RESEARCH BULLETIN 639 SEPTEMBER, 1957

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
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Influence of Temperature on Vitamin Levels in Bovine Blood

A Study of B-Vitamins and Vitamin C Levels in the Blood of Brahman, Santa Gertrudis and Shorthorn Heifers Reared Under Different Environmental Temperature Conditions.

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(Publication authorized September 25, 1957)

COLUMBIA, MISSOURI

SUMMARY

Comparison of the vitamin levels in blood samples taken from Brahman, Santa Gertrudis, and Shorthorn heifers reared under different environmental temperature conditions showed no difference in blood ascorbic acid levels of Shorthorn calves. Brahman and Santa Gertrudis calves reared at 50 and 80° F in the climatic laboratory had lower blood ascorbic acid levels than their controls, which were housed in an open shed.

There was no noticeable effect of environmental temperatures on the pantothenic acid and riboflavin levels in the blood from calves of the three breeds.

There was no definite relation between the blood vitamin levels of calves and their age or season of the year except that all calves showed maximum riboflavin values during July, August, and September.

The Shorthorn calves kept at 80° F had distinctly lower blood levels of niacin and thiamine than those reared at 50° F. Their values were also lower than those for Brahman and Santa Gertrudis calves maintained at the same temperature, 80° F.

ACKNOWLEDGMENTS

Acknowledgments are made to all persons associated with the Environmental Physiology project under the late Dr. S. Brody and especially to A. C. Ragsdale, chairman, Department of Dairy Husbandry, for administrative direction; and to R. G. Yeck for temperature and humidity control. This bulletin reports on Department of Dairy Husbandry Research Project 246, Ruminant Bacteriology.

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INTRODUCTION

Improved western breeds of cattle show a poor heat tolerance, unthriftiness, and low production when imported into tropical regions. The Zebu and Brahmans, which are relatively poor milk producers, show a better heat tolerance. In as much as the environmental temperature can influence the total energy metabolism of cattle, it also might be expected to alter the requirements for the catalytic agents, B and C vitamins, and so, presumably, their levels in blood. High environmental temperature may modify the activity of the organs of digestion, assimilation or excretion, or the activity and function of endocrines, all of which may exert some influence on the requirements for some of the vitamins.

Vitamin Biosynthesis and Requirements of Cattle

Experimental evidence for the rumen microbial synthesis of individual B-Vitamins is based on the following observations:

1. Ability of ruminants to live normally on synthetic diets that are extremely low in these vitamins (Theiler *et al.*, 1915; Bechdel *et al.*, 1926) and ineffectiveness of the vitamins when administered to ruminants (Eckles *et al.*, 1924 and 1925; Marsh *et al.*, 1947).

2. Comparatively high concentrations of the vitamins in the rumen ingesta of animals when they were fed synthetic or natural diets that were poor in vitamins (McElory *et al.*, 1939; 1940a, b and c; 1941a and b; Wegner *et al.*, 1940; Hunt *et al.*, 1941 and 1943; Lardinois *et al.*, 1944; and Agarwala *et al.*, 1953).

3. Increased output of the vitamins in urine, feces, and milk as compared to the intake (McElory *et al.*, 1940a and b; 1941a and b; Winegar *et al.*, 1940; Johnson *et al.*, 1941; Lindahl *et al.*, 1951; and Pearson *et al.*, 1953).

4. *In vitro* synthesis of the vitamins by the rumen microflora (Gall *et al.,* 1951; Howie *et al.,* 1951; Huhtanen *et al.,* 1953a and b; and Hunt *et al.,* 1954).

Many different types of rumen organisms have been isolated, identified and shown to synthesize and require varying numbers of B-vitamins. The synthesis of vitamins in the rumen is thus more a symbiotic activity of the microflora rather than a specific function of any particular type of microorganism.

5. Inability of the young animals, before the development of the rumen function, to subsist normally without the external supply of the individual B-vitamins (Wiese *et al.,* 1946 and 1947a; Johnson *et al.,* 1947a, 1948, 1950, and 1951; Brisson *et al.,* 1951; Draper *et al.,* 1952a, b, and c; and Lassiter *et al.,* 1953).

Characteristic symptoms of deficiencies of biotin, choline, folic acid, niacin, pantothenic acid, riboflavin, thiamine, vitamins B_6 and B_{12}, which are curable by administration of the deficient vitamin, have been produced experimentally in young non-ruminating calves. But niacin deficiency could only be produced when tryptophane was omitted from the diet (Hopper *et al.,* 1954). This, along with the experiments of other workers (Johnson *et al.,* 1947b; and Esh *et al.,* 1948), indicates that the calf possesses the ability to synthesize niacin in its body from dietary tryptophane. The production of such deficiency symptoms also provides evidence that cattle do require these B-vitamins for their metabolic processes.

Though cattle do not require dietary vitamin C, there is no evidence of its synthesis in the rumen. Evidently, it is synthesized in the body because young calves, if fed colostrum for the first 48 hours, can live and maintain normal plasma ascorbic acid levels on a synthetic diet that excludes vitamin C (Wiese *et al.,* 1947b).

Almost all the work reported on vitamin synthesis has been qualitative in nature. Although some attempts have been made to determine the requirements of riboflavin and vitamin B_{12} by young calves, the data are limited and conflicting (Brisson *et al.,* 1951; Draper *et al.,* 1952a; and Lassiter *et al.,* 1953). It has been found that ruminal synthesis of vitamins can occur in young calves at a very early age, particularly when they are given access to roughage; cud inoculations also have been found useful in this respect (Kesler *et al.,* 1951a and b and 1952; Brisson *et al.,* 1951; and Conard *et al.,* 1954). In view of the possibility of such synthesis, an agreement as to the quantitative requirements by the calves is perhaps hardly to be expected.

In animals with a functioning rumen, it is generally assumed that since no visible deficiencies of any of these vitamins occur they are pro-

duced in sufficient quantities to meet the essential body demands. However, there is the possibility that on certain types of rations, these vitamins may not be produced in sufficient amounts to meet the optimum requirements. Some workers have shown that the microflora of the rumen changes with the diet (Gall *et al.,* 1949 and 1951b; Huhtanen *et al.,* 1953a and b). It is therefore reasonable to expect corresponding variations in the vitamin synthesis. The rumen synthesis of certain B-vitamins has been shown experimentally to be affected by the level of readily fermentable carbohydrates, the relative amount of nitrogen, and presence of certain minerals in the ration, and the quality of roughage (Wegner *et al.,* 1941; Hunt *et al.,* 1943 and 1954; Lardinois *et al.,* 1944; Terri *et al.,* 1951a and b and 1953; Kon *et al.,* 1953; Pearson *et al.,* 1953 and Hollis *et al.,* 1954).

The nutritional role of the B-vitamins present in grass and other feeds is not at all clear and needs investigation.

Certain feeds have been shown to contain some vitamin antagonists. Braken, which is known to cause poisoning among cattle, has been found to contain an antithiamine substance (Weswig *et al.,* 1946; Roberts *et al.,* 1949; and Evans *et al.,* 1949a and b). Though the mode of its action in ruminants is not clear, it is possible that the antithiamine factor may alter the metabolism of rumen microorganisms, which, in turn, may lead to deficiencies of essential nutrients for the animal (Kon *et al.,* 1954a).

Antibiotics do not seem to have a significant effect on the synthesis of B-vitamins by the ruminants (Teeri *et al.,* 1950; Chance *et al.,* 1953; Kesler, 1954; Russoff *et al.,* 1954; Smith *et al.,* 1954; and Bohman *et al.,* 1955).

Vitamin Levels in Blood

Data on the value of B-vitamins in bovine blood is quite limited in the literature, although a comparatively large number of studies have been made on the blood and plasma ascorbic acid levels. Albriton (1952) gives the following vitamin values for cows' blood:

Vitamin	Whole Blood Values per 100 ml. Mean	Range
1. Ascorbic acid	0.5 mg.	0.2 - 1.5 mg.
2. Nicotinic acid	0.3 mg.	No values given
3. Riboflavin	45.0 ug.	40 - 50 ug.
4. Thiamine	8.0 ug.	5 - 11 ug.

No values are given for pantothenic acid in cows' blood and except for vitamin C no values are listed for any of the above vitamins in blood

plasma. The mean value quoted for vitamin C in plasma is 0.5 mg. and the range is from 0.2 to 1.5 mg. per 100 ml. of plasma.

Factors Influencing Ascorbic Acid Level in Blood

1. *Feed*—Since cattle very likely meet their requirements for vitamin C by its synthesis in their body and since it is also known that vitamin C is very quickly destroyed in the rumen, feed may not exert any influence on its concentration in blood. Thus, Knight *et al.,* (1941) and Vavich *et al.,* (1945) could not find an effect on blood ascorbic acid values from the administration of large doses of pure ascorbic acid to cows. Wallis (1943) observed that the blood plasma ascorbic acid values were maintained at the normal level in spite of feeding cows a long time on diets that were poor in ascorbic. Although Wallis (1943) did obtain some variations between the four groups of cows fed different diets, these variations bore no relationship to the amount of vitamin C in the diet.

