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Influence of Environmental Temperature Cycles on Vitamin Levels of Milk and Blood of Cows

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SUMMARY

Ascorbic acid, niacin, riboflavin, and pantothenic acid determinations were made on the milk and blood of two Jersey and two Holstein cows maintained under diurnally variable controlled temperature conditions. Increased ascorbic acid levels were found in both milk and blood when the animals were exposed to high environmental temperatures; however, advancing lactation was a complicating factor.

Both niacin and riboflavin values increased in the Holstein milk under the influence of high environmental temperatures; the Jersey milk showed no appreciable change in niacin and riboflavin under these conditions. Riboflavin blood values of all animals were lowered markedly by the 60 to 110° F temperature cycle and moderately depressed by exposure to the 70 to 100° F cycle. High environmental temperatures caused a decline in niacin blood values of the Holstein cows while there was no apparent change for the Jerseys.

Pantothenic acid levels increased in the blood and decreased slightly in the milk when the animals were exposed to the 60 to 110° F temperature cycle.

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INTRODUCTION

The influence of environmental temperature on lactating cows is particularly important in that it may affect both the individual animal's well-being and the nutritional value of the milk produced by the animal. Those general factors affecting the metabolism of vitamins and vitamin levels in bovine blood have been reviewed in a prior publication (Singh and Merilan, 1957); thus, only the literature directly concerned with vitamins in milk will be presented here.

Ascorbic Acid in Milk

Vitamin C is secreted in the milk only in the reduced form, ascorbic acid, but with exposure to light it quickly undergoes photocatalyzed oxidation to dehydro-ascorbic acid (Kon and Watson, 1937; Knight *et al.*, 1939; Woessner *et al.*, 1939). The values quoted for normal fresh milk generally fall around 2 mg. ascorbic acid per 100 ml. of milk.

Some of the factors which have been found to influence the excretion of ascorbic acid in milk are:

1. *Feed*—Experiments of many early workers (Hart *et al.*, 1920; Hess *et al.*, 1921; MacLeod, 1927; Dutcher *et al.*, 1921; and Olson *et al.*, 1924) indicated that feed of the cow may have a marked influence on the vitamin C content of milk. Their findings, in general, were that milk from cows on pasture or ensilage had higher antiscorbutic potency than milk from cows stall or dry fed. However, among these early workers, Hughes *et al.* (1921, 1927) reported that the ration received by the cows had no influence on the antiscorbutic potency of their milk. With the development of chemical methods for determination of ascorbic acid, many further studies were undertaken, which fully substantiated the conclusions of Hughes *et al.* (Riddell *et al.*, 1936; Kon and Watson, 1937; Reedman, 1937; Garrett, 1940).

Whitnah and Riddell, 1937, demonstrated that feeding 65 kg. per day of green rye containing 0.86 mg. of ascorbic acid per gm. for 15 days, to cows that were otherwise fed hay and silage, did not increase the vitamin C content of their milk, although its excretion in the urine increased markedly.

Contrary to the findings of early workers, Holmes *et al.* (1944) and Wallis (1943) obtained lower values for vitamin C in milk from cows on pasture than

from cows stall fed. Wallis (1943) and Vavich *et al.* (1945) found that a very low intake of ascorbic acid in feed by cows for a long time had no influence on ascorbic acid concentrations of their blood and milk.

Knight *et al.* (1941) and Vavich *et al.* (1945) demonstrated that vitamin C is quickly destroyed in the rumen. These workers also showed that oral administration of large doses of ascorbic acid to cows was without any effect on its concentration in their blood and milk, and had only a slight effect on the concentrations of vitamin C in their urine. Erb *et al.* (1947) also confirmed the quick destruction of ascorbic acid in the rumen.

2. *Season of the Year*—Seasonal variations in the vitamin C content of milk have been observed by a number of investigators, although there does not seem to be any definite agreement regarding the seasonal levels. Whitnah and Riddell (1937) observed a rise from October to December, a fall from December to February and another rise from February to March in the vitamin C levels of milk of cows fed a uniform diet. They attributed the low values observed in January and February to continued wintery weather.

Holmes and Holmes (1939) found a steady increase in the ascorbic acid content of milk of Holsteins and Guernseys from the middle of December to the middle of February, followed by a drop from the middle of February until early May; with peaks occurring in November and February.

Trout *et al.* (1939) reported that ascorbic acid in milk was consistently higher in spring and summer than in fall and winter. Jarl (1946) also obtained higher values in summer and autumn than in winter. But Davidov and Gul'ko (1953) observed that the ascorbic acid values of milk in winter were from 30 to 40 percent higher than in summer. The minimum values were recorded in the period from June to August. On the other hand, MacLeod (1927) reported that the milk of stall fed cows given a well balanced uniform feed did not show seasonal variations in its antiscorbutic value. Sharp (1936) and Kon and Watson (1937) also found no influence of season on the vitamin C content of milk.

3. *Stage of Lactation and Pregnancy*—Rasmussen *et al.* (1936) reported that the stage of lactation has a definite influence on the vitamin C content of milk. They observed that colostrum is quite high in vitamin C and that the lowest titer in milk occurs about the second or third month of the lactation period. Kon and Watson (1937) found that the vitamin C content of colostrum was slightly higher than normal milk and the high values dropped quickly after two days to a level lower than the normal range.

