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Contents

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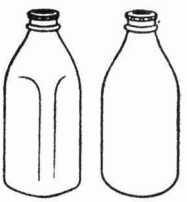

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ALDOLASE IN BOVINE MILK

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The enzyme aldolase which reversibly splits fructose 1,6-diphosphate into dihydroxyacetone phosphate and phosphoglyceric aldehyde was first discovered by Meyerhof and Lohmann (3) in rabbit voluntary muscle. The enzyme probably occurs in all cells, but muscle and yeast are the best sources. More recently, the enzyme has attracted attention because the experiments of Warburg and Christian (6) indicated an increase of aldolase in the serum of tumor-bearing rats. With the known relationship of the serum and milk whey proteins (2) in mind, it was of interest to determine the possible presence of aldolase in normal milk.

EXPERIMENTAL

The procedure described in detail by Sibley and Lehninger (5) was applied directly to milk without any modifications. The method depends on the formation of a 2,4-dinitrophenylhydrazine derivative of the triose phosphate produced by the action of aldolase on hexosediphosphate. In alkaline solutions, the dinitrophenylhydrazine derivative, called chromogen, turns purple. The assay of whole or skimmilk for aldolase activity was complicated by turbidity in the solution of the chromogen after addition of NaOH. This occurred with normal milk or milk inactivated by trichloroacetic acid. The turbidity did not appear with milk that had been dialyzed or with milk aldolase preparations made by salt fractionation. To eliminate the turbidity, the colored solution was centrifuged immediately before it was compared with acid-inactivated milk treated in a similar manner. The aldolase activity of milk determined by this procedure was identical with that of a dialyzed sample of the same milk. No further difficulty was encountered with the aldolase assay.

Aldolase activity may be conveniently expressed as the micromoles of 1,6-fructose diphosphate split by 1 mg. of protein at 37° C. in 1 hr. One micromole of hexosediphosphate is equivalent to 2 micromoles of triosephosphate or 2 micromoles of alkali-labile phosphate. The equivalence between the triose chromogen and alkali-labile triosephosphate was determined with a rat-muscle preparation of aldolase. The aldolase activity, stated in terms of micromoles of hexosediphosphate split, multiplied by the factor 22.4 is equivalent to the Q_{HDP} used by Sibley and Lehninger (5). For comparison with their data, this Q notation is used.

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Protein concentrations were determined by the biuret color reaction described by Kingsley (1). Milk was analyzed within a few hours after collection or after storage overnight at 3° C. in the presence of chloroform. Both mixed commercial skimmilk from a Philadelphia dairy and whole milk from each of six animals were analyzed. The results are summarized in table 1.

TABLE 1

Aldolase activity of normal milk and various milk fractions

	Q_{HDP}	
	Range	Average
Whole milk[a]	0.07–0.13	0.09
Cream[b]	0.14–0.45	0.34
Skim milk[c]	0.07–0.22	0.13
Whey (casein precipitated with acid)	.....	0.00
Whey (casein coagulated with rennet)	0.15–0.35	0.22
Rennet whey salt fractionation at 26° C.		
2.3 *M* ammonium sulfate ppt.[d]		0.41
2.8 *M* " " "		0.00
Salt fractionation at 3° C.[e]		
2.4 *M* ammonium sulfate ppt.[d]		2.3
2.8 *M* " " "		6.8

[a] Whole milk from each of four cows was analyzed.

[b] Whole milk was centrifuged for 10 min. at room temperature (3000 rpm), and the skimmilk was siphoned off. One ml. from the center of the recentrifuged cream layer was used for the aldolase determination.

[c] From two 15-gal. lots of mixed unpasteurized commercial skimmilk and eight lots of milk from six cows.

[d] The fractions were dialyzed free of salt before enzyme assay. The pH was then 6.4 ± 0.1.

[e] Casein was precipitated at 1.5 *M* $(NH_4)_2SO_4$ concentration.

RESULTS AND DISCUSSION

The activity of milk aldolase is of the same order as that reported for blood serum (5). Like xanthine oxidase, this milk enzyme is concentrated in the cream layer. Use of a conventional salt fractionation procedure at room temperature was complicated by the instability of the enzyme in milk. Removal of casein by isoelectric precipitation at pH 4.7 resulted in a complete acid inactivation of the whey aldolase. Although there was an apparent increase of the Q_{HDP} value when the casein was removed with rennet and the enzyme was precipitated at a concentration of 2.3 molar $(NH_4)_2SO_4$ at room temperature, there was a loss of almost two-thirds of the total enzyme activity. The removal of casein with 1.5 *M* $(NH_4)_2SO_4$ and the subsequent fractionation of the whey at 3° C. concentrated the milk aldolase in the fractions that precipitated at concentrations of 2.4 and 2.8 molar salt. Approximately 80 per cent of the total activity in milk could be recovered with this procedure. In the fraction that precipitated at a concentration of 2.8 molar $(NH_4)_2SO_4$ there was about a fifty-fold increase in purity ($Q_{HDP} = 6.8$).

Some explanation for the loss of activity with the rennet whey was obtained in the subsequent study of the effect of temperature on the stability of the milk aldolase. Figure 1 demonstrates a marked instability of the enzyme in milk at 37° C., as compared with the stability of the relatively purified milk aldolase. The

linear relationship for the plot of the log per cent aldolase activity remaining after heating against the time of heating reveals a simple monomolecular inactivation rate for the enzyme in milk at 37° C. A similar curve was obtained with dialyzed milk, indicating that the instability of the aldolase in milk could not be attributed to dialyzable components. Although the purified aldolase fraction showed no inactivation at 37° C., at 48° C. the activity of this fraction diminished rapidly but still followed a monomolecular reaction rate. The complicated inactivation rate of the aldolase in milk at this temperature probably indicated inactivation due to heat and unknown degradative changes.

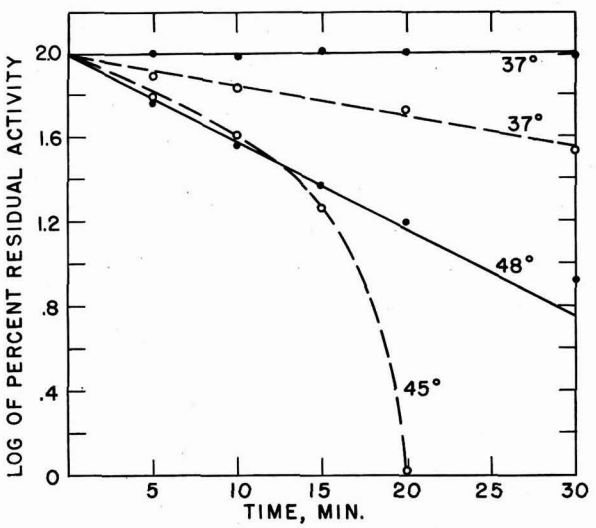

FIG. 1. Aldolase activity of normal milk (○) and a purified milk fraction (●) after heating at various temperatures for progressive time intervals. The pH was 6.4 in both cases. The purified fraction had been dialyzed free of salt.

This rapid inactivation of the aldolase in milk, in the light of the relative stability of the aldolase in fractions from milk, permits the conclusion that milk contains non-dialyzable components capable of destroying or inactivating the aldolase. Similar factors have been found in the crude extracts of muscle aldolase.

The presence of aldolase in milk with an activity level close to that reported for blood serum ($Q_{HDP} = 0.3$) emphasizes further the close relationship of the proteins of serum and milk whey (2). In view of the reported presence of xanthine oxidase in both bovine blood serum and milk and its absence in both human serum and milk (4), the presence of aldolase in both serum and milk constitutes further presumptive evidence of the possible origin of certain milk enzymes.

SUMMARY

With the procedure of Sibley and Lehninger, the enzyme aldolase has been found in normal cow's milk in the same concentration range as in blood serum. The presence of the enzyme in various milk fractions is indicated, and factors affecting the stability of the enzyme in milk are discussed.

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INFLUENCE OF PRE-MILKING PREPARATIONS OF COWS' UDDERS UPON THE LET-DOWN OF MILK

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Improvements in the machine milking of cows have been attained through practical experiences and from findings obtained in experimental work (1, 9, 10, 13, 20). Studies (5, 17) on the anatomy of teats and udders, together with those (14, 15) on the effect of the amount of vacuum at the inflations have contributed valuable lessons regarding mechanical milking. Along with the mechanical features in machine milking, the importance of good management of the cows themselves should be emphasized, as shown by the work of Whittleston and Verrall (19). Since it has been shown (1, 12) that the improper use of milking machines may result in injuries of the teats and udders which, if continued, may cause inflammation of the udder (10), it is important to know and follow the proper procedures.

In a study of milking procedures, Dahlberg (2) showed that with the right management the milking job could be performed more satisfactorily and as quickly with two units as with four. The importance of preparing the cows for the milking act by the use of the strip cup together with washing the udders has been demonstrated by Miller and Petersen (11), Smith and Petersen (16) and Ward and Smith (18). These workers also showed that the milking machines should be attached soon after the pre-milking preparations are made, preferably within 1 min. When the interval was as long as 20 min. after the treatment, lowered milk production resulted. Knodt *et al.* (6) agreed on the short interval but claimed that with special training a 20-min. interval caused no adverse effect on milk production.

A program of "3-min." milking has been recommended (21); this is based on properly preparing the cow and attaching the machine within 1 or 2 min. Several features of this work have been studied at the Ohio Agricultural Experiment Station by the senior author (7) and reported in 1948. During the progress of this work, which was started in 1946, several reports have appeared on the effect of various factors on the let-down of milk.

Dodd and Foot (3) found that the temperature of the water used in washing cows' udders had no effect on the length of the milking period or total milk production. They (4) have confirmed these findings in a more recent study. In their second experiment, an attempt was made to increase the milking rate by removing the milking machine before the milk flow stopped. This practice not only failed to change the rate of milk flow, but caused a drop in milk production.

Korkman (8) showed that the reaction of the cow to pre-milking udder treatments seems to be an individual characteristic. Milk yield was not influ-

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enced by the various methods of udder treatments. The maximum rate of milk flow per minute was greater when stimulation for the let-down was applied 1 or 2 min. before attaching the teat cups. A light massage with a hot (wet) towel for 15 or 30 sec. was the best stimulation. This management treatment of the udders was most important with low-producing cows.

EXPERIMENTAL

The cows used were purebred Holsteins and Jerseys that were in either the fore or middle part of their lactation periods. They represented part of the main dairy herd and, therefore, were accustomed to managed milking procedures. A special effort was made to maintain uniform conditions of feeding and management throughout the work, with the exception of the experimental treatment being used. Each milking trial was for a minimum of 8 days, the

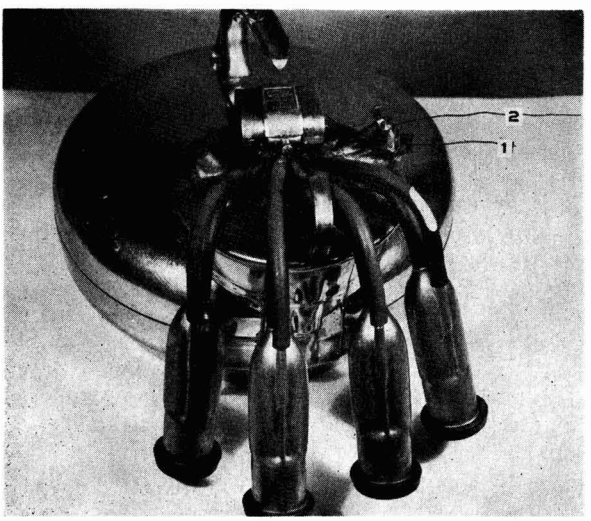

FIG. 1. Experimental milking machine. Wires numbered *1* and *2* are attached to the rotary valve for the control of the first and second latex bag, respectively.

first 4 days of which were considered as preliminary and the second 4 days as experimental. The same procedures were followed for both the morning and evening milkings, but the data presented are for the morning milking, since conditions were more uniform at this time of day.

A Surge milker was used and operated according to the manufacturer's recommendation at 48 to 52 pulsations per minute and with a vacuum of 15 in. of mercury. The pail was so constructed (figures 1, 2 and 3) that the milk obtained in the first and second 45 sec. could be weighed separately. This was made possible by the use of latex bags inside the pail into which the milk was diverted by a valve arrangement on the milking head. After these two 45-sec. periods, the remaining milk was collected in the pail proper. In the first trial,

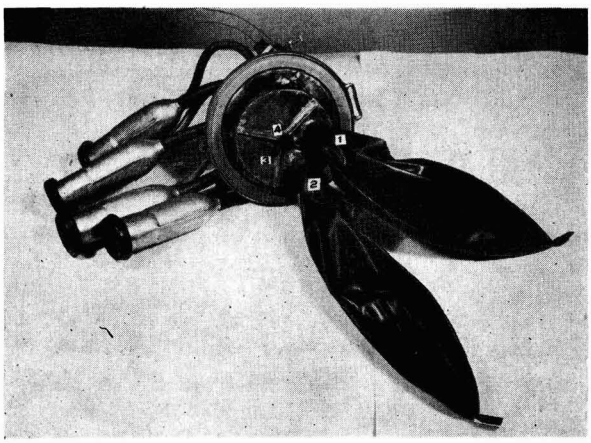

FIG. 2. Lower side of lid and valve with latex bags attached.

only one bag was available so that one measure of let-down was based on the production in the first minute. One man handled the milker and another timed the operations with a stop watch and recorded data.

The time for cleaning or massaging each udder ranged from 10 to 15 sec. A 1-min. period between the preparation of the cow and the attachment of the milking machine was used as standard procedure. The inflations (teat cups) were removed from each teat as soon as the milk flow stopped, and the last one removed determined the length of the milking period.

The response or milking performance of the cows to various pre-milking udder treatments has been measured in three ways. These are explained as follows:

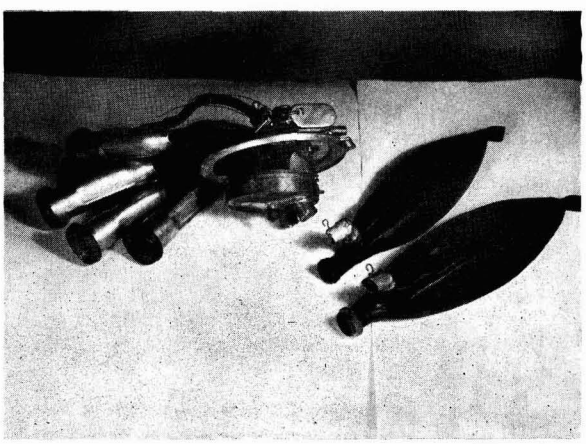

FIG. 3. Pail lid with disc valve and two latex bags.

(a) Pattern of milk let-down. This refers to the percentage (relationship) of the amount of milk removed during the first 45 sec. to that removed during the second 45 sec. of the milking period. The purpose of this measure was to emphasize any production change during the first 1.5 min. of milking. It was reasoned that the speed of let-down and flow of milk of which cows are capable would (in most cases) be established during the second 45 sec. of milking time, regardless of the pre-milking treatment. A high percentage factor indicates that the pre-milking treatment used was an efficient stimulation for speedy let-down. Because it was found preferable to compare cows of similar characteristics as regards ease of milking, the data are presented in this form whenever possible.

(b) One-and-one-half minute production. The pounds of milk obtained during the first 1.5 min. is expressed as a percentage of the total production.

(c) Milking rate. The rate of milk flow equals the milk production (pounds) divided by the milking time (seconds).

The information obtained under *b* and *c* was a further measurement of the efficiency of the pre-milking treatment for the stimulation of speed of let-down, but was somewhat less critical than under *a*.

Experiment I. The influence of temperature of the udder wash water. The 16 cows (Holsteins and Jerseys) used were divided into three groups of five, five and six cows. The first group was milked during the summer season, the second during the spring and the last group in the winter season. Each group was subjected to three different temperatures of udder wash water and use of a strip cup (removing one or two streams of milk from each quarter) previous to milking. The rotation of treatments of the udders for groups 1 and 2 were in the following order: cleaning with a heavy Turkish towel wrung from water at 100° F., cleaning as above except the towels were wrung from water at 132° F., and in the last treatment the towels were wrung from cold water. Temperature of the cold water was 64° F. during the summertime and 50° F. during the spring. Each period of treatment was for 8 days. The third group of six cows (table 3) was arranged and experimentally treated according to the "Latin square" system. Under this system they were divided into three groups of two cows each, thus permitting udder applications with 45, 100 and 132° F. waters to be used simultaneously. The first group received cold water treatment followed by the use of warm and then hot water. The second group received, at the same time, a similar series of treatments with warm, hot and cold waters, while the third group received hot, cold and warm water udder treatments.

The influence of temperature of the udder wash water upon the milking response of cows during the summer, spring and winter is presented in data given in tables 1, 2, and 3, respectively. The results have been classified according to the milking characteristics of the cows, because easy-milking cows require less time to be milked than do hard-milking cows.

Temperature of the water used on the udders previous to milking was of minor importance, at least after the cows had become accustomed to a certain temperature change. In six of the eight comparisons, let-down appeared to be

TABLE 1

Temperature of udder wash water as related to the let-down of milk
(Summer trial, Experiment I, Trial I)

Cows	2			2			1		
Milking characteristics	Slow (hard)			Medium			Rapid (easy)		
Temp. udder wash water (° *F.*)	100	132	64	100	132	64	100	132	64
Milk production, a. m.									
1st. minute (*lb.*)	5.2	4.9	4.8	6.2	6.2	6.8	7.3	7.7	8.3
1st. minute (*% of total*)	24.3	22.1	22.4	37.1	38.2	46.6	38.6	41.4	45.9
Total (*lb.*)	22.2	22.1	21.4	16.7	16.2	14.6[a]	18.9	18.6	18.1
Time (*min. & sec.*)	4–29	4–37	4–28	3–17	3–12	2–31	2–42	2–32	2–16
Milking rate (*lb./sec.*)	0.083	0.080	0.080	0.085	0.084	0.096	0.120	0.120	0.130

[a] Unable to explain this drop. These two cows began to drop in production during later period of hot water treatment, or just before the cold water treatment was started.

just as good using water at 45, 50 or 64° F. as it was with water at 100 or 132° F. Similarly, the response of the cows to warm water (100° F.) was just as good as was the use of hot water (132° F.) in six out of eight comparisons. There were some indications that water at 132° F. was a little too hot for the cows, as shown at times by their stepping around when their udders were being washed and by the lower percentage of the pattern of let-down as shown in the tables. However, one cow in the winter trial seemed to prefer the hot water, as shown by her response (table 3, medium classification). The men doing the milking preferred to use water at 100° F.

The temperature of the udder wash water apparently was without effect on the time required to milk the cows. The time varied slightly with the different temperatures of water used, but there was no definite trend. Use of hot water did not make a fast milker out of a slow one and cold water did not make slow milkers out of fast ones, after the cows had become accustomed to the change in

TABLE 2

Temperature of udder wash water as related to the let-down of milk
(Spring trial, Experiment I, Trial II)

Cows	2			1			2		
Milking characteristics	Slow (hard)			Medium			Rapid (easy)		
Temp. udder wash water (° *F.*)	100	132	50	100	132	50	100	132	50
Milk production, a. m.									
1st. 45 sec. (*lb.*)	2.8	2.8	3.0	3.9	3.5	4.1	5.0	4.7	5.3
2nd. 45 sec. (*lb.*)	3.4	3.6	3.5	4.3	4.2	4.4	5.1	5.5	5.7
Pattern of let-down (%)	82.6	77.7	85.7	90.7	83.3	93.2	98.0	85.4	93.0
1st. 1.5 min., (*% of total*)	43.4	40.2	41.1	42.2	35.3	40.7	53.7	48.3	53.4
Total (*lb.*)	14.3[a]	15.9	15.8	19.4[a]	21.8	20.9	18.8[a]	21.1	20.6
Time (*min. & sec.*)	4–14	4–31	4–24	3–20	3–54	3–40	2–40	2–56	2–36
Milking rate (*lb./sec.*)	0.056	0.059	0.060	0.097	0.093	0.095	0.120	0.120	0.130

[a] Roughage consumption increased by 4.8 lb. of hay, or its equivalent, per cow per day during the latter part of this period and throughout the experiment.

TABLE 3

Temperature of udder wash water as related to the let-down of milk (Winter trial, Experiment I, Trial III)

Cows	3			3		
Milking characteristics	Medium			Rapid (easy)		
Temp. udder wash water (° *F.*)	100	132	45	100	132	45
Milk production, a. m.						
1st. 45 sec. (*lb.*)	3.9	4.1	3.9	4.9	4.7	5.1
2nd. 45 sec. (*lb.*)	4.3	4.4	4.3	5.1	5.3	5.3
Pattern of let-down (%)	90.7[a]	93.2	90.5	96.1	88.6	96.2
1st. 1.5 min. (*% of total*)	37.1	37.6	37.6	47.1	47.0	47.7
Total (*lb.*)	22.1	22.6	21.8	21.2	21.3	21.8
Time (*min. & sec.*)	4–30	4–05	4–26	3–13	3–14	3–08
Milking rate (*lb./sec.*)	0.082	0.092	0.082	0.110	0.110	0.110

[a] Determined by relating the amount of milk produced during the first 45 sec. to that produced during the second 45 sec. of milking time.

temperatures. Some cows milked completely dry after 2 min., while others required 6.5 min., irrespective of treatment.

