OF NORDIHYDROGUAIARETIC ACID IN DAIRY PRODUCTS. IV. ITS ANTIOXYGENIC PROPERTIES IN SPRAY-DRIED WHOLE MILK AND ICE CREAM MIX WITH AND WITHOUT ADDED SYNERGISTS

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The control of the development of off-flavors which are caused by the oxidative deterioration of milk fat and of the phospholipids is a serious problem in dried milk and dried milk products which contain appreciable amounts of these materials. The principal methods which have been proposed for the control of the development of these off-flavors include the use of high heat treatment (4, 5, 9, 11, 16), gas packing (2, 6, 8) and the use of antioxidants (3, 4, 7, 12, 13, 15, 16).

In this investigation, a study was made of the use of nordihydroguaiaretic acid (NDGA) in spray-dried whole milk and ice cream mix with and without added synergists.

EXPERIMENTAL METHODS

The milk used came from the University herd. It was processed in all-stainless equipment except the drying chamber which was made of galvanized iron.

After being condensed to approximately 40 per cent total solids, the milk was spray-dried at 1,000 lb. pressure using a no. 69 nozzle with a no. 20 core. A preheating temperature of 145° F. was selected in order to provide a heat treatment which in itself would not tend to retard the development of oxidized flavor. The air inlet was approximately 310° F. and the outlet was 190 to 200° F.

Preliminary work indicated that there was no significant difference in the antioxidant effectiveness of NDGA when added to the milk before condensing or when added to the condensed milk just prior to drying. It was added to the condensed milk in the preheating tank just prior to spray-drying, using a 15 per cent propylene glycol solution. The concentration of the antioxidant is expressed on the basis of the fat content.

The powder was packed in no. 1 cans immediately after drying. Powder in 150 ± 1-g. quantities was added to each container. The cans then were sealed, half of each lot was nitrogen-packed and all powder stored in a room where the temperature was automatically controlled at 72° F., except during the months when the temperature outdoors was higher than that indoors.

Changes in the oxygen concentration in the headspace were obtained by the manometric procedure of Van Slyke and Sendroy (14).

The flavor scores were determined on the reconstituted powder by two or more experienced judges. The American Dairy Science Association score card for milk was used. Any sample which had an oxidized flavor was assigned a score of 32.0 or below, depending on the relative intensity of this off-flavor.

[1] Now at the University of Arizona, Tucson.

Powder with a flavor score of 22.0 or below was considered unsalable.

A concentration of 0.1 per cent NDGA was selected as being the upper limit for economical use in powdered whole milk and as the amount that would not cause off-flavors in the product. Preliminary trials indicated that concentrations below 0.1 per cent were too low to be effective.

Citric acid was added at a concentration of 0.01 per cent from an aqueous solution, since it has been shown that it acts as a synergist with phenolic antioxidants (10).

RESULTS

The results in table 1 were obtained from powder manufactured from milk produced in August. The results show that 0.1 per cent NDGA will retard the development of oxidized flavor in powdered whole milk and the effect was relatively greater in the air-packed than in the nitrogen-packed samples. For example, the flavor score of the air-packed control sample was zero at the end of 6 mo., whereas the flavor score of the sample containing 0.1 per cent NDGA was 23.0 at this time and it did not have a flavor score of zero until the end of 12 mo. On the other hand, at the end of 12 mo. the flavor score of the nitrogen-packed control sample was 5.0 as compared to 9.0 for the sample containing 0.1 per cent NDGA.

Citric acid acted synergistically with NDGA. At the end of 12 mo., the flavor score of the air-packed sample containing NDGA and citric acid was 19.0, as compared to zero for the sample containing NDGA alone. In the nitrogen-packed samples, the flavor score of the powder containing both NDGA and citric acid was 25.0 at the end of 12 mo. as compared to a score of 9.0 in the powder containing only the antioxidant.

The per cent oxygen in the headspace decreased upon storage as the intensity of oxidized flavor increased. Thus, in the air-packed control sample, the per cent oxygen decreased from 19.78 at the end of 1 mo. to 0.32 at the end of 12 mo. The decrease in the per cent oxygen in the headspace of the powder containing 0.1 per cent NDGA was from 19.92 to 12.36 and for the powder containing NDGA and citric acid it was from 19.57 to 18.0.

The oxygen in the headspace of the nitrogen-packed samples decreased in the control, the powder with 0.1 per cent NDGA and that with NDGA and citric acid from 3.44, 2.68 and 4.47 per cent to 0.52, 2.10 and 2.62 per cent, respectively.

The synergistic action of methionine, ascorbic acid and citric acid in spray-dried whole milk. It recently has been shown by Clausen *et al.* (1) that ascorbic acid and methionine are among the most effective synergists in a large group of compounds and materials which they studied (not including the organic and inorganic acids). Since it had been shown in the foregoing portion of the study that citric acid acted synergistically with NDGA, citric acid was included in the materials which were studied as synergists in spray-dried whole milk.

The procedure followed in this portion of the study was the same as that which was used in the first part of the study. The synergists were added from an aqueous solution at concentrations of 0.1 per cent.

TABLE 1

The antioxidant effectiveness of NDGA in spray-dried whole milk

Sample no.	Treatment	Flavor score[a]							% Oxygen						
		1 mo.	2 mo.	4 mo.	6 mo.	8 mo.	10 mo.	12 mo.	1 mo.	2 mo.	4 mo.	6 mo.	8 mo.	10 mo.	12 mo.
							Air-packed								
1	Control	22.0[b, c]	19.0	11.0	0	0	0	0	19.78	16.53	13.20	11.19	3.40	1.98	0.32
2	0.1% NDGA	34.5	30.0	27.0	23.0	17.0	9.0	0	19.92	19.56	18.90	17.57	17.36	14.95	12.36
3	0.1% NDGA + 0.01% citric acid	36.0	36.0	32.5	28.0	27.0	22.5	19.0	19.57	19.02	19.16	18.85	18.96	18.75	18.00
							Nitrogen-packed								
4	Control	23.0	22.0	17.0	15.0	11.0	9.0	5.0	3.44	3.02	1.79	1.51	0.89	0.91	0.52
5	0.1% NDGA	35.0	31.0	29.0	23.0	19.0	14.0	9.0	2.43	2.68	2.69	2.95	2.76	2.25	2.10
6	0.1% NDGA + 0.01% citric acid	39.5	39.5	36.0	33.0	33.0	29.0	25.0	4.47	4.10	3.92	3.31	2.88	3.01	2.62

[a] Flavor score of fresh powder—40.0.

[b] Powder with oxidized flavor was given a score of 32.0 or below.

[c] Powder with a flavor score of 22.0 or below would be considered unsalable.

The results in table 2 were obtained with powder manufactured from milk produced in December. The results show that citric acid tended to be the most effective synergist of three that were studied. Oxidized flavor did not develop as rapidly in the control sample of this powder as it did in that represented in table 1, due possibly to the higher room temperature at the beginning of the storage period of the powder in the first part of the study.

At the end of 8 mo., the air-packed control sample had a flavor score of 9.0, the sample containing NDGA had a score of 19.0 and those containing the antioxidant plus the synergists methionine, ascorbic acid and citric acid had scores of 22.0, 22.0 and 26.0, respectively.

In addition to oxidized flavor, the powder containing methionine and ascorbic acid had developed objectionable fruity flavors at this stage of the study. These flavors were not taken into consideration, however, in arriving at the flavor scores since the primary objective of the study was to observe the rate of development of oxidized flavor.

At the end of 8 mo., the nitrogen-packed control sample had a flavor score of 19.0, the sample containing NDGA had a score of 23.0 and those containing the antioxidant plus the synergists methionine, ascorbic acid and citric acid had scores of 24.0, 23.0 and 29.0, respectively. The nitrogen-packed sample containing methionine had developed a fruity flavor but that containing ascorbic acid had not.

