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Contents

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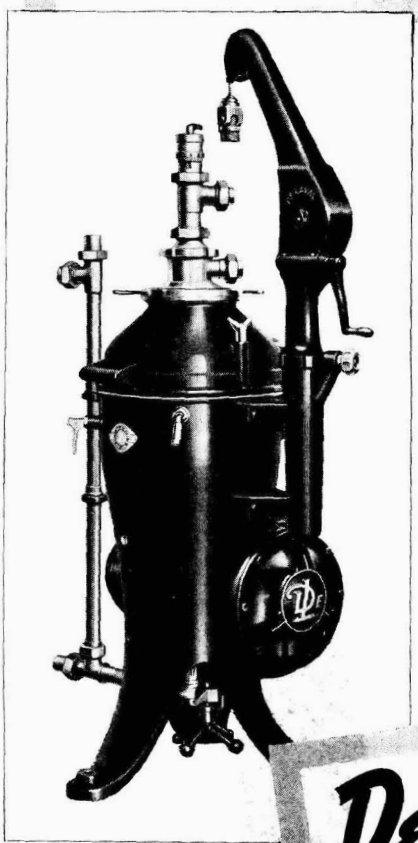

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THE PRODUCTION AND USE OF CONCENTRATED SKIM MILK FOAM

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The foaming of skim milk during various manufacturing operations has long been a source of annoyance to dairy products manufacturers. Many reports deal with prevention and destruction of foam but apparently there have been few attempts to utilize the foaming property of skim milk. While fresh skim milk forms a relatively unstable surface foam, the results presented here will show that reconstituted dry skim milk or concentrated skim milk can often be whipped to a stiff white foam of high stability. Some effects of temperature and time of whipping, concentration of solids, and of some variations in manufacturing procedure upon skim milk foam production and stability are reported. A new food use for dry or concentrated skim milks which whip readily is indicated.

The foaming of milk has been studied by several investigators, a concise discussion being available in Fundamentals of Dairy Science (2). A recent study of Ansbacher, Flanigan, and Supplee (1) is concerned with the substances in milk which cause foam production. Sharp, Myers, and Guthrie (6) were unable to decrease the foaming capacity of skim milk by repeatedly foaming and removing the foam thus formed, nor did they find an accumulation of any major protein fraction in the foam. Studies on the effect of temperature upon the foaming of skim milk (3, 5) indicate that between the temperatures of 20° C. and 30° C. the foaming tendency is at a minimum. Foaming increases above and below this temperature range.

Evaporated milk can be whipped when it is maintained at low temperatures but since this product is manufactured from whole milk, its relatively high fat content inhibits the incorporation of a large quantity of air. Recently Leviton and Leighton (4) have discussed the depressing effect of fat upon skim milk foam.

EXPERIMENTAL

Foam was produced by whipping concentrated skim milk or reconstituted dry skim milk in a small electrically-operated mixer that maintained at high

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speed, 1,000 r.p.m. without a load. When skim milk powder was used, it was added to water in the mixer bowl and beaten at low speed for one minute before whipping at high speed. The time figures given with the data in this paper refer to the number of minutes the sample was whipped at high speed and do not include the one-minute mixing period. Most of the data were obtained by using reconstituted dry skim milks of 30 per cent solids. To prepare these milks 49-½ grams of dry skim milk were added to 110-½ cc. of water. An allowance of 3 per cent moisture was made for all dry milk samples. Different samples were whipped to secure the data for each time period. Percentage overrun was calculated as 100 times the difference between the weight of 175 cc. of milk measured before whipping and 175 cc. measured after whipping, divided by the weight of 175 cc. measured after whipping.

The stability of the foam produced by whipping was determined by observing the time required for the first drainage to appear in the bottom of a 160 cc. glass tumbler filled with the whipped milk. The limit of error in the whipping and stability tests was ± 5 per cent but the actual error in most cases was probably less than half of this figure.

Viscosity measurements were made on the foam drainage obtained from 3 to 6 hours after whipping. A McMichael viscosimeter was used and the wires were standardized with sugar solutions.

The temperature at which all whipping, drainage, and viscosity tests were conducted was 20° C. unless otherwise stated.

The dry skim milks used for the experiments reported in tables 1 and 2 were commercial products prepared for use in bread making. Fresh skim milk from the Beltsville Research Center was used to obtain the data of table 3. The eight samples of skim milk required for the experiments of table 4 and figure 1 were derived from various sources as indicated. On a basis of 9 per cent total solids all skim milks contained less than 0.01 per cent fat by the Babcock test. It was important to use milks of low fat content since fat retarded overrun development.

RESULTS

The data of tables 1 and 2 show that an increase in whipping tempera-

TABLE 1

The effect of temperature upon the whipping properties of dry skim milk reconstituted to 25 per cent solids

Whipping temperature	Overrun after whipping 3 min.	Temperature of room during tests	Stability of foam
°C.	%	°C.	min.
20	368	20	30
30	400	30	25
40	444	30	20
50	489	30	16
70	478	30	15

ture or a decrease in solids cause an increase in overrun but a decrease in foam stability.

TABLE 2

The effect of the concentration of solids upon the whipping properties of reconstituted dry skim milk

Solids content of mix	Overrun after whipping 3 min.	Stability of foam
%	%	*min.*
10	412	1½
15	422	5½
20	402	11
25	331	21
30	307	47

The experiments reported in table 3 show the improvement in whipping properties brought about by the high heat treatment of one skim milk while more detailed data on whipping and related tests with 9 dry skim milks are presented in table 4. Whipping data for 8 of these milks are plotted in figure 1.

TABLE 3

The effect of the heat treatment of a skim milk upon the whipping properties of its condensed or reconstituted dry product. All milks contained 30 per cent solids when whipped

No.	Treatment of skim milk sample	Overrun after whipping 3 min.	Stability of foam
		per cent	*min.*
1	Forewarmed at 65° C. 15 min., condensed to 30 per cent solids	106	0
2	Forewarmed at 95° C. 15 min., condensed to 30 per cent solids	240	7
3	Same as No. 2 but superheated to 100° C. after condensing	259	18
4	Same as No. 2, held cold overnight, heated to 50° C., spray dried	252	> 45

The results reported above were made the basis for experiments on some new food uses for skim milk. High foaming skim milks were whipped to stiffness at concentrations of 25 and 30 per cent solids and when sweetened, used as topping for beverages such as hot chocolate. These whips were used as a base for home frozen ice cream after the addition of flavoring and other fat-free ingredients. For food products requiring a permanent retention of air it was necessary to increase the stability of the skim milk foam. This was done by bringing about a mild coagulation of the casein after development of the whip, thus setting the foam structure. The use of heat, rennet, and acid as stabilizing agents was investigated. Attempts were made to start an incipient coagulation of the casein by heating the reconstituted milks over

TABLE 4

Whipping properties and heat stability of samples of reconstituted dry skim milk. The process used in the manufacture of these milks is given below fig. 1

No.	Overrun after whipping:			Stability of foam			Viscosity of drainage	Time of coagulation at 125° C.
	2 min.	5 min.	10 min.	2 min. whip	5 min. whip	10 min. whip		
	per cent	*per cent*	*per cent*	*min.*	*min.*	*min.*	*centipoises*	*min.*
1	371	436	484	55	56	54	43	17
2	310	400	443	107	120	90	87	60
2S	257	370	411	57	74	67	.....	.....
3	217	353	413	4	21	29	20	156
4	188	294	367	4	9	17	31	160
5	167	244	258	19	28	35	148	37
6	88	154	245	8	18	41	97	.....
7	80	130	214		> 24 hours		.....	15
8	29	93	180		> 24 hours		.....	< 1

The whipped samples contained 30 per cent milk solids, the heat-coagulated milks contained 9 per cent solids, and sample No. 2S was No. 2 milk with 25 per cent milk solids, 15 per cent sugar, and 60 per cent water.

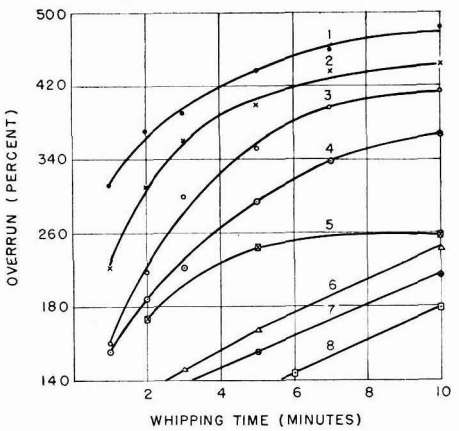

FIG. 1. Showing the development of overrun in dry skim milks reconstituted to 30 per cent solids and whipped for different periods of time. The dry skim milks were made by the methods indicated:

1. Vacuum drum dried commercial sample.
2. Spray dried commercial sample prepared for baking.
3. Fresh skim milk, forewarmed at 65° C., 20 minutes, condensed under vacuum to 30 per cent solids and spray dried.
4. Same milk and treatment as No. 3 but not condensed before drying.
5. Spray-dried commercial sample prepared for baking.
6. Same milk as No. 3, forewarmed at 95° C. for 20 minutes, spray dried.
7. Same milk and treatment as No. 3, but the condensed milk was superheated over a steam bath to 90–96° C. for 10 minutes and dried at once. Total time from start of superheating to end of drying operation was 45 minutes.
8. Atmospheric roll-dried commercial sample.

a steam bath while they were being whipped. The time required for the development of a coagulum was so long that the flavor was adversely affected and the method was not suited for stabilizing whips. The foam could be stabilized well with rennet when the factors controlling rennet coagulation were standardized, but this required exact laboratory methods. Nevertheless, a sweetened, rennet-stabilized, skim milk whip produced an attractive "Junket" type of desert when properly prepared. The foam was easily stabilized by the addition of acid. Pulped fruit from prunes, apricots, or berries was used as a source of acid. The product thus obtained made an excellent fruit whip which was equal to egg white fruit whips in flavor and often superior to them in stability. When pulp such as that of the prune was present, the whip was stable for many hours. Suitable formulas for fruit whips were developed. That for prune whip follows:

Dry skim milk	48 gr.
Powdered sugar	28 gr.
Water ($\frac{1}{2}$ cup)	113 gr.
Prune pulp	150–200 gr.

The skim milk and sugar were added to the water in the bowl of an electric mixer, the preparation whipped for at least 5 minutes and the prune pulp then quickly mixed in at low speed. Excessive mixing or stirring after the addition of acid foods caused some wheying off. Whipped mixtures of this type were frozen without stirring to produce smooth textured frozen desserts.

Fruit pulp was found to be necessary for the adequate stabilization of an acidified skim milk whip. Whips made with lemon juice wheyed off quickly, but when banana pulp and lemon juice were used, greater stability was attained. Excessive quantities of acid produced a whip with curd particles of objectionable size.

A milk powder flavor was sometimes noticeable in the finished whips. Fresh powder of good flavor was necessary for the production of an attractively flavored whip.

DISCUSSION

Foam of high air content and good stability may be secured by whipping properly heat-treated skim milks of 25 to 30 per cent solids content for several minutes. The data, however, are not complete enough to establish manufacturing procedures which will produce uniformly high whipping skim milks. The behavior of milks No. 3, No. 4, No. 6, and No. 7 (table 4 and fig. 1) indicates that increasingly severe heat treatment finally causes a decrease in whipping properties which is accompanied by a lowering in the heat stability of the protein. Milk dried on an atmospheric drum drier (No. 8) is subjected to intense heat when it is in a highly concentrated condition just before all the moisture is evaporated. The result of this drastic treatment is a powder of poor whipping properties and low heat stability.

It is probable that an optimum heat treatment which produces maximum whipping properties exists for each milk and that identical heat treatment does not necessarily produce the same whipping properties in different milks. Milk No. 4, table 3, developed 252 per cent overrun, while a milk obtained from the same source several weeks later and treated in the same way whipped to 367 per cent overrun with a stability of 83 minutes.

Commercial samples of dry skim milk prepared especially for baking whipped well, but some developed greater overrun than others. The preparation of a dry skim milk with high whipping properties should evidently involve high heat treatment somewhat similar to the procedure used in the manufacture of powder for baking purposes.

Particle size of insoluble roller dried powder exerts some influence upon air incorporation during whipping. An overrun of 156 per cent was produced after whipping a commercial powder of this type for 10 minutes. After the product was ground in a ball mill for 2 and 6 hours and whipped under the same conditions, overruns of 200 per cent and 251 per cent, respectively, were obtained.

SUMMARY

1. Reconstituted dry skim milks and condensed skim milks of 25 per cent to 30 per cent solids content were mechanically whipped in a few minutes to a stiff white foam. An overrun of 150 to 450 per cent and a foam stability of 10 to 90 minutes were found for different milks.

2. Wide variations were observed in the whipping properties of skim milks. High heat treatment usually improved whipping properties. Commercial milks prepared for baking purposes generally exhibited excellent whipping ability.

3. Skim-milk-whips were set by rennet or acid, but subsequent disturbance caused wheying off. Fruit-whips similar to an egg white product were prepared by adding sugar to the skim milk foam and stabilizing the whip by stirring fruit pulp into it.

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THE UTILIZATION OF UREA BY RUMINANTS AS INFLUENCED BY THE LEVEL OF PROTEIN IN THE RATION

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INTRODUCTION

The ability of ruminants to utilize simple nitrogen compounds such as urea or ammonium bicarbonate as a source of protein has been explained by bacterial synthetic activity occurring in the rumen and reticulum of these animals (1). A large and varied microflora is known to exist in the rumen and to account for this action. The media in which these microorganisms grow is determined by the ration fed. Since the kind and number of microorganisms are probably influenced by the composition of the medium, the possibility exists that by varying the ration fed, a change of the flora in the rumen could be produced and thereby a change in the synthetic reactions. In a previous "in vitro" experiment (2) it was found that the level of protein in a medium influenced the rate and amount of conversion of the ammonia (urea) when this medium was inoculated with microorganisms from the cow's rumen. As the protein level was increased from 2.5 grams to 5 grams of casein per 100 cc. of medium, the conversion of the added ammonia became negligible. The question arises as to whether this same phenomenon would occur in the rumen of animals fed urea. Acquisition of such knowledge would be important, not only from an academic viewpoint but also from a practical and economic one, since simple nitrogen compounds can be used as protein substitutes in rations of ruminants. With these facts in mind an experiment was set up to determine the effect of the level of protein fed on urea nitrogen utilization in the rumen.

EXPERIMENTAL

The data presented in this paper were obtained through the use of a 1000 pound Holstein heifer with a rumen fistula equipped with a removable rubber plug to facilitate sampling. The animal was fed twice daily at 8 A.M and 8 P.M. The daily ration consisted of corn silage 15 pounds, timothy hay 4 pounds, and a basal grain mixture of 4 pounds. The basal grain mixture was made up of ground yellow corn 50 per cent plus ground oats 50 per cent. The only variable in all the experiments was the grain mixture. These variations are listed in table 1. The heifer maintained its weight on

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the various rations fed throughout the experiment. Since the ration was always completely consumed within one hour after feeding, sampling was

TABLE 1

Variation of protein ($N \times 6.25$) content of the grain mixture

		Protein in basal concentrate theory	% protein increase due to L.O.M. added	% protein equiv. increase due to added urea	Total N as protein in concentrate	
					Theoretical	Actual
Experiment I—Variation of protein and urea levels						
Trial	1	12.0	0	0	12.0	11.3
	2	12.0	0	6	18.0	17.1
	3	12.0	4	2	18.0	17.1
	4	12.0	8	4	24.0	23.3
	5	12.0	12	6	30.0	31.1
Experiment II—Variation of protein level with L.O.M.—urea constant						
Trial	1	12.0	0	0	12.0	11.3
	2	12.0	0	6	18.0	17.1
	3	12.0	6	6	24.0	23.5
	4	12.0	12	6	30.0	31.1
Experiment III—Variation of level of urea added—protein constant						
Trial	1	12.0	0	0	12.0	11.3
	2	12.0	0	6	18.0	17.1
	3	12.0	0	12	24.0	24.0
	4	12.0	0	18	30.0	31.1

initiated at that time and continued throughout the day until the ammonia had returned to the basal level. All trials were repeated on alternate days at least three times before passing on to the next experiment. Table 2 contains the data obtained from three runs of the same trial as an example of the uniformity of results.

The determinations made on each rumen sample were ammonia, non-protein nitrogen (tungstic acid non-precipitable nitrogen), total nitrogen, and dry matter. The methods used in the sampling and in the determinations have been reported in a previous publication (3). With every ration tried it was found that the added urea-nitrogen was always hydrolyzed to ammonia within one hour after feeding. All results are calculated on a dry matter basis. When changing from one ration to another (change of the grain mixture composition) the animal was allowed to equilibrate for a week or more before sampling was started.

Utilization of the urea added was studied from three standpoints: first, varying both the oil meal and the urea added to the grain mixture; secondly, keeping the urea added constant and varying the oil meal; and thirdly, keeping the oil meal constant and varying the urea. In this manner the effect of both variables—urea and oil meal—on conversion could be studied. The results obtained are given in the accompanying charts.

TABLE 2

Data sheet experiment III, trial 3—analysis of rumen contents—urea added to basal grain mixture equivalent to 12 per cent protein

Hour after feeding	NH_3–N % Date				N.P.N. % Date				Total N (as protein) Date			
	12/15	12/17	12/19	Ave.	12/15	12/17	12/19	Ave.	12/15	12/17	12/19	Ave.
1	.317	.294	.276	.296	.522	.480	.470	.490	13.4	12.8	12.5	12.9
3	.192	.185	.206	.194	.348	.332	.322	.334	12.6	12.2	11.8	12.2
6	.044	.064	.066	.058	.222	.189	.183	.198	12.3	11.7	11.8	11.9
8	.....	.033	.039	.036	.....	.....	.....	.....	.....	.....	.....	.....

DISCUSSION

In experiment I the concentrates consisted of mixtures having a protein content comparable to those sold commercially. Therefore it was desirable to know if urea would be utilized when these types of concentrates containing urea were fed with corn silage and timothy hay, a practical dairy ration. In figure 4 it is seen that all of the ammonia (urea) disappears at the end of six hours in all the trials but with one exception, namely, trial 5. In this trial the protein level of the grain mixture was 24 per cent and the urea added equivalent to 6 per cent of protein—a total of 30 per cent protein equivalent. This protein level in the grain mixture is one sometimes used, but is unnecessarily high. If the corresponding curves representing the total nitrogen

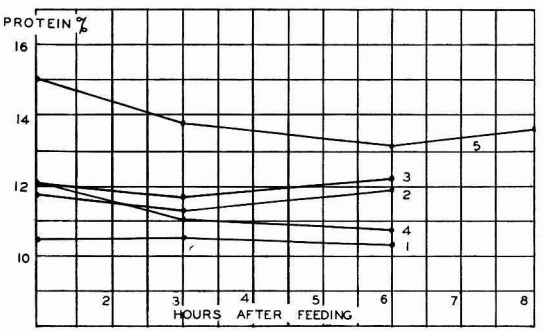

FIG. 1. Exp. I—Protein (N × 6.25) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 4% oil meal protein + 2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
5. " " " " + 12% of oil meal protein + 6% of protein equiv. as urea.

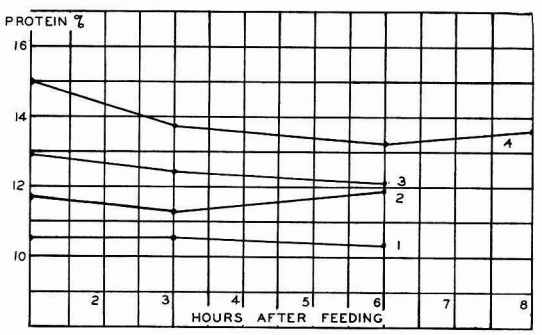

FIG. 2. Exp. II—Protein (N × 6.25) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

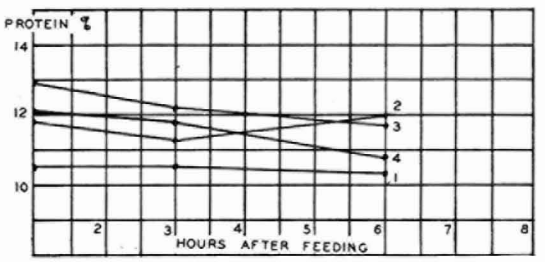

FIG. 3. Exp. III—Protein (N × 6.25) in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " +12% " " " "
4. " " " " +18% " " " "

content of the rumen ingesta are examined (fig. 1) it is seen that here all the curves, again with one exception, lie quite close together (trial 5). In other words, excessively high protein levels found in the rumen will influence the rate of disappearance of ammonia. This verifies the results previously reported in "in vitro" experiments (2), where it was found that as the

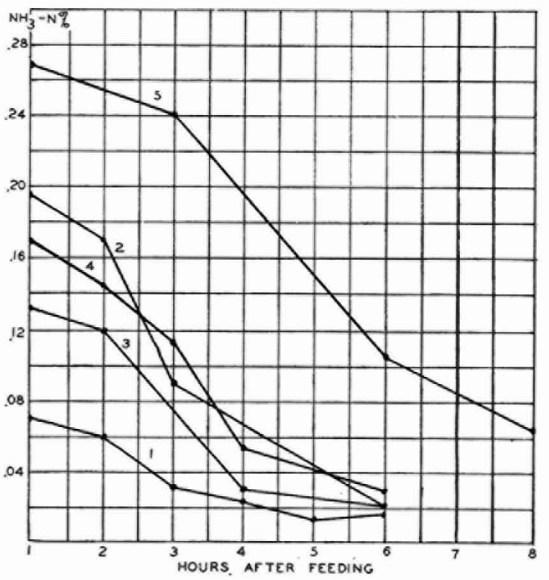

FIG. 4. Exp. I—NH_3–N in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 4% oil meal protein + 2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
5. " " " " +12% of oil meal protein + 6% of protein equiv. as urea.

protein (casein) in the medium was increased above 2.5 grams per 100 cc., the rate of conversion of inorganic nitrogen to protein was decreased. Judging from the results in experiment I, utilization of urea nitrogen will take place with protein concentrate levels as high as found in trial 4 (basal grain mixture plus oil meal to 20 per cent protein plus urea to 24 per cent protein equivalent) other parts of the ration being similar.

Examination of the results in experiment II (figs. 2 and 5, in which the urea added to the grain mixture is constant—6 per cent protein equivalent—and the protein content varied by adding oil meal) again illustrates the point that as the total nitrogen level in the rumen is increased above 12 per cent expressed as protein (fig. 2, trials 3 and 4) by feeding high protein concentrates (trial 3–18 per cent protein plus urea equivalent to 6 per cent protein; trial 4–24 per cent protein plus urea equivalent to 6 per cent protein) the utilization of ammonia is retarded (fig. 5, trails 3 and 4). Under the conditions of this experiment the utilization of urea fed at a level of 2.5 per cent in grain mixture decreased when the protein level of the concentrate was greater than 18 per cent.

When the low protein concentrate (grain mixture) equivalent to 11.3

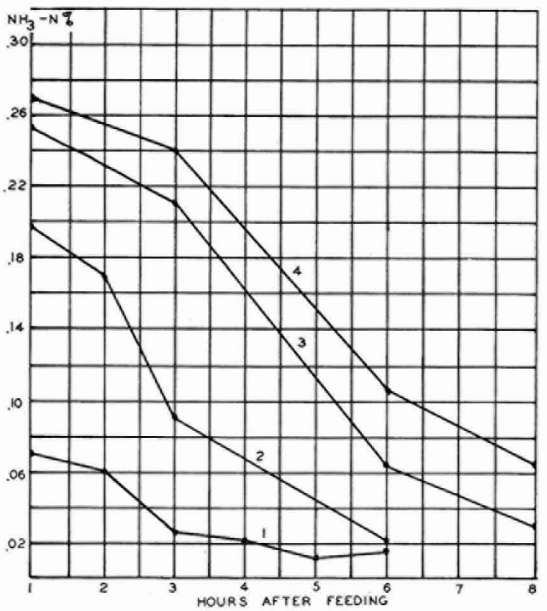

FIG. 5. Exp. II—NH_3-N in rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

per cent protein was fed as in experiment III it was found that the urea added to the concentrate could be raised to a 4.5 per cent level (12 per cent protein equivalent) before there was a pronounced delay in the disappearance of the ammonia from the rumen (fig. 6, trials 3 and 4). It will also be noticed in figure 3 that the protein content of the rumen ingesta remained near the basal level. When large amounts of urea were added to this low protein concentrate as in trial 3 (12 per cent protein equivalent) and trial 4 (18 per cent protein equivalent) the rate of disappearance of the ammonia from the rumen was much faster (fig. 6) than when urea was added to the high protein concentrates as were fed in experiments I and II (figs. 4 and 5).

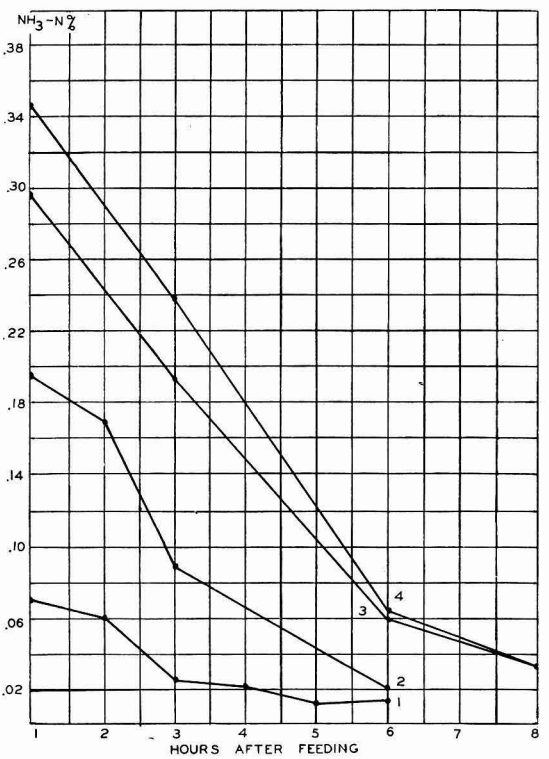

FIG. 6. Exp. III—NH_3–N rumen contents (dry basis).

1. Protein in Conc. 11.3%
2. " " " " + 6% protein equiv. as urea.
3. " " " " +12% " " " "
4. " " " " +18% " " " "

Figures 7, 8 and 9 contain the values obtained from determinations of non-protein nitrogen on several of the trials in each experiment. On

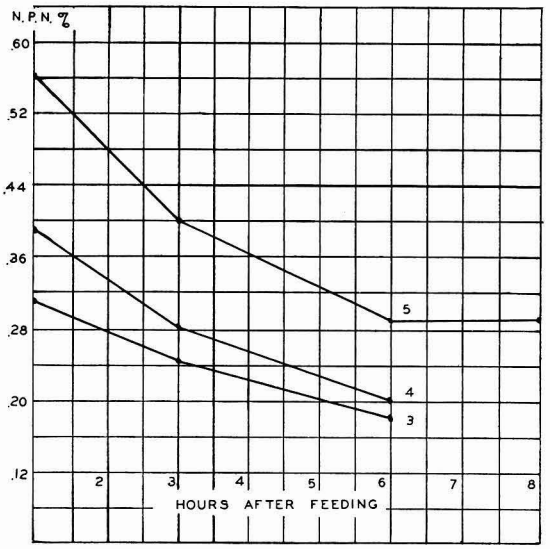

FIG. 7. Exp. I—N.P.N. rumen contents (dry basis).

3. Protein in Conc. 11.3% + 4% oil meal protein + 2% of protein equiv. as urea.
4. " " " " + 8% oil meal protein + 4% of protein equiv. as urea.
5. " " " " + 12% of oil meal protein + 6% of protein equiv. as urea.

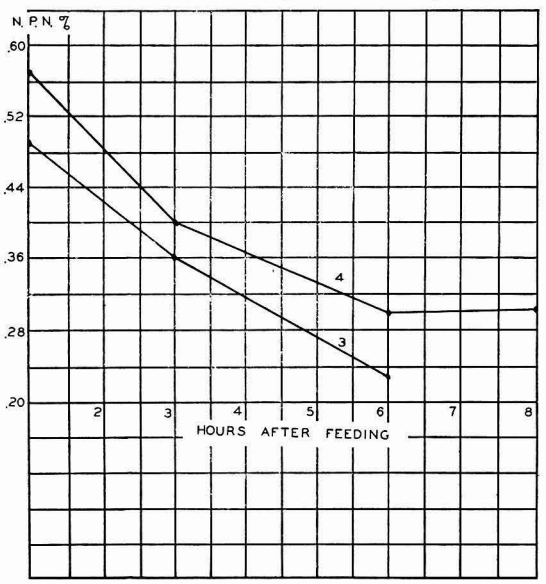

FIG. 8. Exp. II—N.P.N. rumen contents (dry basis).

3. Protein in Conc. 11.3% + 6% oil meal protein + 6% protein equiv. as urea.
4. " " " " + 12% oil meal protein + 6% protein equiv. as urea.

examination it is seen that variations in these curves are directly related to the ammonia variation, since the ammonia is included in the non-protein nitrogen. When the variation in ammonia content is taken into consideration these curves become quite uniform.

The question may be raised as to whether the disappearance of ammonia is due to a conversion to protein by the microorganisms or simply a passage from the rumen unchanged. Some of the inorganic nitrogen may leave the rumen without being converted to protein. However, in view of the present experiments where it was possible to show a decided delay beyond 6 hours in the decrease of ammonia by a high protein content of the rumen ingesta

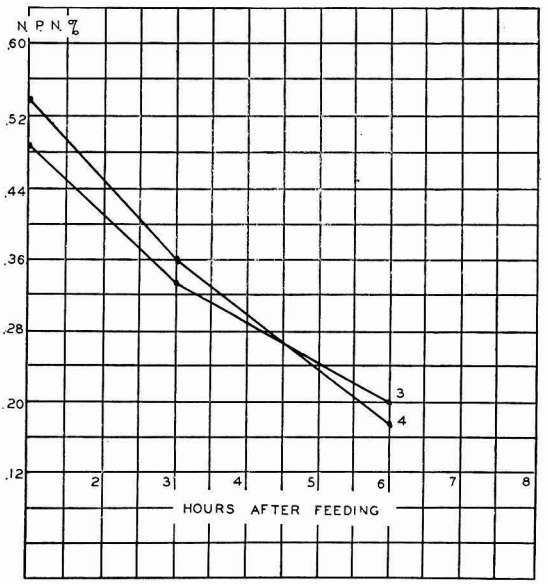

FIG. 9. Exp. III—N.P.N. rumen contents (dry basis).
3. Protein in Conc. 11.3% + 12% protein equiv. as urea.
4. " " " " +18% " " " "

it appears that the disappearance taking place must also be due to a conversion to protein. Also, evidence of protein formation from urea in the rumen has already been reported in a previous publication (3).

Another factor which might easily influence the rate and extent of conversion of ammonia to protein in the rumen is the available carbohydrate fed. Experiments are now in progress to determine how the amount and kind of carbohydrate and the length of time it remains in the rumen will affect the rate of utilization of ammonia.

SUMMARY

1. The protein content of rumen ingesta showed a decided increase when the level of protein in the concentrate fed was increased to 24 per cent.

2. The rate of conversion of urea nitrogen to protein in the rumen decreased as the protein level of the rumen ingesta became greater than 12 per cent.

3. When the level of protein in the concentrate fed was increased to more than 18 per cent the rate and extent of conversion of added urea nitrogen to protein began to decrease.

