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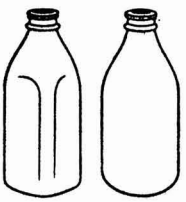

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THE COMPARATIVE VALUE OF CHOCOLATE AND WHOLE MILK AS A SOURCE OF RIBOFLAVIN FOR THE RAT

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Several recent investigations have shown that the photochemical destruction of riboflavin in milk is quite rapid. Peterson *et al.* (1) found losses of riboflavin, as detected by the fluorometric procedure, in pint glass bottles of whole milk exposed to direct sunlight of 28, 50, 66 and 72 per cent after 30, 90, 120 and 210 minutes of exposure, respectively. Similar reports were given by Ziegler (4). Shetlar *et al.* (2) reported that chocolate milks lose riboflavin very slowly when exposed to sunlight, the average loss of riboflavin after 4 hours for five different brands being about 12 per cent as compared to a loss of 80 per cent for whole milk. Warner and Sutton (3) recently reported that calves fed on a photolyzed whole milk diet developed characteristic riboflavin deficiency symptoms. The reports by the above authors indicate the importance of preventing the exposure of milk to sunlight with the resultant inactivation of part of the riboflavin. Loss of riboflavin in whole milk due to light exposure easily could result in at least a partial riboflavin deficiency. Chocolate milk as manufactured compares favorably to whole milk in riboflavin content as determined by the fluorometric procedure developed by Shetlar *et al.* (2). The much slower photochemical loss of riboflavin in chocolate milk perhaps is due to the extra protection afforded by the added chocolate. This would indicate that chocolate milk might prove advantageous if the milk during delivery was subjected to much exposure to light, provided that the chocolate does not interfere with the assimilation of riboflavin after consumption.

The common substitution of chocolate milk for whole milk in the diet, especially of children, has been of concern to nutritionists until it could be established that no vital deficiency resulted from the substitution. Consequently, it seemed desirable to check biologically the comparative value of chocolate milk and whole milk as a source of riboflavin.

EXPERIMENTAL METHODS

The white rats used were selected within an inbred colony descended from a single pair after 3 years of inbreeding. Both males and females were used and

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all were put on experiment at the time of weaning, when weights varied from 48 to 52 g. and average weight of the 22 animals was 48.9 g.

The animals were given a basal diet[4] of the following percentage composition: sucrose, 68; vitamin-test casein, 18; vegetable oil, 10; U.S.P. salt mixture no. 2, 4. The basal diet was a vitamin B-complex test diet nutritionally complete but free of the vitamin B-complex and vitamins A and D. The daily vitamin supplement of 20 γ thiamine, 20 γ pyridoxine, 6 mg. choline chloride, and 200 γ calcium pantothenate was dissolved in 1 ml. of a 25 per cent ethanol solution; this was pipetted into small dishes and given separate from the ration. As a source of vitamins A and D, three drops of halibut liver oil were given once weekly to each animal. One ml. of a 25 per cent ethanol solution containing 20 γ of riboflavin was given daily to the positive control group.

The experimental animals were given 10 ml. of whole or chocolate milk daily as a riboflavin supplement. This compares favorably with the amount of riboflavin given to the positive control group, since whole and chocolate milk contain approximately 2 γ of riboflavin per ml. The animals were housed in individual wire cages having 0.5-inch mesh screen bottoms.

In series 1, six rats which served as the negative control group were fed a complete basal diet except for the absence of riboflavin. This series was depleted of riboflavin for 42 days. The period of depletion was determined by the characteristic symptoms of riboflavin deficiency, such as muscular incoordination, keratitis and alopecia. At the end of the depletion period this series was divided into lots 1 and 2, each containing three rats. Lot 1 was given 10 ml. of whole milk daily as a supplement for 23 more days. Lot 2 was given 10 ml. of chocolate milk daily as a supplement for the same period. Recovery from their characteristic symptoms of riboflavin deficiency would indicate the supplement to be a satisfactory source of riboflavin.

In series 2, six rats, constituting the positive control group, were fed the same basal diet as series 1 except that the diet was supplemented with 20 γ of riboflavin daily. Two of these rats died early in the experiment.

In series 3, six rats were fed the same basal diet as lot 1, except that 10 ml. of whole milk was given daily as a supplement from the beginning of the experiment.

In series 4, six rats were fed the same basal diet as lot 2, except that 10 ml. of chocolate milk was given daily as a supplement from the beginning of the experiment.

The absence of any evidence of riboflavin deficiency in either series 3 or 4 would indicate that the supplements were a satisfactory source of riboflavin.

The rats were weighed three times a week and the average weight of the rats in each series was calculated. Parallel records for each series were made for 39 days, after which series 2, 3 and 4 were taken out of the experiment and series 1, as lots 1 and 2, was continued on experiment for 23 more days. The effect on growth was used as the criterion for the measurement of the availability and assimilation of riboflavin by the rats.

[4] General Biochemicals, Inc., Chagrin Falls, Ohio.

RESULTS

The data from this series of experiments are expressed graphically in fig. 1. The data show that when the rats were weaned, unless milk was given immediately along with the solid food, they lost weight for a few days until they learned to take the solid food. Therefore, each growth curve has to be adjusted for this loss by calculating the rate of growth over the period during which there was a continued increase in weight.

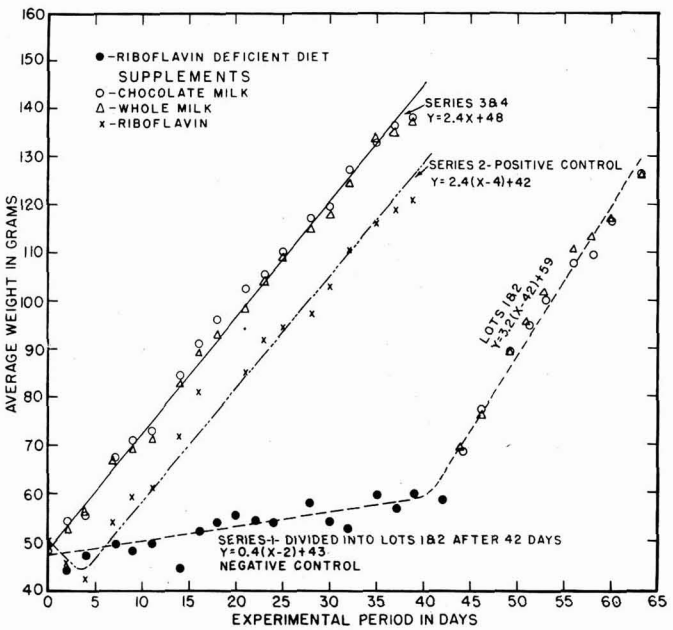

FIG. 1. The value of chocolate and whole milk as a source of riboflavin in the diet of the rat.

While the rate of growth is not a constant but decreases slightly as the growth approaches the limit of maturity, if the sectors covered in the experiment are not too great, a straight line will approximate the curve using the average daily gain as the rate. Using the positive control group, series 2, to obtain the normal growth curve, the equation $y = 2.4(x-4) + 42$ is obtained, where 2.4 is the average daily gain in g., x is the age of the rat in days, $x-4$ adjusts for the initial loss of weight for the ages recorded in days from which the experiment began, 42 is the initial weight and y is the weight in g. of the rat at age $x-4$ in days.

On the basis of the above equation the expected curves for experimental series 3 and 4 can be plotted, provided the necessary riboflavin is in the supplements. A deviation from this expected curve would indicate deficiency of this vitamin.

This equation is: $y = 2.4x + 48$. Superimposing the actual curves on this by plotting the observed data, using triangles for the whole milk, series 3, and circles for the chocolate milk, series 4, both series show a very good fit to the expected curve. The results show that chocolate milk, as well as whole milk, is an adequate supplement as a source of riboflavin in the diet of the rat at the levels investigated. Possibly some difference might have been found at a riboflavin level too low to support optimum growth; however, this possibility was not investigated in this work.

The negative group, series 1, made very little gain during the 42 days they were on the deficient diet and developed symptoms of vitamin deficiency, such as sore eyes and rough coat, indicating a severe deficiency of riboflavin. The average daily gain was only 0.4 g. per day. This period of their life can be represented by the curve: $y = 0.4(x - 2) + 43$. A remarkable change took place when chocolate or whole milk was added to their diet at the end of 42 days. They immediately began to gain at an accelerated growth rate that tended to compensate for the retarded condition. Using the same symbols for the whole milk lot 1 and the chocolate milk lot 2 as before, the average weights coincide a surprising number of times. Both curves fit very closely to that expressed by the equation: $y = 3.2(x - 42) + 59$.

SUMMARY AND CONCLUSIONS

The comparative value of chocolate and whole milk as a source of riboflavin for the rat was investigated. Rats fed chocolate milk had the same rate of growth as rats fed whole milk when both milks were added to a basal diet deficient only in riboflavin. The rate of growth of rats receiving the basal diet supplemented with either milk also was the same as that of rats receiving supplements of crystalline riboflavin. Therefore, at the level of feeding used in this experiment, chocolate milk contains enough biologically available riboflavin to insure maximum growth of the rat. No evidence was found to indicate that the added chocolate interferes with the biological availability of riboflavin.

ACKNOWLEDGMENT

The authors are grateful to the Tullis-Hall Dairy of Sedalia, Mo., for supplying the chocolate and whole milk used in this investigation.

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THE INSTABILITY OF ASCORBIC ACID IN WATER, WITH ADDED COPPER OR HYDROGEN PEROXIDE OR BOTH[1]

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Results obtained in our laboratories caused us to suspect that impurities in the water from a laboratory still were hastening the oxidation of ascorbic acid solutions. They led us to investigate the effect of different factors, particularly the source of the water, upon the stability of ascorbic acid solutions. A water solution of ascorbic acid is used in standardizing the dye, 2,6-dichlorophenolindophenol, with which the amount of this form of vitamin C in milk and in other fluids can be determined.

Water from the laboratory still was obtained by evaporating tap water in a reservoir indirectly heated by steam, collecting the condensate in large earthenware jars, and then drawing it from a tin lined bronze faucet through 12 to 14 feet of tin lined iron pipe. However, the condensate came in contact with brass couplings which contain a high proportion of copper.

The concentration of ascorbic acid in the various water solutions and in the milk was determined by titration in acid solution (sulfuric) with 2,6-dichlorophenolindophenol (4, 14).

In figure 1 are shown the rates of oxidation of ascorbic acid at 30° C. in (a) water distilled in pyrex glassware, (b) water from the laboratory still, and (c) water from the laboratory hot water tap. The amounts of ascorbic acid in these waters, immediately after its addition, were 19.9, 20.0 and 16.5 mg./l., respectively. Also shown in this figure are the rates of oxidation of ascorbic acid dissolved in glassware-distilled water in approximately the same concentration with 0.15 and 0.5 p.p.m. of copper added. The crystalline ascorbic acid was dissolved in a small volume of glassware-distilled water; the copper, in the form of copper sulphate, was added in a standardized solution. These solutions were added to glassware-distilled water at 30° C., made up to the desired volume and mixed by pouring from one flask to another.

The rate of oxidation of ascorbic acid in the tap water was faster than in the glassware-distilled water to which 0.5 p.p.m. of copper had been added. When as little as 0.15 p.p.m. of copper was added to the glassware-distilled water, 83 per cent of the ascorbic acid was oxidized within 60 minutes.

The acid intensity (pH) and oxidation-reduction potential (E_h) of a solution influence the rate of oxidation of ascorbic acid. The former was shown by Barron, DeMeio and Klemperer (2), the latter by Kenny (8). Other things being equal, the rate of oxidation of ascorbic acid is faster in an alkaline than it is in an acid solution. Of two ascorbic acid solutions that are similar except

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[1] This work was done with funds from the Research and Marketing Act of 1946.

that one has a higher E_h than the other, the ascorbic acid in the former will be less stable than in the latter.

Repeated pH and E_h measurements of water from the glassware still, the laboratory still and the hot water tap gave surprisingly constant values, even though the waters were poorly buffered and poised. On the average, the addition of 20 mg. of ascorbic acid to a liter of tap water lowered the pH to 7.5 from 7.7. The E_h was then + 0.41. When the glassware-distilled and laboratory still waters were similarly acidulated their pH values decreased nearly 2 units or from about 6.6 to 4.6. However, their E_h values were + 0.41 and + 0.34, respectively. Because of the lack of buffering and poising of these solutions, these values must be considered only relative rather than absolute.

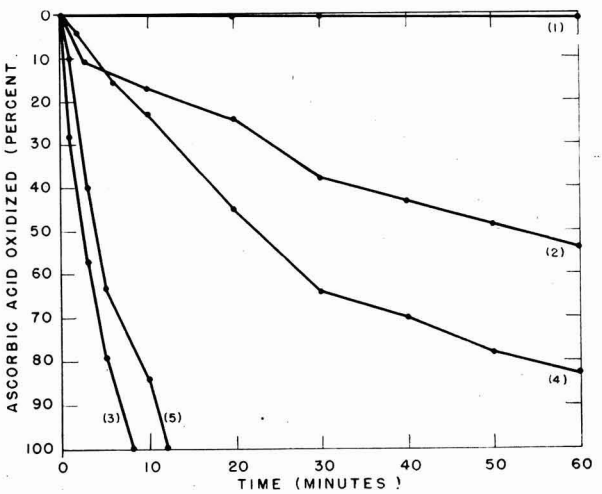

FIG. 1. Rate of ascorbic acid oxidation in 30° C. in pyrex glassware-distilled water, in water from a laboratory still, in tap water, and in glassware-distilled water plus added copper: (1) Glassware-distilled water plus 19.9 mg. of ascorbic acid per l.; (2) water from a laboratory still plus 20.0 mg. of ascorbic acid per l.; (3) tap (hot) water plus 16.5 mg. of ascorbic acid per l.; (4) No. 1 plus 0.15 p.p.m. of copper; and (5) No. 1 plus 0.50 p.p.m. of copper.

Impurities in the water from the hot water tap caused the ascorbic acid to oxidize rapidly. This water contained 0.12 p.p.m. of copper, 0.85 p.p.m. of iron, and, as indicated by the milkiness of the water when it was first drawn, considerable dissolved oxygen. The chlorides test with silver nitrate was positive. The iodine test for chlorine was negative.

The amount of copper in the tap water and in water from the laboratory still was measured by an all-dithizone method using a spectrophotometer (3, 12), and of iron[2] by reduction with zinc and titration with potassium permanganate (1).

[2] We are indebted to H. S. Haller of these laboratories for the copper and iron determinations.

Water from the laboratory still contained only a trace of copper (< 0.01 p.p.m.). The chlorides test was negative.

Barron, DeMeio and Klemperer (2) reported that "among the metallic salts tested (Mn, Ni, Fe, Co, Ca, Cu), copper is the only catalyst for the oxidation of ascorbic acid, its catalytic action being noticed in concentrations as small as 46 micrograms of copper per liter." This is equivalent to 0.046 p.p.m. Mapson (11) has shown that the complete removal of copper from a solution renders ascorbic acid stable to O_2.

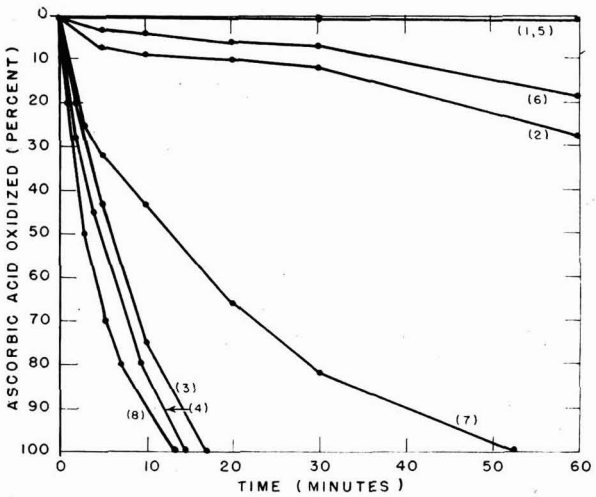

FIG. 2. Effect of deaeration on the rate of ascorbic acid oxidation in pyrex glassware-distilled water at 30° C. when hydrogen peroxide or copper or both were added: (1) Undeaerated glassware-distilled water plus 20.0 mg. of ascorbic acid per l.; (2) same as No. 1 plus 0.03 ml. of 30% H_2O_2 per l.; (3) same as No. 1 plus 0.3 p.p.m. of copper; (4) same as No. 1 plus 0.03 ml. of 30% H_2O_2 per l. and 0.3 p.p.m. of copper; (5) deaerated glassware-distilled water plus 20.0 mg. of ascorbic acid per l. and redeaerated; (6) same as No. 5 plus 0.03 ml. of 30% H_2O_2 per l. after the first deaeration; (7) same as No. 5 plus 0.3 p.p.m. of copper after the first deaeration; (8) same as No. 5 plus 0.03 ml. of 30% H_2O_2 and 0.3 p.p.m. of copper after the first deaeration.

In figure 2 are shown the rates of oxidation of ascorbic acid (20.0 mg./l.) in undeaerated and in deaerated glassware-distilled water, without and with added copper or hydrogen peroxide or both. In preparing the latter solutions, the glassware-distilled water was deaerated by heating it to a temperature (50° C.) at which it would boil when under the vacuum (13); then the vacuum was broken, the warm water poured into a large beaker, a small volume of concentrated ascorbic acid added and the copper or hydrogen peroxide or both, if required, and the solution redeaerated. Thus the fluid was deaerated twice. During the second deaeration and about 30 minutes thereafter the reacting temperature was warmer than 30° C. In order to prevent reincorporation of

air, each 10 ml. for titrating was drawn into a pipette from the deaerating collecting flask.

In the course of an hour there was less than 30 per cent destruction of the ascorbic acid in the undeaerated solution to which hydrogen peroxide had been added. However, the ascorbic acid was oxidized quickly in the presence of as little as 0.3 p.p.m. of copper and at an even faster rate when 0.03 ml. of 30 per cent hydrogen peroxide per liter of water also was added.

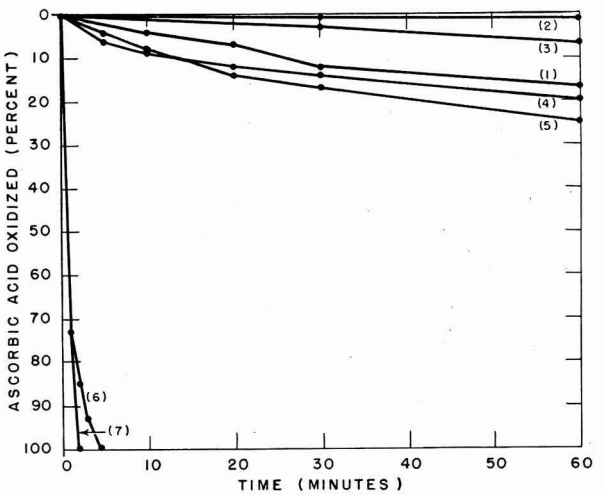

FIG. 3. Rate of ascorbic acid oxidation of 30° C. in fresh raw milk, in pyrex glassware-distilled water, in their mixtures, and in equal parts of milk and water from a laboratory still, and in fresh raw milk with added copper, H_2O_2, or both: (1) Fresh raw milk containing 16.1 mg. of ascorbic acid per l.; (2) glassware-distilled water plus 20.9 mg. of ascorbic acid per l.; (3) equal parts raw milk and glassware-distilled water plus 12.0 mg. of ascorbic per l.; (4) equal parts of fresh raw milk and water from the laboratory still plus 12.0 mg. of ascorbic acid per l.; (5) No. 1 plus 0.3 p.p.m. of copper; (6) No. 1 plus 0.03 ml. of 30% hydrogen peroxide per l.; (7) No. 1 plus 0.3 p.p.m. of copper and 0.03 ml. of 30% H_2O_2 per l.

In figure 3 are shown curves that illustrate the rate of oxidation at 30° C. of ascorbic acid in fresh raw milk, in glassware-distilled water, and in a 1:1 mixture of the two under comparable temperature, indirect light and air incorporating conditions. Others (6, 15) have shown the rate of destruction of ascorbic acid in milk protected from direct light and from copper contamination.

Also shown in figure 3 are the rates of oxidation of ascorbic acid in a mixture of equal parts of fresh raw milk and of water from the laboratory still, fresh raw milk with 0.3 p.p.m. of added copper, fresh raw milk to which 0.03 ml. of 30 per cent hydrogen peroxide per liter of milk had been added and also fresh raw milk to which both copper and 30 per cent hydrogen peroxide had been added in these proportions.

Early investigators of the oxidized flavor in milk (7, 17) believed that the destruction of ascorbic acid and the development of the oxidized flavor was influenced by an enzyme which occurs naturally in milk. In more recent publications Krukovsky (9) and Krukovsky and Guthrie (10) presented evidence to show that a promotor (an enzyme) of ascorbic acid oxidation in milk by hydrogen peroxide might be responsible for quick conversion of ascorbic acid to dehydroascorbic acid. The presence of an enzyme in the water used in our experiments was precluded by the sources of the water. Copper was a key factor in the oxidation of the acid and, when hydrogen peroxide also was added to the solution, the ascorbic acid oxidized rapidly.

Milk produced by cows on a normal ration contains about 0.15 mg. of copper per liter (5). Milk also contains about 5.4 p.p.m. of oxygen (15).

On the first page of their article, "Quantitative Determination of Dissolved Oxygen", Sharp, Hand and Guthrie (16), in describing the standardization of the dye solution to be used for titrating milk to determine its ascorbic acid content, direct as follows: "Weigh accurately approximately 100 mg. of ascorbic acid, place in a 1-liter volumetric flask, and make up to volume with distilled water. Mix thoroughly and use at once for standardizing." In an earlier publication Sharp (14) directs that the ascorbic acid solution be used at once for standardizing the dye (2,6-dichlorophenolindophenol) solution.

The significance and importance of titrating at once is emphasized by the results presented in figure 1. A dye factor based upon the ascorbic acid content of an unstable solution might be inaccurate. Since ascorbic acid is likely to be used in increasing amounts for retaining the fresh flavor of milk (18), the importance of correctly standardizing the dye solution that is used in measuring the ascorbic acid content of milk becomes apparent.

CONCLUSIONS

Water may contain impurities that accelerate the oxidation of ascorbic acid.

Ascorbic acid is stable for more than an hour in pyrex glassware-distilled water and relatively unstable in glassware-distilled water to which hydrogen peroxide has been added. It is unstable when these solutions contain copper.