The per cent oxygen in the headspace of the air-packed control sample decreased from 20.03 at the end of 1 mo. to 15.23 at the end of 8 mo. The per cent oxygen in the headspace of the air-packed powder containing NDGA decreased from 19.98 at the end of 1 mo. to 16.76 at the end of 8 mo. The per cent oxygen in the headspace of the air-packed powder containing the antioxidant and the synergists methionine, ascorbic acid and citric acid decreased from 20.44, 19.69 and 20.03 to 17.03, 18.93 and 19.67, respectively. However, the oxygen values for the first 3 mo. of storage for the powder containing only NDGA, those for the first 4 mo. for NDGA plus methionine, those for the entire storage period for NDGA plus ascorbic acid and those for NDGA plus citric acid show no significant differences, but the flavor scores for the corresponding periods decreased considerably.

The per cent oxygen in the headspace of the nitrogen-packed control sample decreased from 3.04 at the end of 1 mo. to 1.86 at the end of 8 mo. The per cent oxygen in the headspace of the nitrogen-packed powder containing NDGA decreased from 3.75 at the end of 1 mo. to 2.07 at the end of 8 mo. The per cent oxygen in the headspace of the nitrogen-packed powder containing the antioxidant and the synergists methionine, ascorbic acid and citric acid decreased from 3.32, 3.44 and 3.33 to 2.68, 2.10 and 2.13, respectively.

The antioxygenic action of nordihydroguaiaretic acid in spray-dried ice cream mix. Powdered ice cream mix has been received favorably in areas where there is shortage of fresh dairy products, but the development of oxidized flavor in this product decreases consumer acceptability.

TABLE 2

The antioxidant effectiveness of NDGA in spray-dried whole milk with the synergists methionine, ascorbic acid and citric acid

Sample no.	Treatment	Flavor score[a]						% Oxygen					
		1 mo.	2 mo.	3 mo.	4 mo.	6 mo.	8 mo.	1 mo.	2 mo.	3 mo.	4 mo.	6 mo.	8 mo.
						Air-packed							
1	Control	29.0[b]	23.0	22.0[c]	21.0	17.0	9.0	20.03	19.98	19.78	18.53	16.84	15.23
2	0.1% NDGA	34.0	33.0	33.0	29.0	23.0	19.0	19.98	19.89	20.23	.....	18.99	16.76
3	0.1% NDGA 0.1% methionine	35.0[d]	34.0	33.5	30.0	26.0	23.0	20.44	19.91	20.35	19.30	17.27	17.03
4	0.1% NDGA 0.1% ascorbic acid	35.0[d]	34.5	33.5	30.0	26.0	23.5	19.69	.....	19.77	19.21	19.21	18.93
5	0.1% NDGA 0.1% citric acid	37.0	35.0	34.5	32.0	28.0	26.0	20.03	19.89	20.00	19.72	20.69	19.67
						Nitrogen-packed							
6	Control	35.0	34.0	33.0	28.0	23.0	19.0	3.04	2.45	2.62	2.02	2.03	1.86
7	0.1% NDGA	37.0	36.0	34.0	33.0	29.0	23.0	3.75	3.85	3.88	2.48	2.14	2.07
8	0.1% NDGA 0.1% methionine	37.5[d]	35.0	34.0	33.0	30.0	25.0	3.32	2.66	2.79	2.82	2.91	2.68
9	0.1% NDGA 0.1% ascorbic acid	37.0	35.0	34.0	33.0	30.0	26.0	3.44	2.77	2.93	2.43	2.19	2.10
10	0.1% NDGA 0.1% citric acid	38.0	36.0	35.0	34.0	31.0	29.0	3.33	2.37	3.05	2.66	2.43	2.13

[a] Flavor score of fresh powder—39.0.
[b] Powder with oxidized flavor was given a score of 32.0 or below.
[c] Powder with a flavor score of 22.0 or below would be considered unsalable.
[d] Fruity flavor present throughout the entire storage period.

TABLE 3

The antioxidant effectiveness of NDGA in spray-dried ice cream mix

Sample no.	Treatment	Flavor score[a]						% Oxygen					
		1 mo.	2 mo.	4 mo.	6 mo.	9 mo.	12 mo.	1 mo.	2 mo.	4 mo.	6 mo.	9 mo.	12 mo.
						Air-packed							
1	Control	29.0[b]	27.0	23.0[c]	0	0	0	18.46	19.47	17.28	12.49	3.66	2.48
2	0.04% NDGA	34.0	33.0	32.5	31.5	31.5	31.0	20.61	20.05	20.44	20.37	18.19	17.13
						Nitrogen-packed							
3	Control	31.0	29.0	27.0	27.0	23.0	19.0	2.09	2.52	2.08	1.05	0	0
4	0.04% NDGA	34.5	34.0	33.5	32.0	32.0	32.0	3.09	3.43	3.17	3.09	1.67	1.63

[a] Flavor score of fresh powder—41.
[b] Powder with oxidized flavor was given a flavor score of 32.0 or below.
[c] Powder with a flavor score of 22.0 or below would be considered unsalable.

In this investigation, a study was made of the use of nordihydroguaiaretic acid (NDGA) in controlling the development of oxidized flavor in spray-dried ice cream mix. The procedure followed was, in general, the same as that which was used in the preceding portions of this study. A mix was made containing 13.39 per cent fat, 5.60 per cent sugar, 12.20 per cent milk solids-not-fat and 0.22 per cent stabilizer. The remainder of the sugar needed in a mix such as this is incorporated upon reconstitution. The mix was made from condensed skimmilk, skimmilk, fresh sweet cream, sugar stabilizer. The cream was separated from milk produced in December. No flavoring was added since Pyenson and and Tracy (12) have shown that some material in vanilla extracts has antioxygenic properties. The mix was pasteurized at 150° F. for 30 min. and homogenized at a pressure of 2,700 lb. on the first stage and 500 lb. on the second stage. It was cooled to approximately 40° F., held over night and spray-dried the next morning.

The NDGA was added at the preheating vat just before drying. A concentration of 0.04 per cent was selected as being the maximum since higher concentration would give an off-flavor to the reconstituted mix containing approximately 12.0 per cent fat.

The results (table 3) show that 0.04 per cent NDGA will retard the development of oxidized flavor in spray-dried ice cream mix during storage at room temperature for 12 mo.

NDGA gave relatively greater antioxygenic protection in the air-packed powder than in the nitrogen-packed powder. The flavor score of the air-packed control sample was zero at the end of 12 mo., whereas the flavor score of the sample containing NDGA was 31.0 at the end of 12 mo.

The nitrogen-packed control sample had a flavor score of 19.0 at the end of 12 mo. and the sample containing NDGA had a flavor score of 32.0 at the end of 12 mo.

The per cent oxygen in the headspace decreased upon storage as the intensity of oxidized flavor increased. In the air-packed control sample, the per cent oxygen decreased from 18.46 at the end of 1 mo. to 2.48 at the end of 12 mo. The decrease in the per cent oxygen in the headspace of the air-packed powder containing NDGA was from 20.61 per cent at the end of 1 mo. to 17.13 per cent at the end of 12 mo.

The oxygen in the headspace of the nitrogen-packed control powder decreased from 2.09 per cent at the end of 1 mo. to zero at the end of 12 mo. The per cent oxygen in the headspace of the nitrogen-packed powder containing NDGA decreased from 3.09 at the end of 1 mo. to 1.63 at the end of 12 mo.

CONCLUSIONS

A concentration of 0.1 per cent nordihydroguaiaretic acid will retard the development of oxidized flavor in spray-dried whole milk during 12-mo. storage at room temperature.

The retardation of oxidized flavor by nordihydroguaiaretic acid was relatively greater in the air-packed than it was in the nitrogen-packed powder.

Methionine, ascorbic acid and citric acid acted synergistically with nordihydroguaiaretic acid in spray-dried whole milk.

There was an indication that citric acid was the most effective synergist of the three studied.

A concentration of 0.04 per cent nordihydroguaiaretic acid will retard the development of oxidized flavor in spray-dried ice cream mix during storage at room temperature for 12 mo.

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The University of Tennessee is one of the few land-grant colleges located in a large city. On its campus is the new million-dollar layout of dairy buildings, McCord Hall and the Dairy Products Building, dedicated a year ago.