Reedman (1937) concluded from determinations of the ascorbic acid content of milk samples collected from four breeds of dairy cows that there was little variation at different stages of lactation. But Whitnah and Riddell (1937) found that cows produced milk with about 10 percent lower vitamin C content in the first month of lactation than in later months. Holmes *et al.* (1940a) observed that the ascorbic acid content of both Guernsey and Holstein milk rose rapidly during the first two months of lactation and decreased only slightly over

the period from the second to the eleventh month of lactation. Epple and Horrall (1943) noticed a slight gradual increase in vitamin C concentrations of milk with progressing lactation. Jarl (1946) observed lower values between the third and eighth day of lactation.

Davidov and Gul'ko (1953) observed that during the first two or three months of lactation the values for ascorbic acid in milk increased and then fell toward the eighth month; during the last month of lactation there was another slight rise. They failed to observe these changes in animals that were not pregnant. Holmes *et al.* (1941) found that, in general, the vitamin C content of milk from both Holstein and Guernsey cows tended to decrease with the advance of pregnancy, although at all stages of pregnancy different cows showed considerable variations.

While these reports regarding influence of stage of lactation are somewhat contradictory as far as the total daily secretion of ascorbic acid in milk is concerned, there seems to be good agreement that it parallels the daily milk yield. With the advancing lactation, as the milk yield drops, the total daily ascorbic acid secretion also declines (Holmes *et al.*, 1942 and Vavich *et al.*, 1945).

4. *Age of Cows*—Rasmussen *et al.* (1936), in their study with five different breeds, found no consistent relationship between age of the cow and the ascorbic acid content of its milk. Whitnah and Riddell (1937) stated that the relation of vitamin C content of milk to age of the cow was small and unimportant. Holmes *et al.* (1940b) confirmed the finding; however, Jarl (1946) has reported an increase in vitamin C concentration of milk with age.

5. *Diurnal Variations*—Whitnah and Riddell (1937) found that when cows were milked at 12-hour intervals, the evening milk contained about 2 mg. per liter or 10 percent more ascorbic acid than the morning milk. Jarl (1946) also observed that the concentrations were higher in the evening milk than in the morning or at noon. The observations of Davidov and Gul'ko (1953) are also in agreement with the results of these workers, but Wallis (1943) recorded no such differences.

6. *Breed*—Breed does seem to exert some influence. Of the five breeds, Ayrshire, Brown Swiss, Guernsey, Holstein and Jersey, studied by Rasmussen *et al.* (1936), Brown Swiss milk had the highest and Holstein the lowest ascorbic acid content. Whitnah and Riddell (1937) found Jersey milk to be highest throughout the year, but obtained no consistent differences between the other breeds; however, according to the yearly averages of vitamin C content of milk, they were in ascending order: Holstein, Ayrshire, Guernsey and Jersey. Observations of Beck *et al.* (1939) are in agreement with these results. Holmes *et al.* (1939) found Guernsey milk to be consistently higher than that of Holsteins.

7. *Individual and Daily Variations*—Rasmussen *et al.* (1936) reported that cows of the same breed and receiving similar diets produced milk which showed wide variations in ascorbic content; however, they thought such variations to be due in part to differences in stage of lactation.

According to the same workers, ascorbic acid content of milk from the same cow also varies slightly from period to period. Whitnah and Riddell (1937) obtained considerable day to day variation in the vitamin C content of the milk from the same herd.

8. *Relation of Milk Yield*—Some attempts have been made to correlate the vitamin C content of milk to its daily yield, but there does not appear to be such simple and definite relation (Whitnah and Riddell, 1937 and Holmes *et al.*, 1942). However, Holmes *et al.* (1942) found that the daily volume of milk produced has a definite though not wholly consistent influence on the total daily secretion of ascorbic acid in milk. Vavich *et al.* (1945) also observed a similar relationship.

9. *Other Factors*—Udder infections have been found to cause a decrease in the vitamin C content of milk (Kon and Watson, 1937). Reincke *et al.* (1941) concluded that mild mastitis causes a decrease in ascorbic acid of about 10 percent and in severe cases the decrease may be 30 to 50 percent.

Workers are not in agreement regarding the influence of feeding iodinated protein. Hibbs and Krauss (1946) observed no effect of feeding iodinated protein. These results were confirmed by VanLandingham *et al.* (1947) for Holsteins, but not for Jerseys for which they noticed a significant decrease. Booth *et al.* (1947) observed a decrease from 30 to 63 percent in the vitamin C concentrations of both Holstein and Jersey milk as a result of feeding iodinated casein.

Brown *et al.* (1941) reported that feeding 5 grams of potassium iodide daily to cows temporarily lowered the percentage of ascorbic acid secreted in their milk to a marked degree.

Administration of cod liver oil in the form of a drench has been found to cause a rise in the ascorbic acid content of milk, although similar doses in feed were found to cause no change. The effect is attributed to nervous disturbances (Guthrie, 1940).

Niacin

Niacin in milk is usually determined microbiologically since biological and chemical methods of determination have not proved very satisfactory. Several organisms have been found suitable for its microbiological assay, but *Lactobacillus arabinosus* has been used most. Some workers have also used *Lactobacillus casei*. *Lactobacillus arabinosus* responds equally on a molar basis to niacin, niacinamide and cozymase (Snell and Wright, 1941), but *Leuconostoc mesenteroides* responds only to niacin. By using this organism for assay, with or without suitable hydrolysis of niacinamide in skim milk powder samples, Krehl *et al.* (1946) found that about 99 percent of the niacin activity of milk was due to niacinamide. Most of the values reported for total niacin activity of cows' milk fall between 60 to 100 ug. per 100 ml. (Kon and Henry, 1949, and Gregory, 1955).