Hibbs *et al.,* (1949) reported that when calves, approximately 71 days old, were turned out on pasture a temporary increase was noticed in their blood plasma ascorbic acid. The same workers (Hibbs *et al.,* 1948) showed that rumen inoculations of young calves with cud material from cows were effective in preventing the normal drop in blood plasma ascorbic acid levels observed between the seventh and fourteenth day of age, when only alfalfa hay and milk were fed; however, the inoculation was ineffective when a grain mixture was included in the ration.

A number of workers investigated the effect of administering vitamins to young calves on their performance and blood ascorbic acid levels. Thus, Lundquist *et al.,* (1943) reported that calves, though born with adequate ascorbic acid in the blood stream, soon exhausted the supply. They observed that when the calves were fed on skim milk from birth, it was possible to maintain higher levels of vitamin C in their plasma by oral administration of ascorbic acid for the first 10 days of life; thereafter, it had to be injected to be effectively recovered in the blood stream. But Sutton *et al.,* (1946) reported that feeding colostrum over an extended period or feeding vitamin capsules or a combination of the two did not cause an appreciable effect on the ascorbic acid levels in the blood of young calves. Hibbs *et al.,* (1947), like Sutton, did not observe a significant effect of feeding vitamin capsules on the plasma ascorbic acid levels of calves that were allowed to remain on their dams for at least three days and then pail fed on whole milk. Wiese *et al.,* (1947b) found that young calves which were allowed to receive colostrum for the first two days could maintain their plasma ascorbic acid levels at normal on a synthetic diet deprived of Vitamin C.

2. *Relation to Blood Vitamin A Levels*—Boyer *et al.,* (1942) found that with a reduction of vitamin A intake by calves, apart from the decline in their plasma vitamin A levels, a reduction also occurred in the plasma ascorbic acid levels. Hansard *et al.,* (1941), Moore, *et al.,* (1945, 1946, and 1948) and Madsen *et al.,* (1947) reported similar findings.

However, Jensen, *et al.,* (1942) did not observe an influence on the blood ascorbic acid content of cattle from feeding massive doses of vitamin A. Eaton *et al.,* (1952) demonstrated that ascorbic acid levels of calves did not decrease in early uncomplicated A-hypovitaminosis. Administration of vitamin A to severely depleted animals failed to raise the blood ascorbic acid levels. Similarly, Rousseau *et al.,* (1954) found no change in plasma ascorbic acid values of calves fed a low level vitamin A diet. These studies suggest that the influencing factor in the decrease of blood ascorbic acid levels in vitamin A depleted animals may not be due to lack of vitamin A per se; but that some other accompanying mechanism, such as plane of nutrition, may be the direct regulating factor.

3. *Administration of Chlorobutanol*—An unusual factor, oral administration of chlorobutanol, has been shown to significantly increase the blood ascorbic acid levels in cattle (Bortree *et al.,* 1943; Lundquist *et al.,* 1944 and 1945; Moore *et al.,* 1945 and 1946; Scheidenhelm *et al.,* 1942; and Christian *et al.,* 1951). The mechanism of its action is not known.

Lundquist *et al.* (1944) reported that sulfathiazole injections caused an increase in blood plasma level of ascorbic acid in young calves; but when given orally it did not have an influence.

4. *Breed*—Phillips *et al.,* (1941) reported breed differences in the ascorbic acid content of the blood plasma of cows. They found average values of 0.35, 0.48, 0.453 and 0.53 mg. per 100 ml. of blood plasma for Holstein, Guernsey, Jersey and Brown Swiss cows, respectively. The data of Blincoe *et al.,* (1951) indicate that Brahmans may have slightly higher values than the Holstein, Jersey, and Brown Swiss cattle. Christian *et al.,* (1951) did not find a difference between Holstein and Guernsey cows. The results of several other workers (Bortree *et al.,* 1942; Teeri *et al.,* 1946; Long *et al.,* 1952) also show that there may not be any significant breed differences.

5. *Age*—Bortree *et al.,* (1942) observed that the ascorbic acid content of the blood of young calves tended to be lower than that of heifers, and that there was a tendency for the vitamin level to increase as the animal reached maturity. Teeri *et al.,* (1946), who determined the plasma ascorbic acid levels of calves from birth to 23 weeks of age, did not find a difference with age. Blincoe *et al.,* (1951) reported that Brahman and Brown Swiss yearling heifers had significantly higher blood ascorbic acid levels than cows.

Calves neonatally have high concentrations of ascorbic acid in the blood; these concentrations drop rapidly to about normal levels within 24 hours after birth (Lundquist *et al.,* 1943; Sutton *et al.,* 1946; and Hibbs *et al.,* 1947). Lundquist *et al.,* (1943) reported that after the initial rapid drop during the first 24 hours, the blood plasma ascorbic acid level continued to drop at a slower rate, reaching the lowest point at about two weeks of age; then it rose slowly again and leveled off close to 0.3 mg. per 100 ml. of plasma.

6. *Pregnancy and Heat Period*—Bortree *et al.,* (1942) reported that there was no significant difference between the mean values or range of plasma ascorbic acid levels of pregnant cows and those of open cows, although the ascorbic acid values had some tendency to be higher in the blood of pregnant cows.

Phillips *et al.,* (1941) reported that there was a higher concentration of ascorbic acid in the plasma of cows in mid to late estrum than in the diestrum. But Christian *et al.,* (1951) found no such difference. Moreover, administration of estrogen and gonadotropin hormones, both of which are thought to be higher during and immediately after the heat period, have been reported to decrease plasma ascorbic acid levels (Andrews *et al.,* 1942; Erb *et al.,* 1942; and Lardy *et al.,* 1944).

Perhaps, most of the reports regarding the factors influencing the vitamin C content of blood can be best summarized by making a reference to the observations of Bortree *et al.,* (1942). They observed not only great variations in the amount of ascorbic acid in the blood of individual animals when the samples were taken at the same hour each day, but also large fluctuations between samples drawn at three-hour intervals during a 24-hour period. These fluctuations could not be correlated with the normal period of feeding. These workers concluded that random ascorbic acid determinations are of little value in determining its normal concentration in the blood of dairy animals.

Almost all other workers seem to have encountered similar variations, both from animal to animal and from sample to sample. In view of such variations, the interpretations of many results reported in the literature have become of doubtful significance.

Factors Influencing Blood Levels of B-Vitamins

Little work has been done to investigate the causes that can possibly influence levels of B-vitamins in the blood of cattle.

Smith *et al.,* (1954) studied the influence of feeding aureomycin on the blood levels of vitamin B_1, niacin, pantothenic acid, riboflavin and thiamine in young calves (from 4 days to 12 weeks of age). Aureomycin

feeding had no significant influence on the concentrations of these vitamins in blood. No significant difference was observed between the male and female calves. The authors did find some trends with age. The blood levels of niacin, pantothenic acid, thiamine and vitamin B_{12} were high after birth and declined significantly during the first week. Riboflavin did not decline significantly during the first week, but between the fourth and eighth weeks the drop in blood riboflavin levels was quite significant. The ranges of mean values obtained by these workers at different periods of age from 4 to 12 weeks were: for niacin, 15.1 to 6.1 ug. per ml. of blood; pantothenic acid, 1.61 to 0.60; riboflavin, 0.28 to 0.15; and thiamine 0.057 to 0.086.

The results of Teeri *et al.,* (1946) who determined the nicotinic acid levels of calves' blood from birth to 23 weeks of age also showed that the average levels were slightly higher during the first week than at a later age. Their data showed considerable variation, the figures ranging from 0.18 to 1.48 mg. per 100 ml. of blood; however, the mean values for different age groups were fairly constant, ranging from 0.79 to 0.98 mg. per 100 ml. blood.

Evans *et al.* (1949b) found that the thiamine content of blood of cattle affected with bracken poisoning was lower, 3.8 to 6.5 ug. per 100 ml. compared to the normal levels of 8 to 12 ug. per 100 ml. of blood.

Sahashi *et al.,* (1953) estimated vitamin B_1 and B_{12} in the blood of two-year-old twin heifers, one of which was made to carry a load of 20 kg. for 4½ hours on a tread mill, while the other remained at rest. During the period of exertion there was a decrease in both vitamins in the blood. These observations provide additional evidence that these vitamins have some definite role in the energy metabolism of cattle.

Effect of Thermal Stress on Metabolism of Ascorbic Acid

Heat Stress: Most of the experiments on the effect of heat stress on ascorbic acid metabolism have been concerned with the influence of artificially induced fever on urinary excretion of the acid and its concentrations in the blood and tissues of laboratory animals and human subjects.

In addition, some studies have been made on the effect of environmental temperature and season on ascorbic acid levels in the blood and tissues of cattle.

Zook *et al.,* (1938) analyzed the concentrations of vitamin C in tissues—adrenals and kidneys—of guinea pigs which were subjected to induced fever for two to six hours daily and fed a scorbutic diet. They found that the vitamin C stores were depleted faster in animals with fever than in the controls. The same workers found that the 24-hour

urinary excretion of human subjects was lower on the day of induced fever than on the day before. However, no difference was observed in the blood concentrations of vitamin C before and after treatment. Parvis (1941) also reported that the ascorbic acid content of the organs of guinea pigs that had been maintained at 40 to 43° C for some hours was considerably reduced as compared with those to untreated animals. Irwin *et al.,* (1950) observed that when young rats were subjected to heat stress obtained by the use of implanted thermopiles or by the administration of ergotoxine, there was a significant depletion of the ascorbic acid content of the adrenals.