It is quite fitting that the American Dairy Science Association hold its meeting in the southland where the dairy industry has a leading part in the agricultural readjustment program of this section. We are confident that your Association will bring to us much encouragement, information, and inspiration. We trust that this meeting will provide an opportunity for the exchange of the latest scientific information in dairy farming, in breeding of dairy cattle, in the manufacture of dairy products, and in human health and nutrition and related fields.

In addition to the serious part of the program, we trust that you will have a fine opportunity to associate with each other as friends, in making new acquaintances and in having a very happy time together. June is a beautiful month in Tennessee. The Smoky Mountains National Park will be attracting visitors from all over the world. Oak Ridge is nearby. The recreational facilities of the Tennessee River Valley will welcome you.

It is my desire to supplement the invitation which has been extended to you by the Dairy Department and, also, invite you to meet on the Campus of the University of Tennessee. I assure you that the University will do everything possible to make the visit of the members of the American Dairy Science Association on our Campus a pleasant experience.

Sincerely yours,
C. E. BREHM, *President*
University of Tennessee

PAPERS FOR THE 1951 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

The annual meeting of the American Dairy Science Association will be held June 5 to 7, 1951, at the University of Tennessee, Knoxville, Tennessee. 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. Sending the titles and abstracts in promptly is imperative since the abstracts must be made available in printed form for the annual meeting which through necessity is scheduled earlier than usual this year. 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 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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CONTENTS

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ABSTRACTS OF LITERATURE

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International Association of Ice Cream Manufacturers
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BOOK REVIEWS

1. Homogenized milk. G. M. TROUT. Mich. State College Press, East Lansing. 233 pp. $3.75. 1950.

This volume is subtitled a "review and guide." The style is such as to appeal to the milk dealer producing homogenized milk, as well as those more interested in the scientific angles of the subject. A chapter on the effects of homogenization upon the physical properties of milk is followed by another dealing with changes produced specifically in the fat and casein. The milk dealer will be especially interested in the chapter dealing with problems in homogenized milk processing. The laboratory technician will find in the chapter on laboratory control the answer to his questions. The consumer is not overlooked, for the book closes with a discussion of those qualities of homogenized milk which have been responsible for the present popularity of this product. The volume contains 34 illustrations and 13 tables. The citations include 451 references to literature in English and several foreign languages.

The author has been actively interested in homogenized milk throughout its commercial development in this country and is well qualified to prepare this treatise on a product of rapidly increasing popularity. E. F. Goss

2. Annual Review of Microbiology, vol. IV. C. E. CLIFTON, editor. Annual Reviews, Inc., Stanford, Calif. 383 pp. $6.00. 1950.

The chapters included in the new volume are: Electron microscopy of microorganisms and viruses, J. Hillier; Bacteriophages, T. F. Anderson; Constituents of mycobacteria, F. B. Siebert; Mutualisms in protozoa, R E. Hungate; Bacterial metabolism, L. O. Krampitz; Newer antibiotics, W. E. Herrell; Genetics of microorganisms, E. L. Tatum and D. D. Perkins; Genetics of viruses, F. B. Gordon; Current trends of experimental research on the aquatic phycomycetes, R. Emerson; Development of bacterial resistance to chemotherapeutic agents, C. P. Miller and M. Bohnhoff; Chemitherapy of virus and rickettsial infections, M. D. Eaton; Antibiosis in relation to plant diseases, R. Weindling; Immunological reactions in viral diseases, H. Koprowski; Immunology of the human mycoses, A. M. Kligman and E. D. DeLamater; Tularemia, L. Foshay; Brucellosis, M. R. Castaneda; and Influence of nutrition in experimental infection, P. F. Clark.

Many of the reviews will be of value to those whose interests are not primarily microbiological but who are applying in related areas either microbiolgy or technics developed in greater or less degree on microbiological problems. In a number of the chapters evidence of a more critical approach on the part of the reviewers adds to the value of the material. F. E. Nelson

BUTTER

O. F. HUNZIKER, SECTION EDITOR

3. Saltindholdets indflydelse på smørrets holdbarhed ved kølehuslagring. (The influence due to the salt content of butter on its keeping quality during storage.) State Expt. Sta. Creamery, Hillerød, Denmark. Report no. 61, 1949.

In 1944–45, the butter contained up to 1% salt; in 1948, the butter contained up to 2.5 % salt. Unwashed butter was used. The butter was free from leakiness. Butter was examined for quality after about 14 d. storage at 55.4°F. and after about 3 and 6 mo. storage at 10°F. Quality was determined partly by chemical and bacteriological determinations.

The higher the percentage of salt, and the longer the butter was in cold storage, the greater was the tendency to the development of oily flavor. There appeared to be a greater tendency to oiliness in butter in the 1944–45 tests than in the 1948 tests. It was suggested that during these periods of time there could have been a difference in fatty acids and natural content of antioxidants in the milk, due to feedstuffs.

The changes in acidity and pH values were insignificant. The peroxide number, which was 0 immediately after the butter was made, appeared to increase most rapidly in the salted samples. There was a good relation between the peroxide number and oiliness. When butter had been stored at 55.4°F. for 2 wk. there was a considerable increase in bacterial content. During cold storage the count decreased, being in most cases less than it was on the day of manufacture. G. H. Wilster

4. Statens Smørbedømmelser 1949. (The Danish State Butter Gradings 1949). O. S. HANSEN, Ladelund, Denmark. Maelkeritidende, **63,** 23: 487–489. June, 1950.

A total of 5,739 samples of butter were graded during 1949. The right to use the official "Lur" brand was denied 18 creameries. Butter made in Jutland was superior to that made on the islands. The reasons for this were partly due to conditions under which the milk was produced, to withholding of the best milk for consumer trade and to the

feeding of sugar beet waste and other materials that could affect the flavor of milk. The average moisture content of all samples was 15.42%. The salt content averaged 0.81% which was comparable with that for each of 4 previous years (0.79, 0.83, 0.84, 0.81%). There seemed to be no significant relationship between the salt content and the quality of the butter. The unsalted butter had the highest quality average, in spite of poor moisture distribution, than the butter with a salt content of 0.7%. The butter with a salt content of 1.0–0.4% had the lowest quality average. Butter with 0.7–0.9% salt ranked almost as high in quality as the unsalted butter and it had been better worked. Butter with a salt content of 1.0 or higher was slightly lower in quality than butter with a salt content of 0.7–0.9%, in spite of better working and less catalase and mold. The least mold was evident in the butter samples with the highest salt content. Butter made during Dec., June and Sept. was lowest in quality and that made during Nov., Aug. and July was the highest. Crumbly and sticky were common defects in winter (23.1% of the Dec. samples were crumbly). Greasy and soft were summer defects (7.4% of June butter was weak bodied and 4.6% of Sept. butter was greasy). Sour and cheesy were the most common flavor defects. When the butter contained catalase, the defect was usually of bacterial origin and to prevent this, more thorough cleaning and disinfection, higher acidity of cream, better washing of butter granules and better working of butter were recommended.

If the butter was worked while too soft, it often became greasy before sufficient moisture droplets had been incorporated. A churning temperature during summer of 44.6–46.4°F. resulted in firmer butter. Crumbliness has nearly doubled in occurrence since 1946 and is 15 times as frequent now as in 1938. This is due largely to the feed of the cows, consisting partly of oil cake from which the soft oil has been removed. In cases of very firm and crumbly butter it is necessary to employ temperature control during the cream handling and butter making process.

G. H. Wilster

5. Method of producing aromatized butter by cooling rich cream and product thereof. A. L. Stigen (assignor to Aktiebolaget Separator Corp.). U. S. Patent 2,529,232. 6 claims. Nov. 7, 1950. Official Gaz. U. S. Pat. Office, **640,** 1: 277. 1950.

Cream containing a little more fat than butter is cooled and agitated to cause phase reversal. The resulting butter-like product is worked to distribute the moisture in droplets of small size. At a suitable time water-soluble flavoring materials are introduced with water as a carrier, into the mass and the product worked only to an extent to distribute the flavor-rich water in relatively large droplets in the finished product.

R.Whitaker

6. Continuous production of butter from sour cream. P. H. Staaff (assignor to Aktiebolaget Separator Corp.). U. S. Patent 2,526,292. 9 claims. Oct. 17, 1950. Official Gaz. U. S. Pat. Office, **639,** 3: 850. 1950.