Three-tenths p.p.m. of copper is sufficient to cause the rapid oxidation of ascorbic acid in glassware-distilled water.

Only pure water should be used in preparing an ascorbic acid solution that is to be used in standardizing the dye, 2,6-dichlorophenolindophenol, with which the ascorbic acid content of milk and other fluids can be measured in acid solution by direct titration.

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DEFERMENT OF AN OXIDIZED FLAVOR IN FROZEN MILK BY ASCORBIC ACID FORTIFICATION AND BY HYDROGEN PEROXIDE OXIDATION OF THE ASCORBIC ACID OF THE FRESH MILK[1]

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Two outstanding reasons why milk has not been preserved in the frozen state on a larger commercial scale are the development during storage of the flavor defect usually known as oxidized and the deterioration in body as evidenced by uneven distribution of insoluble solids in the thawed milk. The former will be considered here.

Krukovsky and Guthrie (3) found that the reaction which produces the "tallowy" (oxidized) flavor could be inhibited by quick and complete photochemical or chemical oxidation of ascorbic acid in the milk to dehydroscorbic acid prior to pasteurization and storage. Their samples were stored at 0 to 5° C. and were examined usually over a period of about a week.

Whether the characteristic oxidized flavor which develops in frozen milk has its origin in the same source as does the oxidized flavor in unfrozen milk is not known. It has been detected in thawed milk after a storage period of only 2 or 3 weeks (1), and the source is thought to be the same.

The questions which the present investigations undertook to answer were how long the onset of the characteristic oxidized flavor could be delayed in frozen storage by chemical (hydrogen peroxide) oxidation of ascorbic acid in the fresh milk, and whether fortification of the fresh milk with ascorbic acid would be as effective.

The general methods that were used have been described previously (1). The milk was pasteurized in stainless steel containers by holding it at 71° C. for not less than 15 seconds, homogenized at 2,500 lb. pressure per square inch, and cooled at once. During this processing the milk was exposed briefly to copper because some of the tin had worn off the short lengths of pipe leading to and away from the homogenizer and off the small surface cooler. The ascorbic acid was added just prior to packaging the milk, the hydrogen peroxide just before or after pasteurization. Fresh samples were held in pyrex flasks at 2 to 4° C.; others were canned and stored in a room that was maintained at −17° C., and examined as indicated in the tables.

The initial ascorbic acid titrations referred to in table 1 were completed within an hour after the samples were prepared, the oxidation-reduction (E_h) measurements within the next 2 hours. The E_h of the fresh milk was low and the milk was well poised. During the next few days in cold storage the ascorbic acid in the samples held at 2 to 4° C. oxidized, the E_h became more positive and

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TABLE 1

Relationship between the amounts of 30 per cent hydrogen peroxide and/or ascorbic acid added to milk after pasteurization and the development of the oxidized flavor in milk subsequently held at 2° to 4° C. and at −17° C.

Holding temperature and time held	Pasteurized control (A)			Ascorbic acid added to (A) (B)			(C)			0.03 ml. of 30% H_2O_2/l. added to (A) (D)			Ascorbic acid added to (D) (E)			(F)		
	A.A.[a] (*mg./l.*)	E_h[a] (*volts*)	F[a]	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F
2° to 4° C.:																		
1 to 3 hr.	13.8	0.206		33.6	0.189		54.0	0.182		1.2	0.246		14.4	0.235		31.2	0.216	
2 d.	0.0	0.259		1.8	0.239		16.8	0.219		0.0	0.257		0.0	0.240		0.0	0.249	ox
6 d.	0.0	0.316	sl−	0.0	0.309	sl	0.0	0.308	sl	0.0	0.304		0.0	0.301	ox	0.0	0.298	ox+
−17° C.:																		
14 d.	11.4	0.220	tr	30.6	0.198		51.6	0.187		0.0	0.255		0.0	0.243	ox	14.4	0.221	ox+
56 d.	5.4	0.234	st	8.6	0.208	ox	26.4	0.190	tr	0.0	0.269	sl+	0.0	0.256	ox	10.2	0.229	st

Holding temperature and time held	Equal parts of (A) and (D) (G)			Ascorbic acid added to (G) (H)			(I)			(A) + 2(D) (J)			2(A) + (D) (K)		
	A.A.[a] (*mg./l.*)	E_h[a] (*volts*)	F[a]	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F
2° to 4° C.:															
1 to 3 hr.	4.8	0.230		23.4	0.209		41.4	0.195		3.0	0.240		6.6	0.232	
2 d.	0.0	0.259		0.0	0.245	sl	1.8	0.238	sl	0.0	0.270		0.0	0.243	
6 d.	0.0	0.305	tr	0.0	0.301	sl	0.0	0.299	sl+	0.0	0.304		0.0	0.310	sl
−17° C.:															
14 d.	0.0	0.229	sl	10.2	0.217	ox	28.8	0.204	ox	0.0	0.242		0.0	0.244	ox
56 d.	0.0	0.249	ox+	5.4	0.255	ox	22.8	0.208	ox	0.0	0.254	ox−	0.0	0.251	ox

[a] A.A. = ascorbic acid; E_h = oxidation-reduction potential at 30° C.; F = flavor. Oxidized flavor intensity is indicated as follows: tr = trace, sl = slight, ox = oxidized, st. = strongly oxidized. Where there are no flavor notations the samples were considered satisfactory.

some of the samples developed a trace to an almost strong oxidized flavor. As was anticipated by the findings of Krukovsky and Guthrie (3) the milk *D* to which 0.03 ml. of 30 per cent hydrogen peroxide per liter of milk was added did not become oxidized in flavor; samples of this same milk to which ascorbic acid was added (*E* and *F*) did, *F* to a greater intensity than *E*, even though it had a lower initial E_h.

After 14 days at –17° C. the thawed control sample was just beginning to taste oxidized; *B* and *C*, ascorbic acid fortified milks, had a normal flavor; *D*, to which hydrogen peroxide was added, was unchanged; *E* and *F* tasted oxidized. Of the remaining five milks, only *J* was judged normal. However, after 56 days at –17° C. all of the milks in the series including *C* and *D* were oxidized to a greater or lesser degree; *C*, which originally contained 54 mg. of ascorbic acid per liter of milk, being less oxidized than *D*.

It is well known that copper catalyzes changes in milk which cause the oxidized flavor. The data in table 2 re-emphasize this fact and are evidence that hydrogen peroxide delays, but does not prevent, the development of this off flavor in frozen milk. Hand drawn, uncooled morning milk was utilized. The milk was divided into three parts. One (*A*) was pasteurized, homogenized and cooled as usual. The ascorbic acid content of the fresh, raw milk was 21.2 mg./l., that of the pasteurized product immediately after being cooled was 20.6 mg./l. In preparing the second part (*B*) 0.03 ml. of 30 per cent hydrogen peroxide was added per liter of raw milk. In a few minutes the milk contained only 2.1 mg. of ascorbic acid per liter; after pasteurization, it contained none. Part three (*C*) was prepared by first combining equal parts of fresh raw milk and fresh raw milk to which 0.03 ml. of 30 per cent hydrogen peroxide per liter had just been added. Finally, copper in the form of copper sulphate was added at the rate of 1.0 p.p.m. to each of these three pasteurized milks, thus forming milks *D*, *E* and *F*. The ascorbic acid content and E_h of these six milks were determined within 3 hours after the samples were placed in 2 to 4° C. storage.

The "heated" flavor noted in table 2 ought not to be interpreted as "cooked." It was of mild intensity and not readily detectable. At one stage it was described as slight almond. In that part of this experiment (B) in which 30 per cent hydrogen peroxide was added at the rate of 0.03 ml./l. of raw milk, the onset of the oxidized flavor was deferred longer than 55 days. The oxidized flavor that developed in milks *D*, *E* and *F* was particularly objectionable. It was unclean and somewhat rancid.

Table 3 shows the relationship between the amounts of ascorbic acid added to pasteurized milk and of 30 per cent hydrogen peroxide added to the milk before or after pasteurization and the development of the oxidized flavor under two conditions of storage. Heavy fortification with ascorbic acid protected the flavor of the milk more effectively than did the addition of 30 per cent hydrogen peroxide.

In experiment 4, as reported in table 4, pasteurized milk was fortified heavily with ascorbic acid, and 30 per cent hydrogen peroxide was added to separate

TABLE 2

Relationship between the addition of 30 per cent hydrogen peroxide to milk before pasteurization, copper after pasteurization, and the development of the oxidized flavor in the pasteurized milk subsequently held at 2° to 4° C. and at −17° C.

Holding temperature and time held	Pasteurized control (A)			0.03 ml. of 30% H_2O_2/l. of milk (B)			Equal parts of (A) and (B) (C)			Copper added 1.0 p.p.m. Added to (A) (D)			Added to (B) (E)			Added to (C) (F)		
	A.A.[a] (*mg./l.*)	E_h[a] (*volts*)	F[a]	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	E_h (*volts*)	F
2° to 4° C.:																		
2 to 3 hr	18.0	0.239		0.0	0.264		7.8	0.257		15.7	0.279		0.0	0.298		0.0	0.281	
2 d.	0.0	0.306		0.0	0.312	heated	0.0	0.306		0.0	0.316	sl	0.0	0.308	heated	0.0	0.308	heated
5 d.						{ sl. almond						sl						
−17° C.:																		
33 d.	0.0	0.283	sl	0.0	0.272		0.0	0.264	sl −	0.0	0.299	st	0.0	0.302	ox +	0.0	0.293	ox −
55 d.		0.306	st		0.280	heated		0.288	ox		0.314	st		0.318	st		0.306	st
90 d.						ox			st									

[a] Flavor and other designations as in table 1.

TABLE 3

Relationship between the amounts of ascorbic acid added to pasteurized milk and of 30 per cent hydrogen peroxide added to milk before or after pasteurization and the development of the oxidized flavor in milk subsequently held at 2° to 4° C. and at −17° C.

Holding temperature and time held	Pasteurized control		Ascorbic acid added to (A)				0.03 ml. of 30% H_2O_2 /l. of raw milk		Equal parts of (A) & (D) after pasteurization		0.06 ml. of 30% H_2O_2 /l. of raw milk		Equal parts of (A) & (F) after pasteurization		0.03 ml. of 30% H_2O_2 /l. of pasteurized milk		Equal parts of (A) and (H)		0.06 ml. of 30 per cent H_2O_2/l. of pasteurized milk		Equal parts of (A) and (J)	
	(A)		(B)		(C)		(D)		(E)		(F)		(G)		(H)		(I)		(J)		(K)	
	A.A.[a] (*mg./l.*)	F[a]	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F	A.A. (*mg./l.*)	F
2° to 4° C.:																						
2 to 4 hr.	14.4		65.4		117.6		0.0		6.6		0.0		1.2		0.0		4.0		0.0		0.0	
5 d.	0.0		0.0		0.0		0.0		0.0	ox	0.0	sl	0.0		0.0		0.0		0.0	ox−		
−17° C.:																						
27 d.	0.0	st	37.8	tr	75.0		0.0	sl	0.0	ox	0.0	st	0.0	st	0.0	ox−	0.0	sl	0.0	st	0.0	{un-clean
47 d.		st	34.1	ox	70.0	sl		ox		ox		st		st		ox		ox		st		ox−

[a] Flavor and other designations as in table 1.

TABLE 4

Comparison of ascorbic acid and hydrogen peroxide as a means of delaying the development of an oxidized flavor in pasteurized milk preserved in frozen storage

Holding temperature and time held	Pasteurized control (A)				Ascorbic acid added to (A) (B)				(C)				0.015 ml. of 30% H_2O_2 added /1. of raw milk (D)			
	A.A.[a] (*mg./l.*)	Dehy[a] (*mg./l.*)	E_h[a] (*volts*)	F[a]	A.A. (*mg./l.*)	Dehy (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	Dehy (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	Dehy (*mg./l.*)	E_h (*volts*)	F
2° to 4° C.:																
3 to 4 hr.	17.3	3.3	0.219	sl / heated	68.0	4.4	0.177		120.2	4.4	0.168		11.7	5.2	0.230	
5 d.	0.0	4.8		ox	0.0	6.2	0.299	sl	0.0	19.9	0.282		0.0	1.4	0.276	stale
−17° C.:																
27 d.	0.0	3.5	0.260	ox	32.9	4.9	0.214	sl+	86.8	7.0	0.193		0.0	0.0	0.250	st
35 d.		3.0	0.268	st	30.8	3.5	0.246	ox−	74.6	15.7	0.233	tr			0.258	st

Holding temperature and time held	Ml. of 30 per cent hydrogen peroxide added per liter of raw milk: 0.030 (E)				0.045 (F)				0.060 (G)				0.150 (H)			
	A.A. (*mg./l.*)	Dehy (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	Dehy (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	Dehy (*mg./l.*)	E_h (*volts*)	F	A.A. (*mg./l.*)	Dehy (*mg./l.*)	E_h (*volts*)	F
2° to 4° C.:																
3 to 4 hr.	2.9	3.7	0.230		0.0	1.5	0.243		0.0	0.0	0.263	sl / heated	0.0	0.0	0.309	almond
5 d.	0.0	0.0	0.290	stale	0.0	0.0	0.296	stale	0.0	0.0	0.303	stale	0.0	0.0	0.337	stale
−17° C.:																
27 d.	0.0	0.0	0.271	ox	0.0	0.0	0.269	ox−	0.0	0.0	0.277	sl / almond / ox	0.0	0.0		sl / almond / st
35 d.			0.292	st			0.277	ox−			0.280	ox			0.321	st

[a] Flavor and other designations as in table 1. Dehy = dehydroascorbic acid.

portions of the raw milk in carefully graduated amounts rather than by dilution in sequence of milk containing a large amount of added hydrogen peroxide with normal milk. Immediately after each addition of hydrogen peroxide the amount of ascorbic acid was 15.5, 3.7, 4.9, 7.0 and 16.5 mg./l., and of dehydroascorbic acid it was 8.2, 19.8, 18.1, 16.2 and 7.3 mg./l., respectively. Thus the total vitamin C at this time, regardless of the amount of hydrogen peroxide that was added, was approximately the same.

The initial data in the table were obtained 2 to 4 hours after the milks had been pasteurized and stored. Five days later, among the samples maintained at 2 to 4° C., only *A, B, C* and *D* contained any vitamin C. It was the oxidized form (2). Sample *C,* the most heavily ascorbic acid-fortified milk, had a normal flavor at this time. Samples *D* to *H,* inclusive, tasted old and somewhat stale, but not oxidized.

After 27 days at – 17° C., only thawed samples *B* and *C* contained ascorbic acid. Samples *A, B* and *C* contained 3.5, 4.9 and 7.0 mg. of dehydroascorbic acid per liter, respectively; the other samples contained none. Sample *A* tasted oxidized, *B* slightly oxidized, and *C,* the most heavily ascorbic acid fortified milk, had a normal flavor. Samples *D* to *H,* inclusive, to which 30 per cent hydrogen peroxide had been added at the rate of 0.015 to 0.150 ml./l. of raw milk, were oxidized or strongly oxidized. *G* and *H* also had a slight but definite almond flavor due to the relatively large amounts of hydrogen peroxide that were added to the raw milk.

Twelve days later the samples were found to have changed in the usual way. The vitamin C content of *A, B* and *C* was decreasing, their E_h was increasing and, except for *C,* the intensity of the oxidized flavor was the same or somewhat greater. *C* no longer had a normal flavor; an oxidized flavor was detectable.

In this experiment, as well as in others in which 30 per cent hydrogen peroxide was added and a strong oxidized flavor developed, the flavor was also tallowy. These samples were more objectionable than were those which, although fortified with ascorbic acid, became oxidized or strongly so.

DISCUSSION AND CONCLUSIONS

It ought not to be implied from the work of Krukovsky and Guthrie (3, 4) on market milk stored at 0° to 5° C. that a rapid, complete oxidation of vitamin C in milk by hydrogen peroxide prevents the development of an oxidized flavor indefinitely. The results reported here indicate that, when such milk is preserved in frozen storage, this characteristic off flavor will be detectable eventually. From a chemical standpoint this is to be expected, if the flavor is due to a mild oxidative reaction. Whenever a physico-chemical system tends to have a higher oxidation-reduction potential, the more easily oxidizable constituents (for example, ascorbic acid in milk) will tend to oxidize. Free oxygen is not essential because oxidative changes can take place without its participation, merely by transfer of electrons from reductants to oxidants. So long as this can go on, as it can even in frozen milk, the system will be unstable and tend toward a higher poten-

tial. The addition of ascorbic acid to milk lowers the oxidation-reduction potential, the milk is more static and its tendency to develop the oxidized flavor is decreased.

Ascorbic acid, a strong reducing agent, is almost ideal for lowering the E_h of milk. It is a natural constituent of milk; it is one of two equally biologically active forms of vitamin C; it does not alter the flavor, nor does it appreciably increase the acid intensity of the system. Furthermore, it is inexpensive. However, its tendency to oxidize is relatively great. Added to market milk, ascorbic acid is effective in delaying, and usually in preventing, the onset of the oxidized flavor during the life of the product. In frozen milk, which has a longer commercial life than market milk, ascorbic acid defers or delays but does not prevent the defect. This is also true of hydrogen peroxide when it is added to milk that is to be preserved in a frozen state.

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THE RELATION OF PEDIGREE PROMISE TO PERFORMANCE OF PROVED HOLSTEIN-FRIESIAN BULLS[1]

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The items of information which are used as criteria for selection of dairy bulls normally become available in the following time sequence: (a.) pedigree, (b.) individuality, (c.) progeny performance.

The progeny test is theoretically the most accurate method for estimating the production of future daughters of a bull and consequently has been accepted generally as the most accurate from a practical standpoint also. Recent studies by Beardsley (1) indicate that the predictability of the productive performance of a group of daughters in a second herd from the performance of a group of daughters in a first herd is not especially high, the correlation coefficient being +0.34. Thus, except for situations where a proved sire is to be bred in the future to cows known to have the same general genetic make-up and to be kept under identical conditions to those cows with which he was first proved, the accuracy of prediction from first proof is not as high as generally implied.

Even though, for a particular situation, reliance were to be placed solely on the proved sire, all young bulls must be selected on some other basis. Individuality of a bull should be used as a basis of selection to improve milk production or butterfat yield only to the extent that type in the male is correlated with milk production in his daughters. So far as is known, no adequate test of such an hypothesis has been made. Thus, in selecting young dairy bulls, reliance must be placed largely on pedigree information. It has been difficult to determine the effectiveness of pedigree selection in improving the productive performance of dairy cattle, since few data on this question have been reported.

Gowen (5, 6) and Copeland (2, 3), using Advanced Registry or Register of Merit records, have determined the correlations that existed between the average production of the daughters of bulls and different groups of their relatives considered separately. Madsen (9) determined similar correlations from data more comparable to that obtained in D.H.I.A. or H.I.R. testing programs, but he used different groups of bulls and relatives to obtain each correlation.

Lush and Schultz (8) studied the relation between pedigree promise as indicated by Advanced Registry records and the performance of daughters of

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[1] The data in this paper are from a thesis submitted by the senior author to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Feb., 1948. The authors are indebted to Professor S. J. Brownell, Project Leader, Animal Husbandry Extension, New York State College of Agriculture, for permission to obtain these data from the Herd Analysis Extension Project.

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bulls when tested in D.H.I. Associations. Here again different groups of individuals were used to study the relationship of the production of a certain ancestor to the final proof of the sires. These authors point out that the most satisfactory evidence should be obtained from multiple correlation studies where data were available on all groups of ancestors for the bulls under consideration.

The purpose of the study published here was to develop a method and estimate its accuracy for selecting an unproved bull, using the production performance of the ancestors, collateral females and mates, when the information was complete for each pedigree.

EXPERIMENTAL

There were 1,451 Holstein-Friesian bulls proved by D.H.I.A. records in New York prior to Feb. 1, 1947. A number of these proved bulls were sons of proved sires and were born in herds which were cooperating in the New York Dairy Extension Herd Analysis Project. As a consequence, records of butterfat production for relatives on both the paternal and maternal side of the pedigree were available for study.

Two series of proved bulls were selected from those born in analyzed herds. Series A included 158 bulls (average number daughter-dam comparisons, 8.40), each of which had the following recorded information available, in addition to the proof on his sire (average number of daughter-dam comparisons, 21.05)[4]: a. The dam of the bull had at least one record (average number, 4.64), and b. The dam of the bull had at least one daughter with a record (average number of daughters, 1.85).

Series B included 141 bulls (average number daughter-dam comparisons, 8.13), each of which had the following recorded information available in addition to the proof on his sire (average number daughter-dam comparisons, 21.81)[5]: a. The dam of the bull had at least one record (average number, 4.33), and b. The dam of the bull was by a proved sire (average number daughter-dam comparisons, 18.07).[6]

Of the total of 207 bulls meeting either set of established criteria for study, 92 satisfied both sets of requirements and were included in both series. Thus, the two series are not independent samples and any comparisons between the results of the two series should not be made without recognizing the correlation that exists between them.

The average of all butterfat records for each cow, calculated to a mature equivalent, 305-day lactation, twice-a-day milking basis, by D.H.I.A. standards, was used as a measure of the producing ability of each cow. The variables for each bull were means of the production of groups of females in each case, except for the production of the bull's dam which was an average, in most cases, of several

[4] Included among the sires of bulls in series A were 6 bulls with 4, and 7 bulls with 3 daughter-dam comparisons. All others had 5 or more.

[5] Included among the sires of bulls in series B were 1 bull with 4, and 4 bulls with 3 daughter-dam comparisons.

[6] Included were 6 sires having 4, and 17 sires having 3 daughter-dam comparisons, the dam of the series B bulls being one of these daughters in each case.

records of the same individual. The figure for a particular variable of all bulls was given equal weight regardless of the number of production records from which the figure was calculated.

The variables for this study were assigned symbols as follows: X_1 = average production of the mates of the proved bull; X_2 = average production of the maternal half-sisters of the proved bull; X_3 = average production of the dam of the proved bull; X_4 = average production of the paternal half-sisters of the proved bull; X_5 = average production of the dams of the paternal half-sisters of the proved bull; X_6 = average production of the daughters of dam's sire (paternal half-sisters of the bull's dam), and Y = average production of the daughters of the proved bull.