Experiment II. The influence of various pre-milking udder treatments. Trial I. Five cows (three Holsteins and two Jerseys) in the early and middle stages of lactation were used in this experiment according to the following order of udder treatments (table 4): (a) Control period in which no pre-milking treatment of the udders was given, except placing the surcingle over the back of the cows (see management employed for previous herd practices); (b) dry hand rub (brush) of the udder to remove straw and any loose dirt; (c) cleaning the udder with a damp cloth (half of a Turkish towel) that previously was wrung from water and stored in a dry bucket until used at milking time (these towels were cold when used); (d) cleaning the udders with a heavy towel (half of a Turkish towel) wrung from hot water (120° F.); and (e) the same udder treatment as in (d), followed by removing one or two streams of milk from each

TABLE 4

Influence of udder treatment upon the let-down of milk (5 cows; Experiment II, Trial I)

Treatment of udders 1 min. before milking	None	Dry hand	Damp cloth	Towel from hot water (120° F.)	Towel from hot water (120° F.) and strip cup
Milking characteristics	1 slow and 4 medium				
Milk production, a. m.					
1st. 45 sec. (*lb.*)	2.8	3.3	3.8	3.8	3.8
2nd. 45 sec. (*lb.*)	4.1	4.2	4.2	4.2	4.2
Pattern of let-down (%)	68.3[a]	78.6	90.5	90.5	90.5
1st. 1.5 min. (*% of total*)	38.3	41.9	47.3	47.9	49.7
Total (*lb.*)	18.0	17.9	16.9	16.7	16.1[b]
Time (*min. & sec.*)	4–33	4–04	3–30	3–25	3–25
Milking rate (*lb./sec.*)	0.066	0.073	0.080	0.081	0.079

[a] Determined by relating the amount of milk produced during the first 45 sec. to that produced during the second 45 sec. of milking time.

[b] One cow dropped from an average of 20.1 lb. of milk to an average of 17.6 lb. during this trial. Unable to explain this except stage of lactation.

teat (strip cup). Each of these periods were for 8 days duration with the first 4 days being considered as preliminary and the last 4 days, experimental.

Hot water at 132° F., as used in experiment I, appeared to be too hot for cows' udders and so water of 120° F. was resorted to in this second experiment.

The data obtained in this first trial on pre-milking treatments are shown in table 4. With no treatment (control period), the let-down of milk was relatively slow and the average time required to milk a cow was approximately 1 min. longer than with some of the other treatments. Brushing the udder with the hand was of slight benefit in speeding up the let-down and in shortening the time required to get the milk. The other three treatments—the use of the damp cloth, the towel from hot water and this treatment plus the use of the strip cup—seemed to be about equally effective for the efficient simulation for speedy let-down of milk. Thus, the amount of milk obtained in the first 45 sec. with all three of these treatments was 90.5 per cent of that obtained in the second 45 sec. Likewise, the average milking time following these treatments was reduced by 1 min.

To begin the milking act without pre-milking cleaning or massaging of the udder with a damp or wet towel permits the removal of milk from the cisterns and ducts, resulting in the milking of a dry teat before full milk flow started. According to Petersen (12), this may cause teat injury. Under this method the milking act becomes the stimulus for milk let-down as readily experienced when milking is done by hand.

Cleaning udders with cloths removed from 120° F. water failed to change the amount of milk produced during the first 1.5 min. of milking time, pattern of milk let-down or milking rate when compared with the use of a damp cloth that was previously stored in a dry bucket.

Trial II. Completion of trial I revealed that information on the milking response should be obtained upon first, the influence of removing one or two streams of milk from each quarter (strip cup) and, second, a combination treatment of the udders with a bath of hot water (120° F.) and use of a strip cup.

Four cows (three Holsteins and one Jersey) were used in this trial. Pre-milking treatments were used in the following order: (a) no treatment, except placing the surcingle over the cow; (b) use of the strip cup only; (c) massaging the udder with a towel wrung from water at 120° F. and use of a strip cup; and (d) the same as the preceding treatment except that water was allowed to stay in the towel so that this treatment amounted to almost bathing the udder with hot water. Each pre-milking treatment lasted for a period of 8 days, as previously explained.

The cows in this second trial were slower milkers than were those used in the previous trials (table 5). The results agree, in general, with those obtained in trial I. With no treatment previous to milking, the let-down of milk was poor or slow and the milking time was longer than when proper treatments were made. Use of the strip cup slightly improved milk let-down and shortened the milking period. However, desired milking responses were obtained with the use of a damp cloth plus the strip cup. This shortened the milking time by approximately 1 min. and gave a pattern of let-down of 85.7 per cent. The more thor-

ough washing of the udder, which amounted to almost a bath, did not appear to be superior to the use of a wet towel and use of a strip cup; in some instances the cows showed evidences of being disturbed by this more drastic procedure.

As a check upon the milking technique, three special milking experiments were conducted. The influence of increasing the vacuum 20 per cent (to 18 in.) upon the milking response of hard-milking cows was studied. This technical study showed that controlling the vacuum was necessary for satisfactory employment of the procedures used in the experiments.

Since this work was done with a Surge milker, the milking rate conceivably might be slow at the beginning of the milking period because of a lack of weight in the pail. This was investigated and found to be of no consequence.

TABLE 5

Influence of udder treatments upon the let-down of milk (4 cows,[a] Experiment II, Trial II)

Treatment of udders 1 min. before milking	None	Strip cup	Towel from hot water (120° F.) and strip cup	Hot water (120° F.) bath and strip cup
Milking characteristics	2 slow and 2 medium			
Milk production, a. m.				
1st. 45 sec. (*lb.*)	2.7	3.2	3.6	3.4
2nd. 45 sec. (*lb.*)	3.7	4.2	4.2	3.8
Pattern of let-down (%)	73.0	76.2	85.7	89.5
1st. 1.5 min. (*% of total*)	28.4	32.3	37.1	32.7
Total (*lb.*)	22.5	22.9	21.0[b]	22.0[c]
Time (*min. & sec.*)	5–49	5–31	4–55	5–15
Milking rate (*lb./sec.*)	0.064	0.069	0.071	0.070

[a] Data from a fifth cow was not used because 9.75 to 10.75 min. of milking time was required. All treatments failed to shorten the milking time, except when an increase of vacuum was used.

[b] Warm weather retarded appetites, thereby resulting in lowered milk production.

[c] Appetites improved as a result of cold weather.

The influence of administering additional oxytocin to two cows was studied. Results show that the measurements of the milking responses used (in experimental trials) apparently were sound and that desirable stimulation for speedy let-down of milk had been obtained.

DISCUSSION

The milking response was the same with cold, warm or hot water. This is in agreement with the findings obtained by Dodd and Foot (4). This proved to be true for spring, summer and winter. These results were obtained after the cows had become accustomed to a certain temperature of water. There were some indications that the water could be too warm for best results and have a disturbing effect on the cows. In managed milking, great value has been attached to the use of hot water as a means of increasing the milking response or let-down and shortening the total milking time. In this work demonstration of this value for warm or hot water over cold water was not possible.

Various pre-milking treatments of the udder seemed to be about equally

effective in the two trials in which various treatments were compared. Massage with a damp cloth on one extreme proved just as good as the more drastic preparation involving bathing the udder in hot water on the other extreme. However, since one purpose of the preparation is to provide sanitary conditions for milking, one would not want to discourage the use of hot or warm water in properly preparing the cow for the production of clean milk. Two trials (experiment II) show that some preparation of the cow is necessary to encourage the initial let-down of milk and to shorten the milking time. Merely brushing the udder to remove loose dirt or simply using the strip cup did not prove adequate in stimulating maximum initial let-down.

This work indicates that the rate of milk flow after proper stimulation and with continued relaxation of the cow is dependent upon the size of the teat ducts, condition of teat orifice and strength and relaxation of the sphincter muscles, which is in agreement with the work of Dodd and Foot (3, 4).

SUMMARY

The temperature of the udder wash water as used in these trials (45, 100 132° F.) was a minor factor in the stimulation of milk let-down. Proper stimulation of udders at required intervals (1-min. interval as used in these experiments) before milking was necessary for maximum speed of let-down of milk.

A cleaning (massaging) period of 10 to 15 sec. with a cool, damp towel (previously stored in a dry bucket) or a wet towel wrung from water gave the required stimulation for rapid let-down of milk. A similar treatment with the dry hand or the use of a strip cup was inadequate. Bathing udders in hot water (120° F.) for a period of 10 to 15 sec. as a means of pre-milking preparation did not appear to be any more effective than the use of a damp towel. Without pre-milking treatment of the udder, the milking period was prolonged approximately 1 min., as compared with proper preparation and the initial let-down was slow.

Total milk production remained fairly constant throughout these experiments regardless of the method of pre-milking udder treatments.

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THE EFFECT OF BACTERIA ON THE FERTILITY OF BOVINE SEMEN[1]

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The problems associated with the presence of bacteria in semen have received considerable attention in recent years. Various workers (3, 6, 9, 14) have reported on the number of bacteria found in bull semen collected with an artificial vagina. The number in undiluted semen has been found to range from less than 100 to 22,000,000 bacteria per milliliter and in diluted samples from 200 to 3,400,000 organisms per milliliter. Gunsalus *et al.* (14) have shown that cleaning of bulls materially affects the bacterial count, however.

Foote and Salisbury (11) found more bacteria in the first than in subsequent ejaculates and fewer organisms in the semen from bulls of known high fertility than in the semen from other bulls studied. In contradiction to these findings, Almquist *et al.* (6) found no significant differences between the counts on 91 first and 91 second ejaculates and no significant relationship between fertility and the number of bacteria present in the undiluted semen.

The types of organisms which have been isolated from semen by various workers (9, 10, 14) are *Pseudomonas, Streptococcus, Micrococcus, Bacillus,* diptheroids, coliform organisms, actinomycetes and yeast.

Dondero (9) has pointed out that the sources of bacteria in diluted bull semen are many, *i.e.,* the genital tract of the bull, surface areas, non-sterile diluter, etc.

The possibility of certain bacteria having a detrimental, direct effect on sperm has received some attention. Edmondson *et al.* (10) found that hemolytic bacteria decreased the length of time semen could be stored, whereas certain non-hemolytic organisms increased the storage time from 1 to 4 days over the controls. Gunsalus *et al.* (14) noted that *E. coli* improved the motility of spermatazoa in semen samples into which it was inoculated.

Clinical observations by Williams (22) and Gunsalus *et al.* (14) have indicated that bulls harboring bacteria such as *Streptococcus viridans* or *Pseudomonas aeruginosa* often may have a low breeding efficiency. These and other organisms have been isolated by several workers (13, 14, 23) from bulls that were practically or completely sterile. Similarly, the work of several investigators (7, 21, 22) has shown that certain organisms, many of which frequently are found in semen, often are associated with conditions in cows such as vaginitis, cervicitis, metritis, salpingitis, ovarian bursitis or tubo-ovarian abscesses.

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In agreement with this, Moore (18) found disease of the tubular genitialia of the male to be associated with these conditions in cows. Many of the same organisms have been reported (8, 15, 23) to be associated with more severe pathological conditions such as abortions and/or retained placenta in cows. Comstock (8), Gilman (13) and Bartlett (7) have reported that organisms of the *Streptococcus* and *Corynebacterium* types were associated with sterility in cows. Hatch *et al.* (16) isolated *Corynebacterium, Streptococcus, Diplococcus, Micrococcus, Bacillus* and coliform organisms from the reproductive tract of infertile cows.

The effect of several substances upon the motility of sperm and upon the control of bacteria in semen has been investigated by a number of workers (2, 3, 11, 12). The effect of the addition of some of these substances on fertility also has been investigated. Salisbury and Knodt (19) reported that the addition of 300 mg. per cent of sulfanilamide to semen improved the fertility significantly; however, the beneficial effects were thought to be largely metabolic ones, rather than due to bacterial control alone. Almquist (4, 5) has reported that additions of penicillin, streptomycin or a combination of the two improved the fertility of semen from relatively infertile bulls over untreated controls, whereas sulfanilamide failed to show such improvement. The beneficial effects in the case of penicillin, at least, were attributed to the control of bacteria in the semen. However, Almquist *et al.* (1) previously had reported that penicillin did not affect appreciably the fertility of semen from high fertility bulls.

The purpose of the study reported here was to determine whether the number of bacteria present in diluted semen, as used by the technician, or the predominating types of bacteria occurring in the routine semen samples have any relation to fertility.

EXPERIMENTAL METHOD

The material for study included 241 routine samples of diluted semen from 64 bulls in use in two artificial insemination units.[3] This part of the study was conducted in April, 1949. Immediately after collection, the semen was diluted with yolk-citrate diluter to which had been added sulfanilamide at the rate of 300 mg. per 100 ml. of diluter. Plating on blood-agar was done when the semen was approximately 24 hr. old.

Five mg. of para-aminobenzoic acid were added to each 100 ml. of tryptose blood agar base (Difco) to allow the growth of any bacteria which might be susceptible to the sulfanilamide present in the diluted semen. Citrated horse blood was added to this base at the rate of 10 ml. per 100 ml. of agar base. After pouring, and before using, all plates were incubated at 37° C. for 24 hr. to insure the sterility.

The diluted semen was transferred to the blood-agar plates with a sterile pipette and subsequently spread with a glass rod which was dipped in alcohol and flamed between each operation. Tests made to determine whether any bac-

[3] The authors are indebted to the personnel of the Northern Ohio Breeder's Cooperative Assoc. and of the Central Ohio Breeder's Cooperative Assoc., for furnishing the material for study and for assisting in compiling the fertility data for analysis.

teria were being transferred from one plate to another by this procedure showed that the method was satisfactory.

After incubation at 37° C. for 48 hr. the number of colonies on each plate was counted, using a Quebec Colony Counter, and the number of bacteria that would have been present in an entire milliliter of the diluted semen was calculated. Typical predominating colonies were transferred to serum infusion agar slants for further identification. Classification of the bacteria followed the methods described by Merchant (17).

Samples of the diluter used also were examined in a manner identical to that described for the diluted semen. The purpose of this was to determine the extent to which the diluter was the source of the bacteria found in the diluted semen.

Twenty-nine samples of undiluted semen having low motility, or for other reasons not considered good enough for shipment, were plated on blood agar in a manner similar to that described above to determine whether any particular organism consistently was associated with poor quality semen samples.

In a further attempt to determine the source of the various organisms found in the diluted semen, and especially to extend previous observations on the source of *P. aeruginosa* in semen, swabs were taken of the prepuce of nine bulls in use in an artificial insemination unit. These swabs were streaked on blood-agar plates and typical colonies present after 48 hr. incubation at 37° C. were transferred to serum infusion slants for identification.

Analysis of the fertility data was made according to methods described by Snedecor (20).

RESULTS AND DISCUSSION

There was a considerable difference between bulls with respect to the range in the number of bacteria per milliliter of diluted semen. Some bulls consistently had low bacterial counts, while other bulls varied considerably from one ejaculate to another in this respect. A difference was found between bulls with regard to the types of bacteria predominating in the diluted semen samples. Some bulls consistently had one type of bacteria in their semen, whereas other bulls were not consistent with respect to the predominant type. This indicates that, in the case of some of the bulls, the bacterial contamination was due to organisms harbored in the genital tract of the bull; in the case of other bulls, the bacteria represented surface contamination.

The number of bacteria per milliliter of diluter ranged from 0 to 8,000 for 14 samples from one association and from 0 to 60 for five samples from the other insemination unit. Large variation from sample to sample indicated that the precautions taken in the preparation of the diluter may influence markedly the number of bacteria introduced in this manner. *Streptococcus, Micrococcus* and *Corynebacterium* species were isolated; however, the type of bacteria found in the diluter often was not the predominating type found in the diluted semen samples.

For the purpose of statistical analysis the semen samples were grouped on the basis of the number of bacteria per milliliter. The number of cows conceiv-

ing and the number not conceiving were determined for each respective group. A total of 11,912 first services was considered in the analysis. The criterion of fertility was the failure to return for service during a 60- to 90-day period following insemination. A chi-square test of independence to determine whether there was any relationship between the number of bacteria present in semen samples and their fertility was performed. The results are shown in table 1.

TABLE 1

Relation of the number of bacteria to fertility of semen samples

	No. of bacteria/ml. of diluted semen				
	0–100	101–1,000	1,001–10,000	10,001–100,000	Totals
No. cows conceiving	X = 2,335 m = 2,249	3,448 3,445	1,340 1,393	366 402	7,489
No. cows not conceiving	X = 1,242 m = 1,328	2,032 2,035	876 823	273 237	4,423
Totals	3,577	5,480	2,216	639	11,912
X^2 =22.94	X^2 .05 = 7.81		X^2 .01 = 11.34		

A highly significant chi-square value (P = 0.01 or less) was obtained. The highly significant deviations from the theoretical values indicate that there is some association between number of bacteria present in diluted semen samples and fertility, since so large a value of chi-square would occur rarely by chance alone. Furthermore, the deviations were consistent in that conception rate decreased as the number of bacteria increased.

The samples also were grouped on the basis of the types of predominating bacteria and 11,803 first services were divided into the respective groups as

TABLE 2

Relation of predominating types of bacteria to fertility of semen samples

	Type of bacteria predominating						
	A[a]	B	C	D	E	F	Total
No. cows conceiving	X = 764 m = 795	518 555	2,443 2,467	773 782	1,505 1,440	1,416 1,380	7,419
No. cows not conceiving	X = 501 m = 470	364 327	1,482 1,458	471 462	786 851	780 816	4,384
Totals	1,265	882	3,925	1,244	2,291	2,196	11,803

[a] A = *P. aeruginosa;* B = *P. aeruginosa* and *Corynebacterium;* C = *Corynebacterium;* D = *Micrococcus, Streptococcus, Diplococcus;* E = Enteric group; F = Negative samples. X^2 = 21.17 X^2 0.05 = 11.07 X^2 0.01 = 15.08.

above. The results of this analysis on the relation of the types of bacteria to fertility of semen samples are shown in table 2. Here also, a highly significant chi-square value (P = 0.01 or less) was obtained, indicating some association between the types of bacteria predominating in diluted semen samples and their fertility. Apparently certain bacteria, *i.e., P. aeruginosa, Corynebacterium, Streptococcus, Micrococcus* and *Diplococcus* tend to have an adverse effect on

conception rate, while the enteric organisms encountered had no such effect. *P. aeruginosa* was apparently the most detrimental. These results may help to explain some of the findings that have been reported on the use of antibiotics such as penicillin and streptomycin on the semen of low-fertility bulls.

To determine whether insemination with semen containing large numbers of bacteria had any effect on the length of the subsequent estrus cycle, the groups described above were compared in this respect. The mean length of the estrus cycle following insemination with semen containing different numbers of bacteria was determined. The small differences which were found between means were not statistically significant, as was shown by an analysis of variance.

A similar analysis was made on the relation of the predominating types of bacteria in the samples to the length of the estrus cycle following insemination. The mean length of the subsequent estrus cycle was determined for each of the groups. An analysis of variance showed that the small observed differences between means were not significant, even at the 5 per cent level of significance. Even though insemination with semen containing large numbers of bacteria, especially of certain types, may affect fertility, these results indicate that the failure of conception occurs without affecting the length of the subsequent estrus cycle.

No obvious relationship was found to exist between the types of bacteria present in the semen and the distribution of the returns following insemination.

P. aeruginosa and *Corynebacterium* species were the types of bacteria most frequently predominant in the poor-quality semen samples; however, other types predominated in some cases. Although it is possible that some of the bacteria isolated from these samples had some casual relationship to the quality of semen produced, the fact that the same types were found in high quality semen made it difficult to draw any conclusions from these observations.

P. aeruginosa was isolated from both the preputial orifice and the preputial cavity of bulls. Attempts to isolate the organism from some bulls which consistently had shown it in their semen were unsuccessful. On the other hand, the organism was isolated from some bulls which had never shown it in their semen.

SUMMARY AND CONCLUSIONS

A significant relationship was found between the number of bacteria in diluted semen and its fertility when all types of bacteria were considered.

A significant relationship was found between the types of bacteria predominating in the diluted semen and its fertility. Types such as *Corynebacterium, Micrococcus, Streptococcus* and *Diplococcus* species and especially *P. aeruginosa,* tend to have an adverse effect on conception rate, whereas the enteric organisms encountered had no such effect.

No relationship was found between either the number of bacteria or types of bacteria predominating in diluted semen and the average length of the estrus cycle following insemination.

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THE DEVELOPMENT OF CALVES RAISED WITHOUT PROTOZOA AND CERTAIN OTHER CHARACTERISTIC RUMEN MICROORGANISMS

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During investigations concerning the establishment of rumen function in calves, the growth and development were observed of a limited number of animals from birth to 6 mo. or more of age whose rumens were maintained free of usual varieties of rumen microfauna and certain characteristic microflora. Although the importance of rumen microorganisms in ruminant digestion has long been recognized, there is but limited information regarding the effects on young cattle of the absence of these microorganisms.

Protozoa from the rumen, originally described by Gruby and Delafond (9), were summarized by Mangold (14) under more than 30 species. Apparently, all or most of these have been found in the rumens of cattle in North America (5). A complete understanding of their role in the digestive economy of the host animals is lacking, notwithstanding numerous investigations. From their observations of the numbers present and their later destruction in the abomasum, Ferber and Winogradowa-Fedorowa (8) concluded that they had an essential role in the development of the host animals. Mangold (15) states that the proportion of food protein metabolized by infusoria and subsequently digested by the host is considerable. According to Baker (1), protozoa operate as agents in the removal of iodophile microorganisms and so contribute to the maintenance of a balanced population. Hungate (12, 13) found that certain *Diplodinium* species could digest cellulose to some extent. Their presence is considered of little importance by other investigators because sheep and goats can get along without them (3, 4, 6).