4. When no linseed oil meal was added to the basal grain mixture (11.3 per cent protein), the added urea was utilized up to a level of 4.5 per cent (protein equivalent of 12 per cent) of the grain mixture.

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EFFECT OF STILBESTROL ON THE MAMMARY GLAND OF THE MOUSE, RAT, RABBIT, AND GOAT*

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If knowledge of the hormonal causes of the growth and lactation of the mammary gland is to be put to practical use it will be necessary to find cheap sources of the hormones and devise simple and effective methods of administration. This report is concerned with a first step in that direction. Work in this laboratory has shown that the estrogenic hormones stimulate growth of the mammary duct system by increasing the rate of secretion of mammogen by the pituitary (1).

The recent synthesis of a compound called stilbestrol, which has very great estrogen-like physiological activity and still is relatively inexpensive in comparison to the natural estrogens, has stimulated considerable research. Stilbestrol has the further advantages that it is quite effective orally and when formed into pellets is slowly absorbed for months when implanted under the skin (2, 3, 4, 5). This chemical, which simulates the estrogens in its physiological properties, is produced at 1/50 the cost for equal activity. It is sufficiently low in cost at active dosages to consider its practical application in livestock therapy. With this in mind the authors are conducting experiments on the influence of stilbestrol on the mammary gland and milk secretion in laboratory and farm animals.

Many stilbestrol experiments of clinical nature or having no application to mammary development cannot be reviewed here (for these see ref. 6). Assays of the effect of stilbestrol on the genital organs show that one-half gamma produced estrus in 70 per cent of 175 gram spayed rats (7). Stilbestrol was found to be equal to, or five times as active as, estrone in producing vaginal cornification in rats and mice (4, 7, 8). Orally administered stilbestrol was twenty times as active in mice as estrone and 16 times as active as estradiol (7).

Van Heuverswyn *et al.* (9) found that groups of five male mice given 0.05, 0.2, and 2.0 mg. of stilbestrol, respectively, gave plus two, plus three and plus one rating of mammary duct responses after eight injections in 16 days.

Stilbestrol was found to be about one-fifth as active as estrone in causing proliferation of the mammary gland of spayed rats (10), and of guinea pigs (11).

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In contrast to these results, experiments conducted by the present authors showed stilbestrol to be more active than estradiol benzoate in causing mammary duct proliferation in male mice (12).

Mammary development in the human male from oral administration of stilbestrol has been reported (13). A total of 480 mg. were given over 96 days. Hard, firm masses 6 cm. in diameter and 2.5 cm. thick were palpable under the nipples. These masses were harder, and denser than result from the action of the natural estrogens. Similar results, although less development, were obtained in a six-year-old girl. Breast hypertrophy and genital changes were reported (14) in a castrate woman given 20.25 mg. of stilbestrol in 18 days followed by 30 rabbit units of progesterone in six days. MacBryde *et al.* (15) reported similar results with stilbestrol administered orally or by injection.

Proliferation of the mammary epithelium was revealed by biopsy specimens obtained before and after oral administration of 280 mg. of stilbestrol to a castrate woman. One milligram a day given orally for 14 days to a second woman caused painful breast swelling (16).

De Fremery (17) reported in 1936 that inunction of the udders of virgin female goats with estradiol benzoate caused mammary growth. Initially slow udder development increased abruptly with changes of parturient type. Lactogen then produced abundant normal lactation.

Folley *et al.* (18) reported that stilbestrol dipropionate in oil applied to the udders of three virgin female goats, plus daily milking, caused the production of a maximum of 1500 cc. of normal milk daily. A normal lactation curve resulted. A virgin heifer similarly treated responded with colostrum secretion alone. Attempts to induce lactation with stilbestrol in male goats failed even when progesterone was added to the treatment.

Lewis and Turner (19) reported that daily subcutaneous injections of stilbestrol caused the initiation of lactation with a maximum production of about half the normal quantity of milk from two yearling goats during lactation periods of six months. A castrate and a normal kid lactated for 95 days. The effect of stilbestrol injections on two goats already in milk was not good as far as production was concerned.

PROCEDURE

Stilbestrol (4 : 4 dihydroxy α β diethyl-stilbestrol) was obtained in powder form from E. R. Squibb and Sons. For injection it was dissolved in ether, added to the oil carrier and the ether removed before a fan. The daily dosage was administered in 0.05 to 0.2 cc. of oil to mice and rats and 0.1 to 0.4 cc. to rabbits. Two milligram tablets of stilbestrol, from the Geo. A. Breon Co., Kansas City, were put in suspension in the drinking water for oral administration to mice and renewed daily.

The mammary glands were removed in toto at biopsy or necropsy, fixed

in Bouin's fluid and stained in Mayer's hematoxylin. After removal of the paniculus carnosus muscle and overlying connective tissue, the glands of the rabbits were measured and the greatest diameter recorded. Rabbit glands were removed at about 20-day intervals. Pituitary lactogenic hormone was prepared by the Bergman-Turner (20) method in this laboratory from cattle anterior pituitary and was assayed by the McShan-Turner pigeon method (21). One international unit equals 0.8 McShan-Turner units. Three hundred and forty international units (approximately 11 i.u. per 100 gm. body weight) are required to produce lactation in normal pseudo-pregnant rabbits and constitute one rabbit unit (22). It was given to rabbits in 6 equal daily subcutaneous doses. The mammary glands were examined and a gland removed on the seventh day.

Goats were injected subcutaneously in the crops with stilbestrol in 0.2–0.5 cc. of oil daily. Half of the udder was removed under local anesthetic, frozen, cross sectioned by hand, fixed in Bouin's fluid, stained and mounted in isobutyl methacryolate polymer. Thinner, small sections were mounted on slides in clarite. Paraffin sections were also made.

The mice were fed a mixed grain ration containing cod liver oil plus Purina dog pellets. The rabbits were fed a mixed grain ration plus alfalfa hay. Goats were stabled continually and fed a mixed dairy ration plus alfalfa hay.

EXPERIMENTAL RESULTS

Mice. Groups of three to five male mice injected with stilbestrol daily for 14, 21, and 27 days responded with extensive development of the mammary duct system (table 1). Dosages of 0.167 to 0.5 gamma per day caused the production of glands extending 0.5 to 1.5 cm. in length, usually with fewer main ducts than in glands of normal female mice. In most cases early interlobular duct development was evident. In one case isolated clumps of secreting alveoli were found, as reported by Gomez and Turner (23) following anol treatment (fig. 1). This condition has been shown in this laboratory (unpublished) to result from estrone injection. In no case were lobules of alveoli well developed. There was considerable variation in development of glands from the same mouse, but large glands were found in each case. There was progressive development in the groups treated for 14, 21, and 27 days.

Groups of four spayed virgin female mice given 0.167, 0.5 and 1.5 gamma of stilbestrol per day for 11 to 21 days gave a varying response. In some cases only end-buds and duct growth were apparent, in others interlobular ducts had developed. A number had mammary elements expanded with secretion. Two showed small, isolated clumps of alveoli (fig. 1).

Stilbestrol has been reported to be comparatively much more active orally than the natural estrogens (8). Oral administration would appear to be of considerable advantage in practical application of hormone therapy

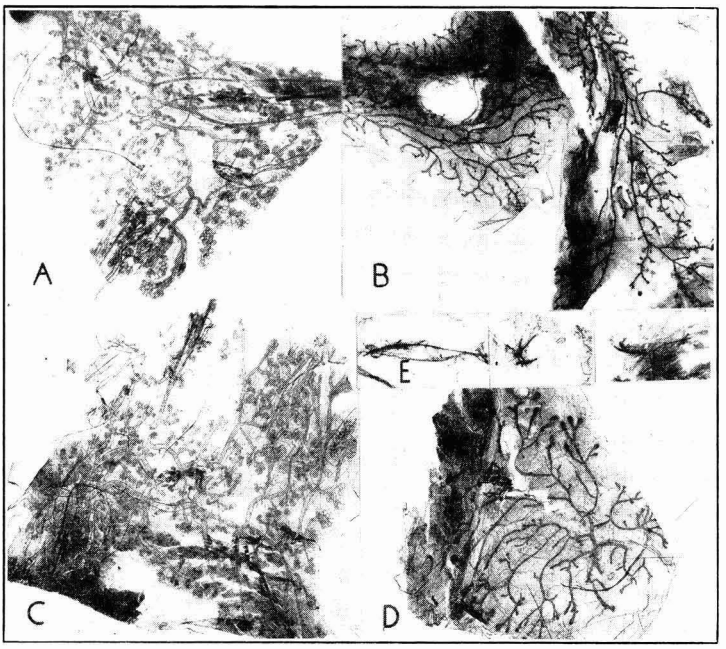

FIG. 1. a. Mammary gland from a spayed virgin mouse after daily injection of 1/6 γ of stilbestrol for 20 days. The ducts were expanded with secretion as were clumps of what appear to be alveoli. ×4.8.

b. Control mammary glands from virgin female mice. Notice the smooth, unexpanded ducts. ×1.5.

c. Mammary gland from a male albino mouse after 21 days treatment with 1/6 γ of stilbestrol daily. This gland was developed similarly to those of the female mouse shown in (a). Only one such case was found among a large number of males treated with stilbestrol. ×4.8.

d. Typical mammary gland from a male mouse treated with stilbestrol for 14 to 27 days. Note active hyperplasia of ducts shown by enlarged, deeply staining end-buds. Some of these mice had ducts made rough in appearance by the beginning of interlobular duct development. This gland was similar to control glands in (e) before treatment. ×4.8.

e. Three typical control mammary rudiments from male mice. ×4.6.

to livestock. Stilbestrol administered in the drinking water to male mice caused extensive duct proliferation similar to that caused by injection. Groups of three and four mice were treated for 14 and 21 days. One-tenth to 0.4 gamma per day gave little or no stimulation while 0.5 to 1.5 gamma per day gave extensive proliferation (table 1).

From these results it is seen that stilbestrol readily caused extensive duct development in male mice either by injection or when administered in the drinking water. Stilbestrol carried the stage of gland development

TABLE 1

Effect of stilbestrol on the mammary gland of the mouse

Mode of adminstration	Number of mice	Days treated	Dosage, gamma per day	Results
Subcutaneous injection, males	5	14	1/6	1 neg. 4 pos. Largest gland in each case 0.6 to 0.9 cm.
	5	21	1/6	5 pos. Most glands 0.5 to 1 cm. diameter. One mouse had isolated alveolar clumps.
	4	27	1/6	Most glands 0.7 to 1.6 cm. extent. Roughened ducts.
Subcutaneous injection, males	5	14	0.5	Glands +1 to .9 cm. Largest .5 to .9 cm. in each case.
	5	21	0.5	Most glands .4 to .9 cm. extent. Roughened ducts.
	5	27	0.5	1 barely positive. Others, most glands 0.5 to 2 cm. extent. Roughened ducts.
Oral, in drinking water	3	14	0.1	One with teats had .4 to .6 cm. glands. Others negative.
	3	21	0.1 0.2 last 7 days	Negative.
	3	14	0.2	One with +1 gland. Others negative.
	3	21	0.2 0.4 last 7 days	Negative.
	3	14	0.4	One with a +1 gland. Others negative.
	3	21	0.4 0.8 last 7 days	2 strongly developed with .5 to .9 cm. ducts.
Males	3	14	0.5	Largest glands 0.6 to .8 cm.
	4	21	0.5	Most glands 0.4 to 1.2 cm. ducts.
	4	14	1.0	0.5 to 1.1 cm. roughened ducts.
	4	21	1.0	0.5 to 1.5 cm.
	4	14	1.5	0.5 to 1.1 cm. roughened ducts.
	4	21	1.5	0.5 to 1.0 cm. roughened ducts.
Injected, spayed females	4	11	1/6	1 with end-buds, 2 interlobular ducts.
	4	20	1/6	2 with clumps of alveoli. 2 with roughened ducts.
	4	14	0.5	2 with end-buds. No lobules. 2 roughened ducts.
	4	21	0.5	Ducts thickened with secretion. Interlobular ducts—No lobules.
	4	14	1.5	Roughened ducts. Glands rather small in 2 cases. End-buds 1 case.
	4	21	1.5	Thick roughened ducts—2 cases. End-buds—1 case. No lobules.

merely to that obtainable with the natural estrogens in the mouse. No true lobule development occurred, such as is found in pregnancy, even in spayed virgin females.

A total dosage of 0.05 γ in six days was reported to cause a mammogenic duct growth mouse unit response (12). In this study 0.167 to 0.5 γ per

day caused extensive duct development. Oral administration appeared to require about six times as much hormone as by injection.

Rats. Subcutaneous injection of stilbestrol into groups of three castrate virgin female rats at 0.004, 0.008, 0.017 and 0.034 gamma per day did not result in any obvious signs of mammary duct growth. Single glands were removed on the 7th, 14th, and 23rd days of treatment. The remaining glands were recovered and examined in toto after sacrificing on the 28th day (table 2). The mammary glands appeared to be in active rather than in regressed condition in most cases, however, staining deeply. Several glands removed had main ducts which were considerably thickened by side development of interlobular ducts and perhaps alveolar buds. No duct end-buds were apparent.

Three groups of similar rats given 0.25, 0.5 and 1.0 gamma per day of stilbestrol for 18 days all showed active proliferation of mammary ducts.

TABLE 2

Effect of stilbestrol on the mammary gland of the rat

Number of rats	Av. weight after treatment	Dosage, gamma per day	Results			
			7 days	14	23	28
	gm.					
3	177	0.004				
3	169	0.008	Glands were all negative for additional duct growth.			
3	191	0.017				
3	169	0.034				
				18 days		
3	111	0.25	Many duct end-buds and considerable proliferation of ducts.			
3	111	0.50				
2	106	1.00				

In this study four times the mouse unit dosage of stilbestrol gave no demonstrable signs of duct proliferation. Duct growth was obtained with a dosage of 0.25 γ per day which is at least 30 male mouse units (12). However, the minimum dosage to secure duct growth in the rat was not ascertained. This is in contrast to results secured with the natural estrogens in which the rat mammary gland appeared to respond to lower dosages than did that of the mouse (24, 25).

Rabbits. Twelve rabbits either injected or implanted with stilbestrol showed extensive mammary proliferation. Glands removed from males after injection of 4 to 32 gamma per day for 20 days had a complex duct system with an average extent of about 3 cm. (tables 3 and 4). This was not extensive development but glands from rabbits given 4 γ were as large as those from rabbits given 32 γ a day, indicating that 4 γ were adequate to secure the maximum rate of development. Since it appeared that the dosage might still be above the optimum, the dosage to four of the rabbits was then reduced to 1/10 that of the first 20-day period (0.4 γ–3.2 γ) and

single glands were removed by biopsy at 40 and 60 days. The 40-day glands averaged 4.4 cm. in extent and the 60-day glands 5 cm. The rabbits were sacrificed at 70 to 80 days of injection when all remaining glands averaged over 6 cm. They compared favorably in size with those of virgin female rabbits but were in several cases more complex. These glands consisted not only of duct systems but in addition had considerable lobule development (fig. 2).

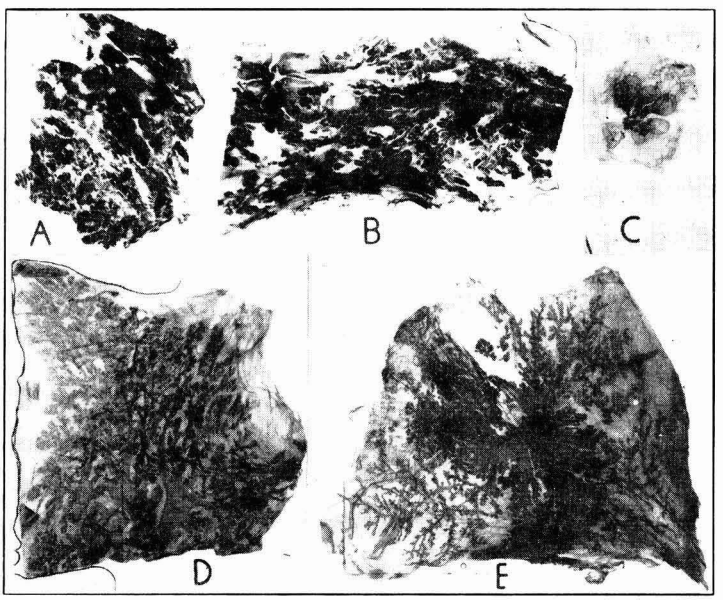

FIG. 2. a. Section of mammary gland from a virgin female rabbit (No. 24) given 32 γ daily of stilbestrol for 20 days. This gland contained copious quantities of milk when removed. There was a well developed lobule system with hypertrophied alveoli. ×1.

b. Section of a mammary gland from a male rabbit (No. 29) with an 18.7 mg. pellet of stilbestrol subcutaneously. This gland was obtained 97 days from implantation of the pellet. The extensive lobule-alveolar system was expanded with milk from administration of a rabbit unit of lactogen. Compare with control gland (c). ×1.

c. A typical control gland from a male rabbit. ×1.

d. Half of a mammary gland from a male rabbit (No. 22) given 16 γ of stilbestrol daily for 20 days and then 1.6 γ for 45 days. Interlobular ducts are well developed and a few areas appear to have small lobules with alveoli. ×1.

e. Mammary gland from a male rabbit (No. 14) given 32 γ of stilbestrol daily for 20 days and then 3.2 γ for 50 days. Lobules have developed in the center of the gland. ×1.

An attempt was made to secure milk secretion from the small duct systems of four of the male rabbits after 20 days treatment with stilbestrol.

TABLE 3

Response of rabbit mammary glands to stilbestrol and lactogen

Sex	Mode stilb. admin.	Dosage stilb. /day 20 days	Lactogen treatment		Dia. 27-day glands	Type of development	Dosage stilb. /day 20 days	Lactogen treatment		Dia. 54-day glands
			Dosage 6 days	Response				Dosage 6 days	Response	
					cm.					*cm.*
Male 5	Injection	32 γ	10 i.u./100 gm. body weight	Serous	2.7, 3.4	Interlobular ducts				
" 14	"	32 γ		Serous	2.8	Early lobules				
" 4	"	16 γ		Serous	3.5	Ducts				
" 3	"	8 γ		Milk	2.7	Lobules	8 γ	10 i.u./100 gm. body weight	Copious milk	5
" 13	"	4 γ		Serous	3.8	Ducts				

Lactogen was given at the rate of 10 international units per 100 gm. body weight in six daily injections. At operation for removal of sample glands, those of three rabbits showed serous secretion and expanded ducts but no milk. Milk could be squeezed from the teats of the fourth rabbit and the small duct system was filled with milk. After 20 more days treatment with stilbestrol this rabbit had a 5 cm. duct system which again filled with milk on lactogenic treatment.

Three 1200 to 2500 gram virgin female rabbits injected with 8, 16, and 32 γ daily of stilbestrol had 5 to 7 cm. glands removed after 20 days of injection. The gland from the rabbit on 32 γ was engorged with milk in a lobule-alveolar system. The glands from the other two rabbits had no milk but the main and interlobular ducts present were swollen with serous secretion.

Glands removed at 40 days of injection did not show such obvious secretion. In two cases interlobular ducts were present while in the third early lobules were present. The glands removed after 60–65 days had in two cases unhypertrophied lobules. In the third only interlobular ducts were present.

Two male rabbits implanted with 16.8 and 18.7 mg. pellets of stilbestrol showed no mammary development in glands removed at 20 days after implantation (table 4). The 40-day glands removed were 4 to 5 cm. in extent, however, and showed what was apparently the beginning of lobules, perhaps consisting of intralobular ducts. Short central ducts were highly cystic. At 60 days the lobules were more apparent. Glands removed at 80 days appeared to have true lobules with alveoli. These were rather scattered and small and did not constitute the pseudo-pregnant gland condition. These glands were approximately 6 cm. in extent. At 90 days lactogen was administered at the rate of 12 i.u. per 100 grams body weight. The mammary glands became swollen with milk so that it could be expressed from the teats. Glands removed on the 97th day were composed of hypertrophied lobules of alveoli and ducts full of milk (fig. 2). Three glands removed from one rabbit at necropsy at 103 days averaged 9.5 cm. in extent. The second rabbit was sacrificed at 122 days when two 10 cm. lobule-alveolar glands were removed. These glands contained isolated areas composed of apparently abnormal lobules with very large alveoli, as seen by Gomez and Turner (23) after anol treatment of rabbits.

Five rabbits given 0.02 γ to 2.0 γ per day per teat applied in alcohol to the shaved skin for 30 days responded with growth of the mammary glands. Two of these rabbits which received 2 γ and 0.2 γ per teat had glands averaging 4.2 cm. and 4.7 cm. in extent. Some of these glands were over 5 cm. in diameter. Development had progressed to the interlobular duct or early lobule stages. The teats were also considerably enlarged over the control condition. This also occurred in the rabbits injected with stilbestrol and in those with pellets.

TABLE 4

Response of rabbit mammary glands to stilbestrol and lactogen

Sex		Mode stilb. admin.	Dosage stilb. /day	Dia. 20-day glands	Type of develop-ment	Dosage stilb.	Dia. 40–43 day glands	Type of develop-ment	Dia. 60–65 day glands	Type of develop-ment	Dia. 70–80 day glands	Type of develop-ment
				cm.		*gamma*	*cm.*		*cm.*		*cm.*	
Male	14*	Injection	32 γ	2.3	Lobules	3.2	4.0	Lobules	2.4	Lobules	5.6, 5.7	Lobules
"	22	"	16 γ	3.2	Ducts	1.6	5.7	Interlobular ducts	5.5	Interlobular ducts	5.7, 6.1 6.6, 7.7	Interlobular ducts
"	23	"	8 γ	2.5	Ducts	0.8	4.0	Ducts	4.4 5.7	Plain ducts Interlobular ducts		Early lobules
"	13*	"	4 γ	3.5	Ducts	0.4	4.0	Early lobules	5.6	Ducts	6.4, 5.0	Interlobular ducts
Virgin female	24	"	32 γ	6.6	Lobules Alveoli small Milk in ducts		5.7	Lobules of alveoli	8.2 7.0 9.0 10.0	Lobules		
" "	25	"	16 γ	5.0	Ducts Serous secretion		6.0	Lobules	8.0	Lobules		
" "	26	"	8 γ	5.0	Interlobular ducts Serous secretion		5.5	Swollen ducts	8.5 6.7	Ducts		
Male	28	Subcutaneous pellets	16.8 mg.	Neg.			5.0	Early lobules	4.6	Lobules	6.0	Lobules
"	29	Subcutaneous pellets	18.7 "	Neg.			3.9	Early lobules	4.3	Early lobules	5.5	Early lobules

	Lactogen treatment at 90 days		Dia. 97 day glands	Type of development	Dia. 103 day glands	Type of development	Dia. 122 day glands	Type of development
	Dosage	Respone	*cm.*		*cm.*		*cm.*	
Male 28 (continued)	12 i.u./100 gm. body weight	Copious milk	6	Cystic ducts Large lobules	8.8, 10.2 9.5	Lobules Small alveoli		
" 29 (continued)		Copious milk	12	Isolated lobules, hypertrophied			9.8 5.8, 10.0	Small alveoli Lobules

* Days elapsed between the two series of injections, rabbit #14–26 days; rabbit #13–40 days.

Male rabbits responded with extensive mammary development on injection of as little as 0.4 γ per day of stilbestrol or on percutaneous application to the shaved skin. It is interesting to note that in the male rabbit the lobule-alveolar system developed to a greater extent than in the female mouse similarly treated. Alveolar lobules began to appear after 20 to 40 days treatment in the rabbit and were well developed in rabbits implanted with pellets of stilbestrol.

Well developed mammary duct systems grown with stilbestrol in male rabbits came into lactation upon administration of lactogen. It was not necessary to terminate the stilbestrol treatment during lactogen administration for male rabbits with subcutaneously implanted pellets responded readily. Stilbestrol was thus shown to have the estrogenic property of preparing the mammary gland to respond to lactogen by the production of milk (26, for review).

A normal adult female rabbit did not require the administration of lactogen, for it responded with copious milk secretion to stilbestrol injection alone. This has also been shown to occur in the dry female goat (18, 19). Stilbestrol appears to cause the release of lactogen from the animal's own anterior pituitary in these cases, for a goat brought into milk with stilbestrol had at least twice the lactogen content in her urine as did normal dry goats (19).

Goats. Six goats were given subcutaneous injections of stilbestrol for periods from 96 days to 8 months. The substantial lactations which re-

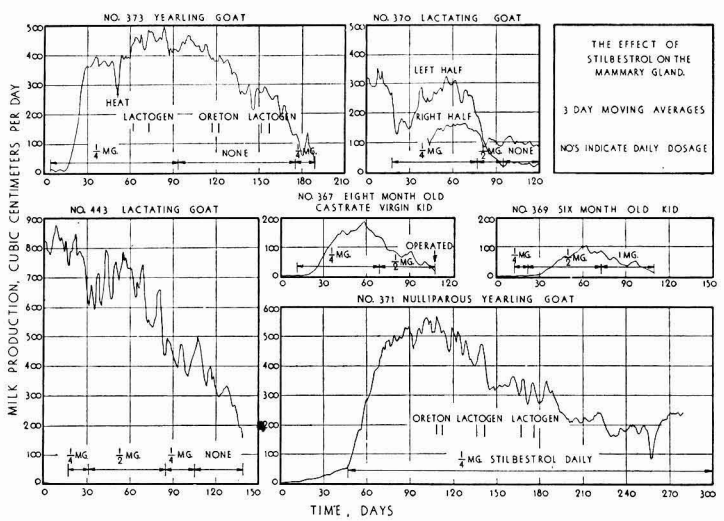

FIG. 3. The effect of daily, subcutaneous stilbestrol injection upon lactation in a castrate and a normal virgin kid, two yearlings goats which had never lactated and two mature goats in milk.

sulted from two kids and two yearlings has been previously reported as was the effect on the milk production of two goats which were already in lactation before the beginning of treatment (19). These lactations have been charted and are reproduced here (fig. 3).

The two virgin female kids received daily injections of stilbestrol for 96 days. The kids were six (No. 369) and eight (No. 367) months old when treatment was begun in July, so presumably they had had no estrous cycles. Goat No. 367 was castrated shortly before stilbestrol treatment was begun. Initial dosage was 0.25 mg. per day raised to 0.5 mg. on the 58th day; a total of 34.25 mg. in 96 days. Goat No. 369 was given 0.25 mg. per day for 12 days, 0.5 mg. for 44, 1.0 mg. a day for 41 days; a total of 67 mg. in 96 days.

At this time half of the udder was removed from each kid. The mammary glands were found to be about 4 cm. in diameter and consisted of ducts with thick clusters of lobules as in normal lactating glands after parturition, although most of the alveoli were no longer secreting heavily. Occasional alveoli or even lobules appeared to be still quite active (fig. 4). The lobules from the normal kid (No. 369) appeared to be more compact and fully developed than those from the castrate (No. 367). The extent of the glands does not appear to have been much increased but the lactation induced had caused the glands to expand into spherical form and had developed the gland cisterns. A mammary gland removed from a 37 day pregnant goat did not have nearly as complete a development of the lobules. Another gland removed from a 74 day pregnant kid appeared to have a complete lobule-alveolar system which was unhypertrophied, however (fig. 4). The alveoli were small masses of cells without lumina. This gland measured 0.5 cm. in thickness and 5.5 by 3.5 cm. in extent.

In contrast to the glands from stilbestrol treated kids Turner and Gomez (27) have shown that the mammary gland of the immature female goat consists of a thin, one centimeter or less, layer of ducts lying at the base of the teat and extending a few centimeters from it. Gland sections showed that the mammary system although rather complex consisted of a two cell layered duct system without lobules. Short, wide branches of the smaller ducts which gave the impression of alveolar buds were also two layered except the distal ends which were composed of solid masses of epithelial mammary cells.

A mammary gland removed from a mature male castrate goat (No. 833) showed no development after extensive stilbestrol treatment (fig. 4). For 78 days 0.25 mg. of stilbestrol was given daily by injection, a total of 19.5 mg. The teats were then enlarged considerably. Twice daily milking was instituted on the 52nd day but the yield was only 0.5 to 1 cc. per day for 10 days. No treatment was given from the 79th to the 118th day. Then three small hard pellets of stilbestrol, a total of 71.75 mg., were implanted sub-

FIG. 4. a. Mammary gland section from castrate virgin kid 367 after 96 days treatment with stilbestrol. There is a well developed lobule alveolar system. The teat is enlarged. ×.64.

b. Enlarged microsection from mammary gland of goat 367 showing hypertrophied alveoli. ×3.

c. Mammary gland section from virgin kid 369 after 96 days treatment with stilbestrol. This gland is similar to that from goat 367 except that it appears more compact partially because no fluid was injected into the gland during fixation. The teat section here is incomplete. The teat from this gland was equal in size to that of goat 367. ×.64.

d. Dorso-ventral section with teat from gland of a 74 day pregnant kid and a lateral section from the same gland. This gland was flat compared with the globular shape of glands from goats 367 and 369. ×.64.

e. Enlarged section from gland of 74 day pregnant kid showing lobules. The alveoli in this gland were unhypertrophied clusters of cells. ×3.

f. Mammary gland and teat from a mature castrate male goat after 78 days injection of stilbestrol and 75 days with 71.75 mg. of subcutaneous pellets. The teat is obviously enlarged but the gland consisted of a short duct and a cluster of mammary cells at the base of the teat. ×.64.

g. Section of teat and mammary gland from an untreated castrate male goat. Compare size of teat with that in (f). The mammary gland consists of two very small clusters of cells at the base of the teat. ×.64.

cutaneously. A mammary gland was removed on the 292nd day which consisted of a teat cistern and a small clump of mammary cells a few millimeters in diameter. The absorption rate of 100 mg. stilbestrol pellets in women was found to be 0.127 to 0.25 mg. per day and they were effective for 400 to 800 days. Pellets were reported to be 5 to 10 times as effective

as injection on a dosage basis (5). Proportionally 0.09 to 0.18 mg. of stilbestrol should have been available to cause mammary development in this male goat but none occurred. The teat had developed, however, for it measured 4.5 cm. in length and was more than twice the length of that found in control male goats.

Turner and Gomez (27) found that the mammary gland of a six-months-old male goat was only one centimeter in diameter. That from a four-year-old male was 5 cm. in extent. The duct system extended little beyond the base of the teat, however. In exceptional males the glands may attain considerable development and may even lactate. A gland removed in this study from a ten-months-old male goat castrated at 8 months of age consisted of a teat cistern and a very small cistern at the base of the teat (fig. 4). This cistern was surrounded by several layers of epithelial mammary cells the extent of which was under 0.5 cm. The teat was two centimeters in length.

This study has shown that stilbestrol caused little duct development but extensive lobule-alveolar hyperplasia in a castrate and a normal kid treated subcutaneously for 96 days. Abundant and prolonged lactation occurred. Extensive stilbestrol treatment both by injection and with pellets in a mature castrate male goat failed to cause mammary development. Only the teats developed as did those of the kids treated.

DISCUSSION

Stilbestrol proved to be a very active mammary duct growth factor in mice, rats and rabbits. It was rather surprising to find that in female goats treated for 96 days instead of extensive duct growth there had occurred the proliferation of rather restricted lobule-alveolar systems. Early lobule development was found in the injected male rabbits and more extensive development in those with pellets but this only occurred after extensive duct growth. The ovaries could hardly have been a primary factor in the lobule development in goats, for one of the kids had been castrated before initiation of treatment. For the same reason the condition of the ovaries was probably not instrumental in initiating, through action on the pituitary, the lactation which occurred in these and the yearling goats. In fact the castrate kid responded more readily than the normal one and produced a greater amount of milk.