Simple correlation coefficients were calculated between all pairs of the above variables (table 1) to determine multiple regression equations after the methods outlined by Snedecor (11), in order to establish the optimum weight to be given each variable in predicting the level of production of Y, the daughters of the proved bulls.

RESULTS

The mean production of the relatives of the bulls and the correlations between these relatives are shown in table 1. The average production of the bulls' dams was from 30 to 80 lb. higher than the average production of the animals constituting any other variable, indicating that the dams were a selected group, not a random group of females from this population. This must be borne in mind in interpreting the results of this investigation.

TABLE 1

Mean production in pounds of butterfat for each group of relatives in both Series A and Series B and the correlations between these groups within each series

	Series	Mates (X_1)	Maternal half sisters (X_2)	Dam (X_3)	Paternal half sisters (X_4)	Dams of paternal half sisters (X_5)	Daughters of maternal grandsire (X_6)	Daughters of bull (Y)
Mean production and standard deviation	A	380 ± 54	421 ± 74	460 ± 78	408 ± 54	402 ± 53		387 ± 65
	B	382 ± 50		455 ± 78	422 ± 50	412 ± 52	406 ± 53	394 ± 63
X_1	A		0.18*	0.18*	0.26**	0.23**		0.66**
	B			0 17*	0 18*	0 08	0.15*	0.65**
X_2	A			0.32	0.47**	0.42**		0.22**
	B							
X_3	A				0.34**	0.47**		0.15
	B				0.25**	0.29**	0.48	0.21*
X_4	A					0.67**		0.35**
	B					0.64**	0.40**	0.26**
X_5	A							0.18*
	B						0.63**	0.11
X_6	A							
	B							0.26**

* Indicates significance at the 5 per cent level of probability.
** Indicates significance at the 1 per cent level of probability.

The complete regression equation for predicting the average production of the daughters of the bulls. Using the correlations of table 1 as the basis for calculation, the following regression equations were developed.

$$(1)\quad \hat{Y}_A = 30 + 0.75^{**}X_1 + 0.03X_2 + 0.01X_3 + 0.34^{**}X_4 - 0.21^{*}X_5$$
$$R = 0.701^{**} \qquad R^2 = 0.491$$
$$(2)\quad \hat{Y}_B = 0 + 0.75^{**}X_1 + 0.01X_3 + 0.23^{*}X_4 - 0.22X_5 + 0.24^{*}X_6$$
$$R = 0.689^{**} \qquad R^2 = 0.475$$

(**Significant at the 1 per cent level of probability; *significant at the 5 per cent level of probability).

The multiple correlation coefficient was highly significant in each equation. The square of these coefficients indicated that from about 47 to 49 per cent of the total variance among the averages of the daughters of different bulls was associated with the regression plane described by these equations.

The effect of the production level of the mates of the bulls. The correlation between Y, the average production of the daughters of a bull, and X_1, the average production of their dams or the mates of the bull, was + 0.66 for Series A and + 0.65 for Series B (table 1). Thus, about 43 to 44 per cent of the total variance among the bulls, in the average butterfat production of their daughters, was associated with the variance among the production averages of their respective groups of mates. The other four variables in each series accounted for only about 5 per cent more of the variance among the bulls in the average production of their daughters than was accounted for by the mates alone. However, when tested statistically, this increase of about 5 per cent was found to be highly significant for Series A and significant for Series B.

The effect of the production level of the dams of the bulls. The record on the dam (X_3) could have been removed from the prediction equation in either series without substantially affecting any of the foregoing results. However, this would have implied that the record of the dam had no significance in the selection of a bull. As was pointed out previously, the bulls in this study were from a selected group of dams and, therefore, the population of bulls to which this equation could be applied most justifiably would be one in which the dams were already a selected group of cattle. There are no data within this study with which to evaluate properly the record of the dam in selecting a bull.

The effect of the production level of the maternal half-sisters of the bulls. In Series A the records on the bulls' maternal half-sisters, X_2, were deleted without affecting the prediction value of the equation as indicated below.

$$(3)\quad \hat{Y}_{A\text{-}x_2} = 32.3 + 0.75X_1 + 0.01X_3 + 0.36X_4 - 0.21X_5$$
$$R = 0.700^{**} \qquad R^2 = 0.490$$

The lack of significance of the maternal half-sisters' records was assumed to be due to the fact that there was an average of only 1.85 *maternal* half-sisters per bull. This is in contrast to the average of over 20 *paternal* half-sisters per bull. The theoretically perfect correlation between the average production of eight daughters of a bull and the average production of two of his maternal half-sisters is only + 0.27. Thus, it is not surprising, when the environmental variation is

considered, to find that the average production of so few maternal half-sisters had no significant value in discriminating between bulls.

The effect of the production performance in the pedigrees of the bulls. In order to obtain a measure of the accuracy with which the average butterfat production of the daughters of unproved bulls could be estimated from information on the production of the bulls' female relatives before the production of his mates was known, multiple regression equations were developed with X_1 (the production level of the mates) deleted.

$$(4)\quad \hat{Y}_{A\text{-}x_1} = 212 + 0.01X_2 + 0.05X_3 + 0.47^{**}X_4 - 0.17X_5$$
$$R = 0.369^{*} \qquad R^2 = 0.136$$

$$(5)\quad \hat{Y}_{B\text{-}x_1} = 212 + 0.06X_3 + 0.39^{**}X_4 - 0.35^{*}X_5 + 0.33^{*}X_6$$
$$R = 0.370^{*} \qquad R^2 = 0.137$$

Approximately 14 per cent of the variance among the bulls, in the butterfat production averages of their daughters, was accounted for by means of equations 4 and 5. This reduction of variance was not large but was found to be significant at the 5 per cent level of probability. Such a small correlation coefficient may appear discouraging to breeders, but it must be recognized that there was a large amount of unaccountable, environmental variation within these data.

DISCUSSION

A significant portion of the differences in average production between the bulls' daughter-groups was determined by consideration of the production information available on the females in the bulls' pedigrees, either alone, as in equations 4 and 5, or in combination with the average production of the herd in which he is to be used, equations 1 and 2.

Production information limited to that found in the bulls' pedigrees has been shown to account for 14 per cent of the variance among bulls in the production averages of their daughters. This same information, when added to the information on the production of the mates, accounted for only a 5 per cent increase over the variance accounted for by the mates alone. This discrepancy of approximately 9 per cent was due to the positive correlation which existed between the production average of the mates and the other four independent variables. The multiple regression of X_1 on the four other variables was determined and the multiple correlation coefficients and their squares were found to be as follows:

Series A	$R = 0.288^{*}$	$R^2 = 0.083$
Series B	$R = 0.251$	$R^2 = 0.063$

Though only approaching the 5 per cent level of significance, these correlation coefficients are indicative of assortative mating and similarities of environment between herds producing and herds using these sires. Because such conditions are not controllable in accumulated data, the correlation coefficients between the several variables in this study may be biased in unknown degree. This condition represents the situation in practice and emphasizes the need for controlled experiments in this field.

On the other hand, it is desirable to derive as much information as possible from the presently available data. Thus, the standard partial regression coefficients (table 2) can be compared with respect to their relative sizes and conse-

TABLE 2

Standard partial regression coefficients for the two series

Series A	Series B
$b'Y1 \cdot 2345 = +0.62$	$b'Y1 \cdot 3456 = +0.60$
$b'Y2 \cdot 1345 = +0.04$	
$b'Y3 \cdot 1245 = +0.01$	$b'Y3 \cdot 1456 = +0.01$
$b'Y4 \cdot 1235 = +0.28$	$b'Y4 \cdot 1356 = +0.19$
$b'Y5 \cdot 1234 = -0.17$	$b'Y5 \cdot 1346 = -0.18$
	$b'Y6 \cdot 1345 = +0.20$

quent effect on the estimated average production of the daughters of a bull (11). The production average of the paternal half-sisters of the bull has the greatest influence, followed rather closely by the production averages of the dams of these half-sisters and the production average of the paternal half-sisters of the bull's dam. Of minor effect are the production averages of the dam and of the maternal half-sisters. From a practical point of view these findings emphasize the proved sire program, since the important items as just cited are in each case parts of sire proofs. From a theoretical point of view the relative importance of the regression coefficients is probably a reflection of differences in numbers comprising the average in each case and differences in selection pressure within each of these groups. The insignificant size of the partial regression coefficient on the dam's record can be explained in one of two ways: (a) the selection of cows for dams of future herd sires is on false premises, or (b) the selection of dams is so effective that relatively little genetic variance exists among them. The true answer is probably a combination of these two explanations.

In the application of these findings to practical sire selection it is evident that the best combination of criteria seems to be a son of a desirably proved sire out of a high producing dam from a desirably proved sire. This combination is already being used by many breeders, but a large proportion of sires are still being selected with the main emphasis on the dam's record. Further study from unselected cow populations will be necessary to determine the true, relative value to be placed upon the record of the dam.

Selection of bulls on the basis of information on production records of direct and collateral female relatives in the immediate pedigree will result in a greater number of successes than random selection, as indicated by the multiple correlation coefficients for equations 4 and 5. Using this correlation, approximately +0.35, and Pearson's "Table for finding the volume of the normal bivariate surface" (10) the following estimates were made:

(a.) If predictions were made on 100 young bulls using equations 4 or 5, and the highest 50 bulls were selected from 100 prospects, 31 of them, or 62 per cent could be expected on the average to produce daughters whose mean production would exceed the mean of the population.

(b.) If 21 bulls were selected from 100 prospects on the same basis, 15 of them, or 70 per cent could be expected to have daughters which would exceed the mean production of the population.

Thus, if all young bulls were evaluated on the basis of the equations formulated in this study in addition to the attention that has always been paid to the dam, and then, for example, only the upper 20 per cent selected for herd sires, a general increase in the average production of dairy cattle could be expected.

This study indicates that under existing circumstances of D.H.I.A. testing the predictability of future average performance of bulls' daughters can be made with approximately the same precision from adequate information on the production of direct and collateral female relatives in the pedigree as can be made on the basis of proof in a first herd (1). This indication is not in agreement with the expected theoretical results assuming that production performance is completely hereditary and influenced by additive genes only. In such a case the average of three or more daughters of a bull would be equivalent or superior to the most perfect set of information possible from the pedigree (7). Since, however, the two studies were based on actual results, both complicated by unmeasurable environmental variations, the comparisons are more applicable to the practical situation facing the average dairyman than would be the theoretical comparisons. When the data become available on a series of bulls, each of which has two or more daughters tested in each of a number of herds, it will then be possible to more accurately account for the portion of the variance directly attributable to environment and consequently obtain a more refined measure of the genetic variance.

SUMMARY AND CONCLUSIONS

The average butterfat production of the daughters of 207 proved Holstein-Friesian bulls was studied in relation to the average butterfat production of the mates of these bulls and that of their direct and collateral female relatives. Two series of multiple regression equations were calculated depending on the records of production available: A for 158 bulls, B for 141 bulls, of which 92 bulls were common to each group. A multiple correlation coefficient of approximately + 0.37 was found in both cases between the direct pedigree estimate and average production of the daughters of the bulls, accounting for approximately 14 per cent of the variance among the daughter groups in their butterfat production. When average butterfat yield of the bulls' mates (the dams of the daughters) was added to the information from the pedigrees the correlation coefficients were approximately + 0.7 and accounted for from 47.5 to 49 per cent of the variance between the butterfat averages of the daughters of the bulls. Non-genetic correlations, presumably due to environmental similarities between herds, contributed to the relationships found.

The study indicates that for selection of young dairy bulls, records of performance of females in their pedigrees are of importance in the following order: (a) the average production of the paternal half-sisters of the bull, (b) the average production of the dams of the paternal half-sisters of the bull, and (c)

the average production of the paternal half-sisters of the bull's dam. The average production of the bull's own dam or of his maternal half-sisters showed no relationship to the average production of his daughters. However, since the dams of the bulls were a highly selected group, the only general conclusion that could be made was that among bulls whose dams average above 450 lb. of butterfat, 2X, M.E., D.H.I.A. conditions, the differences between the records of such dams are of little significance.

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BACTERIOLOGICAL STUDIES OF BOVINE SEMEN. II. THE INCIDENCE OF SPECIFIC TYPES OF BACTERIA AND THE RELATION TO FERTILITY[1, 2]

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Various types of bacteria have been observed in bull semen collected with the artificial vagina. Hatziolos (10) reported that cocci, spore-forming rods and organisms of the coliform, proteus and pseudomonas groups were the main types of organisms present. Gunsalus *et al.* (9) stated that diphtheroids, staphylococci, *Pseudomonas pyocyaneous* and *Escherichia coli* were the predominant types of bacteria in freshly collected bull semen. A report by the United States Department of Agriculture (14) indicated that diphtheroids were the predominating type of organisms. Staphylococci were found to occur next in frequency and *Pseudomonas aeruginosa* and coliform organisms were present occasionally. Ognianov (12) found *Bacillus subtilis, Staphylococcus* and *E. coli.* More recently Edmondson *et al.* (6) isolated streptococci, staphylococci, micrococci, pseudomonas, bacilli, actinomyces and *E. coli* from bovine semen.

There is some evidence that certain types of bacteria can produce toxins and metabolic end products which may be harmful to spermatozoan livability. Several workers (11, 15, 19) have reported observations and opinions supporting this hypothesis but only limited data from controlled experiments are available. Uchigaki (16) found that *E. coli* had a marked deleterious effect on the livability of spermatozoa of the albino rat. Ognianov (12) reported that *E. coli* also was injurious to semen by causing agglutination of bull spermatozoa. Edmondson *et al.* (6) found that hemolytic types of bacteria present in semen caused a decrease in spermatozoan motility during storage. These workers also found that the livability of certain samples containing non-hemolytic types was not as great as the control samples, while in others livability was greater than in the controls.

Perhaps more significant to impaired fertility than the effect of bacteria upon the spermatozoa is infection of the female genital tract by certain organisms following insemination with contaminated semen. There is ample evidence (3, 5, 7, 17, 18) that streptococci and members of the coliform group frequently are associated with ovaritis, metritis, sterility and abortion in females. Although *P. aeruginosa* is generally considered as one of the less virulent of the pathogenic types of bacteria, under favorable conditions the organisms might be capable of establishing infection. Gunsalus *et al.* (8) stated that bulls harboring

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P. aeruginosa in their reproductive tracts are apt to have poor breeding efficiencies and be poor risks for use in artificial breeding. Pounden *et al.* (13) investigated a series of abortions by females previously inseminated with semen from the same bull. Organisms resembling *Staphylococcus albus* were found in the semen of the bull and appeared to be identical to those obtained in large numbers from the pus and exudate surrounding the aborted feti.

The present study was designed to determine the incidence of *P. aeruginosa*, coliform organisms and *Streptococcus* sp. in semen from bulls of known fertility. It also was desired to characterize the predominant types of bacteria present in freshly collected semen and to study their possible relationship to fertility.

METHODS

Semen samples for these studies were obtained from Guernsey, Holstein and Jersey bulls located at the Western Pennsylvania Artificial Breeding Cooperative, Clarion. Previously described collection and shipping techniques (1) were employed in handling the semen samples.

The incidence of *P. aeruginosa* was determined by inoculating tubes of a synthetic asparagine-mineral salts broth with 1 ml. portions of 1 : 10, 1 : 100, 1 : 1,000 and 1 : 10,000 dilutions of semen. A confirmatory test for *P. aeruginosa* was conducted on all asparagine broth tubes that showed turbidity and the presence of a greenish-yellow pigment after 14 days of incubation at 20° C. Confirmation was based on the ability of gram-negative rods to lyse bovine erythrocytes, to liquefy gelatine with the development of a soluble green pigment and to grow at 37° C.

The presence and approximate numbers of members of the coliform group were detected by inoculating lactose with suitable serial dilutions of semen. All positive and doubtful reactions were subjected to standard confirmatory tests (2).

Blood agar plates containing crystal violet were used for detecting the presence of streptococci. Portions of each semen sample were streaked on the surface of previously prepared plates. Bacterial growth which had developed after incubation for 48 to 96 hr. at 37° C. was observed and the Gram morphology was determined in all cases of growth resembling streptococci.

The predominant types of bacteria were isolated from incubated veal infusion blood agar plates which had been employed for plate counts as reported previously (1). Isolated colonies were picked from the incubated plates and streaked on the surface of veal infusion blood agar plates in order to obtain maximum growth upon initial transfer. After incubation at 37° C. the resultant bacterial growth was subcultured and classified according to morphology, Gram reaction and biochemical activity (4).

EXPERIMENTAL

Incidence of P. aeruginosa in semen and the relation to fertility. The approximate numbers of *P. aeruginosa* were determined in 165 ejaculates from 30 bulls at various levels of fertility. The bulls were divided into three groups

based on fertility level. The fertility data were based on the percentage of cows which did not return for service within 90 to 120 days following the last insemination. The inseminations were made during the 4-month period in which the bacterial examination of the samples was conducted.

TABLE 1

The incidence of Pseudomonas aeruginosa in semen from bulls at various fertility levels

Level of fertility	Number of bulls	Number of ejaculates	Ejaculates containing		
			< 2/ml.	At least 2 but < 1,000/ml.	At least 1,000/ml.
Fertile (60–82)[a]	18	79	52	14	13
Relatively infertile (34–59)	10	76	49	15	12
Infertile[b]	2	10	.....	2	8
All levels	30	165	101	31	33

[a] Per cent 90- to 120-day non-returns.
[b] Semen not satisfactory for breeding.

As shown in table 1 the presence of *P. aeruginosa* was confirmed in numbers of at least two per ml. in 64 or about 39 per cent of the 165 ejaculates examined. Eighteen of the 30 bulls yielded at least one ejaculate in which *P. aeruginosa* was confirmed. However, the species was confirmed in all ejaculates examined from only 5 of the 18 bulls. Thus, it appears that, although certain bulls may be classified as "shedders" of *P. aeruginosa,* all ejaculates obtained from these bulls may not invariably contain the organism. Considerable variation in numbers of organisms also was noted between the various ejaculates obtained from the same bull.

Table 1 also shows that the incidence of *P. aeruginosa* was about equal in the fertile and relatively infertile groups of bulls. The organisms were confirmed in all ejaculates from three bulls in the fertile group and from the two bulls comprising the infertile group. The latter two bulls consistently produced semen of unsatisfactory quality for use in artificial breeding. The semen samples were characterized by having a low percentage of motile spermatozoa and a high percentage of morphologically abnormal cells. From these data it appears that although the presence of *P. aeruginosa* even in relatively large numbers is not related to level of fertility, its presence may be associated with individual cases of infertility. Additional data are needed as the fertilizing capacity of any particular semen sample still may be influenced by the presence of large numbers of *P. aeruginosa.*

Incidence of organisms of the coliform group. A total of 209 ejaculates from 35 bulls was examined for the presence of coliform organisms. Table 2 shows that only 24 or about 11 per cent of the ejaculates were found to contain at least 10 coliform organisms per ml. The organisms were found in semen from only 12 bulls and only one of the bulls was found to consistently "shed"

the organisms in all ejaculates examined. As shown in table 2, the organisms were not present in particularly large numbers, since only four ejaculates contained as many as 1,000 organisms per ml. In this study more than one ejaculate frequently was obtained from a single bull on a collection day. There were 109 first, 96 second and 4 third ejaculates collected. The incidence of coliform-positive ejaculates was 13 first, 9 second and 2 third ejaculates.

TABLE 2

The incidence of coliform organisms in semen from bulls at various fertility levels

Level of fertility	Number of bulls	Number of ejaculates	Ejaculates containing			
			< 10/ml.	At least 10 but < 100/ml.	At least 100 but < 1,000/ml.	At least 1,000/ml.
Fertile (60–82)[a]	20	92	78	5	8	1
Relatively infertile (34–59)	13	104	97	4	1	2
Infertile[b]	2	13	10	0	2	1
All levels	35	209	185	9	11	4

[a] Per cent 90- to 120-day non-returns.
[b] Semen considered unsatisfactory for breeding.

Table 2 also shows that the greatest percentage of positive ejaculates was found in the infertile group of bulls and that the percentage of positive ejaculates was greater in the fertile group than in the relatively infertile group. Together with the relatively low incidence of coliform organisms, it appears that there is no significant relation between the presence of coliform organisms in semen and fertility. Nevertheless, their presence definitely was associated with a high bacterial count. The average plate count of 202 of the 209 ejaculates examined for coliform organisms was 200,000 bacteria per ml. The 24 coliform-positive ejaculates averaged 1,400,000 bacterial per ml., as compared to only 91,000 per ml. for the 178 coliform-negative ejaculates.

Thus, coliform organisms were found only occasionally in semen from bulls at various levels of fertility and in relatively small numbers. These results suggest that the presence of organisms of the coliform group is the result of fecal contamination of the semen at the time of collection rather than elimination of the organisms from the genital tract of the bull along with the semen. However, data more recently gathered by this laboratory indicate that semen collected from one bull consistently contains coliform organisms.

Incidence of streptococci in semen. Two hundred and twelve ejaculates from 36 bulls were examined for the presence of streptococci. No evidence of these organisms was found in any of the semen samples examined. This confirms the findings of Gunsalus *et al.* (10).

The predominant types of bacteria in bull semen and their relationship to fertility. The incidence of bacteria in the 19 ejaculates from 8 bulls at various levels of fertility is presented in table 3. Sixty-one per cent of the 457 isolations were gram-positive rods. Species of *Corynebacterium* constituted the

major portion of the flora of this group of bacteria. This genus also represented about 44 per cent of the isolations from the highly fertile group and about 30 per cent of those from the less fertile group. Thus it appeared that the presence of this group was common to nearly all samples of semen and occurred in greatest numbers in semen from the more highly fertile bulls.

TABLE 3

Predominant types of bacteria in bull semen

Level of fertility	Bull	% 90- to 120-day non-returns	No. of ejaculates	No. of cultures	Per cent of cultures classified as			
					Gram-positive rods	Gram-positive micrococci	Gram-negative rods	Others
Fertile (60–69)[a]	H-2	69	2	85	61	26	4	9
	G-7	68	2	34	65	12	15	8
	H-1	65	2	49	76	6	.....	18
Average or total		67	6	168	66	17	5	12
Relatively infertile (40–59)	G-13	54	1	27	45	48	7	.....
	H-10	53	3	68	60	32	6	2
	G- 5	51	3	67	74	10	10	6
	H-14	45	2	48	31	17	52	.....
	H-15	40	4	79	67	14	18	1
Average or total		48	13	289	59	21	18	2
Average or total		61	19	457	61	20	13	6

[a] Per cent 90- to 120-day non-returns.

