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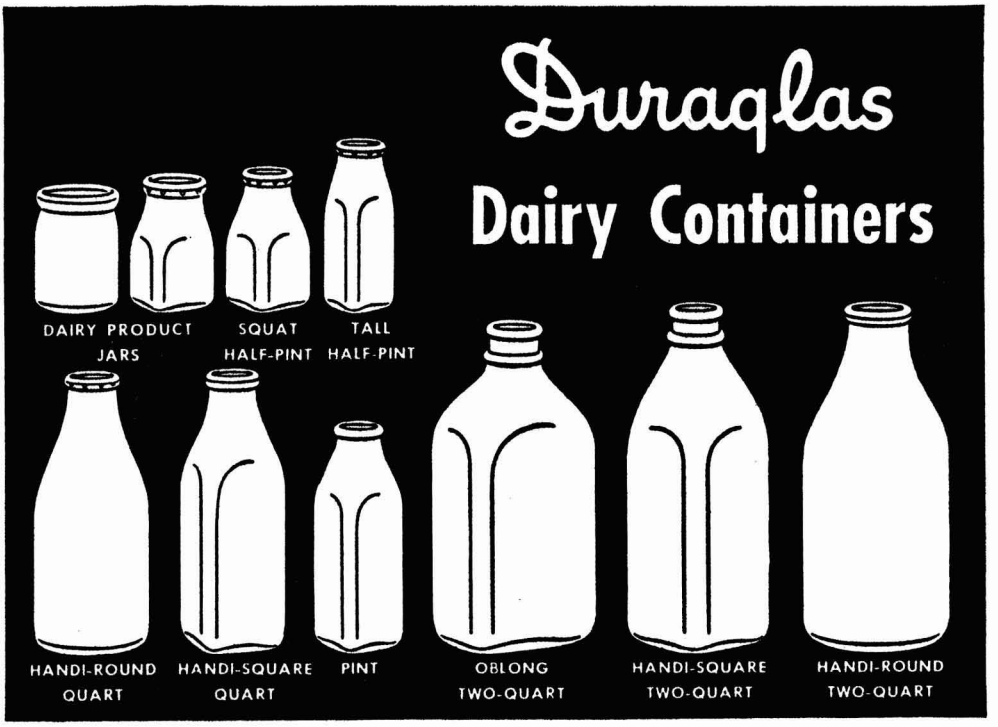

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JOURNAL OF DAIRY SCIENCE

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BLOOD STUDIES OF RED SINDHI–JERSEY CROSSES. I. HEMOGLOBIN, HEMATOCRIT, PLASMA CALCIUM AND PLASMA INORGANIC PHOSPHORUS VALUES OF RED SINDHI–JERSEY DAUGHTERS AND THEIR JERSEY DAMS

L. L. RUSOFF AND J. B. FRYE, JR.

Louisiana Agricultural Experiment Station, Baton Rouge

AND G. W. SCOTT, JR.

Iberia Livestock Experiment Station, B.D.I., U.S.D.A., Jeanerette, La.

A study to develop strains of dairy cattle better adapted to the subtropical climatic conditions existing in the southern part of the United States has been initiated by the Bureau of Dairy Industry, in cooperation with several of the southern state agricultural experiment stations. To accomplish this the Red Sindhi, a milking strain of the Brahman breed, is being crossed with the Jersey, Brown Swiss and Holstein breeds at the Louisiana Station. Studies are being made to determine which breeding methods will best combine the heat tolerance characteristics of the Red Sindhi and the milking qualities of the European breeds. Also, the physiology of heat tolerance in these crosses is being studied.

Studies of animal blood have taken a prominent place in physiological research. Some workers have claimed that the levels of certain blood constituents may be used as a guide in evaluating the adaptability of animals to a given environment. In the Philippines, Manresa *et al.* (9) reported, in 1940, that high hemoglobin values were indicative of the adaptability of animals to tropical conditions. Pure Indian Nellores and native Philippine animals had high hemoglobin values of 9.87 and 9.43 g. per 100 ml. of blood, while imported American Holstein-Friesians and Herefords had lower values of 6.88 and 6.76 g., respectively. The hemoglobin values of Zebu blood and imported temperate breeds decreased with higher air temperatures during the day and increased during the night. Without figures to support their work, Manresa *et al.* also stated that the specific gravity of the blood, the serum P-Ca ratio and erythrocyte count were highest in well-adapted animals, while uric acid, serum phosphate and erythrocyte size were lowest. The reverse claims were true of poorly adapted animals. Bonsma *et al.* (2) also have claimed that in tropical countries (South Africa) a negative relationship exists between high environmental temperature and hemoglobin values of animals. They further reported that the blood chloride content decreases, while the serum Ca increases with higher air temperatures. No data are presented to support these statements. Contrary to the reports of Manresa *et al.* (9)

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and Bonsma *et al.* (2), Akopjan (1) in the Ukraine, in 1941, stated that imported Herefords showed a large increase in hemoglobin value and erythrocyte count as the environmental temperature increased from – 4 in February to + 27° C. in August. Daubney (5) in East Africa reported the hemoglobin content of the blood of native Zebus and imported Shorthorn grades to be similar.

In India, Mullick and Pal (10) claimed that hemoglobin values, erythrocyte count, Fe, Ca and inorganic P values of Indian Hissar cattle were highest in young animals and decreased with age.

Duckworth and Rattray (6) in studying the hemoglobin values of three-quarter Holstein-Zebu heifers found decreasing values with advancing age.

Riek and Lee (11, 12) studied the blood composition of four Jersey cows and four calves at various combinations of controlled temperatures (from 85 to 110° F.) and controlled humidities. At very high temperatures and high humidities they found a marked fall in inorganic phosphate and a slight drop in serum Ca in cows and a similar but only slight change in these constituents in 8-wk.-old calves.

Brody *et al.* (3) found no significant effect of ambient temperature (50 to 100° F.) on serum Ca, hemoglobin, blood cell volume (hematocrit), Mg, plasma protein or glucose values of Jersey and Holstein cows. Increasing the temperature above 85° F. increased the serum inorganic P level.

No previous reports have been found in the literature on the blood composition of Sindhi-Jersey crosses. The purpose of this work was to determine the variation of the percentage of some blood constituents, namely packed blood cells (hematocrit), hemoglobin, Ca and inorganic P of Sindhi-Jersey daughters and their Jersey dams in a semi-tropical environment in order to find (a) any physiological differences possibly due to breed and (b) a level of constituent which might serve as an index to determine heat tolerance or adaptability to Louisiana environmental conditions.

EXPERIMENTAL PROCEDURE

Sixteen purebred Jersey cows with an average age of 3 yr., 8 mo., and their 16 Red Sindhi-Jersey female progeny, averaging 5 mo. of age at the beginning of this study, were bled on 3 consecutive days each month over a 2-yr. period, beginning in October, 1948. Citrated samples were drawn from the animals in the mornings at Jeanerette, La., refrigerated and shipped in ice to Louisiana State University in Baton Rouge, where the 3-day individual samples were pooled and analyzed.

Plasma Ca was determined by the Clark-Collip modification of the Kramer-Tisdall method (4), P by the Fiske and Subbarow method (8) using a Coleman spectrophotometer and hemoglobin by the acid hematin method using a Fisher hemometer standardized for 13.8 g. per 100 ml. of blood equal to 100 per cent. Packed red cells were determined with a Weintrobe hematocrit. Statistical analyses of the data were made according to Snedecor (14).

All animals were on a high plane of nutrition, each group being allowed pasture and being fed grain and hay according to recommended practices.

RESULTS

Table 1 shows the range and mean values of packed red blood cell volume (hematocrit), hemoglobin, plasma Ca and plasma inorganic P for 16 Jersey dams

TABLE 1

The mean hematocrit, hemoglobin, plasma Ca and inorganic P values of Jersey dams and Red Sindhi-Jersey daughters determined monthly over 2-yr. period

	No. of samples		Mean and S. E.		Range	
	Sindhi-Jersey daughters	Jersey dams	Sindhi-Jersey daughters	Jersey dams	Sindhi-Jersey daughters	Jersey dams
Hematocrit (%)	304[a]	304[a]	35.23 ± 0.28	29.95 ± 0.24	17.0 – 58.0	21.90 – 51.00
Hemoglobin (*g. %*)	384	384	10.26 ± 0.06	9.60 ± 0.05	8.50 – 15.60	7.00 – 14.10
Calcium (*mg. % plasma*)	384	384	9.63 ± 0.07	9.60 ± 0.06	8.62 – 13.96	8.70 – 16.11
Phosphorus (*mg. % plasma*)	384	384	7.58 ± 0.08	5.38 ± 0.04	3.75 – 14.29	3.25 – 8.86

[a] Determinations made on 19 mo. only.

and their 16 Red Sindhi-Jersey daughters. The mean values for the various blood constituents, with the exception of plasma Ca, were higher for the Red Sindhi-Jersey daughters than for their Jersey dams. The largest increase was the plasma inorganic P, 7.58 mg. per cent as compared to 5.38 mg. per cent, and the packed red blood cell volume, 35.23 and 29.95 per cent, respectively. A slightly higher value for hemoglobin, 10.26 g. per cent for the Sindhi-Jersey

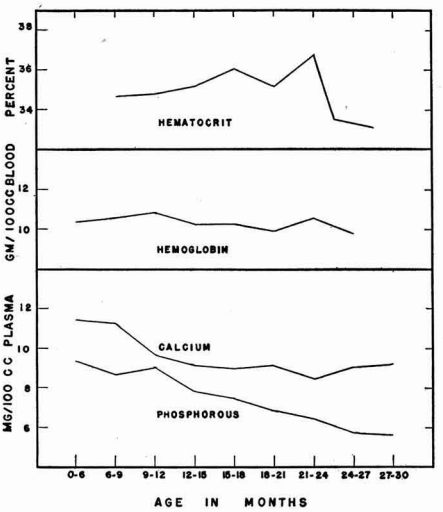

FIG. 1. Trends of blood constituents of Sindhi-Jersey crosses with respect to age.

daughters as compared to 9.60 g. per cent for the Jersey dams, was found. An analysis of variance between daughters and their dams revealed a highly significant difference (P = 0.01) in hemoglobin values and inorganic P values with no significant difference in plasma Ca values. Hematocrit values were not studied for the entire 24-mo. period and, therefore, a *t* test of significance was made between the daughters and their dams. The difference was found to be highly significant (P = 0.01). The effect of month of year on the same animal was highly significant (P = 0.01) for hemoglobin, Ca and P. Since hematocrit values were not studied for the entire 24-mo. period, they were not analyzed. Correlation coefficients between dams and daughters were not significant for any of the four constituents.

The various blood levels of the Sindhi-Jersey daughters were grouped according to age at 3-mo. intervals (figure 1) to determine whether age is a factor in the trend of levels. An analysis of variance for age showed a highly significant difference (P = 0.01) between the various ages of the Sindhi-Jersey daughters for inorganic P, but not for hemoglobin or hematocrit values. Therefore, it appears that the difference between Sindhi-Jersey daughters and their Jersey dams for P values is mostly due to age, while for hematocrit and hemoglobin values the daughter-dam difference appears to be due to a breed difference.

There was no evidence that the season of the year had any effect on the trend of hematocrit, hemoglobin, Ca or inorganic P values of the two groups of animals.

DISCUSSION

Hemoglobin. Investigations by Scott and McDowell (13) have indicated that the Sindhi-Jersey crosses are more heat tolerant than the purebred Jersey breed. Our results, therefore, are somewhat in line with the work reported by Manresa *et al.* (9), who claimed a very wide difference in hemoglobin values for native Philippine cattle (9.4 g. %) over imported breeds (6.8 g. %). Daubney (5) in East Africa, however, reported no difference in hemoglobin content of native Zebus and imported grades of Shorthorns. An analysis of variance showed the age of Sindhi-Jersey crosses was not a factor involved in the hemoglobin differences found for the crosses and their purebred dams. Other workers (6, 10) have stated that hemoglobin values for Indian Hissar cattle and three-quarter Holstein-Zebu heifers were highest in young animals and decreased with age.

Packed red blood cells (hematocrit) values. The mean monthly hematocrit values for the Sindhi-Jersey daughters were higher than those of their Jersey dams by approximately 15 per cent (table 1). An analysis of variance showed that the higher hematocrit values for the Sindhi-Jersey crosses over their Jersey dams was not due to their younger age.

Calcium. Mean plasma Ca values for both Sindhi-Jersey heifers and their Jersey dams were similar (table 1). Mullick and Pal (10) reported that Ca values are highest in young Indian Hissar animals and decrease with age. In this study Ca values of the Sindhi-Jersey crosses for the 2-yr. period showed higher values under 9 mo. of age and then leveled off (figure 1).

Inorganic phosphorus values. The monthly mean inorganic P values for the

Sindhi-Jersey daughters were 29 per cent higher than that of their Jersey dams. Statistical analysis showed that this difference was due mostly to the younger age of the crosses although a breed difference cannot be ruled out. It has been reported previously that inorganic P values were highest in young Indian Hissar animals and decrease with age (10).

Manresa *et al.* (9) have reported a higher P/Ca ratio in well-adapted animals over imported animals. Since age of the animal was found to be a factor in our study with inorganic P values, this P/Ca ratio relationship cannot be used as one of the measures for heat tolerance.

Correlation coefficients between dams and daughters were not significant for any of the blood constituents, and apparently it would not have been necessary to use daughter and dam pairs to determine whether or not blood levels differ between the purebreds and crossbreds.

The literature concerning the levels of various blood constituents of cattle in relation to heat tolerance is at variance. Many factors such as breed, age, growth, gestation, lactation, nutritional plane, number of animals used, methods of blood analyses used and other factors are all involved in the level of a blood constituent for the various breeds. Therefore, it is difficult to compare levels of blood constituents as reported by various investigators.

In this study it appears that high hemoglobin and high-packed red blood cell (hematocrit) levels might be of value in measuring adaptability of Sindhi-Jersey crosses over purebred Jerseys to higher environmental temperatures, provided that the nutritional state of the animal is normal. Further work is necessary to prove this hypothesis.

SUMMARY

Blood analyses for packed red blood cells (hematocrit) hemoglobin, plasma Ca and plasma inorganic P of 16 Sindhi-Jersey daughters and their Jersey dams, determined monthly over a 2-yr. period, are presented.

Plasma Ca values were similar for both groups of animals, while higher values for hematocrit, hemoglobin and inorganic P of the Sindhi-Jersey daughters over their dams were obtained, which were highly significant at $P = 0.01$. The difference between the crosses and purebreds for inorganic P was found to be due mainly to age, while the difference for hematocrit and hemoglobin values probably is due to a breed difference. Correlation coefficients between the blood levels of the daughters and dams were not significant.

There was no evidence of any seasonal variation in any of the blood levels of the Sindhi-Jersey crosses or their Jersey dams. However, a highly significant difference between months was found.

Higher packed red blood cell and hemoglobin levels in the Sindhi-Jersey crosses over their Jersey dams might be an index to their higher heat tolerance. Further research is needed to prove this point.

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THE EFFECTIVENESS OF THE CORNELL PHOSPHATASE TEST FOR DAIRY PRODUCTS

FRANK V. KOSIKOWSKY
Department of Dairy Industry, Cornell University, Ithaca, N. Y.

During the past year, a short note (2) was published by the author on the principle and preliminary development of a simple universal dairy products phosphatase test which employed only one concentration of buffer and precipitating agent for milk, cheese, chocolate milk, butter, frozen desserts and all other dairy products. This method, since called the Cornell Phosphatase test, has now reached its final state of development and is presented here in detail along with some observations as to its accuracy and advantages.

REAGENTS[1] AND MATERIALS

1. Carbonate buffer substrate: Dissolve 11.50 g. anhydrous Na_2CO_3, 10.15 g. anhydrous $NaHCO_3$ and 1.09 g. pure disodium phenylphosphate in water and make up to 1 l. (pH = 9.80).

2. Trichloracetic-hydrochloric acid precipitant: Dissolve 25 g. trichloracetic acid crystals in water, make up to 50 ml. with water; add 50 ml. conc. HCl (approximately 36 per cent) and mix thoroughly.

3. Sodium carbonate solution (8 per cent): Dissolve 80 g. anhydrous Na_2CO_3 in water and make up to 1 l.

4. Copper sulfate-Calgon solution (for milk and all dairy products except ripened cheese): Dissolve 500 mg. $CuSO_4 \cdot 5H_2O$ and 20 g. sodium hexametaphosphate crystals (tech.) in water and make up to 1 l.

5. Calgon solution (10 per cent) (For ripened cheese only): Dissolve 100 g. sodium hexametaphosphate crystals (tech.) and make up to 1 l. (pH of solution approximately 6.3).

6. 2,6-Dibromoquinonechloroimine solution (BQC): Dissolve 50 mg. BQC in 10 ml. absolute ethyl or methyl alcohol and store in dark bottles.

7. Color standards.

Make the following solutions as preliminary to making standards:

(a) Stock phenol solution: Dissolve 1 g. phenol crystals in water and make up to 1 l.

(b) Buffer solution: Make 1 l. of carbonate buffer containing 11.50 g. Na_2CO_3 and 10.15 g. $NaHCO_3$.

(c) Diluted phenol solution: Using 4 ml. of stock phenol solution 7(a) make up to 500 ml. with buffer solution 7(b). This solution contains 8 γ phenol per millimeter.

Preparation of color standards. Place in clean 16 × 150 mm. test tubes 0.5- to 5-ml. portions of diluted phenol solution 7(c). Add enough buffer 7(b) so that

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[1] Unless otherwise directed, use C.P. chemicals and distilled water. Sodium hexametaphosphate (Tech. Grade) may be obtained from The Amend Drug and Chemical Co., Inc., 117–119 E. 24th Street, New York, New York.

total volume of liquid in each tube is 10 ml. Then add 1 ml. of copper sulfate-Calgon solution (reagent no. 4) to each tube. Finally, add two drops of BQC and four drops USP chloroform and mix. Let stand for 15 min. at 37° C. Seal tubes with paraffin wax and store in refrigerator. Tubes containing 0.5, 1.0, 1.5, 2.5 and 5.0 ml. portions of diluted phenol solution will produce standards of 4.0, 8.0, 12.0, 20.0 and 40.0 γ, respectively, after final color development.

Color standards of 2.0 and 5.0 γ can be easily obtained by making 4.0 and 20.0 γ solutions without color development and diluting these with sufficient buffer solution 7(b). Color is then developed in 10-ml. portions by adding copper sulfate-Calgon solution and BQC.

To obtain alcohol color standards, 5 ml. of butyl alcohol are added to a duplicate series of final aqueous color standards. The tubes are inverted 10 times to extract color and the cork stoppers are sealed with wax.

PROCEDURE

Long method. 18 to 24 hr. at 32 to 37 ° C.

1. Sampling and incubating. For milk and other fluid dairy products[2] 1 ml. of milk or product is transferred to a 25 × 150 mm. test tube. This is followed by the addition of 10 ml. of warm (40° C.) carbonate buffer substrate and four drops of USP chloroform.

For cheese and other solid dairy products[3] a representative 0.5-g. portion is placed in a 25 × 150 mm. test tube. The cheese is macerated thoroughly with a glass rod, 1 ml. of warm (40° C.) carbonate buffer substrate is added and the cheese or other solid dairy product is stirred into a paste. Then 9 ml. more of the buffer substrate and four drops of USP chloroform are added and mixed thoroughly.

A piece of parchment paper is fitted over the tube using a rubber band and the milk or cheese solution is incubated at 32 to 37° C. for 18 to 24 hr.

2. Precipitation. After incubation 1 ml. of trichloracetic-HCl precipitant is slowly added to the tube. The resulting protein precipitate is filtered off through Whatman no. 42 paper (11 cm.).

3. Color development. For milk and all dairy products except ripened-type cheese, 5 ml. of the clear filtrate is pipetted into a 16 × 150 mm. test tube. One ml. of $CuSO_4$-Calgon solution and 5 ml. of 8 per cent Na_2CO_3 are added. Then two drops of BQC solution are placed in this solution. The tubes after mixing are inserted in a water bath at 37° C. for 15 min. Color development is measured after this interval against suitable color standards or in a colorimeter.

For ripened cheese only, including fresh curd and green cheese, the same color development procedure as for milk is used except that 1 ml. of plain 10 per cent Calgon solution is substituted for the 1 ml. of $CuSO_4$-Calgon solution.

4. Interpretation of results. All final color readings are, after consideration of control values, multiplied by a factor of 1.2 to convert γ phenol per 0.5 ml. or

[2] Includes all fluid dairy products such as milk, cream, chocolate milk, buttermilk, ice cream mix, exaporated milk, whey, etc.

[3] Includes all solid dairy products such as cheese of all types, butter, milk powder, etc.

0.25 g. Any value over 5 γ per 0.5 ml. milk or other fluid dairy product or over 5 γ per 0.25 g. cheese or other solid dairy product is tentatively considered to indicate either underpasteurization or the presence of raw milk products, or a combination thereof, using the long method.

Short method. 1 hr. at 37° C.

The short method, even to the extent of using the same size samples (1 ml. milk, 0.5 g. cheese), is conducted in the same way as the long method except that (a) incubation is carried out for 1 hr. at 37 to 38° C., and (b) chloroform is omitted.