It remains to be seen whether extensive duct growth can be obtained in the goat with stilbestrol. In laboratory animals the male responds readily to estrogen treatment with proliferation of mammary ducts. The failure with stilbestrol in the male goat treated may have been due to inadequate dosage.

SUMMARY

Subcutaneous injections of stilbestrol at low dosages caused extensive

duct proliferation in male mice in 2 to 4 weeks. Mammary development did not proceed farther in spayed virgin female mice similarly treated. Oral administration of stilbestrol to male mice required approximately six times as high a dosage as by injection to obtain similar results.

Castrate male rats required a higher dosage of stilbestrol than did mice to obtain mammary duct growth.

Four-tenths gamma per day of stilbestrol subcutaneously was adequate to secure extensive mammary duct development in male rabbits. After 40 to 60 days treatment early lobule development was apparent. Percutaneous administration was also effective. Mammary glands from two rabbits with subcutaneous pellets had well developed lobule-alveolar systems and responded well to lactogen treatment at 90 days. Normal females tended to lactate on stilbestrol injection alone.

Subcutaneous injection of stilbestrol into virgin goats caused abundant and prolonged lactation from lobule-alveolar glands. Little increase in extent of glands was apparent. Subcutaneous administration followed by pellet implantation caused no mammary gland development in a castrate male goat, although the teats were hypertrophied.

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THE CONTENT OF GRASS-JUICE FACTOR IN LEGUME SILAGES AND IN MILK PRODUCED THEREFROM*

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In previous publications (1, 2, 5) from this laboratory it has been shown that many plant materials contain an unidentified water-soluble growth-promoting substance for rats and guinea pigs. Because of its abundance in young grass this substance has been called "grass-juice factor." Johnson *et al.* (3) found that this factor can be preserved by suitable methods of ensiling and that cows fed such silage produced a milk rich in the factor. In this paper are reported additional data on the effectiveness of various methods of ensiling for preservation of the factor and also data on the growth-promoting quality of the milk from cows fed a number of these silages.

EXPERIMENTAL

Guinea pigs weighing approximately 300 gm. each were fed a basal diet of mineralized milk supplemented with riboflavin as reported in the previous publication (3). This milk was obtained from cows that had been fed for several months a winter ration which was low in the grass-juice factor.

Silages. The silages fed were preserved in the various ways listed in table 1. Three types of containers were used: quart milk bottles, 40 and 50 gallon barrels, and regular silos.

Samples of the fresh forages at the time of cutting were dried 24 hours at 40° C., ground and stored in the refrigerator. When the bottles, barrels, and silos were opened, samples of the silages were dried, ground and stored in the same way.

Guinea pigs, usually two or more animals per group, were fed 3 gm. per day of the dried materials as a supplement to the basal diet. Orange juice was added to the dried silages to increase the palatability. Weight gains of animals receiving these supplements are given in table 1.

Milks. The growth-promoting qualities of milk produced by cows fed some of the preceding silages were determined. Three groups of five cows each were included in the experiment. Groups I and II each contained two Holsteins, two Guernseys and one Brown Swiss; Group III consisted of two Holsteins and three Guernseys. The ration was 39 lbs. of silage, 8 lbs. of alfalfa hay, and 8 lbs. of grain mixture per cow per day. Oats-peas silage made in different ways was fed to group I for varying periods of time as

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follows: molasses-preserved silage, 4 months; untreated silage, 5 weeks; phosphoric-acid-treated silage, 7 weeks. Alfalfa silage prepared with 20 lbs. of phosphoric acid per ton was fed to Group II for 4 weeks, the same forage prepared with 14 lbs. of acid per ton was fed for 5 weeks, and that with 8 lbs. of acid per ton was fed for 10 weeks. Group III received molasses-alfalfa silage for 5 months.

Each day all the milk from one milking of the cows in a group was mixed together, and a quart sample was taken. This sample was fed to a group of 3 or 4 guinea pigs. A small amount of the milk plus 1 mg. iron, 0.1 mg. copper and 0.1 mg. manganese was fed first in the morning, and after this had been consumed, an excess of milk was given. The average growth curves are plotted in figure 1. The growth curve of a control animal fed the basal winter milk is also included in the figure.

DISCUSSION

The increases in weight of the guinea pigs (table 1) show that the grass-

TABLE 1

Assay of silages for grass juice factor

Forage ensiled	Preservative used in ensiling		Weight gain of guinea pigs in 7 weeks
	Kind	Amount per ton	
			gms.
None	.....	.....	−30
Alfalfa	Fresh (not ensiled)	.....	123
Alfalfa	None	.....	22
Alfalfa	Molasses	60 lbs.	47
Alfalfa	Salt	10 lbs.	44
Alfalfa	Phosphoric acid	15 lbs.	88
Alfalfa	Soured whey concentrate* equal to whey at	600 lbs.	141
Alfalfa	Whey powder	80 lbs.	61
Clover-timothy (1–1)	Fresh (not ensiled)	.....	98
Clover-timothy (1–1)	None	.....	16
Clover-timothy (1–1)	Molasses	60 lbs.	50
Clover-timothy (1–1)	Prosphoric acid	30 lbs.	93
Oats-peas (1–1)	Fresh (not ensiled)	.....	76
Oats-peas (1–1)	None	.....	36
Oats-peas (1–1)	A.I.V. acid mixture	34 litres 2N acid	74
Oats-peas (1–1)	Molasses	60 lbs.	89
Oats-peas (1–1)	Phosphoric acid	20 lbs.	108
Soybean	Fresh (not ensiled)	.....	155
Soybean	None	.....	114
Soybean	Molasses	100 lbs.	103
Sudan grass	Fresh (not ensiled)	.....	93
Sudan grass	Molasses	40 lbs.	70

* Soured by *L. bulgaricus*.

juice factor of the forage was retained in varying degrees by different methods of ensiling. Acid-prepared silages were somewhat superior to molasses-

preserved silages in growth-promoting quality. With the exception of soybean silage, silages prepared without added preservative were rather low in the grass juice factor. Alfalfa preserved with soured whey concentrate gave especially good growth. Of the forages tried, soybean was the richest in the grass-juice factor.

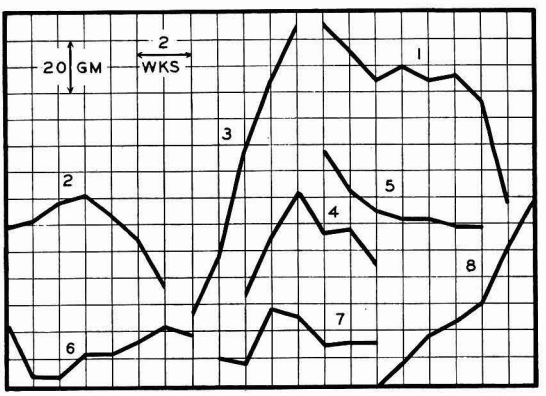

FIG. 1. Average growth curves of guinea pigs receiving various mineralized milks as follows: No. 1, control basal winter milk; No. 2, molasses alfalfa silage milk; No. 3, phosphoric acid alfalfa silage milk (20 lbs. acid per ton); No. 4, as No. 3 at 14 lbs. per ton; No. 5, as No. 3 at 8 lbs. per ton; No. 6, molasses oats-peas silage milk; No. 7, no preservative oats-peas silage milk; No. 8, phosphoric acid oats-peas silage milk (12 lbs. acid per ton).

From figure 1 it can be seen that good growth was obtained with milk produced from alfalfa silage that had been prepared with 20 lbs. of phosphoric acid per ton, but the use of smaller amounts of phosphoric acid resulted in poor quality milks. As reported elsewhere (4) better preservation of other constituents in the silage was obtained with the higher amount of phosphoric acid. With oats-peas 12 lbs. of acid per ton was sufficient to insure the presence of the factor in the milk but when this forage was ensiled with molasses, the milk produced from it was of low potency.

SUMMARY

The grass-juice factor of forages can be preserved in silage but the extent of preservation varies with different methods of ensiling. Silages prepared with phosphoric acid contained somewhat more of the factor than molasses-treated and untreated silages. Addition of soured-whey-concentrate to alfalfa gave excellent preservation.

The quantity of grass-juice factor in winter milk was increased by feeding silages rich in the factor, *e.g.*, silages preserved with adequate amounts of phosphoric acid.

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THE RELATIONSHIP OF pH TO SOME CURD CHARACTERISTICS OF MODIFIED MILKS

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In recent studies of the digestibility of milk several *in vitro* tests have been proposed in which stress has been laid upon the property of the curd particle size or the curd surface area. In the Chambers-Wolman test (1, 2, 3) the curd surface area is the index by which digestibility is gauged. In other methods reported by Hull (4) and Flora and Doan (5) although the measurement of protein degradation has been the basis of determining digestibility the importance of the size of the curd particles has been evident.

Among the factors which may influence the type of curd formation the pH at which coagulation occurs and the range of pH throughout digestion are of significance. This has been noted by several investigators (3, 4, 6). In the *in vitro* tests suggested, however, there have been considerable differences in technique with respect to the pH levels employed and possibly this has been a matter of greater controversy than any other single factor.

Available published reports concerning the conditions of acidity in the human stomach offer little to clarify the situation. The methods employed and the results obtained by several workers (6 to 15 inclusive) have differed so widely that the data appear too variable to warrant the formation of any definite conclusions.

Thus if the effect of pH on the coagula of different types of milk was proportionately the same it would make little difference what pH level was selected for *in vitro* tests. Preliminary work by the author indicated that some modified milks might react differently than others to pH changes. This investigation was undertaken to ascertain the behavior of various commercially modified milks at different pH levels.

EXPERIMENTAL

Samples: All samples used in the study were commercially prepared and included the following types of milk:

Untreated milk, both raw and pasteurized.

Homogenized milk (high pressure, piston type homogenizer).

Enzyme-treated milk (pancreatic enzyme extract).

Base exchange milk.

Evaporated milk, diluted 1:1 with water.

Curd surface area measurements: The Chambers-Wolman test (1, 2) with the modified technique as described by Anderson (3) was used for measurements of curd surface area. The samples were coagulated in thin-

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walled latex sacs, using sufficient coagulant to adjust each to the desired pH level. The coagulant consisted of a mixture of equal parts of 0.6 per cent pepsin solution (U.S.P. 1:3000) and normal hydrochloric acid. After a digestion period of thirty minutes with constant agitation the samples were emptied into individual containers and hardened with formaldehyde. After sieving and weighing the various size fractions of curd particles the relative surface area per gram of curd (S/gm.) was determined by calculation. The results reported are the averages of duplicate tests.

Measurements of total curd formation: In the Chambers-Wolman test the measurement of the total amount of curd formed is a necessary step in the calculation of the curd surface area. The same data were used in this investigation as a method of studying the relative bulkiness and completeness of curd formation at different pH levels. As a further guide to the completeness of coagulation the appearance of the samples at the end of the "digestion" period was observed. Complete coagulation was considered to have occurred only when there was a definite and complete separation of the sample into coagulum and whey.

pH values: A Beckman pH meter with remote electrodes was used and all readings were made at a temperature of 25° C. Each sample was tested at the following pH levels: 6.0, 5.5, 5.0, 4.5 and 4.0.

Total solids: Total solids were determined by means of a lactometer and butterfat tests.

All tests on any given sample of milk were performed on the same day.

RESULTS

Figure 1 shows the average amounts of curd recovered from the different types of milk in the Chambers-Wolman test throughout a pH range from 6.0 to 4.0.

The untreated, homogenized and enzyme-treated milks yielded somewhat similar results with the smallest amount of curd being recovered in each case at pH 5.0. Of these three types of milk the homogenized varied the least and tended to form a somewhat bulkier curd throughout the entire range. The amount of curd formed by the untreated milk was greatest at pH 6.0, dropped to its lowest point at pH 5.0 and then increased slightly as the pH was lowered further. The enzyme-treated milk showed the greatest variation and formed considerably smaller amounts of curd than the other two at pH 5.5 and pH 5.0. As judged by the amounts of curd recovered and the appearance of the samples these milks all coagulated completely throughout the pH range studied.

In base exchange and evaporated milk the effect of incomplete or partial coagulation was noticeably demonstrated. It is an established fact that these milks do not form any appreciable curd under the conditions of the curd tension test which is carried out at about pH 6.0. Likewise, when the

Chambers-Wolman test was performed at pH 6.0 the base exchange milk averaged 7.14 grams of curd while the evaporated milk did not form any curd. As the pH was lowered both types coagulated completely. In the case of base exchange milk this point was reached at about pH 5.5 and with evaporated milk at about pH 5.0.

Table 1 shows a comparison between the total solids content and the average amounts of curd recovered. This was done as a check against the effect of possible variations of the total solids of the different milks upon the amounts of curd formed in the Chambers-Wolman test. It is evident from the data that there was little relationship if any between the solids content of the milks and the amounts of curd recovered.

TABLE 1

The average total solids and the amounts of curd recovered in the Chambers-Wolman test at different pH levels

Type of milk	No. of samples	Total solids	Amount of curd recovered				
			pH 6.0	pH 5.5	pH. 5.0	pH. 4.5	pH 4.0
		%	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
Untreated	11	13.47	40.98	34.35	27.79	31.50	35.33
Homogenized	10	12.98	42.37	36.52	36.07	37.95	40.17
Enzyme-treated	11	13.03	39.21	26.25	23.17	33.62	38.50
Base exchange	10	12.57	7.14	30.13	30.47	32.33	39.08
Evaporated (1: 1)	10	13.78	0.00	27.64	52.54	54.17	52.09

In figure 2 are shown the average values for curd surface area throughout a pH range from 6.0 to 4.0. Also in this respect the untreated, homogenized and enzyme-treated milks followed the same general pattern with the values being low at pH 6.0 and increasing as the pH decreased. At pH 6.0 and 5.5 all three types of milk gave approximately the same low values. It was only at pH 5.0 or lower that there were appreciable differences.

The results with base exchange milk differed from the three just mentioned in that the curd surface area was comparatively high at pH 6.0 and dropped to its lowest point at pH 5.5. Undoubtedly this high surface area was due to partial coagulation of the milk. At pH 5.0 the curd surface area was approximately equal to that of the untreated milk and, while it increased somewhat as the pH decreased, base exchange milk still had the lowest surface area of all samples at pH levels of 4.5 and 4.0.

With respect to curd surface area the evaporated milk was superior to all types tested. No curd was formed at all at pH 6.0 while at pH 5.0 the values were generally very high. As with base exchange milk the high surface area at the upper pH levels coincided with partial coagulation. The lowest curd surface area occurred at pH 5.0 from where it increased slightly at pH 4.5 and showed a much greater increase at pH 4.0. Five different brands of canned evaporated milk were included in the study. There ap-

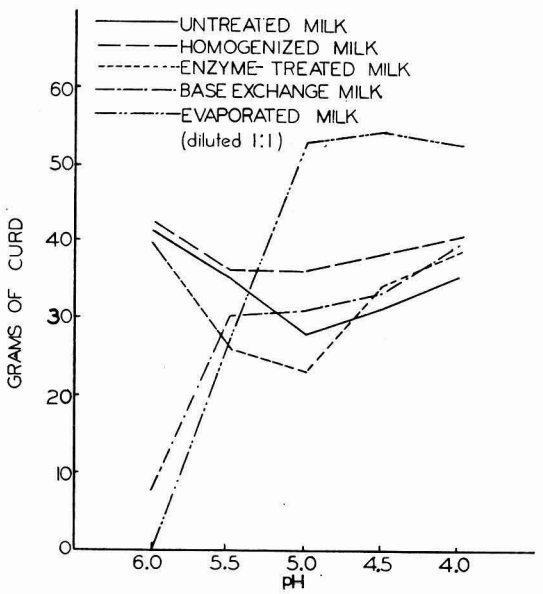

FIG. 1. The total amounts of curd recovered in the Chambers-Wolman tests throughout a pH range from 6.0 to 4.0. (Averages of all samples of each type of milk.)

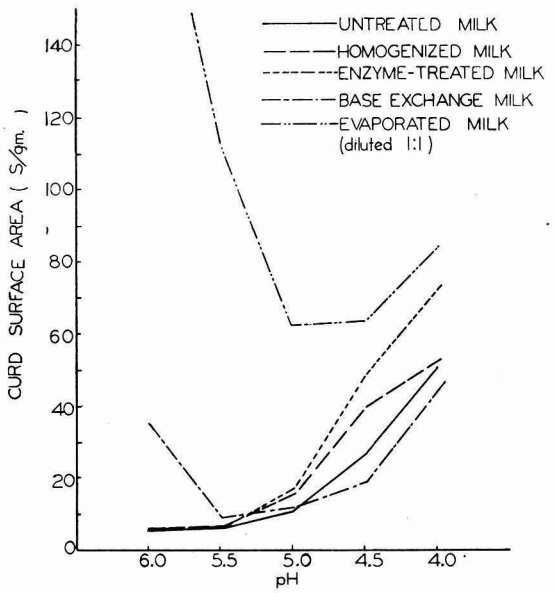

FIG. 2. The average curd surface area of modified milks throughout a pH range from 6.0 to 4.0.

peared to be some similarity of results within brands. However, the numbers of samples of each brand tested were too small to permit the formation of definite conclusions.

DISCUSSION

Considering the data with respect to the total amounts of curd formed in the Chambers-Wolman test it becomes apparent that there are two principal factors which will affect the results: 1) the completeness of coagulation and 2) the hydration of the curds or their affinity for water. Since there were no adequate means available for measuring either of these in a quantitative manner no attempt to do so was made in this investigation.

The completeness of coagulation was judged almost entirely by the appearance of the samples after "digestion" and was interpreted as being "no coagulation," "partial coagulation" or "complete coagulation" depending upon whether there was no curd formation, some curd formation or a complete separation of curds and serum. Under the conditions of the tests incomplete coagulation was observed only with base exchange and evaporated milks within the pH range studied. While the untreated, homogenized and enzyme-treated milks all coagulated completely at pH 6.0 and lower, it seems logical to expect that if even higher pH levels had been employed a zone would have been found in which they too would have exhibited varying degrees of partial coagulation. This statement is based upon the assumption that if coagulant were added to the milk in increasing increments it would be expected that coagulation would occur gradually.

The values reported as the total amounts of curd recovered in the Chambers-Wolman test are "wet" weights and therefore any variations in the hydration of the curd formations would have a corresponding effect upon results. While no specific method of measuring the degree of hydration was employed there seems to be no other logical explanation that could be offered for the variations in the amounts of curd formed at different pH levels in those samples wherein coagulation was complete. Certainly the total solids content (Table 1) was of little importance and it is not likely that at the pH levels employed there was any actual peptic digestion of consequence. With respect to the effect of pH upon the total curd formation, the general reaction seems to be determined by the inherent physical and chemical properties peculiar to each type of milk as a result of the method of modification employed.

When considering tests run throughout a range of pH levels there seemed to be a general relationship between the amount of curd formed in the Chambers-Wolman test and the curd surface area. This was particularly true at the upper pH levels where coagulation of the samples was more likely to be incomplete. Attention has been called to the effect of partial coagulation upon the values for curd surface area in the case of the base exchange and evaporated milks. It is interesting to observe that after these milks had

coagulated completely their values for surface area increased with a decrease in pH in much the same manner as the untreated, homogenized and enzyme-treated milks. Therefore, in the relationship of pH to curd characteristics one fact seems to hold true for all milks, *i.e.*, the curd surface area is lowest at the highest pH level at which complete coagulation will first occur. In the case of the untreated, homogenized and enzyme-treated milks this point is apparently reached somewhere above pH 6.0 and with base exchange and evaporated milk it is at about pH 5.5 and 5.0 respectively. Also, at any pH below that required to bring about complete coagulation, the curd surface area increases as the pH is lowered.

It has already been mentioned that there has been considerable variation in the *in vitro* techniques used by several investigators. This probably has been due to a lack of definite and conclusive knowledge of the coagulating conditions within the stomachs of human beings. Inasmuch as wide variations in the coagulating characteristics of milks occur within the pH limits thus far reported there does not seem to be any single pH level that is satisfactory for comparative tests on all milks.

CONCLUSIONS

In the Chambers-Wolman test the curd surface area of any milk appears to be lowest at the highest pH level at which complete coagulation will first occur. At any pH below that required for complete coagulation the curd surface area increases as the pH is lowered.

The effect of pH upon the bulkiness or completeness of curd formation is variable in milks modified by different processes. The method of modification seems to be the most important factor in determining this relationship.

With our present knowledge there does not seem to be any single pH level suitable for comparative *in vitro* tests on all milks.

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OBSERVATIONS ON DELAYED SALTING OF BRICK CHEESE[1]

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A few Brick cheese manufacturers have attempted to hasten the ripening of Brick cheese by delaying for several days the normal salting operation which usually occurs in the morning of the day following manufacture. Brick cheese must be salted after it is shaped into its characteristic form. Salting is accomplished by exposing the surfaces of the loaves to sodium chloride brine or to dry salt.

Examination of the literature on the salting of cheese shows that the flavor, body, texture and color of cheese, its rate of ripening and its composition can be affected by the method of salting, the amount of salt used, and by the time of salting.

The flavor of cheese can be affected unfavorably by unusual salting methods. If the cheese lacks salt, undesirable flavors may be produced (1, 4, 14). Bitterness in cheese has been attributed to a protein decomposition product, the presence of which can be traced directly to the salt content (20). It has been suggested (13) that the inhibiting effect of salt on the growth of lactic streptococci might explain the relation between salt concentration in cheese and cheese quality. The flavor developed during curing can be decreased by over-salting (1, 9, 17).

The body of cheese is sensitive to variations in salt content. Generally, over-salting tends to produce a hard, harsh body (1, 7, 17) while under-salting gives a pasty, weak body (1, 17). There is a range of concentration through which the salt content can be varied without noticeably affecting the body of the cheese (9); this range probably will vary with other factors affecting body such as type of cheese, moisture content and acidity. Studies of the peptizing effect of salt on rennet casein under different conditions of salt concentration and acidity indicate that the smoothness of Cheddar cheese should be favorably affected by the action of the salt on the paracasein in the pH zone between 5.5 and 6.0 (18). On the other hand, the fact that cheese protein is 100 per cent soluble in 3 to 10 per cent sodium chloride solutions within 7 days after making and remains soluble throughout the life of the cheese seems to justify the conclusion that the influence of salt on cheese quality cannot be caused by variation in the solubility of the protein in brine (12).

The texture of cheese can be made open by light salting and close by heavy salting (1, 4, 17). A white discoloration may be induced in some soft,

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ripened types of cheese by over-salting (14), and a similar fault has also been observed in Brick cheese (1).

Salt affects organisms inside and on the outside of cheese. Bacterial growth inside the cheese is delayed by early salting (3) and by variations in the amount of salt incorporated (4, 14). The presence of salt on the outside of types of cheese not unlike Brick seems to encourage the development of surface flora (7, 8).

The method of salting may influence the results obtained. Dry salting apparently reduces the weight of the cheese more than brine salting (2) and is not apt to be as uniform as brine salting (1). Salting cheese on the outside by either of these methods after the cheese is formed produces results not observed when the curd is salted before it is shaped (9).

The effects of salting on cheese characteristics may be associated with the rate of salt penetration. It has been observed that in Edam cheese 15 cm. in diameter the salt penetrated 5.5 cm. in 10 days (22); in Limburger, 8 to 10 days are required for practically uniform distribution (5, 15). Brine salted, whole milk Trappist cheese weighing 1.2 to 1.3 kg. showed uniform salt distribution in 60 days (16); and salt applied to the surface of Brick cheese requires approximately 8 weeks before it is uniformly distributed (1). Salt diffuses slowly in Cheddar cheese and penetrates most easily along the "grain"; within 12 hours after hooping the salt is essentially uniform (11), although the distribution of salt in cheese from the same vat may be surprisingly lacking in uniformity (10).

Some observations have been made on the influence of the time of salting certain types of cheese. In one study (3), one portion of curd was salted as soon as it was drained while identical curd was pressed 2 days and then salted in a brine bath. The early salting retarded bacterial development; delayed fermentation of lactose and acid development; and when there was incorporated more than 4 per cent of salt in the moisture of the cheese the cheese became hard, brittle and crumbly. The cheese salted after 2 days could absorb 8 per cent salt in the water of the cheese without injury. Salting curd 3 hours after draining gave results similar to those obtained by salting after 2 days. Other workers (8) reported that salting of Camembert cheese 1 day after making increases the dry matter; delays acid formation; increases the salt content; and hastens development of surface flora as compared to salting 2 days after making. The late salting causes slow salt penetration and, because the curd retains the whey, there is induced bitter flavor and whey sourness. When Brick cheese (1) is salted at 4 hours after dipping instead of the usual 20 hours, almost all of the characteristics of the cheese are affected. The acidity development is practically stopped, more moisture is retained; salt penetration is more rapid; and, although the flavor is not materially altered, the body of the early salted cheese tends to be curdy and hard and the texture is closer.

It is clear that salt influences the characteristics of cheese. The growth of organisms is affected by the salting treatments; the chemical substances produced in the course of the ripening process are determined in part by the salt; and the physical properties of the protein are influenced by the combined action of acidity and salt content. The smoothness of body observed after delayed salting treatments and the harshnes of body induced by early salting treatments are particularly significant in the making and curing of Brick cheese because this is a type of cheese which is most popular when the body has smoothness and good slicing properties. A few preliminary trials in this laboratory indicated that delayed salting induced differences which should be studied. The results of this study are reported here.

EXPERIMENTAL PROCEDURES

The manufacturing process used in these experiments was essentially that recommended by Spicer and Price (19). The experiments were made with raw and with pasteurized milk. Milk cultures of *S. lactis* were used for starters and a cooking temperature of 104° F. was therefore adopted.

The loaves of cheese from each lot of milk used in these experiments were divided into groups before salting. The cheese made from raw milk was divided into two groups one of which was salted in the usual manner on the day after making while the experimental group was salted on the 5th day after making. The cheese made from pasteurized milk was divided into three groups; the first or control group was salted the day after making; the second was salted on the 5th day; and the third group was salted on the 9th day after making. Each group was salted by holding in 23 per cent sodium chloride brine for 48 hours. All groups were kept at approximately 60° F. during salting, during the usual washing and until paraffining. All groups were paraffined on the 14th day after making and were then held at approximately 50° F. until the final grading.

Analyses for moisture (21) and acidity (pH) were made at 21 hours, just before salting, at paraffining and again when the cheese was 10 weeks old. Acidity measurements were made with a Leeds Northrup portable potentiometer using the quinhydrone electrode and saturated calomel half cell. Salt determinations (21) were made after salting and at paraffining and again when the cheese was 10 weeks old. These analyses were made by using the whole of a cross section slice of a loaf of cheese after discarding about $\frac{1}{4}$ inch of the rind layer. During the 5- and 9-day intervals before salting, the loaves of cheese were held in the 60° F. curing room and were moistened daily with water and rubbed to prevent mold growth.

All lots of cheese were graded at 14 days and again at 10 weeks of age.

RESULTS

The quality of the cheese is shown by the average grades listed in tables 1 and 2.

In table 1 are shown the grades of the 5 lots of cheese made from raw milk that were subjected to the different salting treatments. At two weeks of age the average quality of the cheese salted on the first day after making was practically identical except perhaps in flavor to that of the cheese salted five days after making. After 10 weeks of aging there was evident a slight margin of difference in favor of salting on the first day after making. The quality of the milk used in these experiments was not very good. All lots of cheese were criticized for off, sharp or unclean flavors after 2 weeks and for very unclean and strong flavors after 10 weeks of curing, regardless of the salting treatments.

TABLE 1

Effect of delayed salting on the quality of five lots of raw-milk cheese

Characteristic	Time of salting	
	1st day	5th day
	Average grades* at 14 days of age	
Flavor	3.5	3.7
Body	2.4	2.5
Texture	3.7	3.6
	Average grades* at 10 weeks of age	
Flavor	3.4	3.8
Body	3.2	3.4
Texture	3.2	3.4

* 1 = Excellent; 2 = Good; 3 = Satisfactory; 4 = Objectionable; 5 = Very Objectionable.

TABLE 2

Effect of delayed salting on the quality of three lots of pasteurized-milk cheese

Characteristic	Time of salting		
	1st day	5th day	9th day
	Average grades* at 14 days of age		
Flavor	2.7	2.7	2.3
Body	2.0	2.3	1.3
Texture	2.5	2.7	1.8
	Average grades* at 10 weeks of age		
Flavor	3.3	3.0	3.7
Body	2.7	2.3	2.3
Texture	2.7	2.3	2.3

* 1 = Excellent; 2 = Good; 3 = Satisfactory; 4 = Objectionable; 5 = Very Objectionable.

Early gas, evidently caused by organisms of the Escherichia-Aerobacter group, was present in every lot of raw-milk cheese regardless of the salting treatment. No definite relation could be observed between the time of salting and the degree of openness in the texture of the cheese. Despite the fact that lack of salt during the first 2 weeks of curing encourages the development of the splitting defect (4) in Brick cheese, this fault was not observed in the raw-milk cheese. The judges found a softness of body in the raw-milk cheese

salted 5 days after making which was not apparent in the cheese salted the day after making. This softness was of such a character that it was regarded as a defect.

The data of table 2 show the grades given the groups of cheese made from pasteurized milk. The grading of the cheese when it was 14 days old showed little difference in quality between those groups salted on the first and 5th day after making. The cheese salted on the 9th day was definitely better than that in the other groups. This improvement was found chiefly in a very desirable smooth, long body and a closer texture in the cheese. The delay in the salting operation for 9 days seemed to cause a more rapid disappearance of the normal curdy characteristics of the cheese. There was a slight improvement in flavor which was evident as a sweet or Swiss-like aroma in some of the lots salted 9 days after making.

After 10 weeks of curing the differences in quality between the groups observed when the cheese was younger had practically disappeared. The quality of all groups of cheese was regarded less favorably by the judges. These trends can probably be attributed to the quality of the original milk from which the cheese was made. The defects observed in the flavor were found in all three groups of cheese but the cheese salted 9 days after making had deteriorated most markedly. Regardless of the salting treatment all groups of cheese showed some mealiness of body. The texture of the cheese salted at 5 and 9 days after making was inferior to that of the control lot. Especially significant was the fact that the cheese salted on the 9th day showed some splitting.

The differences in quality recorded in tables 1 and 2 as the result of delayed salting might be caused by either biological or chemical changes or, more probably, both. There can be little doubt that delayed salting permitted the early and rapid growth of organisms which are ordinarily suppressed by the presence of salt. The marked differences in the body of the cheese, especially in the early stages of curing, reflect the effect of salt on the physical and chemical changes in the protein. It is well known in the industry that salt causes a firmness in cheese that cannot be attributed entirely to a lowered percentage of moisture. In addition to this effect, differences in the experimental cheese may be caused indirectly by the probable influence of salt upon normal acidity changes which must precede the breakdown of cheese curd.

The amount of salt in the cheese. The total amount of salt present in the cheese 10 weeks after curing was only slightly affected by the delay in salting as shown in table 3. The slightly higher concentration in the control lots can be explained by the use of 10 per cent sodium chloride brine to moisten the cheese during the interval of "smearing" before paraffining. Those lots given the delayed salting treatment received brine rubbing only after they had been salted; before salting only fresh water was used on them

for smearing purposes. It seems apparent that delaying the salting treatment influences the final salt content of the cheese so little that differences in the cheese must be primarily associated with the time interval between making and salting.