Although most members of this genus are considered to be non-pathogenic, certain members of the group possess varying degrees of virulence and have been associated with numerous pyogenic infections. However, the cultural characteristics of the majority of these organisms resembled those of the saprophytic forms and these organisms undoubtedly belong to the group commonly known as diphtheroids. The presence of a few of the more pathogenic types was suggested by reactions on blood agar and litmus milk.

Gram-positive micrococci were found in all but one of the semen samples. A greater percentage of these organisms were isolated from the relatively infertile bulls than from the fertile bulls. Many of these organisms appeared as the more inert forms of micrococci. However, certain types were encountered in semen from one relatively infertile bull, H-10, which appeared to be more virulent in nature. These cultures produced a deep golden pigment, hemolyzed red blood cells and were extremely active on the culture media employed. Thus it appeared that certain pyogenic forms were contained within the group.

Included in the group of gram-negative rods were species of the genera *Pseudomonas, Flavobacterium* and *Alcaligenes.* Coliform organisms were not encountered in this particular phase of the study. *P. aeruginosa* was encountered only in semen from two relatively infertile bulls, H-14 and H-15. Although this species was not found during this phase of the experiment in semen from any of the fertile bulls, the presence of the organism was confirmed in two ejaculates from one fertile bull, H-2, when asparagine broth was employed as a

selective medium. In general the organisms of the group appeared to be non-pathogenic, avirulent in nature and consisted of types commonly present on such materials as bedding, soil, feces, etc. However, on the basis of these data, which show a greater percentage of gram-negative rods in semen from relatively infertile bulls than from fertile bulls it is possible that members of this group may be associated with infertility. Further investigations are needed to definitely establish this relationship.

A few other types of organisms were encountered that did not fall into the above classification. About 5 per cent of all the isolates were members of *Actinomycetaceae* and a few false yeasts also were isolated. Due to the low incidence of these organisms it is believed that their presence in semen had no particular relation to fertility.

SUMMARY

By the use of special selective media the incidence of *Pseudomonas aeruginosa,* coliform organisms and streptococci was determined in undiluted semen from bulls used for artificial breeding. In addition, the predominant types of bacteria present in semen were isolated and classified according to morphological and biochemical characteristics.

1. *P. aeruginosa* was confirmed in semen samples from both fertile and relatively infertile bulls. The organism was confirmed in all ejaculates examined from three fertile and two infertile bulls. Even in numbers of at least 1,000 per ml. the presence of the organisms was not indicative of level of fertility. However, the consistent presence of the organism in semen of the infertile bulls indicated that it may be associated with individual cases of infertility.

2. Coliform organisms were found only occasionally in semen from bulls at various levels of fertility. The presence of the group was associated with high plate counts of semen and may represent fecal contamination of the sample at the time of collection.

3. Members of the genus *Streptococcus* were not characteristic of the seminal flora.

4. Gram-positive rods, especially diphtheroids, were found to be predominant in the flora of bull semen. These organisms comprised a greater portion of the flora of fertile bulls than that of relatively infertile bulls.

5. Gram-positive micrococci were found next in frequency to the diphtheroidal flora. It perhaps was significant that the proportion of the flora represented by these forms was slightly greater in the case of bulls of low fertility.

6. Although the gram-negative rods encountered appeared as non-pathogenic types commonly present in nature, a greater percentage was found in semen from the relatively infertile bulls and the presence of certain of these organisms in semen may be associated with infertility.

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THE FERTILITY OF BOVINE SEMEN COOLED WITH AND WITHOUT THE ADDITION OF CITRATE–SULFANILAMIDE–YOLK EXTENDER[1]

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In artificial breeding the procedure of adding extender to semen prior to cooling is now, with several exceptions, routine practice. However, no experimental data have been reported indicating the relative value, as measured in terms of fertility, of cooling bovine semen with or without the addition of extender.

With the exception of Steensma (15), most investigators (2, 3, 4, 7, 8, 12) have found that rapid cooling of unextended bovine semen decreases the survival time of spermatozoa and, consequently, they have recommended that semen be cooled slowly. Easley, Mayer, and Bogart (4) have shown that rates of cooling which decreased the percentage of spermatozoa surviving in unextended semen were without harmful effects when the semen was extended 1:3 with phosphate-yolk prior to cooling. However, none of the cooling rates which they employed was particularly rapid. Phillips and Lardy (13) noted that yolk-buffered semen could be cooled without "undue care". Later Lasley, Easley and Bogart (9), Lasley and Mayer (10), and Mayer and Lasley (11) isolated a factor in egg yolk which increased the resistance of spermatozoa to "cold shock".

Using rapid cooling procedures (30° C. decrease/min.) Willett and Salisbury (16) reported that the motility of spermatozoa in semen extended 1:4 with phosphate-yolk or citrate-yolk as well as the unextended semen was affected adversely. Storage data recently obtained in this laboratory (5, 6) on semen extended at rates of 1:12 and 1:100 before cooling have indicated that, irrespective of the rate of cooling, partial or complete extension in buffered yolk before rather than after cooling was the more effective in maintaining spermatozoan livability. Recently Anderson and Seath (1) have shown that a delay in cooling, and especially a delay in extending semen, results in semen with poorer keeping qualities. This work suggests that semen should be extended immediately. However, in all of these reports the value of the procedures recommended has not been measured in terms of fertility. In view of this fact the experiment reported herein was designed to compare, by using split ejaculates, the fertility of pre-extended semen (semen cooled in citrate-sulfanilamide-yolk extender) with post-extended semen (semen cooled without the addition of extender).

EXPERIMENTAL

Design. The pre-extended and post-extended portions of each ejaculate were shipped to alternate groups of ten technicians each. Thus, any one ejaculate

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[1] In view of the improved livability of spermatozoa in present day media the authors consider the word "extender" more appropriate than "diluter" for describing these media, and therefore have adopted the word "extender" in this report.

was shipped to 20 technicians or multiples of 20 technicians. To insure that all technicians would have approximately equal opportunities to use each treatment, those groups receiving pre-extended semen during the first 8 days received post-extended semen during the last 8 days of the experiment.

Procedure. Immediately following collection, each ejaculate was divided into two equal portions. One portion of semen at 30° C. then was extended on the average of 1 : 4 with citrate-sulfanilamide-yolk extender[2] while the other portion was left unextended. A sterile thermometer was placed in each portion to observe the rates of cooling. Each portion then was placed in a tumbler filled with water at 30° C., and the tumblers placed in a walk-in-type refrigerator maintained at 5° C. After 20 minutes the tubes containing semen were removed from the tumblers and allowed to stand in the cold room air for 55 minutes before the extension to final volume was made. This method of water and air cooling was standard procedure for the New York Artificial Breeders' Cooperative, Inc. Following extension to final volume, 3 ml. sub-samples, which filled to capacity the test tubes used, were taken from each treatment of each ejaculate for the purpose of observing microscopically the motility of the spermatozoa during storage at 5° C. These observations were made after 3, 24, 48, 72 and 96 hr. of storage.

Sixty-four ejaculates from 31 Holstein bulls owned by the New York Artificial Breeders' Cooperative, Inc. were used for insemination. These ejaculates represented nearly all of the semen collected from these bulls during the experimental period, only those ejaculates containing few spermatozoa or otherwise decidedly inferior being discarded. The semen samples initially contained an average of $1{,}647 \times 10^6$ total spermatozoa per ml., of which 70 per cent were estimated to be motile. All ejaculates were extended so as to contain a minimum of approximately 15 million (range 10–25 million) live spermatozoa per ml. of extended semen. The average number for the experiment was approximately 17 million per ml.

Measurement of Fertility. The measurement of fertility was based on the per cent of first and second service cows not returning to service within 60 to 90 days after insemination, these inseminations being performed by the regularly employed technicians affiliated with the New York Artificial Breeders' Cooperative, Inc. The per cent non-returns for each treatment of each ejaculate (treatment × ejaculate sub-group) was considered as the experimental unit. The statistical significance of the differences between treatment means for the per cent non-returns for first, second, and the combined first and second service cows were tested by analysis of variance (14).

RESULTS

The average cooling rates for the pre-extended and post-extended semen are shown graphically in figure 1. Both portions of semen cooled slowly and at

[2] The citrate-sulfanilamide-yolk extender consisted of equal parts of fresh egg yolk and a buffer containing 3.6% sodium citrate dihydrate and 0.6% sulfanilamide.

essentially the same rate while in the tumblers of water. When placed in air at 5° C. the pre-extended semen samples, because of their larger volumes, cooled at a slightly slower rate than did the post-extended samples. Cooling was accomplished in 75 min.

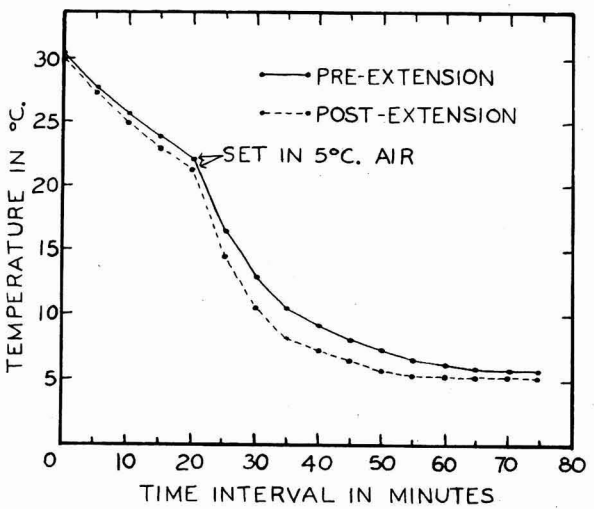

FIG. 1. Comparison of the rates at which pre-extended and post-extended semen were cooled (average of 64 semen samples).

The average per cent of motile spermatozoa and the average rate of progressive motility in the sub-samples after 3, 24, 48, 72, and 96 hr. of storage at 5° C. are summarized in table 1. Even with slow cooling, the proportion of motile

TABLE 1

Average of motility estimations of pre-extended and post-extended semen during storage at 5° C.
(64 ejaculates used for insemination)

Storage time	Pre-extension		Post-extension	
	% Motile	Rate[a]	% Motile	Rate[a]
(*hr.*)				
3	63	2.4	48	2.0
24	56	2.0	41	1.6
48	51	1.7	34	1.3
72	47	1.5	30	1.0
96	42	1.3	23	0.9

[a] =Arbitrary scale indicative of progressive motility with 4.0 as a maximum and 0 as no motility.

spermatozoa was much higher in the pre-extended semen than it was in the post-extended semen. That the detrimental effect was an immediate one is evidenced

by the motility estimations made 3 hr. after the semen was collected. Routine semen examinations made by the laboratory personnel of the New York Artificial Breeders' Cooperative, Inc. and the technicians affiliated with this organization were in agreement with the data in table 1.

The semen shipped in this experiment was used to inseminate 5,769 first-service and 2,749 second-service cows. The fertility results based on 60- to 90-day non-returns to service are shown in table 2. The fertility level of the pre-

TABLE 2

Fertility of pre-extended and post-extended semen (based on 60- to 90-day non-returns to service)

	Pre-extension		Post-extension		Difference[a] between treatment means
	No. serv.	%[a] N. R.	No. serv.	%[a] N. R.	
1st serv. cows	2880	61.8	2889	55.0	6.8[b]
2nd serv. cows	1404	55.1	1345	48.5	6.6[c]
1st and 2nd serv. cows	4284	59.3	4234	52.8	6.5[b]

[a] = % N. R. are the means of the per cent non-returns of each treatment × ejaculate sub-group which was used in the analysis of variance.
[b] = Significant at the 1% level of probability.
[c] = Significant at the 5% level of probability.

extended semen was 6.5 percentage units higher than that of the post-extended semen. Not only is this difference highly significant statistically but it demonstrates experimentally the practical importance of placing spermatozoa in extender before cooling them.

Since presumably there were fewer motile spermatozoa in the post-extended samples at the time they were used for insemination, the authors were interested in determining whether or not the observed difference in fertility could be partly accounted for by the differences in the estimated number of motile spermatozoa per insemination. By covariance analysis using the number of motile spermatozoa inseminated as the independent variate (X) and the per cent non-returns recorded for each treatment × ejaculate sub-group as the dependent variate (Y) it was found that within this experiment the higher per cent non-returns obtained with the pre-extended semen could not be accounted for on the basis of more motile spermatozoa per insemination. Whether or not this increase in fertility should be attributed to the "cold shock" factor of egg yolk or whether it reflects immediate extension *per se*, as suggested by Anderson and Seath (1), has not been elucidated.

SUMMARY

Sixty-four ejaculates of bovine semen were divided and cooled from 30° C. to 5° C. in 75 minutes with and without the addition of citrate-sulfanilamide-yolk extender prior to cooling.

Based on 60- to 90-day non-returns to 8,518 first and second service cows, the fertility level of the pre-extended semen (semen cooled in extender) was 59.3

per cent and that of the post-extended semen (cooled without extender), 52.8 per cent. The difference between treatments of 6.5 percentage units was highly significant statistically.

Motility estimates made after 3, 24, 48, 72 and 96 hr. of storage indicated that the samples cooled without extender had a definitely lower percentage of motile spermatozoa. However, by using covariance analysis the higher per cent non-returns for the pre-extended semen could not be accounted for on the basis of more motile spermatozoa per insemination.

ACKNOWLEDGMENTS

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LIBERATION OF FATTY ACIDS DURING MAKING AND RIPENING OF CHEDDAR CHEESE[1]

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The determination of the chemical changes occurring in the fat of Cheddar cheese during ripening has been the object of numerous investigations. In 1910 Suzuki *et al.* (17) reported the results of partitioning by the Duclaux technique the volatile fatty acids obtained from steam distillation of acidified suspensions of Cheddar cheese. Their results are subject to question since the Duclaux technique is inadequate for the identification and analysis of complex mixtures of fatty acids such as those found in Cheddar cheese.

Since this early study most investigators have measured the free fatty acids of Cheddar cheese as a group and not as individual components. Among the methods most often used are the direct determination of cheese fat acidities (1, 9, 10) and steam or direct distillation of acidified cheese suspensions (5, 8, 11) or extracts (6, 7, 16), followed by distillate titrations. These methods do not give accurate qualitative or quantitative data on the nature and levels of individual free fatty acids present in Cheddar cheese. Undoubtedly these limitations with regard to Cheddar cheese free fatty acid methods have contributed to the failure of many investigators to find a relationship between Cheddar cheese flavor and free fatty acid content (1, 2, 3, 7, 10, 15).

In a previous publication (14) from this laboratory, a rapid partition chromatographic method was reported for the quantitative estimation of formic, acetic, propionic, *n*-butyric, caproic, caprylic and capric acids in biological materials. These fatty acids of short and intermediate chain length are very rich in characteristic flavor and odor and are considered to be important in Cheddar cheese flavor. Naturally-occurring fatty acids having more than ten carbon atoms are quite bland in flavor and odor and consequently are believed to play little or no part in cheese flavor.

The purpose of the present investigation is to determine the nature and levels of individual free fatty acids present during the making and ripening of raw and pasteurized Cheddar cheeses from the same milk. The application of the partition chromatographic method to the estimation of free fatty acids in Cheddar cheese also is described.

METHODS

Cheesemaking procedure. Two 460-lb. identical lots of mixed, raw, whole milk from the University dairy were used. After one lot had been pasteurized

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at 145° F. for 30 minutes, both lots were placed in vats and subjected to identical subsequent treatment. From each lot two 22-lb. non-colored cheeses were made by an experienced cheesemaker according to standard procedures. The green cheeses were kept at 68° F. for 2 to 3 days, then transferred to a cold room (40 to 45° F.) for ripening.

Free fatty acids. The method for the determination of free fatty acids in biological materials previously reported by the present authors (14) was used directly for all analyses of free fatty acids in cheese milk, rennet, starter culture samples and cheeses during the first 6 months of their ripening periods.

In the application of the method to Cheddar cheese older than 6 months or artificially colored, a slight modification of the sample preparation procedure as outlined in the original report (14) was necessary. Substances present carried through the original procedure into the benzene extracts used as the sample on the benzene-33*N* sulfuric acid macro separation column. Their effect on this column was a slow displacement of non-mobile phase. The causative substance in young artificially colored Cheddar cheese was subsequently found to be bixin added as coloring material; that of aged non-colored cheese has not been identified. It was found that both types of substances can be removed successfully from the benzene extracts by the use of an additional macro separation column during the original sample preparation procedure.

The modified procedure is identical with the original procedure prior to the step in which the benzene-33*N* sulfuric acid macro separation column is used. In the modified procedure this column is developed until bixin and bixin-like substances have just reached the column base but not passed into the effluent, as indicated by the erratically-progressing brown zones they form on the column. The benzene effluent, approximately 100 ml. in volume, contains not only all the capric and higher acids but also small amounts of the caprylic and lower fatty acids. The fatty acids are removed from the benzene effluent by several extractions with small volumes of dilute sodium hydroxide. The aqueous solution is concentrated, if necessary, to 100 ml. or less, brought to pH 2 with 5 per cent sulfuric acid, and extracted with three 10-ml. portions of thiophene-free benzene. The residual aqueous solution is combined with the aqueous residue of the original benzene extraction and set aside. The last traces of aqueous phase are separated from the benzene centrifugally and washed once with 5 ml. of fresh benzene. The benzene solution is added without previous equilibration with sulfuric acid to a second benzene-33*N* sulfuric acid macro separation column in which the quantitative separation of the capric and higher acids from the caprylic and lower fatty acids is accomplished without interference from bixin and bixin-like substances.

The contents of the two macro separation columns, including packing, are combined with the aqueous residues from the two benzene extractions. The benzene is removed from the resulting suspension by distillation at pH 8.5. After cooling, the residue is brought to pH 2 with sulfuric acid and the volatile acids are distilled by the magnesium sulfate method described in the original procedure. The bixin and bixin-like substances are non-volatile and remain in the distillation

flask residue which is discarded. From this point on, the distillate which contains all of the lower fatty acids (caprylic and below) of the original sample and the neutralized benzene effluent of the second macro separation column containing the higher fatty acids (capric and higher) subsequently are prepared and chromatographically analyzed exactly as outlined in the original procedure.

FREE FATTY ACID CONTENT OF CHEDDAR CHEESE DURING MAKING AND RIPENING

Free fatty acid content of raw and pasteurized Cheddar cheese after making. In figure 1 the average levels of the individual free fatty acids of two series of

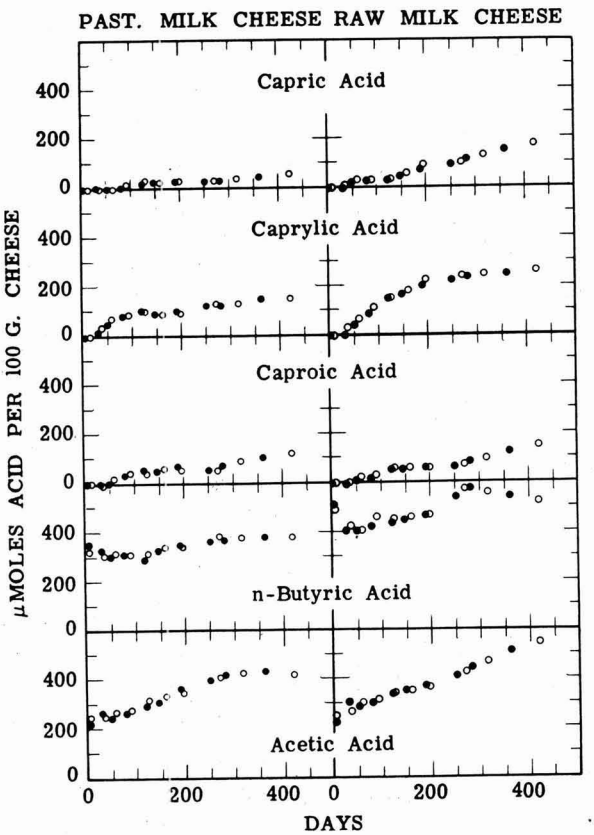

FIG. 1. Free fatty acids in Cheddar cheese during a 420-day ripening period. (The points represent average values for two series each with four pairs of cheese. Series 1 data ●; series 2 data ○.)

pairs of pasteurized milk cheeses and raw milk cheeses made by the procedure described under "Methods" are given. Individual free fatty acids isolated

during the partition chromatographic analytical procedure were checked for identity in two ways. The first of these was their zone positions in column effluents. The second consisted of rechromatographing an isolated fatty acid on a suitable column after adding an equivalent amount of an acid presumably identical. If only one zone was formed and quantitative recovery of the added known fatty acid from that zone was obtained, the two acids were considered identical. By the use of these criteria only straight-chain fatty acids having an even number of carbon atoms, such as are shown in figure 1, have been detected in Cheddar cheese; fatty acids having an odd number of carbon atoms are either absent or present at very low levels.

As may be seen in figure 1, 420-day old raw milk cheese has considerably higher levels of caprylic and capric acids and moderately higher levels of *n*-butyric and acetic acids than corresponding pasteurized milk cheese. Caproic acid levels for both types of cheese are approximately equivalent. The higher flavor generally developed in aged raw milk Cheddar cheese over corresponding pasteurized milk cheese is believed due in part to the higher levels of free volatile fatty acids in the raw milk cheese.

Figure 1 also shows that caproic, caprylic and capric acids are absent in both raw and pasteurized milk cheeses during the first 30 days of ripening, while acetic acid is present at approximately identical high levels in both types of cheese. *n*-Butyric acid also is found at high levels in both types of cheese during this period, although at a slightly higher concentration in the raw milk cheese.

As the two types of cheese age, acetic acid levels increase for both through 300 days of ripening. Since lactose is absent from Cheddar cheese after the first few days of ripening, most of the acetic acid produced during the ripening period is probably due to lactate and protein decomposition by the cheese bacterial flora.

In a previous report (13) the present authors demonstrated that lipases, active at the pH of ripening Cheddar cheese, make their appearance in young Cheddar cheese after 5 to 10 days of ripening. It was suggested that these lipases may represent intracellular lipases of lactic acid bacteria liberated by bacterial autolysis. It is further suggested that part of the free *n*-butyric acid and all of the free caproic, caprylic, and capric acids shown above to be present in 420-day-old raw and pasteurized milk Cheddar cheese are the result of the action of these liberated intracellular bacterial lipases on the milk fat of the cheese. The differences in amounts of these fatty acids in raw milk cheese and corresponding pasteurized milk cheese possibly are due to a large reduction in numbers by pasteurization of bacterial species in milk capable of liberating intracellular lipases through autolysis.