Neutralized sour cream is passed through a special separator which continuously discharges sludge through ports in the outer wall and 80–85% fat cream from the inner portion of the bowl. The cream then is converted to butter.

R. Whitaker

7. Butter flavoring composition. J. W. Armstrong (assignor to Food Technology, Inc.). U. S. Patent 2,527,785. 5 claims. Oct. 31, 1950. Official Gaz. U. S. Pat. Office, **639,** 5: 1482. 1950.

Food products are given a butter-like flavor by incorporating from 1–6 ppm. of Na butyrate.

R. Whitaker

Also see abs. no. 27, 29.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

8. La pasteurization de lait dans la fabrication de fromages de lait de brebis et de chèvre. (Pasteurization of milk in the manufacture of cheese from sheep and goats' milk.) Lait, **30,** 298: 496–499. Sept.–Oct., 1950.

The need for pasteurization of milk for cheese making is discussed and recommended, particularly with regard to sheep and goat milks, which generally are produced under much poorer sanitary conditions than cow milk. Problems arising from pasteurization of cheese milk also are treated and certain recommendations are made concerning their solution. S. Patton

9. Forsøgsmejeriet meddeler: "Saltlagens Surhed" (pH). (Danish State Experimental Creamery reports: "Acidity of the Brine (pH)). Maelkeritidende, **63,** 19. 1950.

Among cheese manufacturers it is of constant concern that the rind of cheese often becomes oily and soft when it has been immersed in freshly-made brine Not until after the brine has been used several times for cheese, does the cheese placed in it develop a firm rind. The reason for this is believed to be due to the degree of acidity of the brine. The acidity of the brine, measured in terms of pH, has to correspond with the pH of the cheese and it should be about 5.2. Freshly-made brine would be about pH 7, and when fresh cheese is placed in it the brine acquires the same pH as the cheese. As a result, the cheese rind becomes soft and some is absorbed by the brine. The rind is of a slimy nature. To prevent slimy rind HCl was added to bring the brine to pH 5.2. The addition of lime for purification of the brine should be done in a separate tank so that the clear brine can be drained off and used again after bringing the pH to 5.2 as previously indicated. Acidity determination by titration can not be recommended because the brine consists of whey and salt, rather than of water and salt. The older the brine the more dissolved organic material is present; these act as a buffer during

titration, so that the determined acidity is higher than actual. G. H. Wilster

10. Cheese and cracker package. L. DRANGLE. U. S. Patent 2,527,919. 2 claims. Oct. 31, 1950. Official Gaz. U. S. Pat. Office, **639,** 5: 1515. 1950.

A thin disc of wrapped cheese is contained in 1 side of a stiff package and 1 or more circular crackers are inclosed in a similar compartment on the other side. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

11. Antibiotics in milk. F. J. DOAN, Dept. of Dairy Husbandry, The Pennsylvania State College. Milk Plant Monthly, **39,** 9: 28–30. Sept. 1950; Milk Dealer, **40,** 1: 102–106. Oct., 1950.

Partial inhibition of acid development results from the addition of 0.05 units of penicillin/ml. of milk. Thus, milk obtained from animals 24 hr. after treatment may contain troublesome amounts of penicillin. To date the only successful methods of neutralizing the effect of antibiotics have been dilution and the use of penicillinase. Since the latter is far too costly for use in the dairy industry, the only satisfactory method of control appears to be withholding milk from treated udders no less than 3 milkings following treatment. J. A. Meiser, Jr.

12. A resazurin starter activity test. H. LEBER. Dairymen's League Coop. Assoc., Inc. Syracuse, N. Y. Milk Plant Monthly, **39,** 9: 40, 42. Sept., 1950.

This test involves inoculation of 9 ml. of reconstituted skimmilk with 1 ml. of the culture to be tested. One ml. of a 0.005% resazurin then is added and the mixture incubated at 98°F. until the resazurin is reduced to a pale pink color. Excellent starters reduce resazurin in less than 35 min.; good starters in from 35–50 min.; and poor starters require more than 60 min. to reduce resazurin. J. A. Meiser, Jr.

13. Recherche dans le lait en nature, de certaines bactéries pathogènes pour l'homme. (Examination of raw milk for certain bacteria pathogenic to man). G. GUILLOT, A. NEVAT and G. THIEULIN. Lait, **30,** 298: 500–507. Sept.–Oct., 1950.

This is a second and final part of a report on detecting certain bacteria, pathogenic to man, in raw milk. Methods for isolating and identifying *S. pyogenes* and staphylococci are presented. S. Patton

14. The effect of fatty acids on the growth of strains of Lactobacillus bifidus. R. M. TOMARELLI, R. F. NORRIS, C. S. ROSE and P. GYORGY, Univ. of Penn., Philadelphia. J. Biol. Chem., **187,** 1: 197–204. Nov., 1950.

Four bifid and 1 straight-rod strains of *Lactobacillus bifidus* were isolated from stools of breast-fed infants for a study of microbial growth inhibition by fatty acids and of factors protective against such inhibition. Cow milk and human milk digested by pancreatin contained toxic factors, associated with the fatty acid fraction, to which the bifid strains were more sensitive than the straight-rod strain. The addition of untreated milk neutralized the toxic factors; dialyzed whey and bovine serum albumin exhibited protective activity; and fatty acid toxicity was reversed by surface-active compounds such as Tween 60, Tween 80 and lipositol.

Both types of *L. bifidus* were sensitive to the presence of lauric, myristic, capric and palmitic acids. Only the bifid strains were inhibited by oleic and linoleic acids if Na acetate buffered the medium. When Na citrate replaced the acetate in the medium, all strains of *L. bifidus* required a source of unsaturated fatty acid; however, free oleic acid inhibited the growth of all strains, but the addition of Tween 80 enabled growth to occur. H. J. Peppler

15. Orotic acid in the nutrition of a strain of Lactobacillus bulgaricus. O. P. WIELAND, JEAN AVENER, E. M. BOGGIANO, N. BOHONAS, B. L. HUTCHINGS and J. H. WILLIAMS, Lederle Lab. Div., Am. Cyanamid Co., Pearl River, N. Y. J. Biol. Chem., **186,** 2: 737–742. Oct., 1950.

Lactobacillus bulgaricus, strain 09, requires whey or related products to achieve maximal growth. Fractionation of whey concentrate led to the isolation of a biologically active crystalline compound ($C_5H_4O_4N_2$) identified as orotic acid. Assay of cells grown in media containing orotic acid failed to show the presence of the growth factor and indicates its transitory existence in the cell. Orotic acid may be converted to pyrimidine nucleosides or nucleotides, as in the rat, but isotopic studies in this direction have not been reported.

Orotic acid is essential to the growth of *L. bulgaricus* 09. Pyrimidines, purines or related compounds could not replace orotic acid. H. J. Peppler

16. The nature of the TJ factor for Lactobacillus lactis Dorner. D. HENDLIN, M. C. CASWELL, V. J. PETERS and T. R. WOOD, Merck & Co., Inc., Rahway, N. J. J. Biol. Chem., **186,** 2: 647–652. Oct., 1950.

Preparations of TJ, the growth factor for *Lactobacillus lactis* Dorner found in tomato juice, were evaluated for pyridoxal phosphate and pyridoxamine phosphate content with 3 species—*L. lactis, L. helveticus* and *L. acidophilus.* The TJ activity of liver paste and *Streptomyces griseus* concentrate was primarily due to pyridozamine phosphate or a pyridoxamine phosphate-like substance. Under the conditions of assay employed, 1 unit of TJ was found to be equivalent to approx. 2γ pyridoxamine phosphate. Quantitative conversion of pyridoxal phosphate to pyridoxamine phosphate is effected by autoclaving in the assay medium. H. J. Peppler

17. Antagonisms in the utilization of D-amino acids by lactic acid bacteria. III. Influence of DL-methionine sulfone and oxidized casein on the

utilization of D-methionine. M. N. CAMIEN and M. S. DUNN, Univ. of Cal., Los Angeles. J. Biol. Chem., **187,** 1: 365–368. Nov., 1950.