Becker (3) noticed that lambs, experimentally defaunated with $CuSO_4$ solutions and starvation, tended to show rotundity of the body as if somewhat bloated when fed alfalfa and ground grain. Calves raised in partial segregation on rations of limited quantities of milk and alfalfa hay alone failed to develop usual rumen varieties of protozoa and certain characteristic indicator rumen bacteria during their first 6 wk. of age (17). They appeared to have rougher hair coats than similarly fed calves which received rumen inoculations. The abdomens of the uninoculated calves also appeared to be deeper than in the inoculated calves. During their first 6 wk. of age, this "pot-bellied" condition was not apparent in other uninoculated calves which received grain (17) or had access to lawn pasture (20). One uninoculated calf which was 2 mo. old when turned on pasture developed a noticeably rougher hair coat than similarly treated but inoculated calves, and it suffered from recurrent mild diarrhea during the 7-wk. experimental period.

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EXPERIMENTAL

Four uninoculated Jersey calves were raised from birth in segregation until they were 6 mo. old and one of them up to 8 mo. of age. Controls were provided by similar calves which received similar feeds from the same lots and which also were given rumen inoculations with cud materials (17, 19).

The calves were fed whole milk at the rate of 0.9 lb. per 10 lb. body weight at birth per day for the first 6 wk. and half this amount during the seventh week. No milk was fed after the seventh week. Fairly good quality alfalfa hay was provided free choice from birth throughout the experimental period. A simple 14.5 per cent protein grain ration consisting of corn, oats, bran and soybean oil meal was added to the ration when the calves were 6 wk. old. They received it in the proportion of half the quantity of hay they were consuming.

Rumen samplies were collected repeatedly by stomach tube and examined in the manner described previously (17) for the presence of usual rumen protozoa and certain characteristic rumen microflora used as indicators of the presence of usual rumen bacteria.

RESULTS

All four calves still lacked usual rumen protozoa at 6 mo. of age and one as late as 8 mo. They all developed large coccoid bacteria, previously designated as making up hay-flora group I (17), in their rumens between the ages of 1 and 2 mo. One calf became inoculated at 9 wk. of age with the large cigar-shaped organism (probably *Oscillospira*) of hay-flora group II (17). This occurred by accident through contact with unsterilized equipment which had been used previously on inoculated calves. Various rumen microflora other than those being used as indicators of the presence of usual rumen microorganisms probably were transferred at the same time. The highest relative concentrations of this large cigar-shaped microorganism ever encountered were in rumen samples from this calf. Limited numbers of the same organism were present in rumen samples from a second calf in the adjoining pen beginning at 4 mo. of age but never were observed in samples from the other two. This microorganism has been observed to disintegrate readily in abomasal fluids, such as occurs in the case of rumen protozoa (2, 16). The remaining two organisms composing hay-flora group II (17), namely the small rods in flat rectangular groups and the thick square-ended rods, never were observed in samples from these four segregated calves.

The average weight of these four calves at 6 mo. of age was 229 lb. as opposed to the 235.5-lb. average of the 12 inoculated calves. Thus, there was only an average difference of 5.5 lb. between the groups.

The calf (fig. 1) having the cigar-shaped organisms present in its rumen was of good appearance both as to hair coat and condition, although possibly it was a little paunchy in comparison with the inoculated calves (fig. 2). The hair coats of the other three calves (fig. 3) appeared rough and they were not as well conditioned as the calves in the inoculated group. They had visibly deeper abdomens that gave them a "pot-bellied" appearance.

The partly digested feed present in two rumens devoid of protozoa, one of

FIG. 1. Partial rumen-inoculated calf (6 mo. old). Rumen devoid of usual rumen protozoa but containing large cigar-shaped rods of hay-flora group II.

which belonged to the calf having considerable numbers of the large cigar-shaped organisms present, was not visibly distinguishable from that present in the rumens of inoculated calves receiving similar feeds.

Uninoculated and segregated calves frequently were noticed to nose down through the bedding and to pick up and eat pieces of wet straw and chaff.

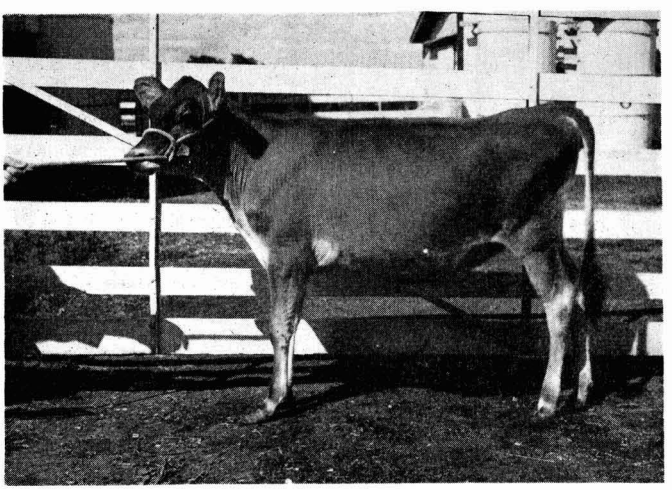

FIG. 2. Rumen-inoculated control calf (6 mo. old). Rumen contained characteristic microorganisms.

Although three of these calves persistently did this, the calf having the cigar-shaped organisms in its rumen seldom was observed to do so.

DISCUSSION

These results obtained with calves devoid of characteristic rumen protozoa further support the opinion that rumen protozoa are not essential to the host animals. However, it must be kept in mind that the experiments so far carried out for an extended period of time with lambs (3) and calves have been under conditions of grain feeding and far removed from those that would exist under primitive conditions. Consequently, more extensive experiments may yet demonstrate a real function and value of protozoa, *e.g.*, as predigestors of undigestible bacteria, if the diet excessively stimulates these. The defaunating treatment used in the experiments mentioned by Becker (3) may have eliminated certain varieties of characteristic microflora besides the protozoa. Thus, the rotundity of their lambs may have been due to reasons similar to those causing the deeper, "pot-bellied" appearance of our calves.

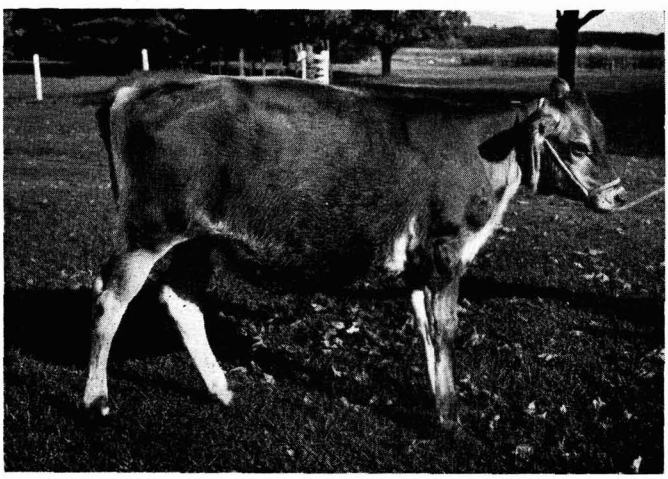

FIG. 3. Uninoculated calf (6 mo. old). Rumen devoid of usual rumen protozoa and hay-flora group II.

Support is provided for the idea that microorganisms that have developed over a period of time in the environment of the rumen would be more likely to function most efficiently in this organ by: (a) the clinical manifestations of rough hair coats and deep, "pot-bellied" middles observed in these uninoculated and segregated calves when compared with inoculated calves on similar fairly high roughage rations; (b) the finding of clinical cases in the field which apparently respond to treatment with rumen inoculations (19); (c) the observed differences in blood plasma ascorbic acid levels between young inoculated and uninoculated calves on milk and hay rations (10); and (d) differences in similar groups of calves in their ability to digest cellulose (7).

A possible explanation for the tendency for the uninoculated calves to nose down into the bedding is that some natural instinct caused them to seek in such locations for substitute microorganisms to assist in carrying on the functions of their rumens. It was interesting to note that the one calf of the four animals whose rumen was devoid of protozoa, but which had the large cigar-shaped bacteria present in large numbers and presumably various other usual rumen microflora, had much less tendency to do this.

Observations such as those reported by Udall (21, 22) that association of calves with nurse cows promotes a more satisfactory condition of health in the calves possibly may be explained in part on the basis of improved transfer of rumen microorganisms. It also is quite possible that calves are stimulated by example to eat more roughage when along with cows. This would be of assistance in promoting an early establishment of usual rumen microorganisms (17, 18).

With reference to rumen inoculations with cud materials as a preventive or cure for abnormal conditions in cattle, Hoflund (11) says the practice was used as much as 100 yr. ago in Sweden. Cuds which were obtained from cattle in other districts were given to cattle which had become debilitated due to existing on forage from an area in which the crops were deficient.

SUMMARY

The growth and development of four Jersey calves which were raised in pens segregated from other cattle were compared with 12 others which were inoculated with cud material from older cattle and raised at the same time on similar rations of alfalfa hay and limited quantities of grain.

The uninoculated calves failed to develop usual protozoa in their rumens and also some varieties of characteristic rumen microflora which were used as indicators of the presence of usual rumen microorganisms. One of the four calves accidentally received a partial rumen inoculation. This resulted in one type of the characteristic indicator microflora which readily is digested by abomasal fluids becoming established in its rumen.

Average gains in weight at 6 mo. of age were 229 lb. for the four uninoculated calves and 235.5 lb. for the 12 inoculated animals, a difference of only 5.5 lb. The calf which received the partial rumen inoculation had a neat and healthy appearance similar to the control inoculated calves, but the hair coats of the other three were much rougher in appearance. Their abdomens seemed deeper and "pot-bellied." The latter three had a persistent habit of nosing down through the bedding to pick up wet bits of straw. It was considered possible that this habit was due to a stimulus to seek inoculation of their rumens with substitute rumen microorganisms in the absence of the usual microflora and fauna.

The authors wish to acknowledge the assistance of John Tate, R. L. Johnson and C. E. Knoop in conducting this investigation.

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RATE OF ABSORPTION OF CAROTENE AND OF VITAMIN A FROM THE ALIMENTARY TRACT OF DAIRY CALVES. I. EFFECT OF METHOD OF ADMINISTRATION[1]

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The importance of vitamin A activity in the nutrition of dairy calves has continued to focus attention on the quantitative dietary needs for carotene and vitamin A. The establishment of optimal allowances of these nutrients is contingent upon a knowledge of factors affecting utilization. Among the multitude of variables that may be related to efficiency of absorption and utilization are the vehicle of the vitamins, the dispersion of the vitamin concentrate and the methods of administration. Of these, only the last will be considered herein.

Lemley *et al.* (5) observed that when vitamin A in an oil medium was injected either subcutaneously or intramuscularly the effectiveness was 35 per cent and 2 per cent, respectively, as great as when taken *per os.* Water-solubilized carotene given intramuscularly, however, was utilized efficiently by rats (11). Aqueous dispersions of vitamin A also were utilized effectively when injected intramuscularly into children (4).

Niedermeier *et al.* (8) found that injections of an aqueous dispersion of vitamin A into the small intestine of the goat effected higher blood plasma levels of this vitamin than did similar injections into either the abomasum or the large intestine. Moreover, when vitamin A was injected into the small intestine of sheep (1), the rate of absorption was more rapid than when placed into the rumen or administered orally. There was little absorption of either carotene or vitamin A from the cecum and the colon.

Since feeding vitamin A and carotene concentrates to calves at different stages of development involves managemental problems as well as nutritional consequences, the objective of this investigation was to compare effects of various methods of administering (nipple feeder, stomach tube and gelatin capsule) supplements on the rates of absorption.

GENERAL EXPERIMENTAL PROCEDURES

Experimental subjects, feeding and management. Dairy calves representing four different breeds, Brown Swiss, Guernsey, Holstein and Jersey, were used in carotene and vitamin A absorption tests. During the first 3 days following birth, each calf received colostrum from its dam. Subsequently, either fresh whole milk or reconstituted milk was fed twice daily at the rate of 10 lb. per day per 100 lb. body weight of calf. The routine method of feeding was from a nip-

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ple pail. At various stages of growth of several of the calves, a concentrate mixture and hay were incorporated in the diet. All the experimental subjects were confined to individual pens bedded with wood shavings. Whenever calves were restricted to milk diets, the individuals were muzzled to minimize consumption of foreign material.

Carotene and vitamin A supplements. In most of the trials the source of carotene was "Carex"[3], a carrot oil that contained 5,000 I. U. of carotene per gram, but during the terminal stages of the investigation, carotene in cottonseed oil[4], 50,000 I. U. per gram, was administered. The source of vitamin A was fish liver oil concentrates. The potency of the product used during the early trials was 25,000 I. U. per gram[5], whereas that given in the later studies was 30,000 I. U.[6].

The quantity of supplement given in absorption tests was 1,000 I. U. per lb. of body weight. The measured amount for each subject was administered either dispersed (by homogenization) in milk or enclosed in gelatin capsules. The milk-dispersed supplement was given either orally from a nipple feeder or intraruminally through a stomach tube. Milk fed from a nipple normally traverses the esophageal groove and enters the abomasum directly (12). In the stomach-tube method of administration the supplements dispersed in milk were passed through a horse catheter into the rumino-reticular cavity. The volume of fluid used for the dispersion medium was approximately the same for either system, nipple or tube. It is possible, however, that in some instances the volume administered by tube might have exceeded the capacity of the rumen and reticulum, thus resulting in an overflow into the abomasum. A balling gun was used to administer the capsules, special care being taken to avoid their rupture before swallowing. Capsules thus administered would be expected to pass into the rumen.

Blood collection and analytical procedures. The criteria of the rates of absorption of carotene and of vitamin A were the levels of these substances in samples of plasma from venous blood collected at the time of feeding and at 2, 4, 8, 12 and 24 hr. thereafter. Blood plasma carotenoids and vitamin A were determined by procedures described by Squibb *et al.* (10).

TRIALS AND RESULTS

Trial I—Nipple feeder vs. stomach tube. At intervals of approximately 1 wk., the milk normally given at the morning feeding was replaced with reconstituted separated milk in which either a carotene or a vitamin A concentrate had been dispersed. The nipple and the stomach-tube methods of administration were alternated from period to period for each calf. In these comparisons eight animals received carotene supplements and six, vitamin A.

The mean pre-absorption carotenoid and vitamin A values in blood plasma are shown in table 1 (trial I). Each initial value was considered as the base

[3] Obtained from Nutrition Research Associates, South Whitley, Ind.
[4] Obtained from General Biochemicals, Inc., Chagrin Falls, O.
[5] Obtained from White Laboratories, Inc., Newark, N. J.
[6] Supplied by the Borden Co., New York, N. Y., courtesy of L. T. Wilson.

level (zero) from which subsequent changes were determined. Mean responses to the respective methods of administering the supplements are depicted in figures 1 and 2.

Although the magnitude of the increases resulting from each method of administration was variable in the different calves and in the same calf at various periods, the nipple system uniformly resulted in more rapid rises than did the stomach-tube procedure. The differences in the levels of carotenoids were more pronounced than those of vitamin A. The maximum values for vitamin A, however, were attained more quickly than those for carotenoids.

TABLE 1

Mean pre-supplementation concentrations of carotenoids and vitamin A in the blood plasma of calves

Trial	Supplement	Method of administration	Mean level in blood plasma	
			Carotenoids	Vitamin A
			(γ/100 ml.)	
I	Carotene in oil	Nipple feeder	15.2	11.7
		Stomach tube	18.9	11.6
	Vitamin A conc.	Nipple feeder	19.6	13.1
		Stomach tube	16.5	12.5
II-a	Carotene in oil	Nipple feeder	19.8	7.4
		Capsule	17.0	6.5
	Vitamin A conc.	Nipple feeder	14.2	9.2
		Capsule	13.6	8.2
II-b	Carotene in oil	Nipple feeder	57.3	16.6
		Capsule	60.0	17.8
	Vitamin A conc.	Nipple feeder	35.0	15.7
		Capsule	42.4	15.7

Small but relatively uniform increases in concentrations of vitamin A in the blood plasma occurred during at least the first 12 hr. following the administration of carotene (fig. 1). Even though the carotenoid level was higher at 24 hr. than at 12, the vitamin A concentration was lower. The true relationship between the values of these constituents in plasma is obscure. Following vitamin A absorption (fig. 2), the carotenoid values in the plasma decreased, the degree and rate of depression being somewhat greater from the nipple administration than from the stomach tube.

In the absence of any well-established law relating concentrations of vitamin A and of carotenoids in the blood plasma to time after feeding massive doses of these substances, it was decided, for the purpose of statistical analysis, to obtain the average (or linear) rate of increase of concentration over the period

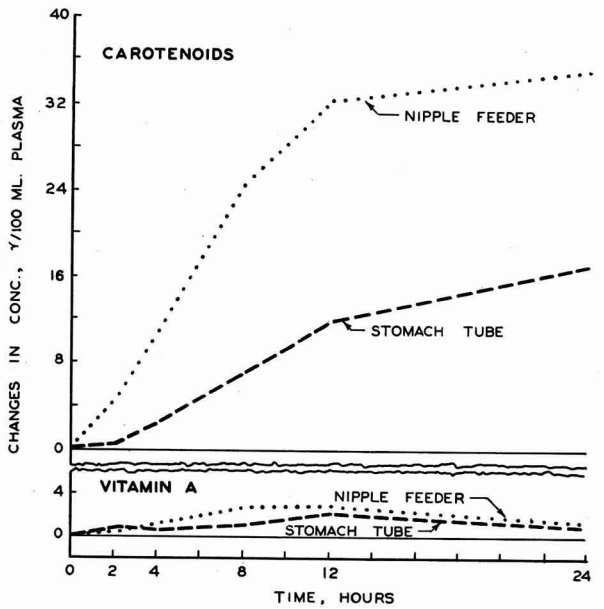

FIG. 1. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight calves that received massive doses of a carotene concentrate homogenized in milk and administered by nipple feeder and by stomach tube.

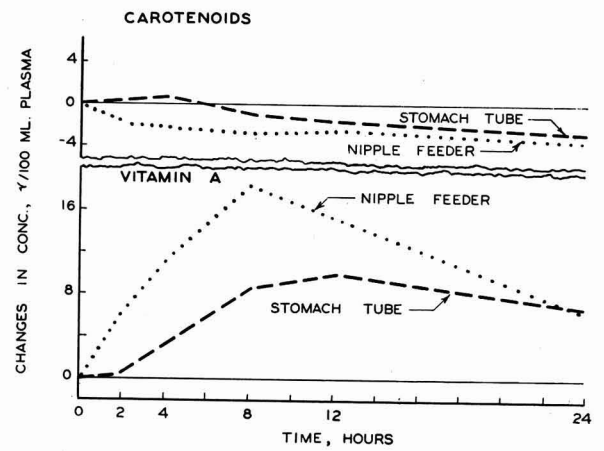

FIG. 2. Mean changes in levels of carotenoids and vitamin A in blood plasma of six calves that received massive doses of a vitamin A oil concentrate homogenized in milk and administered by nipple feeder and by stomach tube.

0 to 12 hr. The curvature in this relationship was examined by evaluating the quadratic component orthogonal to the linear component. If the increases at 2, 4, 8 and 12 hr. are denoted by I_2, I_4, I_8 and I_{12}, the linear rate of uptake L and the orthogonal quadratic component Q are apart from constant numerical divisors, thus

$$L = -8I_2 - 3I_4 + 7I_8 + 17I_{12}$$
$$Q = -20I_2 - 109I_4 - 113I_8 + 115I_{12}$$

The L and Q values were appraised by a simple analysis of variance. The variations in these values were separated into those due to differences between calves, those due to differences between treatments and those due to treatment by calf interactions. The significance of treatment effects on either L or Q was determined by comparing the average effect with a variance which measures the failure of the effect to be the same for all calves. Thus, the test of significance was made by comparing the mean square for treatment with the mean square for calf-treatment interactions.

The analysis of the L values for the first set of data, table 2, revealed a dif-

TABLE 2

Analysis of variance of linear changes of blood plasma carotenoid levels of eight dairy calves following administration of massive doses of carotene homogenized in milk

Source of variation	Degrees of freedom	Sums of squares	Mean square
Calves	7	609,260	87,037
Treatments			
(Nipple pail versus stomach tube)	1	682,235	682,235
Treatments × calves	7	503,927	71,990

$$F = \frac{682,235}{71,990} = 9.28^a$$

[a] Significance $P_{.05} = 5.59$
$P_{.01} = 12.25$

ference significant at the 5 per cent level in the linear rates of increase of blood plasma carotenoid levels following administration of carotene by the two methods. A like analysis of the corresponding Q values also showed a difference significant at the 5 per cent level of probability.

Although the remaining data were analyzed in a manner similar to those illustrated in table 2, only summary statements are presented.

In this first trial the data on vitamin A uptake during the initial 12 hr. following administration of this vitamin showed a difference in curvatures that approached significance at the 5 per cent level, whereas the differences in the linear components were non-significant. This was due, in part, to the marked downward trend in the nipple-fed group after the eighth hour.

Trial II. Nipple feeder vs. capsule. The experimental subjects were 60-day old calves that had been used in a previous study (7) in which all subjects were restricted to a fortified filled-milk diet. Since the calves had not consumed solid feed, it was assumed that the rumen was underdeveloped. To gain

information on the effects of diet and/or rumen development, two series of absorption trials were conducted: the first, while the animals were on a milk diet and, the second, after 2 mo. on a conventional milk, concentrate and hay regime.

a. *Whole milk diet.* The diet of the calves was changed from the filled milk to whole milk. Subsequently, the animals were divided into two units: group A consisted of eight Holsteins and two Guernseys and group B, seven Holsteins and one Guernsey. Each group was divided further into two subgroups (table 3). At approximately weekly intervals, carotene and vitamin A supplements were given at the rate previously indicated. The methods of administration were as outlined in table 3.