In interpreting the results all final color readings, after consideration of control values, are multiplied by a factor of 1.2 to convert γ phenol per 0.5 ml. or 0.25 g. Any value over 2.0 γ per 0.5 ml. milk and other fluid dairy products or per 0.25 g. cheese and other solid dairy product is tentatively considered to indicate underpasteurization or the presence of raw milk products, or a combination thereof, using the short method. Where values of 1.0 to 2.0 γ per unit of sample are attained, it is advisable to confirm using the long method.

Rapid field test. 10 min. at 37° C.

Samples are prepared and incubated with buffer substrate exactly as for the short (1-hr.) test. After 10 min. at 37° C., 1 ml. of incubated solution is removed and placed in a small cup of a white spot plate. One drop of BQC is added and color development noted after 3 min. against that of properly pasteurized samples. Dark grey or blue indicates underpasteurization or the presence of raw products. (Note: Remaining 10-ml. portion in test tubes can continue to be incubated for the 1-hr. or 18-hr. test without loss of accuracy if confirmation of the rapid field test is necessary.)

Controls for long and short methods. One-half ml. of milk or 0.25 g. of cheese, preferably from pasteurized stock, are placed in test tubes, heated to 170° F. for 15 sec. in a water bath and then cooled immediately. These heated controls are tested by the same method as employed for samples of unknown history. All portions of the samples being tested should be heated.

Alcohol extraction. If necessary, butyl alcohol extraction may be used in either the long or short method, especially on critical values. Five ml. of *N*-butyl alcohol is added to the test tube, containing the aqueous colored solutions, and the latter is inverted ten times. The clear layer appears without centrifuging and is compared against alcohol standards. Alcohol extraction is preferred where a colorimeter is not used, as aqueous phenol standards for all methods deteriorate as a rule relatively rapidly.

EXPERIMENTAL RESULTS

In order to obtain information as to the sensitivity of the Cornell method, a collaborative study was conducted involving five laboratories throughout the Northeast. Comparisons were made between the Cornell and the Sanders-Sager phosphatase methods on a series of milks and cheddar cheeses of known

TABLE 1

Average values obtained from 5 laboratories on series of milks and ripened cheddar cheeses of known history using the Cornell and the Sanders-Sager phosphatase tests

Method	No. of samples	Critical value indicating underpasteurization	Properly pasteurized milk	Properly pasteurized milk containing 0.1% Raw	0.4% Raw	1.0% Raw
			Whole milk	(γ phenol/0.5 ml. milk)		
Sanders-Sager[a]	128	> 2.0	0.2	1.9	6.6	14.6
Cornell (1-hr.)	96	> 2.0	0.1	1.9	6.2	12.1
Cornell (18-hr.)	128	> 5.0	0.7	12.7	36.5	> 48.0

Method	No. of samples	Critical value indicating underpasteurization	Properly pasteurized milk	Raw milk in cheese milk 0.1%	0.2%	0.4%	0.7%	1.0%
			Ripened cheddar cheese (1 yr.)	(γ phenol/0.25 g. cheese)				
Sanders-Sager[a]	84	> 3.0	0.5	1.2	2.2	2.9	4.6	5.3
Cornell (1-hr.)	92	> 2.0	0.1	0.7	1.1	2.0	3.3	4.6
Cornell (18-hr.)	92	> 5.0	0.6	6.3	13.2	25.0	36.0	> 48.0

[a] Official directions call for 1-hr. incubation only.

history. Instructions for the latter test recommended only 1-hr. incubation at the time this survey was made (4,5), while the Cornell method advocated both long and short incubation periods.

A condensed version of the results are presented in tables 1 and 2. The Cornell short (1-hr.) test and the official Sanders-Sager method (table 1) are about equally effective in detecting underpasteurized samples. None of the samples tested within this group contained more than 1 per cent raw milk. These two methods missed about 18 to 19 per cent of the milks and 41 to 49 per cent of the cheese samples. On the other hand, within the same category

TABLE 2

A summary of results obtained on whole milk and ripened cheddar cheese using the Cornell and the Sanders-Sager phosphatase methods

Method	No. of individual samples	Missed picking[b] out as pasteurized or underpasteurized (%)	Av. value of control blank γ phenol/0.5 ml. milk or 0.25 g. cheese
	Whole milk		
Sanders-Sager[a]	128	19	0.8
Cornell (1-hr.)	96	18	0.2
Cornell (18-hr.)	128	0	0.1
	Ripened cheddar cheese		
Sanders-Sager[a]	84	49	0.8
Cornell (1-hr.)	92	41	0.3
Cornell (18-hr.)	92	2	0.3

[a] Official directions call for 1-hr. incubation only.
[b] None of milk or cheese samples tested contained over 1% raw milk addition.

the Cornell (18-hr.) test missed none of the milks and only 2 per cent of the cheese. This should effectively serve to demonstrate the high sensitivity of the standard Cornell method and at the same time emphasize the value of using an overnight incubation.

The standard Cornell (18-hr.) test (see table 2) was able to detect 0.1 per cent raw milk additions in either pasteurized milk or cheddar cheese aged at least 1 yr. The average reading for milk containing 0.1 per cent raw milk was 12.7 γ per 0.5 ml. milk. This is considerably over the minimum critical value of 5 γ, and it would be interesting to know what minimum concentration of raw milk this method would detect under routine testing. Recently in a cooperative study between the New York State Department of Health and the Cornell Dairy Department, 100 samples of milk were received over a period of 5 wk. from the department and tested by the 18-hr. test. These properly pasteurized milks had been treated with extremly small concentrations of raw milk but their treatment was unknown to the laboratory analyst. Based upon these hundred samples, as little as 0.04 per cent raw milk could be successfully detected by the Cornell method.

In another survey of 111 samples of commercial pasteurized milk obtained from one New York State county, 5 per cent of these milks were found to be from slightly to grossly underpasteurized by the Cornell method. The average value of the remaining 105 samples of milk which were properly pasteurized was 0.9 γ phenol per 0.5 ml. milk.

Table 2 shows that the control blank values obtained by the Cornell method were considerably lower than those obtained by the method of Sanders-Sager.

DISCUSSION

The Cornell method has been called a universal phosphatase test for dairy products, as it requires only one buffer substrate and precipitant concentration for all dairy products regardless of their history or age. However, the concept of the term "universal" as applied to phosphatase methods might be broadened further to include the time element.

Since the inception of this test, the author has stressed the fundamental importance from the point of view of sensitivity and accuracy of using an 18- to 24-hr. incubation period. Much earlier, as a matter of principle, Kosikowsky and Dahlberg (3) pointed out that an overnight incubation period for any phosphatase method utilizing BQC apparently was superior to the same test employing only a 1-hr. incubation. Yet in order to satisfy the time requirements for universality, such a method also should include provisions for the use of shorter incubation intervals whenever necessary and without requiring any other changes in procedure. It is believed that the Cornell method fufills these requirements adequately as, in addition to the standard 18-hr. test, a short 1-hr. and a rapid 10-min. field test are available and can be used practically interchangeably.

The relative merits and limitations of the 1-hr. and rapid 10-min. tests should be considered. Each of these variations may be extremely useful if their limitations are observed carefully. A public health laboratory undoubtedly would

prefer to use the long method because of its extreme sensitivity and the ease of reading colors on slightly underpasteurized samples. Consequently, a dairy plant wishing to have its results on slightly underpasteurized samples conform to those of the public health laboratory would best use the long method. However, where circumstances dictate a faster test be employed, the 1-hr. test can be used to advantage if it is realized that in practically all 1-hr. phosphatase tests dairy products and especially ripened cheeses containing about the equivalent of from 0.1 to 0.3 per cent raw milk additions may produce negative results. This possibility can be greatly reduced with the short test by providing that where values are obtained from 1.0 to 2.0 γ phenol per 0.5 ml. or 0.25 g., a confirmatory test be run using the 18-hr. incubation test.

The 10-min. test fills the need for a field test which will quickly pick out raw and grossly underpasteurized samples. It has an additional advantage in that one can proceed and utilize, when desirable, the 1-hr. or 18-hr. methods upon the remaining portion.

A few of the reasons for certain steps used in the Cornell method might be considered. The concentration of carbonate-bicarbonate buffer after extensive testing at various levels has been set at its present level because this concentration was found most advantageous when all dairy products of widely different age and buffer capacity are considered. Results in this paper indicate that the Cornell buffer is not so concentrated as to seriously inhibit activity of the phosphatase enzyme. That some buffers may show slightly less inhibition of enzyme when compared to other buffers in other methods has little meaning or importance when the test employing these buffers is considered in its entirety. Precipitants and color developers are not the same for every method and consequently can be adjusted to suit the specific needs of the test. If the phosphatase enzyme is slightly more sensitive in the presence of one buffer than another this may be of little significance in improving the method, as the increased sensitivity most likely exists at all parts of the buffer curve and, as a result, a higher critical value must be used.

The precipitant, trichloroacetic acid plus HCl, is advocated because of the excellent precipitating properties of trichloroacetic acid. Hydrochloric acid was used because trichloroacetic acid will precipitate protein far more effectively under acid conditions and therefore, much less is required if HCl is used to change the highly buffered alkali solution to an acid solution. Also, HCl reduced the pH well below 2, which completely inactivates the enzyme. Inactivation of the enzyme during testing should be a requirement for any phosphatase method. The evolution of CO_2 gas which results upon the addition of the acid precipitant also is advantageous, as it serves as an efficient agitator of the solution at this point.

Earlier instructions for the Cornell method had suggested the use of potassium oxalate to prevent slight turbidity in the final solutions caused by precipitation of excess calcium and carbonate. However, sodium hexametaphosphate (Calgon) as advocated by Neave (1) for the Kay and Graham test has been found to be far more effective in obtaining clear solutions by sequestering these chemi-

cals and, consequently, the use of potassium oxalate is considered only as a supplementary step listed under precautions.

As one of the objectives of this survey was to observe over-all method sensitivity, it would appear completely logical to compare results of an 18-hr. method (Cornell) with that of a 1-hr. method (Sanders-Sager), especially in light of the fact that up to the time of this survey, a 1-hr. incubation was considered adequate for practically all official testing using the latter method (5). The results of this survey show that the great advantage in sensitivity which is obtained by using a longer incubation time cannot be overlooked and that overnight incubation should be considered for any phosphatase test. In addition to sensitivities and especially where they appear to be similar as in the 1-hr. Cornell test and the Sanders-Sager test, the merits of the two methods should be resolved on the basis of simplicity, relative low control values, cost and ability to produce accurate results without prior knowledge of age or past history of samples.

Precautions for this method, though few, should, nevertheless, be pointed out. If the tubes carrying the incubated substrate are of smaller capacity than recommended in the directions, more care should be taken in adding the acid precipitant slowly, as otherwise the liquid may bubble over slightly. The purity and strength of the disodium phenyl phosphate and BQC always should be checked. Reagent blanks always should be run at the start of the day's activities. In order to keep the amount of BQC uniform for all tests, it is suggested that the same size dropper be used as stated in the original directions. This dropper produced 45 drops to 1 ml. at room temperature.

If turbidity occurs in the final tube for a product this would indicate a need for a more highly concentrated Calgon solution for this product. Turbidity in the solution will not interfere with alcohol extraction.

In rare instances in testing extensively ripened cheese, the final color produced may have some yellow due to excessive free nitrogenous compounds. To correct this condition repeat the test but substitute 1 ml. of 5 per cent potassium oxalate (C. P.) for 1 ml. 10 per cent Calgon solution. After color development filter a second time through Whatman no. 42 paper and read color.

In cases of grossly underpasteurized and raw samples far more free phenol is present than is indicated by the color, which is limited to the amount of BQC present. If total phenol concentrations are required, a simple dilution technique on the filtrate can be evolved. Such a dilution technique is better suited for research studies and generally need not be considered for routine laboratory analyses, as the maximum intensity of colors developed on samples of this type in the Cornell method without dilution is well above the critical standards used.

SUMMARY

A simple phosphatase method, utilizing only one buffer substrate concentration and one precipitant concentration for all dairy products, is presented in detail. In this test the dairy product is incubated in a highly concentrated Na_2CO_3-$NaHCO_3$ buffer substrate at pH 9.5 to 9.7 and followed by precipitation with a trichloroacetic-HCl solution. The filtrate then is made alkaline with

Na_2CO_3 and color development proceeds using BQC. Overnight incubation is standard for this test but both a short (1-hr.) and a rapid (10-min.) variation are available whenever circumstances dictate and are practically interchangeable as regards technique.

This universal dairy products phosphatase test is highly sensitive and accurate but many other advantages are indicated by its simplicity of operation, low cost, ability to produce accurate results on samples of unknown age or origin and relatively low control values.

Results in this study point to the importance of using overnight incubation for greatest sensitivity and accuracy on all phosphatase tests utilizing BQC as a color developer.

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A SIMPLIFIED METHOD FOR FILTERING ROLLER PROCESS NONFAT DRY MILK SOLIDS

D. R. STROBEL, W. G. BRYAN AND C. J. BABCOCK

Dairy Branch, Production and Marketing Administration, U. S. D. A.

INTRODUCTION

Strobel and Babcock (3) reported a method of filtering roller process nonfat dry milk solids through a lintine disc by using a pepsin-hydrochloric acid solution. This method gave more accurate results than the tumbler method (1) in determining sediment content, including scorched particles, in roller process dry milks. Microscopic examination of the discs revealed that in all instances a high percentage of the material on the discs was scorched protein particles. This also was true for spray process nonfat dry milk solids.

The development of a reproducible, accurate filtration method for roller process nonfat dry milk solids and the determination that a high percentage of the material filtered from dry milks is scorched protein material indicated the need for new standards. Such standards, consisting of four discs representing 7.5 mg., 15.0 mg., 22.5 mg. and 32.5 mg. of scorched particles, were developed (4). To facilitate their use and availability, a composite sepia photograph of the four discs was prepared (5).

The pepsin-hydrochloric acid method of filtration as reported (3) necessitated holding the sample 20 min. in a 45° C. water bath and bringing the sample to a boil prior to filtering. It was recognized that a more rapid method of filtration was needed.

Development of Testing Procedure. These experiments were undertaken to develop a more rapid method of filtering roller process nonfat dry milk solids than the pepsin-hydrochloric acid method. As reported (3), a preliminary investigation revealed that the procedure using 40 per cent sodium citrate solution as outlined in Standard Methods for the Examination of Dairy Products (2) failed to give a filtrable solution. Modifications of this procedure by increasing the time and temperature of the samples in the water bath also gave unsatisfactory results.

Further investigations, using a new procedure with hot sodium citrate solution, produced a filtrable solution of roller process nonfat dry milk solids. Experiments to determine the minimum amount and strength of solution to use, speed and time of mixing and methods of filtration using 25-g. samples, established a testing procedure comprised of the following steps:

Place 200 ml. of a hot (80 to 90° C.) 10 per cent sodium citrate[1] solution in the mixing jar of a high-speed mixer[2]; turn on the mixer and add 25 g. of sample. Add approximately 0.5 ml. of diglycol laurate (defoaming agent). Mix for

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[1] CP sodium citrate used.

[2] Waring Blendor used.

30 sec. and filter (aspirator or pressure filtering apparatus necessary) immediately through a 1.25-in. lintine disc (1.125 in. filtering surface). Rinse mixing container with hot water and pass all rinsings through the filter disc.

In establishing this procedure, it was found that a low speed mixer was unsatisfactory. When a medium speed mixer was used, more samples failed to filter than when a high speed mixer was used. The scorched particles were of the same character and appearance when either a medium or a high speed mixer was used. It was also found that the character and appearance of the scorched particles were not changed by bringing the sodium citrate solution to a boil or by increasing the mixing time. These factors were, therefore, established at the minimum levels required for a satisfactory test. When the sodium citrate solution was heated to less than 80° C., the temperature frequently dropped during mixing and filtering to a point that caused difficulty in filtering the sample. Difficulty was also experienced with filtering some samples when they were mixed less than 30 seconds. The quantity and strength of solution used were those necessary for adequate mixing and satisfactory filtration.

To determine whether the sodium citrate solution affected the scorched material, discs obtained by filtering 25-g. quantities of 25 spray process samples mixed with warm water and 25 duplicate samples mixed with hot (80 to 90° C.) sodium citrate solution were compared. The sodium citrate solution did not change the color, appearance or quantity of the scorched material.

EXPERIMENTAL RESULTS

The established sodium citrate method was applied to 25-g. quantities of 122 samples of roller process nonfat dry milk solids. Incomplete filtrations were obtained with 23 of the samples, but the discs of the filtered portions of 18 of these contained scorched material in excess of the 32.5 mg. disc of the United States Department of Agriculture Scorched Particle Standards for Dry Milks (5).

To permit a comparison of the solvent-disc method and the sodium citrate method, duplicate 25-g. quantities of 80 of the 122 samples also were filtered by the solvent-disc method. Fifty samples produced the same results by the two methods. The sodium citrate method showed slightly more scorched particles on 14 samples but the differences were less than one disc on the scorched particle standards (5). Nine samples differed by one disc, the sodium citrate method showing more scorched material. The remaining seven samples which failed to filter by the sodium citrate method, were filtered by the solvent-disc method. In all instances, the resulting discs of these seven samples showed scorched particle content in excess of the 32.5 mg. disc of the USDA standards. These comparisons indicate that the sodium citrate method more completely measures the scorched particle content. The color, type, and character of material on the discs of the duplicate samples were comparable.

The practicability and reproducibility of the sodium citrate method was determined by a cooperative project conducted by the authors, the PMA Dairy Branch Laboratory at Chicago, Ill. and the Veterinary Section of the Army Medical Laboratory, Army Medical Center, at Washington, D. C. For this purpose,

100 samples of roller process nonfat dry milk solids examined by the Department in its milk and butterfat price-support program were used. Many of these samples were selected because there was reason to believe that they would have a high scorched particle content. A portion of each sample was filtered by the authors, the Dairy Branch Laboratory at Chicago and the Army Medical Laboratory, using the sodium citrate method. The discs obtained were scored to the nearest disc by using the USDA scorched particle standards for dry milks (5).

The results of tests of the 100 samples of roller process nonfat dry milk solids are shown in table 1. The same results were obtained by the three laboratories

TABLE 1

A comparison of the discs obtained by filtering equal parts of 100 samples of roller process nonfat dry milk solids by the authors, Dairy Branch Lab., and the Army Medical Center Laboratory

A.—Disc scores of the 91 samples on which the laboratories obtained the same results:

Number of samples	Disc score[a] (*mg.*)
14	7.5
3	15.0
7	22.5
67[b]	32.5 or more

B.—Disc scores of the 9 samples on which the laboratories obtained different results:

Authors	Disc score[a] Dairy Branch Lab.	Army Medical Center Lab.
(*mg.*)	(*mg.*)	(*mg.*)
22.5	32.5	32.5
15.0	15.0	22.5
15.0	15.0	22.5
15.0	7.5	15.0
32.5	22.5	32.5
22.5	32.5	32.5
22.5	32.5	32.5
32.5	22.5	22.5
32.5	22.5	32.5

[a] USDA Scorched Particle Standards for Dry Milks used.

[b] Incomplete filtration obtained by all three laboratories on seven samples; incomplete filtration obtained on 12 more by the Army Medical Lab.; 4 more by the Chicago Dairy Branch Lab., and 1 more by the authors.

on 91 per cent of the samples. As compared with the results obtained by the authors, the Army Medical Center Laboratory results contained five discs with more scorched particles and one disc with less; the Chicago Dairy Branch Laboratory results contained three discs with more scorched particles and four discs with less.

Of the 100 samples filtered, all three laboratories obtained incomplete filtrations with seven samples. The Army Medical Center obtained incomplete filtration with 12 more samples, the Chicago Dairy Branch laboratory four more and the authors one more. All of the samples that failed to filter completely contained 32.5 mg. or more of scorched particles on the discs of the filtered portions, thus indicating that the samples contained substantially more than 32.5 mg.

SUMMARY AND CONCLUSIONS

A more rapid and accurate method of filtering roller process nonfat dry milk solids than the pepsin-hydrochloric acid method was developed by using a hot, 10 per cent, sodium citrate solution.

This method produced satisfactory results with 99 out of 122 samples. Eighteen of the 23 samples that failed to filter contained scorched material in excess of the 32.5 mg. disc of the USDA Scorched Particle Standards for Dry Milks.

A cooperative project, between three laboratories proved the sodium citrate method to be practical and reproducible. The three laboratories tested parts of the same 100 samples and obtained the same results on approximately 91 per cent of the samples.

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BUTTERFAT PRODUCTION PER DAY OF LIFE AS A CRITERION OF SELECTION IN DAIRY CATTLE[1]

C. J. LARSON, A. B. CHAPMAN AND L. E. CASIDA

University of Wisconsin, Madison

Economic efficiency or profitability of milk production, in a broad sense, may be considered as the pounds of milk produced per pound of feed consumed. Part of the feed consumed may be utilized for milk secretion and part for body maintenance. Cows usually are fed according to production. Management control then eliminates the necessity of considering the variations in the feed required for milk secretion *per se*. Whether differences in daily maintenance requirements are large enough, intra-breed and intra-herd, to be important is not known. However, it is impractical to measure these requirements of each individual, and body weights usually are not available for reflecting these differences. This leaves as a practical measure of profitability the pounds of milk produced in relation to the number of days maintained, or simply production per day of life or production to a certain age. It is this measure of efficiency which is evaluated as a potential criterion of selection in the study reported here.