Oxidized casein hydrolysate and DL-methionine inhibited the growth (acid production) of *L. arabinosus* and reduced the relative activities of D-methionine, DL-methionine and DL-methionine sulfoxide. The same depression had been previously reported for DL-ethionine. It appears that D-methionine, DL-methionine and its sulfoxide may be converted enzymatically to L-methionine by *L. arabinosus,* but the presence of competitive inhibitors reduces the efficiency of such processes. H. J. Peppler

18. Amino acid derivatives in bacterial metabolism. I. Derivatives of leucine, phenylalanine, tryptophan and valine. C. H. EADES, JR., Univ. of Tenn., Memphis. J. Biol. Chem., **187,** 1: 147–152. Nov., 1950.

The extent to which certain amino acids are replaced by their derivatives and analogues in the growth of lactic acid bacteria was investigated with the synthetic medium of McMahan and Snell. Only 1 of the amino acid substitutes tested, Seitz-sterilized phenylpyruvic acid, supported the growth of *Lactobacillus mesenteroides* P-60.

The acetyl and chloroacetyl derivatives of DL-leucine replaced the amino acid only for *L. arabinosus* 17-5. *L. casei* 7469 utilized acetyl-DL- or chloroacetyl-DL-tryptopan, but the keto analogues of leucine and valine were only 50% as active as the respective L-amino acids for both *L. arabinosus* and *L. casei,* and none of the compounds was utilized by *L. mesenteroides.* None of the test organisms exhibited a growth response (acid production) in the presence of phenylalanine derivatives, the keto analogues of tryptophan, and the acetyldehydro derivatives of leucine, tryptophan and valine.

L. mesenteroides is believed to be more specific than either *L. arabinosus* or *L. casei* in the assay of leucine, tryptophan and valine. H. J. Peppler

19. Purification of coenzyme A from fermentation sources and its further partial identification. W. H. DE VRIES, W. M. GOVIER and J. S. EVANS, Upjohn Co. Research Lab., Kalamazoo, Mich.; J. D. GREGORY, G. D. NOVELLI, M. SOODAK and F. LIPMANN, Harvard Medical School, Boston, Mass. J. Am. Chem. Soc., **72,** 10: 4838. Oct., 1950.

The pantothenic acid derivative, coenzyme A, a co-factor in enzymatic acetyl transfer reactions, has been prepared from fermentation residues of *Streptomyces fradiae* by repeated acid adsorption on charcoal and elution with alkaline acetone-water and further purified on charcoal columns. Analytical data reveals the presence of 15.4% pantothenic acid, 24.4% adenosine, 6.7% P, 4.2% S, 10.8% cystine equivalent, as well as adenine, ribose, glutamic acid and reducing sugar (after acid hydrolysis). The chemical identity of the S fragment, although resembling cystine, remains obscure; however, it is a part of the coenzyme molecule. Chromatographic comparison of the growth factor for *Lactobacillus bulgaricus* (LBF) and the pantothenic acid-S compound suggests that LBF may be, or at least contains, the S fragment of coenzyme A.

H. J. Peppler

20. Molkenverhefung nach dem Waldhof-Verfahren. (Production of yeast from whey by the Waldhof method). English summary. G. DEMMLER. Die Milchwissenschaft, 5, 1: 11–17. Jan., 1950.

Whey was used as growth medium for production of yeast at 32–35°C. with cultures of *Torula utilis* predominating. The pH was held between 3 and 4 by means of adding 3.5 l. of 25% ammonia water/1,000 l. of whey. Nitrogen was supplied in the form of $(NH_4)_2SO_4$ at the rate of 2.5-3 kg./1,000 l.

Although this method offered definite advantages over other methods of similar nature in the production of dry yeast, the low yield of 23–24 kg. of protein/1,000 l. of whey (yeast protein + whey protein), together with the competition against dried milk powder made the process economically unsatisfactory. I. Peters

21. Über den Gehalt der Milchzuckerhefen an Aneurin Lactoflavin und Nicotinsäure. (The thiamin, riboflavin and nicotinic acid content of lactose fermenting yeasts). English summary. J. Christophersen and G. Holzweissig. Die Milchwissenschaft, **5,** 1: 18–19. Jan., 1950.

Eleven tribes of lactose-fermenting yeasts were grown in sterilized deproteinated whey at pH 4.5 for 10 d. at 26°C. The average of 3 trials showed the following concentration of thiamin, riboflavin and nicotinic acid, respectively, in mg./100 g. of dry yeast cells: *Torulopsis kefyr* H70—8.7, 2.7, 6.6; *T.kefyr* H76—5.8, 2.9, 26.6; *T. kefyr* H212—5.9, 7.5, 20.6; *T. kefyr* H222—8.0, 14.8, 36.0; *Saccharomyces* sp. H71—12.4, 10.3, 16.5; *Sacch.* sp. H72—3.8, 7.8, 13.5; *Torulopsis sphaerica* H78—7.9, 7.9, 26.6; *T. sphaerica* H209—3.7, 2.5, 20.0; *T. sphaerica* H217—3.5, 2.4, 21.9; *T.* sp. H216—4.9, 3.4, 16.0; *Zygosaccharomyces lactis* H223—4.9, 3.5, 224.

I. Peters

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

22. The serological relationship of bovine whey albumin to serum albumin. E. J. COULSON and H. STEVENS, Allergen Research Div., U. S. D. A., Washington, D. C. J. Biol. Chem., **187,** 1: 355–363. Nov., 1950.

Crystalline albumin isolated from bovine whey (Polis *et al., ibid.,* **187:** 349–354) was compared serologically by the Schultz-Dale technique with crystalline preparations of serum albumin and β-lactoglobulin. The chief component of the milk albumin preparation was shown to be serologically identical with serum albumin; β-lactoglobulin was distinct from these components of serum and whey. The crystalline milk albumin was found to be contaminated with 0.015%

β-lactoglobulin and a small amount of a substance identifiable with some component of blood serum. H. J. Peppler

23. Isolation of a crystalline albumin from milk. B. D. Polis, H. W. Shmukler and J. H. Custer, Eastern Reg. Research Lab., Philadelphia. J. Biol. Chem., **187,** 1: 349–354. Nov., 1950.

Although present in trace amounts in bovine whey, a crystalline albumin was isolated by fractionation with $(NH_4)_2SO_4$ in the presence of borate and alcohol fractionation followed by crystallization with alkaline $(NH_4)_2SO_4$ in the presence of phosphate and caprylate ions. Electrophoretic comparison of crystalline albumin from milk with crystalline serum albumin established the identity of the 2 proteins. H. J. Peppler

24. Le colostrum et le lait dans leur rapports avec l'immunité du jeune. (Colostrum and milk in their relation to immunity in the young.) E. Lemetayer, L. Nicol, O. Girard, R. Coruazier and M. Cheyroux. Lait, **30,** 298: 474–496. Sept.–Oct., 1950.

The paper constitutes the second and final part of an extensive report on immune activity of blood, colostrum and milk in the mare and cow. S. Patton

25. Action of mineral ion exchange resins on milk constituents. C. W. Gehrke and E. F. Almy, Ohio State Univ., Columbus. Ind. Eng. Chem., **42,** 11: 2344–2347. Nov., 1950.

The action of Zeo-Karb-H (cation exchanger), De-Acidite and Amberlite IR-4B (anion exchangers) on simple solutions of the major anions found in milk and on solutions of lactose and nonprotein nitrogen was investigated. When 0.10 *N* solutions of HCl, H_3PO_4 and citric acid were passed through a column of De-Acidite, the order of adsorption was H_3PO_4 > citric > HCl. This order was changed to HCl, H_3PO_4 citric when binary or ternary mixtures totaling 0.10 *N* solutions of these acids were used. The order of adsorption was in the order of the magnitude of the ionization constant. The Amberlite adsorbed NaH_2PO_4 more effectively than it did Na_2HPO_4. A synthetic cheddar cheese whey was passed through beds of Zeo-Karb-H and De-Acidite in series to completely remove the Ca and Mg ions. Adsorption of the cations from the synthetic whey was in the order $Ca^{++} > Mg^{++} > K^{+} > Na^{+}$. The chloride and phosphate ions were removed and the chloride ion was preferentially adsorbed. Zeo-Karb-H completely adsorbed urea, creatine and creatinine but these substances were not adsorbed by De-Acidite Lactose in 5% solution was not removed from solutions of different pH values by the cation and anion exchangers. B. H. Webb

26. Estimation of amino acids and amines on paper chromatograms. R. J. Block, Dept. Biochemistry, New York Medical College, N. Y. 29. Analyt. Chem., **22,** 1327–1332. Oct., 1950.