Daum *et al.,* (1939) reported that both the level of ascorbic acid in blood plasma and its excretion in urine were lower in a group of patients after they had received electrically induced fever therapy. But, Osborne *et al.,* (1942), could detect no significant changes in the ascorbic acid concentration of blood of arthritic patients as a result of artificially elevating their body temperature to 104° F and maintaining it at that level for four hours. These results are in agreement with those of Zook *et al.,* (1938) as far as blood concentrations of ascorbic acid are concerned. Similarly Craig *et al.,* (1946) were not able to demonstrate any effect on the blood ascorbic acid level of patients with artificially induced fever.

Muira *et al.,* (1951) reported that when two human subjects were kept in a chamber at 37° C and relative humidity of 85 percent for three hours, the urinary excretion of vitamin C diminished, but rose again following a six-hour lag after the subjects had returned to normal temperature. A number of recent studies indicate that the concentrations in sweat of both ascorbic acid and dehydroascorbic acid are negligible with values ranging from 0 to 150 ug. per 100 ml.; in these concentrations the daily loss of ascorbic acid in sweat of men under heat stress is considered to be insignificant (Robinson *et al.,* 1954).

Blincoe *et al.,* (1951) determined the blood ascorbic acid levels of Holstein, Jersey, Brahman and Brown Swiss cows and Brown Swiss and Brahman heifers that were subjected to controlled temperatures ranging from 0 to 105° F. Their data indicate that in general there was a decline in the blood ascorbic acid levels of cows with rising temperature; this was particularly noticeable above 80° F and in the Holstein breed. The blood ascorbic acid levels of yearling heifers were higher than those of cows, but were not affected to an appreciable extent as a result of raising the environmental temperature. Workers at Oklahoma found that the ascorbic acid values of blood plasma from beef cows and heifers—Angus, Hereford, and Shorthorn—were not related to age, breed, environment or diet. The values reported ranged from 79.8 to 456.3 ug. per 100 ml. (Long *et al.,*

1952). Watts (1950) determined the ascorbic acid concentrations of blood plasma and tissues of cattle. Liver values were found to be lowest during May and June while those for kidney and plasma were highest from December to March, which corresponded with the period of turnip feeding.

Foulger (1942) reported that by the administration of vitamin C tablets, the heat tolerance of men working in extreme heat and high humidity could be improved. But Fox (1940), from experiments on a large group of mine workers and also Henschel *et al.,* (1944) by extensive and well controlled experiments, obtained no such evidence.

Cold Stress: With regard to cold stress, the recent experiments of Ryer *et al.,* (1954a and 1954b) and also the earlier studies as reviewed by Mitchell *et al.,* (1951), fail to prove any beneficial effects of supplementation of vitamin C on the ability of human subjects to endure cold. However, Dugal and his co-workers (1952) have obtained evidence for favorable effects on acclimatization to cold environments, from large doses of vitamin C given to rats, guinea pigs and monkeys. It was found that the typical enlargement of the adrenal glands under the influence of stress was completely prevented in rats and guinea pigs that were exposed to cold, if they received large doses of ascorbic acid (Dugal *et al.,* 1949).

Although many studies have been made on the relation of vitamin C to adrenal hormones and stress, the reports on its role in the production of corticosteroids are very conflicting. The subject has been reviewed by Meiklejohn (1953), Sebrell *et al.,* (1954a) and McHenry (1955).

Effect of Thermal Stress on Metabolism of B-Vitamins

Although no study seems to have been made on the effect of environmental temperature on the biosynthesis and metabolism of B-vitamins by ruminants, considerable work has been done on laboratory animals such as rats, rabbits, guinea pigs and dogs.

Heat Stress: Mills, one of the early workers in this field, studied the effect of high environmental temperatures on the thiamine requirements of rats (1941, 1943b). After feeding ad libitum a synthetic diet containing graded levels of thiamine to various groups of rats kept at 95° F (relative humidity 72%) and 65° F, he concluded that for optimum growth, rats require greater concentrations of thiamine per unit weight of feed consumed in a hot environment than in a temperate one. Subsequently, he also demonstrated increased requirements of dietary concentrations of choline for rats subjected to high environmental temperatures, but found no such difference in their requirements for other B-vitamins (1942, 1943a and 1943b). Mills and his co-workers (1947) also demonstrated that chicks kept at 90° F required more thiamine for optimum growth and protection

from polyneuritis than did those kept at 70° F. However, there was no apparent increase in the requirements for choline, nicotinic acid, folic acid and pyridoxine.

Robinson (1943), by determining the thiamine requirements of rats on the basis of minimum amounts required to prevent increased excretion of pyruvate in the urine, found that rats kept at 35° C required more thiamine than those kept at 15° C.

On the other hand, Kline *et al.* (1945) presented evidence for decreased requirements of thiamine by rats at high temperatures. In their experiment, rats previously depleted of thiamine were kept at 78° F and 85 or 90° F., fed ad libitum a thiamine deficient diet, and were given various amounts of thiamine by stomach tube. The decrease in thiamine requirements, based on growth and cure of polyneuritis, was found to be related to the decrease in caloric requirements at the elevated temperatures. Edison *et al.,* (1945) also reported, from experiments similar to those of Kline *et al.,* that the thiamine requirements for growth of rats in a tropical environment (90° F and 70% relative humidity) were not greater and possibly less than under temperate conditions (72° F and 50% relative humidity). They also determined the liver concentrations of thiamine and found that when the thiamine caloric intake ratio was the same, liver thiamine concentrations were comparable to both tropical and temperate conditions in rats fed ad libitum; there was no significant difference between tropical and temperate groups in concentrations of riboflavin, pantothenic acid, or nicotinic acid. Similarly, Williams *et al.,* (1940) from experiments on induced thiamine deficiency in man, concluded that the requirements might be greater in winter than in summer. They found that, while a diet restricted to 0.15 mg. of thiamine was tolerated for 147 days by four subjects who received it during the summer months, a similar diet given to four other subjects in the winter months could be tolerated only for 88 days. The tolerance period was determined on the basis of observations of thiamine deficiency symptoms. One of the limitations of the results of this experiment is the possibility of differences in body reserves of thiamine. Furthermore the experiments of Kline, Edison, and Williams are all subject to criticism in as much as the thiamine intake was kept constant; consequently, the thiamine caloric intake ratio always favored the group fed low levels of thiamine at high temperatures.

Mitchell *et al.,* (1950), in two separate experiments, investigated the riboflavin requirements of growing pigs at 42° F and 85° F. In each experiment, the pigs were first depleted of riboflavin and then fed different levels of the vitamin, but their food consumption was kept equal. Based on the observations of their weight gains and blood picture, the riboflavin

requirements were greater for those kept at the lower temperature.

Squibb *et al.,* (1945) studied the influence of high environmental temperature on the metabolism of vitamins by measuring vitamin serum levels of rats subjected to a temperature of 94° F for 72 hours and a control group maintained at 72° F. In one of their trials the animals were fed ad libitum while in the other they were restricted to 5 gm of feed daily. It was observed that with ad libitum feeding, the rats subjected to 94° F consumed less feed, lost more weight and had blood levels of riboflavin, vitamin A, ascorbic acid and alkaline phosphatase which were significantly lower than those of the controls. With restricted feeding, both groups of rats lost weight and vitamin A and ascorbic acid levels were significantly depressed in those kept at the higher temperatures.

Sarett *et al.,* (1943), approached the problem by measuring the growth, urinary excretion of B-vitamins and nitrogen, and the B-vitamin content in the livers and carcasses of rats maintained at 91° F and 75° F for 22 to 25 days. The rats at both these temperatures were divided into two groups; one was fed the B-vitamins—thiamine, riboflavin, pantothenic acid, nicotinic acid and choline—at a rate only slightly above the minimum requirements while the other received a great excess of vitamins. The food intake of groups at the lower temperature was restricted to that of corresponding groups at the high temperature. The rats at high temperature gained more weight than their controls. Those on both levels of vitamins at the high temperature excreted the same amounts of riboflavin and nicotinic acid but they excreted more pantothenic acid than the corresponding groups at room temperature; excretion of thiamine was not measured. On the other hand, riboflavin, pantothenic acid and thiamine concentrations in the body tissues, were highest in the group at high temperatures and receiving high levels of B-vitamins, and lowest in the low-vitamin, high temperature group; nicotinic acid was essentially the same in all four groups. In view of the differences observed in excretion and tissue storage of vitamins, it is difficult to interpret the results in terms of utilization and requirements of these vitamins at the two temperatures. Williams *et al.,* (1944) also determined the B-Vitamin content of the tissues of rats fed commercial feed and synthetic diets and subjected to environmental temperatures of 90° F and 68° F for over three months, but could not detect a difference. However, it should be pointed out that the rats fed the synthetic diet and kept at a high temperature were given twice as much thiamine and choline as their controls.