Production to a standard age, even though it were a perfect measure of profitability, should not be used as a criterion of merit in a selection program unless it is at least partly under genetic control.

Usually dairy cattle are compared on the basis of lactation production, a character of moderate heritability, without any consideration being given to the age at first calving and the variation in the length of the calving intervals. Profitable animals, in terms of production to a given age, are those which combine a high reproductive rate with a high level of production. Therefore, to select for high total production to a given age, *e.g.*, 84 mo., it is necessary to select animals which are not only good producers per lactation but also good reproducers.

Early first calving has been shown by Chapman and Dickerson (3) to be more efficient than later calving when expressed in pounds of butterfat per month of life after 24 mo. of age. A short service period was found by Chapman and Casida (2) to increase efficiency of production in the accompanying calving interval and have little if any effect on production in the immediately subsequent calving interval. They also found that service periods following short calving intervals are shorter than those following longer calving intervals. Very short calving intervals were found by Tyler and Hyatt (10) to decrease production in subsequent lactations. Therefore, the net effect of decreasing the calving interval beyond an optimum might be to increase the total number of days in which

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a cow milks throughout her life without also increasing total production. It is expected that maximal total production would be associated with optimal age at first calving and optimal length of calving intervals.

The following report is an evaluation of production to various standard ages as selection criteria for genetic improvement of dairy cattle.

EXPERIMENTAL

The data used in this study were obtained from the D.H.I.A. herd records of two Wisconsin State Department of Public Welfare herds. Individual 10-mo. butterfat records were calculated by the simplified method of Tyler and Chapman (9)—30.5 times the butterfat production on the test days of the first 10 mo. of lactation corrected to twice-a-day milking. The total production to a certain age was calculated as the sum of the monthly production figures over all months of production up to and including the month in which the animal was 36, 48, 60, 72 or 84 mo. of age.

The dam-daughter pairs were distributed over the five age groups in the two herds as shown in table 1. All dam-daughter comparisons in each older age class

TABLE 1

Distribution of dam-daughter pairs over five age classes in the two herds

Herd	Age (*mo.*)				
	36	48	60	72	84
I	84	64	47	33	28
II	222	112	70	42	27
Totals	306	176	117	75	55

are first represented in each younger age class.

Two parallel studies were made. One was on 55 dam-daughter pairs having 84-mo. production and the other on 306 dam-daughter pairs (including the 55) having at least 36 mo. production. Correlations between variables on the same individual were calculated on all records of all "daughters" up to and including 84 mo. where available.

Intra-herd correlations between daughter and dam were calculated for each calving sequence of dams. These correlations were tested for heterogeneity and then pooled for all calving sequences and later for both herds. Heritabilities were computed by doubling the intra-sire regressions of daughter on dam.

RESULTS

Individual performance. The extent to which variables measurable in early life are indicative of total production to later ages was first considered.

Correlations of 36-mo. production with total production to 48, 60, 72 and 84 mo. of age indicate that production to 36 mo. of age is a reliable estimate of total production to later ages in the same individual (table 2). The correlations between these variables in the 84-mo. group did not differ significantly from those on the remaining 251 animals, hence only the gross correlations are given. Thirty-

TABLE 2

Correlations of total production to 36 mo. of age with total production to other ages on the same individual

Age of individual	No. of individuals	Correlation
(*mo.*)		
48	176	$+0.83 \pm 0.02$
60	117	$+0.64 \pm 0.06$
72	75	$+0.57 \pm 0.08$
84	55	$+0.62 \pm 0.08$

six mo. production accounted for 38.4 per cent ($r^2 = +0.62^2$) of the variation in 84-mo. production.

Age at first calving and production in the first 305-day lactation were considered as possible major sources of variation in total production to a given age. Intra-herd correlations of first 305-day lactation with 36-mo. (+0.62) and 84-mo. production (+0.57) did not differ significantly (table 3). The correlations of 305-day first lactation production with 48, 60 and 72 mo. also were calculated and found to be +0.58, +0.59 and +0.55, respectively. The negative correlation (−0.27) of age at first calving with production to 84 mo. of age is significantly less than the correlation of age at first calving and production to 36 mo. of age (−0.68). Older animals at first calving showed little if any evidence of higher production during the first lactation ($r = +0.06$).

The multiple regression equations for predicting production to 36 and 84 mo. from age at first calving and also first lactation production are shown in table 3. Age at first calving and production in the first lactation combined accounted for 89 per cent (0.94^2) of the variability in production to 36 mo. of age. These two variables together accounted for 41 per cent (0.64^2) of the variance in total production to 84 mo. The means of the variables used in the regression equations for the two levels were approximately 355 lb. for 36-mo. production, 1,950 lb. for 84-mo. production, 360 lb. for first 305-day lactation and 800 days for age at first calving.

A reliable estimate of 36-mo. production can be made from the age at first calving and production in the first 305-day lactation as shown by the multiple cor-

TABLE 3

Intra-herd correlations and multiple regression equations for predicting production to 36 and 84 mo. of age

Variable	36-mo. production		84-mo. production		Age first calving	
	N	*r*	*N*	*r*	*N*	*r*
First 305-d. lactation (L)	306	+0.62**	55	+0.57**	306	+0.06
Age at first calving (A)	306	−0.68**	55	−0.27*	.....	.....
			R			
Predicted 36 = 695.63 − 0.893 (A) + 1.055 (L)			0.94**			
Predicted 84 = [63.33 − 0.052 (A) + 0.115 (L)] 30.5			0.64**			

** $P < 0.01$. * $P < 0.05$.

TABLE 4

Correlations of total production to each age in daughters with total production to 36 and 84 mo. in the dams

Age of daughters	Age of dams (*mo.*)			
	36		84	
	No. of pairs	Correlation coefficient	No. of pairs	Correlation coefficient
(*mo.*)				
36	306	+ 0.07	55	- 0.14
48	176	+ 0.05	55	- 0.13
60	117	+ 0.16	55	- 0.15
72	75	+ 0.04	55	- 0.16
84	55	+ 0.12	55	- 0.05

relation of 0.94, based on all daughters. The multiple correlation calculated on the 84-mo. group only was of the same order of magnitude as the one based on all but the 84-mo. group. The multiple correlation (R) of age at first calving and first 305-day lactation with 84-mo. production indicates that the first 305-day lactation may be nearly as reliable in predicting 84-mo. production alone ($r = + 0.57$) as is age at first calving and first 305-day lactation combined ($r = + 0.64$).

Heritability. Age at first calving, production in the first 305-day lactation and total production to given ages have just been shown to be interrelated in so far as they are concerned with the individual's own performance. The degree to which each of these is transmissible from one generation to the next is now considered.

Correlation coefficients were calculated for total production to each age in the daughters with total production to each age in the dams. Only one of the 32 correlations was statistically significant. Hence, there is little evidence of an association of production per day of life between daughter and dam. Only those correlations for 36-mo. production in the dam with 36, 48, 60, 72 and 84 mo. in the daughters and for 84 mo. in the dam and 36, 48, 60, 72 and 84 mo. in the daughters are shown in table 4. For all ages at which the 84-mo. group could be compared with those combinations in which both daughter and dam did not reach 84 mo., the correlations did not differ significantly. The consistency in sign of correlations within each age classification of dam is thought to arise from the

TABLE 5

Correlations and regressions of daughter on dam of age at first calving and butterfat production in the first 305-d. lactation.

Source	Degrees of freedom	305-d. lactation		Age at first calving	
		r	b	r	b
Between sires	26	+ 0.81**	+ 0.87**	0	0
Within sires	265	+ 0.15*	+ 0.15*	- 0.08	- 0.88
Total	291	+ 0.25**	+ 0.28**	- 0.07	- 0.06

** $P < 0.01$.
* $P < 0.05$.

partial dependence of the figures used in the calculation of each correlation on those used in the calculation of correlations for all younger ages of the daughters.

The intra-sire regression of daughter on dam for first lactation butterfat production was $+0.15 \pm 0.06$ (table 5). This figure doubled gives an estimate of heritability of first lactation butterfat production of 0.30. The 95 per cent fiducial limits for this estimate would be 0.07 to 0.53. The intra-sire regression of daughter on dam for age at first calving is insignificant, giving no evidence of heritability of age at first calving under the conditions existing in these two herds.

DISCUSSION

Production per day of life up to 48, 60, 72 and 84 mo. of age, as measured by total production to these ages, was significantly and highly correlated with production to 36 mo. of age ($r = +0.57$ to $+0.83$) and 305-day first lactation production ($r = +0.55$ to $+0.62$). None of these correlations differed significantly from

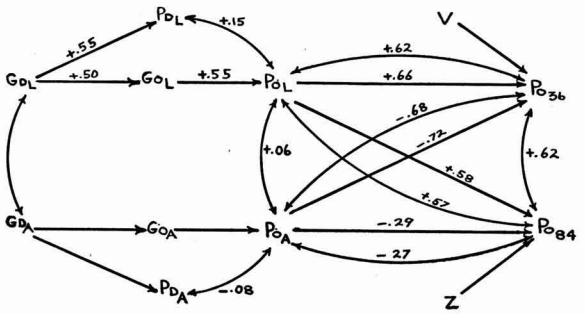

FIG. 1. Path diagram showing the biometric relations between production to 36 mo. of age, production to 84 mo. of age, first 305-day lactation production and age of first calving.

G = Genotype	A = Age at first calving
P = Phenotype	L = First 305-day lactation production
D = Dam	36 = Production to 36 mo. of age
O = Offspring	84 = Production to 84 mo. of age

each other, thus providing little evidence of higher predictive values from any one of these variables to any other.

Some of the reasons for variation in production to 36 and 84 mo. of age are shown in the accompanying path diagram (fig. 1) of the biometric relations between production to these ages (36, 84), age at first calving (A) and first 305-day lactation (L). The curved lines in the diagram represent correlation coefficients and the straight lines, standard partial regression coefficients (path coefficients). The letter V represents all unanalyzed sources of variation in 36-mo. production independent of age at first calving and first lactation production. Z represents all other sources of variation in 84-mo. production not associated with these two variables. Letters P and G represent phenotype and genotype, respectively, whereas D and O designate dam and offspring.

The values shown in the diagram indicate that the combined effect of age at first calving and first 305-day lactation production accounted for 89 per cent of the variation in 36-mo. production and 41 per cent of the variation in 84-mo. production.

The independent influence of age at first calving was found to be significantly less on 84-mo. than on 36-mo. production. It accounted for 51.8 per cent (-0.72^2), and 8.4 per cent (-0.29^2), respectively, of the variance in these two variables. Production in the first 305-day lactation, however, was responsible for approximately the same amount of variation in 36- and 84-mo. production.

The results of this study give no evidence of heritability of production per day of life at any of the ages studied from 36 to 84 mo. Therefore, selection for this character would not be expected to result in genetic gain as far as production per day of life is concerned.

Heritability of butterfat production has been found previously by several workers (1, 6, 7, 8) to be between 0.20 and 0.30. The estimate of heritability of first lactation butterfat production from these data is 0.30 ± 0.12. The genetic basis for correlation of lactation butterfat production in the daughters with lactation production in the dams is shown in the path diagram where the values given on the unidirectional paths connecting P_{D_L} and P_{O_L} are derived from the biometric relationship between parent and offspring, *viz.*, $r_{P_DP_O} = abh^2 = 1/2h^2$ (11).

Delaying first service for certain heifers was considered to be responsible for part of the variation in age at first service but for practically none of the variation in the interval from first service to conception in the data studied by Dickerson and Chapman (4). The results of this study do not indicate that age at first calving is inherited. Dunbar and Henderson (5) report that heritability of fertility estimated from calving intervals and non-returns to first service is negligible.

The extent to which variation in management practices has obscured genetic differences in productive and reproductive efficiency in the studies reported is not known. Data collected under experimentally controlled herd conditions will be necessary to estimate these effects.

SUMMARY

Butterfat production per day of life was evaluated as a possible selection criterion for genetic improvement of dairy cattle.

The data provided little evidence of heterogeneity of the correlations of production to 36 mo. of age (and 305-day first lactation production) with production to 48, 60, 72 and 84 mo. of age. The predictive values of 36-mo. production and 305-day first lactation production to production at these same ages did not differ significantly.

Age at first calving had significantly less influence on total production to 84 mo. than on total production to 36 mo. of age.

Age at first calving and first 305-day lactation production accounted for 89 per cent of the variance in 36-mo. production and 41 per cent of the variance in 84-mo. production.

There was no evidence of heritability of production per day of life at any of the ages studied from 36 to 84 mo., nor was age at first calving indicated to be heritable. The estimate of heritability of first lactation butterfat production from these data was 0.30.

ACKNOWLEDGMENT

The authors are indebted to the Wisconsin State Department of Public Welfare for making available the records on which this study is based.

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A METHOD FOR DETERMINING FREE FATTY ACIDS IN MILK FAT[1]

LYDIA B. TUCKER AND E. W. BIRD
Iowa Agricultural Experiment Station, Ames

The method reported herein is a modification of the one previously presented by Breazeale and Bird (2). The modification was considered necessary since during World War II commercial absolute ethanol, purified as previously indicated (2), yielded ethanolic KOH solutions which turned brown in a short time and in which needle-shaped crystals formed. These crystals were particularly undesirable in the burette. In addition, the former method was difficult to use commercially because of the high cost of absolute ethanol and its virtual unobtainability.

METHODS

Three methods were compared: The A.O.A.C. method for refined oils (1) modified in that a 20-g. sample was employed, the method of Breazeale and Bird (2) and the method herein reported. With high acid samples, the sample sizes were reduced (table 1) so that the 10-ml. semi-automatic burette employed with the Breazeale and Bird method and the modification of this method would not have to be refilled so many times during a titration.

The modified method as finally used is as follows: Weigh 10 g. melted, filtered fat into a 125-ml. Erlenmeyer flask (Torsion moisture scale), add 25 ml. of solvent (800 ml. Skellysolve B + 200 ml. *n*-propanol) and 10 drops of 1 per cent phenolphthalein in purified absolute ethanol or in absolute methanol, and titrate the mixture to the phenolphthalein end point with 0.05 *N* absolute methanolic KOH. Run a blank, including all reagents but no fat, and subtract the blank value from the sample titration. Express the acidity as ml. 0.1 *N* KOH per 10 g. fat.

The NaOH and KOH solutions were standardized against standard HCl solutions. These were prepared from constant-boiling HCl and redistilled water. The c.p. concentrated HCl was refluxed over $K_2Cr_2O_7$ to oxidize Br or I before it was distilled. The standard solutions were standardized gravimetrically by precipitating the Cl^- as AgCl.

Regent grade absolute methanol was found to be satisfactory without purification.

RESULTS

Selection of a solvent. When methanolic KOH was employed to titrate 10 g. of milk fat in 25 ml. Skellysolve B, incipient turbidity began at 3 to 4 ml. additions of the KOH; very marked turbidity was evidenced at a 22-ml. addition of KOH. Butanol has been used successfully as a binder for ethanol in gasoline. It was considered that solvents which were mixtures of Skellysolve B and normal aliphatic alcohols might eliminate the turbidity indicated above.

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Because of the offensive odor of *n*-butanol, *n*-propanol was selected. Solvents containing different proportions of Skellysolve B and *n*-propanol were prepared.

TABLE 1

Comparison of three methods for the determination of the free fatty-acids in milk fat

Sample no.	Method 1[a]			Method 2[b]			Method 3[c]		
	Ml. 0.1 *N* alkali/ 10 g. fat	Max. diff.	Av.	Ml. 0.1 *N* alkali/ 10 g. fat	Max. diff.	Av.	Ml. 0.1 *N* alkali/ 10 g. fat	Max. diff.	Av.
1	0.73 0.74	0.01	0.735	0.76 0.75	0.01	0.755	0.73 0.73	0.00	0.730
2	0.80 0.80	0.00	0.800	0.82 0.82	0.00	0.820	0.82 0.83	0.01	0.825
3	0.66 0.65	0.01	0.655	0.67 0.67	0.00	0.670	0.66 0.67	0.01	0.665
4	1.11 1.15	0.04	1.130	1.17 1.17	0.00	1.170	1.15 1.15	0.00	1.150
5	1.02 1.04	0.02	1.030	1.02 1.02	0.00	1.020	1.01 1.01	0.00	1.010
6	1.09 1.09	0.00	1.090	1.11 1.11	0.00	1.110	1.11 1.10	0.00	1.100
7	0.63 0.62	0.01	0.625	0.68 0.69	0.01	0.685	0.66 0.66	0.00	0.660
8	1.19 1.19	0.00	1.190	1.27 1.26	0.01	1.265	1.23 1.23	0.00	1.230
9	26.94 26.89 26.79 26.79 27.34 27.34	0.55[d] 0.11[e]	27.015	27.42 27.42 26.80 26.90 26.85 26.74	0.68[d] 0.068[e]	27.022	27.17 27.15 26.84 26.89 27.05 27.05	0.33[d] 0.033[e]	27.025
10	24.90 24.90 24.85 24.90	0.05[d] 0.01[e]	24.888	24.89 24.64 24.79 24.89	0.25[d] 0.025[e]	24.803	24.80 24.80 24.75 24.91	0.16[d] 0.016[e]	24.815
11	37.41 37.38 37.44 37.44	0.06[d] 0.03[e]	37.418	37.31 37.53 37.42 37.53	0.22[d] 0.044[e]	37.448	37.26 37.47 37.52 37.52	0.26[d] 0.052[e]	37.443
12	34.82 34.85 34.82 34.82	0.03[d] 0.015[e]	34.828	34.97 34.97 34.97 35.19	0.22[d] 0.044[e]	35.025	34.82 35.07 34.62 34.67	0.42[d] 0.084[e]	34.795

[a] Method 1: A.O.A.C., (1) Sample 9 and 10, 2 g. and sample 11 and 12, 5 g. sample titrated.

[b] Method2: Breazeale and Bird, (2). Sample 9 and 10, 1 g. and sample 11 and 12, 2 g. sample titrated.

[c] Method 3: Modification of method 2, herein presented. Sample 9 and 10, 1 g. sample and sample 11 and 12, 2 g. sample titrated.

[d] Error calculated as of 10 g. fat.

[e] Actual error per titration, *i.e.* (error in footnote 4) $\times \frac{\text{g. titrated}}{10}$.

To 25 ml. of each mixture, 10 g. milk fat were added and the mixture was "titrated" with methanol to a permanent cloud-point at room temperature. The re-

sults of the experiment as presented in fig. 1. It is indicated that mixtures containing from 200 to 400 ml. of *n*-propanol in approximately 1 l. of solvent should yield the best results.

On the basis of the data of fig. 1, solvents containing *n*-propanol : Skellysolve B in the ratios 200 : 800 ml., 300 : 700 ml. and 400 : 600 ml. were used as fat solvents. A mixture of 10 g. milk fat and 25 ml. of solvent was "titrated" with 0.05 *N* methanolic KOH, without added indicator. Slight tendencies toward turbidity were observed with additions of KOH ranging from 3 to 20 ml. The point at which the slight turbidity occurred was a function of the solvent. In no case were these turbidities sufficiently strong to interfere with the end point. The slight turbidity was less intense with the mixture containing 800 ml. Skellysolve B and 200 ml. *n*-propanol. With all three mixtures, methanolic KOH could be added beyond the fleeting turbidity point to 150 ml. without encountering

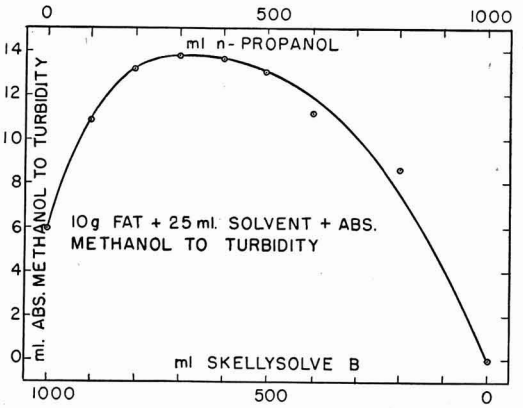

FIG. 1. Ml. absolute methanol required to produce turbidity in a mixture of 10 g. fat + 25 ml. solvent of composition indicated.

turbidity. No check was made beyond the 150-ml. addition. The mixture of 800 ml. Skellysolve B with 200 ml. *n*-propanol was selected as the solvent for use.

Comparison of methods. The data comparing the three methods are presented in table 1. Samples 1 to 8 are representative of good butter made from neutralized, sour cream. Samples 9 to 12 are representative of experimental butters prepared with special lipolytic cultures; they are not considered representative of butters found in the trade.

The results with samples 1 to 8 indicate agreement among the average values by the three methods to 0.02 to 0.03 ml., which is essentially the burette reading error. Checks between duplicates are better by the Breazeale and Bird method and by the modification of this method than they are by the A.O.A.C. procedure, despite the fact that the titration errors are halved in the A.O.A.C. method, since 20 g. of fat were titrated and the result as presented is based on 10 g. fat.

Among samples 9 to 12 the errors are in favor of the A.O.A.C. procedure if the titrations are based on a 10-g. sample (upper figure); the errors are about equal over the four samples among the three methods if placed on a per titration basis (lower figure). The average values indicate that there is little to choose among the methods.

SUMMARY AND CONCLUSIONS

A modification of the Breazeale and Bird method for determining the free fatty acids in milk fat is presented. The modification substitutes a Skellysolve B-*n*-propanol mixture for petroleum ether and absolute methanolic KOH for absolute ethanolic KOH as the titration medium.