TABLE 3

Effect of delayed salting on the salt content of Brick cheese

Time of analysis	Time of salting		
	1st day %	5th day %	9th day %
	Raw-milk cheese*		
After salting	1.43	1.06	.....
At 14 days	1.90	1.76	.....
At 10 weeks	1.90	1.82	.....
	Pasteurized-milk cheese**		
After salting	1.12	1.08	0.84
At 14 days	1.66	1.67	1.56
At 10 weeks	1.73	1.67	1.67

* Average values for 5 lots of raw-milk cheese.
** Average values for 3 lots of pasteurized-milk cheese.

Cheese acidity. The effect of delayed salting on the acidity of the cheese is shown in table 4. These results if considered alone would lend support to the belief that delayed salting would hasten the ripening process. The curing of cheese is accompanied by an increase in pH value; such results are slightly apparent in the trend of data shown for the pasteurized-milk cheese and a little more apparent in the data on acidity changes in the raw-milk cheese. The differences are so small however that they can be disregarded, especially in view of the meager supporting evidence obtained in examining the quality of the cheese.

The pH values shown for the raw-milk cheese are higher at the 10-weeks' interval than those shown for the pasteurized-milk cheese. This result is to be expected in view of the decreased biological activity in the curing process following the heat treatment of the milk.

Losses of moisture during curing. The effects of the delayed salting on moisture losses during the curing process are shown in table 5. There is normally a downward trend in the moisture content of Brick cheese during the first 14 days of curing. This is caused by evaporation losses and by the incorporation of salt. The salt has a double effect in that it tends to decrease the water-holding capacity of the curd and because, as it is absorbed, it increases the dry matter. The combination of these effects as shown in the data of table 5 indicates that the delayed salting of the curd increased the losses of moisture between making and paraffining at 14 days of age. After paraffining the losses were practically identical regardless of the previous

TABLE 4

Effect of delayed salting on the cheese acidity

Time of observation	Time of salting		
	1st day pH	5th day pH	9th day pH
	Raw-milk cheese*		
Before salting	5.08	5.10	.....
After salting	5.03	5.17	.....
At 14 days	5.25	5.26	.....
At 10 weeks	5.39	5.43	.....
	Pasteurized-milk cheese**		
Before salting	5.08	5.06	5.14
After salting	5.05	5.08	5.13
At 14 days	5.13	5.17	5.19
At 10 weeks	5.20	5.26	5.27

* Average values for 5 lots of raw-milk cheese.
** Average values for 3 lots of pasteurized-milk cheese.

salting treatment and regardless of whether the cheese was made from pasteurized or raw milk. It seems probable that the softening of the body,

TABLE 5

Effect of delayed salting on losses of moisture

Losses during the interval:—	Time of salting		
	1st day %	5th day %	9th day %
	Raw-milk cheese*		
Before salting	0.0	1.2	.....
During salting	2.6	2.2	.....
Salting to 14 days	0.6	0.7	.....
14 days to 10 weeks	0.6	0.6	.....
Total moisture losses	3.8	4.7	
	Pasteurized-milk cheese**		
Before salting	0.0	0.8	1.1
During salting	1.6	2.3	1.7
Salting to 14 days	1.4	0.5	0.7
14 days to 10 weeks	0.9	1.0	0.9
Total moisture losses	3.9	4.6	4.4

* Average values for 5 lots of raw-milk cheese.
** Average values for 3 lots of pasteurized-milk cheese.

observed in the lots of cheese salted on the 5th and 9th days after making must therefore be attributed to protein changes rather than to the retention of more moisture.

CONCLUSIONS

Delayed salting as practiced in these experiments seems to have no real benefits to commend it to the practical operator. There is an apparent improvement in the body of the cheese at the end of the 14-day period during which the cheese is retained in the factory. By the time the cheese has been cured, however, this benefit has disappeared and the general quality of the resulting cheese is not as good as that of the cheese salted in the normal manner. The addition of salt soon after making probably establishes a desirable trend in flavor production and body changes in Brick cheese curd that does not happen when salting is delayed.

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THE RELIABILITY OF THE ROOM TEMPERATURE HOLDING TEST AS AN INDEX TO THE KEEPING QUALITY OF BUTTER

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The "room temperature holding test" is understood to include all butter keeping quality tests carried on by incubating small samples of butter for periods of from 6 to 10 days at temperatures of from 67° to 70° F. The actual practices in the various plants differ as to time and temperature but the general idea of predicting the keeping quality of butter in commercial channels on the basis of the flavor and odor developed at relatively high temperatures is common to all.

The need for such a test has been emphasized by a study made by Sprague, Foelsch and Small (1) of the butter offered on the large metropolitan retail markets. The survey was made of selected brands sold in one-pound cartons in New York and Chicago. In their conclusions the investigators stated that the instances in which deterioration had lowered the score more than one full point from the original score were few but still numerous enough to indicate that keeping quality is a serious problem. They state further that "For the purpose of identification of butter which lacks keeping quality and for the prevention of its use in cartons carrying certificates of quality, a wider use of incubation tests for keeping quality is desirable."

The relation of the time elapsed between grading and purchase and the loss in score showed that the time involved in the actual handling and sale of fresh butter supplies ranged up to 25 days, with an average of about 15 days. Since the butter was exposed to many temperature changes during transportation and finally in the retailers hands, the problem of stable butter quality was shown to be a very important factor in marketing.

PREVIOUS WORK

The prediction of the keeping quality of butter in ordinary commercial channels has been given increased attention in recent years probably due to the tendency toward lightly salted butter of higher flavor quality.

Hunziker (2) described two keeping quality or incubation tests involving holding small portions of butter at room temperature or higher. He stated that the tests are effective in revealing relative resistance of butter to flavor deterioration due to bacterial causes. He emphasized the use of the test in the prevention of surface taint by detecting fermentation in the small portions held in the test.

The value of the keeping quality test in the detection of faulty methods

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of production was indicated by Hammer (3). In a discussion of the development of methods of making keeping quality tests he emphasized the fact that the test probably can detect only those defects which are due to organisms rather than those due to chemical action. He states that "Temperature has a definite effect on the growth of organisms in butter, and a close correlation between the deterioration at various temperatures cannot be expected."

The use of a test involving parchment wrapped print butter was described by Parsons (4). The butter was held for fourteen days at 60° F. in a room of controlled humidity of 90 to 100 per cent. He found the test useful in the detection of butter of uncertain handling quality. He indicated that a test involving eight days at 70° F. gave equivalent results but that it required closer temperature control to prevent "oiling off" of the butter in certain seasons.

Sorensen (5) reported on an extensive survey of the keeping quality of both salted and unsalted butter in commercial channels by the use of the holding test. One-fourth pound parchment wrapped samples were held in a thermostatically controlled cabinet at 68° to 70° F. for seven days. The samples were examined for flavor and odor defects at the end of this period and reported as satisfactory, fair or unsatisfactory. A total of 22,060 churnings were examined representing more than 100 plants in eight midwestern states. The author states "a surprisingly close correlation between keeping quality tests and subsequent difficulty with the churnings tested was noted." The putrid-cheesy type of flavor defect was the most frequently encountered defect in the salted butter which showed unfavorable keeping quality. The value of the keeping quality test in locating contaminated water supplies or unsanitary plant conditions was pointed out.

Previous work at this station (6) has shown the relation between the numbers of bacteria in butter and the keeping quality at various temperatures. Similar high points in numbers of bacteria were developed in the incubation test for 7 days at 70° F., after 3 to 4 weeks at 40° F. and after 8 weeks at 32 to 36° F. The types of bacteria growing at these different temperatures varied and as might be expected the type of flavor varied with the holding temperature. It was found that lipolytic and proteolytic bacteria grew best at 40° F. as indicated by the fact that the counts reached higher levels at this temperature than at either the higher or lower temperatures. At 70° F. the lactic acid forming organisms usually predominated and their activity, no doubt, inhibited the growth of more objectionable types. The sour flavor developed at this temperature frequently was sufficient to mask other off flavors which were present and which would develop at lower temperatures.

THE PROBLEM

The holding test has been applied by many of the large butter manufacturers in recent years with good success. There has been a feeling on the

part of the operators, however, that the results of the keeping quality or incubation test has failed in certain cases to sort out properly all of the butter which was of uncertain quality. In some cases butter which failed to show definite deterioration in the incubation test would break down before it could reach the ultimate consumer while in other cases the deterioration developed in the incubation test was of a type which did not occur under the temperature conditions commonly found in butter warehouses and retailers' holding rooms. It was to obtain some information on possible causes of this lack of agreement that the following work was done.

PROCEDURE

The butter in this study was obtained from the educational scoring contests held at the station over a period of three years and included 78 lots representing 25 different creameries in South Dakota. The butter represented regular commercial churnings in some cases while in others it was made especially for the contests. All of the butter was salted with the salt content ranging from 0.5 per cent up to 3.0 per cent and averaging 1.8 per cent.

Samples were obtained with sterile spatulas and placed in 5-ounce glass jars with screw tops protected by parchment paper liners. Two samples were obtained from each tub, one to be held at 70° F. in a thermostatically controlled box, and one at 40° F. in the laboratory refrigerator. These lots were scored and examined for bacteria, yeast and mold when fresh and at intervals during the holding period. The scoring and microbiological analysis were done after 7 days at 70° F. and after 28 days at 40° F. All samples were tempered overnight to approximately 40° F. before scoring regardless of the temperature of holding to make the results of scoring more comparable.

The fresh butter scores were made by the official judges of the contests and by members of the department while the held butter was scored and criticized by members of the dairy department. The yeast and mold and bacteriological studies were made according to the methods suggested by the American Dairy Science Association Committee on Microbiological Analysis of Dairy Products (7).

RESULTS

In order to show the application of the Holding Test to the grading out or sorting out churnings of questionable keeping quality, the 78 lots were divided according to loss in score. In table 1 two methods of classifying the butter are presented. The average scores of the butter when fresh and the loss in score after holding are shown to permit a comparison of the keeping qualities of the two lots.

The results in table 1 under method A show the division of the butters into those showing less than one point loss in the holding test and those

TABLE 1

The comparative keeping quality of butter classified by the holding test

Method	Holding test Loss in score	Number of lots	Fresh score	Loss in score Holding test 7 days—70° F.	Loss in score 1 mo.—40° F.
A	less than 1 point	38	91.68	.10	.28
	1 point or more	40	92.36	2.12	1.44
B	1 point or less	55	91.92	.42	.58
	more than 1 point	23	92.32	2.80	1.91

showing one point or more loss. This method divided the 78 lots almost evenly. A slightly lower average fresh score was obtained in the class which showed less than one point loss. This might be expected because the deterioration in flavor score can be noted more easily when higher scoring butter is involved. The effectiveness of the holding test is indicated by the close relation between the holding test loss in score and the loss in score at 40° F.

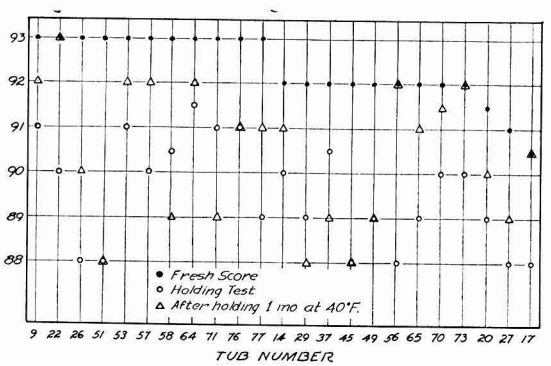

FIG. 1. Relation of "holding test" score to fresh score and to score after holding 1 month at 40° F.

(Lots losing more than 1 point in holding test)

Under method B in table 1 the butters were divided into two groups with only those showing more than one point loss being placed in the low keeping quality group. This division resulted in a smaller number of lots going into the low keeping quality group but the relation between groups was not greatly altered. The twenty-three lots which lost more than one point in the holding test were arranged in Fig. 1 according to the fresh butter scores to show the relationship between the holding test and the score after one month at 40° F. The results with individual lots are shown here to permit a study of the agreement between the test and the score of different lots after holding. In most cases the loss in score in the holding test and a loss during one month at 40° F. compare fairly well. In certain cases such as tubs num-

ber 22, 56, 73, and 17 losses in the holding test were not borne out in the butter held at the lower temperature. In three of these lots, cheesy or rancid flavors developed at room temperature but were not detected at the lower temperature within one month. A longer period at 40° F. might have brought out the flavor deterioration indicated in the holding test. As previously stated, however, it is probably more important to the butter manufacturer to know that the holding test finds the butters of uncertain keeping quality rather than to show absolute agreement with the record of each individual lot. It is apparent from these results that either method of classification results in a general segregation of those butters of low keeping quality from those of more satisfactory keeping quality.

There were exceptions to the general rule as indicated by the fact that seven of the fifty-five which lost one point or less in the holding test showed more than one point loss when held one month at 40° F. Six of these were given a score of 93 when fresh. Also eleven of the butters which lost more than one point in the holding test failed to show more than a point loss in the month storage at 40° F.

The value of the test in selecting butter of poor keeping quality is also indicated by a survey of individual lots in table 2 which showed that every lot which fell below 90 in score at the end of one month at 40° F. was classified in table 1 in the poor-keeping-quality group by the holding test. The greatest discrepancy between results appeared in those cases in which the holding test indicated poorer keeping quality than was found after holding at the lower temperature. Such lots as No. 17 and 56 which became cheesy at room temperature but held their original score at 40° F. were such cases.

The reason for such lack of agreement probably lies in the type of changes taking place in the butter at the different temperatures. The different types of flavors which developed under different holding conditions are shown in table 3. It may be noted that such flavors as feed, old cream and acidy were much more prevalent in the fresh butter than after holding. Flavors such as stale, cheesy or rancid were most marked after the room temperatures holding test, while the flavors developed after one month at 40° F. were storage, coarse, stale, oily and rancid. These results indicate that the room temperature holding resulted in more bacterial deterioration than the lower temperature holding which permitted chemical action but limited the bacterial action. The difference in the flavors produced appears to indicate this conclusion.

The study of the numbers of lipolytic or fat splitting bacteria and proteolytic or casein digesting bacteria supported the viewpoint expressed above. These types of organisms were absent in most of the plates made from fresh butter but after 7 days at room temperatures, large numbers of proteolytic bacteria were sometimes found. The presence of these types was found to be associated with the development of cheesy flavor in most cases. At the

TABLE 2

The "Holding Test" and loss in score after one month at 40° F.

Tub number	Fresh butter		Holding test 7 days at 70° F.		Held one month at 40° F.	
	Score	Criticism	Score	Criticism	Score	Criticism
1	92	old cream	92	sl storage	92	sl storage
2	92	old cream	92	coarse	91	stale
3	92	sl oily	91	sl stale	91	stale, briny
4	93		92	flat	92	
5	93		92	chem	92.5	
6	92	sl feed	92	briny	92	briny
7	93	sl bitter	92	flat, briny	91	stale
8	91.5	old cream	92		91.5	sl stale
9	93		91	tallowy	92	
10	92.5	sl bitter	92	coarse	92	coarse
11	91	bitter	91	tallowy	91	sl stale
12	93		93		92	sl stale
13	93		92	sl stale	92	sl stale
14	91.5	old cream	90	sl cheesy	91	stale
15	92	old cream	92	sl stale	91.5	sl storage
16	91	briny, old cream	91	coarse	91	coarse
17	90.5	briny, old cream	88	sl cheesy	90.5	old cream
18	93		93		93	
19	93		92	sl acidy	93	
20	91.5	stale cream	89	unclean	90	stale
21	89	neutralizer	89	storage	89	storage
22	93	acidy	90	sl rancid	93	
23	93		93		93	
24	90	neut. weedy	89	stale	90	briny, weedy
25	91	neut. coarse	91	coarse	91	briny, grassy
26	93		87	rancid	90	flat, sl oily
27	91	cooked, sl uncl	88	unclean, acidy	89	unclean
28	93		93		91	storage
29	92	flat, sl old cream	89	moldy	88	sl rancid
30	93		92.5	flat	93	flat
31	93		92	sl storage	92	
32	91.5	old cream	92	coarse	91.5	storage
33	91	sl unclean	90	stale, sl rancid	90	storage
34	92	sl coarse, burnt	92.5		91.5	sl malty
35	91.5	acidy	91.5	acidy	91	briny, acidy
36	92	briny, acidy	91.5	briny	92	
37	92	sl acidy	90.5	acidy, stale	89	sl moldy
38	91	acidy	92	coarse	91	coarse
39	92	sl flat	93		92	
40	93		93		91	stale
41	92	briny, acidy	92.5		91	coarse
42	90	briny, metallic	91	briny	91.5	briny
43	92	acidy	91.5	storage	92	
44	92	acidy	92	acidy	91	sl flat
45	92	cooked	88	rancid	88	stale, fishy
46	91	burnt	91.5	stale	91	coarse
47	91	sl unclean	91	sl unclean	91	sl stale
48	92	sl acidy	92		91.5	briny
49	92	acidy	89	oily, acidy	89	stale
50	92	sl utensil	91	flat	93	
51	93		88	cheesy, oily	88	fruity, rancid
52	92.5	sl barny	92.5		92	coarse
53	93		91	sl fruity	92	sl stale
54	92	sl feed	91	sl unclean	92	

TABLE 2—(*Continued*)

Tub number	Fresh butter		Holding test 7 days at 70° F.		Held one month at 40° F.	
	Score	Criticism	Score	Criticism	Score	Criticism
55	93		92	flat, sl tallow	93	flat
56	92	utensil	88	cheesy, rancid	92	
57	93		90	stale	92	
58	93		90.5	bitter, metallic	89	rancid
59	90.5	stale, malty	92	flat	92	
60	90	met., burnt, neut.	91	sl tallow	91	old cream
61	93		92	sl storage	92	sl storage
62	93		93		93	sl coarse
63	91.5	sl musty	91	sl stale	90	oily, stale
64	93		91.5	sl stale	92	sl storage
65	92	wintery	89	sl cheesy	91	stale
66	93		92		91.5	coarse
67	90	burnt, metallic	92	coarse	91	briny, old cream
68	93	heated	92		90	oily, neut.
69	92	briny	91	sl stale	91	storage
70	92	sl coarse, feed	90	sl cheesy	91.5	coarse
71	93	sl heated	91	sl stale	89	woody
72	91	burnt, malty	92	sl bitter	91	burnt, old cream
73	92	coarse, briny	90	stale	92	coarse
74	93		92		91	flat, oily
75	91	malty	92	feed	92	briny
76	93	sl feed	91	sl stale	91	feed, stale
77	93		89	cheesy	91	woody
78	92	stale	91	sl stale	92	sl stale

TABLE 3

Flavor criticisms used in scoring butter

Flavor criticism	Fresh score	Holding test 7 days—70° F.	Held 1 month 40° F.
Number of lots scored	78	78	78
No criticisms	20	16	15
slight old cream or old cream	8	0	3
slight acidy, acidy or coarse	14	13	11
briny	6	4	9
slight feed, feed or weedy	5	1	1
neutralizer	3	0	1
slight unclean or unclean	3	4	1
Cooked, heated	4	0	0
tallowy	0	4	0
slight storage or storage	0	5	9
slight stale or stale	2	15	18
slight cheesy, cheesy or fruity	0	8	1
utensil	4	0	0
slight rancid or rancid	0	5	3
oily, stale	1	2	4
malty	3	0	0
moldy	0	1	1
burnt	4	0	0
fishy	0	0	1
bitter	3	1	0
flat	3	2	0
woody	0	0	2
metallic	3	1	0

lower temperature of 40° F. there was very little evidence of bacterial activity which could be directly associated with flavor deterioration although large numbers of proteolytic bacteria were occasionally found after one month at 40° F. Lipolytic bacteria were absent except in a few lots in which small numbers, usually less than 1000 per ml. were found by the plate method.

Yeast and mold counts on the fresh butter failed to show any relation to the keeping quality of the butter in these trials. This is in agreement with the statements of numerous investigators working on this problem. The number of yeasts was generally high in the butter held at room temperature for 7 days but no correlation with the flavor deterioration could be noted.

CONCLUSIONS

In conclusion, these results indicate that the holding test is useful and fairly accurate as a means of detecting butter of unstable handling quality. The chief factor influencing the reliability of the test appears to be the difference in activity of certain types of bacteria at the incubation temperatures and at lower temperatures.

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A NEW DILUENT FOR BOVINE SEMEN

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A desirable diluent for semen is one that will maintain fecundity of the spermatozoa for many days before it is used for breeding purposes.

Considerable experimental work with diluents versus no diluents for semen has been done in Europe and the United States. The use of egg yolk lecithin in a diluent for semen by Milovanov and Selivanova (2) in Russia, and the use of egg yolk by Phillips and Lardy (4) of Wisconsin has proved helpful in keeping sperm cells viable for some time. The work of Milovanov (2, 3) and Phillips and Lardy (4) suggested an investigation of a combination of gelatin, egg yolk, buffer salts and water, as a diluent material in the artificial insemination studies in progress at the Ohio Agricultural Experiment Station. It is believed that gelatin (Knox) tends to hold sperm inactive, assists in keeping the particles of the egg yolk and the sperm in suspension, supplies extra nutrients, and retards general contamination (bacteria and molds) during storage.

Preliminary work done at this station with the gelatin and the non-gelatin diluents has given encouraging results in favor of the gelatin. Two series of samples have been studied. In the first series 12 samples of bovine semen were diluted four times with a diluent containing 2.14 gm. of gelatin (Knox), 0.2 gm. of KH_2PO_4, 1.325 gm. of $Na_2HPO_4 \cdot 12\ H_2O$, 100 cc. of sterilized distilled water, and 100 cc. of fresh egg yolk. The non-gelatin group consisting of five samples of bovine semen were diluted four times with a diluent containing 0.2 gm. of KH_2PO_4, 1.325 gm. of $Na_2HPO_4 \cdot 12\ H_2O$, 100 cc. of sterilized distilled water, and 100 cc. of fresh egg yolk. The materials other than the egg yolk of both diluents were first dissolved in the water before adding the egg yolk. The range in pH was from 6.7 to 6.85. The vials containing the diluted samples were wrapped in cotton and placed in a refrigerator maintained at 4° to 6° C. Periodical examinations were made with a microscope after a drop of the diluted semen was placed on a glass slide and warmed to 37° C. The gelatin dilutions maintained some sperm motility for an average of $21\frac{1}{2}$ days (range 18 to 30 days), whereas the non-gelatin mixture maintained some sperm life for an average of $14\frac{1}{2}$ days (range 14 to 16 days).

The technique of handling the semen in the second series was as follows: Collection and dilution of the semen was carried out under sanitary conditions; diluted samples were gradually cooled from 30° to 5° C. for storage purposes; and periodical examinations were made of the stored samples with a compound microscope (×440) after a small portion was rediluted and

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gradually warmed from 5° to 37° C. The gelatin and non-gelatin diluents were the same as those used in the first series, except that different amounts of gelatin and $Na_2HPO_4 \cdot 12\ H_2O$ were used from time to time. The range in pH was from 6.45 to 6.9. The results are given in table 1.

TABLE 1

The effect of gelatin and no gelatin in a diluent upon motility of bovine spermatozoa in storage

Diluent contains	Number of samples	Per cent of sperm motile after 2 to 4 days	Average number of days when		
			50 per cent motility was observed	25 per cent motility was observed	All cells were dead
Gelatin (Knox)	26	73 (57–83)*	12.5	17.5	26.5 (16–35)*
No gelatin	14	59 (33–80)*	8.3	14.5	26.0 (14–38)*

* Figures in parentheses represent range.

Maintaining motility in 50 per cent of the spermatozoa an average of four days longer than previously possible and keeping a few alive for 35 to 38 days is stimulating to future work.

According to the literature the previous record for keeping motile bovine sperm in a diluent following collection seems to be 12.5 to 13 days (1, 4).

Artificial breeding of a large number of cows with semen that has been diluted with these two diluents is now in progress.

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SOME OCULAR CHANGES AND DEFICIENCY MANIFESTATIONS IN MATURE COWS FED A RATION DEFICIENT IN VITAMIN A*

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In previous publications (1, 2, 3) a type of blindness has been described which occurred in calves fed low vitamin A rations. The blindness was associated with a constriction of the optic nerve, nyctalopia, and papilledema. The cause of the papilledema was later established as directly due to an increased intracranial pressure (4) in vitamin A deficiency. This type of blindness has never been reported as occurring in the mature bovine because, as previously explained (2, 3), the optic foramina are fully developed and calcified. However, papilledema and nyctalopia develop as well as certain other ocular changes. It is the purpose of this paper to report the ocular changes and deficiency manifestations where the mature bovine was fed a ration deficient in vitamin A.

EXPERIMENTAL

Mature cows were used in this experimental work. They were placed on the low carotene ration previously used with calves which consisted of, 36.0 per cent barley, 27.0 per cent rolled oats, 27.0 per cent wheat bran, 9.0 per cent linseed oil meal, and 1.0 per cent salt. This ration contained from 0.5 to 0.7 micrograms of carotene per gram so that the animals received 2 to 3 micrograms per pound of body weight from this source. Viosterol and sunshine were used as sources of vitamin D. Wood shavings were used as bedding.

Ophthalmoscopic observations were made at various intervals and the animals were tested for night blindness by attempting to run them into objects, and watching their behavior in dim light, a method similar to that used by Guilbert and Hart (5). Blood plasma carotene determinations were made at intervals by a method previously described (6). Carotene extractions on the hays and feeds used were made according to the modification by Peterson *et al.* (7) of the Guilbert method and the concentration of the extract determined by a photoelectric colorimeter.

Before proceeding further it would probably be of assistance to the reader to explain the first three figures. Figure 1 shows the normal bovine fundus. The nerve head or papilla is seen in the center; the tapetum lucidum which consists of the upper yellow part of the retina and the

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tapetum nigrum the lower dark part of the retina are seen surrounding the papilla. There are of course individual variations of the normal fundus in the outline, color and distinctness of the nerve head, the color of the tapetum lucidum arrangement of the vessels, etc. The tapetum nigrum is always of a dark shade. The color of the tapetum lucidum is affected by the amount of exposure to bright light. The normal yellow color as shown in figure 1 will become bleached after the animal has been exposed to bright light such as the sun. These figures were made from animals not exposed to bright light.

Figure 2 shows the mottled appearance of the tapetum nigrum. The tapetum lucidum is bleached and the nerve head shows a cottony white appearance, evidence of papilledema.

Figure 3 shows the mottled appearance of the tapetum lucidum and papilledema. The mottled condition as illustrated in figures 2 and 3 occurs only in the more mature animal or after about 18 months of age.

A1 was a 5-year-old grade Holstein cow which had been receiving a ration in which the sole source of vitamin A was yellow corn. This ration, while apparently adequate for maintenance, had not contained sufficient vitamin A for proper reproduction. The vitamin A reserve for this animal was therefore probably much less than for an animal which had been receiving hay. Further, she had been milking on this ration up to the time she was placed on this experiment. The eyes were normal when placed on the low vitamin A ration except for a narrow violaceous area, $1\frac{1}{2}$ cm. in length, along the temporal vessels.

After 18 days on the low vitamin A ration there was definite nyctalopia, the nerve heads of both eyes were somewhat hazy in appearance, and the tapetum nigrum showed some slight mottling as illustrated in figure 2. By 53 days the margins of the nerve head were definitely indistinct, but not markedly edematous, the mottling of the tapetum nigrum had increased, and the tapetum lucidum was somewhat bleached. At 81 days alfalfa was added, supplying 14 micrograms of carotene per pound of body weight. At 116 days this cow was no longer night-blind and the plasma carotene had increased from 0.18 to 0.4 micrograms per milliliter. The alfalfa was eliminated from the ration at 133 days and at this time the animal got out and obtained a good fill of green grass so that the plasma carotene increased from 0.4 up to 0.9 micrograms per milliliter. This accounts for the relatively long time it took to develop nyctalopia again.

At 236 days this animal again became nyctalopic and the edema of the nerve heads increased so that there was a choking of 2 diopters in each eye. At 249 days carotene was again added in the form of alfalfa at the rate of 14 micrograms per pound of body weight and at 269 days she was no longer night-blind. At 291 days both nerve heads were still edematous but the mottled appearance of the tapetum nigrum had quite largely disappeared.

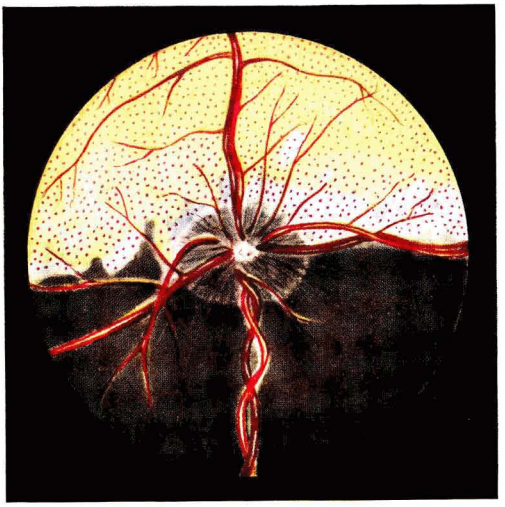


FIG. 1. Normal bovine fundus.

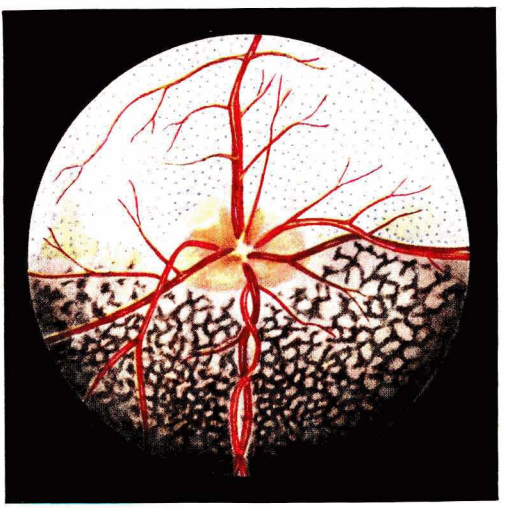


FIG. 2. **Fundus** showing papilledema, bleached tapetum lucidum and a mottled tapetum nigrum.