The causes of variation of niacin in cows milk have been given relatively little attention but those studied include:

1. *Feed and Season*—According to Stefaniak and Peterson (1946), seasonal and feeding changes do not affect significantly the amount of niacin in milk. Although Lawrence *et al.* (1946) also failed to observe any direct effect of the feed of the cow on the niacin content of milk, they reported some influence of the season. The niacin content of milk was found to decrease gradually during the winter and early spring months and then increase progressively during the summer and early fall months. Similar trends with higher values in summer have also been reported by other workers (Kon *et al.*, 1954, and Gregory, 1955).

That nicotinic acid content of the feed may not have any direct influence under normal conditions of feeding has been further confirmed by Marsh *et al.* (1947). They did not obtain a significant increase in its level in milk by feeding 2 grams of pure synthetic niacin daily to cows.

2. *Stage of Lactation*—It is interesting to note that the niacin content of colostrum and milk of the cow is not significantly different (Pearson *et al.*, 1946). The data of Lawrence *et al.* (1946), Luecke *et al.* (1947), and Rao and Basu (1951) also indicate that the values for niacin in colostrum fall within the normal range for milk.

Lawrence *et al.* (1946) reported that the niacin content of milk decreased regularly throughout the lactation until the 22nd week and increased very slightly afterward. A similar trend was observed by workers in New Zealand (Kon *et al.*, 1954).

3. *Breed*—There does not appear to be any clear evidence of the influence of breed on the niacin content of milk. Lawrence *et al.* (1946) found Guernsey milk to be significantly lower in niacin content than milk from Jerseys, Holsteins, and Brown Swiss. There was no significant difference among these latter three breeds. Marsh *et al.* (1947) found milk from Jersey cows to be higher in niacin content than the milk from Holsteins. Other workers have failed to observe any breed differences (Kon and Henry, 1949 and 1954).

4. *Other factors*—Lawrence *et al.* (1946) found considerable variation in the niacin content of milk between individual cows, but the day-to-day variations for a given cow were not so marked. These variations were not found to be correlated with the milk yield, age of the cow and fat, total solids, and solids-not-fat content of milk.

Stefaniak and Peterson (1946) reported great variations between the milk obtained from different herds.

Feeding thyroprotein to cows has been found to result in an increase in the niacin content of milk (Kemmerer *et al.*, 1946)

Pantothenic Acid

Pantothenic acid in milk has been determined microbiologically, mostly by using *Lactobacillus arabinosus* and *Lactobacillus casei*. However, pantothenic acid occurring as coenzyme A is active for both of these organisms (Craig and Snell, 1951). It has been found that pantothenic acid can be released from coenzyme A

by treatment with an enzyme preparation from chick liver and an alkaline intestinal phosphatase (Lipmann *et al.*, 1947). But Gregory (1955) reports that treatment of milk with these enzymes gave only slightly higher values than that obtained from untreated milk. Other workers have used one or a combination of some of the enzymes, takadiastase, papain, clarase or mylase P (Lawrence *et al.*, 1946; Pearson *et al.*, 1946; Stefaniak and Peterson, 1946). But the values they obtained do not seem to differ much from those of Gregory. Most of the values reported for milk cows fall between 300 and 400 ug. per 100 ml., although the extreme values reported range from 80 to 400 ug. per 100 ml. (Gregory, 1955).

Little information is available on causes of variation in pantothenic acid levels of milk but those factors studied include:

1. *Feed and Season*—According to Stefaniak and Peterson (1946), seasonal and feeding changes do not influence the pantothenic acid content of milk. Marsh *et al.* (1947) demonstrated that feeding 2 gm. of pure synthetic pantothenic acid daily to cows resulted in no significant increase in its levels in milk.

2. *Stage of Lactation*—It seems to be well agreed that the pantothenic acid content of colostrum is lower than that of normal milk. The values given for colostrum mostly fall below 200 ug. per 100 ml. (Pearson *et al.*, 1946; Luecke *et al.*, 1947; and Lawrence *et al.* 1946).

Pantothenic acid content of colostrum approaches the levels of normal milk in about a week's time. There seems to be no significant variation in the subsequent lactation (Pearson *et al.*, 1946 and Lawrence *et al.*, 1946). Gregory (1955) reported similar trends.

3. *Breed*—Marsh *et al.* (1947) obtained slightly higher values of pantothenic acid content for Jersey than for Holstein milk. Lawrence *et al.* (1946) found that in order of descending levels of pantothenic acid in milk, the four breeds were: Brown Swiss, Jersey, Guernsey, and Holstein. However, these workers, because of considerable individual variations observed, consider the significance of such breed variations to be questionable.

Apart from large variations in the pantothenic acid content of milk from cow to cow, Lawrence *et al.* (1946) also observed considerable variations between samples obtained from the same cow on successive days.

Riboflavin

Riboflavin in milk has been determined biologically using rats or chicks, chemically by fluorometric methods, or microbiologically using *Lactobacillus casei* as the assay organism. The results obtained by all these methods have been found to agree closely (Kon, 1940; Daniel and Norris, 1944; and Ford *et al.*, 1953c). The values reported in literature fall mostly in the range of 150 to 250 ug. per 100 ml. (Gregory, 1955; Kon and Henry, 1949, 1951 and 1954).