The initial values of carotenoids and of vitamin A in blood plasma are shown in table 1 (trial II-a) and the responses to supplementation in figures 3 and 4. The rate of carotene (oil concentrate homogenized in milk) absorption (fig. 3) following ingestion from the nipple feeder was similar to that

TABLE 3

Grouping of calves and plan of administering supplements (Trial II)

Supplement[a]	Group	Sub-group	No. of calves	Method of administration	
				Period I	Period II
Carotene in oil (carotene)	A	1	5	Nipple feeder	Capsule
		2	5	Capsule	Nipple feeder
Fish liver oil (vitamin A)	B	1	4	Nipple feeder	Capsule
		2	4	Capsule	Nipple feeder

[a] Administered at rate of 1000 I. U./lb. body wt.

in trial I (fig. 1) but more rapid than when the oil was given in a capsule (fig. 3). The differences in linear trends resulting from the two methods of administration were significant at the 1 per cent level, but the differences in curvatures were non-significant statistically.

The rate of absorption of vitamin A (fig. 4) was somewhat greater when the fish liver oil concentrate was fed from a nipple than when given in a capsule, but in the former the maximum level was attained at approximately 12 hr. after ingestion, whereas in the latter the maximum occurred later. During the initial 12 hr., the difference in the linear trends of vitamin A in blood plasma of calves receiving the supplement by the two methods was statistically significant at the 5 per cent level, but the difference in curvatures of rates of uptake was not significant.

When the nipple system of feeding was employed, the corresponding responses in trials I and II-a to carotenoid intake (figs. 1 and 3) and to vitamin A (figs. 2 and 4) were strikingly similar. A comparison of the stomach-tube method (figs. 1 and 2) with the capsule procedure (figs. 3 and 4) indicates that the rate of absorption of the supplements was more rapid when the former of the two methods was used. Moreover, the extent of carotenoid suppression in the blood plasma following vitamin A supplementation was somewhat greater in trial I than in trial II-a.

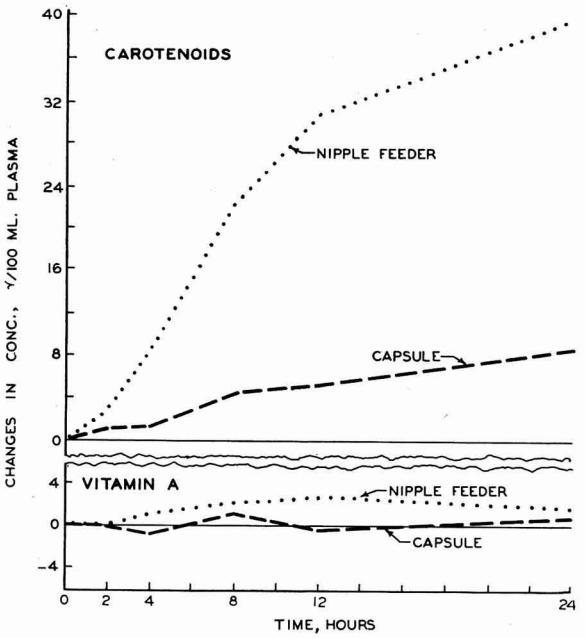

FIG. 3. Mean changes in levels of carotenoids and vitamin A in blood plasma of ten 2-mo.-old calves that received a basal diet of whole milk and a supplement of massive doses of carotene administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

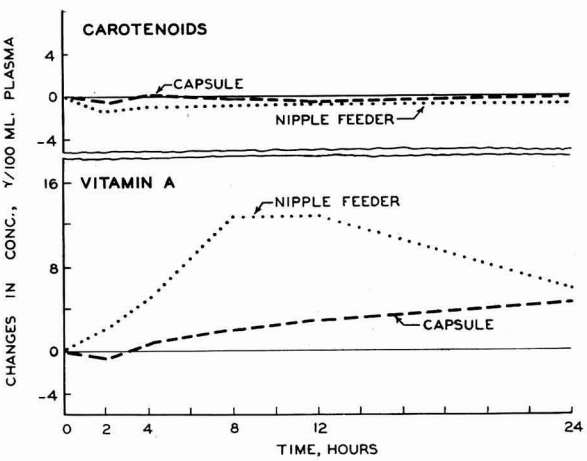

FIG. 4. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight 2-mo.-old calves that received a basal diet of whole milk and a supplement of massive doses of vitamin A administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

b. *Buttermilk (reconstituted), concentrate mixture and alfalfa hay diet.* After this diet was fed to the same calves employed in trial II-a (less one calf in carotene group) for a period of 2 mo., the plan of administering carotene and vitamin A, table 3, was repeated. Since the dry separated milk available was more readily reconstituted and, thus, was a more desirable dispersion medium for the supplement than the dry buttermilk commonly fed, the former was substituted for the latter when the vitamin substances were administered. Other components of the diet, concentrate mixture and hay, were unchanged on the day of the tests.

As a result of hay consumption, the base levels of carotenoids and of vitamin A in the blood plasma of the calves were higher in trial II-b than in II-a (table 1). The post-supplementation changes from these bases are shown in figures 5 and 6.

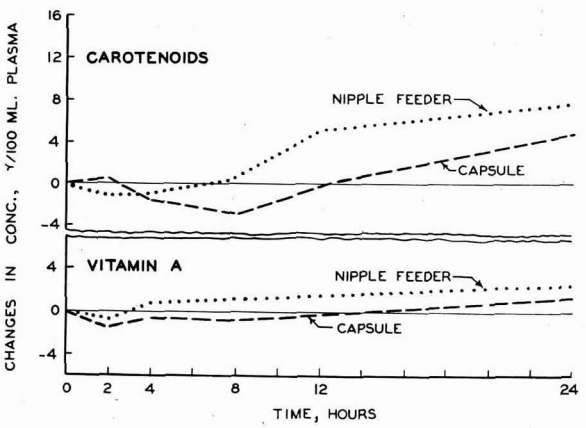

FIG. 5. Mean changes in levels of carotenoids and vitamin A in blood plasma of nine 4-mo.-old calves that received a basal diet of reconstituted buttermilk, alfalfa hay and a concentrate mixture and a supplement of massive doses of carotene administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

The values of plasma carotenoids following carotene administration were slightly greater when the supplement was fed from a nipple than when given by a capsule (fig. 5). During the first 12 hr., the difference in linear trends approached significance at the 5 per cent level, but the difference in curvatures was non-significant. The striking features of the responses in this trial, in comparison with those in trial II-a (fig. 3), were the delayed increases and the subsequent low magnitude. Although, as in preceding trials, the accompanying increases of plasma vitamin A were slight, the higher level of vitamin A corresponded to the higher values for carotenoids.

In contrast to the exceptionally slow rise in carotenoid concentrations in the blood plasma (fig. 5), the increase of vitamin A was rapid (fig. 6). The rate of uptake of this vitamin and the level reached were even greater in this trial than in the preceding (fig. 4). In accord with observations in other trials, vita-

min A was absorbed more rapidly when the nipple procedure of administration was employed than when the capsule method was used (fig. 6). The difference in linear trends, however, during the period from 0 to 12 hr. was not significant, largely due to the precipitous drop in the "nipple" curve after the eighth hour. On the other hand, the difference of curvatures was significant at the 1 per cent level.

A further comparison of responses during the liquid (trial II-a) and the solid (trial II-b) dietary regimes indicates that when vitamin A concentrates were given by capsule, the concentration of this vitamin in the blood plasma was greater in the former trial (fig. 4) at 24 hr. after administration than at

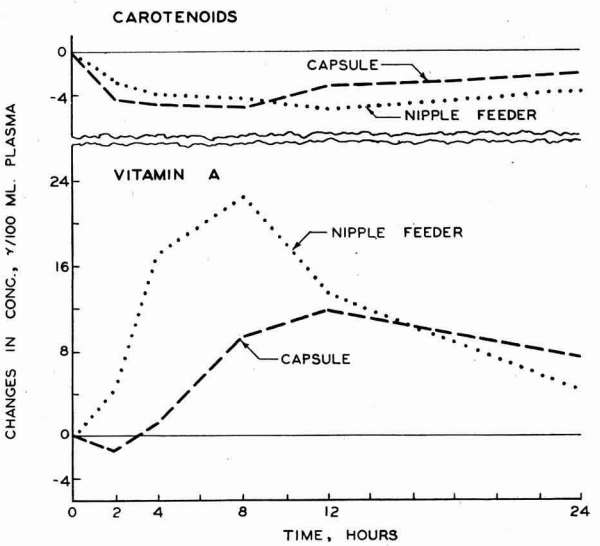

FIG. 6. Mean changes in levels of carotenoids and vitamin A in blood plasma of eight 4-mo.-old calves that received a basal diet of reconstituted buttermilk, alfalfa hay and a concentrate mixture and a supplement of massive doses of vitamin A administered by either nipple feeder (concentrate dispersed in milk) or gelatin capsules.

12 hr., whereas in the latter (fig. 6) the converse was true. This difference in time suggests a more rapid passage of the supplement in the animals having the greater ruminal activity. In trial II-b the depression of carotenoids following vitamin A administration was greater than in trial II-a.

DISCUSSION

Although the concentration of any nutrient in the blood at a given time involves many metabolic processes, the results reported herein seem to indicate a relationship between the methods of administering carotene and vitamin A and the rate at which these substances are absorbed from the alimentary tract of dairy calves. There are several possible explanations for the difference ob-

served when vitamin substances were administered by stomach tube and by nipple. Milk ingested by this latter procedure is mixed with relatively large quantities of oral and esophageal secretions (14). This exposure of the dispersed supplements might have evoked physical and chemical alterations that enhanced subsequent absorption. Moreover, the slower rate of uptake of vitamin supplements following stomach-tube administration may be ascribed to their gradual passage from the rumino-reticular cavity and thence into the other stomach compartments and the small intestine. Inasmuch as it has been demonstrated (2) that vitamin A in oil is absorbed in the bovine largely through the lymph of the small intestine, the rapidity with which this absorptive area was contacted by the vitamin substances used in the present experiment might have affected the rate of transmission to the blood. This delay in the fore part of the digestive tract conceivably also could have resulted in an increased loss of potency of the supplements.

Since the carotene and the vitamin A administered in gelatin capsules presumably passed into the rumino-reticular cavity, the retarded rate of absorption probably resulted, in part, from factors similar to those affecting uptake of vitamin substances dispersed in milk and administered by stomach tube. As the rate of uptake in the latter instance was somewhat more rapid, it would seem that absorption might have been enhanced by dispersion of the vitamin supplements. Frazer and associates (3) found that the average particle size of ingested triglyceride fats in the intestine of the rat is less than 0.5 μ and that paraffin, which normally does not pass through the intestinal wall, is absorbed when similarly dispersed. Since it has been shown (9) that fats and vitamin A are absorbed in a like manner, it seems possible that vitamin A uptake, like fat absorption, may be influenced by dispersion. The need for further experimentation, however, is indicated since Lundbaek and Maaløe (6) were unable to confirm the paraffin absorption observations.

The marked reduction in rate of uptake of carotene following the transition from a diet of whole milk to one composed of reconstituted buttermilk, hay and concentrates is difficult to interpret. Possibly the relatively high initial blood plasma carotenoid values of calves in trial II-b (solid diet) might have masked the effects of supplemental carotene. It would seem, however, that this apparent reduced rate of absorption might have been due, in part, to changes in the amount and the type of oil in the carotene supplement and to the quantity of fat in the milk in which the concentrate was dispersed. It is possible that the reduced absorption might have resulted not from any single factor but rather from the combined effect of several of the foregoing.

The relationship between blood plasma values for vitamin A and those for carotenoids following the administration of massive doses of carotene is obscure. Maximum vitamin A levels, subsequent to carotene administration, usually were reached earlier than the corresponding carotenoid maxima. Since the changes in vitamin A values were small, additional experimentation is necessary before this relationship can be clarified.

The maximum blood plasma levels of the vitamin substances fed were at-

tained earlier after vitamin A administration than after carotene feeding, thus suggesting a possible difference in the metabolism of these materials. Since the rates of administration of these substances were similar on the I.U. basis, the quantity of carotene, in micrograms, was greater, thus possibly affecting the time required for maximum levels to be attained.

The 24-hr. experimental period employed in this investigation was too brief to characterize the entire absorption curves. Limited data (13), however, indicate that the blood plasma carotenoid and vitamin A levels following administration of carotene by stomach tube and by capsule increase over a longer period of time and decline more gradually than those resulting from nipple pail feeding. Studies of this nature, even though conducted over an extended interval, may not indicate the efficiency of utilization of vitamin supplements fed by the various methods. Whether a relationship exists between rate of increase in the blood and total absorption remains to be determined by further experimentation.

SUMMARY

Carotene and vitamin A given at the rate of 1000 I.U. per lb. of body weight of calf were administered, respectively, by nipple feeder, stomach tube and gelatin capsule.

Comparisons of initial blood plasma carotenoid and vitamin A levels with those 2, 4, 8, 12 and 24 hr. after feeding the vitamin substances were employed as criteria of the rates of absorption.

Carotene and vitamin A dispersed in milk by homogenization and fed by nipple were absorbed more rapidly than similar preparations administered by stomach tube. The rates of absorption of carotene and of vitamin A from concentrates administered by gelatin capsules were somewhat less rapid than those resulting from the foregoing procedures.

The rate of absorption of vitamin A by calves restricted to whole milk was less rapid than the rate of uptake by the same calves after having received a diet of reconstituted buttermilk, hay and grain concentrates for approximately 8 wk. Conversely, the rate of absorption of carotene was more rapid under the former dietary regime than under the latter.

ACKNOWLEDGMENT

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DEHYDRATED SWEET POTATOES AS A SUBSTITUTE FOR CORN–SOYBEAN SILAGE

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During the winter months when pasture may be poor or unavailable, silage may constitute a vital part of the dairy cow's ration. Many dairymen in Louisiana and in other parts of the South do not have silage or have herds too small for practical silage feeding. Therefore, the question was raised as to whether a suitable home-grown substitute could replace silage. Some dairy farmers in Louisiana have reported that milk production did not fall when sweet potatoes were used during the winter months when no pasture or silage was available.

Sweet potatoes are plentiful at certain seasons of the year and can be stored after dehydration. It is well recognized that dehydrated sweet potatoes are approximately 90 per cent as valuable as yellow corn meal as a source of carbohydrate in the grain ration for dairy animals (1, 2, 4). Rusoff *et al.* (4) also reported that dehydrated sweet potatoes were approximately 17 per cent more valuable than ground snapped corn including cob and shuck for milk production.

Although dehydrated sweet potatoes are classified as a concentrate, it was decided to compare this material with silage for lactating cows.

EXPERIMENTAL

This study was conducted during the winter months of 1948–1949 and 1949–1950. Dehydrated standard sweet potatoes and dehydrated weevily sweet potatoes (fed wet) were compared with corn-soybean silage for milk production. In a palatability trial using dehydrated "infected" and weevily sweet potatoes, Rusoff and Miller (3) found that animals would consume these culled potatoes as readily as the standard potatoes. Therefore, dehydrated weevily sweet potatoes also were used to determine whether they might affect milk production. Some pasture was available during the 1948–1949 trial, and none in the 1949–1950 trial.

Trial 1. A Latin-square design was used. Three groups of eight milking cows each, (five Holsteins and three Jerseys) were given an 18 per cent protein grain mixture according to production. The animals in each group were similar as to age, production and number of previous lactations. Approximately 8 lb. of alfalfa hay per cow per day were fed. Equal amounts of corn-soybean silage, dehydrated standard sweet potatoes or dehydrated weevily sweet potatoes on an air-dry basis were fed to the groups. Approximately 1 lb. of dehydrated sweet potatoes was equivalent to 1 lb. of air-dry silage. This amounted to approximately 9 lb. of dehydrated sweet potatoes or 30 lb. of fresh silage daily. The trial consisted of three experimental periods of 20 days each with a 5-day change-

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over between periods. The milk produced by each cow was weighed daily and tested for butterfat every 10 days during each period.

The amount of 4 per cent fat-corrected milk (F.C.M.) per cow per day produced by each group while fed silage or dehydrated sweet potatoes is given in table 1. The average daily 4 per cent milk production of all groups was 24.4 lb. on the silage, 25.6 lb. on dehydrated standard potatoes and 26.1 lb. on dehydrated weevily sweet potatoes.

TABLE 1

Pounds of 4% F. C. M. per cow per day produced by the various groups while fed silage or sweet potatoes

Group	Preliminary period (7 d.)	Corn-soybean silage (20 d.)	Dehydrated standard sweet potatoes fed wet (20 d.)	Dehydrated weevily sweet potatoes fed wet (20 d.)
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
A	25.8	24.0	25.2	27.3
B	25.0	24.1	26.6	26.5
C	25.9	25.3	25.2	24.7
Av.	25.6	24.4	25.6	26.1

Table 2 presents the actual feed consumption of silage and dehydrated potatoes on the air-dry basis for each group during each period of 20 days. Any refusals were weighed back. The average amounts of feed consumed were practically the same.

TABLE 2

Feed consumption of silage and dehydrated sweet potatoes for each group on an air-dry basis

Group	Silage	Dehydrated standard sweet potatoes	Dehydrated weevily sweet potatoes
	(*lb.*)	(*lb.*)	(*lb.*)
A	1,328.6	1,374.0	1,336.6
B	1,229.0	1,360.0	1,370.4
C	1,390.0	1,380.0	1,382.4
Av.	1,316.0	1,371.3	1,363.1

Trial 2. In this study (1949–1950) a double reversal plan was used. Two similar groups of nine animals each (five Holsteins and four Jerseys) were used. The same feeding program and length of periods were followed as in the 1948–1949 test. Dehydrated sweet potatoes consisting of an equal mixture of standard and weevily potatoes which were stored for 1 yr. were compared with corn-soybean silage for milk production. Approximately 9 lb. of dehydrated sweet potatoes or 30 lb. of silage were fed per cow per day so that the air-dry amounts were equalized.

Table 3 presents the 4 per cent F.C.M. production per cow per day for the two groups on the dehydrated sweet potatoes or silage for each period. The average milk productions per cow per day for both groups of animals when fed dehydrated sweet potatoes or silage were 21.5 and 22.3 lb., respectively.

TABLE 3

Amount of 4% F. C. M. per cow per day during each period of trial 2

Feed	Preliminary period (10 d.)	Period (20 d. each)			
		1	2	3	Av.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Dehydrated sweet potatoes	22.6	23.1	21.7	19.6	21.5
Silage	21.4	24.5	21.1	21.3	22.3

The amounts of silage and dehydrated potatoes (air-dry basis) eaten during each period were very comparable (table 4).

The chemical composition of the dehydrated sweet potatoes and corn-soybean silage used in both trials is given in table 5. The percentage of crude protein for

TABLE 4

Feed consumption (air-dry basis) of each group on silage and dehydrated sweet potatoes during each period of trial 2

Feed	Period (20 d.)			
	1	2	3	Av.
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
Dehydrated sweet potatoes	1,562	1,451	1,580	1,531
Silage	1,620	1,597	1,586	1,601

the silage in the 1949–1950 trial is 9.75, as compared to 5.77 in the 1948–1949 trial. This higher percentage is due to the greater proportion of soybeans in the silage.

DISCUSSION

In both trials, the amount of 4 per cent F.C.M. produced was similar whether the animals were consuming silage or dehydrated sweet potatoes. The groups

TABLE 5

Chemical composition of dehydrated sweet potatoes and corn-soybean silage (dry matter basis)

Feed	Dry matter	Crude protein	Crude fat	Nitrogen-free extract	Crude fiber	Ash
	(%)	(%)	(%)	(%)	(%)	(%)
1948–49						
Dehydrated sweet potatoes[a]	91.34	5.47	0.38	86.80	4.13	3.21
Dehydrated sweet potatoes[b]	90.35	5.88	0.47	85.45	4.23	3.96
Silage	28.1	5.77	1.21	51.14	33.92	7.96
1949–50						
Dehydrated sweet potatoes[c]	90.1	5.22	0.55	86.57	4.55	3.11
Silage	28.6	9.75	2.55	54.98	27.45	5.27

[a] Standard
[b] Weevily
[c] Mixture of a and b

also consumed approximately the same amount of these feeds on the air-dry basis. The average milk productions per cow per day for both trials when fed corn-soybean silage or dehydrated sweet potatoes were 23.3 and 23.7 lb., respectively.

No evidence of digestive disturbances was observed in any of the animals. Since some of the animals refused part or all of the dehydrated sweet potatoes when this product completely replaced silage, it is recommended that sweet potatoes should be substituted gradually when changing feed.

The cost of the silage was estimated at approximately $10 per ton of fresh material or $1.50 per 100 lb. of air-dry silage, while the dehydrated sweet potatoes cost approximately $3 per 100 lb. on the market. Under existing levels of production and dehydration costs, dehydrated sweet potatoes are uneconomical to use as a substitute for silage in maintaining milk production at a normal level except during the periods when no silage or pasture is available. The cost of using fresh sweet potatoes is much lower than that of dehydrated potatoes but this product cannot be kept for any length of time unless properly protected against cold or dehydrated. One pound of fresh sweet potatoes is approximately equivalent to 1 lb. of fresh silage for milk production, since these products both contain approximately 70 per cent moisture. The dehydrated sweet potatoes were fed wet so as to simulate silage in bulk. Sweet potatoes appear to have a stimulating effect on milk production similar to that of corn-soybean silage.

SUMMARY

Two feeding trials were conducted on substituting dehydrated sweet potatoes for corn-soybean silage during the winter months of 1948–1949 and 1949–1950.

The cows produced the same amount of 4 per cent fat-corrected milk on the dehydrated sweet potatoes as when they were on the silage, the averages for both trials being 23.3 and 23.7 lb. per cow per day, respectively. Apparently dehydrated sweet potatoes may serve as a good replacement for silage for milk production, especially when pastures are poor and silage unavailable.