Free fatty acid content of raw and pasteurized Cheddar cheese during making. Upon completion of the studies on the individual free fatty acids of Cheddar cheese after making, it was found that no information on the sources of the high levels of free acetic and *n*-butyric acids present in both raw and pasteurized milk cheese at the start of the ripening period (figure 1) could be found in the data. Consequently, detailed studies on levels of individual free fatty acids present in

raw and pasteurized milk cheese during making and the early stages of ripening were carried out. The results of these studies are given in figure 2 which shows the average levels of the individual free fatty acids of a series of pairs of pasteurized milk cheeses and raw milk cheeses other than those used in the ripening studies.

At the start of making, only the acetic and *n*-butyric acids of the cheese milk are significant in amount. The levels of these acids present in both the raw and pasteurized milk lots at the time they are placed in the cheese vats are shown in

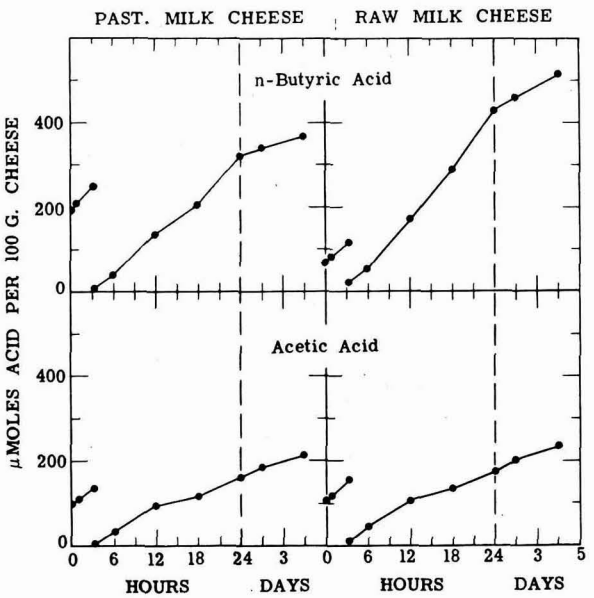

FIG. 2. Free fatty acids in Cheddar cheese during making and the early stages of ripening. (The points represent average values for four pairs of cheese. Breaks in the curves represent times of dipping (200 min.). Of the two fatty acid levels given at the dipping time, the upper represents the acid removed in the whey while the lower represents that remaining in the curd.)

figure 2. Since the free fatty acids contributed by the starter culture and rennet extract added during making are not significant, the small increases in acetic acid concentration during the first 200 min. of making undoubtedly are due to bacterial action on milk lactose while those of *n*-butyric acid are probably the result of rennet lipolytic activity (13) and bacterial activity. It will be noted that the acetic and *n*-butyric acids present in the whey-curd mixture for both types of cheese at the time of dipping (200 min.) can be almost completely accounted for in the whey after its removal from the cheese curd. The levels of the two acids in both raw and pasteurized milk curds increase rapidly during the ensuing 20-hr.

period until the levels of each present in cheese at the start of the ripening period are reached (figure 1). The formation of acetic acid during this period is very probably due in large part to bacterial action on milk lactose carried over into the cheese curd. The rapid rise in free *n*-butyric acid levels in the cheese curd immediately after dipping is not as easily explained. It does not arise from the milk lipase previously studied (12, 13) since this enzyme not only is inactivated in the pH range encountered in Cheddar cheesemaking prior to dipping, but also is destroyed during milk pasteurization (4). The complete absence of any type of lipolytic activity in Cheddar cheese between the time of dipping and the fifth day of the ripening period (13) suggests that the free *n*-butyric acid in Cheddar cheese at the start of ripening probably arises as a fermentation product.

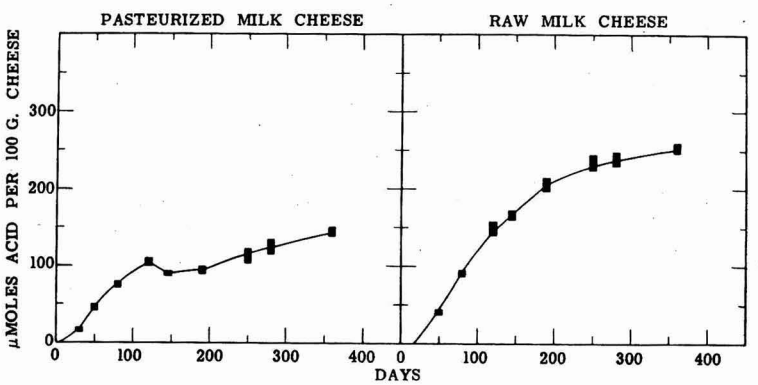

FIG. 3. Variation in free caprylic acid content of Cheddar cheeses of the same age. (The ranges of free caprylic acid content represent the individual acid levels found in the cheeses of Series 1 (figure 1). The intersections of the ranges with the curve show average values.)

Reproducibility of cheeses. The data of figure 1 represent averages of two series of four pairs of raw and pasteurized milk cheeses while that of figure 2 represent averages of a third series of four pairs of raw and pasteurized cheeses. The individual curves for each of the pairs of cheeses in each series are very similar to the average values presented for that series. The variation ranges of free caprylic acid levels at various stages of the four pairs of cheeses making up the first series used in figure 1 are presented in figure 3 and may be seen to be within reasonable limits.

SUMMARY

1. The content of individual free fatty acids of pairs of raw and pasteurized milk Cheddar cheeses has been determined by a chromatographic method at intervals during the making and ripening periods.

2. In both raw and pasteurized milk cheese during the first 30 days of ripening, caproic, caprylic, and capric acids are absent, while *n*-butyric acid is present at slightly lower levels than those of the same cheeses at 420 days. Acetic acid levels for both types of cheese are approximately one half those of the same cheeses at 420 days.

3. Raw milk cheese at 420 days has considerably higher levels of *n*-butyric and acetic acids than corresponding pasteurized milk cheese. Caproic acid levels for the two types are quite similar.

4. Of the free fatty acids contributed by the milk, rennet extract, and starter culture used during cheesemaking, only the acetic and *n*-butyric acids of the milk are significant in amount. There is, however, negligible carryover of these acids into the finished cheese, since they can be accounted for completely in the whey at the time of dipping.

5. The action of intracellular bacterial lipases on the cheese fat is believed to be responsible for part of the free *n*-butyric and all of the free caproic, caprylic, and capric acids present in aged raw and pasteurized milk Cheddar cheese.

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PREPARTUM MILKING. I. THE EFFECT OF PREPARTUM MILKING ON SOME BLOOD CONSTITUENTS OF THE COW[1]

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Prepartum milking has been proposed for reducing the incidence of milk fever and decreasing mammary and umbilical edema (4, 20). However, Smith and Blosser (16) have reported that in a herd of Jerseys, prepartum milking did not decrease the incidence of milk fever. This study was undertaken to determine quantitatively the effect of prepartum milking upon the changes occurring at parturition in total blood hemoglobin, total blood serum calcium, total blood serum inorganic phosphorous, and plasma carotene and vitamin A, and on mammary and umbilical edema. Secondarily these factors were studied in relation to two dietary regimes.

EXPERIMENTAL

Animals. A total of 43 cows of the Ayrshire, Guernsey, Holstein and Jersey breeds in the University of Connecticut herd which calved from November, 1947, through December, 1948, were used in the experiment. They were divided into four experimental groups which were equalized insofar as possible in respect to breed, age, number of previous lactations, anticipated length of dry period, health, ancestry and previous dietary history. Groups 1-A and 1-B were not milked prepartum, Group 1-A receiving the basal ration alone, and 1-B the basal ration + 1 million USP units of vitamin A daily for 30 days prior to the calculated parturition date. Groups 2-A and 2-B were milked prepartum, Group 2-A receiving the basal ration and Group 2-B the basal ration plus the vitamin A supplement.

For 8 weeks prior to the calculated parturition date and for 3 days postpartum, all cows received the same basal ration fed on the basis of liveweight. This consisted per 100 lb. of liveweight of 1 lb. of U.S. No. 2 alfalfa hay, 3 lb. of well matured corn silage, and 1 lb. of a grain mixture consisting largely of cereal grains and containing approximately 13.5 per cent crude protein. The hay, silage and grain contained on an average 10.79, 1.98 and 0.16 mg. of carotene per pound, respectively, as determined by the method of Moore and Ely (10) as modified by Nelson *et al.* (11). The vitamin A supplement was shark liver oil[2] containing 25 per cent by weight of crude soybean lecithin. This oil contained an average of 53,000 USP units of vitamin A per g. as assayed spectrophotometrically against the USP Vitamin A reference standard (vitamin A acetate in cottonseed oil). On the fourth day postpartum, the cows were returned to the milking herd where they received hay and silage *ad libitum* and grain according to milk production.

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[2] This oil was generously supplied by Mr. Melvin Hochberg of the Nopco Chemical Company, Harrison, New Jersey.

In the groups milked prepartum (2-A, 2-B) twice-daily milking was started 10 days prior to the calculated parturition date. Actually group 2-A was milked for an average of 9.6 ± 1.2 days prepartum, the two milkings immediately preceding parturition yielding 19.5 ± 3.8 lb. of milk. Similar values for group 2-B were 10.4 ± 1.4 days and 20.2 ± 3.1 lb. of milk. All cows were milked twice daily after parturition, in most cases beginning within 4 hours. The newborn

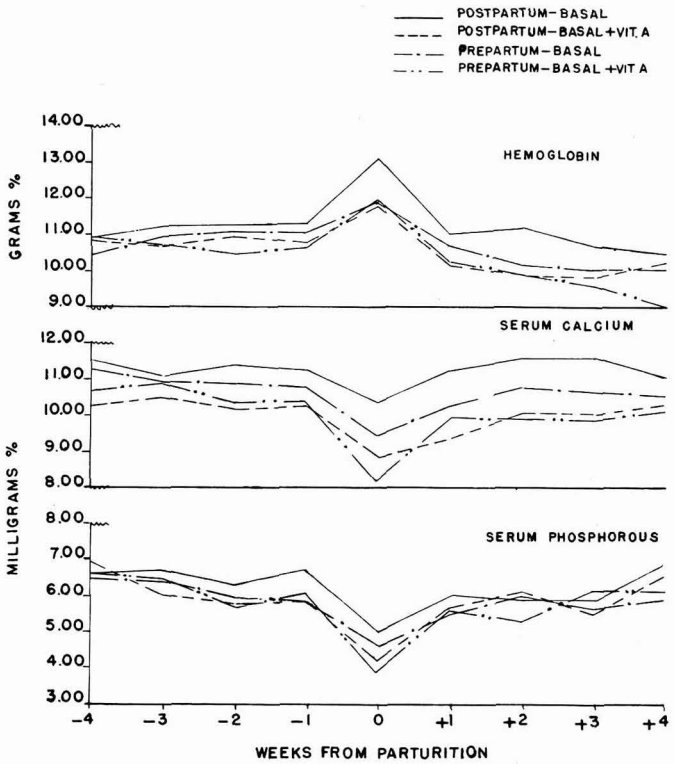

FIG. 1. The effect of prepartum milking on the hemoglobin and serum calcium and phosphorus levels at weekly intervals pre- and postpartum.

calves were removed immediately without nursing to a separate portion of the barn.

Samples and Observations. Venous blood samples were collected from all cows between 7 and 8 a.m. at weekly intervals for 4 weeks prepartum and for 4 weeks postpartum. An additional sample was obtained within 4 hours after parturition from each cow and followed by daily samples for 4 days in 26 of the cows. The sample volumes were 60 ml., of which one-half was citrated and one-half was allowed to clot. All samples were held at 4° C. until analyzed.

Total blood hemoglobin, carotene and vitamin A were determined immediately and the serum calcium and inorganic phosphorus usually within 72 hours.

The mammary and umbilical edema were graded independently on a scale from 0 to 10 by two of the authors (H.D.E. and R.E.J.) at the first milking postpartum. In addition the number of days for the edema to disappear was recorded. The milk fever and ketosis cases were diagnosed clinically by one of the authors (C.F.H.). These were confirmed by determinations of the serum calcium, blood sugar, and urine acetone and histological sectioning of liver biopsies for glycogen and fat. Placentas that were not completely expelled within 12 hours after parturition were recorded as retained. Two cows, one in group 1-B and one in

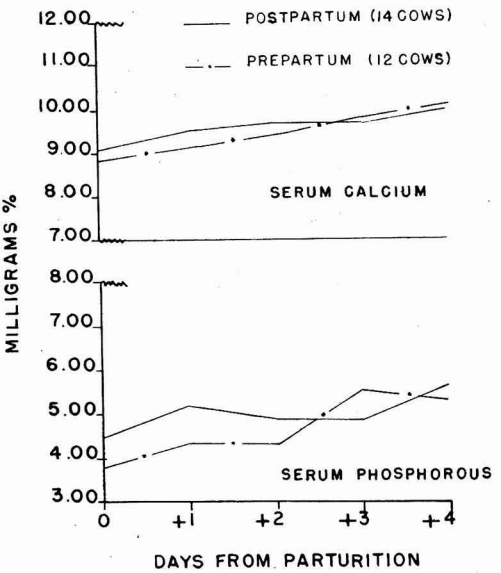

FIG. 2. The effect of prepartum milking on serum calcium and phosphorus levels at daily intervals postpartum.

group 2-B, were given 500 ml. of 20 per cent calcium gluconate intravenously for milk fever after taking the blood sample at parturition. A total of five cows was given intravenously varying amounts of 40 per cent dextrose for ketosis.

Analyses. Total hemoglobin was determined by the method of Evelyn and Malloy (5), total serum calcium by the method of Clark and Collip (2), total serum inorganic phosphorous by the method of Fiske and Subbarow (6) and plasma carotene and vitamin A by the method of Kimble (8). It is recognized that the method of Kimble underestimates the level of vitamin A in the presence of more than 300 γ carotene per 100 ml. plasma (1, 3, 13, 19). Standard statistical procedures (18), such as the analysis of variance and covariance, were used to test for difference between treatments.

RESULTS AND DISCUSSION

Data for all cows are given in figures 1 and 3 on the levels of hemoglobin, of serum calcium and phosphorous and of plasma carotene and vitamin A at parturition and at weekly intervals before and after. Values for serum calcium and phosphorous at parturition and for 4 days postpartum for 26 of the cows are included in figure 2. The scores for mammary and umbilical edema and some

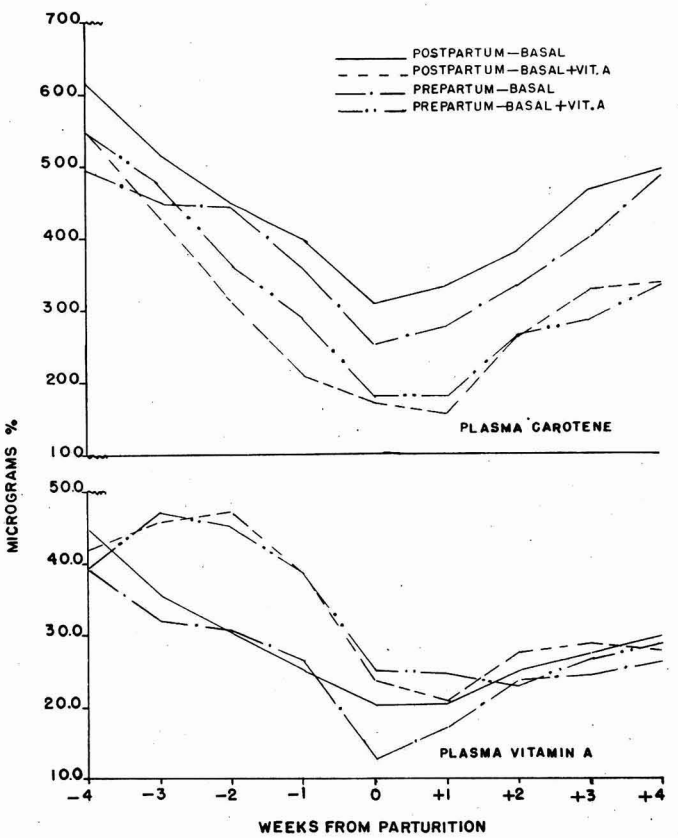

FIG. 3. The effect of prepartum milking on the plasma carotene and vitamin A levels at weekly intervals pre- and postpartum.

other physiological disturbances occurring at or near parturition are contained in table 1. In general prepartum milking did not affect significantly any of these criteria. The supplementary feeding of vitamin A prepartum influenced only the carotene and vitamin A levels in the blood plasma.

Hemoglobin. Total hemoglobin (fig. 1) was not influenced by either prepartum milking or the supplementary feeding of vitamin A. Since the greatest

change in hemoglobin, as well as in calcium and phosphorous, occurred at parturition the difference between the sum of the values observed 1 week before and 1 week after parturition and twice the value at parturition for each cow was used in an analysis of variance. This showed no real differences between treatments. Although not of primary concern in this experiment, the lower levels of hemoglobin occurring after parturition are of interest. With the exception of the group milked only postpartum and fed the basal ration, this decrease was statistically significant ($P > 0.05$).

TABLE 1

The effect of prepartum milking on edema and other physiological disturbances occurring at or near parturition

	Experimental Group			
	Postpartum		Prepartum	
	Basal	Basal + Vit. A	Basal	Basal + Vit. A
	(1-A)	(1-B)	(2-A)	(2-B)
Number of animals	9	11	12	11
Mammary edema score[a]	1.3 ± 0.3	1.9 ± 0 4	2.4 ± 0 4	2 6 ± 0 6
Days for edema to disappear[b]	5.4 ± 0.4	5.5 ± 0.5	10.7 ± 0 4	8.9 ± 0 5
Umbilical edema score[a]	0.4 ± 0.2	0.5 ± 0.3	1.1 ± 0.3	1.0 ± 0.6
Days for edema to disappear[b]	1.2 ± 0.5	1.5 ± 0.5	3.7 ± 0 5	2.0 ± 0.6
Incidence of milk fever[c]	0	1	0	1
Incidence of ketosis[c]	1	3	0	1
Incidence of retained placenta[c]	3	3	4	4

[a] Arithmetric mean ± standard error of mean.
[b] Geometric mean ± standard error of mean.
[c] Total number of cases.

The levels of hemoglobin observed in this study are in essential agreement with those reported recently (9, 14, 15). The marked rise in the hemoglobin at parturition may be due to a decrease in plasma volume associated with parturition. The decrease in the level of hemoglobin after calving may, in contrast, be due to an increase in plasma volume, since lactating cows have been reported (21) to have higher plasma (and blood) volumes per unit of liveweight than nonlactating cows.

Serum calcium and inorganic phosphorous. Neither serum calcium nor inorganic phosphorous (fig. 1 and 2) was affected by prepartum milking or the supplementary feeding of vitamin A. An analysis of the drop in serum calcium and in inorganic phosphorous at the time of parturition, similar to that described for hemoglobin, indicated no real differences between treatments. A more detailed study on 26 of the cows covering the 4 days after parturition gave similar results. This study and those of Smith *et al.* (16, 17, 12) indicate that the changes in serum calcium and inorganic phosphorous occurring at the time of parturition apparently are independent of the initiation of lactation.

Plasma carotene and vitamin A. The plasma levels of carotene and vitamin A were affected significantly by the supplementary prepartum feeding of vitamin A but were not influenced by prepartum milking. An analysis of covariance of the results on carotene used the third week values prepartum as initial levels

to adjust individual differences between cows and an average of the values for the 6 weeks thereafter as the response. This analysis indicated a highly significant ($P > 0.001$) depression in the carotene levels of the plasma of those groups fed supplementary vitamin A. The plasma values of vitamin A, excluding the 4th week prepartum, were higher ($P > 0.05$) in the groups fed supplementary vitamin A than in the groups fed the basal ration alone.

The trends in the levels of both carotene and vitamin A are similar to those reported previously (3, 20) for cows milked only postpartum. Keyes (7) observed a similar trend in plasma carotene for cows milked prepartum. The depression in plasma carotene when supplementary vitamin A is fed also has been demonstrated by others and the literature adequately reviewed by Esh *et al.* (3) and Wise *et al.* (22).

Mammary and umbilical edema. The degree of mammary and of umbilical edema (table 1) and the days for the edema to disappear were higher in those groups milked prepartum, although the difference was not statistically significant. The absence of any beneficial results of prepartum milking on edema does not agree with the report of Turner (20). In the animals reported herein, wide variability was observed between animals within an experimental group, indicating that edema associated with parturition was largely a matter of the individual animal rather than of treatment.

SUMMARY

The effect of prepartum milking for 10 days prior to the calculated parturition date on the total hemoglobin, serum calcium and inorganic phosphorous, plasma carotene and vitamin A, and mammary and umbilical edema has been studied in 43 cows. Secondarily, the effect of feeding daily one million USP units of vitamin A for 30 days prior to the calculated parturition date was measured.

Prepartum milking had no significant effect on the changes occurring at parturition in the several blood constituents, nor did prepartum milking affect significantly the mammary and umbilical edema present at parturition. The prepartum feeding of supplementary vitamin A caused a significant decrease in plasma carotene and increase in plasma vitamin A.

ACKNOWLEDGMENT

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NICKEL IN COWS' MILK.[1]

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This paper is the fifth in a series on the mineral elements of cows' milk (2, 3, 4, 5). Previous investigators (1, 7, 8, 9, 14, 15) are almost unanimous in agreement that nickel, unlike cobalt and certain other trace elements, is not present in milk. Only one group (1) reports its presence (in evaporated milk); the others, with one exception (8), do not make any positive statement about its absence—it simply is not listed among those elements revealed by spectrographic analysis of milk ash. In the exceptional case referred to above (8), the authors state that "no evidence has been obtained of any of the following elements in pure milk". There follows a list of 34 elements in which both cobalt and nickel appear, together with the comment that "the complete absence of cobalt is somewhat unexpected, for it is an element with active biological properities". Cobalt has since been found in cows' milk by several investigators (4, 6, 12, 13), and since cobalt and nickel are so closely related in the periodic system, it has been thought worth while to include nickel in the series of elements under investigation here. The objective has been two-fold: (a) to determine whether nickel is naturally present in milk, and (b) to find out whether, if present, the amount of it can be increased by feeding a nickel compound to cows.