Factors that may influence the riboflavin content of milk are:

1. *Feed and Season*—It has been well established that riboflavin is synthesized by the rumen microorganisms. Cows fed almost riboflavin-free rations have been

shown to produce milk containing approximately normal amounts of the vitamin (McElory and Goss, 1940a). Still studies of many workers indicate that feed of the cow, under certain circumstances, may have some influence. Thus, Hunt and Krauss (1931), by using biological assay on rats, found that milk from cows on pasture had a higher vitamin G (riboflavin) content than milk from dry fed cows. Whitnah *et al.* (1938) reported that the flavin content of milk was slightly higher in July after the cows had access to pasture; however, these workers stated that climate and ration may not be the only factors responsible for these variations. According to the findings of Houston *et al.* (1940) the riboflavin content of cows milk is increased in summer by pasture feeding as compared to that in winter when cows are dry fed.

Virtanen and Holmberg (1938) found that cows when given fresh grass produced 30 percent more riboflavin in milk than cows fed hay and grain. Feeding the cows with phosphoric acid silage along with hay and grain maintained the riboflavin content of their milk at the level obtained from cows on pasture.

Hand and Sharp (1939), in confirming the findings of Virtanen and Holmberg, reported that when cows were fed molasses grass silage or phosphoric acid silage during winter the riboflavin content of their milk was maintained at the pasture feeding level. However, they observed a definite increase of riboflavin content of milk in summer from a herd of cows that were given corn silage in winter.

Kramer *et al.* (1939) found that milk samples collected from a Holstein herd at the end of an abnormally dry summer contained about 25 percent less riboflavin than samples from the same herd collected later in the fall when the cows had had good pasture for about one month. However, the following spring they failed to observe any increase in the riboflavin content of milk of cows that were changed from late winter ration to pasture.

Johnson *et al.* (1941) demonstrated that a ration consisting of good quality hay, acid grass silage, and a grain mixture could maintain the milk riboflavin at the pasture level. When cows were changed from pasture to a ration low in riboflavin, the amount of riboflavin in milk decreased by about 25 percent. Increasing the riboflavin intake of cows considerably by feeding a molasses yeast by-product caused only a temporary increase in the riboflavin concentration of milk.

Theophilus and Stamberg (1945) reported that when a concentrate-dry-roughage ration was supplemented with substantial amounts of sunflower silage, a 36 percent increase in the riboflavin content of milk was obtained within two days. Supplementing further with wheat pasture, or sweet clover and wheat pasture, caused no further significant change in the riboflavin content.

On the other hand, Holmes *et al.* (1944) observed that when cows were changed from a winter ration to early pasture there was a decrease in the riboflavin content of their milk. Marsh *et al.* (1947) obtained no increase in the riboflavin content of milk, as determined microbiologically, by feeding 2 or 16 gms. of pure crystalline riboflavin daily to cows. But the milk from these cows had a

distinct yellow color characteristic of aqueous solutions of riboflavin and gave a high value for riboflavin when measured by fluorometric methods (Pearson and Schweigert, 1957).

Holmes and Holmes (1943) demonstrated that feeding a uniform winter ration to cows under controlled conditions resulted in uniform riboflavin content of their milk throughout the period of study, December to March. Earlier, Dutcher *et al.* (1943), by using biological assay methods, had shown that the vitamin G (riboflavin) potency of raw milk obtained from cows fed uniform diet under controlled conditions was markedly constant throughout the year. Theophilus and Stamberg (1945) also reported that there were no seasonal variations in riboflavin content of cows' milk.

More recent studies have indicated that summer milk contains more riboflavin than the winter milk, possibly due to the change from indoor feeding to pasture (Kon and Henry, 1951, 1954; Gregory, 1955).

2. *Stage of Lactation and Pregnancy*—It seems to be well established that colostrum is several times richer in riboflavin than normal milk (Kramer *et al.*, 1938; Houston *et al.*, 1940; Theophilus and Stamberg, 1945; Pearson *et al.*, 1946; Sutton *et al.*, 1947; and Luecke *et al.*, 1947). It is also agreed that high levels of riboflavin in colostrum decline rapidly to reach the values of normal milk (Theophilus *et al.*, 1945; Pearson and Schweigert, 1946; Sutton *et al.*, 1947). During the remaining period of lactation there does not seem to be any significant variation in the riboflavin content of milk (Houston *et al.*, 1940; Whitnah *et al.*, 1938; Theophilus and Stamberg, 1945).

Oestrus and pregnancy also have little if any influence on either the riboflavin content of milk or the total amount of riboflavin secreted per day (Theophilus and Stamberg, 1945).

3. *Diurnal Variations*—Theophilus and Stamberg (1945) found that the riboflavin content of morning, night, and noon milk from the same Holstein and Jersey cows showed no significant variations.

4. *Breed*—It is generally agreed that Jerseys and Guernseys produce milk richer in riboflavin than Holsteins or other dairy breeds (Kramer *et al.*, 1939; Hand and Sharp, 1939; Whitnah *et al.*, 1938; Theophilus and Stamberg, 1945). Hand and Sharp (1939) and Theophilus and Stamberg (1945) both reported that milk from Jerseys is about 50 percent higher in riboflavin values than milk from Holsteins.

It is interesting to note that the breed differences for riboflavin content of colostrum do not follow the same trend as observed for normal milk. Thus, Theophilus and Stamberg (1945) and Luecke *et al.* (1947) found no significant difference with respect to the riboflavin content of colostrum of Jersey and Holstein cows. However, the data of Sutton *et al.* (1947) indicate that riboflavin concentrations of colostrum were highest for Guernseys, followed by Ayrshires, Jerseys, and Brown Swiss with Holsteins the lowest.