Worden *et al.,* (1955) found that when the environmental temperature of three adult dogs, fed a known amount of riboflavin in standard diet, was raised by about 20° F there was a significant increase in the

total daily urinary excretion of riboflavin. The temperature readings ranged from a minimum of 54° F to a maximum of 78° F for the lower environmental temperature period and from 76° F to 102° F for the higher environmental temperature period. The test period consisted of five consecutive 24-hour periods at each temperature. These results are said to be in agreement with those of Montenero and Frongia (1951), who reported a considerable rise in riboflavin excreted in five healthy subjects after the artificial induction of a temperature of 39° C for three days. Mitchell *et al.,* (1950) also reported, from their experiments with adult swine, that consistently smaller amounts of riboflavin were excreted in the urine of pigs at 42° F than at 85° F. However, Worden *et al.,* (1954), found that there was a drop in the total daily urinary excretion of thiamine by dogs as their environmental temperature was increased by approximately 30° F. Iwamoto (1941) also reported that the urinary output of vitamin B_1 decreased markedly for rabbits when they were kept at 32° C, compared to room temperature or when the body temperature was increased from the normal 38.2° C up to 40° C. Holt (1943) found a tendency for greater excretion of thiamine in the fasting urine of humans on hot days than on cold days. All of the subjects are reported to have been receiving quantities of thiamine close to the minimum requirements. Since this was not a controlled experiment and the urinary excretion as reported did not represent the total daily output, their results are difficult to interpret with regard to the utilization or requirement for thiamine at high temperatures. The reliability of urinary excretion as a measure of the utilization of thiamine has also been questioned by Mitchell *et al.,* (1951) who pointed out that the urinary excretion of thiamine is influenced by the level of its intake.

Spector *et al.,* (1945) determined the pantothenic acid excretion of human subjects kept in a comfortable environment, 28.3° C and 50 percent humidity, and in a hot, moist environment, 38.3° C and 69 percent relative humidity. They found that both the urinary and the dermal excretion of pantothenic acid were greater under hot, moist conditions than under comfortable conditions, whether the subjects were given an ordinary diet or one supplemented with pantothenic acid.

Considerable work has been reported on the excretion of B-vitamins in the sweat of human subjects under hot and humid conditions. However, cattle, unlike man, do not ordinarily sweat when exposed to hot temperatures; thus the usefulness and application of these studies to cattle becomes extremely doubtful. Detailed discussions of this subject have been given by Mitchell *et al.,* (1951) and Robinson *et al.,* (1954). In brief, it may be noted that the B-vitamins studied and found in sweat are:

Thiamine, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, choline, para aminobenzoic acid, inositol, and folic acid. Concentrations of these vitamins in sweat are generally not more than 100 ug. per liter. The amounts present in sweat are so negligible that they do not significantly affect the vitamin requirements of the subjects, even under conditions of profuse sweating (4 to 8 liters per day) in hot weather. (Mitchell *et al.,* 1951; Robinson *et al.,* 1954).

Some studies have also been carried out on the effect of administering extra doses of B-vitamins on the heat tolerance and work capacity of human subjects under hot and humid conditions. Henschel *et al.,* (1944) made physiological and general physical observations of human subjects given high dosage of thiamine, riboflavin and nicotinamide, and doing hard work in hot and humid environment. They concluded that the rate and degree of acclimatization and the ability to do hard work in the heat are not influenced by vitamin supplementation. However, Droese (1942) has provided some evidence on the favorable effects of providing supplementary thiamine for men doing exhausting work in heat. He observed that the endurance of the subjects doing hard work (pedaling on a bicycle ergometer) was increased at cool temperatures (20° C and 57 percent relative humidity) by giving them glucose alone or glucose plus thiamine; at the higher temperature (39° C and 30 percent relative humidity) their endurance was increased only by glucose plus thiamine. The author's conclusion, as reported by Mitchell *et al.,* (1951), is interesting: "The thiamine requirement is increased by work in heat, not because of increased sweating, nor of a diminished efficiency of muscular work, but probably because of some circulatory impairment preventing the rapid removal from the muscle of intermediary products of sugar metabolism that accumulate there in the absence of adequate concentrations of vitamin B_1."

Forni (1952) observed that subcutaneous injections of from 0.01 to 10.0 mg of nicotinic acid caused a temporary reduction in the body temperature of guinea pigs, the fall being greatest with the largest doses. Ferrero (1940) also obtained somewhat similar results. He found that large doses of nicotinamide could increase the resistance of rabbits to heat treatment.

Cold Stress: Hegsted *et al.,* (1950), determined the thiamine requirements of adult rats kept at 78° F and 55° F, using growth after partial depletion as the criteria of adequacy. The thiamine requirement was found to be 50 percent greater at the low temperature than at room temperature. Ershoff (1950, 1951 and 1952) investigated the cold tolerance period of rats, as indicated by the length of survival or the development of deficiency symptoms when they were kept at 2° C on various levels of different

vitamins. This demonstrated that rats deficient in thiamine, pantothenic acid, pyridoxine and riboflavin had a decreased resistance to cold when compared to control animals.

Ralli (1952) reported that after 6 weeks of therapy with 10 gm of calcium pantothenate daily, human subjects were able to stand cold stress —immersion in water at 9° C for 8 minutes—much better than without therapy. Analysis of blood and urine for certain constituents known to be influenced by adrenal cortical function, was used as the criteria for ability to stand stress. Incidentally, there is much evidence for the close relationship of pantothenic acid with the function of adrenal glands which secrete the so-called stress hormones (Sebrell *et al.,* 1954b; Krehl, 1954; McHenry, 1955).

Ryer *et al.,* (1954a and b) made physical observations and certain biochemical and psychological measurements on soldiers residing in a cold environment. They failed to observe any significant effect of supplementation with vitamin B-complex and ascorbic acid tablets on the soldiers' endurance to cold. These results seem to be in line with the conclusions drawn by Mitchell *et al.,* (1951), who concluded from their extensive review of the literature, "The levels in the diet of thiamine, ascorbic acid, riboflavin and niacin, provided they are present in proportions adequate in a comfortable environment seem to exert no appreciable effect upon tolerance to cold."

Among other factors closely related to thermal stress is exposure to sun. It is considered a cause of metabolic disturbances in regard to the requirements for the vitamin B-complex (Fischer 1952). High relative humidity, at room temperature, has also been shown to exert some effect on the metabolism of B-vitamins in rats (Collins *et al.,* 1953, and Schreiber *et al.,* 1954).

EXPERIMENTAL ANIMALS AND METHODS

Animals and Sample Collection

Brahman, Shorthorn and Santa Gertrudis calves were used as the experimental animals. Table 1 gives their birth dates and weights at the start and finish of the experiment.

For this experiment, each of the two chambers of the climatic laboratory was partitioned into three pens. Three calves of each breed were housed together in each of these pens, measuring 11 x 8 feet. The temperature in one of the chambers (No. 1) was maintained at 50° F and that

TABLE 1. EXPERIMENTAL CALVES

Breed	Chain No.	Birth Date 1954	Age-Days as of Nov. 15, 1954	Weight-lb. Nov. 15 1954	Weight-lb. Oct. 24 1955	Gains
			Chamber 1			
Brahman	301	Sept. 20	56	146	639	493
	309	Sept. 18	58	126	623	497
	319	Oct. 3	43	130	728	598
Santa Gertrudis	387	Oct. 1	45	135	850	715
	366	Sept. 22	54	131	800	669
	368	Oct. 2	44	147	750	603
Shorthorn	332	Aug. 15	92	144	723	579
	342	Sept. 28	48	112	739	627
	349	Aug. 7	100	128	734	606
			Chamber 2			
Brahman	302	Sept. 20	56	167	691	524
	315	Sept. 21	55	135	721	586
	321	Sept. 29	47	119	687	568
Santa Gertrudis	384	Oct. 1	45	137	774	637
	393	Sept. 19	57	159	724	565
	396	Sept. 22	54	118	703	585
Shorthorn	338	Aug. 7	100	136	650	514
	354	Aug. 28	79	101	500	399
	355	Sept. 30	46	97	478	381
			Open Shed			
Brahman	313	Sept. 26	50	87	577	490
	361	Sept. 25	51	105	640	535
Santa Gertrudis	371	Oct. 17	29	90	663	573
	385	Oct. 22	24	120	687	567
Shorthorn	334	Aug. 22	85	148	760	612
	344	Oct. 1	45	100	631	531

of the other chamber (No. 2) at 80° F throughout the experiment. The relative humidity in chamber 1 was maintained between 55 and 65 percent and that of chamber 2 between 50 and 60 percent.

The group of calves listed in the table under "Open Shed" were maintained under ordinary housing conditions. The minimum and maximum temperatures and the relative humidities recorded in the shed during the 24 hours prior to collection of samples are given in Table 2.

The calves received milk until December 10 and had access to alfalfa hay throughout the experimental period. They were also fed a calf starter (Ration I) ad libitum until January 21. At this time Ration I was gradually replaced by Ration II, also fed ad libitum. After May 7, the amount of grain mixture was restricted to 6 pounds daily per animal. The two grain mixtures were made up as follows.