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THE FERTILITY OF BOVINE SEMEN IN CITRATE–YOLK EXTENDERS CONTAINING ADDED CATALASE

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Investigations by VanDemark *et al.* (12) have shown that high oxygen tensions are detrimental to the livability of bovine spermatozoa in the 3.6 citrate-yolk extender or diluter. Presumably, the decreased livability reflects an increased H_2O_2 production, as has been demonstrated by Tosic and Walton (11) and Tosic (10). The recent report by Prince and Almquist (5) that agitation of semen in partially filled tubes was harmful to spermatozoan survival also suggests the possibility of oxygen damage due to excessive aeration.

Evans (3) has shown that the fertilizing capacity of *Arbacia* spermatozoa was reduced by treatment with H_2O_2. Retardation of the cleavage time in the fertilized ova resulted. Wyss *et al.* (13) have shown that the mutation rate of *Staphylococcus aureus* increased when the cultures were exposed to H_2O_2 in the media. These effects of H_2O_2 were negated by the addition of catalase to the media.

In view of the earlier work on the improvement in the livability of bovine spermatozoa from additions of catalase, the effects of H_2O_2 on *Arbacia* spermatozoa and certain bacteria, it seems reasonable to postulate that catalase might, through the same process, improve the fertility of bovine spermatozoa used in artificial breeding.

No known reports have been made indicating the relationship between the livability of bovine spermatozoa under conditions of high oxygen tension and their fertility. Similarly, the fertility of bovine spermatozoa in extenders or diluters containing added catalase has not been reported. The results reported by Prince and Almquist (5) and by VanDemark *et al.* (12) were obtained when using 3.6 citrate-yolk extender. The experiment reported herein was designed to compare the fertility of bovine spermatozoa in citrate-yolk and citrate-sulfanilamide-yolk extenders with that of spermatozoa stored in these same extenders but containing added catalase.

EXPERIMENTAL PROCEDURE

The experimental design was a 4×4 Latin square consisting of four experimental extenders, four groups of technician-inseminators and four insemination periods. The insemination periods represented the semen shipped during a 4-day period.

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The compositions of the buffers and extenders are shown in table 1. The buffers were prepared once each week and the extenders were prepared during the afternoon of the day before they were to be used. Catalase was added at the time the extenders were prepared at a rate of one part of Vitazyme Catalase Sarrett to 10,000 parts of extender. This resulted in a lower catalase concentration than was used by VanDemark *et al.* (12), but this concentration was still several times that needed to eliminate the H_2O_2 as fast as it was produced (11), if there were no interfering substances.

TABLE 1

Composition of buffers and extenders

	3.6 CY[a] without added catalase	3.6 CY with added catalase	3.6 CSAY[b] without added catalase	3.6 CSAY with added catalase
Buffer:				
$Na_3C_6H_5O_7 \cdot 2H_2O$ (*g.*)	36.0	36.0	36.0	36.0
Sulfanilamide (*g.*)	.....	.....	6.0	6.0
Water (redistilled over glass) to final vol. (*ml.*)	1000.	1000.	1000.	1000.
Extender:				
Ratio of egg yolk to buffer	1:1	1:1	1:1	1:1
Ratio of added catalase to extender[c]	.....	1:10,000	.....	1:10,000

[a, b] 3.6 indicates the percentage of citrate in the buffers. C = citrate; 8A = sulfanilamide; Y = egg yolk.

[c] The catalase preparation used contained approximately 700–800 units of catalase/ml. (10). (10).

Semen for these studies was obtained from Holstein bulls in the active stud of the New York Artificial Breeders' Cooperative, Inc., and consisted of those ejaculates which contained 500×10^6 or more spermatozoa per milliliter, of which 50 per cent or more were motile, as determined by routine procedures (1, 6).

Immediately after collection, the semen was extended at a rate of approximately 1 to 4 in 3.6 citrate-yolk without added catalase and cooled according to the procedure of Foote and Bratton (4). Final extension to a standard number of motile spermatozoa (approximately 10×10^6 per milliliter of extender) was made at a temperature of approximately 5° C. with the partially extended semen sample and the final extenders at these same temperatures.

An 8-ml. portion from each of the extended semen samples was stored at 5° C. During storage, each sample was mixed and portions withdrawn from it to simulate the field practice of handling semen at the time the technician performs an insemination. During the first day of storage, the samples were mixed five times but no semen was withdrawn. On the second day they were mixed seven times and three portions of 2 ml. each were removed at 2-hr. intervals. Estimations of the per cent of progressively motile spermatozoa were made microscopically at 3, 24, 48 and 72 hr. of storage and used as a basis for comparing the livability of the spermatozoa during storage.

Fertility was estimated from the 60- to 90-day non-returns to first and second service cows and expressed as per cent non-returns.

RESULTS AND DISCUSSION

Forty-seven ejaculates from 24 bulls were used for insemination. The average number of motile spermatozoa per milliliter of extended semen was 11.9×10^6. Table 2 gives the estimated average percentages of motile spermatozoa in the experimental extenders after 3, 24, 48 and 72 hr. of storage at 5° C. The differences between spermatozoan livability in the different extenders were not significant. These results are in contrast to those previously reported by VanDemark *et al.* (12) for semen extended in 3.6 citrate-yolk with and without catalase and mixed at regular intervals.

TABLE 2

Livability of bull spermatozoa during storage at 5° C. in extenders with and without added catalase

Duration of storage	% motile spermatozoa			
	3.6 CY[a] without added catalase	3.6 CY with added catalase	3.6 CSAY[b] without added catalase	3.6 CSAY with added catalase
(*hr.*)				
3	66	66	66	67
24	62	64	63	64
48	58	58	58	57
72	53	55	52	53

[a, b] See footnotes for table 1.

Table 3 gives the number of first, second and the combined number of first and second service cows inseminated and the mean per cent 60- to 90-day non-returns for these groups of cows. The means for the per cent non-returns to both first service cows and second service cows show that the fertility level of the semen with added catalase varied but little from the level of the semen without added catalase. On the basis of the combined first and second service cows, the average per cent non-returns for extenders containing catalase was 61.2 and for those not containing catalase, 61.8.

Since no improvement from added catalase was shown in either spermatozoan livability or fertility in the present study, it is possible that the H_2O_2 level of the extended semen during the 2 days in which the majority of inseminations were being made was not high enough to be detrimental. This may have been a consequence, in part, of the procedure used in cooling the semen. In the studies by VanDemark *et al.* (12) in which oxygen damage to spermatozoa was alleviated by catalase, the semen was gradually cooled to 5° C. and then extended with cold (5° C.) citrate-yolk containing added catalase. Since that time Foote and Bratton (4) have shown an improvement in fertility from partially extending the semen with the citrate-yolk before cooling. This cooling procedure is in routine use at the New York Artificial Breeders' Cooperative and was used in this experiment.

The change in cooling procedure also may have been responsible for the small difference in the fertility level shown between semen extended with citrate-yolk and that extended with citrate-sulfanilamide-yolk. Earlier investigations (2, 7, 8), in which the fertility of semen in extenders with and without added sulfanilamide was compared, showed an increase of approximately five percentage units in 60- to 90-day non-returns to first service cows in favor of the samples with added sulfanilamide.

SUMMARY

Using the split sample technique, the spermatozoan livability and the fertility of 47 semen samples from 24 Holstein bulls were studied when extended to contain approximately 11.9×10^6 motile spermatozoa per milliliter in citrate-yolk and citrate-sulfanilamide-yolk extenders with and without added catalase.

TABLE 3

Fertility level of bull semen extended with and without added catalase. (Based on 60- to 90-day non-returns to 1st and 2nd service cows)

	3.6 CY[a] without added catalase	3.6 CY with added catalase	3.6 CSAY[b] without added catalase	3.6 CSAY with added catalase
Total number of:				
1st service cows	1205	1168	1172	1140
60- to 90-day non-returns (*mean* %)	62.0	60.8	63.0	63.1
2nd service cows	575	554	528	569
60- to 90-day non-returns (*mean* %)	61.0	58.7	57.9	57.3
Combined 1st and 2nd service cows	1780	1722	1700	1709
60- to 90-day non-returns (*mean* %)	61.4	60.7	62.2	61.7

[a, b] See footnotes for table 1.

On the basis of the average per cent 60- to 90-day non-returns to service to approximately 1,700 first and second service cows per treatment, the extenders compared as follows: 3.6 citrate-yolk without added catalase, 61.4; 3.6 citrate-yolk with added catalase, 60.7; 3.6 citrate-sulfanilamide-yolk without added catalase, 62.2; and 3.6 citrate-sulfanilamide-yolk with added catalase, 61.7.

Differences in spermatozoan livability and fertility in the various extenders were not significant.

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PAROTID GLAND LESIONS IN EXPERIMENTAL BOVINE VITAMIN A DEFICIENCY

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The study was concerned with clinical, biochemical and pathologic manifestations of vitamin A deficiency in young dairy bulls which had been maintained on a diet low in carotene but otherwise of sufficient caloric value to permit normal growth. The experiments were terminated after an average period of 105 days, when convulsive symptoms were well established. The aim was to elucidate the basic lesions in rapidly developing, uncomplicated A-hypovitaminosis.

The pathology of bovine vitamin A deficiency of different intensity and duration has been studied by a number of workers, but the reports vary with respect to the significance and specificity of the lesions observed.

Ocular changes simulating infectious keratitis were found frequently by Hart and Guilbert (9) under natural conditions ascribed (10) to pinching of the optic nerve by a sphenoidal stenosis. Blindness without observable lesions was shown by Wetzel and Moore (22) to be due to edema of the optic papilla, resulting from increased cerebrospinal fluid pressure, according to Moore and Sykes (18).

The seminiferous tubules of young bulls were found, by Guilbert and Hart (7), to exhibit structural changes, a fact confirmed in detailed studies of Hodgson *et al.* (11), Erb *et al.* (5) and Bratton *et al.* (2).

Nephritic changes interpreted as parenchymatous nephritis were found to be associated with fatally terminating spontaneous cases in the experience of Hart (8). The corresponding experimental lesions were characterized by Langham *et al.* (13) as degenerative in the form of hydropic and necrobiotic alterations in the proximal portions of the nephron and as inflammatory in the form of cellular infiltrations and proliferations in the interstices. There was occasional metaplasia with rare hyperkeratinization of the transitional epithelium of the minor calices and the ureters. In a similar study Thorp *et al.* (21) confirmed these findings and reported only 2 of 25 animals as showing metaplasia in the calices.

The pituitary has been found to present cystic degeneration by Moore (16) or increased fluid between the anterior and posterior lobe by Sutton *et al.* (20). The latter authors also found an increase of "alpha" cells (acidophils) and believed the change to be similar to that in A-deficient rats, although their original studies on this species (19) showed an increase of "beta" cells (basophils). The cellular changes are interpreted as compensatory to the testicular degeneration, paralleling the so-called castration effect. On the basis of 10,000 slaughter-

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ing house specimens, Madsen *et al.* (15) considered cystic pituitaries in young cattle as a pathologic expression of A deficiency.

Anasarca or edema of subcutis and adjacent musculature has been described by Creech and Seibold (3) and was used as a criterion by Madsen and Earle (14) in characterizing "old corn" disease as vitamin A deficiency.

Pneumonic lesions were frequently observed by Hart (8) in natural, fatal cases and occasionally under experimental conditions by Thorp *et al.* (21). The latter authors also reported mild hyperplastic lesions in the small intestine and necrobiotic changes of like intensity in the liver.

On the whole, it may be seen that the now universally recognized basic lesion of A-hypovitaminosis, namely squamous metaplasia with varying degrees of hyperkeratinization (23), has been reported in the kidney only and even there as a distinctly minor alteration.

TABLE 1

Age, hemoglobin and carotene and vitamin A liver storage of experimental animals

			Age in days			Hemoglobin (*g./100 ml.*)		Final liver storage (γ/*g.*)	
	No.	Breed	Start	Finish	Difference	Start	Finish	Carotene	Vitamin
First expt.	1	Jersey	339	403	64	11.5	11.5	0.7	0.2
6–15–48	2	Guernsey	53	134	81	8.5	8.4	0.1	0.0
to	3	Guernsey	210	312	102	8.5	10.5	0.3	0.1
9–25–48		Av.	201	283	82	9.5	10.1	0.4	0.1
		Control							
	4	Guernsey	208	310	102	9.4	10.7	0.4	25.7
Second expt.	5	Guernsey[a]	240	375	135	10.0	11.4	0.8	8.2
2–15–49	6	Ayrshire	139	274	135	9.8	10.9	0.4	3.4
to	7	Holstein	148	231	183	10.4	10.1	0.4	0.1
6–30–49		Av.	176	293	118	10.1	10.8	0.5	3.9
		Control							
	8	Holstein	171	306	135	9.9	10.4	0.6	103.0
	Av. Deficient		188	288	100	9.7	10.7	0.5	2.0
	Av. Controls		189	308	119	9.7	10.6	0.5	64.4
	Av. Totals		189	293	105				

[a] Freemartin.

In a recent comprehensive treatise on the pathology of nutritional diseases, Follis (6) emphasized the importance of differentiating between specific and nonspecific damage due to deficiency of a single nutrient.

MATERIALS AND METHODS

The experiments were conducted on two groups of four bull calves each, except for one freemartin, representing four standard dairy breeds (one Jersey, four Guernseys, one Ayrshire, two Holsteins). In average terms, the first group, aged 204 days, was treated for 92 days during the summer of 1948, and the second group, aged 173 days, was treated for 126 days during the spring of 1949. The details are presented in table 1.

During treatment, each animal received a daily allowance of 4 lb. of grain mixture[1] containing less than 350 μg of carotene per lb. and beet pulp *ad libitum*. One control calf in each group received a daily supplement of 100,000 I.U. vitamin A from dogfish oil containing 25 per cent crude soybean lecithin. Clinical observations were made daily, and hemoglobin, plasma carotene and vitamin A content were determined weekly. Spinal fluid pressure readings according to Moore (17) and liver biopsies for histologic study were obtained approximately once per month. All of the animals were sacrificed when they showed daily convulsions, except for one which died on the 64th experimental day. The livers were frozen for later carotene and vitamin A determinations and the tissues subjected to thorough gross and microscopic examination.

CLINICOPATHOLOGIC RESULTS

Symptoms of spasmodic convulsions became manifest in the animal which later died, after about 45 days on experiment, in the others after about 75 days. The spasms increased in frequency until they occurred three to four times every day and were accentuated by sexual excitement. Bloat and diarrhea occurred occasionally. Some animals manifested impaired eyesight and exophthalmus.

The average clinicopathologic data for six treated and two control animals were as follows:

Hemoglobin. Expressed in grams per 100 ml. both the treated and control groups averaged 9.7 at the beginning of the experiment and 10.7 *versus* 10.6 at the end. There was no significant difference between groups, but all of the hemoglobin values increased slightly during the course of the experiments. The details are presented in table 1.

Spinal fluid pressure. Expressed in millimeters of water, the average values obtained in the first experiment were 307 in the treated group as against 322 in the control group at the start and 260 in the treated against 100 in the control group at the end. Later experiences showed that these values probably had been exaggerated by excitement. In the second experiment, the measurements averaged 107 for the treated against 91 for the control groups at the start and 190 *versus* 155 at the end.

The total averages were 207 for both the treated and control groups at the start and 225 for the treated *versus* 128 for the control groups at the end. There was a relative increase in spinal fluid pressure in the deficient group as compared with the control group, in accordance with the literature (18).

Liver biopsies. Narrow cylinders of hepatic tissue, obtained with an instrument designed for human prostatic biopsy, were fixed in Zenker's fluid, formol-saline and absolute alcohol, respectively. Special strains were applied to bring out cellular detail, neutral fat and glycogen. In general, both the deficient and the control groups failed to show any uniform structural changes or fatty meta-

[1] Grain mixture: Ground barley, 419.5; crimped oats, 500; wheat bran, 500; linseed oil meal (solvent process), 150; soybean oil meal (solvent process), 150; molasses, 200; 500-potency B-Y dried fermentation solubles, 40; steamed bone meal, 20; salt, 20; irradiated yeast (Standard Brand, type 9-F, 9,000 I.U. vitamin D per gram), 0.5; total, 2,000 lb.

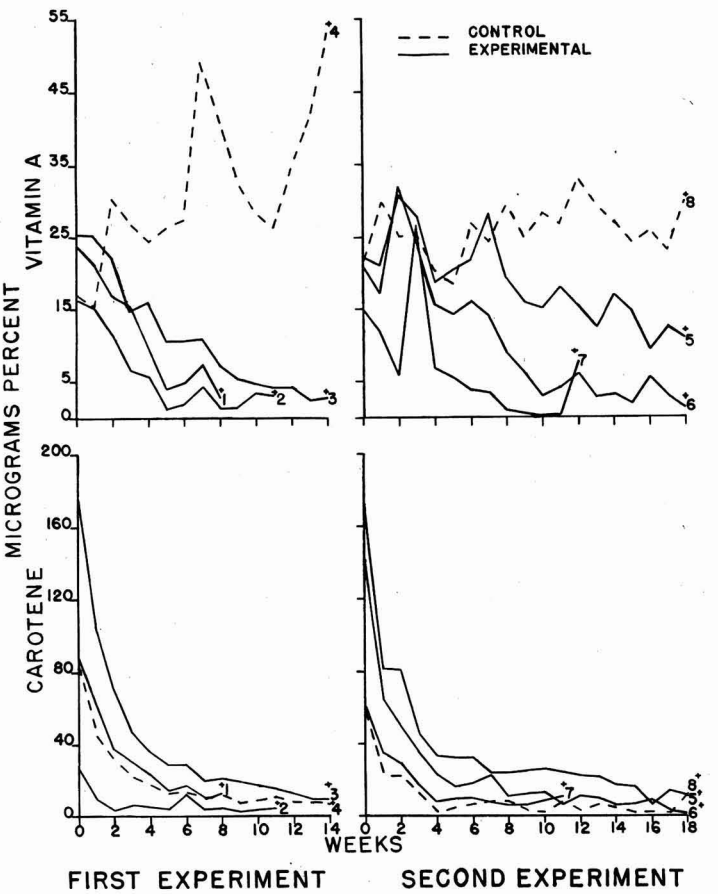
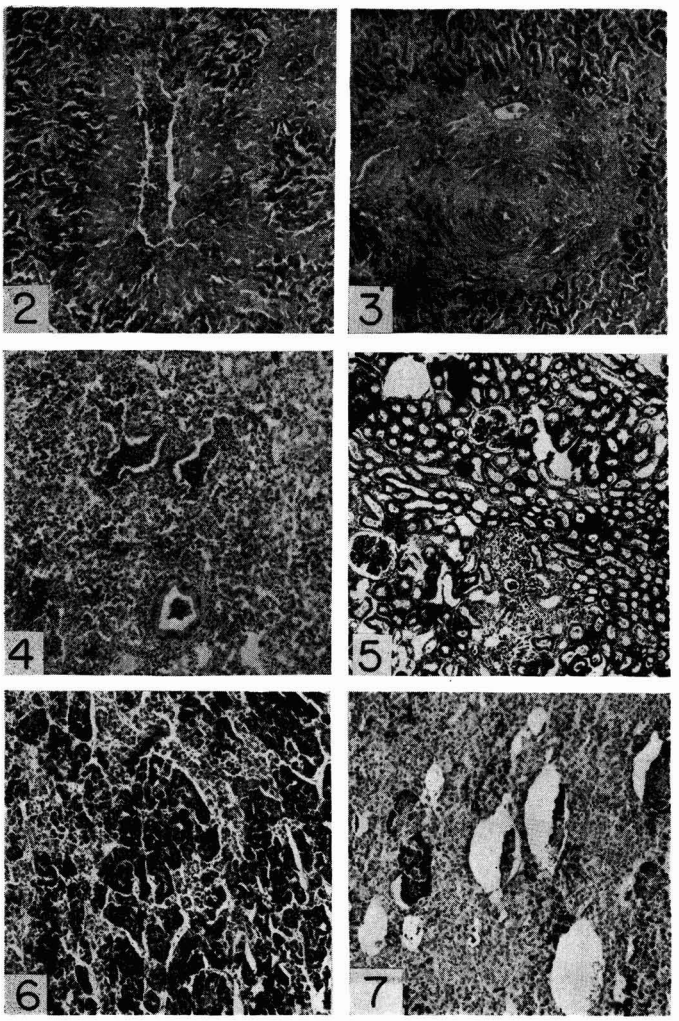


FIG. 1. Blood plasma carotene and vitamin A values obtained by weekly determinations during the course of the first and second experiment. The numbers at the end of each line refer to the no. of the animal as listed in table 1.

morphosis. There was fair-to-good glycogen storage throughout the course of the experiments.

Blood plasma carotene. Expressed in micrograms per 100 ml. the treated groups averaged 184 and the controls 132 at the beginning and 8 *versus* 9 at the end. Thus, there was no significant difference between groups. Both the deficient and the supplemented animals showed an approximately equal regression of plasma carotene under the conditions of these experiments. The details are presented in figure 1.

Blood plasma vitamin A. Expressed like carotene, the treated groups averaged 22.6 and the controls 22 at the start and 3.6 *versus* 42.5 at the end. Thus,



the deficient animals exhibited a marked decrease and the supplemented animals a corresponding increase in plasma vitamin A levels (fig. 1).

Final liver storage. Expressed in micrograms per gram of liver, carotene in both the treated and control groups averaged 0.5 thereby failing to show differences due to treatment, in line with the corresponding plasma values.

Vitamin A, on the other hand, averaged 2.0 in the treated group, as against 64.4 in the control group. There was, therefore, a significantly higher storage in the supplemented groups in comparison with the deficient ones, as was to be

expected from the corresponding plasma values. The details are presented in table 1.

PATHOLOGIC RESULTS

On gross examination, animal no. 1 (table 1) which died presented significant hepatic changes in the form of multiple poppy-seed sized yellowish areas, which were interpreted as focal necrosis.