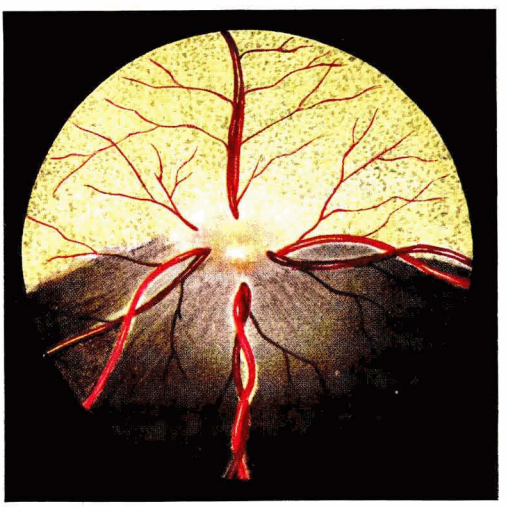


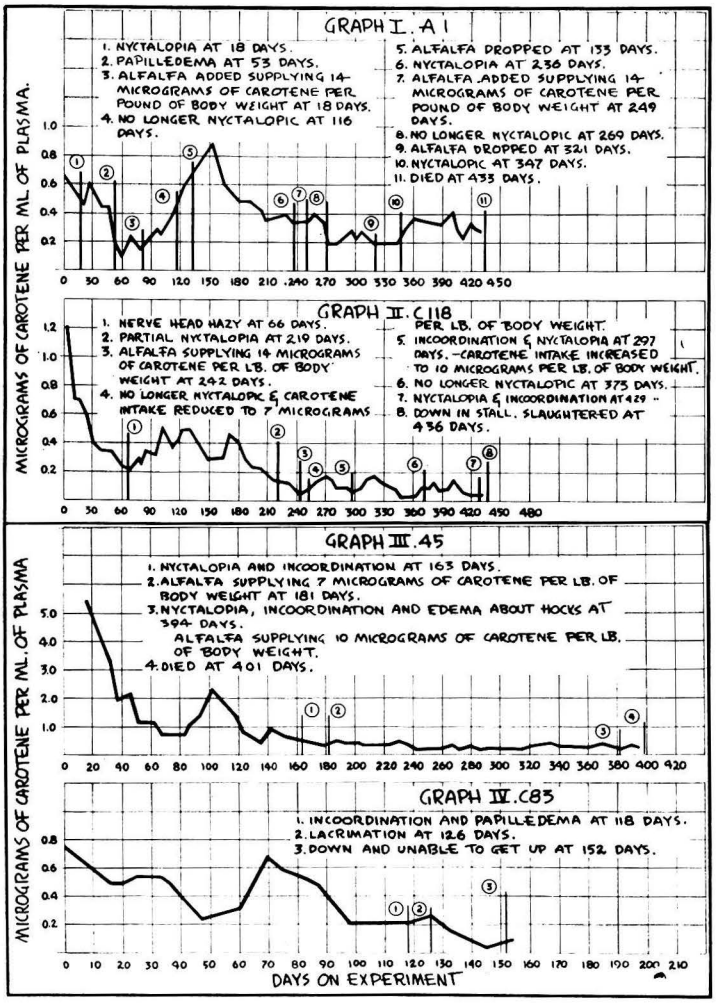
FIG. 3. Fundus showing papilledema and a mottled tapetum lucidum.

At 321 days the alfalfa was again eliminated from the ration. At 347 days she was again night-blind and by 421 days the edema had increased so that there was a choking of 3 diopters in both eyes and marked mottling of the tapetum nigrum. The cornea was slightly opaque and there was considerable lacrimation. At 411 days she was quite weak, showed marked incoordination and died at 433 days. The principal results along with the variations in level of blood plasma carotene are shown in graph I. Post-mortem examination revealed considerable pneumonia and other lesions associated with vitamin A deficiency which will be reported in a subsequent paper.

Cow C118 was a six-and-one-half-year-old Holstein cow which had been on a grain ration containing corn gluten and yellow corn as a source of vitamin A. This cow had been dry for a considerable period and calved about three weeks before being placed on the low vitamin A ration. This animal was dried up about three weeks after being placed on the low vitamin A ration so that she was milked for only about six weeks. Consequently, she probably had some storage when placed on the low carotene ration. The principal results are shown graphically in graph II.

Twenty-nine days after being placed on the low carotene ration, the tapetum nigrum of each eye showed definite mottling. At 66 days the papillae were quite hazy in appearance, but showed no elevation. At 95 days both nerve heads were hazy, but the margins could still be seen and there was no apparent elevation. She did not appear quite so active. At this time C118 got out of the pen at night and obtained some green material which probably delayed somewhat the later changes. At 165 days the tapetum lucidum was bleached and the tapetum nigrum quite mottled. At 219 days there was some indication of nyctalopia. At 230 days the animal showed considerable incoordination and a poor appetite. The nerve heads showed slight edema, but the margins were still discernible. There appeared to be little or no elevation. Alfalfa was added at 242 days to suply 14 micrograms of carotene per pound of body weight because of the extreme incoordination. At 262 days she was no longer night-blind, and the alfalfa intake was reduced to supply 7 micrograms of carotene per pound of body weight. At 276 days she no longer showed indications of incoordination and was fairly active although there was some edema in the rear legs. At 297 days she again manifested night-blindness, incoordination and had a rough appearance. At this time, the alfalfa was increased to 10 micrograms of carotene per pound of body weight. The incoordination largely disappeared at this level of intake but she remained partially nyctalopic till the 373rd day. The plasma carotene, however, remained exceedingly low at this level, and at 429 days nyctalopia and incoordination were again manifested. At 433 days the nerve head showed some edema, but the margins were still discernible. There was marked mottling of the tapetum nigrum and slight mottling of the lucidum as shown in figure 3. The tapetum lucidum was also

bleached. At 436 days the animal was quite weak, showed marked incoordination, got down in her stall and was unable to get up. The next day she was slaughtered in order to save the tissue for pathological study.



Cow 45 was a three-and-a-half-year-old Guernsey cow which had received a normal ration. She was placed on the low carotene ration the middle of June and had received some pasture so that the plasma carotene was quite high. After 163 days on the low carotene ration this animal showed nyctalopia, a slight bleaching of the tapetum lucidum, and was unsteady on her feet. The carotene in the blood plasma had decreased to 0.5 micrograms per

milliliter at this time as shown in graph III. After 172 days there was some yellowish discoloration just dorsal to the nerve heads. At 181 days alfalfa leaf meal was added to supply 7 micrograms of carotene per pound of body weight or one-half the minimum requirement. At this level she continued to show considerable incoordination and remained nyctalopic. At 349 days the tapetum lucidum was bleached somewhat but the nerve heads showed no evidence of papilledema. At 381 days the animal was unsteady on her feet, and there was considerable edema about the feet and legs. The carotene intake from the hay was then raised to 10 micrograms per pound of body weight.

At 394 days the tapetum lucidum of both eyes was entirely bleached and she walked slowly because of the edema about the feet. She died after 401 days on the low carotene ration without showing evidence of papilledema.

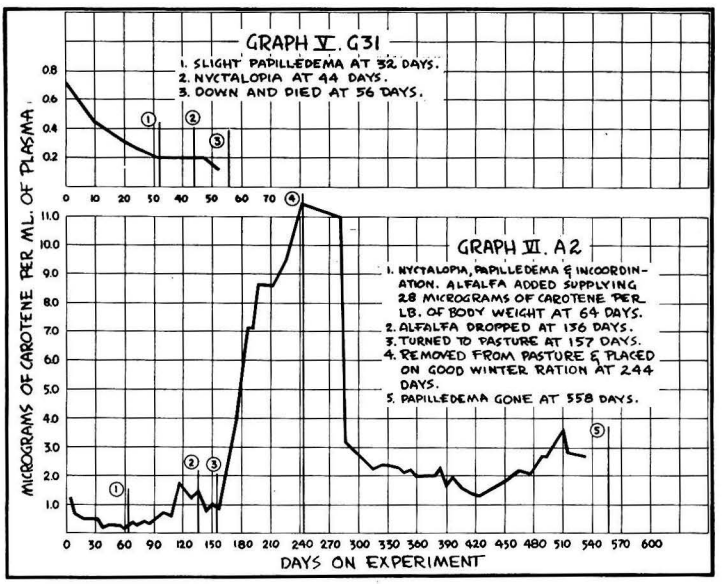
At post-mortem, aside from the changes due to vitamin A deficiency, the most notable phenomenon was the yellow color of the fat in the various parts of the body. A sample of the fat was weighed out, saponified with alcoholic KOH and extracted with petroleum ether. The petroleum ether was extracted with 92 per cent methyl alcohol to remove any xanthophylls. The extract showed a carotene content of 19 micrograms per gram. Further, this animal was in good condition and showed considerable deposition of fat in the mesentery. Post-mortem examination also showed the presence of pneumonia.

Cow C83 was an exceptionally fat seven-year-old Holstein which had previously been receiving a ration of skimmed milk, yellow corn, oats and viosterol. The ration had always been kept rather low in vitamin A during most of this animal's life. At the time this cow was placed on the deficient ration no routine ophthalmoscopic examinations were being made, nor was she tested for night blindness.

After 118 days on the low carotene ration marked incoordination was noted and examination of the eyes revealed an extreme papilledema. At 126 days excessive lacrimation was noted. At 145 days the appetite was poor and the plasma carotene had decreased to a 0.05 level as illustrated in graph IV. At 152 days she got down and was unable to get up. It was necessary to sacrifice the animal two days later because she could not get into position to eat.

G31 was a seven-year-old cow which had previously been receiving a ration in which the sole source of vitamin A was yellow corn. She was in excellent condition when placed on the low carotene ration and the eyes appeared normal. Thirty-two days after being placed on the low carotene ration the nerve heads were blurred in appearance. There was one diopter of papillary edema at 44 days in the right eye and 2 diopters in the left and the nerve head showed considerable vascularity. At this time she was also nyctalopic and walked rather slowly and stiffly. At 52 days there were 2

diopters choking in each eye. At this time she got down in the yard and was unable to get up alone. At 56 days she got down in the stall and was unable to get up. She was removed to a box stall and carotene in oil and linseed meal was administered by stomach tube. Cod liver oil was also given subcutaneously. She never regained her feet, however, and died during the night of tympanites. These observations as correlated with the level of blood plasma carotene are shown in graph V.



A2 was a five-year-old Holstein cow and had been receiving a grain mixture containing yellow corn as the only source of vitamin A. Ophthalmoscopic observations were not made on this animal until 64 days after she had been placed on the low carotene ration. At this time there was a choking of 2 diopters in the right eye and 4 diopters in the left and the nerve margins were entirely covered with the edema. She also showed considerable incoordination at this time and there was some suspicion that she was night-blind. The plasma carotene had decreased to 0.15 micrograms per milliliter. At this time alfalfa supplying 28 micrograms of carotene per pound of body weight was added to the ration. The alfalfa was dropped from the ration at 136 days. At 157 days the eye conditions remained the same and she was turned to pasture for the purpose of noting how long it would take these changes to clear up. It will be noted that the plasma carotene increased with this change as shown in graph VI. At 244 days she was removed from pasture and placed on a ration of alfalfa hay and corn silage which was followed by a rapid decline in plasma carotene. By 273 days the edema of

the nerve head had receded some and the nerve fibers could be seen.[1] At 341 days the nerve head had lost most of the cottony white color associated with the edema and had taken on a darker color which was more nearly normal. By 422 days the right eye was about normal but the margins of the left eye were still somewhat indistinct. By 558 days no edema could be seen in either eye and the nerve margins were fairly distinct. The nerve head, however, was still somewhat elevated since the readings were one diopter in the right eye and 2 diopters in the left. This was probably a residual effect of the long continued papilledema.

DISCUSSION

The results obtained with the animals of this experiment show that none developed the permanent type of blindness due to constriction of the optic nerve such as occurs in calves (1, 2, 3) on vitamin A deficient rations. Several of these animals were permitted to develop extreme deficiency symptoms yet never developed the blindness. Insofar as the author is aware, blindness has never been reported as developing in a mature bovine due to a constriction of the optic nerve associated with vitamin A deficiency. The explanation of this observation as previously set forth (2, 3) appears to be due to the fact the bony optic canal grows in length from one-fourth inch in a young calf to about one and a half inches in a mature animal. In vitamin A deficiency in a calf the normal growth processes are affected in such a manner as to cause a stenosis of the bony canal with a consequent constriction of the optic nerve (1, 2, 3). Wolbach and Bessey (8) have noted an overgrowth of the central nervous system in vitamin A deficiency in young rats. They noted the presence of herniations of the cerebrum, cerebellum and posterior colliculus with changes in the contours of the fossae of the floor of the skull due to bone resorption. If such an overgrowth of the central nervous system takes place in the bovine species it could easily account for the increased intracranial pressure reported from this station (4).

The results likewise show that papilledema does not develop as readily in mature animals as in young calves. Animal 45 did not develop evidence of papilledema while the nerve head of C118 showed only a slight hazy appearance. Usually the papilledema did not develop until considerable incoordination was present. Unpublished data indicate that the differences are probably explained by individual and age variations of intraocular tension. It would seem that a higher intracranial pressure would be necessary to overcome a high intraocular tension than a low one in order to permit the development of papilledema.

Besides the presence of papilledema in cows fed vitamin A deficient

[1] Papillary edema existing for any length of time results in secondary atrophy (postpapillitic atrophy).

rations, certain other changes were observed with the ophthalmoscope. These consisted of a mottled appearance of the tapetum nigrum and occasionally a mottled appearance of the tapetum lucidum. These two alterations are illustrated in figures 2 and 3 and may be compared with the normal fundus shown in figure 1. Both these conditions were cleared up by administration of some source of vitamin A. Usually the changes were more easily observed and were more marked in the nigrum than in the lucidum. The micropathological alterations of the retina associated with these changes have not been investigated.

The papilledema of animal A2 took considerable time to recede even though she was turned to pasture or kept on good winter feed. The observation was duplicated in another animal not considered in this report. In older calves the same was true but not to such an extent. It has also been noted in calves that the intracranial pressure takes considerable time to return to normal after the return of carotene to the ration (4).

It is interesting to note that animal 45, a Guernsey, had a very yellow fat at autopsy. The pigment was epiphasic between petroleum ether and 92 per cent methyl alcohol so that it was most likely carotene. These results seem paradoxical in view of the fact that the animal showed marked symptoms of vitamin A deficiency. One must conclude that the animal was unable to draw extensively from this store of carotene.

Another interesting observation was that the more flesh the animal carried at the time the deficiency started to show up, the quicker the animal succumbed to the deficiency. Warm weather also seemed to be hard on the deficient cows. C83 and G31 were in exceptionally good flesh and were able to withstand the effects of the deficiency for only a short time. On the other hand C118 and A1 which were poor when the deficiency symptoms were first observed seemed to withstand the deficiency much better. C83 and G31 both got down in the stall and were unable to raise their heads. It is thought that this acute condition is due in part to an abnormally high intracranial pressure. In another experiment the intracranial pressure of a young male on the deficient ration was found to be equal to 500 millimeters of saline while the normal is about 100 millimeters. This animal was just able to get up and draining the spinal fluid gave a short period of relief.

In calves when the level of plasma carotene had decreased to about 0.13 micrograms per milliliter nyctalopia and papilledema and other evidences of vitamin A deficiency began to appear (9). In mature cows this level would appear to be somewhat higher. Animal 45 showed evidence of nyctalopia at 0.5 micrograms level so that a range of 0.2 to 0.5 level should be considered. Davis and Madsen (10) reported a level of 0.25 for heifers of the Shorthorn and Hereford breeds.

From the results obtained with animals 45 and C118 it would appear that an intake of 9 to 12 micrograms of carotene per pound of body weight was

not sufficient to prevent the development of symptoms of vitamin A deficiency. This intake was made up of the carotene fed in the alfalfa meal at the rate of 7 to 10 micrograms and the 2 to 3 micrograms present in the basal ration per pound of body weight. A total intake of 16 micrograms, however, appeared to be sufficient as shown by the results for A1 and C118. These observations agree with our previous observations (9) and those of Guilbert and Hart (5) who found that an intake of about 30 micrograms per kilo was necessary to prevent nyctalopia. However, it seems questionable whether this intake is the physiological minimum as stated by Guilbert and Hart since it is not sufficient for proper reproduction, or to prevent the development of an increased intracranial pressure in calves as shown by unpublished data from this station.

It will be noted in this paper that no cases of xerophthalmia are recorded even though extreme vitamin A deficiency was permitted to develop. In some cases considerable lacrimation and some clouding of the cornea were noted. The absence of xerophthalmia was confusing in the early work at this station on vitamin A deficiency and led to considerable doubt as to whether the deficiency seen was actually due to lack of vitamin A (1). However, the explanation of the apparent discrepancy probably lies in the environmental conditions under which these experiments were conducted. The eyes of the animals during the deficiency period were probably not subjected to the presence of large amounts of abrasive dust particles and possibly the proper type of bacteria.

SUMMARY

1. Mature cows on a vitamin A deficient ration failed to develop blindness due to constriction of the optic nerve such as has been reported in calves.

2. A definite papilledema failed to develop in two cows out of six in these experiments. Once the papilledema develops it takes considerable time for it to recede.

3. Mature cows did develop nyctalopia, incoordination, and an edema of the legs on the A deficient ration.

4. The tapetum nigrum and lucidum developed a mottled appearance.

5. When the plasma carotene values receded to a 0.2 to 0.5 microgram level deficiency symptoms usually followed in a short period of time.

6. The fat of a Guernsey cow which died with symptoms of vitamin A deficiency showed the presence of a pigment which was most likely carotene since it was epiphasic between petroleum ether and 92 per cent methyl alcohol.

The author wishes to express his appreciation to Dr. J. O. Wetzel of Lansing for his criticisms in preparing this paper.

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Announcement

Translations of a number of Danish and Swedish articles of interest to readers of the JOURNAL OF DAIRY SCIENCE have been completed in a W.P.A. project sponsored by the University of Minnesota. These translators assigned to W.P.A. Official Project No. 65–1–71–140, Sub-Project No. 484, have been supervised by Dr. Harold Macy. Copies of these translations are available in the Office of the American Documentation Society, 2101 Constitution Avenue, Washington, D. C.

Translations of the following Danish dairy articles are now available.

Smørrets Vandindhold og Saltning (Water content and salting of butter). H. Hendemann. 15de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1938.

Forsøg med Silkeborg Stassano apparat Model 1937. (Experiments with Silkeborg Stassano apparatus, Model 1937). Joho. Jensen and others and Sv. Horning. 16de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1938.

Forsøg med "Spirala" til Varmebehandlung af Konsummaelk. (Experiments with "Spirala" for heat-treatment of consumers' milk). H. Jörgensen. 17de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1939.

Forsøg med "A.P.V." Apparat til Varmebehandling af Konsummaelk. (Experiments with the "A.P.V." apparatus for heat-treatment of consumers' milk). A. Petersen and K. Rasmussen. 18de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. 1939.

Forsøg med Pladeapparat "Kolding" Type B.P.K. til Varmebehandling of Konsummälk. (Experiments with the plate apparatus "Kolding," type B.P.K. for heat treatment of consumers' milk.) N. Kjärgaard-Jensen. 20de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1–26. 1939.

Af prøvning af Victoria-Kubus Kaerneaelter. (Testing of the Victoria-Kubus churn-worker.) N. Kjärgaard-Jensen. 22de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1–21. 1939.

Forsøg med Victoria-Kubus Kaerneaelter. (Experiments with the Victoria-Kubus churn-worker.) N. Kjärgaard-Jensen. 24de Beretning fra Statens Forsøgsmejeri, Hillerød, Denmark. pp. 1–28. 1939.

Translation of the following Swedish article is also available.

Inverkan av vissa Konserveringsmedel på Mögel—och Jastsvampar från Ost. (Effect of certain preservatives on moulds and yeasts from cheese.) K. E. Thome. Meddeland No. 3 fran Statens Mejeriförsök Särtryck ur Arsskrift for Alnarps lant-bruksmejeri-och trödgårdsinstitute, pp. 1–20. 1939.

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ABSTRACTS OF LITERATURE

ADVANCE ABSTRACTS OF REPORTS ACCEPTED FOR PUBLICATION

658. The Bacteriology of Brick Cheese. I. Growth and Activity of Starter Bacteria. JOHN C. GAREY, EDWIN M. FOSTER AND WILLIAM C. FRAZER, Department of Agricultural Bacteriology, University of Wisconsin.

Brick cheese was manufactured by the conventional method with *Streptococcus lactis* and *Streptococcus thermophilus* starters used singly and in different combinations. The growth and activity of the starters were followed by bacteriological, chemical and physical methods.

When 0.6 per cent of *Str. lactis* starter was used alone and the curd cooked to 106° F., the development of the lactic streptococcus was very slow until the third or fourth hour after dipping; thereafter the numbers increased rapidly and reached their maximum at one to two days. If the curd was cooked at 112° F., the growth of the *Str. lactis* bacteria was decreased as evidenced by a slower rate of multiplication and a lower maximum number. Because of the lack of activity of the lactic streptococcus until the latter part of the draining, it was necessary to dip the curd in a relatively dry condition otherwise the cheese would retain too much moisture and those defects characteristic of an acid cheese would develop.

When 0.6 per cent *Str. thermophilus* starter was used alone and the curd cooked to 106° F., growth of the starter bacteria was most rapid during the cooking and the first three hours after dipping; thereafter the rate of multiplication decreased sharply because of the unfavorably low temperature in the cheese. A cooking temperature of 112° F., in comparison with 106° F., increased the growth and activity of the thermophilic streptococcus. When *Str. thermophilus* starter was used alone, all the lactose was not fermented in the cheese. This was evident from the high pH at one day, the later development of *Str. lactis* in large numbers and the development of undesirable bacteria which produced gassiness and fermented flavors in the cheese.

Alone, neither of the starters produced a Brick cheese of satisfactory quality. The cheese manufactured with *Str. lactis* starter developed a sour flavor and a short and crumbly body, that with *Str. thermophilus*, a fermented flavor and a very open texture. Of the different combinations of starters tried (cooking temperature—106° F.), a mixture of 0.3 per cent each of *Str. lactis* and *Str. thermophilus* produced a Brick cheese of more desirable quality than that with the other combinations.

If the moisture was higher than 42 per cent after salting, it was a practical guarantee of an acid or sour cheese with characteristic defect. This

meant that the moisture of the ripened cheese had to be 2 to 4 per cent lower than the legal limit in order to produce a desirable Brick cheese.

659. Lipolytic and Proteolytic Activities of Various Penicillia. C. JENSEN, North Dakota Agricultural Experiment Station, Fargo, North Dakota.

Studies were made of the lipolytic and proteolytic activities of the penicillia employed in the ripening of blue veined cheeses. The report deals with a study of 23 strains of penicillia including one *P. chrysogenum,* 3 *P. gorgonzola,* 15 *P. roqueforti,* one *P. stilton* and 3 unidentified strains.

There was considerable variation in the lipolytic activities of various penicillia on butterfat and cottonseed oil, as determined by the nile blue sulfate technique; the intensity and uniformity of lipolysis of the cultures ranged from nonlipolytic to very pronounced lipolytic.

There was considerable variation in the lipolytic activities of various penicillia on different triglycerides according to the nile blue sulfate technique. Only a few cultures hydrolyzed tripropionin, while all readily hydrolyzed tributyrin and trivalerin. As the molecular weights of the triglycerides increased, variation in lipolytic activities became more conspicuous. Some cultures showed gradual declines in their lipolytic activities, whereas others declined sharply on the triglycerides beginning with tricaproin.

There was considerable variation in the toxic effect of different triglycerides on various penicillia. In general, the triglycerides that exhibted the most pronounced toxicity, in declining order of their effect, were tripropionin, tributyrin, trivalerin, tricaprin, trilaurin, trimyristin and tripalmitin. The least toxic were triheptylin, tricaproin and triolein.

There was considerable variation in the proteolytic activities of various penicillia as determined by the acidified milk agar and the carbon dioxide techniques. There was a general agreement between the results obtained with the two techniques.

The rates of growth and of proteolysis of certain penicillia were affected by different growth conditions. The cultures grew more slowly but showed greater proteolytic activities in air at 28° C. than at 19° or 12° C.; the cultures were somewhat retarded in growth but proteolysis was unaffected when grown at 28° C. in an atmosphere in which 10 per cent of the air had been replaced by carbon dioxide; culture growth and proteolysis at 28° C. were almost stopped in an atmosphere saturated with carbon dioxide; growth usually was unaffected but proteolysis was accelerated at 28° C. in an atmosphere consisting for the most part of nitrogen.

660. A Test for the Protein Stability of Milk. ARNOLD B. STORRS, American Seal-Kap Corporation, Long Island City, N. Y.

A test for protein stability which requires a minimum of equipment, reagents and technical skill is described. Increasing amounts of N/10 HCl

are added to 10-ml. portions of the milk to be tested. The mixtures are placed in a boiling water bath for 10 minutes and then examined for coagulation. The stability number is equivalent to 100 × the ml. of HCl required to produce coagulation under the conditions of the test.

The average stability number of untreated fresh milk has been found to be about 60 to 70 as indicated by the test. Pasteurization tends to increase the protein stability of milk while copper contamination tends to lower the stability.

661. **A Method for the Estimation of Nicotinic Acid in Milk.** E. A. BAILEY, JR., W. J. DANN, G. HOWARD SATTERFIELD, AND C. D. GRINNELLS, Duke University and North Carolina State College of Agriculture.

A chemical method for the estimation of nicotinic acid, the pellagra-preventive factor, in milk is reported. The essentials of the method are: Acid hydrolysis, removal of interfering salts and colored material by treatment with Lloyd's reagent and lead hydroxide, and development of a colored complex by treatment with cyanogen bromide and metol (p-methylaminophenol sulfate). The reproducibility of results and the recovery of added nicotinic acid by this method are found to be satisfactory.

The analysis of milk from Ayrshire cows during the month of January 1941, shows the normal nicotinic acid content of the milk from these cows during this period to have been 1.46 micrograms per ml.

The low value obtained for the nicotinic acid content of milk is of interest in view of the fact that milk has long been considered of great importance in pellagra-preventive diets and has been shown to have pellagra-preventive value. The possibility is suggested that when considerable quantities of milk are included in the diet, the intestinal flora are so altered that a significant amount of nicotinic acid is synthesized by the intestinal microorganisms.

662. **The Effect of the Administration of Shark Liver Oil on the Butter Fat and Milk Production of Cows.** H. J. DEUEL, JR., Department of Biochemistry, University of Southern California Medical School, Los Angeles.

Shark liver oil was administered to six Guernsey cows in amounts of 30 cc. daily (700,000 I.U. of vitamin A) and a comparison in milk production was made with that of six other cows receiving the same basal diet without the supplement. All cows received the same basal diet which included large amounts of fresh-cut alfalfa. In a five-week preliminary period, the average milk production was practically identical in the two groups. With the feeding of the shark liver oil, an immediate rise in milk production of approximately 10 per cent over the control level was noted which continued

for 11 weeks; this gradually increased to a value of over 20 per cent by the 16th week of the test. An increase in butter fat given by the cows receiving the oil supplement over that of the control animals varied during this period between 507 and 794 grams per week per cow. These alterations are not ascribable to season, lactation cycle, or to food and are believed to be caused by the administration of the shark liver oil.

663. Age, Live Weight and Milk-Energy Yield—A Correction. W. L. GAINES, C. S. RHODE AND J. G. CASH, University of Illinois.

This correction removes certain systematic errors in the live weight estimates of a previous paper (this journal Oct., 1940). The most important change thereby effected is that the new (more correct) live weight data show that within herd milk-energy yield is proportional to the 1.02 power of live weight in the Holstein records; and proportional to the 0.98 power of live weight in the Jersey records. Live weight is measured within the first 31 days after calving and yield is for the first 8 months of the lactation.

BACTERIOLOGY

664. The Bacteriological Analysis of Creamery Waters. H. WOLOCHOW, Science Service, Dept. of Agr., Ottawa, Ont.; H. R. THORNTON, Univ. Alberta, Edmonton, Alberta, AND E. G. WOOD, Science Service, Dept. of Agr., Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 2: 23. 1941.

Creameries in many parts of Canada have experienced very serious trouble in the past few years from butter deteriorations caused by the use of contaminated water supplies, although in many cases these waters were of potable standard. Eighty-five samples of water from 37 Alberta creameries were examined for specific types of organisms. A surprising number of the waters contained large numbers of bacteria capable of growth at 10° to 15° C. Many of the bacteria were proteolytic according to the criteria applied. Further investigation may indicate the necessity of stricter bactericidal treatment of all creamery waters. O.F.G.

665. Burri Technique Discussed. H. H. WEISER, Ohio State Univ., Columbus, Ohio. Amer. Milk Rev., *2,* No. 7: 157–158. July, 1940.

The Burri agar slant method offers an inexpensive, rapid, simple procedure for determining relative numbers of bacteria in milk, and may be modified for use with butter, cheese, and ice cream. The original method has been considerably modified. Directions for making the test are given. The method gives better differentiation of colonies than the ordinary plate method because all colonies are on the surface; the count is lower because

clumps are not completely broken up; composition of the medium may be varied to suit the growth of particular types of bacteria; and it offers a good means for cultural examination of mastitis milk. P.S.L.

666. Heat Resistant Bacteria. A. C. MAACK. Amer. Milk Rev., *3*, No. 1: 1–2. Jan., 1941.

Survival of heat resistant bacteria after pasteurization presents a serious problem in attempts to regulate plate counts of pasteurized milk. Both thermophilic and thermoduring bacteria compose the groups surviving, and both rod and spherical forms are found. Feed, bedding, and soil harbor thermophilic organisms and utensils and milking machines often are a source of thermoduric types. Milk from suspected farms may be plated to locate sources. Exposure to chlorine of 100 p.p.m. for 2 or 3 minutes is necessary to kill these organisms. Repasteurization is often a cause of trouble as is incomplete pasteurization of foam, and milk stone in vats is strongly suspected as acting to harbor bacteria of these strains. In controlling infection all utensils must be sterilized. Infections are more common during cold weather when carelessness in cooling is more apt to occur. P.S.L.

667. Is Zero To Be the Limit. M. E. PARKER. Amer. Milk Rev., *3*, No. 6: 128–130. June, 1941.

In this article the author has reviewed action of the Coordinating Committee on Standard Methods of the American Public Health Association to better the method of making plate counts of milk. The author suggests that emphasis be placed on qualitative rather than quantitative methods for evaluating quality of milk. He believes that such quantitative emphasis will result in lowered palatability, that it will gradually reach a point where cost would not be justified by the safety attained, and that the plate count for evaluating quality is futile and confusing. Adoption of cultural media designed to develop the maximum number of bacteria does not give a true picture of the numbers of bacteria in milk causing spoilage. It will cause reduction of counts by increasing the care given to milk production but will not necessarily increase quality. Lowering bacterial counts has increased susceptibility to oxidized flavor in milk and surface taint in butter, due to the upsetting of the balance in the normal bacterial flora. If the goal is zero it means reduction in quality with consequent reduction in consumption. P.S.L.

BREEDING

668. Eine volle Laktationsperiode umfassende Amidfutterungsversche mit eineiigen Rinderzwillingen. J. SCHMIDT AND J. KLEISCH, Univ. Berlin. Züchtungskunde, *15*, No. 16: 169–174.

A pair of identical twin heifers were fed through a whole lactation period

with one of them receiving in the form of amide nitrogen almost half of the protein judged necessary by present feeding standards. The fat percentage was identical and the production for the heifer receiving the amide nitrogen was 3755 kg. of milk and 126.5 kg. of fat, as compared with 3936 kg. of milk and 132.6 kg. of fat for the other heifer. No differences in the general health of the animals were observed. J.L.L.

669. Vergleichende Untersuchungen über den Zusammenhang zwischen Alter und Leistung bei verschiedenen Rinderrassen, durchgeführt an Kühen des Deutschen Rinderleistungsbuches (RL). J. LANGE, Univ. Berlin. Züchtungskunde, *16*, No. 4: 123–126. 1941.

Whether dairy breeds differ in the regression of milk and fat production on age was studied on data from the German "Rinderleistungsbuch." These data correspond somewhat to the data from the Herd Improvement Registry testing in the United States, except that the German data are reported by association testing year instead of lactations, and the cows are selected cows. The lifetime records of 2,113 cows of the lowland races and 452 cows of the highland breeds of Germany were studied. Each of these cows had at least 7 years of records. By this restriction it was thought that the effects of selection on the age curve would be avoided, but the possibility of the opposite error arising because of the imperfect repeatability of the records on which past selection may have been based is not considered.