EXPERIMENTAL

The procedure was similar to that described in an earlier paper (2). The work was carried on during two winter feeding seasons, eight cows being used in 1948 as in earlier work, and six in 1949. The four breeds represented in each sub-group of cows in 1948 were the Ayrshire, Guernsey, Holstein and milking Shorthorn. In 1949 the last three mentioned were used. Each breed pair was quite closely matched with respect to age and stage of lactation. The supplement fed was nickel (ous) chloride ($NiCl_2 \cdot 6H_2O$) in approximate daily amount of 500 mg., which is the equivalent of 145 mg. of elemental nickel. One group received the supplement during December and January in 1947–48; the other group received it during February and March. In 1949 the feeding trial was started a month later, so that one group received the supplement during January and February and the other during March and April. The reason for repeating the trials will appear in a moment.

Composite 2-day milk samples of 2 l. each were taken from each cow once a month. Nickel was determined in triplicate on 500-ml. portions of each sample by the method of Alexander *et al.* (1). This method, when used in conjunction with a quartz spectrophotometer, working with standard solutions of nickel chloride and known amounts of added nickel, gave good results. Results the

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first year were obtained with the aid of a grating type of spectrophotometer and were not uniform. Admittedly the grating type of instrument is not as delicate as the quartz type, and it was therefore deemed advisable to repeat the work a second season using an instrument[2] that would more adequately resolve the very minute differences involved. In addition, as a preliminary to work of the second season, each step in the analytical procedure was subjected to rigorous inquiry in order to refine technique wherever possible.

RESULTS

The values obtained are summarized in the accompanying tables, table 1 containing the results for 1948, and table 2 those for 1949. In the 1948 results, al-

TABLE 1

Effect on nickel content of the milk of feeding cows nickel chloride (season of 1947–48) (*γ of nickel/l. of milk*)

Month	Cows on control ration					Cows receiving supplemental nickel				
	1st half of the season									
	[a]A232	G639	H447	S74	Av.	A205	G629	H444	S62	Av.
December	12.8	20.2	10.6	1.6	11.3	8.8	22.6	20.0	21.1	18.1
January	10.0	5.2	1.2	2.8	4.8	3.2	2.8	0	25.0	7.8
Av.—1st half	11.4	12.7	5.9	2.2	8.1	6.0	12.7	10.0	23.1	13.0
	2nd half of the season									
	A205	G629	H444	S62	Av.	A232	G639	H447	S74	Av.
February	1.2	0.5	10.4	0	3.0	6.4	0.8	0.6	6.0	3.5
March	1.4	1.4	0.8	0.3	1.0	1.8	1.8	20.4	1.6	6.4
Av. 2nd half	1.3	1.0	5.6	0.2	2.0	4.1	1.3	10.5	3.8	4.9
Av.—entire season	6.4	6.9	5.8	1.2	5.1	5.1	7.0	10.3	13.5	9.0

[a] The initial letter prefixed to each cow's number indicates the breed.

though the trend was toward a higher concentration of nickel in the milk when the supplement was fed (9.0γ/l. on the average, in contrast to 5.1γ), as already noted the individual values were entirely lacking in uniformity (5 of the 16 possible comparisons were in the reverse direction) and because of the wide variations the difference in the general averages (3.9γ/l.) was not significant statistically. It therefore was decided to repeat the work, and it will be noted from table 2 that the results were even more inconclusive than in 1948. The cows showed a slightly higher average amount of nickel in their milk when not receiving the supplement than they did when they received it; there was even greater variability in the individual results; and the small average difference was statistically not sig-

[2] The instrument used in this later work was a Model DU Beckman spectrophotometer. Measurements were made at a wave length of 385μ, using Corning filter #9863 (red purple Corex A).

nificant. The values for the 1949 season are in general of a much lower magnitude than those for 1948. This is attributed to refinements of technique and method and to the use of a more precise spectrophotometer.

Obviously these inconclusive and conflicting results in 2 successive years mean one of two things; either cows do not secrete supplemental nickel uniformly into their milk as they do cobalt (4), or else natural milk does not contain nickel at all, in which case the widely varying amounts found represent varying amounts of nickel dissolved from the milking machine. It was decided to either eliminate or confirm this latter possibility.

TABLE 2

Effect on nickel content of the milk of feeding cows nickel chloride. (season of 1949) (γ *of nickel/l. of milk*)

Month	Cows on control ration				Cows receiving supplemental nickel			
	1st half of the trial							
	G692	H567	S74	Av.	G686	H444	S68	Av.
January	0.48	1.36	0.48	0.77	0.20	1.12	0.40	0.57
February	0.52	1.04	1.76	1.11	0.96	0.56	0.48	0.67
Av.—1st half	0.50	1.20	1.12	0.94	0.58	0.84	0.44	0.62
	2nd half of the trial							
	G686	H444	S68	Av.	G692	H567	S74	Av.
March	0.24	2.24	1.12	1.20	1.40	2.20	0.88	1.49
April	0.32	0.72	1.54	0.86	0.24	0.72	0.66	0.54
Av.—2nd half	0.28	1.48	1.33	1.03	0.82	1.46	0.77	1.02
Av.—entire season	0.39	1.34	1.23	0.99	0.70	1.15	0.61	0.82

At the conclusion of the feeding trial in April, 1949, composite samples for two milkings from each sub-group of cows were obtained without any contact with metal by milking by hand directly into 2-l. glass jars after the fore-milk had been first drawn off and discarded. These samples when carried through the usual analytical procedure were found to contain not even a slight trace of nickel.

The conclusion from this final phase of the investigation hardly needs stating, but it raises an interesting question. Here are two elements—cobalt and nickel—side by side in the periodic system; their atomic weights and atomic numbers are respectively: 58.9 and 58.7, 27 and 28. The only known difference in structure is a difference of one electron in the "M" orbit; cobalt has 15 electrons in this orbit, while nickel has 16. In the past few years it has been shown by numerous investigators that cobalt has a remarkable biological significance and within a year it has been proven to be a constituent of vitamin B_{12} (11). Nickel on the other hand is not known to be essential to biological systems, and here is further evidence of the same sort; viz. that either it is not absorbed into the blood stream or else the mammary gland excludes it while permitting cobalt to pass into

the milk. A similar situation with respect to iron and manganese was noted and commented on in an earlier paper of this series (5).

SUMMARY

Nickelous chloride was fed as a supplement (500 mg. daily) to the rations of six cows for a period of 2 months by the double reversal method and the milk was analyzed for nickel. Although varying amounts of nickel were found in the numerous milk samples taken, it was shown that when the milk was kept from contact with metal by milking directly into glass jars, nickel was not present.

Therefore it was concluded that nickel is not a constituent of natural milk and that the varying amounts found in the course of the investigation came from the milking machine.

The difference in this respect beween cobalt and nickel, two closely related elements, is discussed briefly.

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THE METABOLISM OF THE LACTOGENIC HORMONE[1]

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The lactogenic hormone secreted by the anterior pituitary gland is essential for the secretion of milk. It has been suggested that the initial increase in the secretion of this hormone at about the time of parturition is due to the action of estrogen upon the pituitary gland, and that the maintenance of lactogen secretion is largely due to the regular stimulation of the teats at the time of milking (16).

The mode of action of the lactogenic hormone upon the epithelial cells of the mammary gland is not understood, but it is presumed to act upon the enzyme systems of the cells which cause the milk precursors coming from the blood to be transformed into the several constituents of milk (21).

Since the intensity of lactogenic hormone secretion by the pituitary undoubtedly is one of the important factors governing the inheritance of the capacity for large milk and fat production, it is important that methods be developed to measure the secretion rate of this hormone. In experimental animals where they may be sacrificed, the determination of the amount of the lactogenic hormone present in the pituitary under varying physiological conditions has been studied extensively. In these studies, there has been observed a high degree of relation between the amount of lactogenic hormone present in the pituitary and the rate of milk secretion.

In the larger domestic animals it is impossible to sacrifice the animals, and other methods must be developed to measure lactogenic hormone secretion rate. Ehrhardt and Voller (3) assayed the blood and urine for lactogen during the menstrual cycle. They claimed that parallel peaks in both blood and urine were observed at the beginning of menstruation and at the time of ovulation. It has been shown, also, that the lactogenic hormone is present in the blood of dairy cattle, goats and rabbits (20), and in the serum of mares during pregnancy and lactation (9). Meites and Turner (14) assayed the whole, untreated blood of rabbits before and after the injection of estrone. There was a definite increase in the lactogen content of the blood associated with an increase in the pituitary.

During recent years, the problem of hormone metabolism has been under intensive investigation. There are many aspects of the problem including the question of the use of the hormone by the cells which are activated, the inactivation of the hormone in other parts of the body but especially in the liver, and the paths of elimination.

Lyons and Page (13) were the first to report the presence of lactogenic hormone in postpartum human urine. They claimed that at least as much of this hormone is excreted daily as is present in the bovine pituitary. Lyons (12) also showed that lactogen was present in the urine of four new-born male and

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female babies, who were secreting witches milk. Tesauro (19) found that the injection of untreated or extracted postpartum urine from women gave a definite increase in the crop-gland weight of pigeons. Hoffmann (6) reported no hormones could be detected in women's urine before parturition in 10 of 11 cases. Following parturition no hormone was detected for the first 2 days, but beginning on the third day, with the inflow of milk, lactogen could be demonstrated in the urine. Hoffmann (7) further found that in seven of eight cases of deficient lactation, the excretion of lactogen was considerably less than in the urine of women with ample lactation.

Langecker and Schenk (8) detected lactogen in the urine of women during lactation, and also at the end of pregnancy. One and one-half liters of urinary extract was found to be as effective as 1.8 g. of fresh cattle pituitary.

Liard (11) reported that there was sufficient lactogen in 50 to 150 ml. samples of urine from lactating women to produce milk secretion in guinea pigs primed with large doses of estrogen.

The lactogen content of the urine of ten lactating women during the first 2 weeks postpartum was determined by Meites and Turner (15). They observed a daily range in hormone excretion of from 4.05 to 12.50 I. U. From 0.8 to 3.0 ml. of urine was required for a micro-unit response. In three cases of definite or suspected hypogalactia less lactogenic hormone was found than in mothers with adequate lactation. The three best lactators excreted the highest average amount of hormone.

Coppedge and Segaloff (2) found that the lactogenic hormone content of urine collected during 24-hour periods from normal women ranged from 20 to 100 I. U. and from normal males ranged from 50 to 100 I. U.

Stimulated by the discovery of rather large amounts of male hormone in dried cow manure [for references see (23)], it seemed of interest to determine whether or not other hormones might be excreted into the digestive tract. Since the lactogenic hormone is a protein hormone, it might be thought unlikely that it would be present in the feces for two reasons. First, if the hormone passed into the digestive tract it might be partially or totally digested and rendered inactive. Second, that protein would pass unaltered through the digestive mucosa in the reverse direction. That the lactogenic hormone passes into the urine in a biologically active form is proof of the passage through the kidney epithelium.

The object of the present paper is to describe a method which successfully extracted lactogen from dried cow manure, and to report upon the content of the hormone in the feces of cows in varying stages of lactation. Its presence in the feces of bulls also was observed.

MATERIALS AND METHODS

The fresh cow manure free from urine, straw and extraneous material was collected and dried by spreading it in a thin layer in an electrical drying oven at a temperature of 45° C. for about 48 hours. The sample then was ground in a mill to the consistency of alfalfa meal and then was ready for extraction.

Bergman and Turner (1) made a study of four methods frequently employed

for the extraction of lactogen. They found aqueous alkaline ethyl alcohol was satisfactory for the extraction of lactogen, and also for the other pituitary hormones. It was decided to use ethyl alcohol as the solvent for the extraction of the hormones which might be present in the cow manure.

Initial attempts were made to obtain the protein hormones qualitatively from the manure of the lactating cow by successive extraction with 90, 80, 70, 60, and 50 per cent ethyl alcohol. These extracts were divided into several portions for further study. The concentration of alcohol was increased in most cases and an attempt was made to precipitate any hormones which might be present by adjusting the pH to their individual isoelectric point.

A positive qualitative response for the lactogenic hormone was obtained. Negative assays for the adrenocorticotrophic, thyrotrophic and gonadotrophic hormones were obtained. It was decided to extract the manure for the lactogenic hormone without regard to the other hormones and to compare the lactogenic hormone excretion by way of the digestive tract from bulls and high and low milk producing cows.

In the beginning a few attempts were made to precipitate the active material from the alcohol extract suspension by tannic acid, benzoic acid as used for precipitating the protein hormones from urine, and by increasing the concentration of the alcohol solution. It was found that increasing the alcohol concentration was the most effective method. It was also found that extraction with 40 per cent ethyl alcohol was most effective. The steps are as follows:

1. A 1 kg. sample of dried cow manure which was collected individually was mixed thoroughly with 10 l. of 40 per cent ethyl alcohol.

2. The suspension was brought to pH 10 (Beckman glass electrode) by the addition of 1 *N* NaOH solution, and was kept at room temperature for 4 hours with frequent stirring.

3. The residue was removed by the aid of a centrifuge fitted with a perforated disc and a draining chamber. One-fifth of the original quantity of alcohol can be obtained by this method.

4. The fine particles in the suspension were removed by centrifuging in large centrifuge bottles.

5. The combined clear supernatant fluid was then filtered through a no. 1 filter paper on a Buchner funnel with suction.

6. A volume of 90 per cent alcohol was added so as to increase the alcohol concentration to 65 per cent and brought immediately to pH 6.5 by the addition of 1 *N* HCl solution. It then was kept at a temperature of -1 to $-5°$ C. for 12 hours, at the end of which period the precipitate had settled down to the bottom of the container.

7. The supernatant solution was sucked out by the aid of vacuum. The precipitate together with a small quantity of fluid was poured into large centrifuge bottles which were kept at -1 to $-5°$ C.

8. The solution was centrifuged and the precipitate discarded. A one-half volume of 95 per cent ethyl alcohol was added to the combined supernatant solution so as to increase the alcohol concentration to 75 per cent. The mixture was

brought to pH 5.7 by the addition of 1 *N* HCl and kept at a temperature of -1 to $-5°$ C. for 12 hours, at the end of which period the active material had settled down to the bottom of the container.

9. The supernatant solution was sucked out and a small amount of fluid was separated from the precipitate by centrifuging under cold conditions and was discarded.

10. The precipitate was dissolved in distilled water by adjusting the pH to 9 and was dialyzed against distilled water for 24 hours. For dialyzing, a cellulose casing was used. It was soaked in distilled water for 5 to 10 minutes, tied at one end, filled with solution, and then the other tied, and placed in a large beaker containing distilled water which was changed several times. The beaker was kept under cold conditions. By this step the active material loses much of its toxic and inflammatory properties.

11. After dialyzing, 5 volumes of 95 per cent alcohol were added to the dialyzed solution which was brought to pH 5.7 by the addition of 1 *N* HCl and kept at -1 to $-5°$ C. for a few hours.

12. The precipitate was collected by centrifugation and was washed four times with 95 per cent alcohol and three times with ether.

13. After the last washing, the precipitate was easily dried by holding the centrifuge bottle in front of an electrical fan. The grayish dry material was ground to pass through an 80 mesh sieve and was then ready for biological assay.

To determine the amount of lactogenic hormone present, the very sensitive "micro" assay was used (15). It is based upon the proliferation of the crop gland of pigeons after intradermal injection over the crop glands. The dried extracts which were screened through an 80-mesh sieve were dissolved in distilled water and adjusted to pH 9 with NaOH solution. After the extract was completely dissolved the solution was brought to the isoelectric point with HCl so as to precipitate the protein and to slow down the absorption rate from the site of injection. The volume of fluid injected daily amounted to 0.1 ml. In all quantitative assays, preliminary tests were made at three dosage levels in a few pigeons in order to approximate the unit. The "micro" unit is defined in our laboratory as that amount of hormone which, when injected intradermally over the crop gland of 20 pigeons, will elicit a minimum but definite response in 50 ± 10 per cent of the pigeons. This unit is equivalent to 1/160 I. U. of lactogenic hormone.

RESULT OF QUANTITATIVE ASSAY OF LACTOGENIC HORMONE

Quantitative assays for lactogen were made on the extracts of cow manure from individual cows. It is shown (Table 1) that in general the cows producing considerable milk execreted more lactogenic hormone through their digestive tract than the low producing cows except in the case of H 139, which was a relatively good milk producer, yet execreted less lactogenic hormone than the low producer H 95. The lactogenic hormone content of 1 kg. dry manure of high-producing cows ranged from 47.9 to 200 "micro" units (0.29 to 1.25 I. U.),

TABLE 1

Correlation between milk record and the lactogenic hormone content of bovine manure

Animal no.	Sex	Records of Dairy Cattle			Yield of Extract	Lactogenic Hormone Content of cow manure		
		Monthly milk production when sample was collected	From freshening to the time of sample collection		Av. total yield/kg. of sample	Mg./ micro unit	Av. total unit/kg. of sample	
			Total milk production				Micro unit	International unit
		(*lb.*)	(*lb.*)	(*d.*)	(*mg.*)			
J977	F.	645.0	645.0	27	3200.0	20	160.0	1.00
H139	F.	1064.6	2599.6	68	1150.0	24	47.9	0.29
H46	F.	460.2	7362.3	247	2400.0	24	100.0	0.62
H63	F.	1468.0	5456.9	104	2020.0	24	84.1	0.52
H84	F.	1030.0	5964.0	130	1300.0	16	81.2	0.50
H81	F.	1427.0	1625.0	35	3600.0	18	200.0	1.25
J987	F.	494.6	4095.0	243	1180.0	32	36.8	0.23
J994	F.	440.9	2600.3	186	1208.0	28	43.1	0.26
H95	F.	383.7	6729.0	265	1156 0	24	48.1	0.30
H	M.				1022.0	36	28.0	0.17
H	M.				1000.0	32	31.0	0.19

while the low producers ranged from 36.8 to 48.7 "micro" units (0.23 to 0.3 I. U.). The two bulls execreted nearly as much as the low milk-producing dairy cows, *i.e.* 31.2 and 28.3 micro units (0.19 and 0.17 I. U.), respectively.

DISCUSSION

The lactogenic hormone content of the pituitary remains low during most of pregnancy in experimental animals, but there is an abrupt increase shortly before or after parturition. Also the urinary lactogen content of lactating women increases after parturition and usually is related to the ability to secrete milk. In most experimental animals except guinea pigs, the pituitary lactogenic hormone content of females is higher than the males.

Although no experimental data are available on the urinary lactogenic hormone content of lactating dairy cattle, this investigation of the lactogenic hormone content of dried manure has shown that there may be a relationship between the milk producing capacity and the lactogenic hormone excretion from the digestive tract.

The discovery of lactogenic hormone in dairy cow manure raises the question of how and where this hormone is secreted into the digestive tract. The routes of excretion of metabolic by-products and the hormones in their active or inactive forms from the living body are:

(a). The urinary system through which the water, minerals, protein metabolic products, pigments, toxic substances, protein hormones such as gonadotrophin, adrenocorticotrophin, thyrotrophin, and steroid hormones such as estrogen, androgen and inactivated progesterone known as pregnandiol are execreted.

(b). The respiratory system through which water and carbon dioxide are eliminated.

(c). The sweat glands by which the sweat is secreted. The composition of sweat is nearly the same as the urine, but to what extent the hormones are excreted by the sweat glands is still a question.

(d). The digestive tract and its accessory organs such as the liver. The liver secretes bile which is poured into the digestive tract. In the bile the steroid hormone estrogen (17) has been found. Considerable amounts of estrogen (10) and androgen (23) have been found in cow manure.

Of great interest is the fact that the epithelial cells of the intestine have both absorptive and secretory functions. Nutrients are absorbed by these cells into the blood, while digestive enzymes, mucin and several hormones such as secretin, cholecystokinin, enterogastrone, villikinin, etc., are formed by these cells and poured into the lumen of the intestine.

The lactogenic hormone is protein in nature and is manufactured by the anterior pituitary from where it is carried by the blood stream to the mammary gland. As it has been found in colostrum (5) of cows lactating normally, it is known that this hormone can be picked up by the mammary gland from the blood and execreted together with milk, so it is possible for the epithelial cells of the intestine to function in a similar manner. There might be another possibility for lactogenic hormone to reach the intestinal lumen if it were present in the bile.

Due to the fact that lactogenic hormone is inactivated by both pepsin and trypsin, it would be reasonable to suggest that in case it is excreted by the epithilial cells of the intestine, only that part which is excreted in the lower portion of the intestinal tract would be present in the feces in an active form unless it were protected from the digestive enzymes in some way.

It would be of interest to estimate the daily excretion of lactogenic hormone through the digestive tract. A dairy cow weighing 1000 lb. has been reported to excrete daily 59 lb. (4) of fresh manure. As the dry matter content of fresh manure is about 15 per cent (22), this would equal about 4.02 kg. dry matter per day. The average lactogenic hormone content per kg. of dry matter as indicated by these data is 0.54 I. U., so the daily lactogenic hormone excretion would be 2.17 I. U. The average lactogenic hormone content of the pituitary of the dairy cow has been estimated to be about 56.28 I. U. (18). Thus, 3.8 per cent of the lactogen contained in an average bovine pituitary is excreted in an active form through the digestive tract.

SUMMARY

1. Cow manure free from urine and extraneous materials was collected and dried in an electrical drying oven at a temperature of about 45° C. for about 48 hours.

2. Forty per cent aqueous alkaline ethyl alcohol extracts of individual samples of lactating cow and bull manure were precipitated by increasing the alcohol concentration to 75 per cent at pH 5.7 under cold conditions.

3. The average yield of lactogenic hormone from one kilogram dry manure ranged from 0.29 to 1.25 international units for the high milk producers, from

0.23 to 0.3 international units for the low milk producers, and from 0.17 to 0.19 international units for the bulls.

4. It was concluded that there might be a relationship between the amount of lactogenic hormone excretion into the digestive tract and the milk producing ability.

5. Possibly lactogenic hormone is secreted into the digestive tract, but only that part which is excreted in the lower portion of the intestine is excreted together with the feces in an active form.