Histopathologically, animal no. 2 showed focal necrosis (fig. 2), portal cirrhosis (fig. 3) of the liver and early exudative pneumonia (fig. 4). Animal no. 6 showed focal interstitial nephritis. Brain sections often showed perivascular and perineuronal edema, so-called lamina cribrosa. Because of their irregular occurrence, these lesions were considered as due to mild intercurrent diseases, not necessarily associated with treatment.

Microscopic changes of probable significance were found in the pituitary and the thyroid. The pituitary, especially the anterior lobe, has been stated in the literature to show both cystic (15) and cellular changes (20). In the present material, microcysts were found in both the treated and the control groups and, therefore, not accorded significance. The differential cellular picture, as presented in Bouin's fixed Masson's trichrome preparations, showed in the controls massive ribbon-like accumulations of acidophils in the periphery, leaving a narrow central area composed primarily of chromophobes and basophils (fig. 6). The principal differences in the treated animals were an apparent reduction of both chromatic cellular elements and a consequent predominance of chromophobes. In some sections from affected animals it was impossible to demonstrate any appreciable number of basophils (fig. 7). Although these numerical differences were based on estimates and not differential counts, they were contrary to expectations from the literature (19, 20) and suggested that this subject requires reinvestigation.

The thyroid of treated animals showed mild hyperplasia, while control animals presented more or less uniformly sized and well filled follicles lined by low cuboidal epithelium (fig. 8). Treated animals exhibited many small follicles with high cuboidal or nearly columnar epithelium which had a tendency to encroach upon the lumen (fig. 9). Other follicles varied widely in size and contained colloid with markedly scalloped margins. Hyperplasia of the thyroid

FIG. 2. Liver of no. 2. Peripheral necrosis—Karyolysis of liver cells around interlobular vein.

FIG. 3. Liver of no. 2. Portal cirrhosis—Marked increase of connective tissues in portal island accompanied by proliferation of bile ducts.

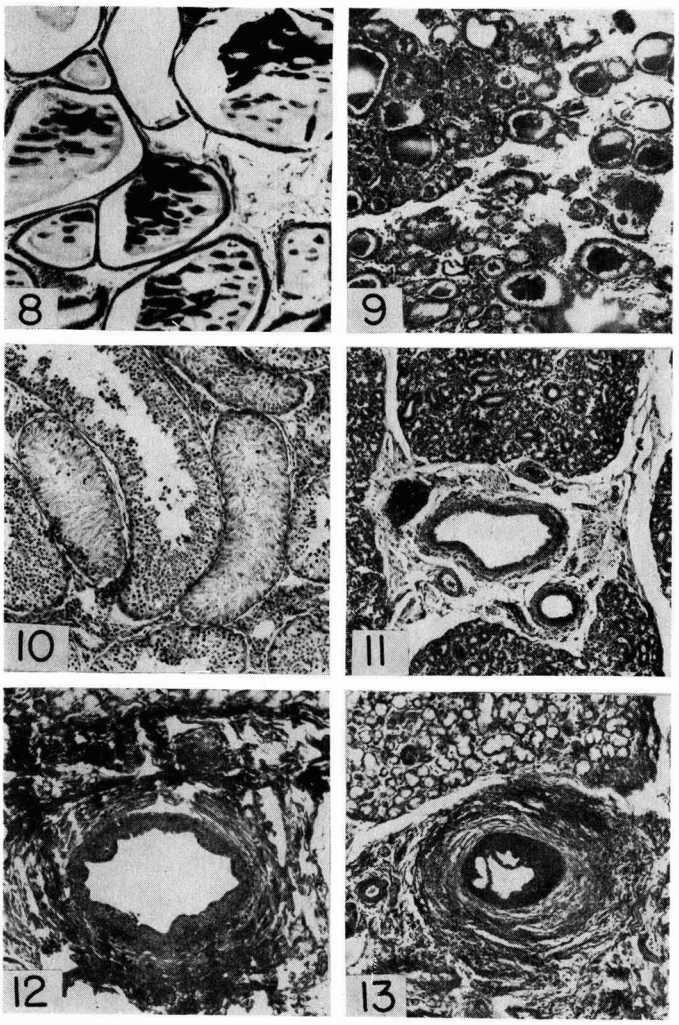
FIG. 4. Lung of no. 2. Exudative pneumonia—Polynuclear and mononuclear cells in alveoli, bronchiole (low center) and alveolar ducts (high center).

FIG. 5. Kidney of no. 6. Interstitial nephritis—An atrophied glomerulus in low center surrounded by interstitial round cell infiltration.

FIG. 6. Anterior pituitary of no. 8. Normal—Massive cords of acidophiles (dark) separated by narrow cords of basophiles and chromophobes (light).

FIG. 7. Anterior pituitary of no. 5. Vitamin A deficiency—Broad bands of chromophobes (light) and islands of acidophiles (dark). Many microcysts.

All figures are photomicrographs of paraffin sections stained with hematoxylin-triosin, 80 ×. The numbers refer to the experimental animals listed in table 1.



in vitamin A deficiency may be compensatory to increased stress upon this organ, which is known to have an important function in the conversion of carotene to vitamin A (4).

Specific microscopic changes were observed in the testes and the parotid gland. The testicular changes, which have been reported frequently in the literature (2, 5, 7, 11), consisted in the present material of various degrees of retardation in spermatogenesis. In the most advanced cases, the seminiferous epithelium in certain tubules was extremely cell-poor, with only a few Sertoli

cells near the basement membrane. In most instances spermatogenesis had not progressed beyond the spermatogonial stage. There were only isolated primary and secondary spermatocytes, but there was no evidence of any orderly progressive maturation. However, the abnormalities often were confined to certain selected tubuli with adjacent ones appearing almost normal (fig. 10).

The parotid gland, which, as far as the authors are aware, has not been mentioned in the literature, proved to be the only organ that regularly showed pathognomonic changes of vitamin A deficiency.

The parotid as the largest, chiefly serous salivary gland has a complex duct system which terminates in the oral cavity (Stenson's duct) opposite the second upper molar. The serous alveoli drain into prominent intralobular and intercalated ducts which are lined by a single layer of columnar cells with centrally located nuclei. Where the ducts reach the interlobular connective tissue septa, the epithelium changes to a pseudostratified columnar epithelium with the nuclei in two or more layers (fig. 11) and maintains this architecture to its termination (1).

In vitamin A deficiency the specific changes were confined to the interlobular ducts of both small and large diameter. There the normally columnar epithelium in some of these ducts had changed to squamous epithelium (fig. 12) accompanied occasionally by hyperkeratinization. The pathologic epithelium was markedly hyperplastic and built up in irregular layers. The germinal layers were relatively rich in mitotic figures with the cytoplasm of some hypertrophied prickle cells occasionally containing round bodies, suggestive of dyskeratotic degeneration. The innermost surface cells not infrequently formed loops or bridges over vacuolar spaces (fig. 13) presumably containing retained secretion. Cross sections of affected interlobular ducts showed the narrowing effect of the pathologic process on the ductal patency and obviously suggested a pathogenetic relationship between stenosis of the parotid duct and vitamin A deficiency. On the whole, the lesions reflected the squamous metaplasia considered to be the basic lesion of vitamin A deficiency in mammals and birds.

Apparently the parotid gland in the bovine is one of the organs of predilec-

FIG. 8. Thyroid gland of no. 4. Normal—Large, moderately filled follicles, lined by low cuboidal epithelium. Slightly hypoplastic state.

FIG. 9. Thyroid gland of no. 7. Vitamin A deficiency—Small follicles lined by high cuboidal to columnar epithelium, sometimes obliterating lumen. Colloid has scalloped margins and stains deeply. Hyperplastic state.

FIG. 10. Testis of no. 1. Vitamin A deficiency—Cessation of spermatogenesis in two lateral seminiferous tubules (uniformly gray), other tubules normal.

FIG. 11. Parotid gland of 6-week-old calf affected with pulmonary abscesses, caused by *Spherophorus necrophorus*. Normal—Peripheral alveolar tissue; central H-like interlobular connective tissue with normal interlobular ducts lined by two-layered pseudostratified columnar epithelium.

FIG. 12. Parotid gland of no 1. Vitamin A deficiency—Interlobular connective tissue with large interlobular duct lined by irregularly built-up metaplastic squamous epithelium. Stratum corneum is nucleated (parakeratotic).

FIG. 13. Parotid gland of no. 7. Vitamin A deficiency—Alveolar tissue in upper third. Large interlobular duct in center with thickened wall and advanced squamous metaplasia of lining epithelium showing interepithelial bridges and microcysts.

tion for exhibiting specific lesions of vitamin A deficiency, in distinction from chickens where the corresponding locus seems to be in the mucocutaneous junction of the nasal septum (12).

SUMMARY

Two groups of four dairy bull calves, averaging 189 days in age were fed a grain mixture containing less than 350 γ per lb. of carotene and beet pulp *ad libitum* for about 105 days. One control calf in each group received a daily supplement of 100,000 I.U. of vitamin A from dogfish oil containing 25 per cent crude soybean lecithin. In weekly determinations, the hemaglobin values showed no significant changes for treated and supplemented animals, plasma carotene regressed in both groups, while plasma vitamin A was markedly higher in the supplemented animals. The same relationship was reflected in the final liver storage of carotene and vitamin A. Monthly readings of spinal fluid pressure indicated a relative rise in the treated animals, while simultaneous liver biopsies failed to manifest changes in glycogen and fat storage.

Pathologic studies of the animals killed after convulsions occurred daily failed to show consistent gross lesions. Irregularly occurring microscopic lesions of focal necrosis and/or cirrhosis in the liver, pneumonia and mild interstitial nephritis suggested intercurrent diseases. Consistent changes were found in the anterior pituitary showing a decrease in the chromatic cells and in the thyroid showing mild hyperplasia. The testes manifested retarded spermatogenesis in some seminiferous tubuli. The parotid gland showed a high incidence of specific squamous metaplasia in the interlobular ducts.

The parotid gland appears to be especially prone to exhibit specific histopathologic alterations of A-hypovitaminosis and is the only organ so far ascertained that lends itself to specific morphologic diagnosis of vitamin A deficiency in the ox.

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SELECTION OF SAMPLE IN DETERMINATION OF THE STREPTOCOCCAL FLORA OF THE UDDER[1]

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The selection of samples of milk for study of the udder bacterial flora has been a subject of controversy (4). Several investigators have observed the composition of milk obtained at various stages of milking (5) but few have reported on the relative usefulness of such samples in the determination of the udder flora. Murphy (6) compared four successively drawn 10-ml. quantities with the strippings and reported that, in general, the same flora appeared throughout. Numbers of microorganisms were found, however, to decrease progressively with successive samples and the leucocyte count likewise decreased. Cunningham *et al.* (2) arrived at the conclusion that strict foremilk should be used in a determination of udder flora, as it contained essentially the same flora and in larger quantities than samples from a later stage of milking. Bull *et al.* (1), however, observed that the milk in the teat canal contained a wider variety of organisms than did milk from the udder. Little (3) in a study of an animal inoculated with a hemolytic streptococcus, found that mid-milk and strippings showed the presence of relatively few streptococci in comparison with the large numbers found in strict foremilk. Little and Plastridge (4) have suggested that in experimental work the strict foremilk be used, while in routine examinations 5 ml. may be discarded before sampling.

The rather scant and conflicting data on the selection of a sample for the determination of the udder flora led the authors to the conclusion that further study of the sampling procedure was necessary. Inasmuch as the authors were interested primarily in the streptococcal flora of the udder at the time this study was conducted, this report was limited to the choice of a sample for the determination of streptococci present.

EXPERIMENTAL

Forty-seven animals in the College herds were chosen for the purpose of this study. Various breeds, ages, stages of lactation and levels of production were represented. Five animals were suffering from chronic mastitis and several others had past histories of udder trouble. Many of the animals were termed normal on the basis of periodic laboratory examination of the milk and no history of clinical mastitis.

From these animals, 940 samples of milk were obtained for this study. Prior to sampling, the udder and flanks of each animal were washed with a fresh solution of hypochlorite containing approximately 200 ppm. available

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[1] Authorized for publication as Paper no. 1507 in the journal series of the Pennsylvania Agricultural Experiment Station.

chlorine. Special care was taken to insure that the ends of the teats were well cleansed. Samples then were drawn into sterile bottles containing 0.2 ml. of a sterile solution of the following composition: 0.2 g. brilliant green, 0.75 g. sodium azide, 10.0 g. glucose and 200.0 ml. distilled water. This solution had been filtered and then sterilized in the autoclave at 15 lb. pressure for 20 min. and had been placed aseptically in the previously sterilized bottles.

Five 15-ml. samples were obtained from each quarter and labeled A to E, inclusive. Sample A comprised the strict foremilk, B and C represented, respectively, the second and third 15-ml. portions of milk drawn from the quarter. The machine then was placed on the animal and, in the judgment of the operator, removed in order to obtain the fourth sample, D, at the time one-half the milk had been removed from the udder. Sample E was obtained following the final removal of the machine from the animal and represented strippings.

The samples were incubated for a period of 16 hr. at 37° C., following which microscopic examinations were made by means of a modification of the standard direct count. Using a loop delivering 0.01 ml. and a surface area of 2 cm.^{2}, smears were made which were air dried, defatted with xylol and stained

TABLE 1

Comparison of flora and leucocyte content of samples of milk obtained at various stages of milking

	Number of positive samples among the 188 of each group examined				
	A Foremilk	B 2d 15 ml.	C 3d 15 ml.	D Mid-milk	E Strippings
Long chain streptococci	17	16	17	11	8
Streptococcus agalactiae	7	7	6	5	5
Beta hemolytic colonies	27	30	32	23	24
Leucocyte content of more than 1,000,000/ml.	41	43	42	53	66

with the Newman-Lampert formula II. Actual counts of bacteria were not made but the following information was obtained: leucocyte count, relative numbers of long chain streptococci, medium chain streptococci, short chain streptococci, rods and staphylococcal clusters.

From all samples streaks were made on Edward's medium (4). These plates were incubated for a period of 48 hr. at 37° C. and examined for nature of growth, if present. Further examinations of isolates from these plates were made in those cases in which it proved necessary to determine the nature of the streptococci present.

RESULTS

Examination of the data obtained reveals certain pronounced differences in bacterial flora and leucocyte content in the several samples drawn from a quarter. The significant differences found are presented in table 1. Of particular significance is the progressive decrease in the number of samples showing long chain streptococci and the increase in samples containing more than 1,000,000 leucocytes per milliliter as successive samples are obtained in the milking. It

also would appear that the presence of *Streptococcus agalactiae* is more likely to be observed in foremilk than in later sampling.

In table 2 is presented an approximation of the relative numbers of long chain streptococci noted in the positive samples and of leucocytes in those samples containing more than 1,000,000 per milliliter. It will be noted that not only do the number of samples showing long chain streptococci decrease in successive sampling (table 1), but there is a similar decrease in the number of chains noted

TABLE 2

Relative numbers of long chain streptococci and leucocytes present in the positive samples shown in table 1

	Relative numbers in positive samples				
	A Foremilk	B 2d 15 ml.	C 3d 15 ml.	D Mid-milk	E Strippings
Chains of long chain streptococci	26	23	18	2	1
Leucocytes, as millions/ml.	2.8	2.9	3.4	4.1	6.4

in microscopic examination of the incubated milk. Likewise, the increase in the number of quarters showing excessive leucocytes (table 1) is accompanied by progressive increases in numbers per milliliter in the questionable samples.

In table 3 are presented detailed data obtained in the study of the strict foremilk (A) and the second 15 ml. (B) drawn. The data suggest little actual difference in the two series.

TABLE 3

Comparison of microflora and incidence of excessive leucocytes in strict foremilk and the following 15-ml. sample

Incidence of	Numbers of positive samples among the 188 of each group examined	
	A Foremilk	B 2d 15 ml.
Micrococci in smears	157	158
Pairs of cocci in smears	130	136
Short chain streptococci in smears	99	104
Medium chain streptococci in smears	24	19
Long chain streptococci in smears	17	16
Staphylococci in smears	10	12
Beta hemolysis on plates	27	30
Gamma hemolysis on plates	6	5
Streptococcus agalactiae on plates	7	7
Leucocytes in excess of 1,000,000/ml.	41	43

SUMMARY

The streptococcal flora and the leucocyte content of the quarters of 47 dairy cows were determined for five different stages of milking. These stages were as follows: first 15-ml. drawn, second 15-ml., third 15-ml., mid-milk and strippings. Little difference was noted between the strict foremilk and the second 15-ml. sample. It would seem that either would be useful in a determination of the streptococcal flora. On the other hand, not all quarters found shedding *Str.*

agalactiae by the use of these samples would have been detected had either midmilk or strippings been used as the basis of the tests. The presence of long chain streptococci other than *Str. agalactiae* likewise would have been missed. Excessive numbers of leucocytes appeared more often and in larger numbers in the later samples.

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THE METHYL KETONES OF BLUE CHEESE AND THEIR RELATION TO ITS FLAVOR[1]

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The interest of research workers has been focused upon the origin and nature of mold-ripened cheese flavor for a number of years. According to Currie (1), early investigators attributed the characteristic flavor of the cheese to esters or ketones. From his own extensive work, Currie concluded that the flavor of roquefort cheese is due to the presence of certain fatty acids or their readily hydrolyzable salts. Hammer and Bryant (2) believe that one or more methyl ketones, heptanone-2 in particular, are responsible for part of the characteristic flavor of blue cheese. These workers demonstrated the conversion of *n*-caprylic acid to heptanone-2 in a milk medium inoculated with *Penicillium roqueforti.* The relationship of certain chemical analyses to the flavor of blue cheese has been studied recently by Parmelee and Nelson (4).

The odor of blue cheese strongly suggests the presence of ketones. Evidence from the literature also supports this contention. However, insofar as is known, no ketones have been conclusively identified as constituents of a mold-ripened cheese. The present investigation was conducted to amplify this point.

EXPERIMENTAL

The blue cheeses used in these experiments were representative of the type produced at the Pennsylvania State College Creamery. They were 6 mo. old, of good saleable quality and averaged approximately 5 lb. in weight.

Preliminary experiments. Considerable preliminary experimentation was necessary in order to develop effective methods of recovering the ketones in good yield. In these initial experiments, one cheese constituted the starting material. Several similar trials were made during which it was possible not only to improve the steam distillation method of isolation but to collect a considerable amount of presumptive data relative to the specific ketones present in the distillate from blue cheese. This distillate was observed invariably to give positive results with certain tests for methyl ketones. These tests included the color reaction with nitroprusside reagent, the iodoform reaction and the reaction with semicarbazide or 2,4-dinitrophenylhydrazine reagents to form carbonyl derivatives.

The ketones were removed from the distillate by extracting several times with equal volumes of ethyl ether. The ether solution was dried with anhydrous sodium sulfate and the ketones concentrated by removing the solvent on a warm

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water bath. The solvent-free residue had a very potent aroma of blue cheese. The yield of crude mixed ketones obtained under these conditions was improved somewhat with each experiment but never exceeded 1.5 g. per 5 lb. of cheese. Attempts to separate the ketones by distillation were only partially successful because of the small yields. However, data on the fractions obtained indicated the presence of heptanone-2 and nonanone-2 rather conclusively. These data are omitted for the sake of brevity, since similar data from a large scale experiment are presented hereafter in detail.

The distillate still gave positive nitroprusside and iodoform reactions after extraction with ether. Semicarbazone (melting point, 190° C.) and 2,4-dinitrophenylhydrazone (melting point, 125–126° C.) derivatives were prepared from it. The melting points of these derivatives agreed well with values reported for the same known derivatives of acetone (3). In addition, these two derivatives showed no depression in melting point when admixed with corresponding authentic samples. Thus, the presence of acetone was demonstrated consistently in several trials.

Procedure for a large-scale experiment. The principal problem encountered in the preliminary trials concerned insufficient yield of mixed ketones which complicated fractionating of the mixture and obtaining reliable fraction boiling ranges and refractive indices. To overcome these difficulties, the amount of starting material was increased from one to three cheeses (16 lb., 3 oz.). A continuous ether extraction procedure, which also appeared to improve the yield, was substituted for extraction of the distillate in a separatory funnel.

The procedure employed for isolating and concentrating the ketones in this experiment was as follows: Three cheeses were mixed with 5 l. of cold water in a blending bowl until a homogeneous mass, free of lumps, was obtained. This mixture was transferred to an 18-l. pyrex jug. The jug and contents were fitted into a steam distillation apparatus of conventional design. The steam was conducted first through a trap, then through the cheese mixture. Effective condensation of the vapors was accomplished by means of two Allihn condensers connected in tandem. The condensate was conducted through a small quantity of ice water by extending a piece of glass tubing from the end of the condenser to the bottom of the receiving flask. The receiving flask was immersed in crushed ice as an additional precaution against loss of volatile material. The end point of the distillation was determined by the time necessary to exhaust the ketonic odor from the cheese mixture. This required distillation for slightly less than 1 hr. during which time 2 l. of condensate were obtained. The bulk of the odorous material appeared to have distilled within the first 15 to 20 min.

The distillate thus obtained was transferred to a continuous extraction apparatus[2] of 2-l. capacity and extracted for a period of 72 hr. with ethyl ether. This ether had been redistilled several times and rendered free of carbonyl compounds by treatment with 2,4-dinitrophenylhydrazine reagent. The extracted distillate was observed to contain acetone as previously noted. The ether extract was dried and the solvent evaporated as described heretofore. The extract residue (5.2 g.) was transferred to a 25-ml. Erlenmeyer flask from

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

which it was fractionally distilled. The fractionating column used in this distillation was a paper-jacketed piece of 10-mm. pyrex tubing containing a 10-in. section packed with $\frac{1}{16}$-in. glass helices and fitted with a sidearm delivering from above the column packing to a micro condenser. Boiling ranges were measured with a 360° C., partial immersion thermometer. Refractive indices were determined at 25° C. with a Spencer refractometer. Carbonyl derivatives were prepared from the various fractions according to customary procedures (5), but on a somewhat micro scale.