Ration I		Ration II	
Ground yellow corn	24.04%	Ground yellow corn	33.05%
Ground oats	29.83	Ground oats	20.65
Ground grain sorghums	.33	Wheat bran	13.77
Wheat bran	7.67	Wheat middlings	10.50
Linseed oil meal	8.33	Linseed oil meal	1.72
Soybean oil meal	9.67	Soybean oil meal	7.23
Dehydrated alfalfa meal	4.67	Reinforced cod liver oil	.17
Dried beet pulp	2.33	Vitamin D_2 supplement	.86
Dried skimmilk	3.33%	Vitamin A oil supplement	6.88%
Dried whey	1.00	Steamed bone meal	.69
Blood flour	1.83	Calcium carbonate	1.72
Cane molasses	2.22	Defluorinated phosphate	1.72
Cod liver oil	.18	Trace mineral and salt mix	1.04
Vitamin D_2 supplement	.17		
Vitamin A oil supplement	1.67		100.00%
Riboflavin supplement	.04		
Aurofac 2A	.08		
Steamed bone meal	.67		
Calcium carbonate	.58		
Defluorinated phosphate	.33		
Trace mineral and salt mix	.92		
	100.00%		

The feeding of calves was generally done between 6 a.m. and 7 a.m. and 4 p.m. and 5 p.m.

Thirty-milliliter blood samples were drawn from each calf through an indwelling polyethylene catheter in the jugular vein. The blood was collected and citrated in opaque bottles to prevent harmful effects from exposure to light, and immediately taken to the laboratory. Aliquots then were removed for ascorbic acid and thiamine determinations. The remainder of each sample was transferred to a tube for storage under toluene at −10° F until analyzed for the other vitamins.

TABLE 2. TEMPERATURES AND RELATIVE HUMIDITIES (IN OPEN SHED) RECORDED DURING THE 24 HOURS PRIOR TO SAMPLE COLLECTIONS

Sampling Date (1954-1955)	Nov. 30*	Jan. 11*	Feb. 15	Mar. 22	Apr. 26	Jun. 7	Jul. 19	Aug. 30	Oct. 11
Temperature: °F.									
Maximum	43	32	56	41	76	75	88	81	80
Minimum	34	25	38	27	59	62	74	67	57
Relative Humidity: %									
Maximum	69	82	78	80	79	86	87	92	76
Minimum	43	64	42	40	34	46	51	36	36

* The data for November 30 and January 11, have been taken from the Columbia Weather Station records.

The following schedule for collection of samples from individual calves was repeated throughout the experiment, from November 16, 1954, until October 25, 1955.

Week	Chamber and Environ. Temp.	Calves Chain Numbers		
		Brahmans	Shorthorns	Santa Gertrudis
1st	I	309,319	342,349	366,368
2nd	II	302	338	384
3rd	Open Shed	313,361	334,344	371,385
4th	I	301	332	387
5th	II	315,321	354,355	393,396

Thus, each calf was bled at an interval of five weeks. The samples were collected each Tuesday at either 11 a.m. or 2 p.m. Collections before June, 1955, were made mostly at 2 p.m. and after that at 11 a.m.

Vitamin Assay Methods

All determinations were made in 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 and 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 Kmieciak 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 two hours at 40° C. The samples were further acidified and heated in a boiling water bath for ten 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 one percent potassium ferricyanide solution. Thiochrome was extracted by shaking with isobutyl alcohol and then centrifuging. The extracted samples were treated with sodium sulfate and 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; *Lactobacillus casei* and the media recommended by the Association of Vitamin Chemists (1951) were used 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. 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 more than 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.

Recovery checks for these assays were made occasionally and the recoveries were within satisfactory limits, between 90 and 110 percent.

RESULTS AND DISCUSSION

(Tables 3 to 7 in Appendix)

Blood ascorbic acid levels (Fig. 1 and Table 3) of Brahman calves maintained at 80° F were higher than those of Brahman calves kept at 50° F, and lower than those of calves housed under ordinary conditions. The ascorbic acid levels in the blood of Santa Gertrudis calves subjected to high temperatures also were lower when compared with levels in the controls kept in the open shed. In view of the idea that the stress of high environmental temperatures on an animal may cause a decrease in its blood ascorbic acid level, the lower ascorbic acid blood values for Brahman and Santa Gertrudis calves at both 50 and 80° F implies that these conditions were more stressful than the open shed environment. Indeed, this may have been true because of abnormal conditions such as lack of exercise; however, there was no appreciable intrabreed difference in the weight gains or general performance of either Brahman or Santa Gertrudis calves under these experimental conditions. In contrast, there was no appreciable difference in the blood ascorbic values of the Shorthorn calves exposed to the various experimental conditions even though the group at 80° F had the lowest weight gains and showed general symptoms of stress. Perhaps this can be explained by the third stage, the exhaustion stage, of Selye's Adaptation Syndrome concept (Selye 1955). It may be that, because of the stress being severe in their case, their adrenals became exhausted and failed to respond further to the continuous stress. Or it may be that the adrenals of Shorthorns, as a breed, do not show as much response to heat stress, since heredity can affect the production of adaptive hormones (Selye 1955).

On the other hand, the evidence regarding the role of ascorbic acid in the function of adrenals is contradictory; there are various indications that it may not be involved in the secretion of adrenal hormones (Meiklejohn 1953, Sebrell 1954a and McHenry 1955).

There does not appear to be a correlation between the ascorbic acid levels in the blood and age of the calves or season of the year. The values for all the calves are within the range given by Albriton (1952).

High environmental temperatures of 80° F seemed to have an adverse effect on the blood niacin levels (Fig. 2 and Table 4) of Shorthorn calves. The group of Shorthorn calves at 80° F had distinctly lower values when compared to either of the other two groups of Shorthorn calves which were housed at 50° F and in the open shed, or the Brahman and Santa Gertrudis calves maintained in the same chamber, 80° F. After the second sampling period, Brahman calves at 80° F showed clearly higher values than both Shorthorn and Santa Gertrudis calves at the same temperature.

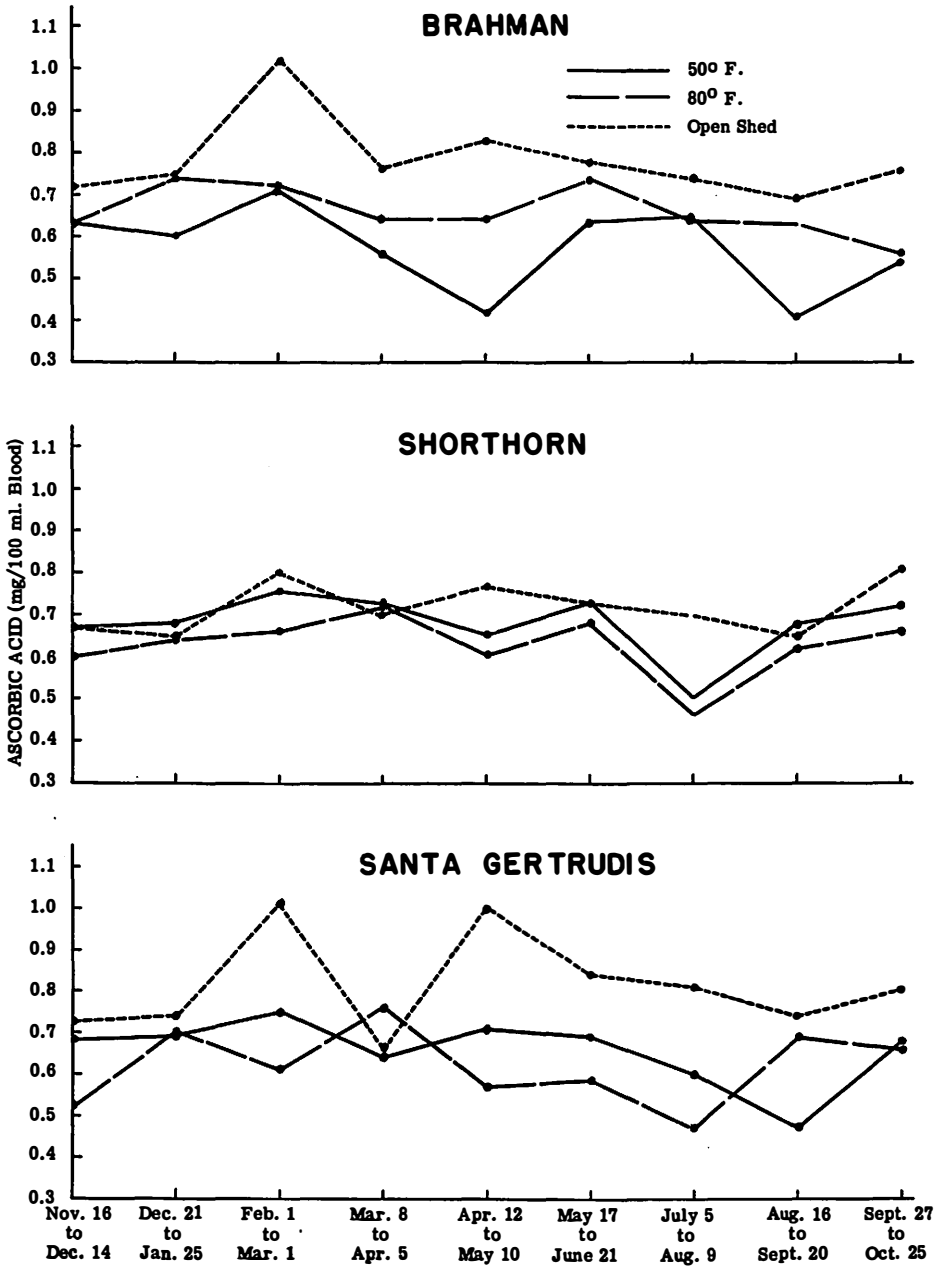

Figure 1—Intrabreed comparison of ascorbic acid blood levels in heifers as influenced by environmental temperature.