The modified method checks its original satisfactorily; it likewise checks the A.O.A.C. method (for refined oils) within reading accuracy of the burette for normal butterfat samples. The checks between duplicates are better with this method than are those for the A.O.A.C. method, for normal samples.

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HORMONAL DEVELOPMENT OF MAMMARY TISSUE IN DAIRY HEIFERS

J. F. SYKES AND T. R. WRENN
Bureau of Dairy Industry, ARA, USDA, Washington, D. C.

In continuation of previously reported work (7) on hormonal development of the mammary glands of young dairy heifers, eight heifers have been injected with hormone combinations similar to those used in the previous study.

Two changes in technique were made. In the present work, injections were started when the heifers were 6 mo. rather than 1 mo. of age. It seemed possible that differences due to hormone treatment might be more evident in heifers of this age than in younger calves. The second change involved an increase in the progesterone dosage used. In the earlier work with heifers (7) no effect of progesterone on the type of tissue developed was observed. On the other hand, Mixner and Turner (6) and Folley (2) have indicated that progesterone is necessary for histologically normal development of mammary tissue in goats.

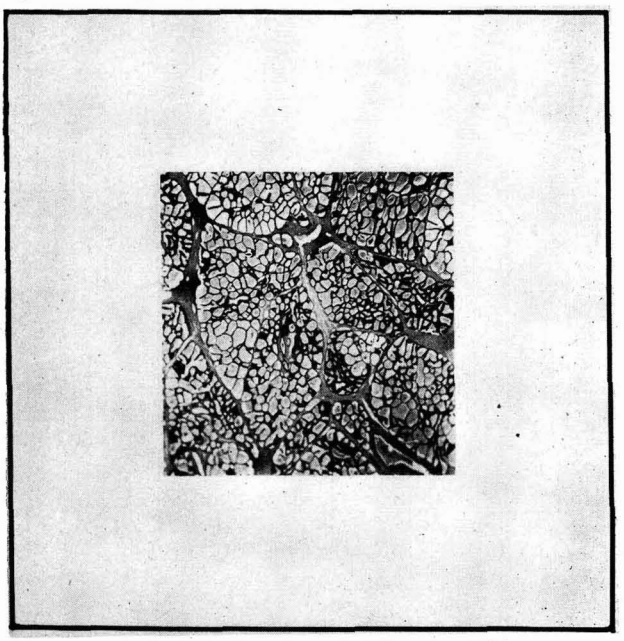

FIG. 1. Udder tissue from a lactating cow. (13×)

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The progesterone: estrogen ratio used by Mixner and Turner (6) in their work with goats was 1 mg. progesterone to 5 γ of stilbestrol, whereas a ratio of 1 mg. of progesterone to 50 γ of stilbestrol had been used in the USDA experiments. In the present study the dosage of progesterone was increased to give a ratio of 1 mg. of progesterone to 25 γ stilbestrol. This ratio had been shown to be satisfactory for experimental mammary gland development in mice (5).

PROCEDURE

Four pairs of heifers were injected with the following hormone preparations: Pair 1, stilbestrol; pair 2, stilbestrol and pituitary gland extract; pair 3 stilbestrol

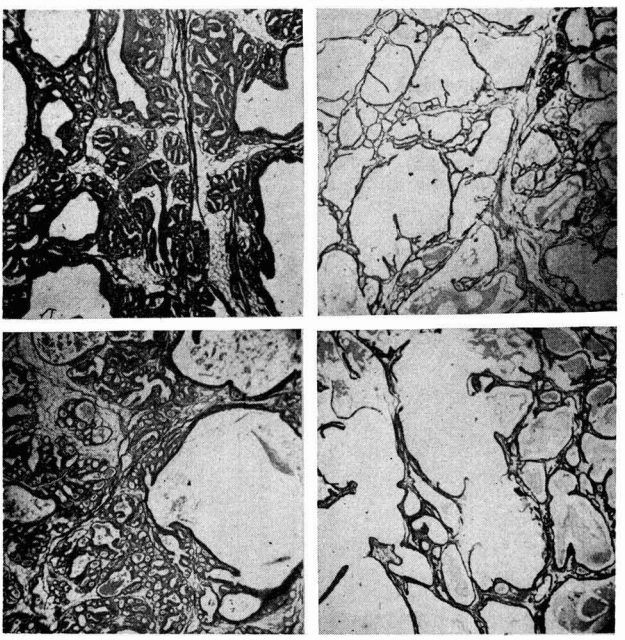

FIG. 2. Representative sections of udder tissue from heifers injected with stilbestrol. (13 ×)

and progesterone; and pair 4, stilbestrol and progesterone and pituitary gland extract. All preparations were injected subcutaneously. Stilbestrol was injected at the rate of 6 mg. per week, progesterone at the rate of 240 mg. per week and pituitary extract at the rate of 90 mg. per week. The pituitary extract was prepared from fresh frozen anterior pituitary tissue by the method of Bergman and Turner (1) for their "initial extract." Injections were given for 5 mo., from 6 to 11 mo. of age.

Examination of the mammary glands for secretion was made at monthly in-

tervals. At autopsy the udder tissue was fixed in Bouin's fixative. After removal of the udder, the fixative was injected into the teat canals until the gland was firmly distended and the teats were tied off to prevent escape of fixative. The whole gland then was immersed in fixative. After fixation the gland was sliced into sections and representative samples of tissue from all parts of the gland were obtained for histological examinations.

RESULTS AND DISCUSSION

Marked differences occurred in the type of udder tissue which resulted from the several hormone treatments. Abnormal tissue was present in varying degree

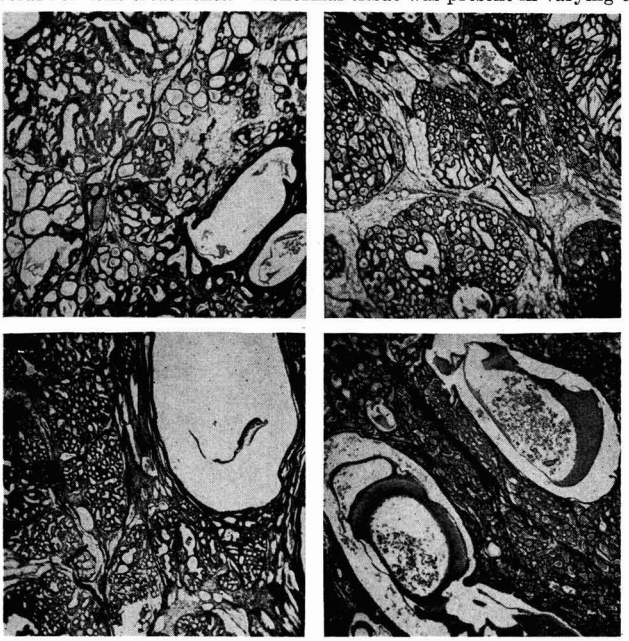

FIG. 3. Udder tissue from heifers injected with stilbestrol plus pituitary extract. (13×)

depending on the hormone treatment. For purposes of comparison a section of normal tissue from the udder of a lactating cow is presented in fig. 1.

When stilbestrol only was injected, the type of tissue developed was definitely abnormal, as seen in fig. 2. Distension of ducts and alveoli was very marked. The fixed udders were very porous on gross examination. Few of the alveoli were normal. Lobule formation was indistinct and lobules frequently appeared crowded and distorted by the widely dilated ducts. The general picture suggested mainly marked stimulation of ducts.

When pituitary extract was injected in addition to stilbestrol, some improvement in lobule formation and in the normality of the alveoli resulted. The udders still were moderately porous on gross examination. The types of tissue seen are shown in fig. 3. Duct distension and crowding and distortion of lobules were seen frequently. Many lobules consisted largely of terminal ducts. There was considerable distension of alveoli, but this was much less marked than in the previous pair of heifers. Areas showing thickened alveolar walls with papillae were frequent.

On the other hand, when progesterone or progesterone plus pituitary extract

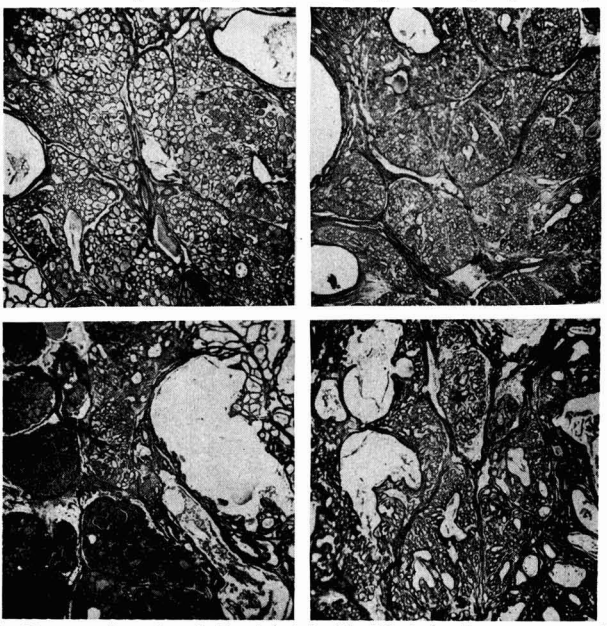

FIG. 4. Mammary tissue from heifers injected with stilbestrol and progesterone. (13 ×)

was injected with stilbestrol, many areas of normal tissue were observed. With one exception, the udder tissue seemed compact on gross examination and the udders were closely attached to the body wall and were less pendulous than those of the two previously described pairs of heifers. The types of tissue seen are shown in fig. 4 and 5. The essential normality of the lobule formation and of the alveoli which was frequently seen in these udders can be noted by comparison with fig. 1. There were, however, sections which showed abnormal development, consisting of distended ducts, moderate alveolar distension and distortion of lobules; in one heifer which received stilbestrol plus progesterone, considerable

tissue of this sort was seen. Areas of moderately distended alveoli with thickened walls and papillae were seen in the udder tissue of those heifers that were injected with pituitary extract in addition to stilbestrol and progesterone.

These results definitely indicate that progesterone is necessary for normal mammary gland development in dairy heifers. The fact that abnormal tissue was found in the udders of heifers receiving progesterone may indicate that the optimal progesterone to estrogen ratio was not attained in these experiments. The results further indicate that pituitary hormones tend to prevent the development of abnormal tissue induced by stilbestrol. (Compare figs. 2 and 3.)

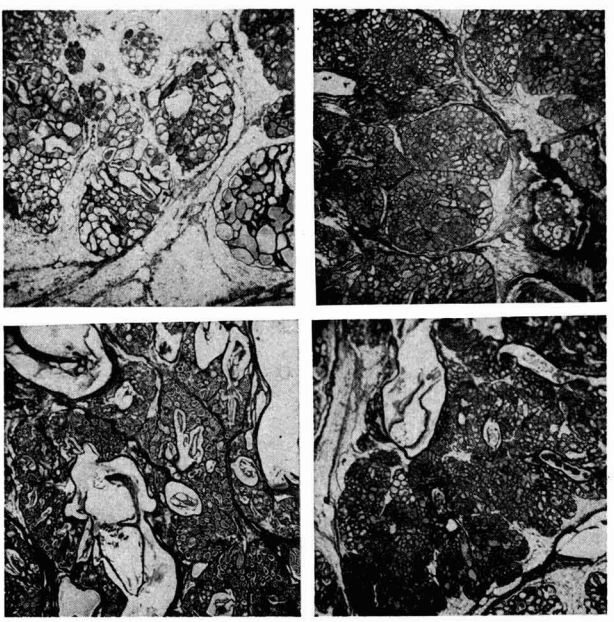

FIG. 5. Mammary tissue from heifers injected with stilbestrol, progesterone and pituitary extract. (13 ×)

The amount of milk secretion obtained from these hormonally developed udders was always small. This might be expected since the heifers were continuously under hormone influence. Folley and Malpress (3) and Hammond and Day (4) have shown, in experiments in which mammary growth and lactation were induced by estrogen treatment, that maximum lactation responses frequently were not obtained until after estrogen withdrawal. Mixner and Turner (6) also observed that no secretion was obtained in goats as long as progesterone was being injected. The maximum amount of milk obtained from these experimental

heifers varied from 200 to 700 ml. With one exception—this in a heifer with fairly large amounts of abnormal tissue—no secretion beyond 25 ml. of serum-like fluid was obtained from any of the heifers which were injected with progesterone. The amount of secretion obtained therefore was inversely proportional to the normality of the tissue developed.

Folley and Malpress (3) have suggested that the lactation responses obtained from estrogen implants were limited by incomplete mammary development due to a deficiency of progesterone. The present experiment indicates that a more normal development occurs when progesterone is administered in addition to estrogen. The conditions of this experiment, for reasons noted above, have made it impossible to fully determine the relative lactational ability of the udders developed under the influence of progesterone as compared to those developed without progesterone. Further experiments to settle this point are desirable.

SUMMARY AND CONCLUSIONS

Mammary gland tissue in dairy heifers that had been given stilbestrol injections was observed to be abnormal in structure.

Progesterone, and to a lesser extent pituitary extract injected in conjunction with stilbestrol, resulted in more normal development and thus demonstrated the necessity of these hormones for normal udder development in heifers.

The ratio of progestrone to stilbestrol necessary for normal development of the udders of dairy heifers appears to be somewhat in excess of 1 mg. of progesterone to 25 γ of stilbestrol.

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SUSTAINED ELEVATION OF BLOOD LIPIDS AND EFFECT UPON MILK PRODUCTION OF RUMINANTS GIVEN A SURFACE-ACTIVE AGENT INTRAVENOUSLY[1]

A. C. CHUNG AND J. C. SHAW

Department of Dairy Husbandry, Maryland Agricultural Experiment Station, College Park

Kellner *et al.* (2) reported that intravenous injections of a detergent resulted in marked elevations of the blood cholesterol and blood phospholipids of rabbits on a normal diet. It was stated that a single injection of Triton A-20 increased blood cholesterol and phospholipids to more than five times normal. No reference was made to other lipid fractions.

It was deemed important to determine whether the same blood lipids of ruminants would react in a similar fashion and also to find out whether blood lipids other than cholesterol and phospholipids would be affected. Since the neutral fat of blood is important in the formation of milk fat (1, 3, 4, 7), it appeared that if the blood lipids, particularly neutral fat, could be maintained at a high level for an extended period of time it would provide a means of studying the possible relationship between the level of blood lipids and the secretion of milk.

EXPERIMENTAL

A surface-active agent (Triton WR 1339[2]) was diluted to 12 per cent in distilled water after each had been sterilized separately in a steam sterilizer. This solution was injected intravenously into two non-lactating goats and one lactating cow. The total blood plasma lipids and the various lipid fractions were determined daily by the extraction and acidometric procedure described by Saarinen and Shaw (6).

The first goat received 80 ml. of the 12 per cent solution of 320 mg. of Triton WR 1339 per kilogram of body weight. The blood plasma remained clear and there was a 30 per cent decrease in plasma cholesterol within 24 hr. The second goat was injected with 100 ml. of the 12 per cent solution or 430 mg. of Triton WR 1339 per kilogram of body weight. Within 24 hr. the plasma presented a milky appearance. The effect of Triton WR 1339 upon the various blood plasma lipids of the second goat during the 3 days following the injection is shown in figure 1. The total lipids increased by more than six times normal. The greatest increase was in the lipid fraction made up of neutral fat, other glycerides and free acids, which will be referred to as the neutral fat portion. This fraction increased to 20 times normal. Total blood plasma cholesterol increased about to four times and ester cholesterol about to three times normal. The free fatty acids in phospholipids increased about 80 per cent.

The lactating cow received 110 per cent of her net energy requirements computed according to the Morrison (5) standards for 2 wk. prior to and throughout

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[2] Furnished by Rohm and Haas Co., Philadelphia.

two injection periods. Milk weights were recorded and butterfat tests made at each milking. In the first study 1,000 ml. of the solution were injected followed by another injection of 1,280 ml. 3 days later. These were equivalent to 194 and 248 mg., respectively, of Triton WR 1339 per kilogram of body weight. The results are shown graphically in figures 2 and 3. There was some decrease in all blood plasma lipid fractions except fatty acids in phospholipids within the first 24 hr. and these fractions remained below the initial levels until after the second injection. The slight increase in total fat during the second day was due to the phospholipids. During this period the blood plasma became translucent. Following the second injection the plasma became milky and all of the fractions except ester cholesterol exceeded the pre-injection levels. The greatest increase was in neutral fat which increased to six times normal.

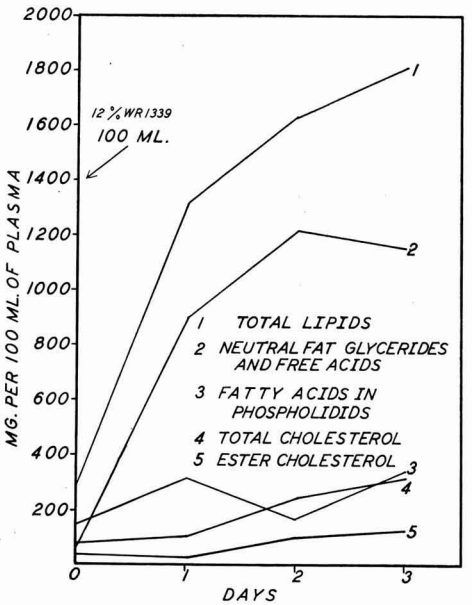

FIG. 1. Blood plasma lipids of a goat given 100 ml. of 12% Triton WR1339 intravenously.

Twenty days after the first study with the cow was initiated, 1,790 ml. of the 12 per cent solution were injected into the same animal, followed by 700 ml. within 24 hr. These were equivalent to 328 and 128 mg., respectively, of Triton WR 1339. The blood plasma became milky and opaque and remained so for 5 days, after which it became translucent for several days. There was a sharp drop in all lipid fractions except phospholipids within 3 hr. (figs. 4 and 5).

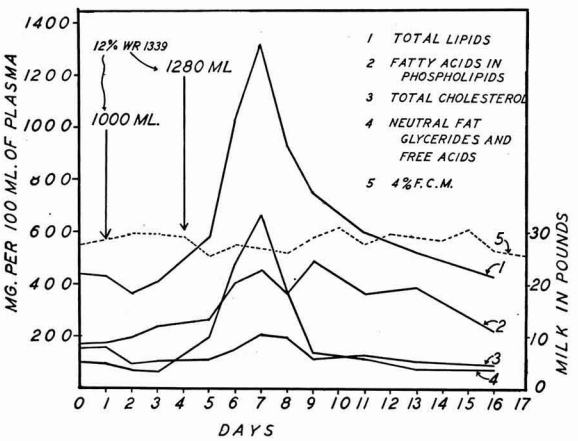

FIG. 2. Blood plasma lipids and 4% F. C. M. of a cow given intravenous injections of Triton WR1339 (series 1).

Twenty-four hr. after the first injection all fractions except cholesterol had increased above the pre-injection levels. Following the second injection, all lipid fractions except the phospholipids exhibited a marked rise. The neutral fat fraction increased to 17 times normal, both free and ester cholesterol to four

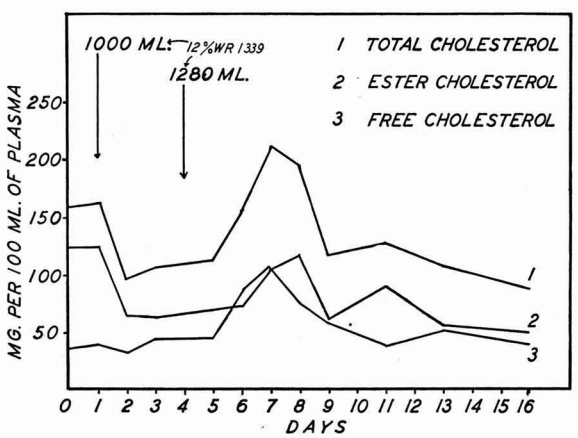

FIG. 3. Blood plasma cholesterol fractions of a cow given intravenous injections of Triton WR1339 (series 1).

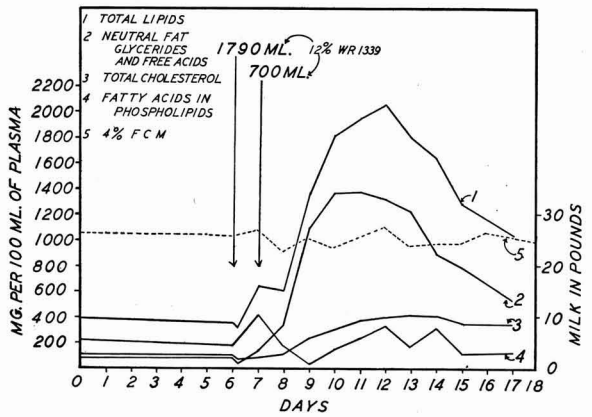

FIG. 4. Blood plasma lipids and 4% F. C. M. of a cow given intravenous injections of Triton WR1339 (series 2).

times normal and the total lipids to five times normal. The phospholipid values were erratic, the highest level being a 50 per cent increase above normal.