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VENOUS CATHETERIZATION OF DAIRY COWS

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In studying the phosphorous metabolism of dairy cows by the use of radioactive tracers (16), it was necessary to develop a suitable injection and bleeding technique which would fulfill the following requirements: (a) It must permit complete injection of the radioactive material for hazard control; (b) it must provide for accurate and rapid injection of the material, followed immediately with the withdrawal of blood samples; (c) it must allow for the bleeding at frequent intervals over long periods (4 to 5 weeks); and (d) it must minimize the disturbance and apprehension of the cow, conditions which have been shown to affect the blood phosphorous values.

REVIEW OF LITERATURE

Forssman (9) in 1929 catheterized the right auricle on himself, after exposure of a vein of the arm by a surgeon. From 1930 to 1939 this technique (4) of catheterizing the right heart was widely used in Europe for injecting contrast substances in order to visualize the right chambers of the heart and the pulmonary vascular tree. Cournand and Ranges (2) modified the Forssman technique. They used a specially made gauge (no. 10) Lindeman type of needle; a three way stopcock with a Leur lock; a tightly fitted adapter; and a No. 8 F flexible, X-ray opaque, varnished, silk catheter with two holes, one at the rounded tip and another about 1 cm. from the tip. A saline reservoir with rubber tubing and a clamp for controlling the rate of flow was used to supply a constant flow of saline, at the rate of 15 drops per minute as an anticoagulant. The catheter was introduced into the median basilic vein of either arm. The passage of the catheter through the vein was accomplished while the patient was on a fluoroscopic table. There was no evidence of blood clotting or thrombi in the holes of the catheter.

Since the Cournand and Ranges (2) paper, venous catheterization has been employed to obtain blood samples from the coronary sinus of man (1, 12, 20), of the dog (8, 10, 11, 12, 13), the right auricle of man (2, 3, 4, 6, 12, 23, 24), of the dog (21), the hepatic vein of man (24), the portal vein of the dog (5), the jugular vein of man (18), of the dog (25), and the pulmonary artery of the dog (8, 10, 15), as well as in the measurement of cerebral blood flow in man (14), in the monkey (6, 22) and in the dog (14).

Catheters made of various materials have been used: steel or other metal cannulae (13, 14, 21), a soft ureteral catheter (9), a flexible radiopaque ureteral catheter (1, 2, 3, 4, 8, 10, 15, 24), and a modified, tapered tipped ureteral catheter with a woven, shellacked nylon core covered with a heavy X-ray-opaque

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plastic covering. This latter catheter has been modified (12) by the addition of two small side eyes, with shallow grooves on the outer surface of the catheter leading forward from the side eyes to the terminal eye. Unspecified plastic catheters (25), vinylite (18) and polyethylene (5) catheters have been used for intravenous work. Polyvinyl chloride tubing (19) was used for prolonged intramuscular injections.

Several thousand short-time catheterizations of the right auricle, to determine the cardiac output in man, have been performed in various laboratories without serious ill effects. However, it is a potentially dangerous procedure and should only be carried out with considerable care and thoughtfulness (20). Occasionally linear mural thrombosis occurred in patients near the site of incision when the catheters were left in place for 24 hours (3). Plastic intravenous catheters, used for protracted administration of various drugs to patients caused a transient phlebitis about the tube when left in the vein for several days (18). This condition may have been due to the injected fluids rather than the presence of the tube.

Dogs with the external jugular vein catheterized for 4 to 5 weeks showed that the veins were thrombosed around the tube in about half of the cases (25). In patients, the injection of materials into the median basilic vein for 12 days was satisfactory. This author felt that thrombosis of the veins occurred when concentrated or irritating substances were infused. Subendocardial fibrosis, mural thrombi and subendocardial hemorrhage were found in the right auricle after catheterization of the right heart (10). Following catheterization of the pulmonary artery, the right ventricle and the tricuspid valves showed some injury. Dogs were catheterized (11) seven times during 4 months, with only three failures out of 68 catheterizations. The catheters were left in the dogs up to 5 hours. Blood samples were obtained from the portal veins of dogs (5) for an average of 21 days and a maximum of 34 days after insertion of the catheter.

It is apparent from the foregoing summary that blood can be obtained satisfactorily by the use of catheters. Further investigations, however, are needed to improve the technique and to evaluate the effect of the infusion of various materials on long term blood sampling.

EXPERIMENTAL PROCEDURE

A smooth, flexible, transparent tubing made of polyvinyl chloride was used as a catheter. This tubing ("spaghetti") is used as insulation by electricians and can be obtained from electrical supply houses. Our catheters were 40 to 45 cm. in length with an inside diameter of 1.4 mm. and an outside diameter of 2.2 mm.

In our experiments catheters were placed in both external jugular veins of cows. Radioactive substances were injected into one jugular vein, while blood samples were drawn from the other. Prior to insertion of the catheter, the skin area over the jugular veins was closely clipped and local anesthetic (procaine) injected at the site of insertion. A jugular tourniquet was applied. Then a

sterile hypodermic needle of 10 to 12 gauge was thrust into the vein. A needle with a relatively short beveled point was used with the short edge of the bevel turned in the direction which the catheter was to travel. The sterile catheter was inserted through the needle into the vein, leaving 6 to 8 cm. protruding. The needle then was removed. A small clamp halted the flow of blood through the catheter and held the tube in place to prevent it from being drawn into the blood stream. Finally, the catheter was filled with a sodium heparin solution (4 mg. sodium heparin per 100 ml. of 0.9 per cent saline) to wash out the blood and prevent the formation of a clot. The catheter then was closed with a plug. A sterile, heavy silk suture was placed in the skin to form a loop close to the catheter. The catheter then was tied to the suture my making three single knots.

A syringe fitted with a 16 gauge needle was used to draw blood samples. To reduce the apprehension of the cow and to prevent her from pulling out the catheter during bleeding or injecting, the catheter was lengthened by using the tubing from a 16 gauge needle connected to an additional piece of "spaghetti" 15 to 20 cm. in length.

Prior to drawing a blood sample, the catheter was washed with heparinized saline to remove any clot which may have partially closed the tube. Occasionally considerable force was necessary to open the catheter. Often a valve-like clot formed in the catheter which permitted injection but not withdrawal. Following the bleeding, the injection of heparinized saline was repeated.

EXPERIMENTAL RESULTS

Nineteen catheters were used in twelve trials on five different cows. Blood was obtained through these catheters at the rate of 1 ml. per sec. Bleeding caused very little disturbance to the cows. One sample was secured while one cow was lying in a paddock chewing her cud. Numerous samples were drawn when the cows were eating or ruminating. The length of time a catheter remained opened varied from 2.1 hours to 14.5 days (table 1).

From one cow 24 samples were obtained in 14 hours. During a 38-day trial, another cow had five catheters inserted into the right jugular vein from which 135 samples of blood were taken. In the same period only one catheter was placed in the left jugular vein for repeated injections of radioactive material at 12-hour intervals.

Preliminary trials with calves as well as catheterization of the carotid artery of one dairy cow for 4 days show promise.

In two instances catheters were drawn into the blood stream, in one cow when the needle was being withdrawn and in the other cow when the tubing was being placed in the opposite vein. No deleterious physiological effects were observed during the following 66 and 48 days respectively, at which time the cows were slaughtered. At autopsy, one catheter was found in the right pulmonary artery. No gross abnormalities were observed.

Cresson and Glenn (5) report that polyethylene catheters caused a clotting

reaction in some of their dogs. Only in one out of twenty dogs did a complete thrombosis occur and then this followed three separate catheterizations of the portal vein at 2 and 3 week intervals, the catheter having been left in the vein

TABLE 1

Time venous catheters remained functional and number of samples obtained

Trial no.	Cow no.	Catheter	Blood samples per catheter	Functional time	Condition of catheter
1	798	a	4	3.3 hr.	occluded
2	890	a	4	2.1 hr.	occluded
3	798	a	9	15.6 hr.	open
4	890	a	6	1.1 d.	open
5	890	a	21	4.0 d.	open
6	798	a	17	1.0 d.	open
7	890	a	7	2.2 hr.	occluded
		b	10	3.8 d.	occluded
8	798	a	9	1.0 d.	open
9	890	a	13	3.0 d.	open
10	853	a	8	10 0 d.	occluded
11	834	a	8	3.1 d.	occluded
		b	25	4.3 d.	occluded
		c	43	8.5 d.	occluded
		d	35	7.0 d.	occluded
		e	24	14.5 d.	occluded
12	757	a	45	11.0 d.	occluded
		b	10	1.3 d.	occluded
		c	3	2.0 d.	occluded

for 2 months. In one of the cows, slaughtered 18 days after the catheters had been inserted, a complete thrombus was found in both the right and left jugular veins. The occluded portion corresponded approximately to the length of the catheters in the veins.

SUMMARY

A technique of venous catheterization has been adapted to dairy cows, which allows numerous blood samples to be taken at short intervals and the frequent, accurate intravenous injection of substances (particularly radioactive material) over a long period with a minimum of disturbance to the animals.

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A METHOD FOR ESTIMATING THE FEED-REPLACEMENT VALUE OF PASTURE FORAGE

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Improved methods for determining the yields of pastures have been given much attention by both agronomic and livestock investigators. Agreement has been reached with regard to many of the procedures which should be followed and an outline of pasture techniques (1) has served as a useful guide for many research workers. An excellent review and appraisal of methods used in evaluating the results of pasture studies has been presented by Ahlgren (2).

The determination of yields by agronomic methods proves satisfactory under many conditions but has the limitation that yields alone do not indicate the value of the forage for livestock production. The investigation reported herewith had as its objective the designing of a simple method for the measurement of the feed-replacement value of pasture forage.

EXPERIMENTAL

Two experimental groups, each containing 13 registered Holstein cows, were established. Division into groups was made by selecting two cows as nearly alike as possible with respect to weight, stages of lactation and gestation, and level of milk yield, and then assigning at random one cow to the "Pasture Group" and the other to the "Dry-lot Group." The cows of the pasture group had been in milk for 109 days (group average) and those of the dry-lot group 116 days (group average) since the last calving date. Five cows of the pasture group were pregnant, the average stage of gestation being 31 days. One dry-lot cow had been pregnant for 22 days at the beginning of the trial.

The pasture group was allowed access to pasture and was given grain mixture (barn-fed) as the only supplementary feed. The dry-lot group was barn-fed on silage, alfalfa hay, moistened beet pulp and the same kind of grain mixture as that given to the pasture group. The silage feeding program was: corn silage (dry-matter content, 30.1 per cent) for 1 week; sorghum silage (dry-matter content, 22.3 per cent) for 5 weeks; corn silage (dry-matter content, 30.1 per cent) for 4 weeks; Sudan grass silage (dry-matter content, 29.3 per cent) for 4 weeks; and grass-legume silage (dry-matter content, 26.1 per cent) for 4 weeks. The alfalfa hay was chiefly second-cutting hay with a dry matter content of 84.2 per cent (average of determinations). The grain mixture consisted of ground corn, 350 lb.; ground oats, 300 lb.; wheat bran, 150 lb.; brewers' dried grains, 50 lb.; soybean meal, 120 lb.; bonemeal, 15 lb.; and salt 15 lb.

It originally was planned that the cows of the pasture group would be fed the same amount of grain mixture as the cows in the dry-lot group so that the amounts of feed saved by pasture could be measured in terms of roughages. It

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was soon found, however, that the cows at pasture would not eat as much grain mixture as the ones in the dry-lot group.

The experimental period extended from May 17 to September 19, 1948, inclusive, a total of 126 days. Both groups of cows were started on experimental feeding at the same time and were carried throughout the entire period without change. After the cows had been on trial for 1 week and had become adjusted to the experimental feeding, they were weighed for 3 successive days. They also were weighed on 3 successive days at monthly intervals and at the end of the trial period.

The amounts of feed (other than pasture forage) which were offered and refused were carefully weighed and recorded. The amount of milk produced by each cow at each milking was weighed and butterfat tests were made of composite samples of milk made up from the milkings of each cow on 4 days of each week. Production records were computed to an F.C.M. (fat-corrected milk) basis (3).

The pasture consisted of grass-legume mixtures seeded in the spring of 1947 and grazed for the first time at the beginning of this trial. The crops included bromegrass-Ladino clover, bromegrass-alfalfa, bromegrass-birdsfoot trefoil and orchard grass-Ladino clover. Rotational grazing was practiced for a part of the season. The dry-matter yield of the pasture forage as determined by the protected-cage and clipping method was 9,370 lb. per acre, of which 1,320 lb. remained on the pasture at the close of the trial.

RESULTS

A summary of the feed consumption, milk production, and liveweight gains is given in table 1. The average daily milk production during the trial was

TABLE 1

Milk production, liveweight gains and feed consumption of cows kept in dry lot and at pasture

	Dry lot group		Pasture group		Feed saved per acre of pasture[a]
	Per cow daily	Per 100 lb. F.C.M.	Per cow daily	Per 100 lb. F.C.M.	
	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)	(*lb.*)
F.C.M. produced	34.1		35.1		
Liveweight gain	0.8		1.3		
Feed consumed:					
Silage	31.9	93.8	.....	.....	3,739
Hay	17.2	50.6	.....	.....	2,017
Beet pulp	1.5	4.4	.....	.....	175
Grain mixture	11.9	34.9	7.8	22.1	510

[a] Or feed-equivalent value per acre of pasture.

practically the same for each group. The cows in the pasture group made slightly greater liveweight gains than the dry-lot cows, thus showing that the pastured cows were as well fed as those in the dry-lot group.

The amount of feed saved by pasture is found from table 1 by subtracting the amounts of feed other than pasture forage consumed by the pasture group from the amounts eaten by the dry-lot group. For each 100 lb. of F.C.M. it was 93.8 lb. of silage, 50.6 lb. of hay, 4.4 lb. of dried beet pulp, and 12.8 lb. of grain mixture. These amounts multiplied by the yield of F.C.M. per acre (39.86 cwt.) gives 3,739 lb. of silage, 2,017 lb. of hay, 175 lb. of beet pulp and 510 lb. of grain mixture, respectively, as the feed saved, or the feed-equivalent value, of an acre of pasture (table 1).

The figures also furnish the basis for calculating the money value of an acre of pasture. The receipts from the sale of milk produced per acre (3,986 pounds F.C.M.) less the cost of the grain mixture fed per acre (881 lb.) gives the cash value of the feed obtained from pasture.

ANALYSIS AND DISCUSSION OF DATA

The yield of F.C.M. in pounds for each cow was calculated for each of the 18 weeks. The yields of one pair of animals for 1 week were called a weekly pair. Table 2 gives the analysis of variance of the weekly pairs on the basis of the variation between and within weekly pairs.

TABLE 2

Analysis of variance of F.C.M. per cow per week

Source	Sum of squares	Degrees of freedom	Mean square
Total	2,435,835	467	
A—Between weekly pairs	1,711,064	233	
Weeks	520,846	17	30,638.00**
Cow pairs	1,085,634	12	90,469.50**
Weeks × cow pairs	104,584	204	512.67
B—Within weekly pairs	724,771	234	
Rations	6,030	1	6,030.00
Rations × weeks	24,965	17	1,468.53
Rations × cow pairs	506,998	12	42,249.83**
Rations × weeks × cow pairs	186,778	204	915.58

** Significant at the 0.01 level of probability.

The sum of squares between weekly pairs was broken down into three parts. One part was associated with the differences between cow pairs. Another was associated with the differences brought about by the time trend as measured in weeks. The third part was associated with the interaction of the two former sources. Using the mean square for interaction between weeks and cow pairs as an error term, the mean squares for weeks and cow pairs were significant at the 0.01 level of probability. This was expected and hardly needs an explanation.

The sum of squares within weekly pairs was broken down into four parts. These four parts were associated with the differences between rations and the two first-order interactions and the one second-order interaction involving rations.

Using the second-order interaction, ration × weeks × cow pairs, as an error term, the two first-order interactions were tested. The interaction, ration × cow pairs, was significant at the 0.01 level of probability. This may indicate a differential rate of response to the two rations from one pair of cows as compared to another. It may also be a fact that the members of a pair just failed to perform similarly irrespective of the ration fed. The intraclass correlation of the weekly pairs was 0.407, which shows that the pair members were far from being identical.

The first-order interaction, rations × weeks, was not significant. This points out that one ration was as consistent as the other in maintaining F.C.M. yield for the 18 weeks. There was no appreciable differential response from the two rations from one week to another. In figure 1 the regression of F.C.M.

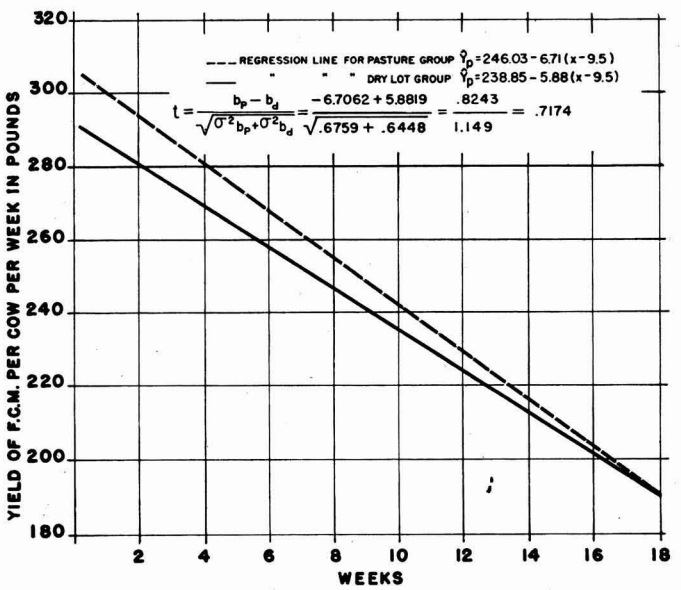

FIG. 1. The regression of F.C.M. per cow per week on time as measured in weeks.

per cow per week on weeks for the pasture group is slightly larger than the corresponding regression for the dry-lot group. Both regression coefficients are negative, and are not significantly different from each other.

The mean square for rations when tested by the interaction, ration × cow pairs, was not significant. Since the mean square for ration was smaller than that for the ration × cow pair interaction, the interaction was divided by the main effect in making the F test. From the analysis of variance and regression lines one can say that the two rations have not produced significantly different effects on the yield of F.C.M.

Table 3 gives the analysis of variance for the monthly weights of the cows on experiment. The weight at each interval or month was recorded as the average of three successive daily weights. The sum of squares for weights was broken down into the two parts, between monthly pairs and within monthly pairs. The differences associated with months and cow pairs were highly significant. The difference between the weights of the cow pairs was expected because of the pairing of similar animals. The changes in weight from one month to the next were mainly a manifestation of gain in weight. Figure 2 shows that in only

TABLE 3

Analysis of variance of the monthly weights

Souree	Degrees of freedom	Sum of squares	Mean square
Total	129	2,012,315	
Between monthly pairs	64	1,245,752	
Cow pairs	12	931,829	77,652**
Months	4	284,871	71,218**
Months × cow pairs	48	29,052	605
Within monthly pairs	65	766,563	
Rations	1	2,669	2,669
Rations × months	4	18,405	4,601*
Rations × cow pairs	12	681,117	56,760**
Rations × months × cow pairs	48	64,372	1,341

* Significant at 0.05 level of probability.
** Significant at 0.01 level of probability.

one case was there a loss in weight, and this was during the second month. The cause of this loss of weight is not known.

The sum of squares within monthly pairs was broken down into four parts. That part attributed to differences between rations was not significant while the first order interaction mean squares rations × months and rations × cow pairs were significant at the 0.05 and 0.01 level of probability, respectively. The ration × cow pairs interaction is an expression of a differential response of the cow pairs to the two rations; this may be nothing more than the failure of members of pairs to perform similarly in changes in weight irrespective of ration. The ration × months mean square denotes a differential response to the two rations from one month to another. Figure 2 gives the average weight of the two groups at monthly intervals, and the interaction is clearly shown in the third and fourth monthly periods. The regressions of weight on time as measured in monthly intervals (fig. 2) were not significantly different for the two rations. A t test of the gains of the pair members showed no difference in the total gains made under each ration.

The analyses made make it possible to accept the hypothesis that there was no difference in the F.C.M. yield and weight gains of the two groups on the two different rations. Approximately the same amount of energy was consumed by the two groups, so the pasture has replaced the amount of grain, hay, and silage

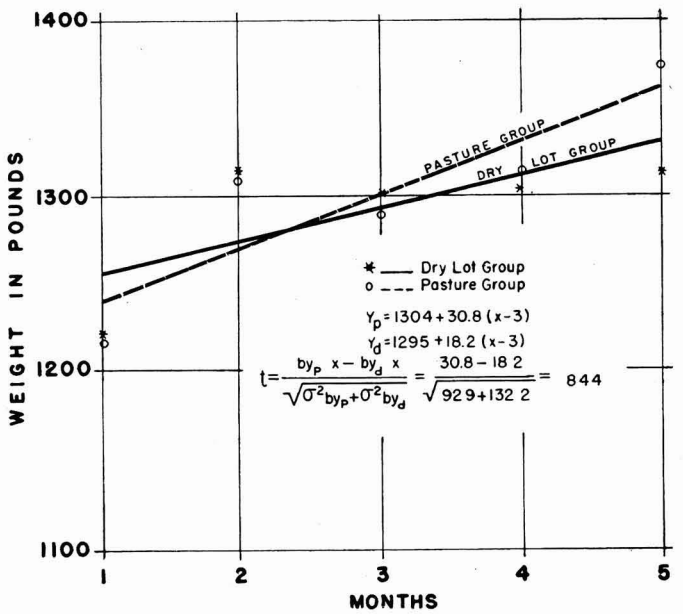

FIG. 2. Regression of weight in pounds as measured in monthly intervals.

which the cows on dry lot had in excess to that given the pasture group. This is probably a minimum estimate of the replacement value of pasture, because of more exercise of the pasture group, and because of the slightly larger, but not significantly so, yields of F.C.M. and gains in weight of the pasture group.

SUMMARY AND CONCLUSIONS

The milk-production value of pasture in terms of the amount of feed replaced by pasture forage was studied by means of a group of 13 cows fed only grain mixture and given access to pasture and a comparable group of 13 cows kept continuously in dry lot and barn fed on silage, hay, beet pulp and grain mixture over a period of 18 weeks. The amounts of milk produced were large and were practically the same for each of the two groups throughout the experimental period. Live weight gains for both groups were good, indicating that the amounts of nutrients supplied were ample.

Statistical analyses of the data indicate that there were no significant differences in the F.C.M. yield and weight gains of the two groups on the different rations. The feed-equivalent value of an acre of the pasture used in this investigation was found to be 3,739 lb. of silage, 2,017 lb. of hay, 175 lb. of beet pulp and 510 lb. of grain mixture.