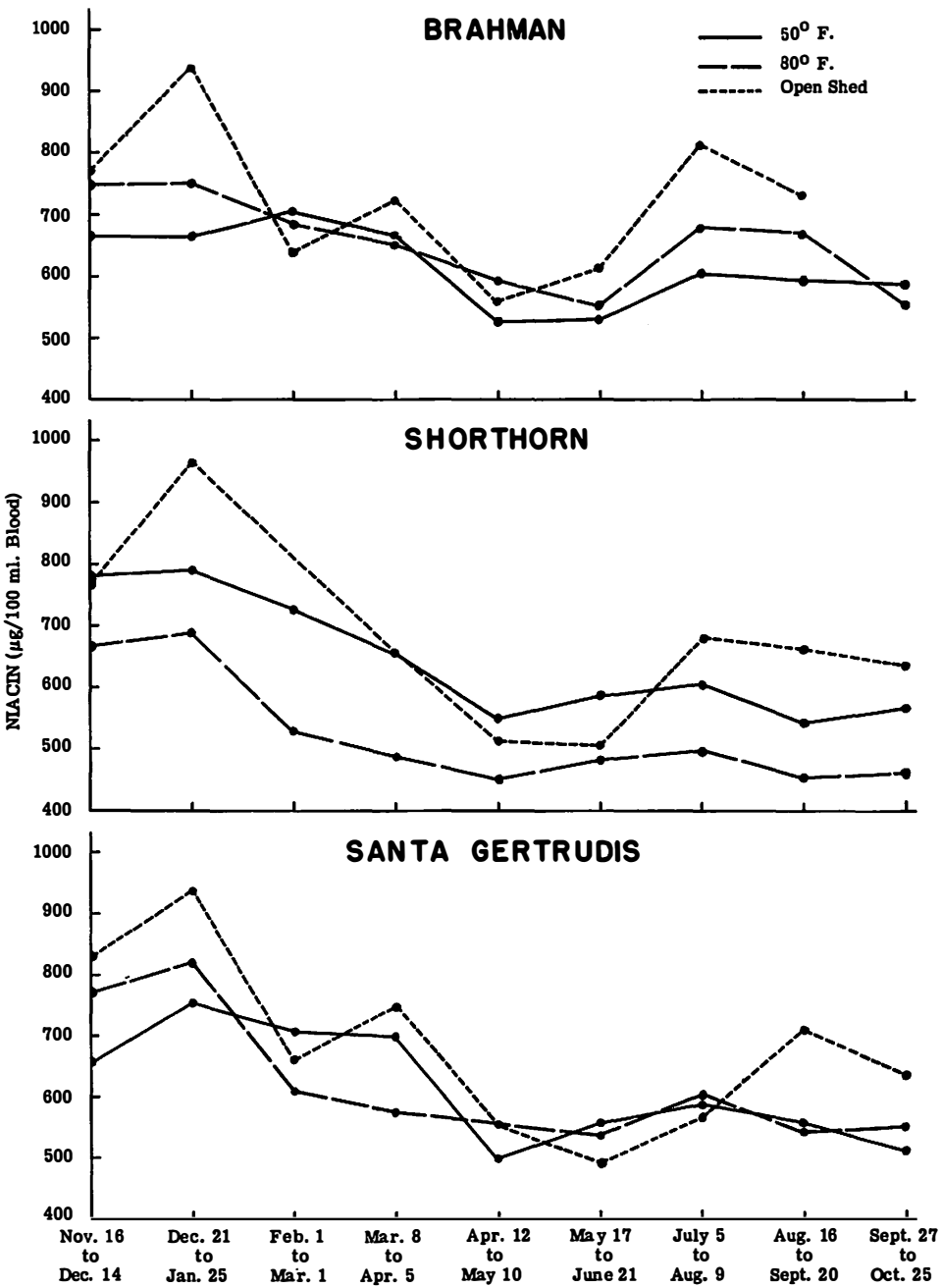

Figure 2—Intrabreed comparison of niacin blood levels in heifers as influenced by environmental temperature.

The niacin content of blood of all the three breeds of calves that were kept at 50° F was high until the fourth sampling period, then the niacin levels dropped off markedly and remained relatively constant at this decreased value. At 80° F or in the open shed, the decrease in blood niacin occurred earlier, after the second collection of samples. At 80° F, as at 50° F, the blood niacin values for all calves tended to remain at the decreased level after this drop; however, in the open shed the blood niacin values showed a marked rise again sometime in June-July.

The values for blood niacin levels of calves agree favorably with those reported by Teeri *et al.,* (1946) and Smith *et al.,* (1954).

Environmental temperatures apparently did not influence pantothenic acid (Fig. 3 and Table 5) or riboflavin (Fig. 4 and Table 6) levels in the blood of Brahman, Shorthorn, or Santa Gertrudis calves. No breed differences were apparent under any of the environmental temperature conditions. The values for both of these vitamins, particularly for riboflavin, for all the calves at different periods of collection of samples, were quite variable. However, variations between individual animals during the same period of collection were comparatively small. All calves, irrespective of breed, showed increased amounts of riboflavin in the blood for the samples collected between July 5 and September 20, compared to the values obtained during other periods.

The values obtained for pantothenic acid were generally lower than those quoted in the literature for young calves (Smith *et al.,* 1954); those for riboflavin compared favorably with the values quoted for cows (Albriton 1952) but were much higher than those given for young calves (Smith *et al.,* 1954).

The average concentrations of thiamine (Fig. 5 and Table 7) in the blood of Shorthorn calves kept at 80° F were distinctly lower than the values for the calves of the same breed kept at 50° F; to some extent, they were also lower than the corresponding control (open shed) group of calves. The thiamine blood levels of Shorthorn calves under hot environmental temperatures (80° F) were lower than those of Brahman and Santa Gertrudis maintained under the same conditions; however, the difference between Shorthorn and Brahman calves was less both in magnitude and regularity than the difference between Shorthorn and Santa Gertrudis calves.

Santa Gertrudis calves tended to have somewhat lower thiamine blood values at 80° F than at 50° F or under "natural" conditions in the open shed. In the cold chamber, Santa Gertrudis calves had distinctly higher blood thiamine levels than the Brahman or Shorthorn calves. Brahman calves tended to have the lowest values.

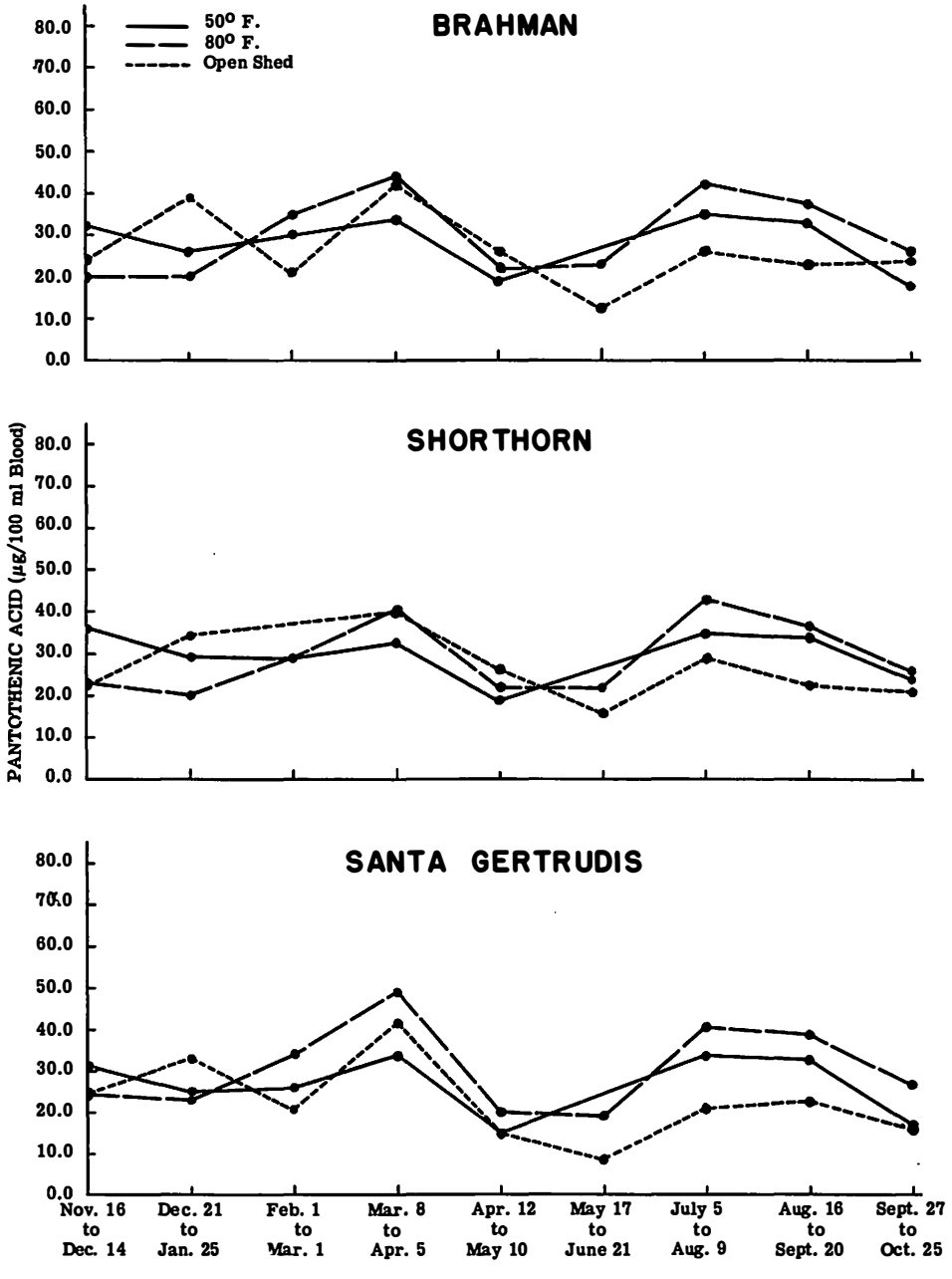

Figure 3—Intrabreed comparison of pantothenic acid blood levels in heifers as influenced by environmental temperature.