The milk production expressed as 4 per cent fat-corrected milk did not show any appreciable change during either of the two studies, as will be noted in figures 2 and 4. The total milk production and per cent fat in the milk during the experimental periods are shown in table 1, along with the total plasma lipids. It will be noted that the large increase in total plasma lipids exerted very little influence on milk and milk fat production. In the second period there was a de-

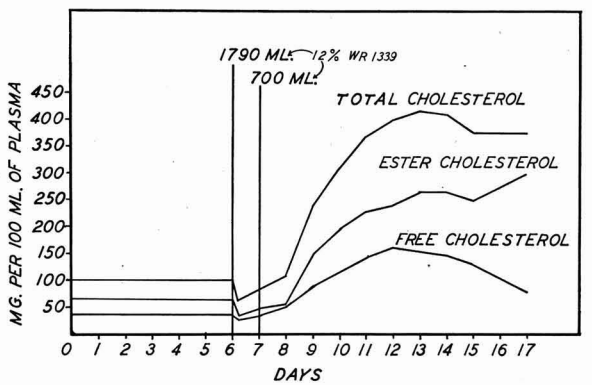

FIG. 5. Blood plasma cholesterol fractions of a cow given intravenous injections of Triton WR1339 (series 2).

pression of total milk amounting to 3 to 4 lb. for a few days but no appreciable change in total fat.

DISCUSSION

The Triton WR 1339, an alkyl aryl polyether alcohol, used in this study is the same surface-active agent which was used by Kellner *et al.* (2). The results reported herein differ from those reported by Kellner *et al.* on rabbits in that the phospholipids of cows' blood was not affected nearly as much as blood cholesterol. Also, the greatest effect was upon the neutral fat fraction.

The level of blood plasma lipids obtained in the cow (2,049.2 mg. per cent) was far greater than any recorded to date. During the second series, the blood

TABLE 1

The effect of a sustained elevation of the blood lipids of a cow upon milk production and per cent fat in milk

	Series 1				Series 2			
Days before and after injection	Surface active agent[a]	Total plasma lipids	Milk	Fat	Surface active agent[a]	Total plasma lipids	Milk	Fat
	(*ml.*)	(*mg. %*)	(*lb.*)	(%)	(*ml.*)	(*mg. %*)	(*lb.*)	(%)
−3	.....	.....	31.0	3.40	.....	392.0	.....	.....
−2	.....	.....	29.7	3.55	.....	.....	.....	.....
−1	.....	431.2	.....	.....	.....	.....	27.6	3.60
0	1,000	428.9	32.4	3.40	1,790	347.6	27.8	3.55
1	.....	360.2	29.3	3.60	700	628.7	27.7	3.75
2	.....	406.0	29.4	3.70	.....	606.0	24.0	3.60
3	1,280	.....	32.0	3.55	.....	1,347.7	25.3	3.95
4	.....	575.9	31.2	3.68	.....	1,811.5	23.5	3.96
5	.....	1,027.6	31.4	3.55	.....	1,945.0	25.9	3.85
6	.....	1,322.9	28.8	3.20	.....	2,049.2	26.6	4.15
7	.....	930.1	31.7	3.13	.....	1,795.6	23.9	3.95
8	.....	743.8	28.6	3.70	.....	1,614.3	25.4	3.70
9	.....	598.6	29.6	3.20	.....	1,271.23	24.4	4.0
10	.....	565.6	30.8	3.68	.....	.....	26.4	4.1
11	.....	425.2	31.6	3.90	.....	1,031.5	26.2	3.8
12	.....	.....	28.8	3.73	.....	.....	.....	.....
13	.....	.....	31.8	3.60	.....	.....	.....	.....
14	.....	425.2	30.1	3.80	.....	.....	.....	.....

[a] 12% solution of Triton WR1339.

plasma lipids were maintained above 1,000 mg. per cent for at least 9 days. This high level of blood lipids did not exert any marked effect upon milk or milk fat production. There was some decrease in milk production but not in milk fat during the second series. It could be postulated that the surface-active agent inhibited milk fat secretion due to a deleterious effect upon the enzyme systems involved in milk fat formation. However, in this case it would have been expected that total milk fat production would decrease. Perhaps an effect on milk fat production could have been brought about by maintaining the high blood lipid level for a longer period of time.

CONCLUSIONS

The intravenous injection of a surface-active agent resulted in a marked increase in all plasma lipids except phospholipids in one of two goats and a cow.

In one case the total plasma lipids of the cow increased to five times normal, neutral fat increased to 17 times normal and both free and ester cholesterol increased to four times normal. Total plasma lipids reached 2,049.2 mg. per cent and remained above 1,000 mg. per cent for 9 days without producing an increase in milk fat production.

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THE EFFICIENCY OF ARTIFICIAL BREEDING WITH BULL SEMEN ONE TO THREE DAYS OLD

ERIC W. SWANSON
University of Tennessee, Knoxville

There is considerable variation in the length of time bull semen is used routinely in artificial breeding. One recent survey (2) showed that out of 70 associations, semen was collected and shipped daily by 24, while 46 used each day's collection for 2 to 3 days. One of the main reasons usually given for daily collection is that it will maintain a higher conception rate. At the same time this practice requires keeping many more bulls than would less frequent collection. In such instances there may be an economical choice between fewer, higher quality sires with lower upkeep expense and slightly higher conception rate. In order to evaluate these factors properly, accurate information concerning the effect of age of semen upon breeding efficiency is required.

The early observations concerning the effect of age of semen upon conception rate have been reviewed by Schultze *et al.* (3). The usual observation was a progressive decrease in breeding efficiency with age of semen. The actual differences found in early reports are hard to evaluate in terms of present-day artificial breeding because of the few services and the different methods of handling semen. Schultze *et al.* (3) compared the 4-mo. non-return rate of semen used 1 to 4 days after collection in 13 Nebraska artificial breeding locals. They found 2-yr. averages of 63.2, 57.9, 53.3 and 48.7 per cent for 1 to 4 days, respectively; their data indicated a linear regression of conception rate on age with 4.61 per cent decline per day. Bulls averaging below 55 per cent declined 6.3 per cent per day, while those averaging above 60 per cent declined only 4.3 per cent daily. As experience and improved methods were progressively raising 6-mo. average non-return rates from 52.5 to 59.2 per cent, the average daily decline decreased from 5.3 to 3.7 per cent. A similar study conducted in Washington by Erb *et al.* (1) showed no difference between non-return rates of 1- and 2-day-old semen, both of which averaged 64 per cent, but a drop to 59 per cent for 3-day-old semen. In neither of these studies did all locals use semen on each of the 3 and 4 days averaged.

MATERIALS AND METHODS

Data for this investigation were secured from the records of the East Tennessee Artificial Breeders' Association, Inc. This association began inseminating operations in November, 1947, with three bulls each of Jersey, Guernsey and Holstein-Friesian breeds. Semen was collected 3 days each week, and rotation was arranged so that each bull was not used the same day every week. Semen was collected before 6 a.m. and was used by all associations the day of collection as well as the first and second day after collection. After little more than 1 yr.

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of operation, this association experienced a rapid increase in growth and a change in management. Semen then was collected in late afternoons and shipped early the next morning every other day so that all associations used semen the first and second day after collection, but services with 3-day-old semen were negligible. For this reason an analysis has been made of the data from each period separately.

Semen was collected with a short artificial vagina into a warm sterile glass tube and diluted immediately after collection with egg yolk-citrate diluter at 90 to 95° F. The diluted semen was placed in paper-insulated test tubes for shipment and cooled in these tubes in air at 45° F. for 2 hr. before packaging with a can of ice in insulated cardboard boxes. No bactericidal substances were added to the semen the first year. Penicillin and streptomycin were used during the second period. Semen was shipped in separate tubes for use each day.

TABLE 1

Services and non-return rates by bulls of semen used for insemination 3 days after collections

Bulls	Semen used 1st day (5–13 hr.)		Semen used 2nd day (29–37 hr.)		Semen used 3rd day (53–61 hr.)	
	Serv.	Non-ret.	Serv.	Non-ret.	Serv.	Non-ret.
	(*no.*)	(%)	(*no.*)	(%)	(*no.*)	(%)
J-1	298	58.4	273	56.4	332	52.7
J-2	299	64.2	305	61.0	88	54.5
J-3	267	62.5	321	62.9	117	52.1
G-1	173	64.2	190	64.2	177	65.0
G-2	182	63.2	185	56.8	101	46.5
G-3	124	62.9	130	47.7	58	41.4
G-4	38	71.0	47	63.8	22	50.0
H-1	116	65.5	181	65.2	135	58.5
H-2	126	71.4	126	81.0	36	80.6
H-3	130	61.5	194	66.5	98	60.2
Totals	1,753	63.3	1,952	62.0	1,164	55.7

All first and second services in the periods studied were recorded. Rebreedings to those services were recorded up to 150 to 180 days. The number of non-returns to first and second services was converted to percentage, giving a 5- to 6-mo. non-return rate. Tests of statistical significance included the Chi-square test applied to the returns and non-returns, and the analysis of variance of percentages converted to angles according to Snedecor (4).

RESULTS

During the period of early morning collections, all locals received semen in time to use it the same day, and in most instances the first-day inseminations were completed before the semen was 12 hr. old. A comparison of the first, second and third day breeding under this regime is presented in table 1, with the data separated according to bulls. The 1.3 per cent difference between the first and second day is not statistically significant. However, the 6.3 per cent decline the

third day is highly significant. Although the average non-return rates of the bulls did not vary much the first day, differences became quite marked with the older semen. Semen of bulls G-1, H-2 and H-3 was practically as efficient the third day as it was the first day, but others dropped nearly 20 per cent in non-return rate in the 3 days.

Following management changes, it was of interest to compare first and second-day service efficiency again. The body of data also was large enough to try to evaluate differences between locals and seasons as well as bulls. A period was selected during which ten well-established locals serviced by the association had experienced inseminators. The monthly differences observed, therefore, would not be due to training. The services and non-returns are summarized by locals in table 2.

TABLE 2

Services and non-return rates of 10 locals using all semen for 2 days after collection, December, 1949, to December, 1950, inclusive

Local assoc.	Semen used 1st day (18–26 hr.)		Semen used 2nd day (42–50 hr.)		Mo. (of 13) that 2-d. N.R. exceeded 1-d. N.R.
	Serv.	Non-ret.	Serv.	Non-ret.	
	(*no.*)	(%)	(*no.*)	(%)	(*no.*)
Blount	547	66.7	504	60.7	5
Bradley	1,179	57.5	1,046	60.8	11
Greene	739	59.4	721	57.7	5
Hamblen	412	66.0	371	60.9	4
Hawkins	428	70.8	437	59.3	3
Jefferson	816	60.3	822	59.4	7
Knox	932	64.5	1,017	56.8	3
Monroe	616	63.5	581	60.9	6
Sullivan	773	60.0	974	53.9	2
Washington	497	52.7	461	52.9	6
Totals	6,941	61.5	6,934	58.2	40%

The average difference in non-return rate between first- and second-day semen (here about 13 hr. older than in the earlier study) was 3.3 per cent, compared to 1.3 per cent the first year. This difference was highly significant statistically ($P < 0.01$). The difference between locals was statistically significant. It was postulated that some of the local differences might be due to shipment of the semen or to mishandling by the inseminator. Although this is a plausible explanation of local differences, it probably is not significant in this study because the local (Knox) with the second greatest daily decline in efficiency received semen at the bull stud. The local with the least decline (Bradley) was at one of the furthest shipping points.

The differences between months were not statistically significant. There was no indication of a seasonal trend in differences between first- and second-day services. The greatest difference was in June and the least in August. In 40

per cent of the local-month averages the non-return rate of second-day services exceeded or equalled that of first-day services.

The differences between bulls were statistically significant. Four of the 21 bulls had higher efficiencies with second-day services than with first-day services. Five bulls also had more than 6.0 per cent decline from first-day to second-day non-returns. Ten bulls with first-day non-return averages below 60 per cent averaged 56.93 per cent the first day and 53.47 per cent the second. The remaining eleven bulls averaged 65.37 per cent non-return the first day and 61.73 per cent the second. The difference in decline between the low group (3.46 per cent) and the high group (3.64 per cent) was not significant.

DISCUSSION

Good quality bull semen can be used for 2 days with only negligible differences in non-return rates if the second-day inseminations are completed within 37 hr. of collection. These observations are in agreement with those of Erb *et al.* (1) when the semen was collected in early morning and used the first day. When use of the semen the first day was delayed until 18 hr. past collection, the decline in non-return rate the second day was more than twice as large and statistically significant. The decline in this instance was not as great, however, as was the decline from the second to third day where the third-day semen was used during 53 to 61 hr. after collection, and was not as great as the 3.7 per cent average daily decline noted by Schultze *et al.* (3) in the last 6 mo. of their study. This is an indication that the decrease in breeding efficiency of bull semen is not a direct linear function of time from collection, but that the differences due to age are very small in the first hours and become progressively greater as the semen exceeds 36 hr. in age.

If semen can be distributed for use the same morning it is collected, there will be no significant increase in average breeding efficiency by using semen only 1 day instead of two. However, if semen cannot be delivered for use until the major part of a day following its collection a slight advantage in breeding efficiency would result from using semen only the first day.

SUMMARY

The effect of age of semen upon breeding efficiency has been investigated in an artificial breeding association during two management periods. During the first period, semen of all bulls was used for 3 days at ages of 5 to 13, 29 to 37 and 53 to 61 hr. and resulted in respective non-return rates of 63.3, 62.0 and 55.7 per cent. During the second period, all semen was used 2 days at ages of 18 to 26 and 42 to 50 hr. with respective non-return rates of 61.5 and 58.2 per cent. Differences between bulls and associations were significant, but no effect of season was noted. Bulls varied greatly in the decline of their breeding efficiency with age of semen but rapid decline was not associated with either a low or a high first day non-return rate.

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EXPERIMENTAL ALTERATION OF THE SUGAR AND KETONE LEVELS OF THE BLOOD OF RUMINANTS IN RELATION TO KETOSIS[1, 2]

L. H. SCHULTZ

Department of Animal Husbandry, Cornell University, Ithaca, N. Y.

AND

VEARL R. SMITH

Department of Dairy Husbandry, University of Wisconsin, Madison

Ketosis in ruminants is characterized by two major changes from the normal in the blood composition. One is an increase in ketone bodies; the other is a decrease in blood sugar. This inverse relationship has been demonstrated by numerous workers (15, 16, 19).

The purpose of this study was to determine the effect of experimental alteration of the blood level of each of these substances upon the blood level of the other in an attempt to clarify some of our ideas regarding the underlying causes of ketosis in ruminants and the relative importance of the changes of the two blood constituents. Part of this work was reported upon earlier in abstract form (18).

Many workers have experimentally altered the blood sugar levels of ruminants. It is well known that insulin will cause a decrease in the blood sugar level. Brown *et al.* (4) produced an artificial hypoglycemia in cows by injecting 200 units of insulin every 4 to 6 hr. Cutler (6) produced hypoglycemia in goats with insulin injections and noted that large doses were needed to produce hypoglycemic shock. Laboratory animals and humans have been shown to develop a ketonuria when made hypoglycemic with insulin. Collip (5) was the first to make this observation with rabbits. Somogyi (21) suggests that, in the human, insulin exerts two opposite effects upon the blood ketone level; for some time after injection it causes a decrease, but after protracted states of hypoglycemia it effects a rise. Drey (7) has reviewed this same phenomenon in human diabetics.

Alloxan, a drug which produces lesions of the pancreas, is known to cause a permanent hyperglycemia in ruminants. Dye and Wood (8) and Jarret (11) produced hyperglycemia in sheep by the intravenous injection of alloxan in dosages of 90 to 116 mg. per kilogram of body weight. The response was a triphasic one, with an initial hyperglycemia, a secondary hypoglycemia and a subsequent maintained hyperglycemia. Ketosis of varying degrees accompanied the condition. McCandless and Dye (12) were not able to produce a typical diabetic condition in the calf with alloxan.

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[1] Part of these data were taken from a thesis submitted by L. H. Schultz to the Graduate School of the Univ. of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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

The two methods used in this study for experimentally increasing blood ketones involved the administration of ketogenic fatty acids and the direct administration of the ketone bodies themselves. The authors (17) have previously shown that blood ketones can be increased in ruminants by the administration of certain fatty acids, particularly butyric and caproic. The effect of the direct administration of ketone bodies upon blood sugar has been studied in laboratory animals with limited and contradictory results. Nath and Brahmachari (14) found that injection of acetoacetic, betahydroxybutyric or pyruvic acid into rabbits caused an increase in blood sugar in all cases. Tidwell and Axelrod (22), working with rats, found that the administration of large doses of acetoacetate caused hypoglycemia even after a single dose.

EXPERIMENTAL

Animals. Because of their adaptability to this type of experimentation, goats were used in all of the studies. Part of the work was done at the University of Wisconsin and part at Cornell University. This report involves a total of 43 trials using ten different goats. Animals were used for more than one trial only after complete recovery from any previous treatment. The goats used were all females weighing 70 to 95 lb. Some were milking and others were not. All were fed normal rations.

Analytical. All blood samples were taken from the jugular vein. Blood proteins were precipitated by the method of Folin and Wu (9). Blood sugars were determined by the Benedict (2) method. Blood ketones were distilled according to the method of Behre and Benedict (1) and the acetone was determined colorimetrically by the method of Block and Bolling (3).

Alteration of blood sugar levels. Blood sugars were lowered experimentally by means of insulin. Preliminary trials were conducted on two goats using unmodified insulin (Lilly). A dosage of 20 units was injected subcutaneously into each goat every 6 hr. for the first 2 days and every 8 hr. for 5 more days. Later, the same experiment was repeated using protamine zinc insulin (Lilly) injected once a day for a period of 10 days. Dosage varied from 20 to 80 units, depending upon the response. Blood sugar and blood ketone levels were followed, blood samples being taken just prior to the insulin injections.

Alloxan was used to increase the blood sugar level experimentally. Injection was made intravenously at the rate of 90 mg. per kilogram of body weight. Blood sugar and blood ketone levels were followed at short intervals the first day and daily thereafter. Only one goat was used on this trial because this treatment necessitates eventual sacrifice of the animal.

Alteration of blood ketone levels. The first method used to increase blood ketone levels experimentally was the administration of certain ketogenic fatty acids, namely butyric, caproic, caprylic and capric. Acetic and propionic acids were used for comparison, since previous experiments had shown them to be essentially non-ketogenic for ruminants. The dosage level was 15 g. for the single doses and 100 g. for continuous administration. Acetic, propionic, butyric and caproic acids were diluted with water and administered by stomach tube or

rumen puncture. Caprylic and capric acids were given by capsule. For the single dosages, blood samples were taken prior to administration and at short intervals afterward for a period of 4 hr. Only blood sugars were determined on most of these samples, since previous work (17) had established the effect on blood ketone level. Acetic, propionic and butyric acids were used for continuous administration over a 2.5-hr. period. One hundred g. of acid were diluted to 1 l. with water. A rumen puncture was made with a 16-gauge needle. A 6-in. blunt needle was inserted through the needle used to make the puncture. The solution administered was allowed to drip into the rumen from an intravenous outfit and an overhead bottle. Blood sugar and blood ketones were followed at short intervals during and after the administration.

The second method used for experimentally increasing blood ketone levels was the administration of the ketone bodies themselves. In the first trial sodium acetoacetate was used. It was prepared by hydrolyzing the ethyl ester with NaOH. Five hundred ml. of solution containing 40 g. of acetoacetate (by analysis) were administered by stomach tube. Blood sugar and ketone levels were followed at regular intervals for an extended period of time. Since this material proved rather toxic, the experiment was repeated using 50 g. of ethyl acetoacetate instead of the sodium salt. The ethyl ester also was injected subcutaneously at a dosage level of 20 g. for one injection. Betahydroxybutyric acid also was given by stomach tube after dilution with water and was administered subcutaneously in one injection of 10 g. In another series of experiments acetone was used. In the first trial, 50 g. of acetone were diluted with water and administered by stomach tube. In another trial, the acetone was injected directly into the blood stream without dilution. Fifteen ml. were injected every 8 hr. for a period of 56 hr. Blood sugar and blood ketones were followed at regular intervals in all of these studies.

RESULTS

Insulin. Results of the administration of protamine zinc insulin appear in table 1. Goat no. 1 was a milking goat being fed fairly heavily on grain. Except

TABLE 1

Blood sugar and total blood ketone levels following daily subcutaneous injection of protamine zinc insulin

Days	Goat no. 1			Goat no. 2			Goat no. 3		
	Dosage	Sugar	Ketones	Dosage	Sugar	Ketones	Dosage	Sugar	Ketones
	(*units*)	(*mg. %*)	(*mg. %*)	(*units*)	(*mg. %*)	(*mg. %*)	(*units*)	(*mg. %*)	(*mg. %*)
0	80	47.3	2.3	60	43.9	2.4	60	46.5	3.0
1	80	40.2	.....	60	17.8	2.6	60	13.3	2.2
2	80	16.8	2.4	50	14.8	2.7	50	12.1	2.3
3	80	16.0	2.8	40	14.3	1.7	40	9.0	1.5
4	80	15.2	2.2	40	31.8	1.4	40	17.3	1.8
5	80	56.9	2.0	20	26.4	2.0	20	12.1	2.2
6	80	43.8	1.9	40	59.0	1.6	20	12.3	1.4
7	80	8.6	2.0	40	14.6	2.2	20	20.7	1.1
8	80	25.1	1.5	.....	27.9	2.0	Died		
9	.....	11.4	1.4	.....	63.7	1.8			
10	.....	58.0	1.8	.....	55.2	2.5			

for one day, sugar levels were kept at low ketosis levels for a period of 9 days with a daily injection of 80 units of protamine zinc insulin. There was no increase in blood ketones. Goat no. 2 was a non-lactating goat and was fed only fair quality hay during the trial. Less insulin was needed here to maintain the sugar at low levels. Insulin dosage was regulated by the appearance of the animal, incoordination being one of the first symptoms of insulin overdosage. Goat no. 3 was handled in the same manner as the previous goat, except that she was given less insulin as the trial progressed. Despite this, the animal died of insulin shock on the eighth day of the trial. In none of these goats did the blood ketone level exceed the normal, despite the prolonged low blood sugar. The same thing was true in the preliminary trials using unmodified insulin. The data on these trials are omitted because the response in blood sugar was more erratic than with protamine zinc insulin.