The procedures followed provide a dependable method for estimating the value of pasture forage consumed by dairy cows.

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ABSTRACTS OF LITERATURE

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ANIMAL DISEASES

W. D. POUNDEN, SECTION EDITOR

741. Brucella infections. F. M. POTTINGER, JR., I. ALLISON AND W. A. ALBRECHT. Merck Report, **58,** 3: 13–14. July, 1949.

Enterically coated tablets containing 2 g. Mn, 2 mg. Cu, 2 mg. Co., 60 mg. Mg and 15 mg. Zn were taken by humans at the rate of not more than 3/d. in conjunction with carefully regulated high-protein, low-sugar diets for 12 wk. or more. Many cases of relief from symptoms and improvement of blood picture are reported. Trace element administration to a herd of cattle is reported to have increased the number of viable calves, increased milk production and improved the breeding record. Data are not presented.

F. E. Nelson

742. The milk ring test for detecting Brucella agglutinins in cow's milk. I. F. HUDDLESON AND C. CARRILLO, Michigan State College, East Lansing. Vet. Med., **44,** 6: 240–243. June, 1949.

A complete description is given for the preparation of *Brucella* antigen for the detection of *Brucella* agglutinins in milk by the Fleishhauer ring test. A transparent plastic block bored with 0.25 in. holes serves as test tubes and rack. Soda straws were used instead of glass pipettes. The milk samples mixed with antigen are incubated at room temperature and the test read 1 to 6 hr. later. The ring test may be a valuable aid in conjunction with the blood agglutination test for detecting brucellosis. B. B. Morgan

743. Procaine penicillin G levels in udder during treatment of chronic mastitis. W. D. BOLTON, J. M. FRAYER, J. H. CADY AND E. F. WALLER, Vermont Agr. Expt. Station, Burlington. Vet. Med., **44,** 6: 244–245. June, 1949.

Twenty-eight cows infected with mastitis were divided into 3 groups and treated with procaine penicillin G bougies. Of these groups, 18 received 25,000 units/quarter, 6 received 50,000 units and 6 received 75,000 units. A total of 112 quarters were treated. Milk samples for bacterial colony counts and penicillin assays were collected before treatment and 1, 4, 6 and 8 hr. after treatment. The number of colonies were reduced from a maximum of 36,000 to 10 and the maximum number of units of penicillin/ml. dropped from 225 to 10/quarter. The amount of penicillin remaining at the end of 8 hr. was in excess of that required to inhibit streptococci.

B. B. Morgan

744. Aureomycin in the treatment of staphylococcic mastitis in cows. E. C. MCCULLOCH, J. S. KISER AND H. MIGAKI, Washington State College, Pullman. Vet. Med., **44,** 6: 253–258. June, 1949.

A group of 16 cows representing 61 staphylococcic mastitis infected quarters was treated. Three cows received 100 mg. of aureomycin in 20 ml. sterile saline/quarter; 3 others received 100 mg. in 7.5 g. of an ointment base (10% lanolin, 22.5% mineral oil and 67.5% white petrolatum). Additional treatments were given to several flare-up cases. Twenty-four quarters were treated with 12 quarters (50%) apparently cured. A second group of 6 cows was treated with 150 mg. of aureomycin in 30 ml. sterile saline/quarter by intramammary infusion. The treatment was repeated after 48 hr. Four cows received sulfamethazine orally. Of 23 quarters involved, 60.8% were cured. The last group of 4 cows was given 200 mg. of aureomycin in 15 g. of ointment; treatment was repeated at 72 hr. Of 14 quarters treated, 12 (85.7%) apparently were cured. Assays of aureomycin in the milk revealed between 12 to 50 γ/ml. at 24 hr., 6 to 25 at 48 hr. and 1 to 2 γ at 72 hr. No evidence of irritation or reduction in milk flow due to the drug was observed. B. B. Morgan

745. Preliminary report on use of sulfamethazine and penicillin in bovine mastitis. R. W. FULLER, State Institution Farms Bureau, Batavia, N. Y. Vet. Med., **44,** 3: 103–107. Mar., 1949.

Infected quarters of approximately 127 cows were determined by physical examination, strip

cup and bromthymol blue tests. The routine treatment was the infusion into each infected quarter of 50 ml. of a 10 to 25% sterile solution of sodium sulfamethazine by weight/volume with 25,000 or 50,000 units of penicillin. Two infusions at 24 hr. intervals or 4 infusions at 12 hr. intervals were given. A 94.6% clinical recovery of mastitis by the sulfamethazine-penicillin treatment was obtained. Milk was available for human consumption 3 to 5 d. after treatment. Toxic symptoms or udder irritation were not observed.

B. B. Morgan

746. Mastitis in dairy cattle. J. W. CUNKELMAN, Fort Dodge, Iowa. Vet. Med., **44,** 5: 207–209. May, 1949.

A brief review of mastitis pointing out that during the past 1.5 yr. most of the research has been toward treatment of the disease. Various control measures are discussed. B. B. Morgan

747. Veterinary practitioners and community health. J. H. STEELE, U. S. Public Health Service, Atlanta, Ga. Vet. Med., **44,** 5: 192–195. May, 1949.

A brief review of the health policies involving man and animals in the United States is presented. Emphasis is placed on the role of the veterinarian in regard to milk sanitation and food inspection. The U.S.P.H.S. has established veterinary research units in research and communicable disease control. The main objectives of this program are the pasteurization of dairy products from disease free animals and veterinary inspection of all meat and dairy products.

B. B. Morgan

748. Diagnosis and control of mange in dairy cattle. H. H. SCHWARDT, Cornell Univ., Ithaca, N. Y. J. Econ. Entomol., **42,** 3: 444–446. June, 1949.

Dairy cattle mange diagnosis during early stages is important. Then prompt treatment can prevent serious damage. Cattle died from mange; others lost condition and fell far below normal production. In the north most serious cases occur during cold weather.

Mange may be confused with several other skin disorders. Definite diagnosis may be made by microscopic examination of deep skin scrapings at the edges rather than the center of active lesions. Scrapings must be deep enough to draw blood if sub-dermal Sarcoptic mites are to be found. Technic for concentrating a possibly few mites is described. There is discussion of Sarcoptic, Chorioptic, Psoroptic and Demodectic mites.

High pressure (400 lb.) spraying equipment with at least 2 gal./animal, for coverage of all external body parts, is described. Four applications at weekly intervals with either (a) lime sulphur solution at 1:15 dilution, or (b) 20 lb. wettable sulphur in 100 gal. water gave mange-free herds 8 mo. after treatment. Benzene hexachloride was used, but no conclusive control evidence presented.

Milk from cattle sprayed with 6 lb. of 6% *gamma* benzene hexachloride/100 gal. water contained about 4 p.p.m. of benzene hexachloride a few hr. after treatment. No residue was detected after about 1 wk. E. H. Fisher

749. Poisoning of farm animals by the marsh ragwort. W. C. EVANS AND E. T. R. EVANS. Nature, **164,** 4157: 30. 1949.

The death of cows, horses, etc. on pastures or cured hay containing marsh ragwort (*Senecio aquaticus*) is caused by an alkaloid named aquaticine by the authors. R. Whitaker

BUTTER

O. F. HUNZIKER, SECTION EDITOR

750. Observations on butteroil. C. W. DECKER, State College of Washington. Natl. Butter Cheese J., **40,** 8: 32–34. Aug., 1949.

A description of the American method of processing butteroil from cream and the Australian and New Zealand method of processing it from butter is given. A high quality cream is essential to avoid off-flavors in the finished product. A good quality butteroil can be made from butter of low quality caused by physical defects, high free fatty acids, neutralization or protein decomposition. Butteroil of satisfactory quality cannot be made from butter that is defective due to either metallic flavors, fishiness, tallowiness or excessive oiliness. Butteroil can be kept for a period up to a year at ordinary warehouse temperatures or for a longer period if held at 0° F. This product may be used for almost any food product where cream or butter is used. Butteroil may be used in the manufacture of preserved butters. Another possible use is for overseas shipments as a source of butterfat in reconstituted milks.

H. E. Calbert

DAIRY CHEMISTRY

H. H. SOMMER, SECTION EDITOR

751. Production of devitaminized casein by solvent extraction. S. M. WEISBERG AND J. GREENSPAN (assignors to National Dairy Research Labs., Inc.). U. S. Patent 2,477,505. 6 claims. July 26, 1949. Official Gaz. U. S. Pat. Office, **624,** 4: 1211. 1949.

Casein is devitaminized by maintaining the pH in the range 4.7 to 6.0 and extracting the vitamins with methanol. R. Whitaker

DAIRY ENGINEERING

A. W. FARRALL, SECTION EDITOR

752. Thermometer with mounting well. R. J. WINNING (assignor to Sheffield Farms Co.). U. S. Patent 2,475,211. 3 claims. July 5, 1949. Official Gaz. U. S. Pat. Office, **624,** 1: 232. 1949.

To overcome the delay in reaching the correct temperature of a thermometer inserted in a fixed well in the wall of pasteurizing and other tanks, vats, etc., 2 holes are provided in the well on the outside of the tank. One hole, located on the bottom side of the well is for draining while the other hole, located on top, is for introducing a liquid into the well to provide better contact between the thermometer and the well.
R. Whitaker

753. Meeker tube holder. H. J. EASTON. U. S. Patent 2,477,366. 2 claims. July 26, 1949. Official Gaz. U. S. Pat. Office, **624,** 4: 1175. 1949.

An adjustable spring arm is attached to the handle of the milk receiver of a milking machine and holds the rubber tubes from dragging in the litter on the floor of the stall. R. Whitaker

754. Hoist. S. H. HALL (assignor to DeLaval Separator Co.). U. S. Patent 2,476,192. 5 claims. July 12, 1949. Official Gaz. U. S. Patent Office, **624,** 2: 615. 1949.

This hoist is designed for lifting milk cans in dairy barns equipped with a vacuum line. The vacuum is employed to operate a piston in a cylinder, which, through a system of pulleys, raises and lowers the milk can into cooling tanks, on trucks, etc. The whole mechanism is mounted on a vertical cylindrical support fastened to floor and ceiling. R. Whitaker

FEEDS AND FEEDING

W. A. KING, SECTION EDITOR

755. The influence of corn starch upon roughage digestion in cattle. W. BURROUGHS, P. GERLAUGH, B. H. EDGINGTON AND R. M. BETHKE, Ohio Agr. Expt. Station, Wooster. J. Animal Sci., **8,** 2: 271–278. May, 1949.

The effects of adding varying amounts of mineralized starch to basal rations of either corncobs, alfalfa hay, or corncobs and alfalfa hay on the digestibility of the dry matter in the roughage were determined in 5 series of digestion trials.

Apparent digestion of the dry matter of basal rations of corncobs, or of corncobs and alfalfa hay was decreased with each increment of starch. The addition of starch had no significant effect on the digestibility of the dry matter of alfalfa hay basal rations in one series. In 2 of the alfalfa hay series only slight decreases in dry matter digestibility were noted when starch was added to the ration. F. C. Fountaine

756. The nutritive value of green berseem (Egyptian clover), hay and silage. A. GHONEIM, M. T. EL-KATIB AND A. A. BADR. Fouad I Univ., Giza, Egypt. J. Animal Sci., **8,** 2: 279–285. May, 1949.

Chemical analyses and digestion trials with sheep were used to determine the nutritive value of berseem (*Trifolium alexandrinum*) fed as fresh forage, hay and as silages made as follows: (a) untreated-unwilted, (b) 1% molasses, (c) wilted, and (d) A. I. V. The starch equivalent and digestible protein were higher in green berseem than in hay or any silage. A. I. V. silage was superior to hay in starch equivalent and equal to hay in digestible protein. Hay was superior to silages made by other methods, in both starch equivalent and digestible protein.
F. C. FOUNTAINE

757. The effect of crude soybean lecithin on the absorption and utilization of Vitamin A fed prepartum to the ewe and sow. H. D. EATON, J. A. CHRISTIAN, F. C. DAUGHERTY, A. A. SPIELMAN AND L. D. MATTERSON, Univ. of Connecticut, Storrs. J. Animal Sci., **8,** 2: 224–233. May, 1949.

Supplementation of a normal prepartal ration for sows and ewes with Vitamin A with or without soybean lecithin increased the Vitamin A content of the blood of the dams, and in the blood and livers of their offspring at birth and at 30 d. of age. The addition of lecithin to the Vitamin A supplement did not materially increase the content of Vitamin A in the blood or livers over that of animals receiving Vitamin A concentrate alone. F. C. Fountaine

Also see abs. no. 749.

GENETICS AND BREEDING

N. L. VAN DEMARK, SECTION EDITOR

758. A study of the metabolic activity of bull semen and spermatozoa in relation to their fertilizing ability. D. GHOSH, L. E. CASIDA AND H. A. LARDY, Univ. of Wisconsin, Madison. J. Animal Sci., **8,** 2: 265–270. May, 1949.

The respiration rate (cmm. of O_2 uptake/10^8 cells/hr.) of 4 mature Guernsey and 4 mature Holstein bulls ranged from 3 to 12.55, a lower rate than obtained in previous studies. There was no correlation between respiratory metabolism of spermatozoa and their fertility.
F. C. Fountaine

759. An hereditary digital anomaly of cattle. S. W. Mead, P. W. Gregory and W. M. Regan, Univ. of California, Davis. J. Heredity, **40,** 6: 151–155. June, 1949.

Hoofs that were small and slightly malformed were associated with probable modifications in the metacarpals, carpus and tarsus in 5 Jerseys of both sexes. Pain was manifested when affected animals were on their feet, with more discomfort in the fore than in the hind feet. The condition was observed at ages of 2 to 4 mo. and became progressively worse. Feeding, health and management all were excluded as possible causative factors. All affected animals descended in one or more lines from each of 2 bulls that had a common great-grandparent. A single autosomal recessive gene is indicated as the genetic conditioner for this anomaly. L. O. Gilmore

760. Dwarf cattle for the tropics. C. G. Arrillaga, Univ. of Puerto Rico. J. Heredity, **40,** 6: 167–168. June, 1949.

Proportionate dwarfism has been observed in a few cases in the cattle of Puerto Rico. Inherited dwarfism (recessive) is distinguished from the condition produced by environmental causes. The possible significance of genetic dwarfs possessing high performance ability for use in the mountainous regions of tropical America is indicated. L. O. Gilmore

HERD MANAGEMENT

H. A. HERMAN, SECTION EDITOR

761. Milking parlor. H. B. Babson and C. A. Thomas (assignors to Babson Bros. Co.). U. S. Patent 2,477,035. 7 claims. July 26, 1949. Official Gaz. U. S. Pat. Office, **624,** 4: 1091. 1949.

This milking parlor is characterized by having the cows on a level above the operator's floor for convenience in milking. R. Whitaker

762. Stock watering fountain. A. F. Klinzing. U. S. Patent 2,476,876. 1 claim. July 19, 1949. Official Gaz. U. S. Pat. Office, **624,** 3: 906. 1949.

A bowl shaped fountain for watering farm animals, such as cows. A hinged member, depressed by the animal's nose, actuates a valve and admits water to the bowl. R. Whitaker

ICE CREAM

C. D. DAHLE, SECTION EDITOR

763. Carton. B. A. Rafoth (assignor to Marathon Corp.). U. S. Patent 2,475,294. 1 claim. July 5, 1949. Official Gaz. U. S. Pat. Office, **624,** 1: 254. 1949.

A paper carton for ice cream and other food products similar in shape to the regular pail type container with a spoon or dispensing implement held to the top cover by an extension panel, so cut and folded to both hold the spoon and lock the cover of the carton. R. Whitaker

MILK SECRETION

V. R. SMITH, SECTION EDITOR

764. The effect of the plane of nutrition on the composition of sow's colostrum and milk. J. P. Bowland, R. R. Grummer, P. H. Phillips and G. Bohstedt. Univ. of Wisconsin, Madison. J. Animal Sci., **8,** 2: 199–206. May, 1949.

Colostrum from sows fed on pasture during gestation was higher in fat, and lower in S.N.F., protein and lactose than that of sows carried on dry lot during gestation. There was no difference between groups in the T.S. and ash of the colostrum. Milk sampled weekly from the 1st to the 8th wk. from sows maintained on pasture was lower in T.S. but was not appreciably different in S.N.F., protein, ash and lactose than milk from sows fed on dry lot. No breed differences were noted. F. C. Fountaine

NUTRITIVE VALUE OF DAIRY PRODUCTS

R. JENNESS, SECTION EDITOR

765. Tocopherol (Vitamin E) deficiency among sheep fed natural feeds. F. Whiting, J. P. Willman and J. K. Loosli, Cornell Univ., Ithaca, N. Y. J. Animal Sci., **8,** 2: 234–242. May, 1949.

Colostrum and milk of ewes fed a prepartal ration of alfalfa hay and cull beans, even when supplemented with tocopherols, contained insufficient tocopherol to prevent stiff lamb disease. Administration of tocopherols to the lambs cured the condition. The incidence of muscle dystrophy was higher in lambs of ewes fed a prepartal ration including alfalfa hay than in those of ewes fed mixed or grass hay.
F. C. Fountaine

766. Supplemented milk diets for young pigs in cages. J. A. Weybrew, H. A. Stewart, G. Matrone and W. J. Peterson. North Carolina

Agr. Expt. Station, Raleigh. J. Animal Sci., **8,** 2: 207–223. May, 1949.

Three whole milk diets supplemented with minerals and cod liver oil were compared for pigs from 2 d. to 8 wk. of age. When measured by growth, reconstituted whole milk solids was equal to reconstituted skim milk solids plus butter, and both were superior to evaporated milk. There was no difference in efficiency of the 3 diets. Pigs on each of the 3 diets made better gains than those nursing well fed sows.

F. C. Fountaine

SANITATION AND CLEANSING

K. G. WECKEL, SECTION EDITOR

767. Residual action of organic insecticides against stable flies. G. W. EDDY AND W. S. MCGREGOR, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., **42,** 3: 547–548. June, 1949.

Laboratory tests with 11 recently developed organic insecticides were made on stable flies to determine speed of knock-down and kill, and length of effectiveness. Screen cages were dipped in 1% solution of toxicant in acetone. One cage was held indoors while the other was exposed to the weather. Tests were made 1, 7, 14, 50 and 126 d. after treatment. Flies were held in cages up to 48 hr.

DDT and methoxychlor were fastest acting, and toxaphene and chlordan were slowest, in 24 hr. check. Methoxychlor and the bromine analog of DDT were superior to others in knock-down and duration of effectiveness. Parathion, compound 118 and toxaphene retained toxicity longer than dichlorodiphenyl dichloroethane, *gamma* benzene hexachloride (95%), compound 153, heptachlor or chlordan. E. H. Fisher

768. Toxicity to house flies of synthetic compounds of the pyrethrin type in relation to chemical structure. W. A. GERSDORFF, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., **42,** 3: 532–536. June, 1949.

Laboratory tests with house flies showed some new synthetic compounds related to pyrethrins to be more toxic than a mixture of pyrethrins contained in the ordinary pyrethrum-kerosene extract. E. H. Fisher

769. Fumigating properties of several new insecticides. R. A. HOFFMAN AND A. W. LINDQUIST, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., **42,** 3: 436–438. June, 1949.

Laboratory tests with house flies showed several residual, contact insecticides to have a fumigating effect. A 100 mg./ft.^{2} treatment on the inside of wide-mouth qt. jars was made. Flies were held in similar untreated jars immediately above these for 6 hr. Benzene hexachloride, parathion, chlordan and fluorine analog of DDT caused complete kill of flies within 24 hr. after the 6 hr. exposure to their vapors. Toxaphene and TDE vapors gave high mortalities, but DDT killed none. A 10 mg./ft.^{2} treatment was also tested.

E. H. Fisher

770. The residual property of DDT as influenced by temperature and moisture. A. F. BURGESS AND H. L. SWEETMAN, Univ. of Massachusetts. J. Econ. Entomol., **42,** 3: 420–423. June, 1949.

House fly mortality was used to measure the effects of moisture and temperature on DDT, applied in kerosene solution to screens. Treated screens held at 37° C. and 60 to 75% relative humidity decreased in toxicity more rapidly than those held at 23° C. and 25 to 40% humidity.

E. H. Fisher

771. Failure of DDT to control houseflies. W. V. KING AND J. B. GAHAN, U.S.D.A., Agr. Res. Adm., Bur. of Entomology and Plant Quarantine. J. Econ. Entomol., **42,** 3: 405–409. June, 1949.

House flies from several natural sources, including dairy barns, were compared with laboratory-reared ones for resistance to some insecticides. Laboratory tests with only 25 mg. DDT/ft.^{2} of treated panel showed 70% mortality in 8 to 67 min. for flies from natural sources, and 1.1 to 3.19 min. for laboratory flies.

Chemical analysis of several DDT preparations revealed no inferior quality. Biological tests were not reported.

With cage tests on dairy barn walls, variation in mortality showed uneven DDT application.

Deposits of DDT, technical benzene hexachloride, and partially refined benzene hexachloride showed fly repellency when used at rates much greater than usually recommended, chlordan and methoxychlor did not. Tests indicated there was greater fly resistance to DDT than to methoxychlor, chlordan or benzene hexachloride.

E. H. Fisher

772. Insecticides and the food law. C. W. CRAWFORD, Deputy Commissioner of Food and Drugs. J. Econ. Entomol., **42,** 3: 564–566. June, 1949.

The Federal Food, Drug and Cosmetic Act of 1938 prohibits interstate traffic in adulterated foods. Some adulteration tolerances in or on foods are in effect, and others may be set, if it

can be shown that "—such substance is required in the production thereof or cannot be avoided by good manufacturing practice." Interpretation of the Act indicates there should be insurance that the total of toxic substances in all items constituting our diets will be held at safe levels. This is in contrast to tolerance considerations regarding safety of a single adulterated food.

Since the advent of new insecticides, beginning with DDT, information on public health problems related to these materials has not kept pace with the development and use of them. In addition to proving chemicals which may be used in connection with food production to have excellent insecticidal value, it is also necessary to devise quantitative analytical methods, determine toxicity to man and other animals and determine the quantities to which man and other animals may be exposed. Among other answers needed are whether the insecticide is translocated in plants, excreted in milk of animals which consume it, or cumulative in animal tissue. E. H. Fisher