Although the milk from Jerseys has a higher riboflavin concentration than

milk from Holsteins, the Holstein cows, because of their higher milk production, secrete more total amount of riboflavin in milk daily than do the Jersey cows (Theophilus and Stamberg, 1945).

It has been reported by several workers that the riboflavin content of milk tends to be inversely related to the milk yield (Johnson, *et al.*, 1941; Holmes *et al.*, 1944; Theophilus and Stamberg, 1945). Perhaps the breed differences observed can, to a great extent, be explained by this factor.

Large variations between individual cows of the same breed have also been observed (Hand and Sharp, 1939; Theophilus and Stamberg, 1945). Hand and Sharp (1939) reported that the variations among individual cows in a given season can be more than 500 percent.

5. *Other Factors*—A direct correlation between the riboflavin content and the fat content of milk was suggested by Whitnah *et al.* (1938) but this possibility has been ruled out by the experiments of Johnson *et al.* (1941). They showed that by feeding cod liver oil to cows, while both milk yield and fat percentage decreased, the riboflavin content actually increased.

The riboflavin content is decreased in milk from udders infected with mastitis (Kon *et al.*, 1954).

The influence of feeding thyroprotein is controversial since Hibbs and Krauss (1946) and Kemmerer *et al.* (1946) reported that the riboflavin content of milk is slightly decreased by feeding thyroprotein, while Chanda *et al.* (1952) observed no effect of thyroxine and thiouracil injections.

EXPERIMENTAL ANIMALS AND METHODS

The data were collected from lactating Holsteins and Jerseys, two of each breed. All animals were subjected to various diurnally variable temperature rhythms in the animal psychroenergetic laboratory as given in Table 1.

TABLE 1--TEMPERATURE SCHEDULE FOR COWS

Diurnal* Temp. Range °F.	Average Temp. °F.	Average Relative Humidity %	Date (1954)	
			From 3 p.m.	To 3 p.m.
40.2- 70.7	55	56	Mar. 18	Apr. 22
62.1-108.0	83	51	Apr. 22	May 7
**	63	57	May 7	May 13
69.3-100.7	84	52	May 13	June 3

* Average daily minimum and maximum.

** Constant temperature.

Details of the psychroenergetic laboratory have been described by McCalmont (1946) and Ragsdale *et al.* (1948). Briefly, the laboratory consists of two insulated chambers, 26 x 18 x 9 feet. These two chambers are located within an insulated 40 x 60 foot steel frame structure covered with galvanized iron. The temperature, humidity, air movement, light, and ventilation rate of each chamber can be varied independently.

The diurnal temperature rhythm patterns adopted in this experiment were such as to imitate some of the natural diurnal variations in different seasons of the year. The minimum temperatures in each experimental cycle occurred between 5 and 7 a.m. and the maximum between 3 and 4 p.m. Throughout the experiment the air velocity in the chamber was maintained at about 0.5 m.p.h. Between changes of diurnal temperature rhythms an adjustment period at a constant temperature of about 60° F was allowed or the temperature was changed gradually within a few days. Other details of the temperature cycles used in the experiment have been described by Brody *et al.* (1955).

The cows were fed alfalfa hay *ad libitum*, and a grain mixture consisting of corn, oats, bran, soybean oil meal, cod liver oil supplement, steamed bone meal, and salt. The grain mixture was fed at the rate of 1 pound for each 3 pounds of milk production for Jerseys and for each 4 pounds for Holsteins. In addition, each cow received 2 pounds of beet pulp daily. Water was available to each cow in an individual cup.

Table 2 gives additional details concerning the individual cows.

Jugular venous blood was collected and citrated in opaque bottles to prevent harmful effects from exposure to light. It was immediately taken to the laboratory. Aliquots then were removed for ascorbic acid determinations and the remainder of the samples were transferred to tubes for storage under toluene at -10° F until analyzed for the other vitamins. The samples were drawn during each tempera-

TABLE 2--EXPERIMENTAL COWS*

Cow No.	Birth Date	Date of Last Calving	Number of Previous Lactations	Date of Last Breeding	Beginning of Expt.		Ave. During Feb. 1954	
					Approx. Age Years	Approx. Body Wt. Lbs.	Milk Lbs/Day	B.f. %
<u>Jersey</u>								
274	Oct. 25, 1948	Aug. 19, 1953	2	Apr. 19, 1954	5	846	16.7	6.0
564	Jan. 16, 1950	Sep. 20, 1953	1	Nov. 30, 1953	4	860	14.6	5.9
<u>Holstein</u>								
144	June 11, 1945	Sep. 3, 1953	5	June 14, 1954	8½	1288	36.3	3.5
178	Dec. 12, 1946	Apr. 1, 1953	3	----	8	1297	29.1	3.7

* Data taken from University of Missouri Agriculture Experiment Station Research Bulletin 578.

J-564 -- Aborted five month fetus May 7 and consequently was removed from the experiment on May 24.

ture range, after the cows had a few days to adjust to that environment. Specific collection dates are given in the tables.

Both morning and evening milk samples were collected separately in half pint opaque bottles. The milking hours were 5 a.m. and 3 p.m. The samples were stored in an ice bath until removed to the laboratory where they were analyzed for ascorbic acid and transferred to tubes for storage at -10° F.