Procedures are described in detail for the preparation of 1- and 2-dimensional paper chromatograms for the separation of all common α-amino acids and a number of nonvolatile amines. Preparation of standard solutions, a chromatograph chamber and paper for chromatograms are discussed. The quantity of color, as measured by photoelectric densitometer, that is developed by ninhydrin or other reagents in each spot on the chromatograms is proportional to the concentration of the substance at that spot. The standard color ratios of 4 concentrations of mixtures of amino acids on phenol-lutidine 2-dimensional chromatograms on Whatman no. 4 paper are given. The amino acids in casein have been determined on 2-dimensional chromatograms using less than 0.1 mg. of casein nitrogen.

B. H. Webb

Also see abs. no. 3.

DAIRY ENGINEERING

A. W. Farrall, Section Editor

27. Afprøvning af Titan Centrifuge, Type C 368 og Alfa-Laval Centrifuge, Type 171 M. Undersøgelser over Renkaerningens Afhaengighed af Renskumningen. (Experiments with a Titan cream separator, Type C 368 and an Alfa-Laval cream separator, Type 171 M. Experiments to determine how much churning efficiency depends upon skimming efficiency.) State Expt. Sta. Creamery, Hillerød, Denmark. Report no. 54, Nov., 1947.

Two pressure-type, foamless cream separators, each with a capacity of 3,000 l. of milk/hr., were tested. With the Titan separator, in the comparative tests on the same lots of milk, there was an average of 0.045% fat (Röse-Gottlieb) in the skimmilk, and in the 2-hr. tests, 0.044% fat. The cream showed air contents up to 6%. The skimmilk was completely free from foam. The power consumption was 3.1 kw. under normal conditions. With the Alfa-Laval separator, in the comparative tests, the skimmilk contained 0.044% fat and in the 2-hr. tests it also contained 0.044% fat. The cream and skimmilk were free from foam. Power consumption was 2.8 kw. Almost no fat globules exceeding 2 μ in diameter were present in the skimmilk from either separator. In order to determine how much churning efficiency depended upon skimming efficiency, 45 parallel skimming and churning tests were made. With an average fat content of 0.0465% in the skimmilk, when the skimming efficiency was high, the buttermilk from the different lots of cream churned contained an average of 0.466% fat. This was an efficiency churning no. of 1.063 (1.063% of the total fat churned lost in the buttermilk). With inefficient skimming, the average fat content of the skimmilk was 0.0617% and the buttermilk had a fat content of 0.415%, with an average churning efficiency no. of 0.986. When the fat loss in the skimmilk and buttermilk were totaled, there was a lose of 2.10% of the total fat in the milk when the skimming efficiency was high and 2.37% when the skimming efficiency was low. When the skimming efficiency was good, 76.5% of the additional fat that was ob-

tained during separation, as compared with the fat lost during poor skimming, was recovered during churning. G. H. Wilster

28. La pasteurization du lait par les radiations infra-rouges. (Pasteurization of milk by infra-red radiations.) J. KEILING, A. BARRETT and P. BIDAN. Lait, **30,** 298: 465–474. Sept.–Oct., 1950.

Equipment for pasteurizing milk or cream by infra-red radiations is described. Essentially, the processing involves heating the milk or cream as it passes in a thin film down the inside wall of a circular vat. Heating is effected by radiations from a number of infra-red lamps which are lowered into the center of the vat. After leaving the vat, the milk is cooled in a conventional manner. The unit described has a capacity of 1,000 l./hr.

Coli and total counts on milk and cream pasteurized by the equipment were satisfactory. Phosphatase tests were negative. A minimum temperature of approximately 80°C. (176°F.) and a maximum exposure of 6 sec. are indicated for processing. According to the authors, economic considerations and convenience of application are the limiting factors of this method of pasteurization. S. Patton

29. Continuous buttermaking. I. G. WIECHERS and B. DE GOEDE. Worth-Holland Pub. Co., Amsterdam. 103 pp. $3.50. 1950.

This volume contains a description of all the important methods for continuous buttermaking (Penn, Fritz, Alfa, Cherry-Burrell, Creamery Package and Kraft) as well as of some less known ones. The American methods are described mainly from letters patent, but the discussion of European processes is well documented with results of experiments, practical experience and theoretical considerations. The text is supported by a number of drawings showing the various systems in their entirety, as well as details of equipment.

Apart from the title page, only a few incorrectly applied words and phrases betray that this volume in the English language was written and published abroad. It is otherwise very readable and succeeds in summarzing much of the information to date on a subject which currently demands so much attention. V. H. Nielsen

30. Milk-sampling apparatus. N. C. KOTTKAMP, P. J. BAILEY, J. M. TRIMBLE and R. M. LANGSENKAMP (assignors to Langsenkamp-Wheeler Brass Works Co.). U. S. Patent 2,529-397. 12 claims. Nov. 7, 1950. Official Gaz. U. S. Pat. Office, **640,** 1: 320. 1950.

An electrically controlled vacuum-operated sampler for collecting a constant volume sample of milk from weigh tanks, etc. is described. R. Whitaker

31. Refrigerated trucks for retail milk delivery. Anonymous. Milk dealer, **39,** 12: 40–41, 59. Sept., 1950.

The refrigerated milk delivery trucks of Petan Dairy Farms, Santa Barbara, Cal., may be setting the pattern for future milk delivery equipment. Of a fleet of 15 refrigerated trucks, 12 are used for milk delivery, mostly retail. There are 3 types of vehicles: wholesale ice cream trucks, combination wholesale ice cream and wholesale milk, and house to house retail milk delivery trucks. Temperatures of 0 to −5°F. are maintained for ice cream and 35–45°F. for milk. Some of the advantages are: (a) A large percentage of milk goes direct from the filler to trucks, eliminating the rehandling of the milk from refrigerator to trucks. (b) The trucks are ready to leave the dock immediately after the driver-salesman checks in, since they are loaded the afternoon before. (c) There is no problem with the return milk. The temperature of this milk averages about 45–48°F. when the trucks come in and it stays on the truck for the next day's delivery. (d) When the drivers return, they park their trucks and check in as soon as they have made their reports to the office. The trucks are serviced on the dock. The loaders remove the empty cases and load the full cases. This saves from 40–60 min. of each driver-salesman's time. For refrigeration the trucks are equipped with a holdover-type plate. Inside the truck plate, but outside of the coil, is eutectic solution, *i.e.*, a solution which freezes at a known freezing point. Ammonia from the plant is circulated through the truck plates while the trucks are idle during the night. The temperature of the circulating ammonia approximates −25°F. and freezes the eutectic solution in the plates solidly. C. J. Babcock

32. 5 ways refrigeration operators can get stuck. G. HOLMAN. Operating Eng., **3,** 10: 42–43. Oct., 1950.

Equipment manufacturers, plant designers and installing contractors sometimes make mistakes which may harm new refrigeration systems before the operator takes over. Five common mistakes which are discussed briefly are: (a) equipment improperly designed—difficult to repair; (b) unsuitable materials used; (c) poor plant layout; (d) mismatched equipment; and (e) damage during installation. H. L. Mitten, Jr.

33. Refrigerant-drum hints. R. K. COLLIER. Operating Eng., **3,** 9: 36–37. Sept., 1950.

Refrigerant-drum hazards can be reduced by proper handling and storage operations. Hand trucks can be used to move drums weighing up to 220 lb. Above that weight the hand truck is inadequate. Ten drums can be moved on jack-type trucks.

Storage rooms for refrigerant-drums should be cool, well ventilated and free from open flames. Masks and emergency equipment should be kept outside entrance to storage. Drums stored upright against a wall should be chained in place. Empty drums should be kept separated from those containing refrigerant to avoid confusion.