The data for this experiment are presented in table 1. All fractions reported in the table gave positive iodoform and nitroprusside reactions. They also formed derivatives with 2,4-dinitrophenylhydrazine or semicarbazide.

TABLE 1

Some properties of fractions obtained by the fractional distillation of material from blue cheese containing a high concentration of methyl ketones

Fraction no.	Physical properties			Melting points of derivatives		Identity of ketone component
	Major boiling range	Refractive index (n_D^{25})	Wt.	2,4-DNPH[a]	Semicarbazone	
	(° C.)		(g.)	(° C.)	(° C.)	
1	78–80	1.3721	0.850	140–141	105–106	Pentanone-2
2	100–111	1.3895	0.340	140–141	105–106	Pentanone-2
3	116–127	1.3992	0.045	[b]	[b]	.....
4	150–154	1.4075	0.680	72, 90[c]	121–122	Heptanone-2
5	155–175	1.4075	0.640	55–60	105–110	[d]
6	185–192	1.4160	0.940	38, 55[c]	118–119	Nonanone-2
7	200–255	1.4290	0.660	38	105–110	[e]
8	non-distilling	.....	0.610	.....	.....	.....

[a] 2,4-dinitrophenylhydrazone.
[b] Insufficient of the derivatives to permit purification.
[c] Preparations of these derivatives resulted in two forms which had different melting points.
[d] The components of this fraction were a mixture which could not be resolved.
[e] This fraction contained very little methyl ketone.

In order to confirm the identity of the ketones indicated for fractions 1, 2, 4 and 6 in table 1, mixed melting points were performed with the semicarbazones prepared from the respective fractions and the corresponding known derivatives. None of these mixtures showed any depression in melting point. In order to test the validity of this procedure as a confirmatory test, equal quantities of octanone-2 (melting point, 122° C.) and heptanone-2 (melting point, 123° C.) semicarbazones were intimately mixed and the melting point determined. This mixture gave a melting point of 107° C. or a depression of approximately 15° C. Thus, it would appear that the mixed-melting point procedure was a suitable confirmatory test.

Control experiment. It seemed advisable to determine whether compounds of the type isolated in this investigation were present in blue cheese which had not been subject to steam distillation. Blue cheese (0.5 lb.) was macerated with a small volume of ethyl ether and the mixture allowed to stand for several hours,

after which time the ether extract was decanted. This extract was concentrated by evaporating the solvent on a warm water bath. The residue had a strong aroma of blue cheese and gave positive reactions with nitroprusside, iodoform and 2,4-dinitrophenylhydrazine reagents. The results of these tests would indicate that methyl ketones are present in blue cheese prior to steam distillation of the cheese.

DISCUSSION

The data of table 1 for fractions 2, 4 and 6 adequately demonstrate the identity of pentanone-2, heptanone-2 and nonanone-2 in the steam distillable material from blue cheese. The data concerning boiling ranges, refractive indices and derivatives are in good agreement with those reported in the literature (3) for the indicated ketones. Fraction 1 contained in addition to pentanone-2, ethyl alcohol which was carried over in the ether used for extraction. Results from the control experiment denote that the methyl ketones identified are present in the cheese prior to steam distillation. The high concentration of methyl ketones observed in the first portion of steam distillate from blue cheese also suggests that heat decomposition of the cheese during distillation is a minor consideration. However, it is conceivable that beta-keto acids, possible intermediates in the formation of methyl ketones from fatty acids, are converted to methyl ketones during steam distillation of the cheese. The extent to which these acids contribute to the total acetone bodies of blue cheese will bear further investigation. Beta-oxidation of fatty acids by molds has been studied and elucidated by Stokoe (6) among others, and interpreted in terms of blue cheese flavor, as related to methyl ketones, by Hammer and Bryant (2). The stages in the beta-oxidation of fatty acids to methyl ketones which have been proposed are first to the beta-hydroxy acid and then to the beta-keto acid which is decarboxylated to yield a methyl ketone and carbon dioxide. The isolation in these experiments of methyl ketones with only odd numbers of carbons is in keeping with characteristics of the beta-oxidation mechanism. Thus, the precursors of acetone, pentanone-2, heptanone-2 and nonanone-2 may be butyric, caproic, caprylic and capric acids of butterfat, respectively.

The fact that no appreciable quantity of any ketone boiling above 200° C. could be recovered in this study would seem to warrant some discussion. Although fraction 7 (boiling range, 200–255° C.) contained small quantities of ketone, it was composed mainly of other materials. These materials might well have been fatty acids, since no measures were taken to remove such compounds. Presence of the high boiling material appears to have been advantageous, since it served as a "pusher" for the methyl ketones during fractional distillation. A further consideration is that only 2 l. of steam distillate were taken from 16 lb., 3 oz. of blue cheese. It is possible that steam distillation to this limited extent was not sufficient to permit recovery of the higher boiling ketones. It also is quite possible that little or no additional ketones were present in the cheese. According to Stokoe (6), no acids above lauric in molecular weight are absorbed by molds; consequently, no ketones above undecanone-2 (boiling point, 223 to 226° C.) are formed. Since these investigations of blue cheese are being

continued, the above matters will receive adequate study in the future. For the present, recovery of the ketones without prolonged and rigorous steam distillation seemed justified.

SUMMARY

By means of a steam distillation and ether extraction procedure, it has been possible to recover material from blue cheese containing a high concentration of methyl ketones. By fractional distillation of this material relatively pure fractions of pentanone-2, heptanone-2 and nonanone-2 were obtained. Acetone was identified as a constituent of the ether-extracted steam distillate from blue cheese. These methyl ketones would appear to be formed from the fatty acids in the cheese by beta-oxidation.

Similarity in odor between these ketones, particularly heptanone-2 and that of blue cheese was noted by a number of observers during the course of this investigation. It seems probable that minute quantities of these methyl ketones are the constituents which make the flavor of mold-ripened cheeses distinctly different from the flavor of other types of cheese.

ACKNOWLEDGMENTS

The author is indebted to J. H. Jones of the Petroleum Refining Laboratory, The Pennsylvania State College, for samples of pentanone-2 and its 2,4-dinitrophenylhydrazone; to E. F. Price, graduate student of this department, for his counsel and assistance in the preparation of carbonyl derivatives.

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ABSTRACTS OF LITERATURE

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ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

625. Pathogenesis of bovine mastitis. II. The significance of hypersensitivity in streptococcic infection. G. R. SPENCER and D. M. ANGEVINE, Wis. Agr. Expt. Sta., Madison. Am. J. Vet. Research, **11,** 40: 317–323. July, 1950.

Normal cows were distinctly less sensitive to intradermal injections of antigens prepared from a culture of *Str. agalactiae* than were cows infected with *Str. agalactiae*. Cows with clinical mastitis had greater reactions than infected cows without clinical manifestations. Some cows with streptococcic mastitis failed to react, and irregularities in the reaction make the method of little value in diagnosis. Two formerly infected hypersensitive cows given intramammary injections of antigens developed rapid inflammatory reactions, while injections of distilled water caused no appreciable swelling. Intramammary injections of streptococcic polysaccharide also caused severe inflammatory response. Intramammary injections of polysaccharide in a normal cow produced a mild reaction similar to that following distilled water. Similar results with intramammary antigens were observed in normal and hypersensitized rabbits. These studies indicate that hypersensitivity may be an important factor in clinical bovine mastitis.

E. W. Swanson

626. A practitioner treats mastitis. R. CURTIS, Portage, Wis. Vet. Med., **45,** 7: 283–285. July, 1950.

This is a brief review of mastitis based on the experiences of a veterinary practitioner. The veterinarian is primarily interested in proper diagnosis and treatment of this disease. A differential diagnosis is essential. Various methods of treatment are discussed. Several herds of dairy cows are on an annual check basis. The cows are tested with the Hotis test and the results suggest proper control and sanitary measures. Good herd management is one of the most valuable factors in controlling mastitis. B. B. Morgan

627. The treatment of bovine pyelonephritis. E. V. MORSE, Univ. of Wis., Madison. Vet. Med., **45,** 5: 221–224. June, 1950.

An excellent review on the treatment of pyelonephritis in cattle is given. Several treatments are descibed. Until the advent of penicillin, most treatments were of doubtful value. Successful therapy depends upon early diagnosis and prompt, proper treatment. Symptomatic treatments alone, including the use of dextrose, saline and blood transfusions are ineffective. The sulfonamides have not shown much promise in the treatment of this condition when employed as the only therapy. Penicillin has given the most encouraging results. Doses of 2–3 million units have been used. Most practitioners use 10 million units of penicillin/cow. Therapy should cover an interval of about 10 d. Cows which recover clinically should be examined every 6 mo. for 18 mo. in order to determine if the animal has permanently recovered.

B. B. Morgan

628. Sulfamethazine and blood transfusion in experimental treatment of bovine brucellosis. R. E. WATTS, L. E. BOLEY and W. A. GREIG, Ill. Agr. Expt. Sta., Urbana. Am. J. Vet. Research, **11,** 40: 304–307. July, 1950.

Repeated courses of treatment with 1.5 gr./lb. of sulfamethazine intravenously followed by 0.75 gr./lb. *per os* for 4–7 d. accompanied by a transfusion of 1 l. of citrated whole blood or 300 ml. of normal cow serum were given to brucellosis-infected cows. Three infected cows were used as controls. Changes in blood titers were insignificant and brucella were still shed in the milk of 3 of the treated cows following the experiment. One treated cow and 1 control became negative to the blood test before the end of the experiment. Death of 2 of the treated cows during

the experiment was attributed to the treatment. Reaction to the blood transfusion frequently was marked. E. W. Swanson

629. The treatment of retained fetal membranes and their sequelae in the bovine. W. L. Boyd, Univ. of Minn., St. Paul. Vet. Med., **45,** 7: 263–266. July, 1950.

A brief review is presented on the various conditions of cattle in which retained fetal membranes may be involved. These included brucellosis, vibriosis and trichomoniasis. In other instances no microorganisms can be incriminated. Cows which give birth to twins frequently fail to expel the placenta. A review of the anatomy and physiology of the uterus also is presented. Symptoms, lesions and treatment of retained fetal membranes are discussed. Important sequelae of placentitis included metroperitonitis, pyometritis and abscess formation with pelvic adhesions.

B. B. Morgan

630. The clinical use of tyrothricin-B.F.I. uterine tablets in cows. J. L. McAuliff, W. V. Phillips and J. R. Steele, Cortland, N. Y. Vet. Med., **45,** 6: 241, 245. June, 1950.

Tyrothricin-B.F.I. uterine tablets were used in 210 cows to prevent infections and promote healing of the uterine wall. Two to 4 tablets were inserted at each treatment. The tablet consisted of tyrothricin (0.05 g.), bismuth-formic-iodide (0.5 g.), bismuth subgallate (2.0 g.), boric acid (2.15 g.), and urea (1 g.). The cows treated were divided into 4 groups: (a) 110 retained placentas removed manually after calving, (b) 20 cows with partially removed placentas, (c) 50 cows with retained placentas after abortion and (d) 30 cows which developed metritis about 1 wk.–10 d. after calving. The results indicated that the tablets were a safe and effective material for treating retained placentas. B. B. Morgan

631. A quantitative study of Trichomonas foetus in preputial samples from infected bulls. D. V. Hammond, V. R. Bishop, G. Jeffs and W. Binns, Utah Agr. Expt. Sta., Logan. Am. J. Vet. Research **11,** 40: 308–314. July, 1950.

Six bulls known to be infected with **T. foetus** were sampled at frequent intervals (1–2 d.) over periods as long as 6 mo. Samples of fluid were secured from the glans penis and surrounding preputial membrane by means of a glass pipette and rubber bulb. The number of *T. foetus* organisms per ml. of fluid was determined undiluted in a hemacytometer. The average collection of fluid was 0.52 ml. Of 241 examinations, 217 (90%) were positive and 3 of the bulls were positive at every examination. One bull sampled on alternate days with pipette and swab exhibited only 6% of the organisms from the swab as found by the pipette. Wide variations in concentration of organisms were observed with each bull. The highest average was 44,000/ml. and the lowest bull averaged 80/ml. The highest single count was 488,000/ml. E. W. Swanson

632. Allergic response to johnin and tuberculin of various skin regions of cattle. A. B. Larsen, A. H. Groth and H. W. Johnson, Reg. Animal Disease Research Lab., Auburn, Ala. Am. J. Vet. Research, **11,** 40: 301–303. July, 1950.

Five steers made hypersensitive to johnin and 1 hypersensitive to tuberculin were used in an experiment designed for statistical analysis to measure the reaction on various parts of the body to intradermal injections of johnin or tuberculin. The size of reaction was measured with a dermal thickness gauge. The regions in order of decreasing sensitivity were neck, back, side and caudal fold. The mean size of reaction at the neck was more than twice that at the caudal fold. Results were similar with johnin and tuberculin.

E. W. Swanson

Also see abs. no. 639.

CHEESE

A. C. DAHLBERG, SECTION EDITOR

633. The design and operation of the cheese trommel—its use in cheddar cheesemaking. J. M. Sharkey, Kraft Walker Cheese Co., Melbourne, Australia. Australian J. Dairy Technol., **4,** 1: 3–6. Jan.-Mar., 1949.

The construction details and the operation of a cheese trommel are described. The unit consists of a stainless steel perforated drum 15 ft. long and 5 ft. in diameter, with ends tapering to openings 18 in. in diameter. At about 0.2% acidity the curd with a portion of the whey is pumped into the trommel, where firming of the curd is completed. The whey drains into a specially constructed trough which conducts it to a sump vat from which the whey is pumped to separators. The trommel is mounted on rails and thus may be moved from vat to vat. The unit is claimed to be labor saving and to allow increased manufacturing output. J. C. Olson

634. Cheese press. N. J. Peters (assignor to Damrow Bros. Co.). U. S. Patent, 2,514,007. 1 claim. July 4, 1950. Official Gaz. U. S. Pat. Office, **636,** 1: 278. 1950.

To provide uniform pressure on cheese in hoops in the conventional horizontal type cheese press, a heavy coil spring is inserted between the end plate and the first hoop. R. Whitaker

635. Making a quality cottage cheese. N. C. ANGEVINE, Meyer-Blanke Co., St. Louis, Mo. Milk Dealer, **39,** 9: 62, 83–89. June, 1950.

See abs. no. 557.

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

636. Production of casein yarn. R. F. PETERSON (assignor to U. S. A.). U. S. Patent 2,512,674. 2 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635,** 4: 1104. 1950.

An alkaline solution of casein is extruded into a heated hardening bath of a metal salt and formaldehyde, followed by a final stabilization of the fibres in a concentrated buffer solution at a pH of 6–8. R. Whitaker

637. Water paste paints. B. O. NEWMAN (assignor to National Gypsum Co.). U. S. Patent 2,511,782. claim. June 3, 1950. Official Gaz. U. S. Pat. Office, **635,** 2: 640. 1950.

A water base paint is described, consisting of peptized casein, pigments, fillers, water and 1 of the following acids: gluconic, arabonic, mannonic, gulonic, galactonic and talonic. R. Whitaker

638. Animal food manufacture. R. R. HAUGH (assignor to Kraft Foods Co.). U. S. Patent 2,508,112. 4 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, **634,** 3: 920. 1950.

An animal feed in the form of small pellets, made by extruding a moist plastic mass of lactose and protein feed materials is described.
R. Whitaker

Also see abs. no. 654, 655, 674.

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

639. The bactericidal effect of various disinfectants on Str. agalactiae on the skin and in the environment of the cow. A. CHODKOWSKI, Vet. Lab., New Haw, Weybridge, Surrey, England. Brit. Vet. J., **106,** 5: 181–196. May, 1950.

The effect of different disinfectants at various concentrations on *Str. agalactiae* was tested. *Str. agalactiae* survived for as long as 3 wk. on various objects in the barn and up to 26 d. on the skin of cattle. The organism can multiply and persist in sores on the teats of non-infected udders, thus providing a constant source of infection. Fourteen different substances were tested *in vivo* and *in vitro*. A drug mentioned only as CTAB was found to be satisfactory. CTAB in an aqueous solution (0.5–1%) or in cream and iodine solution was a good disinfectant for the skin, while CTAB in aqueous solution (2%) and formaldehyde were best for barns. CTAB in an aqueous solution was the most satisfactory for dairy utensils while CTAB and penicillin creams were the most efficient for the treatment of teat sores. A 0.1–0.2% CTAB aqueous solution was suggested for the routine washing of the udder before milking. B. B. Morgan

640. On the contamination of the milk supply of the city of Pretoria with tubercle bacilli. M. W. HENNING and W. G. VAN ASWEGEN, Inst. of Onderstepoort, Pretoria, So. Africa. J. So. African Vet. Med. Assoc., **21,** 1: 27–29. Mar., 1950.

The authors outlined a method which may be used for detection of tubercle bacilli in cows' milk. Approximately 100 ml. of each composite sample were centrifuged at 3,000 r.p.m. for 30 min. One ml. of the gravity cream from each sample was injected into separate pairs of guinea pigs. The same procedure was followed with the sediment from each sample, except the sediment was emulsified in 1 ml. of normal saline before injection. The guinea pigs were killed from 6–8 wk. following the injections and microscopic observations for tubercle bacilli were made. Of the herds producing milk for the Pretoria market, 4% produced milk contaminated with tubercle bacilli. K. M. Dunn

Also see abs. no. 625.

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

641. Method of replacing cations in milk. R. J. MYERS (assignor to Rohm and Haas Co.). U. S. Patent 2,511,825. 3 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635,** 2: 651. 1950.

The Na of milk is exchanged for the NH_4 radical by contacting it with ammonium sulphonated phenol formaldehyde cation exchange resin. The NH_4 ions then are displaced by the addition of Ca and K hydroxides to the milk.
R. Whitaker

642. Détermination de l'eau incorporée au beurre par une méthode de controle rapide. (Determination of water incorporated in butter by a quick method.) E. POZZI-ESCOT. Lait, **20,** 295–296: 225–228. May–June, 1950.

The method consists of weighing a 10–20-g. sample of butter into a graduated centrifuge tube, adding sufficient fat solvent (kerosene or gasoline are recommended) to dissolve the butterfat and

then centrifuging to obtain the aqueous layer. Readings are corrected for casein and soluble salt content and then converted to per cent moisture. The procedure is convenient and rapid. A Babcock centrifuge may be used but it is necessary to provide special cups to hold the centrifuge tubes. For production control work and most other purposes the results appear to be sufficiently accurate. S. Patton

643. Modifications apportées a la methode de diagnostic du lait de vache dans le lait de femme. (Modifications applied to the method of detecting cow's milk in mother's milk.) P. ROMEYER. Lait, **30,** 295–296: 249–252. May–June, 1950.

Certain imperfections in the original method (Lait, 29: 576. 1949.), which is based on measurement of difference in phosphorus content of mother's milk and cow's milk, are remedied.
S. Patton

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

644. Power requirements in the churning of cream to butter by the normal buttermaking process in New Zealand. F. H. McDOWELL, W. R. CRAIG, M. E. MARTIN and B. W. HARVEY, The Dairy Research Inst., New Zealand. Australian J. Dairy Technol., **3,** 4: 137–141. Oct.-Dec., 1948.

Power requirements expressed as K.W.H./ton of butter were obtained from 3 New Zealand butter plants. The requirements included power used in operating the churns during washing of butter. Average requirements in the 3 factories were sufficiently uniform to indicate that 15 units (K.W.H.)/ton of butter may be used as a working basis for calculating power costs for churning of butter in N. Z. Variations of from 10–20 K.W.H./ton of butter were observed. Most of the data were obtained from observations on 100-box capacity churns. The rate of power utilization at various stages of the churning process was studied in 1 plant. Two to 4 units/min. were required during the first 15 min., increasing to 10 at time of maximum viscosity before the breaking stage, followed by a decrease during granule formation to the time of draining. During working, the power requirements remained constant at 4.04 units/min. J. C. Olson

645. Diesel vs. purchased power. R. UMBACH, R. Umbach & Assoc., Selem, O. Milk Dealer, **39,** 9: 80–81. June, 1950.

When good diesel fuel oil could be purchased for 6¢ or less/gal., diesel power could produce a K.W.H. for less than 0.5¢ in the larger installations and for less than 1¢ in the smaller installations. The increased cost of electric power from the utilities is not as phenomenal as the increase of the price of fuel anl lubricating oil and, therefore, comes close to competing with diesel power at present prices, especially in small installations from 50–500 h.p. Wasted heat recovery with the diesel and development of the gas diesel should not be overlooked. Sheer thermal efficiency is not always a sound yardstick for measuring economy of operation and the adoption of either diesel or purchased power should be closely investigated. C. J. Babcock

646. Firetube boilers. J. R. McDONNELL. Operating Eng., **3,** 6: 24–25. June, 1950.

A brief procedure for preparing a firetube boiler for boiler inspection is given. On the fireside, remove soot from the tubes, remove soot from the furnace, and inspect grates and setting. On the waterside, drain water from boiler, remove handhole manhole plates, remove oil and wash. Boiler accessories should be checked. H. L. Mitten, Jr.

647. How to handle ammonia safely. L. BRANDT, Pa. Salt Mfg. Co. Power, **94,** 7: 85–87. July, 1950.

Safe handling of anhydrous NH_3 depends on how thoroughly the handler understands the three potentially hazardous properties of the refrigerant: (a) toxicity, (b) flammability of oil-ammonia mixtures and (c) rapid expansion of liquid.

NH_3 odor is so irritating that no one purposely inhales dangerously high concentrations. NH_3 is not cumulatively toxic. It gives warning of its presence by its irritating properties. Gas masks should be stored outside NH_3 equipment rooms and be used when it is necessary to work in areas of high concentration.