Vitamin Assay Methods for Whole Blood

Ascorbic Acid: Total ascorbic acid, both the reduced and oxidized forms, was determined by the method of Roe and Kuether, as described by Gyorgy (1951). Briefly, the blood was deproteinized with trichloroacetic acid and the extract shaken with norit. The norit filtrate was treated with 2,4, dinitrophenylhydrazine, and thiourea and incubated for three hours at 37° C. Eighty-five percent sulfuric acid was then added. After one-half hour the tubes were read in a photoelectric colorimeter at 540 mu. against suitable blanks prepared by adding the reagent, 2, 4, dinitrophenylhydrazine, to the aliquot samples after, instead of before, incubation. Final calculations were made with the help of a standard calibration curve prepared by the same procedure from various levels of standard vitamin solution.

Thiamine: Thiamine was determined by the method of Friedman and Kmiecik for blood, as described by Gyorgy (1951). However, instead of 5 ml. samples as prescribed in their method, 10 ml. were taken and all reagents were increased proportionally. Briefly, the bound thiamine in the blood was hydrolyzed by treatment with the enzyme takadiastase at pH 4.5 to 5.0 and incubation for 2 hours at 40° C. The samples were further acidified and heated in a boiling water bath for 10 minutes. The cooled samples were treated with metaphosphoric acid, centrifuged and filtered. The filtrate, after adjustment of pH to about 3.5, was passed through suitably prepared Decalso columns. The columns were washed and eluted with acidified 25 percent potassium chloride solution. Thiamine in the elute was oxidized to thiochrome by an oxidizing reagent consisting of nine parts of 10 N sodium hydroxide and one part of 1 percent potassium ferricyanide solution.

Thiochrome was extracted by shaking with isobutyl alcohol and then centrifuging. The extracted samples, after treatment with sodium sulfate, were read immediately for fluorescence against suitable blanks prepared by addition of sodium hydroxide instead of the oxidizing reagent to the eluted aliquots. The amounts were calculated from standard curves prepared by the addition of different amounts of thiamine to blood samples which were analyzed in parallel with experimental samples.

Niacin, Pantothenic acid and Riboflavin: These vitamins were determined microbiologically using *Lactobacillus arabinosus* and the media described by Flynn *et al.* (1951) for niacin and pantothenic acid, and *Lactobacillus casei* and the media recommended by the Association of Vitamin Chemists (1951) with slight modifications for riboflavin. For extraction, blood samples laked with sufficient sodium

acetate buffer were digested with a combination of enzymes, mylase P and papain, under toluene, for 24 hours at 37° C. They were then autoclaved for 15 minutes, cooled, made to known volume and filtered. The pH of the known amount of filtrate was adjusted to about 6.8 and then diluted 1:10 for both pantothenic acid and riboflavin and 1:100 for niacin. Six levels of each diluted sample, four of them in duplicate, were added to culture tubes, sterilized, inoculated and incubated for 72 hours, prior to titration for acidity. The amounts were calculated by interpolation from a standard curve prepared from data obtained by the analysis of eight duplicated levels of standard vitamin solution.

For riboflavin, the tubes containing over 2.5 ml. of the final sample generally gave inconsistent and lower results. Readings and calculations for riboflavin were therefore made from tubes containing lower levels. It should be mentioned that Strong *et al.* (1941) also encountered some inhibiting factor in tubes containing larger volumes of blood.

All samples of cows' blood were run in duplicate.

Vitamin Assay Methods for Milk

Ascorbic Acid: Only the reduced form of ascorbic acid was determined in freshly drawn milk samples. The proteins in milk were precipitated by a combination of metaphosphoric acid and trichloroacetic acid. An aliquot from the filtered and suitably diluted extract was titrated against a solution of 2,6, dichlorophenolindophenol (standardized against a standard solution of ascorbic acid in metaphosphoric acid) solution.

Niacin, Pantothenic Acid and Riboflavin: Procedures used for determination of these vitamins in milk was essentially the same as described for blood, except that a combination of papain and takadiastase was used for extraction of niacin and riboflavin.

Only four levels, 1, 2, 3, and 4 ml. of the final diluted sample (all in duplicate) were added to the culture tubes. According to the estimated amounts of vitamins in milk, the final dilutions made were different from those used for blood.

All milk samples were run in duplicate.

RESULTS AND DISCUSSION

The ascorbic acid content of evening milk from both the Holstein and Jersey cows increased under the influence of both high temperature cycles (Table 3). The ascorbic acid values for milk agree favorably with those given by Kon and Henry (1951 and 1954); however, it is possible that the high values in the 70 to 100° F temperature cycle and the gradual increase in ascorbic acid throughout the experimental period may be the result of a summation effect of environmental temperature and advancing stage of lactation.

TABLE 3--ASCORBIC ACID IN MILK (mg. per 100 ml.) (P. M. SAMPLES ONLY)

Environmental Temperature	40-70° F.			60-100° F.			70-100° F.			
	Apr. 5	Apr. 6	Apr. 14	Apr. 15	May 5	May 6	May 17	May 18	May 19	May 20
Cow No.	H. 144	H. 178	J. 274	J. 564	Holstein Averages	Jersey Averages				
Sampling Date	2.0	2.1	2.1	2.2	3.0	2.3	2.7	2.9	2.9	2.9
	2.1	1.6	1.8	2.0	2.0	2.2	2.3	2.5	2.5	2.5
	1.2	1.7	1.5	1.8	1.8	2.2	2.4	2.2	2.2	2.5
	1.2	1.5	1.5	1.8	1.7	2.1	1.8	2.0	2.0	1.9
	2.1	1.9	2.0	2.1	2.5	2.3	2.5	2.7	2.7	2.7
	1.2	1.6	1.5	1.8	1.8	2.2	2.1	2.3	2.1	2.2

Table 4 presents the results of ascorbic acid determinations on blood from these cows during the 40 to 70° F and 70 to 100° F temperature cycles. All cows showed higher ascorbic acid levels in their blood during the high temperature cycle; statistically this difference was significant at the 1 percent level. These results vary from those obtained in trials where the animals were exposed only to constant environmental temperatures (Blincoe *et al.* 1951). Blincoe and associates observed a consistent decrease in the ascorbic acid content of the blood of cows subjected to high temperatures, particularly above 80° F. The blood ascorbic acid values for the cows exposed to the 70 to 100° F temperature cycle are within the range obtained by Blincoe under thermoneutral conditions; the values at the 40° to 70° F range are somewhat lower.