Storage near charging connections minimizes

handling. Small drums may be placed on a scales when a system is being charged from it. Drum records should be kept.

H. L. Mitten, Jr.

34. Notwendigkeit der energiewirtschaftlichen Überprüfung der Molkereien. (Necessity for supervision of use of energy in dairy plants). English summary. K. A. ZIEGLER. Die Milchwissenschaft, **5,** 1: 6–11; **5,** 2: 44–51. Jan., Feb., 1950.

An attempt has been made to obtain the energy requirements, such as steam, electricity and water, of each operation in a dairy plant. The ease or difficulty with which such measurements can be made at each operation are pointed out, together with the possible savings in energy in each operation. Operations dicussed include can washer, plate pasteurizer, care of butter cultures, cheese vats, care of cheese starters, cheese-mat sterilizer, cheese hoop washing machine, condenser, general cleaning operations and steam heating in the plant. I. Peters

35. Low voltage is a killer. W. DAVIS, Natl. Safety Council. Operating Eng., **3,** 9: 46–47. Sept., 1950.

The human body acts as a conductor of variable resistance of electrical currents. The resistance it offers depends upon moisture, pressure at contact point and area of contact. 110 milliamperes with 110 volts can cause ventricular fibrillation—muscular contraction of the heart—which results in instant death.

Use 3-conductor cords on small portable tools or add a ground line. Keep insulation in good condition. Use a fuse puller to remove cartridge-type fuses. H. L. Mitten, Jr.

36. Why let metal waste away? D. E. CARROLL. Operating Eng., **3,** 9: 26–27. Sept., 1950.

Boiler-metal corrosion has as its usual sources dissolved O_2 and CO_2, acidic boiler water and electrolysis. Corrosion by O_2 is recognized by a brittle, black crust which covers pits. This type attack usually occurs at or below the water line Corrosion from CO_2 occurs most often in the steam space or return systems, thinning out the metal. A pH above 7.0 should be maintained if attack by acidic water is to be prevented. Where copper fittings and plugs are used, electrolysis may occur.

Deaerating feedwater prevents most of the corrosion by dissolved gases such as O_2 and CO_2. Na_2SO_3 can be added after deaeration to remove remaining traces of O_2.

Acid corrosion can be stopped by adjusting the pH of the boiler water to 10.5 with soda ash, caustic soda or phosphates.

Protective coatings of slaked lime, silicate of soda, cement wash or a special preparation can be used to prevent corrosion. Coatings should be thin and applied where they will not be exposed to radiant heat. Scale should be removed before these coatings are applied.

H. L. Mitten, Jr.

37. How big a safety valve? Anonymous. Operating Eng., **3,** 10: 32–33. Oct., 1950.

Safety valves installed on the low pressure side of steam pressure-reducing valves must be of greater size than the low side piping or must be installed in multiple units if safe relieving capacity is to be maintained. A chart shows the correct safety valve size to protect the low side in case of reducing valve failure. H. L. Mitten, Jr.

DAIRY PLANT MANAGEMENT AND ECONOMICS

L. C. THOMSEN, SECTION EDITOR

38. Effect of weigh can design on butterfat samples. V. SCHWARZKOPF, Lathrop-Paulson Co., Chicago, Ill. Milk Dealer, **40,** 1: 176–182. Oct., 1950.

Increase in the volume of milk produced per farm, the use of mechanical refrigeration and the installation of flat, shallow weigh cans in receiving rooms have made it more difficult to get representative samples for the butterfat test. The following suggestions will greatly minimize this problem: (a) Milk should be emptied into the weigh can in the downhill fashion to provide maximum mixing force. (b) Strainer in weigh can should have 3/16″ perforations and free from louvers. (c) Use oversize weigh can to provide added depth for better mixing. (d) Weigh can should be of the type to provide good mixing. Broad, flat, shallow weigh cans should be avoided. The oblong type weigh can with steep bottom pitch equipped with a blender has given excellent results quite consistently around the calendar in a large number of plants. (e) Mechanical agitation is a step in the right direction but it may provide false security unless ample time is given for mixing. A good mixing weigh can with proper dumping and sampling methods has been found to be more satisfactory than mechanical agitation. (f) Samples should be taken near the center of the weigh can well below the surface of the milk, to avoid churned fat in the samples. (g) Proper sampling technique and careful handling of samples should be practiced.

C. J. Babcock

39. How Arden saves $30,000 annual plant loss. Anonymous. Milk Dealer, **40,** 1: 48–49, 70–72. Oct., 1950.

The receiving-measurement problem has been solved by installing 3, 3,500-gal. rectangular storage tanks, using a liquid level gauge measurement system, controlled by a unique photoelectric eye mechanism. The liquid level gauge actually weighs the product and renders the weight in easy-to-read lb. graduations. The system operates with amazing simplicity, since it is based on the weight of a column of liquid of known specific gravity. C. J. Babcock

40. Your taxes are a year round operating problem. F. MERISH. Milk Plant Monthly, **39,** 10: 68–70, 72, 74. Oct., 1950.

A discusson of the mounting tax problem and how plants should revise methods of handling tax expense is presented. J. A. Meiser, Jr.

41. An accounting guide for milk-plant operators. F. MERISH. Milk Plant Monthly, **39,** 9: 43–44, 46. Sept., 1950.

The author presents a discussion of frequently misunderstood accounting terms.

J. A. Meiser, Jr.

42. Route break-even points for the small dairy. A. SEARLES, JR. Cornell's Dairy Prod., Endicott, N. Y. Milk Plant Monthly, **39,** 10: 54, 56, 58. Oct., 1950.

A simple method of determining the break-even point for delivery routes necessitates assigning 3 different expense accounts to the individual routes. The first assessment is the routeman's salary which also includes the cost of the relief man. The second is a division of manufacturing expenses and the final allocation is a division of general expenses. Once the above assessments have been made, it is a simple matter of addition and subtraction to obtain individual route's profit and loss figures. J. A. Meiser, Jr.

43. Year round incentives build sales. Anonymous. Milk Plant Monthly, **39,** 10: 102–104. Oct., 1950.

All milk and by-products sold are given a point rating. Operating from the previous month's base, each routeman is credited with additional points monthly which are accumulative but can be "cashed in" for merchandise prizes or cash whenever the participant so desires. Emphasis on the sale of certain products is obtained by increasing the point value of that particular product for a predetermined period.

J. A. Meiser, Jr.

44. How bargaining co-ops operate. D. E. HIRSCH, Farm Credit Admin., U.S.D.A. Milk Dealer, **40,** 1: 52–62. Oct., 1950.

A dairy co-op is an economic tool for self-help that the milk producer may use to increase the efficiency of the handling of milk produced on his farm and to improve the conditions under which he lives. A survey covering 150 associations showed that, in addition to the primary function of price negotiation or representation, most co-operatives perform one or more of a number of secondary services These are discussed in detail. They are: (a) Assurance of accurate and prompt payment for milk. (b) Advice on general production problems and on making necessary adjustments in milk production to comply with consumption requirements. (c) Provision for a dependable market outlet for milk.

C. J. Babcock

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

45. Stability of carotene in alfalfa. Effect of feed ingredients. H. L. MITCHELL and R. E. SILKER. Kansas Agr. Expt. Sta., Manhattan. Ind Eng. Chem., **42,** 11: 2325–2327. Nov., 1950.

The stability of carotene in dehydrated alfalfa meal was affected by other feed ingredients that often are found in mixed feeds. Expeller soybean and cottonseed meals mixed in a 1: 1 ratio with alfalfa meal had a stabilizing influence on the carotene. Carotene stability was improved as the percentage of expeller meals in the mixtures was increased. Cottonseed glands and rice bran also reduced destruction of carotene. Solvent meals were low in stabilizing activity. Presence of hops and linseed meal caused carotene to be destroyed more rapidly than the carotene of undiluted alfalfa meal. Some other materials had little effect on carotene retention.

B. H. Webb

46. The occurrence of vitamin B_{12} and other growth factors in alfalfa. E. M. BICKOFF, A. L. LIVINGSTON and N. S. SNELL, Western Reg. Research Lab., Albany, Cal. Arch. Biochem., **28,** 2: 242–252. Sept., 1950.