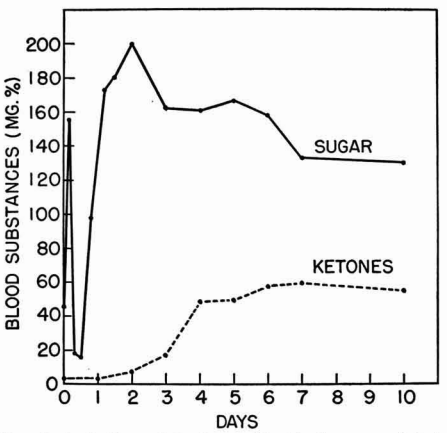

FIG. 1. The effect of alloxan injection on the blood sugar and ketone levels.

Alloxan. The results of alloxan administration are shown in figure 1. The typical triphasic response found by other workers in sheep was observed. First, there was an increase in blood sugar, then a decrease and finally, a sustained high blood sugar. There was a gradual but marked increase in blood ketones. For the first few days the animal ate normally and appeared normal but then the appetite began to fail and a weakness and lethargy developed. On the 11th day insulin was given but it had very little effect and the animal died on the 14th day post injection. These data illustrate the fact that it is possible to have a typical diabetic condition in the ruminant, with both blood sugar and ketones at high levels. This situation resembles the ketosis of humans and laboratory animals, but apparently none of the field cases of ketosis in ruminants are of this type.

Fatty acid administration. The effect of the oral administration of single doses of various acids upon the blood sugar levels of goats is shown in table 2.

TABLE 2

Blood sugar levels following administration of 15 g. of various acids to goats

Time after administration	Acids administered					
	Acetic	Propionic	Butyric	Caproic	Caprylic	Capric
	Blood sugars[a]					
(*hr.*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
0	48.8	49.2	47.7	46.0	47.9	44.1
0.25	51.8	72.0	83.2	65.3	49.9	47.2
0.5	52.2	53.3	58.0	61.1	41.9	43.1
1	51.1	43.4	22.5	34.8	37.9	41.8
1.5	51.7	49.8	24.8	30.0	46.5	43.8
2	52.0	50.3	33.8	36.5	48.9	48.0
3	55.3	52.7	63.1	45.3	48.9	48.9
4	58.0	54.7	74.9	48.9	52.9	51.5

[a] Average of 5 trials for each acid.

Acetic acid caused no appreciable change in blood sugar. Propionic caused an increase in blood sugar. The ketogenic acids all caused similar changes in blood sugar, the degree of change corresponding in general to their ketogenic activity. There was an initial increase in blood sugar, then a decrease and finally, an increase to somewhat higher-than-normal levels again at the end of about 3 hr.

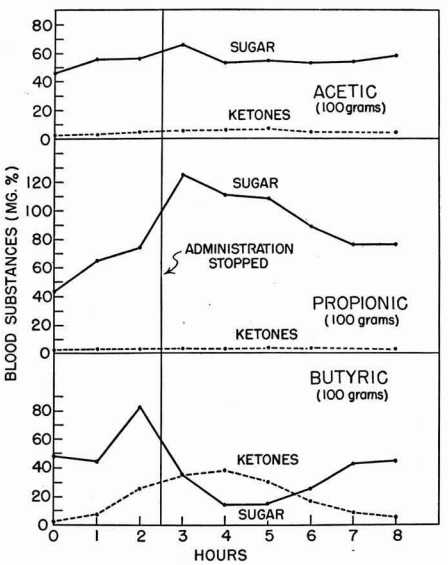

FIG. 2. The effect of prolonged administration of acetic, propionic and butyric acids on the blood sugar and ketone levels.

Figure 2 shows the results of continuous administration of acetic, propionic and butyric acids to three non-lactating goats of approximately the same size. Acetic acid caused a slight rise in blood sugar and at this high dosage level did cause a slight increase in blood ketones. Propionic acid caused no change in blood ketones and a marked increase in blood sugar. Butyric acid caused an initial increase in blood sugar followed by a depression to ketosis levels for a prolonged period. Blood ketones were markedly increased to ketosis levels. This goat exhibited symptoms of listlessness and grinding of teeth. These trials all were repeated with essentially the same results.

TABLE 3

Effect of ketone body administration on blood sugar and ketone levels

Time after administration	Acetoacetic acid					
	Orally (40 g. sodium salt)		Orally (50 g. ethyl ester)		Subcutaneously (20 g. ethyl ester)	
	Sugar	Ketones	Sugar	Ketones	Sugar	Ketones
(*hr.*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
0	51.9	1.8	49.3	3.3	41.7	0.4
0.5	47.2	4.4	.....	.....	50.7	9.4
1	50.3	4.3	44.7	28.8	99.3	9.6
2	82.3	6.5	80.8	16.2	110.6	3.0
3	171.8	7.1	77.1	7.4	112.3	1.5
4	189.4	8.4	77.1	6.3	81.9	2.0
6	138.1	10.8	70.4	5.7	44.9	1.8
10	117.0	10.8	83.6	6.5	.....	.....
20	76.8	7.7	58.9	2.6	.....	.....
30	60.2	4.8	.....	.....	.....	.....

Time after administration	β-Hydroxybutyric acid				Acetone	
	Orally (15 g.)		Subcutaneously (10 g.)		Orally (50 g.)	
	Sugar	Ketones	Sugar	Ketones	Sugar	Ketones
(*hr.*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)	(*mg. %*)
0	47.2	2.7	40.4	0.4	47.2	2.4
0.5	45.2	2.6	41.7	0.8	47.5	49.0
1	51.0	3.7	47.1	0.5	45.2	56.3
1.5	56.0	3.9	42.0	0.7	42.6	61.1
2	50.0	2.7	42.3	0.7	47.5	61.1
3	56.0	2.4	44.9	0.8	52.5	63.0
4	54.7	1.6	49.3	0.8	42.6	42.3

Ketone body administration. The results of ketone body administration appear in table 3. Acetoacetic acid, given orally as the sodium salt, produced a gradual increase in both blood ketones and blood sugar. This material proved rather toxic and the goat went off feed. Using the ethyl ester, either orally or subcutaneously, also caused increases in sugar and ketones with no apparent adverse effect upon the animal. Betahydroxybutyric acid, in the amounts given, caused no appreciable change in either ketones or sugar. Oral administration of acetone caused a marked increase in ketone bodies, as would be expected, but no

appreciable change in blood sugar levels. The effect of direct intravenous injection of acetone over a long period of time also was tested. The graph is omitted for brevity, since the results were similar to those observed with oral administration. During a period of 56 hr., 120 ml. of acetone were injected and, although blood ketone levels were maintained above 40 mg. per cent for most of this period, there was no appreciable change in blood sugar levels.

These results indicate that the blood sugar depression observed with fatty acid feeding was not due to the ketone bodies themselves but to some mechanism operating during their formation.

DISCUSSION

It is recognized that these studies represent laboratory conditions. Consequently, any attempt to apply the results to the problem of ketosis in the field should be done cautiously. It is felt, however, that certain fundamental observations have been made which contribute to basic knowledge of ketosis in ruminants.

Under the conditions of these experiments, it was not possible to produce conditions similar to field ketosis by experimental alteration of blood sugar levels. A ketonemia was produced by means of alloxan, but this apparently was a typical diabetic condition, since blood sugar levels also were high. A prolonged hypoglycemia was produced by means of insulin, but this was not accompanied by an increase in blood ketones. It is beyond the scope of this paper to discuss the theoretical implications of these insulin effects, but the results suggest that a deficiency of blood glucose alone is not sufficient to cause ketosis in ruminants.

The results of fatty acid feeding introduce a slightly different aspect into this problem of ketosis, namely, the effect of fatty acid production in the rumen. It has been well established that the lower fatty acids are important products of normal bacterial fermentation in the rumen, and that the ratio is about 65 per cent acetic, 20 per cent propionic and 15 per cent butyric. It also has been established by Tyznik and Allen (23) that under certain feeding programs this proportion is altered. One of the most common theories regarding ketone body formation is that they are formed by the condensation of two molecules of acetic acid, derived from the oxidation of longer chain fatty acids (13). This idea suggests that excess acetic acid production would be undesirable as far as ketosis is concerned. The work reported here does not support this idea. Large excesses of acetic acid were introduced into the rumen with only slight increases in blood ketones. There also was a slight increase in blood sugar, which would tend to be beneficial. Results of introducing propionic acid into the rumen indicate that an excess production of this acid in the rumen would be desirable in relation to ketosis, since there was a marked increase in blood sugar with no increase in blood ketones. Propionic acid commonly is considered to be glycogenic in all species. Results of butyric acid feeding suggest that an excess production of this acid in the rumen would be undesirable. Introduction of large amounts of butyric acid produced a typical ketosis blood picture with high ketones and low sugar. A good explanation for the initial increase observed in blood sugar is lacking. The subsequent low blood sugar apparently was the result of some mechanism operat-

ing during the formation of ketone bodies from the fatty acids, since the ketone bodies themselves produced either no change or increases in blood sugar. Glycogenolysis and gluconeogenesis, as well as ketone body formation, are considered to take place primarily in the liver. It is possible that there is some interference with these normal glucose-forming processes when large amounts of ketone bodies are being formed from fatty acids. Whether this same picture would be found when large amounts of ketone bodies were being formed from body fat has not been established. Other ketogenic acids do have the same effect, however, when fed. Further study is needed to determine whether abnormal production of fatty acids in the rumen is of any practical importance in the development of ketosis.

Shaw (20) has suggested that ketosis is due to improper functioning of the adrenal and pituitary glands. It seems clear that there is damage to certain endocrine glands in severe ketosis and that usually there is a favorable response to cortisone treatment. The exact cause of the endocrine disturbance, however, has not been completely established. Further work is being conducted along the lines of this study in an attempt to get at this fundamental issue.

SUMMARY

A study was made of the interrelation of the sugar and ketone levels of the blood of ruminants by means of experimental alteration of each of these substances.

Under the conditions of these experiments, it was not possible to produce conditions similar to field ketosis by alteration of blood sugar levels. Alloxan injection produced a typical diabetic condition, with high levels of both blood sugar and ketones. By means of insulin injections it was possible to maintain the blood sugar at low levels for periods of several days, but in no case was there any increase in blood ketones.

Alteration of blood ketones by the administration of ketogenic fatty acids produced temporary changes in blood composition resembling ketosis. Blood ketones were high and blood sugar was low. Non-ketogenic fatty acids did not cause these changes. The administration of the ketone bodies themselves likewise caused no depression of blood sugar.

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FACTORS AFFECTING THE INCIDENCE OF CYSTIC OVARIES IN A HERD OF HOLSTEIN COWS[1]

L. E. CASIDA AND A. B. CHAPMAN[2]

University of Wisconsin, Madison

Various manifestations of nymphomania in cows have been set forth by Zschokke (9), Albrechtsen (1), Hess (4), Williams and Williams (8) and Quinlan (7). The same authors also have described the conditions in the ovaries and genital tract which accompany nymphomaniac behavior.

Different points of view have prevailed from time to time on the causes of cystic ovaries. Albrechtsen (1) and Quinlan (7) have held that the formation of ovarian cysts is a sequel to disease of the uterus. Hess (4) has considered the ovarian condition as primary and most of the accompanying uterine conditions as secondary.

Pearl and Surface (6) suggested that the cystic condition might be a result of an endocrine disturbance. Casida *et al.* (2) gave evidence of the ability of pituitary extracts to bring about the formation of corpora lutea in nymphomaniac cows, thus emphasizing the likelihood that the condition is basically a derangement of the relatonship between the pituitary gland and the ovary.

Garm (3) listed infections of the genital organs, high protein diet, insufficient range feeding, hereditary predisposition and high milk yield as potential etiological factors. He concluded from his own studies that infections of the genital organs did not seem to play any part, but that nymphomania is a multiglandular syndrome for which an hereditary predisposition usually exists. He also saw evidence in his material of a positive relationship between nymphomania and high milk yield.

The present study is an examination of some of the possible causes of variation in the incidence of cystic ovaries in the purebred Holstein herd of the Pabst Farms, Oconomowoc, Wis. The incidence of the cystic condition in this herd is judged to be quite comparable to that in other herds of a similar level of milk production. This particular herd was studied because of the unique thoroughness of daily observations on the animals and the completeness of the reproduction records. These records covered the interval from July, 1932, to November, 1942, during which time the care of the herd was under the direction of the late Howard Clapp. The careful observations and the detailed records used in this study are the work of this outstanding herd manager. They have been made available for this study through the courtesy of Fred Pabst.

Records on 341 cows were collected during this interval. The number of service periods these cows spent in the herd ranged from one to 13. The occur-

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rence of cystic ovaries was determined in two ways: first, from abnormal estrual behavior of the cows and second, by direct palpation of the ovaries. It was the practice to treat all animals showing evidence of nymphomania and cystic ovaries; the main treatment was the manual rupture of the cysts, *per rectum,* with repetition of the treatment after a short interval if cyst development recurred. Treatment by intravenous injection of an unfractionated pituitary extract (2) was used during the later years.

The majority of the animals in this herd was placed on the Holstein official milk and butterfat production tests during the first lactation. Another test was made on most animals when they reached maturity. The official test required milking four times daily for those animals tested during the interval before February, 1940, and three times daily after that date. Cows, when not on official test, were milked twice daily. The general level of feeding and management given animals in this herd is considered to be above average.

RESULTS

Cystic ovaries occurred in 18.8 per cent of the 341 cows that were recorded in this 10-yr. interval. Altogether, 1280 service periods were recorded for these cows; of these, 7 per cent showed the occurrence of cystic ovaries.

TABLE 1

Cows showing initial occurrence of cystic ovaries by number of service periods already spent in herd

Comparison	Service period no.												
	1	2	3	4	5	6	7	8	9	10	11	12	13
No. cows passing through a given service period, excluding those which had become cystic prior to that service period	341	266	174	106	79	50	35	25	16	8	7	1	1
Proportion of cows not previously cystic becoming cystic for first time in a given service period	0.035	0.064	0.069	0.066	0.114	0.080	0.029	0.040	0	0	0.143	0	0

Mean proportion 0.058 (weighted by number of cows passing through a given service period).

Test of heterogeneity of proportion of cystic cows in different service periods: $P \cong 0.13$.

Number of service periods in the herd and the occurrence of cystic ovaries. The proportion of cows (not previously cystic) which became cystic for the first time in a given service period fluctuated around a mean of 0.058 (table 1). There was little evidence of heterogeneity of the proportions in the different service periods ($P \cong 0.13$).

The proportion of cows that had been cystic sometime during their lifetimes should increase in the older age groups if at least some of the animals recover from the cystic condition sufficiently to allow conception. The theoretical proportions (table 2) that should have been cystic by the end of the fifth service

period approximates one-fourth and by the end of the eleventh or twelfth service period approximates one-half, assuming a constant probability of 0.058 for non-cystic cows becoming cystic in each service period and that culling of cystics and non-cystics is proportional to their numbers in the herd. The theoretical proportion for the herd as a whole weighted by the proportion of cows with each of the different numbers of service periods was 0.191. This is very close to the observed proportion of 0.188 for the herd as a whole and is consistent with the assumption that culling of the animals was independent of the cystic condition. Only nine of the 64 animals that were cystic some time during their lifetimes actually went out of the herd while cystic. The remainder bore at least one calf after being cystic. Occurrence of cystic ovaries was shown in two service periods by 12 cows, in three service periods by four cows and in four service periods by two cows.

TABLE 2

Proportion of cows that were cystic in relation to total lifetime in herd

Comparison	Total no. of service periods in herd													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Totals
No. cows with a given total number service periods in herd	63	84	61	26	31	21	13	18	9	4	9	0	2	341
No. cystic	0	8	6	6	11	10	4	10	1	3	4	0	1	64
Proportion cystic	0	0.095	0.098	0.231	0.355	0.476	0.308	0.556	0.111	0.750	0.444	.....	0.500	0.188
Theoretical proportion[a]	0.058	0.113	0.164	0.212	0.258	0.301	0.342	0.380	0.416	0.450	0.482	0.512	0.548	0.191[b]

[a] Proportion of all cows in the herd that would have been cystic at least once by the end of a given service period assuming a constant probability of 0.058 for non-cystic cows becoming cystic in each service period and assuming that culling of cystics and non-cystics is proportional to their numbers in the herd.

[b] Weighted by the proportions of cows in the herd with each number of service periods.

Heritability of cystic ovaries. There were 245 daughters out of 144 dams. These dams were classified on the basis of whether or not they had been cystic; 43 had been cystic sometime during their life, whereas 101 had never shown the condition. The 43 cystic dams had a total of 82 daughters, of which 26.8 per cent had been cystic at least once each. The 101 non-cystic dams had a total of 163 daughters, and of these only 9.2 per cent had shown cystic ovaries. Thus, there was a difference of 17.6 per cent between the daughters of the two kinds of dams.

It was noted above that the probability of a cow having shown the cystic condition sometime during her lifetime is increased markedly the longer the span of life she has spent in the herd. Therefore, an examination was made to determine the degree of association between the total number of service periods that a dam had spent in the herd and the total number of service periods that her daughters had spent in the herd. The daughter-dam correlation for length of time in the herd was 0.157 ($P = 0.01$–0.05). This correlation may explain a part of the dam-daughter association in the proportion of cystic ovaries.

An attempt was made to evaluate the daughter-dam association for the occurrence of cystic ovaries in relation to the number of service periods each individual spent in the herd. This was done by comparing the proportions of cystic daughters from cystic and non-cystic dams within service-period groups. The service-period group into which a daughter-dam pair fell was fixed by the member of the pair which was in the herd for the shorter period of time. The data in table 3 give no evidence of heritability of cystic ovaries from those animals which stay in the herd less than four service periods. For animals that stay four or more service periods, however, cystic dams had 48.1 per cent of 27 daughters that were cystic, whereas non-cystic dams had 21.7 per cent of 46 daughters that were cystic, a difference of 26.4 per cent.

TABLE 3

Daughters from cystic and non-cystic dams using equal numbers of service periods in herd for dam and daughter

No. of service periods used in classifying daughters and dams	Cystic dams		Non-cystic dams	
	Total no. daughters	Proportion cystic	Total no. daughters	Proportion cystic
1	3	0	61	0
2	9	0	60	0.100
3	3	0	36	0.083
4	7	0.429	15	0.200
5	6	0.333	16	0.250
6	7	0.714	5	0.200
7	1	0	5	0.200
8	4	0.500	3	0.333
> 8	2	0.500	2	0
All no. of service periods	42	0.310	203	0.094

The weighted mean of the percentages of the daughters from cystic cows that were cystic in the different service periods is 31.0 and for those from non-cystic cows is 9.4. Twice the difference between these two values, 43.2 per cent, gives an estimate of the heritability (5) of the occurrence of cystic ovaries in a population in which the dams and daughters have spent the same number of service periods in the herd and in which the proportion of different aged animals and other conditions are as they were in this herd.

Productivity and the occurrence of cystic ovaries. The more productive cows in a herd are sometimes considered to be more subject to nymphomania. The policy of management in this herd, as noted earlier, was for most animals to go on official test in the first lactation. Classification of the animals on the basis of butterfat production during this test gave no evidence of an association between level of production and cystic ovaries ($P = 0.3–0.5$). Likewise, for butterfat percentage, the Chi-square test gave no evidence of differences in the proportions of animals with cystic ovaries at the different levels of butterfat percentage ($P = 0.2–0.3$).

The mean butterfat production for cystic cows was 578 ± 12.3 and for non-cystic cows, 562 ± 7.9. The average percentage of butterfat for cystic cows was 3.56 ± 0.13 and for non-cystic cows, 3.60 ± 0.03.

Environmental factors which might possibly effect a relationship between milk production and cystic ovaries were next examined. There were 358 service periods of cows during a time when they were not milked (table 4). Three hundred and forty-one of these were of heifers before their first parturition. There were 457 records made during the time the cows were in the milking herd and being milked twice daily. There were 359 service periods of cows on official test when they were being milked three or four times daily. There were 105 records on animals that were started on test but were moved to the milking herd before conception. Thus, we have four different conditions in terms of the number of times the cows were milked daily, and also, to a certain extent, in age and the level of feeding and management. The incidence of the cystic condition varied from 3.4 per cent in the animals not milked, to 6.8 while they were in the milking

TABLE 4

Proportion of cows showing cystic ovaries in relation to milking and official testing

Service period number	Not milked		In milking herd		Started on test[a]		On official test	
	No. cows	Proportion cystic	No. cows	Proportion cystic	No. cows	Proportion cystic	No. cows	Proportion cystic
1	341	0.035	.....	.....	.....	.....	.....	.....
2	14	0	22	0.046	31	0.032	211	0.081
3	2	0	110	0.046	25	0.120	57	0.123
4	1	0	75	0.053	18	0	39	0.180
5	.....	.....	60	0.117	14	0.214	33	0.182
6–13	.....	.....	190	0.074	18	0.110	19	0.053
Total	358	0.034	457	0.068	106	0.085	359	0.106

[a] Removed to regular milking herd before conception.

herd, to 8.5 if they were started on test and then removed to the milking herd, and finally, to 10.6 while they were on official test. The over-all differences in proportions of non-cystic to cystic cows under these four different management and milking conditions are significant ($P = 0.01$).