In general the breed-to-breed differences were small and it is doubtful whether they were statistically significant. The middle German red breed did not decline as rapidly after maturity as the others and this is attributed to innate resistance and hardiness. The Oldenburger division of the black and white lowland cattle showed an earlier rise to the peak of production and a quicker decline afterwards, which is attributed to the influence of some Shorthorn blood introduced long ago. The Angler breed showed less extreme changes than the black and white lowland cattle. These differences

Year on test	Milk quantity		Fat (per cent)	Total fat	
	Kg.	Relative		Kg.	Relative
2nd	4111	76.3	3.57	146.7	77.0
3rd	4632	85.8	3.59	166.1	87.2
4th	5082	94.3	3.57	181.5	95.3
5th	5268	97.8	3.57	188.0	98.7
6th	5373	99.7	3.55	190.5	100.0
7th	5388	100.0	3.53	190.0	99.7
8th	5296	98.2	3.54	187.6	98.5
9th	5212	96.2	3.51	182.8	96.0
10th	5109	94.8	3.52	179.8	94.4
11th	5049	93.7	3.48	175.5	92.1
12th	5150	95.6	3.38	174.3	91.5

were slight and for practical purposes it may almost be said that there were no breed differences in the relative changes of production with age.

The change in fat percentage with advancing age was small and no breed differences were found in the shape of that curve. The maximum production of total fat was reached in the sixth association year which would be at an age of about 8 or 9 years. The following table shows the averages by year on test. The year the cow first came on test was omitted, since that would be fragmentary in nearly all cases.

J.L.L.

BUTTER

670. The Yeast and Mold Service in Relation to Quality Improvement. W. H. SPROULE, Dairy Dept., Ontario Agr. College, Guelph, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 1: 50. 1941.

Yeast and mold counts have been recognized for a considerable period of time as playing an important part in laboratory control of butter quality. While butter containing numerous yeasts and molds might give good commercial satisfaction at times, as shown by some of the work accomplished, nevertheless, the larger creameries recognized that butter with a low yeast and mold content was a better risk for storage purposes than butter made in a less sanitary way. In reviewing the progress made during 8 years, D. B. Shutt reported marked improvement as the work progressed. In 1928, 34.8 per cent of submitted samples had counts of 10 yeasts, or less, per cc. as compared with only 1.2 per cent in 1921. The mold counts also showed improvement; 61.8 per cent of the samples showed counts of 10 or less in 1921, whereas, in 1928, 89.9 per cent fell in this class. The butter laboratory is at present making analyses for yeast and mold on samples submitted by creameries which have applied for the service. O.F.G.

671. The Experimental Error in the Plate Count Examination of Butter. E. G. PONT, Dept. of Agriculture, Sydney, Australia. Jour. Dairy Res. *12,* No. 1: 24–34. 1941.

In an investigation into the experimental error of the plate count of butter, 154 boxes of butter were examined by plating in triplicate, in a dilution of 1/500, each of three 1-gram samples per box. The means of the triplicate counts on any one sample, judged by conformity to the Poisson distribution, were considered to give reasonably satisfactory estimates of the bacterial content of the samples. The between-sample variability was shown by transforming the counts to logarithms and calculating the coefficient of variation in respect of each box. In the distribution of the coefficients approximately 50 per cent was found to lie on either side of a 4 per cent level, while 10 per cent gave values higher than 14 per cent.

In a further study 12 boxes of butter were selected for quality and uni-

formity and data were secured from the examination of 7 1-ounce samples selected at random from each box. Using the method of analysis of variance, the results indicate that the estimates of within sample variance obtained would be regarded as estimates of a common variance. High significant differences were found, however, among the between-sample mean squares and the variability was found to be excessive in six of the twelve boxes examined.

The author points out that owing to the excessive between-sample variance found, the result of a single plating used as an index of the mean bacterial population of a box of butter may be quite inaccurate. It is only when a high estimate is encountered (*e.g.,* several hundred thousand or more per gram) that any real degree of significance can be attached to the result. Errors arising from technique would be unlikely to influence a normally low count to this extent. The occurrence of butter giving rise to such a count, even though it appeared only in parts of a box or a churning, would, from the standpoint of quality control, indicate the need for remedial measures. S.T.C.

672. **Facts to Know about Packaging Butter.** L. C. THOMSEN. Amer. Butter Rev., *2,* No. 4: 114, 116, 128. 1940.

Figures from several localities in this country show surprisingly large quantities of bulk butter are still sold to the public. Most of this comes to the dealer in spruce tubs, notorious for their tendency to transmit wood flavor to butter. Paraffin retards, but does not prevent, absorption of these flavors. Anti-oxidants applied to parchment liners, according to preliminary work, do not prevent absorption. The casein-formalin treatment, while perhaps effective, has not been looked upon favorably in this country. Avoidance of air pockets in packing butter reduces aerobic conditions necessary for the growth of bacteria causing spoilage; careless storage conditions for sterile parchment may result in mold and yeast contamination; and treatment of parchment circles and liners with salt brine and calcium propionate further reduces mold. The use of the latter alone in 10 per cent solution has been reported as responsible for surface mottling of butter.

The author predicts as the next great advance in packaging butter, a continuous churn with packaging following directly from the churn. Wholesaler's demands for amount of overweight per 64 pound tub, he states, varies from 6 to 12 ounces. From two to fifteen per cent of butter reaches the market underweight. On the average when butter is cut the overweight per uncartoned pound print is $\frac{1}{4}$ ounce. Losses in cutting and in overweight may amount to $\frac{3}{4}$ to one pound per 64 pound tub. Overweights given per pound of butter amounts to $\frac{2}{3}$ pounds per hundredweight of butter cut. Shrinkage of pound prints of dry wrapped butter is about $\frac{1}{16}$ ounce more than for prints wet wrapped. Machine wrapping and cartoning costs for

one pound prints vary from 0.76 to 1.35 cents per pound; and for quarter pounds, from 1.0 to 1.36 cents per pound. The latter, uncartoned, costs 0.75 cents per pound. P.S.L.

673. **Manufacture and Use of Butter Culture.** N. E. FABRICIUS. Amer. Butter Rev., *2*, No. 3: 74, 92. 1940.

The author reviews the arguments for and against the use of starter, the organisms responsible for flavor in starter, the products formed by starter bacteria, and the problems involved in the successful carrying on of cultures. He recommends heavy inoculation of cultures, one pint or quart per 10 gallons of milk; the addition of two ounces of citric acid crystals dissolved in ¼ pint of hot water per 10 gallons of milk; ripening the culture to a higher degree of acidity than ordinarily practiced, and using taste or the creatine test rather than acidity test for determining the degree of ripeness; quick cooling with vigorous stirring; and cooling to a low temperature to prevent reducing diacetyl to flavorless, 2, 3-butylene glycol. As between addition of starter to cream at 70° F. allowing to grow a few hours and cooling, or addition of starter to cooled cream and holding 8 to 12 hours before churning, the author prefers the latter procedure because a greater quantity of the acetylmethyl-carbinol is oxidated to diacetyl. P.S.L.

674. **Testing Cream for Mold Mycelia.** C. H. PARSONS. Amer. Butter Rev., *2*, No. 11: 382–384. 1940.

The details of procedure for the Parsons Visual Mold Test together with a list of required equipment is given in this article. The author suggests classification of cream into four grades according to mold content. Being both inexpensive and simple the test is recommended as a routine measure in cream stations and butter plants. P.S.L.

675. **On the Receiving Line.** V. SCHWARTZKOPF. Amer. Butter Rev., *2*, No. 12: 406, 408, 410, 412. 1940.

Quality of cream will be maintained if it is handled promptly at the plant, graded and churned by grade, contamination avoided, metallic taint prevented through use of well-tinned cans, and return to the farm of clean, dry cans. For cleaning cans the use of soft water will prevent reduction of washing machine efficiency by preventing formation of sludge which clogs pipes, tank and pumps. Washing powder that produces no bacteria harboring sludge is best. It must be free rinsing and capable of removing all deposits quickly and completely and be a good water softener if hard water is used. Temperature of water should be 140°–145° F. and alkalinity, 0.3 per cent or less. After washing, the can should be rinsed in clean hot water at 190° to 200° F., although the rinse, if hard water, leaves a film of mineral

on the can. Use of softended water eliminates this difficulty. After steam or hot water sanitization it is desirable to dry the can with air heated to 250° F. or higher. Such procedure seldom gives an absolutely sterile can. Sterilizers such as 1:100 Zephrin are efficient but costly. Sterilization by light gives little promise. Straight-side cans are more easily cleaned.

P.S.L.

676. Counting Mold Mycelia in Butter. G. W. SHATTUCK, JR. Amer. Butter Rev., *3*, No. 1: 10, 12. 1941.

A complete outline of the method for counting mold mycelia is given. Recommendation is made that the microscope be standardized to cover a field 1.382 mm. in area and that no field is reported positive unless one filament or the combined length of the two longest filaments exceeds $\frac{1}{6}$ the diameter of the field.

Filters aid in picking out the filaments. Use of five per cent aqueous solution of crystal violet as a stain in preparing the sample outlines the mycelia sharply making possible their identification as single or broken filaments and making their measurement more conclusive. Efforts to simplify mold mycelia counting procedure have been disappointing. In case the count is low or high, the counting of 50 fields is satisfactory, but where the count is close to 60 per cent positive, the examination of 100 fields is preferable. A tolerance of 10 per cent difference in counting between technicians is allowable. Observation leads to the theory that length of the mold filament is correlated with age of cream. P.S.L.

677. Cream Grading and the Future of the Butter Industry. C. E. LACKNER, Dept. of Agr., Toronto, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 62. 1941.

The author points out that, although the fear of surplus Canadian butter is passed now, there is likely to be a surplus again when world conditions become normal. The quality of Ontario cheese has been good but a considerable portion of the butter has been of low grade due primarily to poor quality in the cream. Looking ahead, therefore, to normal market conditions when a surplus of butter can be expected it is essential that a sound cream-grading program be promulgated now. O.F.G.

678. Bacteria in Well-Waters. C. H. CASTELL AND E. H. GARRARD, Ontario Agricultural College, Guelph, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 18. 1941.

As pasteurization of cream and improved sanitation has decreased certain types of butter and cream spoilage, the importance of trouble from what appears to be minor sources has become more significant. One of the most important of these is water used by creameries and owing to the peculiar

characteristics of water bacteria, the milder and less salty butter is made, the more their activity will be noticed. Ten per cent of the samples examined were found unfit for human consumption, 30 per cent showed the presence of butyric acid forming anaerobic bacteria, a majority of the waters contained organisms which were oxidase-positive, and approximately 85 per cent contained organisms capable of growing at a temperature within 4 or 5 degrees of freezing and at the same time capable of decomposing fat and curd. Results suggested the presence of *Pseudomonas fragi* and *Pseudomonas fluorescens.* O.F.G.

679. Factors Influencing Mold Mycelia in Cream. P. R. ELLIKER, Purdue Univ. Natl. Butter and Cheese Jour., *32,* No. 7: 8. 1941.

The velvety, white growth commonly found on the surface of sour milk or cream is *Oospora lactis,* "milk mold." It gets into milk and, eventually, cream and butter through dust, dirt, manure and utensils. Some strains grow as low as 40° F., others as high as 100° F., but ideal temperatures approximate 75° F. It is destroyed by proper pasteurization. Growth of *Oospora lactis* is favored by a slightly acid reaction and is retarded or inhibited by high acidity, lack of air, presence of salt and possibly propionic acid or its salts. Its presence in butter with yeasts and other molds indicates unclean churns or equipment. It forms part of the surface flora of some cheese. Delivery of cream 3 times weekly eliminates the mold problem if clean utensils and separators are used and if cream is stored at 60° or lower. The mold content of gathered cream decreases as the fat increases from 30 to 50 per cent, probably because it is machine- rather than hand-separated but perhaps because rich cream may be a less favorable medium for mold growth. Frequent stirring of cream decreases the mold count on butter but may injure the quality of the butter because it encourages undesirable changes in the cream. A large surface area of cream in relation to amount of cream increases mold growth. Delays in neutralizing and pasteurizing cream permit mold growth. During the processing of cream and butter, mold filaments are broken. Although some of the mold may go into the buttermilk, still a high mold content in the cream causes a high mold content in butter. W.V.P.

680. Safeguarding Butter Quality. B. F. MCKIBBEN. Amer. Butter Rev., *2,* No. 3: 78, 92. 1940.

In a discussion of the thirty-five off flavors listed in the government score card for butter this writer emphasized the danger of transmitting lubricating oil flavors to cream from oil lubricated pumps, and cites the work of Turgasen as to the frequency of cheesy flavors from contaminated water.

P.S.L.

681. Recent Trends in Neutraliziation. LEE H. MINOR. Amer. Butter Rev., *3*, No. 3: 90, 92. 1941.

Calcium in lime neutralizers has a greater affinity for casein or curd than for lactic acid and tends to increase viscosity of cream, produce lime flavor, and clog filters; sodium in neutralizers, while excellent for neutralizing lactic acid, has a tendency to create foam and has been accused as being responsible for soapy flavor. Combination of the two lessens the effect of each. With the vacuum process of pasteurization neutralizer may be added to the cold cream in the forewarmer, allowing it to act on the acid before pasteurization temperature is reached, reducing foaming and danger of saponification of fat. Dilution of the neutralizer with water is important, the amount increasing with the causticity of the alkali. P.S.L.

CHEESE

682. "Phage" in Cheesemaking. C. K. JOHNS, Div. Bacteriology and Dairy Res., Science Service, Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 18. 1941.

Most makers of Cheddar cheese have had experience with slow acid development, an occurrence which is generally accepted as one of those things that cannot be explained and about which nothing can be done except to get a new starter. In New Zealand considerable use is made of single strain lactic acid bacteria starters. When such a starter becomes contaminated with bacteriophage the bacteria are destroyed practically 100 per cent and acid development ceases abruptly. In Canada, on the other hand, the starter is usually a mixture of several different strains or species of desirable bacteria. Since a phage generally attacks only one strain, or a few closely related strains, the result is usually a slow development of acid. The author, however, found a phage different from that reported in New Zealand which attacked every one of 10 different organisms found in the starter in use. Several outbreaks of slow acid development were found each of which disappeared when the plant and equipment had received a thorough housecleaning and sterilizing treatment. O.F.G.

683. The Drying of Cheese Whey and of Acid Casein Whey by the Roller Process. R. WAITE, The Hannah Dairy Res. Inst., Kirkhill, Ayr. Jour. Dairy Res., *12*, No. 1: 71–77. 1941.

Sodium or potassium compounds were found to be unsuitable for neutralizing cheese whey prior to drying. Neutralization with calcium hydroxide gave a satisfactory product. Reduction of the acidity of hydrochloric acid casein whey to 0.18 per cent by the addition of calcium hydroxide allowed satisfactory neutralization. S.T.C.

684. Starter Cultures for Cheese Manufacture. Further Attempts to Eliminate Failures Due to Bacteriophage. H. R. WHITEHEAD AND G. J. E. HUNTER, Dairy Res. Inst. (N.Z.), Palmerston North, New Zealand. Jour. Dairy Res., *12*, No. 1: 63–70. 1941.

Bacteriophages for lactic streptococci were found to occur in the atmosphere of commercial cheese factories. This was established in three ways: (a) aspiration of air, (b) exposure of sterilized skim milk, and (c) exposure of inoculated agar surface. Finely divided particles of whey emitted from the whey separator appeared to be the main vehicle for the air-borne phage although whey contaminated dust probably also played a part. Protection of the starter from air-borne phage eliminated phage failures. A separate building for starter propagation is suggested as the only means of insuring this at present. S.T.C.

685. The Consistency of Cheese Curd at the Pitching Point and Its Bearing on the Firmness and Quality of the Finished Cheese. G. W. SCOTT BLAIR AND F. M. V. COPPEN, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., *12*, No. 1: 44–54. 1941.

Further studies are reported on the use of the method previously described (JOUR. DAIRY SCI., *24*, No. 2: A26. 1941) for measuring the consistency of cheese curd at the "pitching" point. The value determined W/h was found to be an excellent criterion of the properties of the cheese curd assessed by an expert cheese maker.

No significant relationship was found to exist between the firmness of the curd at "pitching" and acidity. The most usual values (medians) for "pitching" consistency were compared for 4 different factories and it was shown that differences in technique may be associated with the same "pitching" consistency and produce very similar cheese, but that in other cases good cheese may be produced from very different "pitching" consistencies. S.T.C.

686. "Slowness" in Cheesemaking. J. HARRISON AND D. V. DEARDEN, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., *12*, No. 1: 35–43. 1941.

Two cases of "slowness" in cheesemaking are reported in which the trouble was eliminated by changing the source of the starter. The principal cause of the "slowness" studied appears to have been the inability of the streptococci in the starter cultures used to grow at scalding (cooking) temperatures. The necessity of using a starter capable of normal growth at 40° C. (104° F.) is thus demonstrated.

Attempts to isolate either bacteriophage or "non-acid" organisms failed. S.T.C.

687. The Problem of Rancidity in Cheddar Cheese. E. G. Hood, I. Hlynka and C. A. Gibson, Dept. of Agr., Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 26. 1941.

The odor and flavor of rancid cheese are characteristic of those of butyric acid. It has been shown experimentally that it is possible to produce rancidity by the addition of butyric acid to the cheese milk. Lipase gives rise to butyric and other fatty acids when added to cheese milk. Raw milk contains lipase. Free butyric acid may be produced from casein, lactose, glycerol or butterfat. The most likely cause of rancidity is the action of lipase on butterfat to produce free butyric acid. In an experimental study of the problem it was found:

1. Typical rancid cheese were reproduced with the addition of lipase or homogenized milk to the cheese milk. Homogenization activates the naturally present lipase.

2. Rennet and pepsin partially inactivated additions to cheese milk of lipase or homogenized milk.

3. A number of the cheese made from inactivated milk fell in grade after a storage period of 6 weeks.

4. A relation between unclean, dirty, etc., flavors and rancidity in cheese was suggested. O.F.G.

688. Some Micro-organisms Associated with Gassy Swiss Cheese. Harry H. Weiser, Ohio State Univ., Columbus, Ohio. Natl. Butter and Cheese Jour., *32*, No. 7: 20. 1941.

Splitting rinds were observed on the edge and extending 4 to 5 inches toward the center of the Swiss cheese. The defect was accompanied by gas formation, lack of characteristic eye formation, poor body, bitter flavor, sometimes yeasty odor and lack of Swiss flavor. Bacteriological examination disclosed that associated with the occurrence of this defect were considerable numbers of lactose fermenting yeasts and anaerobic or facultative anaerobic bacteria of the *Clostridium perfringens* group. Other organisms may be involved. Practical control lies in the use of good milk and good active starter. W.V.P.

689. Pasteurization for Cheesemaking. G. S. Bixby, Cherry-Burrell Corp., Chicago, Ill. Natl. Butter and Cheese Jour., *32*, No. 7: 14. 1941.

Three types of pasteurizers commonly used are: the internal tube heater with tubular surface regenerator; a flash pasteurizer with a tubular surface type regenerator; and the plate pasteurizer. All are of the "flash" type and use maximum temperatures of 160°–165° F. frequently with a 16 to 20 second hold for increased bacterial destruction. Costs of pasteurizing

100 lbs. of milk approximate 1.5 cents for steam and 0.35 cents for power. Two to four man hours daily are required for cleaning. Extra costs are offset by benefits of increased yield, uniformity and quality. Health authorities, eventually, will demand that all cheese milk be pasteurized as Kentucky does now. W.V.P.

690. **Survey of Cheese Preferences.** EDITORIAL. Amer. Butter Rev., *3,* No. 5 : 162, 196. May, 1941.

This editorial presents results secured in a survey representing 3.5 per cent of the families in Milwaukee, Wisconsin, as to their cheese preferences. Families purchasing packaged cheese in 1937 were 59.9 per cent and in 1940, 56.1 per cent, the numbers being 112,701 in 1937, and 110,569 in 1940. Of package cheese users, 48.8 per cent preferred the 8-ounce package, 26.5 per cent a smaller size, 12.4 per cent a 2-ounce carton, and 12.3 per cent preferred a one-pound package. In 1937, 82.6 per cent of the population surveyed regularly used bulk or loaf cheese; in 1940 the number had increased to 92.9 per cent. Of bulk cheese purchased 64.2 per cent was American type of cheddar, 22.9 per cent brick, and 12.9 per cent Swiss. Average consumption of bulk cheese per family has shown a variation of only one-tenth of a pound since 1934, monthly consumption per family being 2.1 pounds. P.S.L.

691. **Manufacture of Acid-rennet Type Cottage Cheese.** D. W. GLOVER AND L. H. BURGWALD, Ohio State Univ., Columbus, Ohio. Milk Dealer, *30,* No. 8 : 42–50. May, 1941.

Directions are given for the manufacture of acid-rennet type cottage cheese. Directions for using homogenized milk returns are included. The authors draw the following conclusions:

1. Start with sweet, clean skim milk and pasteurize it by heating to 143° F. for 30 minutes. Higher pasteurizing temperatures may be detrimental to the texture of the resulting curd.

2. Temper the milk to 70° F. if the long set is to be used or to 85° if the short set is to be employed.

3. Add starter at the rate of 0.5 per cent of the weight of the milk used for the long set or 3.0 per cent to 5.0 per cent for the short set. A choice between the long and short set methods will depend upon the work schedule of the particular plant.

4. Add rennet at the rate of 1.0 cc. per 1,000 pounds of milk in the vat.

5. Utmost care should be exercised in taking the whey sample for the acidity test to insure that no curd particles are present. The use of the whey well affords a satisfactory method of obtaining the sample.

6. Cut the curd when the acidity of the whey reaches 0.52 per cent.

7. The cooking may be accomplished by the use of hot water in the jacket of the vat and by adding hot water directly to the curd. The use of water in the cooking process enhances the firming of the curd, thus saving time in the cooking process.

8. The use of tempered wash waters prevents matting and the breaking up of curd made brittle by too sudden cooling.

9. The curd should be thoroughly chilled before creaming and packaging. Unchilled curd has a tendency to be tender and is prone to break up in the creaming process.

10. The use of homogenized milk results in a product with superior quality, and at the same time provides a method for utilizing returns.

C.J.B.

CHEMISTRY

692. Analysis of Proteins. 13. Caseo-Phosphopeptone. J. LOWNDES, T. J. REW MACARA AND R. H. A. PLIMMER, St. Thomas's Hospital Medical School, London, S. E. Biochem. Jour., *35*, No. 3: 315–319. March, 1941.

Judging from its N and amino-N contents, caseo-phosphopeptone is an octapeptide containing two H_3PO_4 groups, 2 mol. glutamic acid and 2 mol. serine. Its acidity indicates the presence of another mol. of a dicarboxylic acid, leaving 3 mol. of simple amino-acids. V.C.S.

693. Methods of Measuring the Rate and Extent of Oxidation of Fats. FRANK C. VIBRANS, Amer. Meat Institute, 59 East Van Buren St., Chicago. Oil and Soap, *18*, No. 5: 109. May, 1941.

This paper discusses the following tests and methods for measuring the rate and extent of fat oxidation: 1. Kreis test; 2. Issoglio-Kerr test; 3. Aeration methods; 4. Photochemical methods; 5. Oxygen absorption; 6. Oxygen absorption-Peroxide method. V.C.S.

694. A Convenient and Efficient Method for the Determination of the Digestibility of Fats with Pancreatic or Other Lipases. J. R. KOCH AND SISTER M. DOLOROSA DUELLMAN, Marquette Univ., Milwaukee, Wis. Oil and Soap, *18*, No. 4: 86. Apr., 1941.

A convenient and easy to operate method for carrying out hydrolysis experiments with pancreatic lipase is described. The authors list the following advantages for this method:

1. It allows five determinations to be carried out at one time.
2. It shows a smooth course of reaction in every case.
3. It gives more complete hydrolysis since the acids are used up as formed.

4. It keeps the pH on the alkaline side under constant control and in the range where the enzyme is most active.

5. It eliminates the removal of aliquots and the killing of the enzyme.

6. It makes it possible to make determinations directly in the digestion mixture. V.C.S.

695. Antioxidants for Edible Fats and Oils. H. S. OLCOTT, Mellon Institute, Pittsburgh, Pa. Oil and Soap, *18*, No. 4: 77. Apr., 1941.

Ascorbic acid, vitamin C, possesses antioxidant activity of the acid type while the tocopherols, that is vitamin E, possess the properties assigned to the phenolic inhibitors. Theoretically, combinations of the two should be particularly advantageous antioxidants, and actually the data confirm this assumption.

Purified lecithin possesses no antioxidant activity but with the commercial product the cephalin fraction carries the inhibitor action.

Cereal flours and particularly oat flour possess antioxidant properties. Phospholipids may account for part but not all of the effective principle of oat flour.

Cottonseed meal is an excellent antioxidant in fats and oils. V.C.S.

696. The Mechanism of the Autoxidation of Fats. H. A. MATTILL, State University of Iowa, Iowa City, Iowa. Oil and Soap, *18*, No. 4: 73. Apr., 1941.

Although the chemical changes during the induction period are very different and less obvious than those that follow it, they are, from a practical point of view, more important because once the induction period is past the damage is done. During oxidation the fats pass through two stages: a latent or induction period of variable length during which the amount of oxygen absorbed is small, followed by a period of rapidly accelerating oxygen absorption. The end of the induction period usually coincides with or immediately precedes the first appearance of the products of organoleptic rancidity.

Numerous tests have been devised for detecting the degree of susceptibility of a fat toward oxidation. These tests are based upon the estimation of some chemical change, yet the order of reason for the reaction is not clearly understood.

This paper deals with some of these chemical changes. No all-inclusive theory of autoxidation can yet be formulated. V.C.S.

697. A Convenient Method for the Rapid Estimation of Carotene in Butterfat. WILLIS D. GALLUP AND A. H. KUHLMAN, Oklahoma Agr. Expt. Sta., Stillwater, Okla. Oil and Soap, *18*, No. 4: 71. Apr., 1941.

A simple method is described for determining the carotene content of

butterfat under conditions where extreme accuracy is not required. A direct comparison is made of the color of the melted fat with that of known concentrations of potassium dichromate solution.

The dichromate solutions are prepared by dilution of measured amounts of a 0.2 per cent stock solution to a volume of 25 ml. These dilute solutions and the fat samples are contained in cylindrical sample bottles of uniform diameters and their color matched with the aid of a comparator block placed before a "daylight" lamp.

The authors give a table showing the carotene content of butterfat in micrograms per gram corresponding to the color produced by various concentrations of potassium dichromate. The carotene content may be calculated from the formula $X = \frac{Y - b}{a}$ in which X is the micrograms of carotene per gram of fat, Y is the concentration of the matching dichromate solution in per cent, and b and a are factors, 0.012 and 0.018, respectively.

V.C.S.

698. The Melting Points of Binary Mixtures of Oleic, Linoleic, and Linolenic Acids. H. W. STEWART AND D. H. WHEELER. Oil and Soap, *18*, No. 4: 69. Apr., 1941.

The oleic-linoleic acid system has eutectics for the alpha and beta forms of oleic acid of 75.2 and 76.3 mole per cent linoleic acid, at – 10.0° and – 9.8°, respectively.

Linoleic and linolenic acid mixtures show only melting points intermediate between the pure acids.

The oleic-linolenic acid system has eutectics for the alpha and beta forms of oleic acid of 82.7 and 85.5 mole per cent linolenic acid, at – 15.7° and 15.1°, respectively. V.C.S.

699. Enzymic Proteolysis. 4. Amino-Acids of Casein Phosphopeptone. M. DAMODARAN AND B. V. RAMACHANDRAN, Univ. Biochem. Lab., Chepank, Madras. Biochem. Jour., *35*, Nos. 1 and 2: 122–133. Jan., 1941.

By digestion of "paranuclein" from casein with trypsin an enzyme-resistant phosphopeptone of constant composition had been isolated in the form of its barium salt.

The phosphopeptone was shown to contain 10 amino-acid units, viz., 3 mol. glutamic acid, 3 mol. of isoleucine and 4 mol. of serine. The absence of other hydroxy- or dicarboxylic-amino-acids has been demonstrated by indirect methods.

A method is described for the approximate estimation of serine in the absence of other hydroxyamino acids. V.C.S.

700. The Component Acids of Phosphatides Present in Cow's Milk Fat. THOMAS PERCY HILDITCH AND LIONEL MADDISON, Dept. Indust. Chem., Univ. Liverpool. Biochem. Jour., *35*, Nos. 1 and 2: 24–30. Jan., 1941.

The typical milk fat glyceride acids of low molecular weight are wholly absent from the phosphatide acids. The component fatty acids of milk fat phosphatides have little in common with those of the milk fat glycerides; on the other hand, they bear more general similarity to those of the phosphatides of the ox liver.

The authors report the following acids and amounts in the phosphatides separated from Swiss and English butters:

Acid	Per cent by weight	
	"Swiss"	"English"
Myristic	3.2	5.5
Palmitic	21.0	13.4
Stearic	7.3	9.0
As Arachidic	12.3	20.9
As $C_{20}H_{32}O_2$	5.2	10.0
Hexadecenoic	4.3	4.9
Oleic	32.5	23.5
As Octadecadienoic	6.4	.....
As C_{20-22} unsaturated	7.8	12.8

V.C.S.

701. Preliminary Experiments on the Vapor Pressure of Dairy Products. G. W. SCOTT BLAIR, F. J. DIX AND A. WAGSTAFF, Natl. Inst. Res., in Dairying, Univ. Reading, Reading. Eng. Jour. Dairy Res., *12*, No. 1: 55–62. 1941.

The vapor pressure was determined as follows: A large number of air-tight tobacco tins of diameter about 4.5 in. were fitted with a simple wire clip to hold a pad of cotton wool about 2 in. square, against the lower side of the lid. The cheese were spread thinly on the bottom of the weighed tin, which was then reweighed as quickly as possible to avoid evaporation. A pad of cotton wool was then soaked in a salt solution of known concentration and vapor pressure and was lightly pressed out to prevent dripping. The pad was quickly clipped against the lid and the tin tightly closed. After 48 hours at a constant temperature, approximately 60° F. (15.6° C.), the lid was removed and each tin and cheese reweighed immediately. The change in weight per gram of cheese was then plotted against salt concentration and that concentration corresponding to no change in weight was obtained by interpolation.

Vapor pressure moisture curves are given for a number of different varieties of cheese. The vapor curve was found to be influenced by the

amount of salt in the cheese, but differences between varieties cannot be accounted for entirely in terms of differences in salt content.

A preliminary experiment on the relationship between vapor pressure of Stilton cheese and amount of blueing indicated that such a relationship does in fact exist, but that a much larger experiment is required before the connection is fully understood.

Preliminary experiments on the measurement of the vapor pressure of milk showed that additions of 2–3 per cent of water in milk can be detected. S.T.C.

702. Electrical Testing Device. NATHAN SCHNOLL. Amer. Milk Rev., *3*, No. 5: 106, 107. May, 1941.

As a method for checking the percentage of free caustic in a washing machine the solubridge is a modified Wheatstone bridge for measuring conductivity of caustic solutions. By using a rotary switch different tanks of caustic solutions may be checked with the same bridge. Used with a total alkalinity test it gives positive knowledge of the strength of the washing solution. P.S.L.

703. Autoxidation Measurements on Fatty Oils Using Barcroft-Warburg Apparatus. W. R. JOHNSON AND CHARLES N. FREY, Fleischmann Labs., Standard Brands, Inc., New York, N. Y. Jour. Indus. and Engin. Chem., Analyt. Ed., *13*, No. 7: 479–481. 1941.

The Barcroft-Warburg equipment was used to measure induction periods of sesame and cottonseed oils at temperatures from 50° C. to 100° C., most of the determinations being made at 100° C. in an atmosphere of oxygen. The data show that the Barcroft-Warburg technique can be extended to elevated temperatures with convenience and precision. B.H.W.