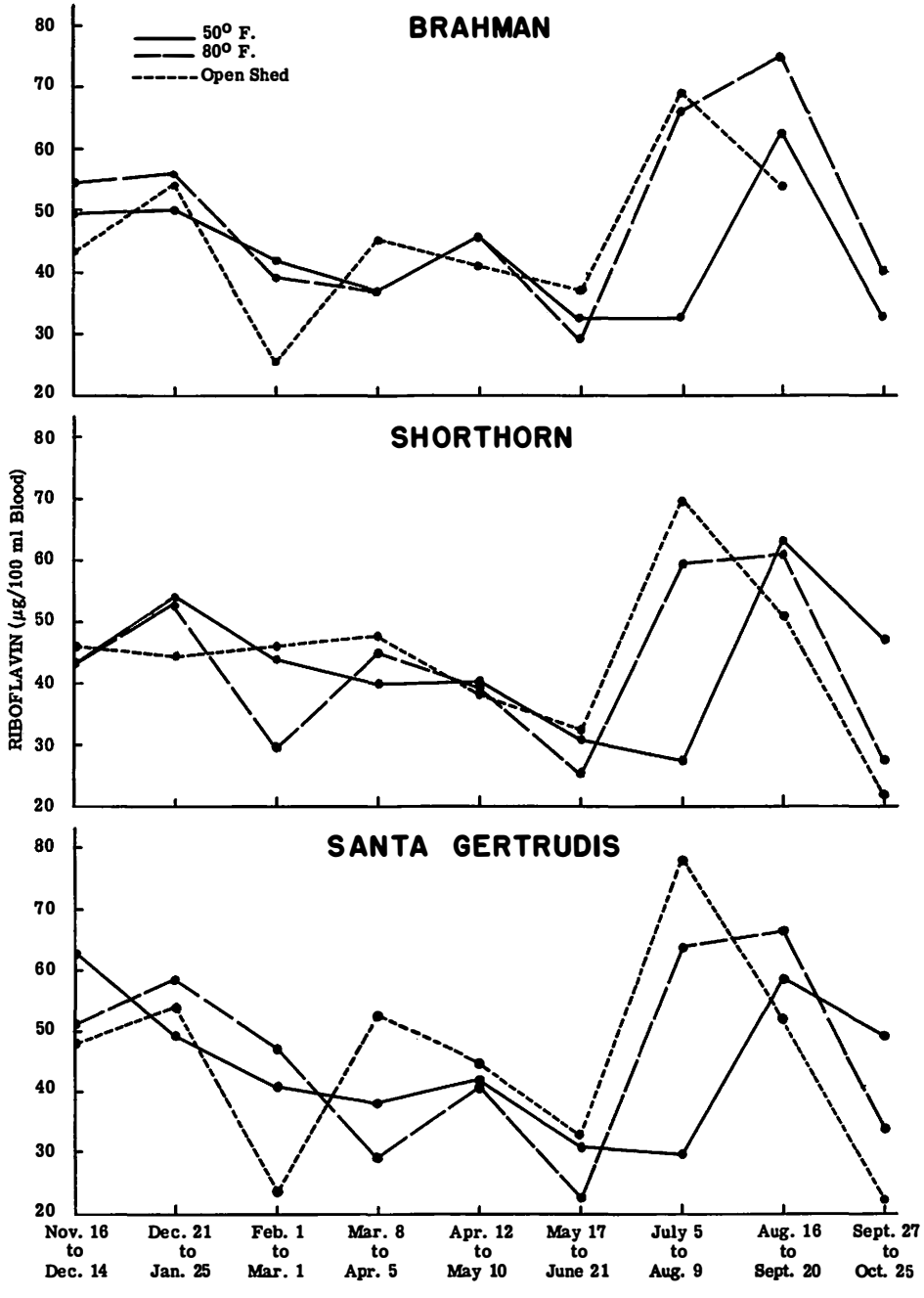

Figure 4—Intrabreed comparison of riboflavin blood levels in heifers as influenced by environmental temperature.

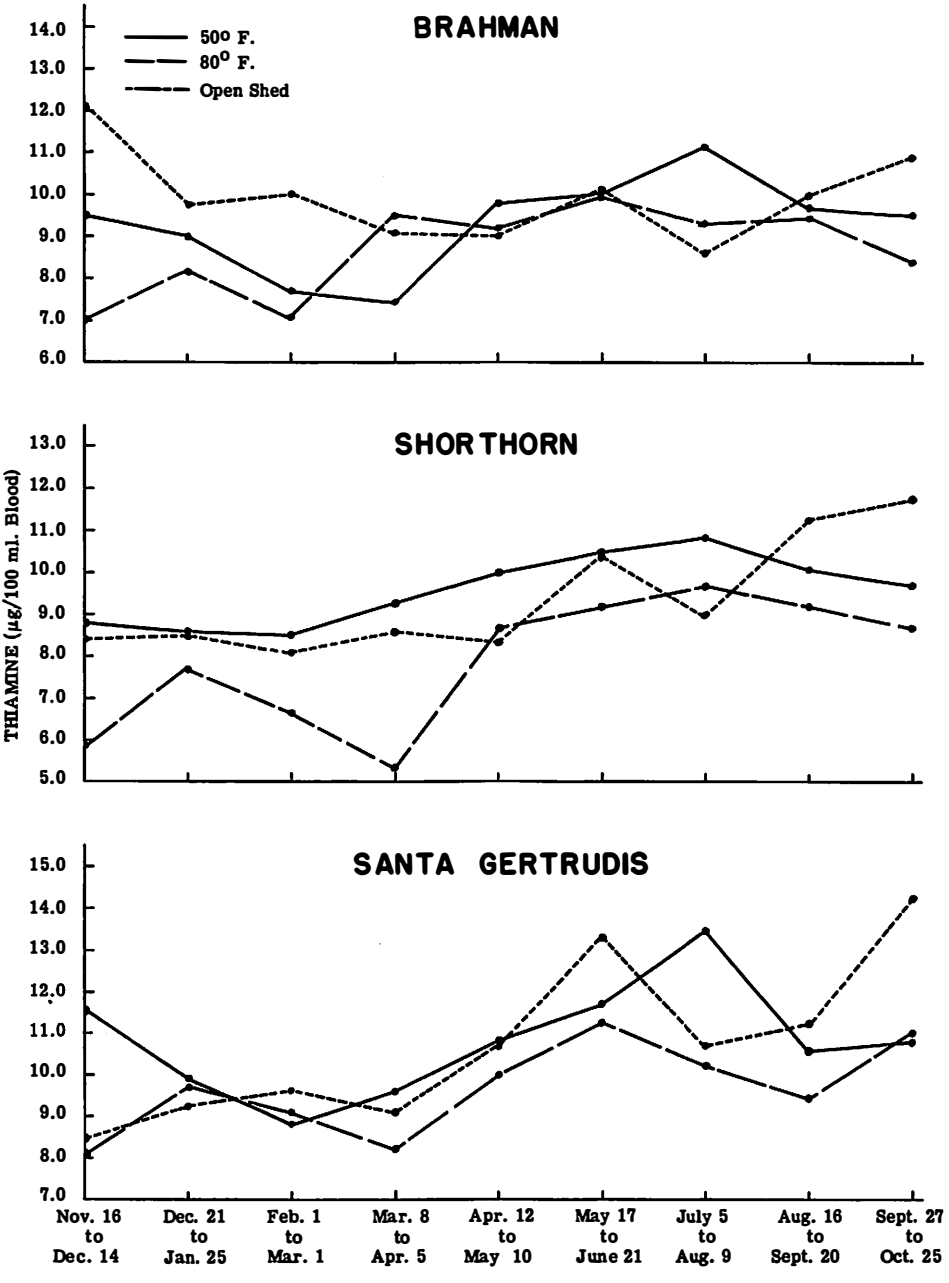

Figure 5—Intrabreed comparison of thiamine blood levels in heifers as influenced by environmental temperature.