The vitamin B_{12} content of alfalfa, as measured by *Lactobacillus leichmannii* assay, ranges from 50–62 parts/billion for fresh alfalfa and from 12–45 parts/billion for dehydrated alfalfa meal. Differential assay techniques, such as alkali digestion and paper-partition chromatography, reveal that not more than 15% of the total apparent B_{12} activity of alfalfa actually is due to vitamin B_{12}. The high proportion of other factors, probably naturally-occurring desoxyribosides, to which *L. leichmannii* responds, dictates cautious interpretation of results obtained with alfalfa.

Various procedures were attempted to isolate the factors responsible for the apparent B_{12} activity of alfalfa; none of the techniques preferentially concentrated either the pure B_{12} or the non-B_{12} activity. H. J. Peppler

47. Hydrolysis and esterification of vitamin A during absorption. E. EDEN and K. C. SELLERS, Univ. of Cambridge. Biochem. J., **46,** 3: 261–266. 1950.

Ten adult sheep and 11 calves (4 of which were newborn) were used in a study of the fate of vitamin A alcohol and vitamin A ester during absorption. Blood levels of both forms of vitamin A were determined before dosing and 4 hr. later when the animals were sacrificed. Circulating vitamin A in normal blood is mainly in the alcohol form, but after dosing the surplus absorbed is there as the ester. The surplus in the intestinal lymph also was in the ester form irrespective of whether dosed as the alcohol or the ester. A. O. Call

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

48. Metabolism of spermatozoa. The formation and elimination of hydrogen peroxide by spermatozoa and effects on motility and survival. J. TOSIC and A. WALTON, Agr. Research Council Unit of Animal Reproduction, Cambridge. Biochem. J., **47,** 2: 199–212. 1950.

The metabolism of washed suspensions of bull spermatozoa was measured on a Barcroft manometer. Details are given of a procedure used for the fractionation of egg yolk. Respiration

and mobility measurements were made on the buffered spermatozoa suspensions, adding the various egg yolk fractions. The non-dialyzable portion of egg yolk did not contain the inhibitory factors found in the dialyzable portion. It was concluded that the inhibitor itself was not present originally in either the egg yolk or the dialyzable portion, but was formed under aerobic but not anaerobic conditions in the presence of respiring spermatozoa. The spermatozöon has an enzyme system which acts on the aromatic amino acids, L-tryptophan, L-phenylalanine and L-tyrosine, in the presence of O_2, to give rise to H_2O_2 by dehydrogenation and deamination. The H_2O_2 depresses both respiration and motility. Considerable discussion is devoted to reasons for the conclusions reached and possible applications to the improved preservation of spermatozoa *in vitro* and survival *in vivo*. A. O. Call

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

49. Vacuum milking system. S. J. ERLING (assignor to Aktiebolaget Separator Corp.). U. S. Patent 2,526,212. 1 claim. Oct. 17, 1950. Official Gaz. U. S. Pat. Office, **639,** 3:829. 1950.

A milker operated by pulsating vacuum created by a rotating valve which alternately connects the milker line with the source of vacuum and an atmospheric vent is described. R. Whitaker

50. Milk can cart. J. R. STOLTZFUS. U. S. Patent 2,526,295. 1 claim. Oct. 17, 1950. Official Gaz. U. S. Pat. Office, **639,** 3: 851. 1950.

A low 2-wheeled cart for moving cans of milk is described. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

51. Apparatus for making variegated ice cream. L. F. MARKS (assignor to G. G. Balch). U. S. Patent 2,527,273. 2 claims. Oct. 24, 1950. Official Gaz. U. S. Pat. Office, **639,** 4: 1238. 1950.

This device consists of a sanitary fitting enclosing 2 tubes, by means of which chocolate syrup, fruit puree and other flavoring materials are injected into a continuous stream of ice cream from the freezer, to form variegated ice cream. R. Whitaker

52. Method of making frozen food products. A. J. TACCHELLA (assignor to Steady-Flow Freezer Co.). U. S. Patent 2,527,894. 1 claim. Oct. 31, 1950. Official Gaz. U. S. Pat. Office, **639,** 5: 1508. 1950.

A horizontal freezer for the manufacture of soft ice cream at point-of-sale has a foot-operated pedal which opens the gate for removing the ready-to-serve product and also admits a proportional amount of mix to the freezing chamber. R. Whitaker

53. Ice cream cone extension. A. C. HABLER. U. S. Patent 2,527,993. 21 claims. Oct. 31, 1950. Official Gaz. U. S. Pat. Office, **639,** 5: 1534. 1950.

On top of a regular ice cream cone carrying a spherical portion of ice cream, is placed this edible extension cylindrical in shape and with top and bottom ends flared. Another scoop of ice cream is placed in the flared top of the extension. R. Whitaker

54. Process for accelerating the freezing of foods. G. HAMMOND and R. E. JACKE (assignors to Reynolds Metals Co.). U. S. Pat 2,529,388. 2 claims. Nov. 7, 1950. Official Gaz. U. S. Pat. Office, **640,** 1: 318. 1950.

Foods, including various dairy products, packed in containers of aluminum foil 0.003–0.005 in. caliper are rapidly frozen due to high thermal conductivity of the package wall. R. Whitaker

55. Apparatus for inserting sticks in stick holders. L. W. VON LOSBERG and A. FRIEDMAN (assignors to Joe Lowe Corp.). U. S. Patent 2,527,471. 6 claims. Oct. 24, 1950. Official Gaz. U. S. Pat Office, **639,** 4: 1288. 1950.

A machine for placing wooden sticks in a stick holder, which in turn holds the sticks in molds for making brine-tank frozen novelties is described. R. Whitaker

MILK SECRETION

V. R. SMITH, SECTION EDITOR

56. Studies on the synthesis of lactose by the mammary gland. I. Precursors. F. H. MALPRESS and A. B. MORRISON, Queen's Univ., Belfast. Biochem. J., **46,** 3: 307–312. 1950.

Cow and guinea pig mammary gland tissues were incubated in various buffered substrates. Synthesized lactose was measured by the Somogyi reduction method and also by a methylamine color reaction said to be more specific for lactose. Synthesis of lactose from glucose and glycogen by guinea pig mammary tissue was demonstrated; however, when cow mammary tissue was used no such synthesis could be demonstrated. This is in disagreement with what has been reported by others. There was no evidence that maltose or lactates were substrates for lactose synthesis. A. O. Call

57. The intermediary metabolism of the mammary gland. 3. Acetate metabolism of lactating mammary gland slices with special reference to milk fat synthesis. S. J. FOLLEY and T. H. FRENCH, Univ. of Reading. Biochem. J., **46,** 4: 465–473. 1950.

Mammary gland slices from ruminants (cow, goat and sheep) and non-ruminants (rat and rabbit) were studied manometrically for their possible utilization of acetate for milk-fat synthesis. The glands were taken at various periods of lactation, and in all cases the lapsed time from slaughter to the manometric flask was kept at a minimum—about 10 min. In some cases, determinations were made on tissues taken by biopsy. It was concluded that ruminant mammary tissue can actively use acetate as contrasted to little or no utilization of acetate by non-rumi-

nant (rat) tissue, rabbit tissue being somewhat intermediate in this regard. In all cases, added glucose stimulated the utilization of acetate. Some discussion is devoted to milk-fat synthesis by mammary tissues utilizing acetate.

A. O. Call

58. Utilization of acetate for milk-fat synthesis in the lactating goat. (Abs.) G. POPJAK, T. H. FRENCH and S. J. FOLLEY, Univ. of Reading. Biochem. J., **46,** 5: xxviii. 1950.

A saline solution containing C^{14} in the form of Na acetate was injected intravenously in a lactating goat, following which milk samples were collected at intervals for 48 hr. The fatty acids were separated from the milk and then fractionated. The radioactivity of the volatile fatty acids was several times that of the long-chain non-volatile fatty acids, indicating that the smaller volatile acids could not have originated from a degradation of the long-chain acids, but rather from a synthesis from acetate. It was concluded that milk-fat is synthesized from small molecules in the udder.

A. O. Call

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