704. Determination of Thiamin by the Thiochrome Reaction. R. T. CONNOR AND G. J. STRAUB, Central Labs., General Foods Corp., Hoboken, N. J. Jour. Indus. and Engin. Chem., Analyt., Ed., *13*, No. 6: 380–384. 1941.

This method for the determination of thiamin first proposed by Jansen is based on the measurement of the florescence produced by thiochrome formed by the oxidation of thiamin with potassium ferricyanide in an alkaline solution. This paper defines more exactly than has been done previously, the optimal conditions for carrying out the thiochrome procedure and suggests some improvement in the equipment used. Extraction and hydrolysis of the sample are carried out in the same vessel and for the enzymatic hydrolysis of cocarboxylase, the enzyme clarase is introduced. Optimal conditions for the oxidation of thiamin and for the extraction of the thio-

chrome formed are given. The method is in close agreement with biological assays and has been applied to various types of natural products including whey and skim milk powders. B.H.W.

705. Combined Determination of Riboflavin and Thiamin in Food Products. R. T. CONNOR AND G. J. STRAUB, Central Labs., General Foods Corp., Hoboken, N. J. Indus. and Engin. Chem., Analyt. Ed., *13*, No. 6: 385–388. 1941.

A rapid and accurate procedure is described which makes possible the determination of both vitamins on the same sample. The method is an extension of the one proposed for thiamin and gives results closely agreeing with biological assays. It has been applied to grains, dairy products, fresh and frozen vegetables. Rapid destruction of riboflavin in aqueous solutions by light was found. Destruction by diffused light of the laboratory occurred irrespective of pH but in artificial light destruction was slower and dependent upon pH. The pH range from 2 to 8 was studied. Ferrebee's procedure for adsorption of riboflavin on Supersorb was modified to use a smaller extraction column and a study was made of Corning glass filters suitable for the fluorometric determination of riboflavin. B.H.W.

706. Distribution of Nitrogen and Protein Amino Acids in Human and Cow's Milk. ELIOT F. BEACH, SAMUEL S. BERNSTEIN, OLIVE D. HOFFMAN, D. MAXWELL TEAGUE AND ICIE, G. MACY, Res. Lab. Children's Fund of Michigan, Detroit. Jour. Biol. Chem., *139*, No. 1: 57. May, 1941.

The amounts (in milligrams) of seven amino acids contained in the proteins of 100 ml. of human and cow's milk were calculated to be as follows:

	Cow's milk	Human milk
Histidine	59	12
Arginine	127	40
Lysine	223	50
Tyrosine	197	50
Tryptophane	43	19
Cystine	23	20
Methionine	104	18

In the proteins of cow's milk the preponderance of sulphur is in the form of methionine, with very little in the form of cystine, while in the proteins of human milk the sulphur is about equally divided between cystine and methionine. V.C.S.

DISEASE

707. Lancefield Group B Streptococci (Str. agalactiae) on the Hands of Milkers and Others. J. HARRISON, Natl. Inst. Res. in Dairying,

Univ. Reading, Reading, Eng. Jour. Dairy Res., *12,* No. 1: 18–23. 1941.

Lancefield group B streptococci were recovered from the hands of all but one of the milkers (eight in all) on three different farms. A routine method of disinfecting the milkers' hands consisting of a soap and water wash followed by a rinse in sodium hypochlorite solution containing about 800 parts per million available chlorine was used on the farms. Following, in most instances, the routine cleaning, the hands were scrubbed with a hard nail brush in sterile milk and the milk examined for the group B streptococci using Edwards medium.

Since the organisms recovered from the hands of the milkers had the biochemical reaction of *Str. agalactiae,* and since none was recovered from the hands of non-milkers, the milkers' hands were considered to be a potential source of infection to cows. S.T.C.

708. Die Agglutinationsmethode nach Stableforth und Willems zur Feststellung der Rinderbruzellose. (The Agglutination Test of Stableforth and Willems in the Diagnosis of Brucellosis in Cattle. R. ENDRESS, (Aus der vet.-med. Abteilung d. Reichsgesundheitsamtes, Zweigstätte Dahlem.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *56,* No. 4: 297–320. 1940.

Since the agglutination test is the most important and widely used test for the diagnosis of this disease, attempts have been made to standardize it for international use. A standard test is necessary because of the great variation in methods used by different workers. Stableforth in London, and Willems in Brussels, have suggested standard methods for making the tests and this investigation was an attempt to evaluate them.

This writer used five strains of *B. bovis.* The cultures were grown on 2 per cent glycerine agar, pH 7.5, for 3–4 days at 37° C. The suspension was made in phenolized salt solution, filtered through gauze, and heated at 70° C. to kill the organisms. It was examined culturally and microscopically for purity. This suspension was standardized by a Leitz photometer to the density recommended by Stableforth (Brown No. 4 = 97 cc. 1 per cent H_2SO_4 plus 3 cc. 1 per cent $BaCl_2$ solution). The suspension was placed in the refrigerator 2–3 weeks at 5–6° C. and the agglutinability tested with a known serum. Stableforth kept a dried serum to which was added sterile saline at time of use to give dilutions of 1:25 to 1:750 in 30 tubes. To the various serum dilutions was added an equal volume of the antigen, thus doubling the dilution. Appropriate controls were included. The tests were incubated at 37° C. for 24 hours and for one hour at room temperature.

As a guide in the evalution of the antigen, Willems' modification of the formula of Stableforth was used. This consists of determining the agglu-

tination constant and the agglutinability index. In the agglutinability index the titer limit of a suspension of the antigen and a standard serum is observed. The agglutination constant is the relation between the diagnostic titer of the infection and the agglutinability index. According to Willems the former is 50 and the agglutinability index for his particular antigen was 700; therefore, the agglutination constant was $50/700 = 0.07142$. With the aid of a standard serum the agglutinability index of other antigens can be determined. By multiplying the numbers thus obtained by the agglutination constant the infection titer can be determined. The agglutinability index for the English strains of the organism studied was 500, for the German strains 550. The infection titer for the latter strain was $550 \times 0.07142 = 39.3 = 40$. A 25 per cent agglutination in a 1:40 dilution was considered as a positive reaction.

The sensitivity of all cultures used in preparing the antigens was tested before they were used. In routine testing, dilutions of the serum are made by using 1 cc. of serum and 4 cc. of 0.5 per cent salt solution. This is diluted to 1:80 or above. In this investigation 1:40 was considered as the infection titer.

From this study it was concluded that the agglutination method of Stableforth and Willems for the diagnosis of brucellosis in cattle is useful and reliable.

The advantage of the method lies in the accuracy and greater ease of determining the results. For the diagnosis in doubtful cases the method has an advantage.

The utilization of several strains of bacteria in preparing the antigen was considered necessary in order to make it as active as possible.

The difference obtained by the use of the Stableforth-Willems method and those methods now commonly used in Germany and the complement fixation test are negligible. L.D.B.

709. **The Role of Milk in Tuberculosis.** H. A. Reisman, Queens General Hospital, Jamaica, N. Y. Certified Milk, *16*, No. 179: 5. March, 1941.

Milk not only plays an important role as the vehicle through which this disease is transmitted to man, but it also plays an important role in the treatment of infected individuals, because of the availability of its calcium. The author concludes that the bovine strains of tuberculosis in man can be completely eliminated in two ways:

(1) The eradication of the disease at its origin by tuberculin testing, and the slaughtering of all positive reactors.

(2) By the universal pasteurization of all market milk. Since there is evidence to show that pasteurization does alter the milk, it would seem that the interest of health should demand a good, fresh, clean milk at the start.

W.S.M.

710. What an Inspector Should Look for in Making Dairy Cattle Physical Examination. C. U. DUCKWORTH, State Dept. Agr., Sacramento, Calif. Jour. Milk Technol., *4*, No. 1: 48. 1941.

An inspector should form the habit upon entering the stable to observe each animal carefully. The physical examination should take in manifest evidence of disease, such as enlarged glands, mastitis, pyometra, tuberculosis, ulcerated teeth, abscesses, and any abnormality in general.

Veterinary council is to be advised where any question occurs with respect to specific nature of any condition found. L.H.B.

711. Experimentelle Bruzellose beim Hunde. (Experimental Brucellosis in the Dog.) WALTER DOMKE (Aus dem Hygienischen institut der Tierärztlichen Hodhscule Hannover.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *56*, No. 4: 321–328. 1940.

The views concerning the abortus bacillus infection in the dog and the possibility of this animal being a disseminator of the organism are conflicting. The serological evidence on this point is very limited. Some writers on the subject consider that there may be certain factors which will influence the sensitivity of these animals to this infection.

Dogs of different ages were fed and inoculated with the organism. Clinical observations and agglutination tests were made for three-month periods when the animals were killed and cultural and pathological studies made. In order to test the localizations of the organisms suspensions of the sex organs were made in salt solution and 2 cc. injected into guinea pigs. These animals were then examined for 6 weeks when they were killed and cultural and serological examinations made.

From this investigation it was concluded that the dog is susceptible to *Brucella bovis* infections by feeding and injection. Clinically the course of the disease in the dog is much the same as in man. Pathological changes were observed in the genital organs. The agglutination titer followed very closely the fever curve. Gravid females were more susceptible than others.

Pathologically the disease in dogs is similar to that in cattle in that the organisms localized in the placental membranes.

An agglutination titer of 1: 80 was observed in some of the infected animals. L.D.B.

712. Erfahrungsbericht über die amtlichen tierärztlichen Milchuntersuchungen aus dem Jahre 1937–1938 in Regierungsbezirk Oberschlesien. (Report of the Official Veterinary Milk Investigations for 1937–1938 in Upper Silesia.) OTTO SCHIEL (Aus dem Staalichen Veterinär-Untersuchungsamt Oppeln). Ztschr. f. In-

fektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *57*, No. 2: 159–176. 1941.

This issue contains only the first part of the paper; the final part will appear in the next issue of this journal.

The report is detailed and contains considerable information on the results of official investigations of the milk supply of the area together with a scheme for control of milk-borne diseases. The report deals with the finding of tubercle bacilli, the Bang's disease organism and mastitis streptococci.

It has not been possible to control tuberculosis entirely in these larger areas and the official veterinary control service has developed plans for a more detailed study of the disease producing organisms and methods of control in a limited area. The study covered the years 1937 and 1938. Udder tuberculosis was found in 1.92 per cent of 3,381 samples from 61 different sources. Abortion bacilli were found in 8.21 per cent of these samples. Microscopic examination revealed 1.16 per cent contained mastitis streptococci. There was considerable variation in the results obtained with milk from different sources.

Conclusions will appear in the next issue. L.D.B.

713. **Zur Feststellung von Tuberkelbazillen in Ausscheidungsproben vom Rind. (A Study to Determine the Presence of Tubercle Bacilli in the Excreta of Cattle.)** W. STOCKMAYER (Aus dem Württ. Tierärztlichen Landesuntersuchungsamt.) Ztschr. f. Infektionskrank., Parasitäre Krank. u. Hyg. der Haustiere, *57*, No. 1: 75–84. 1940.

The older methods of detecting the presence of tubercle bacilli in excreta by animal inoculation is tedious, time-consuming and costly. The writer has developed other methods which he considers as satisfactory substitutes. The methods here described are microscopic, cultural and animal inoculation.

The microscopic methods may be direct, using fresh material or following an enrichment. A combination of the two appears preferable to either alone. The writer examined 202 samples of bronchial slime from tuberculous animals by means of the antiformin method using 2.5 per cent concentration of the chemical. The material was obtained with a tracheal cannula and swab. The sample thus obtained by means of a swab was spread directly on slides. The swab was then placed in 2.5 per cent antiformin for 20 minutes and squeezed out with sterile forceps. The fluid thus obtained was centrifuged and the sediment stained by the Ziehl-Neelsen method and examined. Of the 202 samples studied, 119 were positive by direct examination and 113 by the so-called enrichment process. In 41 instances the direct examination was most strongly positive and in 24 instances the

enrichment method gave better results. The reason the direct examinations gave more positive results was that the organisms were in clumps and more easily found. Also, the antiformin treatment tends to reduce the intensity of the staining.

The usual method of injecting animals and holding them for eight weeks with frequent examinations and removal of regional lymphatics is time-consuming. An attempt was made to reduce this period. The material obtained on a swab was placed in 1 cc. salt solution and centrifuged and the sediment suspended in 1 cc. of the cream obtained from 60 cc. of milk. This material was injected into guinea pigs and the animals examined at weekly intervals. When enlarged lymph nodes could be observed they were recovered under local anaesthesia and examined microscopically for the presence of tubercle bacilli. All negative glands were examined histologically and the presence of giant cells was considered as positive. The writer concludes that holding the animals for six weeks is not sufficient.

For cultures the writer used sediments obtained by suspending the slime in an HCl solution (15 cc. HCl sp. gr. 1.122 plus 83 cc. aq. dist.) from five to 15 minutes with shaking followed by centrifugation for 10 minutes. The sediment was then placed on Patragnani-Witte media. It was found that this concentration of HCl did not destroy the tubercle bacilli in the time used. There was good agreement between the results of culture and animal inoculation. The culture usually gave 20–30 per cent more positive results than the microscopic examination.

As a result of this investigation the writer concludes that animal inoculation gives the highest results and that the guinea pigs used in such investigations should be held for eight weeks or longer. L.D.B.

FOOD VALUE OF DAIRY PRODUCTS

714. Effect of Milk on Gizzard Erosion and Cholic Acid in the Chick. H. J. Almquist, E. Mecchi and F. H. Kratzer, College of Agr., Univ. California, Berkeley. Soc. Expt. Biol. and Med. Proc., *47:* 525. 1941.

Evidence was presented for the existence in cows milk of a labile substance which acts like cholic acid. Dried milk products were fed by mixing in the diets while liquid milk products were given to the chicks in place of the drinking water. Liquid milk products reduced the severity of gizzard erosion, increased the gall bladder bile volume per chick and increased the quantity of cholic acid per chick. There was no effect on the characteristics measured following the feeding of dried milk products. Attempts to detect cholic acid in skim milk produced only negative results.

R.P.R.

715. **The Effect of Vitamin A Intake on Vitamin A Content of Butterfat.** HARRY J. DEUEL, JR., NELLIE HALLIDAY, LOIS HALLMAN, CORNELIA JOHNSTON AND ALBERT J. MILLER, Dept. of Biochemistry, Univ. of Southern California, Los Angeles, Calif. Jour. Biol. Chem., *139*, No. 1: 479. May, 1941.

The supplementary feeding of vitamin A in the form of Shark liver oil greatly increased the vitamin A content in the butterfat. The milk production of the cows receiving the vitamin A supplement promptly rose and continued at a level approximately 10 per cent higher than the cows not receiving the vitamin A during the test. V.C.S.

716. **Proteins and Our Dairy Products.** F. H. PLETCHER, Lab., Borden Farm Products, Brooklyn, N. Y. Milk Dealer, *30*, No. 8: 36, 56–60. May, 1941.

Proteins are discussed under the following headings: Why Stress Proteins? What is Protoplasm? Chemical Composition. Amino Acids. Milk Proteins. Needs of Adults. The author summarizes his discussion as follows:

Why stress the importance of proteins in our dairy products when there are also present the all important minerals, and excellent carbohydrate, butterfat and vitamins in varying quantities? Possibly because we seldom realize we handle this food nutriment—when we think of milk our cothought is usually fat and sometimes total solids.

Proteins, however, are highly important in the diet—especially the milk proteins, for in milk we have the "complete" proteins, capable of maintaining life and supporting growth. Other foods are rich in protein, but they are usually lacking in some essential amino acid and are therefore either "incomplete" or "partially incomplete." An example of a food rich in "incomplete" proteins is gelatine, which will neither maintain life nor support growth; cereals, on the other hand, are generally classed as "partially incomplete" since they are usually lacking in at least one essential amino acid which is necessary for the promotion of growth, although they will maintain life.

But in milk or other dairy products such as cottage cheese we have protein foods containing all of the known 23 amino acids which when included in the dietary, in sufficient amounts, have been proved by experimentation to possess outstanding properties.

It's up to the men in the dairy industry to educate consumers on this point. C.J.B.

717. **Effect of Supplemented Raw and Pasteurized Milks upon Growth and Well-being of Rats.** ALICE M. BAHRS, St. Helen's Hall Junior

College, Portland, Ore., AND ROSALIND WULZEN, Orgeon State College, Corvallis, Ore. Certified Milk, *16*, No. 180: 5. Apr., 1941.

Rats fed raw milk rations showed a superior growth and certain tissues of the body showed distinct differences. W.S.M.

718. **The Soil Basis of Better Milk Production.** L. A. MAYNARD, Cornell Univ., Ithaca, N. Y. Certified Milk, *16*, No. 181: 5. May, 1941.

The author discusses ways in which the soil is definitely related to milk quality. It is generally understood that the nature of the feed of the cow affects the quality of the milk as well as its quantity. But, it is less appreciated that the quality factor in the feed depends upon how the feed crop is produced and particularly upon the fertility of the soil. The influence of the soil on milk quality is an indirect one, expressing itself through the food crop, yet it has some advantages over adding deficient nutritive essentials directly to the feed. W.S.M.

719. **Possibilities of Improving Milk by Increased Nutritive Qualities in Feeds.** D. B. HAND, Cornell Univ., Ithaca, N. Y. Certified Milk, *16*, No. 182: 7. June, 1941.

The author concludes that iodine and a few of the fat soluble constituents, notably vitamins A and D, are the only substances in milk which are greatly dependent on feed. From the practical standpoint for some of the other constituents, it is possible to improve the quality of milk by selection of individual cows. However, selection of cows offers many complicated problems. The quality of milk with respect to color and flavor can be improved by feeding. How many compounds are present in milk and which of these are essential to human diet is still unknown. W.S.M.

720. **Nutritional Restoration and Fortification of Foods.** Jour. Indus. and Engin. Chem., *33*, No. 6: 707–722. 1941.

Several papers on this subject which are of general interest were presented in a symposium at the 101st meeting of the American Chemical Society, St. Louis, Missouri. These papers are as follows:

Nutritional Requirements of Man. C. A. Elvehjem, Univ. of Wisconsin, Madison, Wis.

Cereal Products. R. T. Connor, General Foods Corp., Hoboken, N. J.

Fortification and Restoration in the Baking and Dairy Industries. James A. Tobey and William H. Cathcart, Amer. Inst. of Baking, New York and Chicago.

What the Consumer Should Know about Fortified Foods. Helen S. Mitchell, Nutrition Div., Health, Welfare and Activities Affecting National Defense, Washington, D. C.

Fortification and Restoration of Processed Foods. R. R. Williams, Bell Telephone Labs, New York, N. Y.

Control Problems of the National Nutrition Program. E. M. Nelson, U. S. Food and Drug Admin., Washington, D. C. B.H.W.

ICE CREAM

721. Helping the Ice Cream Retailer Stay in Business. JOHN KIRKWOOD, Advertising and Marketing Counsellor, Toronto, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 52. 1941.

The author discusses rather lengthily the principles of a retail business. Although he points out that 75 per cent of retailers do not last 7 years, he states that retailing can be a "safe" business if business principles are charted and the chart used as a guide. O.F.G.

722. Some Suggestions for Stepping up Winter Sales of Ice Cream. ANONYMOUS. Ice Cream Rev., *24*, No. 7: 94. 1941.

Suggestions are given for increasing the use of ice cream during the winter months by means of health appeal, colored mailing pieces and magazine advertisements. J.H.E.

723. Ice Cream Production by Regions for the Years 1930–1939. E. E. VIAL, Bureau of Agr. Econ., U. S. D. A., Washington, D. C. Ice Cream Rev., *24*, No. 7: 84. 1941.

Data, gathered by the Agricultural Marketing Service, is compiled showing ice cream production and per capita consumption for principal geographic areas. These data indicate that per capita consumption of commercial ice cream is highest in the North Atlantic states, amounting to 3.02 gallons in 1939. The South Central states were lowest with 1.17 gallons per capita in the same year. J.H.E.

724. Sugars That Can Be Used in Ice Cream Making. P. H. TRACY, Univ. Illinois, Urbana, Ill. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 68. 1941.

Sugars, which compose approximately 20 per cent of the weight of ice cream, perform several rather important functions when used in ice cream. They are high in energy value and greatly increase the palatibility of the ice cream. They sufficiently lower the freezing point of the mix to permit incorporation of the desired amount of air without the semifrozen mass becoming too stiff to be removed from the freezer. Granulated cane and beet sugars are the most important sources of sweetness for ice cream. Generally the dry sugar has been used but in recent years a sugar syrup which contains about 68 per cent solids has found some favor. The advantages of the syrup are that it can be transported in tank trucks and

handled by pumps. Corn sugar, known as dextrose, glucose or cerelose, is a monosaccharide and differs in some of its properties as compared to cane or beet sugar, known as sucrose, which is a disaccharide. Dextrose is manufactured by hydrolyzing corn starch. It has a sweetening value of approximately 70 as compared to 100 for sucrose but its sweetening power is increased when used in conjunction with sucrose in ice cream. A new type of corn syrup, known as "Sweetose," recently has been perfected which has a much higher dextrose equivalent and better flavor than the older syrup. "Sweetose" has a beneficial effect upon the body of ice cream, sherbets and ices. A dry corn sweetening agent, known as "Fro-dex," has recently been introduced. In the selection of a sweetening agent the manufacturer of ice cream should be governed by cost as well as by the effect of the product upon the ice cream. O.F.G.

725. Some Pointers in Making Sherbets and Water Ices. P. H. TRACY, Univ. Illinois, Urbana, Ill. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 20. 1941.

Desirable features of a good stabilizer for ices and sherbets are given as follows: (1) Stabilizing qualities should not be impaired by citric acid, (2) should be easily dispersed, (3) should be a desirable effect upon texture and resistance, (4) should have sufficient effect upon viscosity to prevent settling out of unfrozen syrup, (5) should not cause high overrun, (6) should be tasteless.

The merits and methods of using such stabilizers as gelatin, gums, pectin and carob bean products are discussed. The amount of a monosaccharide sugar, such as honey, dextrose or sweetose, which can be used in conjunction with sucrose is limited to about 7 per cent. The following defects are discussed: bleeding, surface crustation, crumbly body, hard body, snowy body, coarse body and sticky body. O.F.G.

726. Ice Cream Sales Index for 1941. Statistical and Accounting Bureau. Internatl. Assoc. Ice Cream Mfrs., Washington, D. C. July, 1941.

This bulletin contains an analysis of ice cream sales in the United States and Canada for the first four months of 1941. The increase in sales over 1940 for the first four months of 1941 is as follows:

Month	Per cent of increase	
	United States	Canada
January	31.42	49.07
February	9.57	39.07
March	15.14	45.08
April	27.66	78.44
Average increase	21.05	56.52

The Central Eastern States led the increase for the four-month period with a gain of 28.4 per cent, the North Atlantic States, followed with 21.67 per cent increase over the previous year, the Midwestern States enjoyed a gain of 17.59 per cent, the Southern States 15.77 per cent, while in the Western States the increase was 4.76 per cent.

The bulletin also contains an analysis of business and weather conditions in the different sections of the country. In the supplement to the bulletin the final ice cream statistics for 1939 of the Agricultural Marketing Service of the U.S.D.A. are given. The total production of ice cream for 1939 was 304,522,000 gallons. M.J.M.

727. **The Frozen Desserts Code Recommended by the Public Health Service.** A. W. FUCHS, Sanitation Section, U. S. P. H. Service, Washington, D. C. Jour. Milk Technol., *4*, No. 1: 26. 1941.

The need for public health control of frozen desserts is cited. Prior to 1928 there have been 36 outbreaks of milk borne disease reported in the literature as having been traced to ice cream. In the five-year period, 1934 to 1938 inclusive, ten outbreaks have been reported.

The Public Health Service urges states not already doing so to adopt a frozen desserts control program similar to the milk control program.

L.H.B.

728. **Chocolate Malted Milk.** C. E. HENDERSON, Bastian-Blessing Co. Ice Cream Rev., *24*, No. 6: 31. 1941.

The popularity of chocolate malted milk drinks is due to their delicious taste, high food value and the quick energy they provide. The variable factors are the chocolate flavor and the consistency of the finished drink. The consistency depends not only upon the proportions of milk and ice cream used, but also upon the temperature of the ingredients and the mixing time. Blending is done by whipping air into the milk as the ingredients are mixed. Milk at 32° F. can hold approximately 90 per cent air. Electric mixers accomplish mixing and aeration in less than two minutes. If the mixing is too rapid, the ice cream will be broken down instead of blended. If not removed from the mixer when the maximum aeration of the milk has been accomplished, the air will escape as the temperature of the milk rises. Success consists largely in keeping the ingredients as near the freezing temperature as possible during mixing.

A number of formulas are given. J.H.E.

729. **New Developments in the Science of Ice Cream Making.** W. H. MARTIN, Kansas State College, Manhattan, Kan. Ice Cream Rev., *24*, No. 6: 34. 1941.

This is a review of the recent problems and developments in ice cream

manufacture. Shrinkage, ingredients, melting qualities, stabilizers, flavors and sanitary quality are some of the subjects discussed. J.H.E.

730. Sugars That May be Used in the Ice Cream Industry. P. H. TRACY, Univ. of Illinois, Urbana, Ill. Ice Cream Rev., *24,* No. 5: 29. 1940.

When the ice cream manufacturer replaces sucrose with other sweetening agents he should expect some differences in results. The type of sweetness may not be the same, but this is not necessarily a disadvantage. Some of the present replacement agents contain prosugars of high molecular weight so that the lowering of the freezing point of the mix is not a serious problem. It is claimed that in some cases a better flavor and a better body may result from the use of a sweetening agent made from corn. J.H.E.

731. The Control of Shrinkage in Ice Cream. J. H. ERB, Ohio State University, Columbus, Ohio. Canad. Dairy and Ice Cream Jour., *20,* No. 3: 60. 1941.

The mechanism of ice cream shrinkage is fundamentally a problem of a destabilized protein or a protein sensitive to coagulation. Factors which operate to influence protein stability are, (1) salt balance of the original milk, (2) added salts, (3) acidity, (4) composition of the mix, (5) homogenizing pressures, (6) stiffness of freezing, and (7) hardening temperature and fluctuations in storage temperature. Another factor which influences shrinkage is the ease of air transfer. Mechanical factors which influence air transfer are (1) type of container, (2) jolting of truck, (3) amount of overrun, (4) size of air cells, and (5) external pressures. Factors making for a stable, or highly hydrated plastic protein are desirable in correcting shrinkage since such a protein better holds the air within the cells. O.F.G.

732. Is There a Place for Substandard Products in the Ice Cream Industry? C. H. SNOW, Snow and Palmer Co., Bloomington, Ill. Ice Cream Rev., *24,* No. 5: 74. 1940.

There are dairy products which are meritorious for which standards are desirable. Among these are "cereal cream" and a frozen malted milk mixture. Standards are created by custom of consumers and of the trade generally or they may be created by law.

The test of value in any specific product would seem to be whether or not its production and sale resulted in benefit to the producers of milk and the consumers of the product. Substandard or inferior commodities should not be substituted for the genuine, but there are places for additional standards for special products that fill a real need which is beneficial to industry.

J.H.E.

733. **Use of Vanilla and Other Flavors in Ice Cream.** ANONYMOUS. Ice Cream Rev., *24*, No. 7 : 114. 1941.

This is a review of a paper written by Dr. A. Katz of Florasynth Laboratories, Los Angeles, California. In the inception of the ice cream industry, the ice cream was flavored with chopped vanilla beans, as the art of making extract was not known. In the early days of making vanilla extract 95 per cent alcohol was used. This destroyed the fine vegetable aromatic principles present in the vanilla beans. Best results are now obtained using 30 to 35 per cent alcohol.

To obtain proper results in citrus flavorings it is necessary to utilize not only the juice of the fruit but also flavor obtained from the peel. These should be combined together in a vegetable gum media.

Interesting facts about other flavors for ice cream such as butterscotch, English toffee, grenadine, etc., are included. J.H.E.

734. **Dextrose and Corn Syrup for Frozen Desserts.** A. C. DAHLBERG AND E. S. PENCZEK, New York Agr. Expt. Sta., Geneva, N. Y. Ice Cream Rev., *24*, No. 7 : 38. 1941. (This is a review of the original New York Agr. Expt. Sta. Bul. No. 696, "Dextrose and Corn Syrup for Frozen Desserts" by the authors of this article.)

Good results can be secured when 25 per cent of the sucrose in ice cream is replaced with dextrose or corn syrup to give comparable sweetness. Based upon securing comparable sweetness the weight of the dry corn sweeteners required to replace one pound of sucrose is as follows: Enzyme-converted corn syrup, 1.5 pounds; corn syrup solids, 2.0 pounds, and dextrose, 1.1 pounds. J.H.E.

735. **Understanding Improves Consumer Friendship.** RACHAEL REED, The Borden Co., Chicago, Ill. Ice Cream Rev., *24*, No. 7 : 34. 1941.

It is possible for the ice cream industry to profit by the experience of fluid milk and other industries in giving the consumers the information they would like to have about the ice cream industry and its products before they become too critical. A number of things consumers are interested in about ice cream are discussed. The article contains several good tables on the comparative food value of ice cream and other desserts. J.H.E.

736. **High Operating Costs.** C. F. BAKER, Atlanta, Ga. Ice Cream Rev., *24*, No. 7 : 29. 1941.

Some of the common causes for high operation costs of the refrigeration system are malpractices in connection with pumping condenser water, low ammonia charge, high condensing pressures and oil in evaporating coils. Examples of these conditions and their remedy are given. J.H.E.

737. Cherries, the Luxury Fruit. HOWARD BLACK, Traverse City, Mich. Ice Cream Rev., *24*, No. 7: 26. 1941.

The author sketches some interesting history of cherries. Cherries are a valuable ice cream flavor because of the eye appeal. J.H.E.

738. Formulas for Combination Flavors of Pineapple and Other Flavors. ANONYMOUS. Ice Cream Rev. *24*, No. 6, 52. 1941.

A number of new ice cream flavors combining pineapple and other popular flavors has been developed by Dr. C. D. Dahle, of Pennsylvania State College. Detailed formulas and other helpful instructions are given for a number of combinations. J.H.E.

739. Pasteurizing the Ice Cream Mix. J. M. BRANNON, Univ. of Illinois, Urbana, Ill. Ice Cream Rev., *24*, No. 6: 28. 1941.

The ice cream industry is endeavoring to fix a standard temperature and time for pasteurization of ice cream mix. It has been shown that a temperature of 150° F. for three minutes will kill *Eberthella typhi*, beta hemolytic streptococci, *Corynebacterium diphtheria* and bovine tubercular bacilli in ice cream mix. Twelve states have set bacterial standards for ice cream.

The results of a survey of Illinois ice cream made a few years ago by the author are cited which indicated only 17 per cent of the samples had bacterial counts of 100,000 or less. Sixty per cent of these samples gave a positive test for the coli aerogenes group. The author concludes that in the average plant the ice cream mix is generally sufficiently pasteurized but that large numbers of organisms are picked up from equipment after pasteurization. J.H.E.

740. Cutting Truck Refrigeration Costs. ANONYMOUS. Ice Cream Rev., *24*, No. 6: 26. 1941.

The experience of a southern ice cream company with a new group of five refrigerated trucks is discussed. Three of the five trucks are used for city delivery and are quipped with ammonia refrigeration coils. These are hooked up each day to take-off lines at the plant. Trucks on country routes, which do not return to the main plant every day, are equipped with compressors as well as power take-off units on the drive shaft. Savings have been especially realized for the country trucks because they do not lose efficiency when kept in the territory overlong. They can spend more time in actual service than formerly. J.H.E.