TABLE 4--ASCORBIC ACID IN COWS' BLOOD (mg. per 100 ml.)

Environmental Temperature	40-70° F.			70-100° F.	
	Apr. 5	Apr. 6	Apr. 15	May 27	Jun. 3
Cow No.					
H. 144	0.26	0.48	0.37	0.60	0.73
H. 178	0.52	0.30	0.62	0.63	0.73
J. 274	0.30	0.41	0.45	0.72	0.65
J. 564	0.52	0.23	0.30	---	---
Holstein Average	0.39	0.39	0.49	0.62	0.73
Jersey Average	0.41	0.32	0.38	---	---

Niacin (Table 5) and Riboflavin (Table 7) values for the morning milk of Holstein cows were markedly higher in both high temperature cycles than those in the low temperature cycle of 40 to 70° F. While the riboflavin content of their evening milk also showed similar trends, the influence of high environmental temperatures on the niacin content of evening milk was less marked and more irregular. There was no appreciable effect of high temperature conditions on the concentrations of these vitamins in the Jersey milk.

The difference between the influence of environmental temperatures on the niacin content of morning and evening Holstein milk may be explained by the fact that the maximum temperature in the diurnal rhythms occurred between three and four p.m. and the maximum rectal temperatures of Holstein and Jersey cows were observed after 1 to 2 and 2 to 3 hours lag, respectively (Kibler and Brody, 1956). Since the evening milking was done at three p.m., the influence of high temperature stress would probably be more marked on milk drawn in the morning.

In general, it appears that there is an inverse relationship between the concentration of these vitamins—niacin and riboflavin—and the daily milk production. Thus, for Holstein cows, while the milk production was highest in the

TABLE 5--NIACIN IN MILK (ug. per 100 ml.)

Environmental Temperature	COW No.		Sampling Date	Cow No.		Cow No.		Cow No.		Cow No.	
40-70° F.	H. 144	A.M.	Apr. 5 and 6	29	56	31	33	33	35	40	39
			Apr. 6 and 7	56	31	33	35	40	39	33	35
60-100° F.	H. 178	A.M.	Apr. 14 and 15	41	60	31	45	45	52	52	47
			Apr. 15 and 16	41	60	31	45	45	52	52	47
70-100° F.	J. 274	A.M.	May 5 and 6	31	32	37	38	32	39	42	44
			May 6 and 7	31	32	37	38	32	39	42	44
40-70° F.	J. 564	A.M.	May 17 and 18	54	68	47	68	47	47	47	55
			May 18 and 19	54	68	47	68	47	47	47	55
40-70° F.	Holstein	A.M.	May 19 and 20	35	58	31	39	39	42	52	60
			May 20 and 21	35	58	31	39	39	42	52	60
40-70° F.	Jersey	A.M.	May 27 and 28	43	65	37	37	42	46	46	44
			May 28 and 29	43	65	37	37	42	46	46	44
40-70° F.	Averages	P.M.	Apr. 5 and 6	38	37	37	38	32	39	42	44
			Apr. 6 and 7	38	37	37	38	32	39	42	44
40-70° F.	Averages	A.M.	Apr. 5 and 6	43	65	35	35	42	46	46	44
			Apr. 6 and 7	43	65	35	35	42	46	46	44

temperature cycle 40 to 70° F, lowest in 60 to 110° F and intermediate in 70 to 100° F, the niacin content of their milk in general was highest in the 60 to 110° F temperature cycle, lowest in 40 to 70° F and intermediate in the 70 to 100° F temperature rhythm. Jerseys did not show an appreciable change in their daily milk yield with change of environmental temperature. There was no significant difference in the niacin content of their milk in the different temperature cycles.

The inverse relationship between the daily milk and its riboflavin content has been observed by several workers (Johnson *et al.* 1941; Holmes *et al.*, 1944; Theophilus and Stamberg, 1945). In general, the trends observed for the riboflavin content of milk with change of environmental temperatures are also in accordance with this relationship.

From Table 6 it seems apparent that the niacin blood levels of the Holstein cows were depressed under the influence of diurnally variable high temperatures, particularly the 60 to 110° F range. However, no such effect was evident for the Jersey cows.

The influence of environmental temperature on the riboflavin content of blood (Table 8) seems to be quite regular and marked for all cows. There are no breed differences since all of the cows have similar values in each temperature range and show similar trends with change of temperatures. The riboflavin levels of their blood are highest in the low temperature cycle of 40 to 70° F, lowest in the high temperature cycle of 60 to 110° F, and intermediate in the temperature cycle of 70 to 100° F. The differences in blood riboflavin values between temperature cycles are statistically significant.

The blood riboflavin levels of cows reported here are somewhat lower compared to those given by Albritton (1952), but are higher than those reported by Smith and Allen (1954) for young calves.

The effect of high environmental temperatures on the pantothenic acid content of milk is variable between individual cows (Table 9). The decrease, if any, was more marked under the influence of the 60 to 110° F temperature cycle than the 70 to 100° F cycle.