Why the conditions of official testing should increase the probability of a cow becoming cystic is not known. There is a whole complex of conditions which might explain the relationship. There is a higher feed consumption during official testing than under the more usual herd conditions; there also is higher milk production and more frequent daily milking when the cows are on test. These factors will have to be separated and tested one-by-one and in combinations under controlled experimental conditions in order to arrive at the basic cause of these results.

SUMMARY

Factors affecting the incidence of cystic ovaries have been examined in a herd of Holstein cattle. The records covered 341 cows and 1,280 cow-service periods over a 10-yr. period. Cystic ovaries occurred in 18.8 per cent of the cows and 7 per cent of the service periods.

The proportion of the cows becoming cystic for the first time averaged 0.058 per service period and no evidence was obtained for significant variation in this rate between service periods.

Heritability of the occurrence of cystic ovaries sometime during life was estimated for this herd as 0.43.

No evidence was obtained for an association between inherent level of butterfat production or test and the occurrence of cystic ovaries.

Cows when not milked (mainly in the heifer service period) showed an incidence of cystic ovaries of 3.4 per cent; those in the milking herd, 6.8 per cent; those started on test and then removed to the milking herd, 8.5 per cent; and those on official test, 10.6 per cent.

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COLLEGIATE STUDENTS' INTERNATIONAL CONTEST IN JUDGING DAIRY PRODUCTS

DETROIT, MICHIGAN—OCTOBER 22, 1951

Teams from twenty-three (23) land grant colleges, participated in this, the seventeenth annual contest sponsored by the Dairy Industries Supply Association, Inc., and the American Dairy Science Association.

Following is list of those who won high standings in the Contest:

ALL PRODUCTS

Individuals

1. Austin A. Christensen, Iowa State College
2. Robert J. Christie, University of Connecticut
3. David E. Watts, University of Connecticut
4. Claude J. Blackburn, Mississippi State College
5. Bruce H. Collins, Ohio State University
6. Richard Bassette, University of Maryland
7. Lawrence D. Custer, Mississippi State College
8. Albert W. Hosner, Michigan State College
9. Robert A. Brooks, Mississippi State College
10. Delmar L. Andersen, Iowa State College

All-Products Teams

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

* Tied for third place. Tie broken in favor of Iowa State College with a grade of 279.11 compared with 301.81 for Maryland.

BUTTER

Individuals

1. William F. Krueger, Michigan State College 12.34
*2. Calvin Bolton, Iowa State College .. 13.00
*3. Bruce H. Collins, Ohio State 13.00
4. Austin A. Christensen, Iowa State College 13.50
5. Delmar L. Andersen, Iowa State College 14.00
*6. Robert J. Christie, University of Connecticut 14.50
*7. Bill Kemp, University of Tennessee 14.50
*8. Claude J. Blackburn, Mississippi State College 15.00
*9. Sanford C. Downs, University of Nebraska 15.00
10. Robert M. Hamilton, University of Massachusetts 16.00

* Ties broken on basis of flavor score.

Teams

1. Iowa State University 40.50
*2. Mississippi State College 54.67
*3. University of Connecticut 54.67
4. Michigan State College 56.01
5. Ohio State University 56.67
6. University of Nebraska 59.50
*7. University of Minnesota 60.50
*7. University of Tennessee 60.50
9. Purdue University 63.51
10. University of Georgia 64.67

* Ties broken on basis of flavor score.

CHEESE

Individuals

1. Austin A. Christensen, Iowa State College 17.17
2. Calvin Bolton, Iowa State College .. 18.01
3. Delmar L. Andersen, Iowa State College 20.67
4. Robert E. Lockwood, University of Connecticut 21.00
5. David E. Watts, University of Connecticut 21.01
6. Allen Wood, State College of Washington 21.17
7. Robert J. Christie, University of Connecticut 22.36
8. Richard Bassette, University of Maryland 22.52
9. Claude J. Blackburn, Mississippi State College 22.98
10. Bruce H. Collins, Ohio State University 23.76

Teams

1. Iowa State College 58.35
2. University of Connecticut 64.37
3. University of Maryland 74.58
4. Mississippi State College 77.86
5. Oklahoma A & M College 80.41
6. University of Georgia 80.90
7. University of Massachusetts 81.75
8. State College of Washington 82.74
9. Purdue University 85.07
10. University of Wisconsin 86.51

ICE CREAM

Individuals

1. S. R. Allen, A & M College of Texas 19.68
2. David E. Watts, University of Connecticut 21.67
3. T. M. Huston, A & M College of Texas 23.01
4. William G. Hoffmann, Cornell University 23.84
5. N. A. Rysavy, University of Maryland 24.01
6. Austin A. Christensen, Iowa State College 24.17
7. George M. Kloser, Cornell University 24.34
*8. Robert Hicks, Texas Technological College 24.50
*9. James P. Gracy, University of Tennessee 24.50
10. Robert F. Going, Purdue University 24.67

Teams

1. Cornell University 72.85
2. University of Connecticut 75.52
3. A & M College of Texas 76.86
4. University of Maryland 82.36
5. Mississippi State College 82.68
6. Michigan State College 83.52
7. University of Georgia 87.01
8. University of Tennessee 87.09
9. Oklahoma A & M College 87.67
10. Purdue University 90.17

* Ties broken on basis of flavor.

MILK

Individuals

1. Albert W. Hosner, Michigan State College 18.60
2. Simns Jacquette, University of Maryland 19.77
3. Rodrigo Montealegre, Cornell University 20.48
4. Robert J. Christie, University of Connecticut 21.42
5. Claude J. Blackburn, Mississippi State College 21.63
6. Hartl R. Jones, Ohio State University 21.83
7. Fred Williams, University of Massachusetts 22.26
8. Frank Whigham, University of Georgia 22.50
9. Bruce H. Collins, Ohio State University 22.53
10. Lawrence D. Custer, Mississippi State College 22.73

Teams

1. Mississippi State College 69.68
2. Cornell University 70.83
3. Ohio State University 71.53
4. Iowa State College 75.25
5. University of Wisconsin 78.17
6. State College of Washington 78.79
7. University of Maryland 79.20
8. Michigan State College 79.36
9. University of Georgia 79.51
10. University of Illinois 83.21

NATIONAL INTERCOLLEGIATE DAIRY CATTLE JUDGING CONTEST
NATIONAL DAIRY CATTLE CONGRESS—1951
WATERLOO, IOWA

TEAM RANK—ALL BREEDS

1.	Iowa	2027	6.	Missouri	1987
2.	Kansas	2015	7.	Ohio	1972
3.	West Virginia	2006	8.	Tennessee	1968
4.	Oklahoma	2002	9.	Cornell	1966
5.	Kentucky	2001	10.	Illinois	1961

HIGH INDIVIDUALS IN JUDGING ALL BREEDS

1.	Eugene Weise, Iowa	698—70	6.	Fred Ledlow, Ontario Agr. College	687—54
2.	Edsel Gainer, West Virginia	697—30	7.	Robert Coleman, Ohio State	685—52
3.	Maurice Core, Iowa (tie)	690— 8	8.	Bernard McKean, Illinois (tie)	680—38
3.	Jack Snyder, Colorado (tie)	690—35	8.	Albert Rinehart, Missouri (tie)	680—48
5.	Jane Robens, Cornell	689—36	10.	William Baker, Kansas	678—40

AYRSHIRE

	Teams			*Individuals*	
1.	Cornell	437	1.	Jane Robens, Cornell	149
2.	Calif. Poly.	430	2.	Wayne Rauch, Ohio State	148
3.	Kentucky	427	3.	Maurice Core, Iowa	147
3.	Maryland	427	3.	Geo. Thomas, Missouri	147
5.	Kansas	426	3.	Gordan Hueter, Maryland	147
6.	Missouri	425	3.	John Gorski, Wisconsin	147
6.	Iowa	425	7.	Geo. Collins, N. Carolina	146
8.	Texas Tech.	423	7.	Raymond Sis, Kansas	146
9.	Oklahoma A & M	422	7.	Edsel Gainer, W. Virginia	146
10.	L. S. U.	421	10.	Jack Albright, Cal. Poly.	145
			10.	George Payne, Cornell	145
			10.	Bob Basse, Oklahoma	145

BROWN SWISS

	Teams			*Individuals*	
1.	Calif. Poly.	413	1.	Fred Ledlow, Ontario	148
2.	Ontario Agr. College	401	2.	Herbert Norry, Ontario	147
3.	Oklahoma A & M	398	3.	Jack Snyder, Colo. A&M	144
4.	Missouri	397	4.	John Gorski, Wisconsin	142
5.	Purdue	392	5.	Jack Albright, Cal. Poly.	140
6.	West Virginia	389	5.	Gordon Hueter, Maryland	140
7.	Tennessee	388	7.	Marion Masters, Missouri	139
8.	Wisconsin	386	7.	Blaine Menning, Cal. Poly.	139
9.	L. S. U.	384	9.	Robert Meyer, Wisconsin	138
10.	Iowa	383	10.	Bill Pickett, Oklahoma	137
			10.	Jack Burke, N. Dakota	137
			10.	Warren Poage, W. Virginia	137

GUERNSEY

Teams		*Individuals*	
1. Kentucky	387	1. Robert Coleman, Ohio State	149
2. Iowa	383	2. Wm. Baker, Kansas	146
3. Ohio State	379	3. Eugene Weise, Iowa	142
4. Kansas State	378	4. Jane Robens, Cornell	140
5. Illinois	376	4. Fred Warren, Connecticut	140
6. Cornell	368	6. Earl Smith, Tennessee	139
7. Oklahoma A & M	364	7. Ted Howard, Kentucky	138
8. West Virginia	361	8. Edsel Gainer, W. Virginia	137
9. Connecticut	357	9. Bernard McKean, Illinois	135
10. Michigan	352	9. Jack Snyder, Colorado	135

JERSEY

Teams		*Individuals*	
1. North Carolina	433	1. Jane Robens, Cornell (perfect)	150
1. Ontario	433	2. Fred Ledlow, Ontario	149
3. Iowa	429	2. John Collins, N. Carolina	149
4. Oklahoma A & M	424	4. Edsel Gainer, W. Virginia	148
5. Missouri	423	4. Albert Rinehart, Missouri	148
6. Cornell	419	6. Maurice Core, Iowa	147
7. North Dakota	416	6. John Speicher, Kansas	147
7. Kentucky	416	8. Keith Harrison, Tennessee	145
9. Connecticut	410	8. Richard Fuchs, Kentucky	145
10. Illinois	409	8. Wayne Rauch, Ohio	145
10. West Virginia	409		

HOLSTEIN

Teams		*Individuals*	
1. West Virginia	432	1. Edsel Gainer, W. Virginia	149
2. Tennessee	424	2. Eugene Weise, Iowa	146
3. Kansas	421	3. Bernard McKean, Illinois	145
4. North Carolina	418	3. J. M. Fussel, L. S. U.	145
5. Illinois	416	5. John Collins, N. Carolina	144
6. Ohio State	410	5. Ray Spann, Tennessee	144
7. Colorado A & M	405	7. Robert Coleman, Ohio State	143
8. Missouri	404	8. Robert Rupp, Texas Tech.	142
9. Texas Tech.	399	8. Warren Poage, W. Virginia	142
9. L. S. U.	399	10. John Speicher, Kansas	141
9. South Dakota	399	10. Keith Harrison, Tennessee	141
		10. Wm. Baker, Kansas	141
		10. John Galford, W. Virginia	141

AUTHOR INDEX OF ORIGINAL ARTICLES
(Prepared by D. D. Deane)

*Abstract of paper presented at Annual Meeting.

ERRATA

The sentence beginning in line 3 of the third paragraph on page 687 of volume XXXIII, 1950, should read: "It is evident that there is a slight bias in favor of the higher testing milks and a slight bias against the lower testing ones (table 2), as indicated by theoretical calculations."

On page 1109 of volume XXXIV, reference 2 should read: J. Dairy Sci., **34**: 1119–1127. 1951.

SUBJECT INDEX OF ORIGINAL ARTICLES
(Prepared by D. D. DEANE)

* Abstract of paper presented at Annual Meeting.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

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

BOOK REVIEW

662. Milchkunde und Milchhygiene *(Dairy Science and Hygiene).* M. KLIMMER and F. SCHÖNBERG. 6th ed. M. & H. Schaper, Hanover. 348 pp., 84 figs. DM 28. 1951.

The 6th ed. constitutes a thorough revision, taking into acount new literature and recent developments in milk production. It contains sections on the economic importance of milk, general properties of milk, constituents of milk, milk sanitation, milk supervision, a short section on products produced from milk and regulations controlling milk production in Germany.

The sections on milk hygiene and supervision of milk production are of major interest to veterinarians, doctors and public health officials. Under the section on milk hygiene, the diseases of the cows, as well as those of dairy personnel likely to be transmitted in milk, the care and feeding of cows, cleanliness in milking, conditions of dairies and markets are discussed. In the section on supervision of milk production, techniques are given for microscopic, bacteriological and serological examinations covering all the important phases of the work.

The book is printed on good stock in clear, easily read type. It contains an extensive subject index. No references to the literature are given. Chapter headings at the top of each page would facilitate the use of the book.

O. W. Olsen

BUTTER

O. F. HUNZIKER, SECTION EDITOR

663. Newly developed unit checks pH right on line. L. E. SLATER, Food Eng. Food Eng., **23,** 9: 98, 99, 155, 156, 161. Sept., 1951.

Continuous pH standardization of cream is achieved by means of equipment designed by Robichaux. Post-war improvements in devices for measuring pH, associated with a technique of adding reagents under vacuum were utilized to provide continuous neutralization. The instruments, together with jars of alkali, are mounted on a mobile panel. Connections to the Vacreator pasteurizer are made with flexible sanitary tubing. In operation, the desired pH is set on the controller and all connections are opened. The pH of the cream flow is indicated and recorded. Controlled air positions the valve on the alkali supply and permits the required amount of neutralizer to enter the Vacreator automatically. The process eliminates the time and errors involved in batch neutralizing and prevents batch-to-batch variation. It gives more accurate pH control, resulting in improved product quality.

T. J. Claydon

664. Continuous process for producing butter. A. L. STIGEN (assignor to Aktiebolaget Separator). U. S. Patent 2,569,203. 5 claims. Sept. 25, 1951. Official Gaz. U. S. Pat. Office, **650,** 4: 1134. 1951.

High-concentration cream is cooled to approximately 14° C. where phase reversal is affected, followed by heating the butter to a temperature of 18–30° C. and again cooling. R. Whitaker

665. Guarded knife for cutting butter and cheese. J. F. HARTMAN (assignor to J. B. Reinhard, in part). U. S. Patent 2,570,671. 3 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 500. 1951.

A knife for cutting butter and cheese has a guard over the sharpened edge. When in use the guard is turned on a pivot to expose the cutting edge. R. Whitaker

CONDENSED AND DRIED MILKS; BY-PRODUCTS

F. J. DOAN, SECTION EDITOR

666. Condensed milk composition and its preparation. F. F. HANSEN. U. S. Patent 2,570,231. 8 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 382. 1951.

Heat coagulation and age thickening are inhibited in sweetened condensed milk by the addition of a small amount of an edible Ca salt, such as Ca acid lactate. R. Whitaker

667. Process for sterilizing canned foods. G. K. VIALL (assignor to Chain Belt Co.). U. S. Patent 2,569,645. 6 claims. Oct. 2, 1951. Official Gaz. U. S. Pat. Office, **651,** 1: 123. 1951.

A continuous HTST sterilizer suitable for evaporated milk and other liquid foods in cans, consists of a chamber in which are located 2

roller-type conveyors. The cans are introduced through a star wheel lock, rolled forward on 1 conveyor under a spray of heated liquid, submerged in a cooling liquid on a 2nd conveyor and finally ejected from the sterilizer through another star wheel. The chamber is maintained above atmospheric pressure but below that developed in the cans at the maximum sterilizing temperature. R. Whitaker

668. Food products and method of making the same. L. A. SCHOLZ. U. S. Patent 2,568,369. 9 claims. Sept. 18, 1951. Official Gaz. U. S. Pat. Office, **650,** 3: 809. 1951.

A mixture of butter-fat, milk solids-not-fat, sugar and sufficient gelatin to gel the product below 85° F. for use in making hot drinks is discussed. R. Whitaker

DAIRY BACTERIOLOGY

P. R. ELLIKER, SECTION EDITOR

669. De vorming van diacetyl in zuursels (The formation of diacetyl in starters). (English summary) N. EVENHUIS, J. LERK and H. BROUWER, Cooperative Fabriek van Melk producten, Bedum, Holland. Neth. Milk and Dairy J., **5,** 2: 110–117. Apr.-June, 1951.

An investigation was made to obtain more information about the oxidation resulting in diacetyl formation during the cream-ripening procedure. According to Pette's scheme (Neth. Milk and Dairy J., 2, 12. 1948.), the oxidation is a chemical process. To check this, the authors studied the diacetyl formation with and without inactivation of the microorganisms. Starter was shaken with air during 0.5 hr. and the diacetyl content determined before and after shaking. Normally, the last value was about twice as high as the first. The amount of diacetyl did not increase when the microorganisms were inactivated by adding excessive amounts of HCl, by pasteurization or by the addition of quaternary ammonium salts (cetavlon) before the shaking procedure. For complete inactivation of microorganisms the acidity had to be increased to about 140° D (= ml. N/9 NaOH per 100 ml. of starter), or pasteurization during 5 min. at 70° C., or addition of 5 g. of cetavlon in 25 ml. of water to 200 ml. of starter was needed. Shaking at 0° C., addition of 100,000 units of penicillin/225 ml. of starter, or addition of sublimate to a concentration of 0.5% had no influence on diacetyl formation. They concluded that the oxidation reaction probably is a bacteriological oxidation of a decomposition product of citric acid. A. F. Tamsma

670. Lactaatvergisting door Coli-aerogenes bacterien (Fermentation of lactate by coliform bacteria.) (English summary) J. L. LIEBERT, Nederlands Instituut voor Zuivelonderzoek, Hoorn, Holland. Neth. Milk and Dairy J., **5,** 2. 118–124. Apr.-June, 1951.

When KNO_3 is used as an inhibitor of the early gas production in cheese, it is theoretically possible to imagine also a detrimental effect of the nitrate because it promotes the growth of coliform bacteria at the expense of the lactate. Experiments were made on the lactate fermentation of coliform organisms in culture media. Under aerobic conditions lactic acid salts could be used as a carbon source, and also under anaerobic conditions in the presence of nitrate as a hydrogen acceptor, confirming the literature. However, under anaerobic conditions the amount of gas formed was too low to explain gas production in cheese as the result of an anaerobic decomposition of lactic acid with the aid of KNO_3.
A. F. Tamsma

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

671. Evaluation of the thiobarbituric acid test as a measure of oxidized flavor in milk. W. L. DUNKLEY, Univ. of Cal., Davis. Food Technol., **5,** 8: 342–346. 1951.

The test outlined for the chemical estimation of oxidized flavor is based on a reaction involving the formation of a red color when oxidized milk is acidified and heated with 2-thiobarbituric acid. Cu also causes an increase in optical density in the thiobarbituric acid (TBA) test but a correction factor can be applied, if desired, for the amount of Cu present in the milk. A close correlation was found between the results of the TBA test and the adjusted numerical flavor scores of milk samples having an oxidized flavor of varied intensity. Regression lines showing the relation between adjusted flavor scores and the TBA test were calculated from the data obtained.
E. R. Garrison

672. Das Lab und seine Wirkimg auf das Casein der Milch. IV. Die Proteolyse des Caseins durch kristallisiertes Lab. (Rennet and its action on the casein of milk. IV. The proteolysis of casein by crystallized rennet). Hs. NITSCHMANN and R. VARIN, Universität Bern, Switzerland. Helv. Chim. Act., **34,** 5: 1421–1430. Aug., 1951.