MILK

741. Milk in the Schools. F. W. HAMILTON, Royal Oak Dairy, Ltd., Hamilton, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 1: 17. 1941.

The primary objective in the sale of milk in schools is not the sale of milk

but the development of the milk drinking habit. The school is the best place to develop in young people the habit of drinking milk instead of soft drinks, tea and coffee. A plan was worked out through the Board of Education whereby the dairies agreed to furnish refrigeration in the schools and in return were to be allowed to sell "free" milk to school children. The schools were apportioned to those competing dairies which wished to enter the plan. For the year 1938–1939, before the plan was put into effect, the amount of free milk given out was 273,000 half pints. For the year 1939–1940, after the plan went into effect, the free milk totaled 378,000 half pints and the sale milk totaled 285,000 half pints. O.F.G.

742. Cooked Flavor in Milk, a Study of its Cause and Prevention. I. A. GOULD, Michigan State College, East Lansing. Internatl. Assoc. Milk Dealers, Assoc. Bul., 33rd yr., *21:* 553–564. Apr., 1941.

That the "sulfurous-like" flavor produced in milk by high temperature heat treatment is related to sulfur compounds is shown by producing the defect by adding to milk a sulfite salt or glutathione which contains the sulfhydryl (–SH) linkage and artificially producing a cooked flavor. Two methods were employed to determine if sulfur were involved (a) liberation of sulfides from the milk, (b) the nitro prusside test which detects the presence of sulfhydryl (–SH) groups for which a slight modification of the technique of Jacobson and Doan was used. The temperature at which the flavor appears is 76–78° C. (169–172° F.) for momentary heating and 70–72° C. (158–162° F.) for 30 minute holding.

The momentary temperature required to produce the cooked flavor is raised to 84–86° C. (183–187° F.) when 1 p.p.m. of copper was added after heating. A somewhat closer relationship was found between the cooked flavor and the sulfhydryl groups than heat labile sulfides. A lower critical temperature for cream than skim milk suggests that the proteins are associated with the fat, also these proteins were not removed by 3 washings of the cream. The retardation or prevention of oxidized flavor in cooked milk may be due to the creation of a reducing system unfavorable to oxidation or through direct combination with the metals which are oxidative catalysts. Copper is more effective in preventing or dispelling cooked flavor when added after heating. Two p.p.m. of copper has been used to dispel heated flavor in heat treated soft curd milk. E.F.G.

743. Developments in Production and Reports in 1940. J. F. SINGLETON, Assoc. Director of Marketing Service, Dairy Products, Ottawa, Ont. Canad. Dairy and Ice Cream Jour., *20,* No. 1: 24. 1941.

The production and marketing of dairy products in Canada for 1940, has been conditioned greatly by the needs of the United Kingdom. There has been some diversion of milk supplies from butter to cheese and evaporated

and condensed milks. Butter prices dropped while cheese prices increased. In order to conserve supplies of cheese for the Ministry of Food, the Board has taken action to restrict exports to non-Empire countries. Production of condensed milk, evaporated milk and skim milk powder was higher than in 1939. Greater total milk production for 1941 could be used to advantage.
O.F.G.

744. **Operating a Credit Bureau in the Milk Industry.** E. J. LeBoeuf, Windsor Milk Distributor's Assoc., Windsor, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 1: 44. 1941.

The Credit Collection Agency results in a control bureau which does away with loose credits by keeping accounts from becoming inactive. The co-operative method promotes good will to all. O.F.G.

745. **Co-operation for Efficient Milk Production.** H. B. Ellenberger, Univ. Vermont, Burlington, Vt. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 20. 1941.

Milk can be made more cheaply when approved modern and efficient methods are practiced more generally on farms. Both co-operatives and proprietary distributors can well afford to lend a helping hand to producers, for in the long run both would profit. Many reasons exist why distributors and producers should co-operate. It is net income rather than gross income that is usually important and efficiency is the master key to better net income. Dealers and producers can accomplish more through working jointly than through independent action. O.F.G.

746. **The Determination of the Viscosity of Human Milks and the Prenatal Secretions.** George W. Scott Blair, Natl. Inst. Res. in Dairying, Univ. Reading, Reading, Eng. Biochem. Jour., *35*, No. 3: 267–271. March, 1941.

An apparatus, quite similar in principle to the Ostwald pipette, is described by which the viscosity and viscous anomalies of small (1 ml.) samples of human mammary secretions may be quickly and accurately determined.

These secretions, tested at blood heat, behave in general, surprisingly like true fluids, *i.e.*, their viscosities differ but little with varying shearing stress. V.C.S.

747. **Methods of Producing High Quality Table Cream.** O. J. Schrenk, Bowman Dairy Co., Chicago, Ill. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 58. 1941.

All the rules and regulations laid down for the production of good milk should apply to cream. Cleaned, rinsed and sterilized equipment made of

metals that cause no off-flavors is essential to good flavor in cream. Good viscosity is essential to table cream and methods of producing a higher viscosity are described. The chief contributing factor to undesirable cream plug formation is agitation at temperatures within the churning range. Cream feathering is increased by, (1) coffee made with hard water, (2) long-time contact of coffee with the grounds, (3) high acid cream, (4) high calcium and magnesium content of cream, (5) low pasteurization temperature, (6) high homogenization pressure, (7) low homogenization temperature, and (8) single-stage homogenization. Feathering is reduced by (1) soft water, (2) short-time contact with coffee grounds, (3) low acid in cream, (4) addition of sodium citrate, (5) high pasteurization temperature, (6) low homogenization pressure, (7) high homogenization temperature, and (8) two-stage homogenization. The formation of a skim milk layer may be inhibited by high pasteurization temperature or careful homogenization. To the average consumer yellow color in the cream is an indication of richness and therefore is important. O.F.G.

748. A Discussion of Public Relations in the Milk Industry. H. L. GARNER, Ontario Daily Newspaper Assoc., Peterborough, Ont. Canad. Dairy and Ice Cream Jour., *20*, No. 2: 26. 1941.

A public relations program really means a program for building public goodwill, or favorable public opinion. To sell your milk products, you must, first, win the goodwill of the public and, second, win the good opinion of the workers in your plant. Goodwill is based on good "works" and on good "words." O.F.G.

749. Public Relations in the U. S. Milk Industry. G. G. DIFFENBACH, Abbott's Dairies, Philadelphia, Pa. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 23. 1941.

A vast amount of work by the industry along nutritional and consumption lines is essential. An intensive type of activity must be pursued to acquaint the public with the unique position of the fluid milk distributor and the industry he represents. People must be informed more fully about the functions and operation of the dairy industry. O.F.G.

750. Mechanical Principles and Problems of Vat Pasteurization. A. H. RISHOI, Cherry Burrell Corp., Chicago, Ill. Dairy World, *20*, No. 1: 22. June, 1941.

This is a detailed discussion of the problem of heat transfer in vats used for pasteurizing milk products particularly with reference to conductivity of the material separating the heating or cooling medium and the fluid being heated or cooled, the temperature gradient and the importance of agitation

on either side of the material through which the heat flows. The following conductivity table is presented:

Material	Temperature °F.	Conductivity
Aluminum	64	0.504
Brass	64	0.260
Copper	64	0.918
German silver	32	0.700
Iron	64	0.161
Steel	64	0.115
Stainless steel	212	0.039
Nickel	64	0.142
Tin	64	0.155
Glass	68	0.002
Water	68	0.00143
Air	32	0.0000568

F.J.D

751. **Pasteurization of Modified Milk Products.** K. G. WECKEL, Univ. Wisconsin, Madison, Wis. Dairy World, *20,* No. 2: 17. July, 1941.

Pasteurization treatments for milk "by-products" differ from that for milk since body or viscosity, texture, flavor, solution of ingredients, color, bacteriological effects, etc., are often extremely important considerations, whereas with milk the main concern is to render the product free of pathogens. The author discusses the pasteurization or heat treatment problem involved in the manufacture of buttermilk, cultured cream, chocolate milk, pasteurized cream, homogenized milk, cheese spreads, ice cream mix and some less important products. F.J.D.

752. **Oxygen Constant Variants.** E. S. GUTHRIE, PAUL F. SHARP AND DAVID B. HAND, Cornell Univ., Ithaca, N. Y. Amer. Milk Rev., *2,* No. 6: 131, 132. June, 1940.

Milk absorbs from 3.84 to 9.74 p.p.m. of oxygen during hand milking but contains none while in the udder. It is absorbed slowly when the surface is quiet, is driven out during vat pasteurization, and reabsorbed during cooling and bottling. If deaerated it may be bottled without additional air by admission to the bottom of the bottle through a tube, taking care to cause a minimum of agitation. P.S.L.

753. **Testing Pasteurized Cream.** HERBERT JENKINS. Amer. Milk Rev., *3,* No. 3: 58–60. March, 1941.

Seeking to cut the time required for plate counts of pasteurized cream, many tests using resazurin dye were run, the time required being 6 to 7 hours. Those creams having a reduction time of 6 to 7 hours, when plated, had a plate count of less than 40,000 bacteria, the average being 17,000. Such cream occasioned no complaints from customers. P.S.L.

754. Studies on Soft Curd Milk Prepared by the Enzyme Treatment Method. A. W. TURNER, University of Illinois, Urbana, Ill. Soc. Expt. Biol. and Med. Proc., *46:* 593. 1941.

An investigation was made of the characteristics of enzyme-treated milk. The enzyme treatment was carried out by adding one part of pancreatic concentrate to 10 to 15 thousand parts of cold raw whole cow milk followed by immediate pasteurization. Enzyme-treated milk contained more 70 per cent alcohol-soluble protein and proteose peptone nitrogen but only slightly more amino nitrogen than ordinary pasteurized milk. Casein prepared from enzyme-treated skim milk was more soluble in 70 per cent alcohol than was casein prepared from pasteurized skim milk. R.P.R.

755. Are You Encouraging the Love Life of a Fly? ANONYMOUS. Milk Dealer, *30,* No. 8: 76–80. May 1941.

A discussion is given on how to control the fly in dairy plants. The article is summarized as follows: The best arrangement for controlling the fly menace, it would seem, is first to clean up and keep clean the premises; second, screen all doors and windows; third, supplement screens with electric screens to kill flies seeking admission; fourth, use a good power spraying system to get rid of the flies which get into the plant. C.J.B.

756. The Contribution of Industrial Milk Service to National Defense. JAMES R. HUDSON, Baker-Stuber Dairy, Peoria, Ill. Milk Dealer, *30,* No. 8: 116–121. May 1941.

A discussion is given of how the dairy industry can contribute to national defense by supplying factory workers with enough milk to keep up their efficiency, reduce absenteeism, and maintain their morale. C.J.B.

757. The Instantaneous Heat Treatment of Milk. G. C. SUPPLEE AND O. G. JENSEN, Borden Co., Bainbridge, N. Y. Jour. Milk Technol., *4,* No. 1: 5. 1941. (Also published in the Annual Proceedings of the New York State Assoc. of Dairy and Milk Insp., 1940.)

Using a flowing film electric pasteurizer the authors studied the effect of momentary exposure periods at various temperatures on bactericidal effectiveness, flavor, cream line and phosphatase test.

Exposure periods as short as 0.8 second between the lethal temperature range of 145° F.–185° F. were found effective in bactericidal reduction. No automatic controls were used on the equipment either to regulate the temperature or the flow of current. Variations in temperature from the average operating level did not exceed about 3°, and it was believed that the major variations may have been due to surges in the feed line voltage.

It was found that 76 per cent of the milks exposed to 180° F. and above showed a percentage reduction in bacterial count of over 99 per cent.

Data was obtained on the phosphatase test using Gilcreas and Davis' modification for average or mean temperatures of 163°, 173°, 177°, 181°, and 186° F.

At 163° F. all samples were classified as grossly underpasteurized, showing values of 0.15 and above. At 173° F. they varied from 0.03 to 0.15. At 177° F. they varied from 0.00 to 0.09; about 70 per cent of the samples were classified as satisfactorily pasteurized and the remainder as slightly underpasteurized. At 181° F. and above they were all classified as satisfactorily pasteurized having values of 0.04 or less.

The characteristic heated milk flavor was practically undetectable at operating temperature of 185–186° F. Improvement in the flavor was even noted at operating temperature of 185° F. and lower. At 190° F. the heated flavor was detected by experts, but was not as pronounced as is frequently observed in milk pasteurized by usual methods.

Reduction in creaming ability when compared to the raw milks ranged from 6 to 20 per cent through the temperature range of 160° F. to about 180° F. Creaming ability was destroyed most rapidly per degree increase in temperature through the 185–190° F. range. L.H.B.

758. A Simplified Procedure for Laboratory Examination of Raw Milk Supplies. R. P. MEYER AND J. A. PENCE, Sealtest, Inc., Baltimore, Md. Jour. Milk Technol., *4*, No. 1: 18. 1941.

For the purpose of testing producers' milk to find if it contains thermoduric organisms, the use of the loop measurement, oval tube technique saves time and material in making the laboratory pasteurization tests.

Test briefly is as follows:

Pasteurize 5-cc. samples of milk in screw-capped vial at 143° F. for 30 minutes, in a constant temperature bath, cool, shake vigorously 50 times. With standard loop needle (0.001 or 0.01 cc.) transfer loopful of milk to an oval culture tube (size 152 mm. in length and 23 mm. by 11.5 mm. in diameter) containing approximately 4 cc. of sterile melted T.G.E. agar cooled to 45° C. Mix contents by swinging tube through a small arc for about 5 seconds. Tube is then laid on table with open end raised about $\frac{1}{8}$ inch so tube is slanted to permit agar to flow to a point 2.5 to 3 inches from bottom of tube. Allow agar to harden. Place tubes in special wire rack in horizontal position with agar adhering to upper side of tube. Incubate for 48 hours at 37° C. Count colonies in usual manner by placing tubes over a well lighted colony counter. This method is reported to save one-half the time, uses only $\frac{1}{3}$ as much agar, and requires no dilution blanks and pipettes.

Results checked very closely with standard agar plate count using T.G.E.M. agar. L.H.B.

759. To What Extent Should Bacterial Counts of Milk be Given Publicity. C. C. PROUTY, Agr. Expt. Sta., Washington State College, Pullman, Wash. Jour. Milk Technol., *4*, No. 1: 32. 1941.

Bacterial counts should not be given publicity on the ground that the milk consuming public is not aware of the limitations of making bacterial counts, and therefore, not qualified to interpret them properly in relation to the sanitary quality of the product.

Equal ratings should be given to all samples falling into the same count bracket.

A discussion of the paper by M. E. Parker, of the Beatrice Creamery Company, Chicago, Illinois, is also given. L.H.B.

760. "Approved Milk" for New York City in Place of Grade A and Grade B. J. L. RICE AND SOL PINCUS, N. Y. City Dept. Health. Jour. Milk Technol., *4*, No. 1: 38. 1941.

A history of New York City's milk regulations are given reviewing the events in the development of milk control which leads up to the latest step of eliminating A and B grades and replacing with "Approved Milk."

The standards for the "approved milk" are stricter than formerly called to grade B milk which was the bulk of their supply. A chart showing the former requirements for grade A and B is given comparing the standards with those of "approved milk."

Advantages expected to be gained by simplification of grading system are:

1. It will be possible to concentrate all energies upon improving general supply without regard to grading.

2. With the elimination of dual grading the consideration of grade A as the only safe milk was removed as was the inferiority implication given grade B. Confidence of public in their milk supply will be encouraged.

3. Sanitary control will be simplified and the industry will be enabled to eliminate some plant duplication, where formerly they were required to maintain separate equipment for handling grades A and B. L.H.B.

761. Report of Committee on Applied Laboratory Methods. T. H. BUTTERWORTH, San Antonio, Texas. Jour. Milk Technol., *4*, No. 1: 44. 1941.

The committee presents a program of work for the coming year. At least one member of the committee is actively interested in one or more of the subjects which are as follows:

1. A study of requirement recommendations for officially certified milk and milk products analysis laboratories.

2. A study of the relationship of laboratory tests to field inspection work and an evalution of the emphasis to be placed on each.

3. A study of the use of the reductase test in controlling raw-to-plant milk supplies.

4. A study of the tentative 32° C. temperature requirements for milk and milk produce incubation.

5. A study of the numerical bacterial content of city supplies of raw-to-plant milk and the best tests for estimating same.

6. By means of a questionnaire to the industry and control officials, a study of the present usefulness of the phosphatase test and the most valuable modification for general use. L.H.B.

762. Some Practical Applications of Milk Technology. E. EUGENE CHADWICK, Acting City Sanitarian, Astoria, Ore. Jour. Milk Technol., *4*, No. 1: 45. 1941.

A discussion is given of how two cities in Oregon improved the sanitary conditions of their milk supplies. Milk consumption has increased from 0.7 pint per person to 1.3 pints. L.H.B.

PHYSIOLOGY

763. Growth of the Lobule-Alveolar System of the Mammary Gland with Pregneninolone. JOHN P. MIXNER AND CHARLES W. TURNER, Univ. of Missouri, Columbia. Soc. Expt. Biol. and Med. Proc., *47*: 453. 1941.

The injection of pregneninolone either alone or in conjunction with estrone into spayed virgin mice caused the development of the lobule-alveolar system of their mammary glands a property similar to progesterone. Injected estrone enhanced the activity of the pregneninolone about 5 times while under similar conditions progesterone was found to be about twice as effective. R.P.R.

764. Death of Embryos in Guinea Pigs on Diets Low in Vitamin E. ALWIN M. PAPPENHEIMER AND MARIANNE GOETTSCH, Columbia Univ., New York. Soc. Expt. Biol. and Med. Proc., *47*: 268. 1941.

A diet low in vitamin E supplemented by 5 to 10 mg. of alpha-tocopherol protected 5 guinea pigs against muscular dystrophy but the amount of supplied vitamin was not adequate to insure successful pregnancy. Three pigs receiving 5 mg. of alpha-tocopheral had resorption at about 30 days and of 2 pigs receiving 10 mg. one gave birth to a living young, followed by resorption (initial fertility) and the other one went beyond mid-term, dying on the 47th day. R.P.R.

765. Effect of Desoxycorticosterone on Pituitary and Lactogen Content. C. W. TURNER AND JOSEPH MEITES, Univ. of Missouri, Columbia. Endocrinology, *47*: 232. 1941.

Three groups of female and 2 groups of male guinea pigs were injected

with 7 to 20 mg. of desoxycorticosterone acetate over a period of 10 to 20 days to determine its effect on pituitary lactogen content and pituitary weight. The treatment produced a significant increase in pituitary weight but the lactogen content was not altered. R.P.R.

766. Biennial Reviews of the Progress of Dairy Science. Section A. Physiology of Dairy Cattle. I. Reproduction and Lactation. Jour. Dairy Res., *12*, No. 1: 78–107. 1941.

A review of recent literature under the subheadings: Hormones, Biochemical Aspects, Anatomical Aspects, Clinical Chemistry, Climatic and Other Factors Affecting Milk Secretion is given with 208 references. S.T.C.

767. Some Experiments on the Chemical Enrichment of Cows' Milk by the Administration of Diethylstilbestrol and Its Dipropionate. S. J. Folley, H. M. Scott Watson and A. C. Bottomley, Natl. Inst. Res. Dairying, Univ. Reading, Reading, Eng. Jour. Dairy Res., *12*, No. 1: 1–17. 1941.

The results secured were extremely variable as is shown by the following summary:

1. Diethylstilbestrol administered orally to a Shorthorn cow had no marked effect on milk yield or composition.

2. A series of injections of the dipropionate in oily solution led to a slight rise in non-fatty solids in the same cow as in (1). Single larger injections were followed by a rise in milk solids accompanied by a rapid fall in milk yield in two other Shorthorns.

3. Inunction with an ointment containing the dipropionate led to a marked increase in milk solids in a Shorthorn cow, with no change in milk yield. The effect subsided rapidly when treatment was stopped. No significant effects were produced by similar treatment of four pregnant British Friesians; on increasing the dose two of these aborted. A Guernsey cow showed a slight increase in non-fatty solids and a slight, but temporary, fall in milk yield.

4. Subcutaneous implantation of crystalline diethylstilbestrol led to a striking and prolonged increase in milk solids, with no fall in milk yield, in a Shorthorn cow.

5. Subcutaneous injection of an aqueous suspension of diethylstilbestrol (1 g.) was equally successful when applied to the same cow as 4, but in the next lactation. In three Ayrshires the increase in solids was accompanied by an appreciable decline in milk yield. A Shorthorn receiving 375 mg. showed a temporary rise in solids, while one receiving 225 mg. showed no effect.

6. In all cases where milk yields declined the milk solids percentage rose, but the converse did not hold. Hence, the threshold dose for inhibition is apparently higher than for enrichment.

7. The threshold doses may depend on the breed; the most successful results were obtained with Shorthorns.

8. Treated cows may be difficult to get in calf subsequently, especially those treated twice.

9. Administration of large doses of diethylstilbestrol to cows in advanced pregnancy results in abortion.

10. The enrichment of the milk in favorable cases represented a true increase in the yield of solids secreted, and not merely a concentration due to reduced secretion of water. S.T.C.

768. Comparison of Assay Methods Using International Standard Lactogen. J. MEITES, A. J. BERGMAN AND C. W. TURNER, Univ. Missouri, Columbia. Endocrinology, *28:* 707. 1941.

Three methods of assay of International Standard lactogen were compared, all assays based upon a 50 per cent minimum crop gland proliferation response in 20 common pigeons weighing 300 ± 40 gm. The subcutaneous route of administration required 0.1 mg. of the International Standard to equal the International Unit. The shallow intrapectoral method required 1.25 International Standard Units and the intradermal (micro) method required 1/160 of an International Unit. In connection with the intradermal method it was shown that a 2, 3 and 5 fold difference in injection volume containing the same amount of hormone caused no change in effectiveness of the crop gland responses. R.P.R.

769. Influence of Lactogenic Preparations on Production of Traumatic Placentoma in the Rat. HERBERT M. EVANS, MIRIAM E. SIMPSON AND WILLIAM R. LYONS, Dept. Anatomy, Univ. California, Berkeley, Calif. Soc. Expt. Biol. and Med. Proc., *46:* 586. 1941.

Experiments were conducted which demonstrated that the lactogenic hormone was the only pituitary preparation which would stimulate the production of progestin by either normally occurring or artificially induced lutein tissue in the rat. R.P.R.

770. Local Responses of the "Sexual Skin" and Mammary Glands of Monkeys to Cutaneous Applications of Estrogen. T. L. CHAMBERLIN, W. U. GARDNER AND E. ALLEN, Yale University, New Haven, Conn. Endocrinoloy, *28:* 753. 1941.

Small doses of estrogen in alcohol applied cutaneously induced local responses in the sexual skin of immature female monkeys. One to 3 gamma of estrone in alcohol daily for 8 to 12 days on one side produced a unilateral reaction while alcohol alone on the other side served as a control. Similar

treatment on one breast of young male monkeys induced considerably more growth in both nipple and glandular tissue of that side. The other mammary gland showed slight growth. R.P.R.

771. **Rumen Synthesis of the Vitamin B Complex on Natural Rations.** M. J. WEGNER, A. N. BOOTH, C. A. ELVEHJEM AND E. B. HART, Univ. Wisconsin, Madison, Wis. Soc. Expt. Biol. Med. Proc., *47:* 90. 1941.

Six members of the vitamin B complex were determined in the rumen ingesta of a heifer fed a ration composed of natural feeds. In most cases higher values were found in the rumen ingesta than in the ration fed. With the exception of riboflavin, variation of the amount of urea or protein in the grain mixture of the ration had little if any effect on the vitamin content of the ingesta. The authors were of the opinion that the increase in B vitamins in the ingesta as contrasted with the ration fed was due to a synthesis and not to a concentration effect. R.P.R.

772. **Inability of Desoxycorticosterone to Maintain Lactation.** ROBERT GAUNT, Washington Square College of Arts and Science, New York Univ., New York City. Soc. Expt. Biol. and Med. Proc., *47:* 28. 1941.

A study was made of the effect of desoxycorticosterone acetate on the lactation of rats adrenalectomized within 24 hours after parturition. The necessity of adrenal cortical secretions for the support of lactation in rats was confirmed. Desoxycorticosterone acetate, unlike adrenal cortical extract, was of no benefit at all in correcting this deficiency and might have further depressed lactation. R.P.R.

773. **The Interrelation of Oxidative and Glycolytic Processes as Sources of Energy for Bull Spermatozoa.** HENRY A. LARDY AND PAUL H. PHILLIPS, Univ. Wisconsin, Madison, Wis. Amer. Jour. Physiol., *133:* 602–609. 1941.

In spermatozoa the energy requirement for the maintenance of their vital activity can be obtained from the oxidation of intracellular phospholipids or from glycolytic processes. When sugars are available to the spermatozoa the energy obtained from their breakdown to lactic acid lessens the demand on the intracellular lipids. D.E.

774. **Brunsterzeugung bei Schafen mit Geschlechtshormonen.** H. M. AUGUST, Office of Animal Health, Breslau. Züchtungskunde, *16,* No. 2: 41–60. 1941.

In an attempt to cause sheep to come in heat in the spring so that fall lambs could be produced, 4,281 ewes in 28 flocks in German Silesia in the spring of 1938 were given intravenous injections of follicular hormone,

either alone or together with Prolan. Of the treated ewes 72.5 per cent accepted service and showed other signs of estrus and 43.1 per cent, (59.5 per cent of those which were served) produced lambs. Results appeared to be the same whether the folliculin was accompanied by Prolan or not. The methods used are not yet satisfactory for widespread practical application. The prospects for success are too uncertain, good results being obtained in some flocks but completely unsatisfactory results in others. Satisfactory explanations for these discrepancies were not found, but the problem is of enough practical importance and the present results are promising enough to deserve further investigation. J.L.L.

MISCELLANEOUS

775. Selling and Advertising Dairy Products. 1. "Let the Taste Tell and Sell." H. D. BURBIDGE, Jersey Farms, Ltd., Vancouver, B. C. Canad. Dairy and Ice Cream Jour., *20*, No. 1: 56. 1941.

Since nothing the dairy can say or write or picture about its products equals a taste of the product itself, sampling is an important sales aid to the progressive dairyman. The salesman should give the housewife a sample of all his products. Advice to the salesman is, (1) arouse interest, (2) cut down length of sales story, (3) arrange the sales story in the best possible sequence, (4) close with a bang. Let taste, not a description of taste, do your selling. O.F.G.

776. Selling and Adveristing Dairy Products. 3. "Selling Through the Kiddies." H. D. BURBIDGE, Jersey Farms, Ltd., Vancouver, B. C. Canad. Dairy and Ice Cream Jour., *20*, No. 3: 46. 1941.

The author suggested that an excellent approach to dairy sales is through children. Organize children's clubs, sports and contests, birthday clubs and write the children friendly letters, is his advice after having tried them. Other means of approach are through child talent radio programs and educational school films. O.F.G.

777. Utilization of Skim Milk and Whey in Precooked Dried Soup. G. A. RAMSDELL AND B. H. WEBB, Div. Dairy Res. Labs., Bur. Dairy Indus., Washington, D. C. Food Res., *6*, No. 3: 265. May–June, 1941.

Skim milk and whey were found to improve both the flavor and the body of spray dried soup mixtures when used in quantities up to 25 per cent of the weight of the dry mix. F.J.D.

778. Corrosion of 18–8 Stainless Steel in Sodium Chloride Solutions. H. H. UHLIG AND M. C. MORRILL, Mass. Inst. of Tech., Cambridge, Mass. Indus. and Engin. Chem., *33*, No. 7, 875–880. 1941.

A detailed study is reported of the effect of temperature, concentration,

and pH of aerated sodium chloride solutions, on the nature and rate of corrosion for 24 hour periods of 18-8 stainless steel. Corrosion increases sharply with temperature, reaching a maximum with 1 per cent to 10 per cent NaCl solutions at 90° C. and above but at the boiling point corrosion decreases to nearly zero due to lack of dissolved oxygen. Maximum corrosion occurred at 90° C. with 4 per cent NaCl solution. Maximum pit penetration in 4 per cent NaCl at 90° C. was at pH 6 to 7 and this fell to a minimum at pH 2.9 to 4.5 with a sharp increase in corrosion below pH 2.9. There was a drop in corrosion above pH 7 to a minimum at pH 12. B.H.W.

779. Fleet Maintenance. ANONYMOUS. Milk Dealer, *30,* No. 8: 38–39, 87–90. May, 1941.

A description is given of the efficient system of fleet maintenance as carried on by the Alfar Creamery Company in West Palm Beach, Florida. To get an accurate account of what it costs to operate the trucks for the benefit of figuring cost of delivery, as well as the garage department expense, an analysis is made of each truck on separate cards. Samples of the cards used are presented. C.J.B.

780. An Apparatus for Comparison of Foaming Properties of Soaps and Detergents. JOHN ROSS AND GILBERT D. MILES, Colgate Palmolive Peet Co., Jersey City, N. J. Oil and Soap, *18,* No. 5: 99. May, 1941.

A simple apparatus and procedure is described for measuring the foaming properties of soaps and detergents. By this method the relative stability of foams is compared by measuring the effect of an arbitrary standard destructive mechanism acting upon the volume of foam during production under standard conditions and protected from adventitious destructive forces. V.C.S.

781. The Use of Neoprene in Refrigeration Equipment. H. LOGAN LAWRENCE, E. I. duPont de Nemours and Co., Wilmington, Del. Refrig. Engin., *41,* No. 6: 404. 1941.

Details of the use of neoprene, a substitute for rubber, in small type refrigeration equipment, it being little effected by the usual refrigerants. It is an excellent rotary sealing material and is especially efficient as an insulating material for motor windings of sealed-in units even for motor sizes up to 7½ h.p. It is also used for refrigerator door gaskets and machine gaskets. While the price of a neoprene gasket is 25 per cent greater than that of a high-grade rubber gasket, the increased service life much more than offsets the higher first cost. L.M.D.

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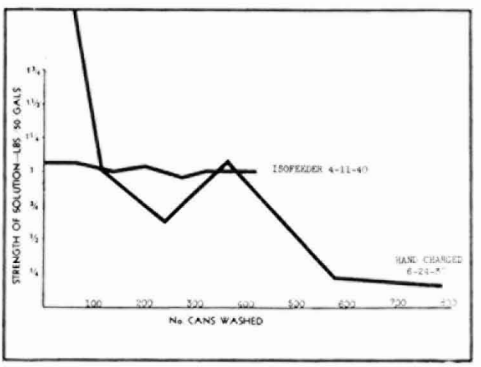

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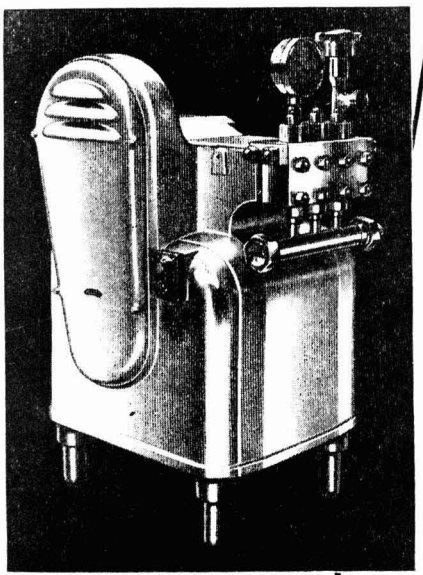

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