The significance of the opposite trend observed for pantothenic acid, compared with that for niacin and riboflavin, which in general showed an increase under the influence of high environmental temperature, is obscure. It may be noted that the effect of 60 to 110° F high temperature cycle on the pantothenic acid levels of the blood of these cows (Table 10) was, in general, opposite to that observed for niacin and riboflavin. Could the explanation be that under the influence of very high temperatures, the requirements for pantothenic acid are reduced and, consequently, its uptake by the body tissues, including the mammary gland, is reduced, with the result that there is an increase in its concentration in blood and a decrease in milk?

Except for riboflavin, no definite diurnal variations were observed for the vitamins. Evening milk of both Holstein and Jersey cows was richer in riboflavin than the morning milk. This contradicts the findings of Theophilus and Stamberg (1945) who failed to observe such variations for the riboflavin content of milk.

TABLE 6--NIACIN IN COWS' BLOOD (ug. per 100 ml.)

Environmental Temperature	40-70° F.			60-110° F.		70-100° F.			
Sampling Date	Apr. 5	Apr. 6	Apr. 15	May 5	May 6	May 17	May 20	May 27	Jun. 3
Cow No.									
H. 144	647	574	488	402	402	380	394	442	461
H. 178	639	589	350	358	369	563	338	542	525
J. 274	600	682	338	574	539	544	495	636	594
J. 564	485	508	375	489	472	256	324	---	---
Holstein Average	643	582	419	380	385	472	366	492	493
Jersey Average	543	595	356	531	505	400	410	---	---

TABLE 7--RIBOFLAVIN IN MILK (ug. per 100 ml.)

Environmental Temperature		40-70° F.				60-110° F.		70-100° F.			
Cow No.	Sampling Date	Apr. 5 and 6	Apr. 6 and 7	Apr. 14 and 15	Apr. 15 and 16	May 5 and 6	May 6 and 7	May 17 and 18	May 18 and 19	May 19 and 20	May 27 and 28
	H. 144	A.M.	144	138	136	134	163	151	148	163	159
P.M.		178	189	201	203	208	210	194	239	188	212
H. 178	A.M.	157	117	150	140	147	170	178	171	144	166
	P.M.	203	199	190	200	206	228	205	235	321	191
J. 274	A.M.	171	204	217	230	176	215	181	206	170	159
	P.M.	238	263	267	237	229	271	262	267	202	214
J. 564	A.M.	163	175	173	---	171	170	172	188	---	---
	P.M.	215	252	259	243	203	236	246	254	202	---
Holstein Average	A.M.	151	128	143	137	155	161	163	167	152	164
	P.M.	191	194	196	202	207	219	200	237	210	202
Jersey Average	A.M.	167	190	195	---	174	192	177	197	---	---
	P.M.	227	257	263	240	216	254	254	261	202	---

TABLE 8--RIBOFLAVIN IN COWS' BLOOD (ug. per 100 ml.)

Environmental Temperature	40-70° F.			60-110° F.		70-100° F.			
	Apr. 5	Apr. 6	Apr. 15	May 5	May 6	May 17	May 20	May 27	Jun. 3
Cow No.									
H. 144	38	35	36	23	21	24	24	26	26
H. 178	36	39	40	22	21	27	22	29	28
J. 274	32	38	48	27	19	26	26	30	28
J. 564	49	35	27	21	15	28	22	--	--
Holstein Average	37	37	38	22	21	26	23	28	27
Jersey Average	41	37	38	24	17	27	24	--	--

TABLE 9--PANTOTHENIC ACID IN MILK (ug. per 100 ml.)

Environmental Temperature		40-70° F.				60-110° F.		70-100° F.			
Cow No.	Sampling Date	Apr. 5 and 6	Apr. 6 and 7	Apr. 14 and 15	Apr. 15 and 16	May 5 and 6	May 6 and 7	May 17 and 18	May 18 and 19	May 19 and 20	May 27 and 28
	H. 144	A. M.	485	551	476	445	436	362	540	538	531
P. M.		514	473	491	440	348	352	502	503	490	496
H. 178	A. M.	225	318	280	247	268	253	230	269	285	250
	P. M.	320	292	349	382	200	190	281	265	283	257
J. 274	A. M.	410	499	516	438	360	335	365	387	376	320
	P. M.	435	420	423	423	278	268	388	391	363	272
J. 564	A. M.	365	417	456	520	412	340	480	495	515	---
	P. M.	410	412	354	420	368	294	548	574	547	---
Holstein Average	A. M.	355	435	378	346	352	258	385	404	408	390
	P. M.	417	388	420	411	274	271	392	384	387	377
Jersey Average	A. M.	388	458	486	479	386	338	423	441	446	---
	P. M.	423	416	389	422	323	281	468	483	455	---

TABLE 10--PANTOTHENIC ACID IN COWS' BLOOD (ug. per 100 ml.)

Environmental Temperature	40-70° F.			60-110° F.		70-100° F.			
Sampling Date	Apr. 5	Apr. 6	Apr. 15	May 5	May 6	May 17	May 20	May 27	Jun. 3
Cow No.									
H. 144	48	45	37	43	40	23	23	25	23
H. 178	12	11	13	31	30	14	14	16	15
J. 274	12	14	11	29	27	14	15	18	16
J. 564	26	26	20	20	20	11	15	--	--
Holstein Average	30	28	25	37	35	19	19	21	19
Jersey Average	19	20	16	25	24	13	15	--	--

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