The action of large quantities of crystallized rennet on dissolved casein at 25° C. and pH's 6.8 and 2.3 was followed by titrating the increase in acid and basic components. The large rennet concentrations caused complete casein hydrolysis within a few hours; in these short periods the action of milk-proteinase present in the casein could be neglected. Acid groups were titrated according to Willstätter and Waldschmidt-Leitz (B., **54:** 2988. 1921.) in the micromodification of Grassmann and Heyde (Z. physiol. Ch., **183:** 32. 1929.); however, 50% alcohol was used. Basic groups were titrated according to Linderstrom-Lang (Z. physiol. Ch., **173:** 32. 1928.) and Holter *et al.* (Z. physiol. Ch., **206:** 85. 1932.). The casein employed was Mercks Hammersten casein, the rennet was prepared by N. J. Berridge of the University of Reading, England (Biochem. J., **39:** 179. 1945.). At pH 6.8, an average of 0.25 milliequivalent titratable groups/gram of casein were set free, or 1 bond/4000 molar weight units of casein or/33 amino acid residues (their average molar weight figured at 120). At pH 2.3 one bond/8 amino

acids was hydrolysed. They concluded that this hydrolysis is concerned with the peptide bonds. Other bonds like acid amide, ester, phosphamide, guanidine bonds may contribute to the effect; however, these can only cause part of the effect. At the moment of coagulation of milk by rennet, only a small part of the hydrolysis can be completed. This leaves the primary reaction of the coagulation process with rennet still an open question. A. F. Tamsma

673. Methods for the determination of water. Methods for determination of moisture-oven drying. C. O. WILLITS, Eastern Reg. Research Lab., Phila., 18, Pa. Anal. Chem., **23,** 8: 1058–1062. 1951. **Determination of moisture by distillation.** W. R. FETZER, Clinton Foods, Inc., Clinton, Ia. *Ibid.* pp. 1062–1069. **Karl Fischer Reagent Titration,** J. MITCHELL, JR., Polychemicals Dept., Research Div., E. I. duPont de Nemours & Co., Inc., Wilmington, Del. *Ibid.* pp. 1069–1075. **Electrical measurement of water vapor with a hygroscopic film.** E. R. WEAVER, Natl. Bureau of Standards, Wash., D. C. *Ibid.* 1076–1080.

A series of symposium papers which review methods for water determination is presented. The important factor in thermal drying is the differential between the vapor pressure of the substance to be dried and the vapor pressure of the atmosphere of the drying chamber. Guides are given for establishing thermal drying conditions of most organic and biological materials. The distillation procedure for moisture determination measures the water as such and thereby establishes a primary or reference method for other moisture methods. Applications of the distillation procedure and some basic designs in apparatus are given. The Fischer reagent titration is rapid and widely applicable. A visual or an electrometric end-point may be employed. Methods generally are available to eliminate the effect of interfering substances. The development of the electrical conductance method is described and the characteristics of various kinds of hygroscopic films are presented. B. H. Webb

674. Quantitative determination of sugars on filter paper chromatograms by direct photometry. E. F. MCFARREN, K. BRAND and H. R. RUTKOWSKI, Natl. Dairy Research Labs., Inc., Oakdale, L. I., N. Y Anal. Chem., **23,** 8: 1146–1149. 1951.

Lactose, glucose and galactose were determined in the presence of each other. The method consisted of separating the sugars in an ethyl acetate-pyridine-H_2O solvent system containing $AgNO_3$, air-drying, exposing the chromatograms to NH_3 vapors and developing the sugar spots by heating in an oven. The maximum densities of the spots were determined by means of a densitometer. A standard curve was prepared with which the concentrations of the unknowns were determined from their observed densities. The sugars present in a mixture may be determined with a maximum error of 5%. Unknown reducing substances were found which under usual conditions of analysis apparently would be calculated as lactose or total monoses. B. H. Webb

675. Small scale filter paper chromatography. Filter papers and solvents. L. B. ROCKLAND, J. L. BLATT and M. S. DUNN, Univ. of Cal., Los Angeles. Anal. Chem., **23,** 8: 1142–1146. 1951.

Systematic investigations relating to filter papers and miscible and immiscible solvents are reported. Thirteen filter papers were studied and listed in the order of their suitability for use in amino acid chromatography as determined on the basis of 7 physical and chemical characteristics. The influence of the water content of 8 water-miscible solvents on the R_f values of amino acids has been investigated. The most common and fixed sequences of the amino acids, the solvents yielding inverted sequences and the solvents that separated individual pairs and groups of amino acids must effectively were determined. B. H. Webb

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

676. Permanent welded pipelines. C. R. HAVIGHORST, Food Eng. Food Eng., **23,** 9: 74–79. Sept., 1951.

Permanent welded pipelines are in operation in several plants of the Lucerne Milk Co., Oakland, Calif. The sanitary condition of these lines has been satisfactory as shown by records of the company and of the Oakland Health Dept. In addition, the system reduces processing and maintenance costs. The key to its success is the welding technique employed. Details are given for obtaining smooth, strong joints. There are approximately 300 welds in the lines of a 4,000-gal. milk plant. Permanent welded pipelines must be carefully planned in advance, since revisions are expensive. The sanitizing procedure used generally is the same as for HTST units involving turbulent circulation of rinses, detergents and sterilizing solutions. T. J. Claydon

677. Heat exchange receptacle. T. MOJONNIER and O. W. MOJONNIER (assignors to Mojonnier Bros. Co.). U. S. Patent 2,568,653. 6 claims. Sept. 18, 1951. Official Gaz. U. S. Pat Office, **650,** 3: 883. 1953.

An insulated jacketed cylindrical tank for heating and cooling fluid dairy products is described. Parallel channels built in the jacket cause the heating and cooling medium to spiral downward around the tank to provide efficient exchange of heat. R. Whitaker

678. Tunnel freezer. H. W. KLEIST (assignor to Dole Refrigerating Co.). U. S. Patent 2,570,250. 5 claims. Oct. 9, 1951. Official Gaz. U. S. Patent Office, **651,** 2: 387. 1951.

A freezing tunnel consisting of an insulated cabinet equipped with a series of shelves built on horizontal refrigerating coils is described. The air is circulated across the tunnel by fans placed on 1 side wall, so situated that the air passes

between several shelves and is returned back to the fans between the remaining shelves.
R. Whitaker

679. Ice cream freezer. D. C. WILLIAMS. U. S. Patent 2,568,839. 4 claims. Sept. 25, 1951. Official Gaz. U. S. Pat. Office, **650,** 4: 1036. 1951.

Details are given for construction of an ice cream freezer of the vertical type with the freezing chamber revolving in the refrigerant. The dasher is attached to the motor by means of a friction clutch which does not engage the dasher at the start of the operation, but only after some ice has formed and the viscosity increased.
R. Whitaker

680. Falling film evaporator. J. A. CROSS (assignor to Mojonnier Bros. Co., Inc.). U. S. Patent 2,570,211. 4 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 376. 1951.

Details are given for a series of vertical tube chests for use in evaporating liquids under reduced pressure. R. Whitaker

681. Milk evaporation process. J. A. CROSS (assignor to Mojonnier Bros. Co., Inc.). U. S. Patent 2,570,212. 4 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 376. 1951.

Essentially the same as Abs. 680, designed especially for producing concentrated milk with no heated flavor. R. Whitaker

682. Milk evaporation process and apparatus. J. A. CROSS (assignor to Mojonnier Bros. Co., Inc.). U. S. Patent 2,570,213. 4 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 377. 1951.

Equipment is described for continuously evaporating milk at low temps., employing an ingenious and efficient system of heat balances.
R. Whitaker

683. Evaporation method and apparatus. J. A. CROSS (assignor to Mojonnier Bros. Co., Inc.). U. S. Patent 2,570,210. 23 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 375. 1951.

An efficient continuous low-temp, evaporator is described for concentrating liquids which are heat sensitive. R. Whitaker

684. Device for straightening milk can covers. E. C. ANDERSON. U. S. Patent 2,570,420. 1 claim. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 433. 1951.

A hand-operated device for restoring distorted milk can lids to their proper shape is described.
R. Whitaker

685. Machine for opening hinged closure members on containers. R. E. J. NORDQUIST (assignor to American Can Co.). U. S. Patent 2,570,267. 8 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 392. 1951.

An attachment for the bottle filler is described for lifting at the time of filling the hinged flap of the container described in Abs. 705 (U. S. Pat. 2,570,266). R. Whitaker

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

686. Structures for self-feeding of hay and ensilage. C. H. REED. Agricultural Eng., **32,** 7: 375. July, 1951.

Results from the New Jersey Expt. Sta. indicate that self feeders for chopped hay can be recommended where farm operators are willing to make minor improvements. Cost of improvements should not exceed cost of labor saved by their use.

Trials with 5 self-feeder silos for corn silage show that the diameter should not exceed 14 ft. nor the height exceed 45 ft. Silos must be designed to meet load requirements and lateral outward pressures.

Self-feeding grass silage trials have not proved satisfactory; however, a new silo having a slightly smaller diameter at the top than at the bottom is under test. This may function satisfactorily with grass silage and improve feeding with corn silage.

Because of the compression of the silage column, the grass silage at the bottom of the column develops resistance to flow over a cone. It is desirable to incorporate into the design a means of cutting into the silage to induce freer flow.

There is more top spoilage of silage than in conventional silos because of longer exposure and the development of fissures when the last portion is being emptied. H. L. Mitten, Jr.

687. Stability of dry vitamin A concentrates. M. J. BURNS and F. W. QUACKENBUSH, Purdue Univ., Lafayette, Ind. Ind. Eng. Chem., **43,** 7: 1592–1593. July, 1951.

A study of the stability of 7 dry, commercial, vitamin A preparations showed that they all lost some of their initial vitamin potency during storage. The samples retained 60–85% of their original vitamin A content during a 6-mo. storage period in the dark at room temperature. The vitamin products were improved in stability when they were mixed with corn and soybean meal.
B. H. Webb

688. Livestock feed. R. J. BLOCK (assignor to The Borden Co.). U. S. Patent 2,569,282. 6 claims. Sept. 25, 1951. Official Gaz. U. S. Pat. Office, **650,** 4: 1154. 1951.

A feed supplement for ruminants contains in the finished feed 10–25 parts nitrogen in the form of urea to 1 part of non-protein sulfur.
R. Whitaker

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

689. Fertility and bacterial content of diluted bull semen treated with various concentrations of dihydrostreptomycin sulfate. H. L. EASTERBROOKS, P. HELLER, M. LIEBERMAN, W. N. PLASTERIDGE and E. L. JUNGHERR, Storrs Agr. Expt. Sta., Storrs, Conn. Am. J. Vet. Research, **12,** 44: 191–193. July, 1951.

Four separate semen collections from each of

8 bulls were each treated with 0, 100, 300 and 900γ of dihydrostreptomycin/ml. of diluted semen. The semen was used alternately by 4 groups of inseminators in routine artificial breeding. Non-returns to 1st services averaged 61.4, 69.2, 76.5 and 72.0%, respectively. Bacterial counts were made of all samples and general types of bacteria were identified. Counts were inversely proportional to the concentration of streptomycin, and bacteria-free plates occurred 20 of 32 times at the 900γ level. Fertility was not correlated with numbers or types of bacteria in this study. The 500γ level of streptomycin was recommended because of its inhibitory effect against *Vibrio fetus*. E. W. Swanson

690. Southern dairy breeding project. C. F. COLLISSON. Am. Dairy Prod. Mfg. Rev., **13,** 9: 2–4. Sept., 1951.

In order to develop dairy cattle better able to withstand high summer temperatures of the southern states, Red Sindhi cattle from India have been crossed with several popular dairy breeds. The experiments have been conducted by the Bureau of Dairy Industry, USDA. The results obtained so far are encouraging and some of the cross-breds have exceeded the production of their dams. T. J. Claydon

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

691. The dairy stable ventilation problem. C. N. TURNER. Agricultural Eng., **32,** 7: 377–8. July, 1951.

There must be a continuous change of air in the stable in order to provide optimum environment for dairy cows and maximum life of the dairy structure. The most tangible impressions for showing the need for better ventilation are visible moisture on walls and ceiling, frost on masonry walls and under the roof in hay lofts, white mold and black rot on wood surfaces, rust on underside of roofs and nails, loss of paint on outside walls and loss of feed in the hay loft.

Major functions of a ventilating system are to control the outgoing air and to control the incoming air. Too many fan manufacturers have considered only control of outgoing air as being of consequence. Fresh air with less moisture, free of odors and cooler than stable air must be supplied in proportion to animal weight. Drafts must be avoided. Moisture given off by the cows must be removed continuously to prevent deterioration of the building and spoilage of feed. The temperature of the stable should be maintained between 45–60° F.

Recommendations for stable ventilation should be prepared which can be adopted by manufacturers of ventilating equipment, and dealers should be trained to tailor their equipment to each job. H. L. Mitten, Jr.

692. Portable milking machine. J. R. ORELIND. U. S. Patent 2, 569,187. 3 claims. Sept. 25, 1951. Official Gaz. U. S. Pat. Office, **650,** 4: 1129. 1951.

A milking-machine timer which blows a whistle when milking is complete is described.

R. Whitaker

693. Pulsator for milking machines. C. B. BARBER. U. S. Patent 2,570,749. 13 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 522. 1951.

A device is described for causing pulsations in the vacuum line operating a milking machine.

R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

694. Experiments with stabilization of fruit for ice cream. C. KOERVER, Borden Co., New York City. Ice Cream Rev., **35,** 2: 50, 52, 97–102. Sept., 1951.

When the use of the continuous freezer was established, the quality of strawberry ice cream suffered because of the necessity of separating the juice from the defrosted berries and then injecting the drained berries into the ice cream. Berries treated thus are mostly cellulose and seeds with little or no flavor. Such ice cream is definitely lacking in eye appeal.

Addition of a suitable stabilizer to the fruit has been suggested for preventing iciness in fruit ice cream and improving the body of the fruit so that it can be drained and injected into ice cream without completely disintegrating. Suitable materials for stabilizers include colloidal materials similar to some of the present ice cream stabilizers. If stabilizers are used, the best place to add them would be at the fruit packers before the fruit is frozen. Actual gel formation should be prevented in the use of a stabilizer or the natural flavor of the fruit will be masked. Any thickening action should not take place until the sugar is completely dissolved and absorbed by the fruit so the sugar concentration in the fruit will not be less than 21%.

Stabilization of fruit shows considerable promise of overcoming some of the difficulties encountered with the present methods of using frozen fruits in ice cream. W. J. Caulfield

695. Effects of package design, use of treated paper upon ice cream shrinkage. J. A. MEISER, JR., Mich. State College, East Lansing. Ice Cream Rev., **35,** 3: 52–54, 56, 58. Oct., 1951.

Weight losses in ice cream stored for periods up to 12 wk. at cabinet temperatures, in different types of ice cream cartons emphasize the importance of using a properly designed moisture-resistant carton for storage of ice ceram.

Cartons which proved most satisfactory in preserving the natural flavor and appearance of ice cream were constructed of heavy weight paper, designed with a minimum of surface area. The lids as well as container bodies should be made impervious to moisture by either a heavy coating of paraffin or other moisture-proof coating.

W. J. Caulfield

696. Consumer preference on flavors. R. J. RAMSEY, Ramsey Labs., Cleveland, O. Ice Cream Rev., **35,** 2: 120–122. Sept., 1951.

Type and amount of flavor used in ice cream should be determined by the consumer and not by selected small groups of individuals within a given plant. Test markets generally have proved more reliable for evaluating consumer preference for any food product than basing an opinion upon a selected taste panel acting for a brief period of time.

Trends toward lower overrun ice cream and use of higher solids mixes usually necessitates an increase in amount of flavor employed. Since corn sugar solids do not detract from flavor to the same extent as do excessive amounts of solids-not-fat, the amount of serum solids should be limited to approximately 11% and the additional solids desired be supplied by corn syrup.

Introduction of high-temperature continuous methods of processing with little or no aging time may result in more cooked flavor being apparent in the finished ice cream. This would work to the detriment of delicate flavors such as vanilla.

Use of inferior quality dairy products in compounding ice cream mixes has been a frequent cause of off-flavored ice cream. Stale or oxidized frozen cream and stale milk powder have been responsible for many of the off-flavors in ice cream associated with the mix ingredients.

Mild delicate flavors generally are more acceptable than strong flavors. Over-flavoring is a serious mistake made by some manufacturers. Variegated ice cream is preferred by many consumers over solid flavors because of the pleasant flavor contrast between the vanilla ice cream and the syrup used. Certain flavors will be acceptable for only short periods of time. When consumption of a particular flavor shows a rapid decline, it is a wise policy to discontinue the flavor rather than continue with its production.

W. J. Caulfield

697. Ice-cream disher. S. J. POPLAWSKI (assignor to J. Oster Mfg. Co.). U. S. Patent 2,568,300. 3 claims. Sept. 18, 1951. Official Gaz. U. S. Pat. Office, **650,** 3: 790. 1951.

A hemispherical bowl-shaped disher with a wiper blade for discharging the portion of ice cream from the bowl is described.

R. Whitaker

698. Ice-cream dispensing machine. E. NELSON. U. S. Patent 2,568,293. 5 claims. Sept. 18, 1951. Official Gaz. U. S. Pat. Office, **650,** 3: 788. 1951.

Ice cream in a bulk container is forced, by means of a piston, into a tube which conducts the ice cream to a cutting device where cylindrical-shaped individual portions of ice cream are cut off as required. The ice cream container, tube and butting device are located in a refrigerated chamber. R. Whitaker

699. Canvas insulated containers cut shipping costs. Anonymous. Ice Cream Rev., **35,** 3: 100, 102. Oct., 1951.

Canvas insulated containers have proved satisfactory for shipping ice cream within a radius of 30–40 mi. by the Cresthaven Farm., Inc., St. Paul, Minn. The bags are pre-cooled in the hardening room for 2 hr. prior to use. Ice cream has been maintained in perfect condition and the cost has been much lower than that of dry ice.

W. J. Caulfield

700. Frozen confection and method of making the same. H. C. GIBSON. U. S. Patent 2,570,031. 7 claims. Oct. 2, 1951. Official Gaz. U. S. Pat. Office, **651,** 1: 228. 1951.

A frozen stick novelty having a waved center layer of stabilized fruit puree, chocolate or other non-dairy food material is described.

R. Whitaker

701. Ice cream and method of making the same. H. T. SPANNUTH (assignor to Wilson & Co., Inc.). U. S. Patent 2,568,666. 5 claims. Sept. 18, 1951. Official Gaz. U. S. Pat. Office, **650,** 3: 887. 1951.

Ice cream mix is stabilized with 0.01–1% of a higher fatty alcohol having at least 12 carbon atoms in the molecule. R. Whitaker

702. Regulating air-charging means for ice cream freezers. T. GIUSTI and R. B. GIUSTI. U. S. Patent 2,569,235. 3 claims. Sept. 25, 1951. Official Gaz. U. S Pat. Office, **650,** 4: 1142. 1951.

A batch ice cream freezer is so constructed that the freezing chamber is placed under constant air pressure, providing any desired overrun when a controlled quantity of mix is introduced.

R. Whitaker

MILK AND CREAM

P. H. TRACY, SECTION EDITOR

703. Tank truck pick-up of milk in Connecticut. A. C. FISHER, Bryant and Chapman Co., Hartford, Conn. Milk Dealer, **40,** 12: 49–50, 53–55. Sept., 1951.

This system first was installed in April, 1948, and at present the truck makes a daily run of approximately 200 mi. collecting 350 cans of milk from 16 producers. One other tank run is operated close to Hartford, picking up milk from 3 certified farms and taking it to a special certified milk bottling plant located at one of the farms. Another large-volume tank truck run in eastern New York and western Connecticut, is being established to haul approximately 300 cans daily. The company bought the first 7 or 8 tanks that were installed on the farms but now the farms are buying their own tanks. On a farm producing 15 cans of milk daily this means an investment of something over $2,000. The milk is cooled immediately to 38° F. in these tanks and maintained at that temperature until collected by the tank trucks. The advantages of the system are: (a) Elimination of milk cans; (b) quick and permanent cooling on the farm; (c) probably reduced hauling rates; (d) elimination of disagreements between dealers and producers brought about by differences of fat con-

tent and daily weight of milk delivered; (e) elimination of farm labor; (f) elimination of plant labor; and (g) flexibility in diverting milk from one plant to another. C. J. Babcock

704. Tank truck collection of milk from farms. R. L. PERRY. Agricultural Eng., **32,** 9: 478–480. Sept., 1951.

The systems of tank truck collection of milk from farms make use of cold-wall type farm milk tanks or aerators and insulated tanks. The tank truck is the usual type equipped with an ice cooled, insulated compartment for sample bottles and a dust-tight compartment for a sanitary pump and hose.

Advantages of the system for patrons include simpler milk house routine, lower transportation costs where volume is large, elimination of losses through milk house spillage and milk sticking to cans and the opportunity to check volume and fat test daily. For the processor, advantages include better quality milk through the elimination of contamination from imperfect cans, lower plant handling costs and elimination of can maintenance. Driver labor is reduced.

Tank truck collection requires greater responsibility on the part of the driver and appropriate sanitary procedures by producers. Good all-weather roads are necessary.

Tank truck collection is more economical than can collection for dairies averaging over 300 gal./day. H. L. Mitten, Jr.

705. Container. R. E. J. NORDQUIST (assignor to American Can Co.). U. S. Patent 2,570,266. 3 claims. Oct. 9, 1951. Official Gaz. U. S. Pat. Office, **651,** 2: 392. 1951.

The paper milk bottle manufactured by American Can Co. is improved by having the closure mounted in a paper flap hinged along a diagonal on the container top. The flap provides sanitary protection for container top. R. Whitaker

706. Home pasteurizer with removable inner receptacle and sealing means therefor. S. L. STRUVE and NELSON O. WELCH. U. S. Patent 2,569,958. 2 claims. Oct. 2, 1951. Official Gaz. U. S. Pat. Office, **651,** 1: 209. 1951.

A design for a small volume jacket-type pasteurizer is presented. R. Whitaker

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(Prepared by D. D. DEANE)

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(Prepared by D. D. DEANE)

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