Although limits of flammability are from 16–27% by volume in air, the range is so narrow that a flame cannot be made self-sustaining. Investigations of NH_3 fires usually reveal that they were caused by leakage of oil-NH_3 mixtures rather than by NH_3 alone. The high side NH_3 contains oil from the compressor. When the high side of an NH_3 system must be emptied, it is preferable to store the charge in another part of the system rather than to replace it in the cylinders. In case the charge is placed in cylinders, the cylinders should be carefully weighed to prevent overfilling and danger from bursting on heating.

Because NH_3 attacks nonferrous alloys, the universal construction material is steel and the piping is extra-heavy steel. Valves usually are of the backseating type which can be packed under pressure. H. L. Mitten, Jr.

648. The indicator-card story. T. MITCHELL, Frick Co., Waynesboro, Pa. Operating Eng., **3**: 36–37. June, 1950.

A method for attaching a card indicator system to large NH_3 and Freon compressors is suggested. The cards tell the refrigerating work being done by each cylinder. They also indicate defective operation. A number of cards are presented to show typical curves for various types of malfunction. H. L. Mitten, Jr.

649. Process and apparatus for cooling milk and other liquids. G. GRINDROD. U. S. Patent 2,511,582. 19 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635,** 2: 588. 1950.

An internal tube cooler is described in which the milk is pumped at high velocity to permit rapid cooling without freeze-on, thus permitting the use of a low temperature refrigerant. The amount of refrigerant used is adjusted automatically to compensate for variations in pressure and velocity of the milk. R. Whitaker

650. Ice cream freezer. L. N. YOHE. U. S. Patent 2,511,313. 6 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635,** 2: 519. 1950.

This invention is concerned with a means for removing separately, the front end and the cylinder or barrel of a horizontal freezer. Suitable adjustments are provided to prevent leakage at the joints and to compensate for wear.
R. Whitaker

651. Apparatus for freezing desserts. L. N. YOHE. U. S. Patent 2,511,314. 8 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635,** 2: 519. 1950.

The cylinder of a horizontal freezer is extended to form an elongated tubular portion beyond the dasher; it contains a device for varying the size of the outlet from which the frozen ice cream is withdrawn. R. Whitaker

652. Sonic method for control of air in ice cream. R. FRIEDMAN (assignor to Westinghouse Electric Corp.). U. S. Patent 2,508,152. 7 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, **634,** 3: 931. 1950.

The overrun of ice cream is controlled automatically by varying the amount of air admitted to a continuous freezer. The overrun is measured from changes in sound velocity resulting from passing sound waves through a layer of given thickness of the ice cream as it leaves the freezer.
R. Whitaker

653. Apparatus and method for deaeration of liquids. W. McK. MARTIN (assignor to Schwarz Eng. Co.). U. S. Patent 2,507,797. 10 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, **634,** 3: 839. 1950.

Milk and other liquid food products may be continuously deaerated by this device, which consists of a rotor operating in a vacuum chamber. The product is introduced into the center of the rotor, whence it travels outwards past baffles that break it up into small droplets and then collect the deaerated droplets and discharge the liquid from the chamber. R. Whitaker

654. Apparatus and method for the evaporation of liquids. G. G. ZAHM (assignor to Hurd Corp.). U. S. Patent 2,512,513. 6 claims. June 20, 1950. Official Gaz. U. S. Pat. Office, **635,** 3: 949. 1950.

A vacuum concentrator for milk and other liquid food products has a method for collecting the condensable volatile flavor forming materials from the vapor proportionally returning them to the concentrate after pasteurization.
R. Whitaker

655. Evaporator and separator. R. O. HENSZEY. U. S. Patent 2,512,938. 11 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635,** 4: 1172. 1950.

An entrainment separator for vacuum pans is described. R. Whitaker

656. Emulsifying apparatus. H. S. BROCHNER (assignor to International Morfat Co.). U. S. Patent 2,509,288. 5 claims. May 30, 1950. Official Gaz. U. S. Pat. Office, **634,** 5: 1438. 1950.

Stable oil-in-water emulsions or creams may be made in this equipment, which consists of a chamber in which are located 2 perforated cone-shaped nozzles pointing toward each other and separated by a distance about 1/3 of the diameter of the chamber. The continuous phase liquid is introduced under pressure through the lower nozzle, the dispersed phase liquid through the upper nozzle. The emulsion leaves the chamber through an outlet in the top. R. Whitaker

657. Apparatus for heat-treating and stabilizing liquid food products. C. O. BALL (assignor to Owens Illinois Glass Co.). U. S. Patent 2,508,212. 11 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, **634,** 3: 948. 1950.

Milk and other liquid food products may be heated continuously in this equipment, consisting of a 3-compartment cylindrical chamber containing steam under pressure. The liquid to be heated passes through a spiral channel located in the wall of the cylinder. R. Whitaker

658. Art of pasteurizing milk, etc. G. H. BROWN (assignor to Radio Corp. of America.). U. S. Patent 2,510,796. 4 claims. June 6, 1950. Official Gaz. U. S. Pat. Office, **635,** 1: 280. 1950.

Milk is pasteurized by the high-temp., short-time method by passing through a high frequency electric field. The hot milk is rapidly cooled by spraying into a vacuum chamber. The hot vapors are employed to heat the incoming cold raw milk.
R. Whitaker

659. Pumping system for milk processors. R. H. STEINBERG and H. COHEN. U. S. Patent 2,512,045. 1 claim. June 20, 1950. Official Gaz. U. S. Pat. Office, **635,** 3: 829. 1950.

In a milk pasteurizing unit containing a milk to milk regeneration step, an auxiliary milk pump is provided ahead of the raw milk inlet to maintain milk pressures throughout the system within a carefully selected range. The system is electrically controlled and operated.
R. Whitaker

Also see abs. no. 633, 634, 672.

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

660. Efficient mechanical milking. W. G. WHITTLESTON, Dept. of Agr., Hamilton, New Zealand. Australian J. Dairy Technol., **3,** 2: 45–72. Apr.–June, 1948.

This is a review article to acquaint practical workers in the dairy industry with the theory and practice of machine milking. There are 3 parts: Part I, "The cow," in which milk let-down, reflexes, stimulation, stripping, milking machines and mastitis, and speed of milking are discussed; Part II, "The milking machine," presenting pumps, pulsators, relief valves, teat cups and claws, and rubber ware; and Part III, covering the various aspects of installation and servicing.
J. C. Olson

661. Claw for milking machines. G. H. GASCOIGNE (assignor to Gascoignes, Ltd.). U. S. Patent, 2,507,969. 3 claims. May 16, 1950. Official Gaz. U. S. Pat. Office, **634,** 3: 883. 1950.

This manifold for milking machines has 4 openings for teat cups, so placed as to permit easy attachment to the udder. A finger-operated valve cuts off the vacuum and milk outlet and vents the manifold for removal from the udder.
R. Whitaker

662. Teat cup holder for milking machines. C. B. FINN. U. S. Patent 2,512,926. 3 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635,** 4: 1169. 1950.

A holder for positioning teat cups under the cow's udder is described. R. Whitaker

663. Flow indicator for milking machines. L. DINESEN (assignor to Perfection Mfg. Co.). U. S. Patent 2,513,627. 2 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636,** 1: 179. 1950.

An attachment for a milking machine, inserted in the lines leading from the teat cups, indicates when milk is flowing through the lines to the milk-collecting reservoir. R. Whitaker

664. Milking machine. E. T. JANSSON (assignor to Akiebolaget Separator Corp.). U. S. Patent 2,510,581. 4 claims. June 6, 1950. Official Gaz. U. S. Pat. Office, **635,** 1: 225. 1950.

A vacuum-operated pulsating type of milker is constructed as part of the lid of a milk pail.
R. Whitaker

665. Means for milking and handling the milk of farm animals. G. R. DUNCAN. U. S. Patent 2,512,094. 13 claims. June 20, 1950. Official Gaz. U. S. Pat. Office, **635,** 3: 841. 1950.

A milking parlor arrangement, including a system of entrance and exit for the animals, a 2-cow milking platform with the animals standing tail to tail and provisions for cooling and storing the milk is described. R. Whitaker

666. Cow tail holder. A. J. KLINE. U. S. Patent 2,513,494. 2 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636,** 1: 144. 1950.

A cow is prevented from swishing her tail by this device which grips the tail and one rear leg. R. Whitaker

667. Self-cleaning drinking bowl for animals. W. H. SHELDON (assignor to Michigan State Board of Agr.). U. S. Patent 2,513,753. 5 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636,** 1: 213. 1950.

A siphon arrangement flushes out this animal drinking bowl as soon as the animal's nose ceases to press a lever in the bowl. R. Whitaker

668. Stock feeding appliance. J. A. POWELL. U. S. Patent 2,512,260. 2 claims. June 20, 1950. Official Gaz. U. S. Pat. Office, **635,** 3: 884. 1950.

A feeding trough for cattle consists of a cylindrical reservoir holding dry feed and a feeding trough protected by an overhang to prevent water from collecting in the trough. A mechanism in the trough, actuated by the animal's nose, agitates the feed in the reservoir to keep it uniform and

conveys it from the reservoir into the trough as it is consumed. R. Whitaker

669. Comparative evaluation of rotenone formulations for cattle grub control. J. R. Douglas and D. P. Furman, Univ. of Cal. at Davis and Berkeley. J. Econ. Entomol., **42,** 6: 884–887. Dec., 1949.

Nine spray formulations were applied by power sprayer at 250–400 lb. pressure, and compared in efficiency of cattle grub (*Hypoderma lineatum* and *H. bovis*) control. About 2 qt. of spray/animal were used, and control data were determined 7 d. after treatment.

A wetting agent increased the effectiveness of ground derris formulations but sulfur did not. Five lb. of derris (5% rotenone content) to 100 gal. of water was about half as effective as a 10-lb. rate. Piperonyl butoxide and N-octyl bycycloheptene dicarboximide did not increase rotenone efficiency. *H. bovis* was more resistant to insecticides than *H. lineatum*. E. H. Fisher

670. La brebis productrice de lait et facteur économique. (The sheep as a producer of milk and an economic factor.) V. De Sa. Lait, **20,** 295–296: 245–248. May–June, 1950.

It is contended that sheep could be better utilized as producers of milk than is presently the case. The point is made that wool production is not antagonistic to milk production in the sheep and that high production of wool and milk usually go hand in hand. Recommendations are made concerning methods for making better use of the sheep as a milk producing animal.

S. Patton

Also see abs. no. 686, 687, 688.

ICE CREAM

C. D. DAHLE, SECTION EDITOR

671. Edible food product. A. Rubin. U. S. Patent 2,511,082. 2 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635,** 2: 459. 1950.

An ice cream novelty consisting of a split doughnut and ice cream is described. One half the doughnut is placed flat side down on the bottom of a round package of approximately the same diameter. Ice cream then is filled into the package and the remaining half doughnut, also flat side down, is placed on top. The ice cream fills the holes in the doughnut halves.

R. Whitaker

672. Measuring dispenser for filling ice cream containers and the like. K. P. Herbold (assignor to Eskimo Pie Corp.). U. S. Patent 2,510,576. 7 claims. June 6, 1950. Official Gaz. U. S. Pat. Office, **635,** 1: 223. 1950.

A device is described for delivering a measured quantity of ice cream into a container, using a reciprocating piston, the volume of which may be easily changed manually to compensate for changes in overrun. R. Whitaker

673. Coin-freed ice cream vending machine. W. H. Partridge. U. S. Patent 2,511,076. 5 claims. June 13, 1950. Official Gaz. U. S. Pat. Office, **635,** 2: 458. 1950.

A vending machine for wrapped pieces of ice cream, cooled by air refrigerated by a small motor driven compressor, is described. R. Whitaker

Also see abs. no. 650, 651, 652.

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

674. What to do with surplus milk. D. Armerding, Mojonnier Bros. Co., Chicago, Ill. Milk Dealer, **39,** 9: 68–69. June, 1950.

The dairy industry should take care of its own surpluses by enriching its own products instead of expecting the baking industry and others to use its surplus milk powder. Increasing the serum solids content of the fluid milk consumed annually from 8.5–10% automatically would remove 750 million lb. of nonfat dry milk solids from the market. This enrichment would increase the nutritional ingredients by 12.5% with only a 5% increase in selling price. Increasing the solids-not-fat in evaporated milk from 18–19% would absorb another 27–30 million lb. of nonfat dry milk solids and the serum solids of other products could be increased. C. J. Babcock

675. La méthode de controle et de conservation du lait maternal au lactarium. (Quality control and preservation of mother's milk at the "lactorium.") A. Roissier and J. Bertrand. Lait, **20,** 295–296: 252–256. May-June, 1950.

Methods of determining acidity, bacteria count, density, fat, solids-non-fat, watering and adulteration of mother's milk are given. Procedures for bottling, sterilizing and refrigerating the milk also are presented. S. Patton

676. Bottle crate. H. Kershaw. U. S. Patent 2,512,096. 2 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635,** 4: 1212. 1950.

A milk bottle crate, having metal bound wooden sides and metal rod partitions, is described.

R. Whitaker

677. Milk bottle carrying case. E. R. ERICKSON (assignor to C. E. Erickson Co.). U. S. Patent 2,512,855. 6 claims. June 27, 1950. Official Gaz. U. S. Pat. Office, **635,** 4: 1151. 1950.

An all metal milk bottle crate is described.
R. Whitaker

678. Pasteurizer. E. K. MALME (assignor to Guard-It Mfg. Co.). U. S. Patent 2,513,577. 6 claims. July 4, 1950. Official Gaz. U. S. Pat. Office, **636,** 1: 165. 1950.

A few gallons of milk in a can may be pasteurized automatically by placing the can in this electrically heated and agitated farm or home pasteurizer. R. Whitaker

679. Rapport over de Zuivelindustrie in de U.S.A. (Report on the dairy industry in the U.S.A.). Publication of the General Netherlands Dairy Union of Cooperative factories, The Hague, Holland. 78 pp. 1949.

Report of a Dutch Committee which visited the U.S.A. in April, 1949. A. F. Tamsma

Also see abs. no. 649, 653, 658, 659.

PHYSIOLOGY AND ENDOCRINOLOGY

R. P. REECE, SECTION EDITOR

680. The paralytic action of histamine on the ruminal musculature. R. CLARK, Inst. of Onderstepoort, Pretoria, So. Africa. J. So. African Vet. Med. Assoc., **21,** 1: 13–15. Mar., 1950.

Merino sheep with permanent ruminal fistulas were used for the trials. The ruminal cavity was connected to a rubber diaphragm tambour for the registration of pressure changes.

Complete rumen stasis followed the intravenous injection of 1–2 mg. of histamine. This cessation of ruminal movement lasted for a period of 30 min. The sheep defecated repeatedly from 5–10 min. following the histamine administration, indicating a constriction of the intestines. The ruminal stasis and intestinal constriction were prevented or cured by the administration of antihistamine drugs prior to or following the histamine administration.

The authors point out that their findings may give a physiological basis for the use of antihistamine drugs for the treatment of various types of bloat in ruminants. It was shown that the rumen paralyzed with histamine was still capable of responding to faradic stimulation of the vagus nerve. This response showed that the paralysis caused by the histamine was of myogenetic origin. K. M. Dunn

681. Purification of hyaluronidase. H. TINT and R. BOGASH, Wyeth Inst. Applied Biochem., Philadelphia, Pa. J. Biol. Chem., **184,** 2: 501–509. June, 1950.

The effects of variations of pH, ionic strength and alcohol concentration on the solubility of hyaluronidase and their influence on purification procedures were studied employing a crude hyaluronidase obtained by extracting decapsulated, ground bovine testes in the cold with acetic acid followed by precipitation with $(NH_4)_2SO_4$. Optional recovery and purity of hyaluronidase were obtained in the pH range of from 6–8, 0.15 ionic strength and ethanol concentration between 20–40%. Refinement of the single stage ethanol precipitate by 1 salting-out operation with $(NH_4)_2SO_4$ brought 50–60% of the recovered solids to a purity of about 40 units/mg. The over-all recovery of starting material obtained was above 30% with a 20-fold increase in activity.
H. J. Peppler

682. The competitive interaction of organic anions with bovine serum albumin. F. KARUSH, Sloan-Kettering Inst. for Cancer Research, N. Y. J. Am. Chem. Soc., **72,** 6: 2714–2718. June, 1950.

The competitive binding by bovine serum albumin of the detergent sodium dodecyl sulfate and dye p-(2-hydroxy-5-methylphenylazo)-benzoic acid was investigated to determine the relative heterogeneity of the binding sites. Interpreting the data in terms of a comparison of self-competition *vs.* detergent competition, it was concluded that the group 1 sites, which bind the dye most strongly, also bind the detergent most effectively and are able to assume structures complementary to a wide range of configurations. Group 2 sites bind less strongly and are more restricted in the range of configurations assumed.
H. J. Peppler

683. Heterogeneity of the binding sites of bovine serum albumin. F. KARUSH. Sloan-Kettering Inst. for Cancer Research, N. Y. J. Am. Chem. Soc., **72,** 6: 2705–2713. June, 1950.

The binding of the anionic dye p-(2-hydroxy-5-methylphenylazo)-benzoic acid by bovine serum albumin was studied at 5 and 25° C. over a wide range of concentrations. An assumption of 22 binding sites/protein molecule affords accurate description of the data. The dye binding sites can be divided into 2 groups: Group 1 contains between 4 and 5 sites and is characterized by a high binding constant; Group 2, with about 17 sites, has a relatively low binding constant. The ability of serum albumins in solution to exist

in many molecular configurations of approximately equal energy is discussed. An hypothesis of configurational adaptability has been advanced to account for the distinctive binding properties of serum albumins. H. J. Peppler

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

684. An evaluation of hypochlorites and cleaner-sanitizers. N. E. LAZARUS, Lazarus Lab., Buffalo, N. Y. Milk Dealer, **39,** 9: 50–59. June, 1950.

The relatively low cost, rapid action and wide effectiveness at high dilutions are some of the advantages provided by hypochlorites; if they are used properly on organically clean surfaces, they are effective chemical sanitizing agents. Testing methods for hypochlorites are given. The favorable qualities of quaternary ammonium compounds are summarized as follows: (a) not affected by extremely high temperatures and efficient down to 50° F.; (b) nontoxic, nonirritating, stable, colorless, odorless and nonvolatile in use-dilution; (c) neither a primary irritant nor a sensitizer when applied to the skins of humans or animals in a dilution as strong as 1:1,000; (d) exhibit good germicidal properties in the presence of large amounts of organic matter, such as horse-serum, skimmilk and ice cream mix; (e) contain no mercury compounds, hypochlorites, phenols, or formaldehyde, hence are not classified by FDA as a poison when used as recommended dilutions as specified by the manufacturers; (f) relatively constant in their rate of kill and on short exposures will destroy over 95% of vegetative cells in concentration as great as 1:15,000; (g) exhibit desirable qualities of surface activity, hence have greater penetration; (h) generally, extremely selective for Gram-positive bacteria, particularly thermodurics and, to a slightly less degree, for Gram-negatives; (i) long residual action which is essential in practical operation. Testing methods are given. The use of cleaner-sanitizers is a step in the right direction for securing better results with lower operating costs, labor and time. C. J. Babcock

685. Sanitation in the food industries, with special reference to chemical cleaners and sanitizers. C. G. DUNN, Mass. Inst. of Technol., Cambridge. Wallerstein Lab. Comm., **13,** 41: 121–140. June, 1950.

In addition to reviewing the principles of cleaning and sanitizing operations in food plants, the more common detergents and sanitizing agents are classified and their roles are described briefly. Some applications of detergents and chemical sanitizers are cited; bibliography of 136 references is provided. H. J. Peppler

686. Comparative effectiveness of DDT, methoxychlor and dichlorodiphenyl dichloroethane residues against house flies and Aedes floodwater mosquitoes. A. R. ROTH and A. W. LINDQUIST, U.S.D.A., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., **42,** 6: 871–873. Dec., 1949.

Laboratory tests were made with acetone solutions of 3 insecticides to determine their effectiveness in knockdown and kill of the house fly when used at concentrations of from 1–80 mg. of toxicant/ft.,^{2} with exposures of from 1–10 min. At low exposure periods with low dosage of toxicant, DDT was slightly more toxic than dichlorodiphenyl dichloroethane and several times as toxic as methoxychlor. Higher toxicant dosages plus longer exposure periods eliminated these differences in toxicity. E. H. Fisher

687. Effect of temperature on knockdown and mortality of house flies exposed to residues of several chlorinated hydrocarbon insecticides. R. A. HOFFMAN and A. W. LINDQUIST, U.S.D.A., Bureau of Entomology and Plant Quarantine. J. Econ. Entomol., **42,** 6: 891–893. Dec., 1949.

Laboratory tests with the house fly compared the residues of several insecticides at relatively low to high dosages, exposure periods and temperatures. DDT, DDD and methoxychlor caused faster knockdown and greater kill at 70°F. than at 90° F. Heptachlor, parathion, chlordane, dieldrin and toxaphene were more effective at 90° F.

It was surmised that the house fly is controlled with low dosages of DDT in cool climates, whereas a higher dosage is needed where it is warm. E. H. Fisher

688. Development of resistance to organic insecticides other than DDT by house flies. R. B. MARCH and R. L. METCALF, Univ. of Cal., Riverside. J. Econ. Entomol., **42,** 6: 990. Dec., 1949.

Field and laboratory tests with the house fly revealed that flies resistant to DDT also may be resistant to each benzene hexachloride and dieldrin. There is no evidence that resistant fly strains will become susceptible after non-exposure to the insecticide for several generations.

Presently accepted fly control procedures with residual insecticides may need to be revised with emphasis on sanitation, use of repellents and space sprays. E. H. Fisher

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