The blood thiamine values obtained for all calves were within the range quoted for cows by Albriton (1952).

With regard to the decrease observed in blood levels of some vitamins for animals with poor heat tolerance, several possible causes can be postulated. Feed intake may be one of the contributing factors, since it is known that the feed consumption of cattle with poor heat tolerance is considerably reduced under thermal stress. Although it has been established that cattle are independent of external sources of B-vitamins, the exact role of the vitamins present in their feed is not known. It is reasonable to assume that some of the vitamins present in the feeds may be absorbed through the digestive tract. Secondly, the reduced feed intake may also lead to decreases in the amount of vitamins synthesized in the rumen. Further, the increased body temperature and abnormal physiological behavior of these animals may modify the conditions in their rumen and disturb the microbial biosynthetic processes. The reduced ruminal synthesis of vitamins and, consequently, their decreased availability to the animal may adversely affect their metabolic functions as well as the animal's appetite. The decreased appetite may lead to the so-called hunger stress and production of large amounts of abnormal metabolites such as ketone bodies and thus cause alterations in the biochemical pathways of metabolism.

Nath *et al.,* (1953a) demonstrated that the administration of sodium acetoacetate and a B-hydroxybutrate to rabbits caused an increase in the pyruvate and a decrease in the vitamin B_1 excretion in their urine. They also demonstrated by *in vitro* experiments, considerable destruction of vitamin B_1 in the presence of acetoacetate. These workers (1953b) also observed a decline in the blood levels of riboflavin and nicotinic acid of rabbits to which sodium acetoacetate was administered. Although Dale *et al.,* (1954) observed no increase in the concentration of ketone bodies in the blood plasma and their excretion in urine of Holstein and Jersey cows subjected to thermal stress for short periods, the possibility of their increased production under prolonged periods of chronic heat stress is not ruled out. Then it is possible that the distinct increase in the rectal temperature of such cattle and to some extent the pH of their blood (Dale *et al.,* 1954) may cause a rapid and increased destruction of thiamine in their system, as it is sensitive to both of these conditions.

With regard to the synthesis of niacin, which may also occur in the body tissues of cattle, at least of calves (Hopper *et al.,* 1955), it is very likely that the increased body temperature may have an adverse effect.

Other possibilities include alterations in the rate of absorption and excretion of some of the vitamins in such a way as to cause a decline or increase in their blood levels. With an increase in body temperature the

pathways and rate of certain metabolic reactions may be altered so that the requirements for certain particular vitamins may be increased or decreased accordingly. Droese (1942) concluded from his experiments with human subjects that the vitamin B_1 requirement is increased by work in heat, probably because of some circulatory impairment preventing the rapid removal from the muscles of intermediary products of sugar metabolism that accumulate there in the absence of adequate concentrations of vitamin B_1. Thus, it is possible that the requirements for niacin, riboflavin and thiamine may be increased for cows and calves under heat stress causing decreased blood levels.

There does not appear to be an appreciable relation between the blood levels of any of the vitamins and the age of the calves or season of the year except in the case of riboflavin which increased in the blood of all calves during July, August, and September.

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APPENDIX

Tables 3 to 7 give the breed averages for ascorbic acid, niacin, pantothenic acid, riboflavin, and thiamine concentrations in the calves' blood under various environmental conditions.

TABLE 3. ASCORBIC ACID IN CALVES' BLOOD; BREED AVERAGES
(Mg. per 100 Ml.)

Collection Periods / Calves (1954-1955)	Nov. 16 to Dec. 14	Dec. 21 to Jan. 25	Feb. 1 to Mar. 1	Mar. 8 to Apr. 5	Apr. 12 to May 10	May 17 to Jun. 21	July. 5 to Aug. 9	Aug. 16 to Sept. 20	Sept. 27 to Oct. 25
					Chamber I. 50°F.				
Brahmans	0.63	0.60	0.71	0.56	0.42	0.64	0.65	0.41	0.54
Shorthorns	0.67	0.68	0.76	0.73	0.65	0.73	0.50	0.68	0.72
Santa Gertrudis	0.68	0.69	0.75	0.64	0.71	0.69	0.60	0.47	0.68
					Chamber II. 80°F.				
Brahmans	0.63	0.74	0.72	0.64	0.64	0.69	0.64	0.63	0.56
Shorthorns	0.60	0.64	0.66	0.72	0.60	0.68	0.46	0.62	0.66
Santa Gertrudis	0.52	0.70	0.61	0.76	0.57	0.59	0.47	0.69	0.66
					Open Shed				
Brahmans	0.72	0.75	1.02	0.76	0.83	0.78	0.74	0.69	0.76
Shorthorns	0.67	0.65	0.80	0.70	0.77	0.73	0.70	0.65	0.81
Santa Gertrudis	0.73	0.74	1.01	0.66	1.00	0.84	0.81	0.74	0.80

TABLE 4. NIACIN IN CALVES' BLOOD; BREED AVERAGES.
(Ug. per 100 Ml.)

Collection Periods / Calves (1954-55)	Nov. 16 to Dec. 14	Dec. 21 to Jan. 25	Feb. 1 to Mar. 1	Mar. 8 to Apr. 5	Apr. 12 to May 10	May 17 to Jun. 21	Jul. 5 to Aug. 9	Aug. 16 to Sept. 20	Sept. 27 to Oct. 25
					Chamber I. 50°F.				
Brahmans	662	662	704	667	525	529	604	596	587
Shorthorns	783	791	729	654	550	587	604	542	567
Santa Gertrudis	657	758	708	700	500	556	591	558	513
					Chamber II. 80°F.				
Brahmans	746	750	683	650	591	550	679	670	554
Shorthorns	668	691	531	488	450	483	500	458	462
Santa Gertrudis	771	821	609	576	558	537	604	541	554
					Open Shed				
Brahmans	769	937	644	725	556	612	812	732	---
Shorthorns	769	965	---	656	512	506	675	662	637
Santa Gertrudis	831	937	662	750	556	494	569	712	637

TABLE 5. PANTOTHENIC ACID IN CALVES' BLOOD; BREED AVERAGES.
(Ug. per 100 Ml.)

Collection Periods / Calves (1954-55)	Nov. 16 to Dec. 14	Dec. 21 to Jan. 25	Feb. 1 to Mar. 1	Mar. 8 to Apr. 5	Apr. 12 to May 10	May 17 to Jun. 21	Jul. 5 to Aug. 9	Aug. 16 to Sept. 20	Sept. 27 to Oct. 25
					Chamber I. 50°F.				
Brahmans	32	26	30	34	19	--	35	33	18
Shorthorns	36	29	29	33	19	--	35	34	24
Santa Gertrudis	31	25	26	34	15	--	34	33	18
					Chamber II. 80°F.				
Brahmans	20	20	35	49	23	23	43	38	26
Shorthorns	23	20	29	41	23	22	54	37	26
Santa Gertrudis	25	23	34	49	20	19	41	39	27
					Open Shed				
Brahmans	24	38	21	42	26	13	26	23	24
Shorthorns	23	34	--	40	26	16	29	23	21
Santa Gertrudis	24	33	21	42	20	9	21	23	16

TABLE 6. RIBOFLAVIN IN CALVES' BLOOD; BREED AVERAGES.
(Ug. per 100 Ml.)

Collection Periods Calves (1954-55)	Nov. 16 to Dec. 14	Dec. 21 to Jan. 25	Feb. 1 to Mar. 1	Mar. 8 to Apr. 5	Apr. 12 to May 10	May 17 to Jun. 21	Jul. 5 to Aug. 1	Aug. 16 to Sept. 20	Sept. 27 to Oct. 25
					Chamber I. 50°F.				
Brahmans	49	51	42	37	46	33	33	62	33
Shorthorns	43	55	44	40	41	31	28	63	47
Santa Gertrudis	63	48	41	38	42	31	30	58	49
					Chamber II. 80°F.				
Brahmans	55	56	39	37	46	29	67	75	40
Shorthorns	43	53	30	45	39	26	60	62	28
Santa Gertrudis	51	58	47	29	41	23	64	66	34
					Open Shed				
Brahmans	43	54	26	48	41	38	69	54	--
Shorthorns	46	44	--	48	38	33	70	51	22
Santa Gertrudis	48	54	24	53	46	33	78	52	23

TABLE 7. THIAMINE IN CALVES' BLOOD; BREED AVERAGES.
(Ug. per 100 Ml.)

Collection Periods Calves (1954-55)	Nov. 16 to Dec. 14	Dec. 21 to Jan. 25	Feb. 1 to Mar. 1	Mar. 8 to Apr. 5	Apr. 12 to May 10	May 17 to Jun. 21	Jul. 5 to Aug. 9	Aug. 16 to Sept. 20	Sept. 27 to Oct. 25
					Chamber I. 50°F.				
Brahmans	9.5	9.0	7.7	7.4	9.8	10.0	11.1	9.7	9.5
Shorthorns	8.8	8.6	8.5	9.3	10.0	10.5	10.8	10.1	9.7
Santa Gertrudis	11.6	9.9	8.8	9.6	10.8	11.7	13.5	10.6	10.8
					Chamber II. 80°F.				
Brahmans	7.0	8.2	7.1	9.5	9.2	9.9	9.3	9.4	8.4
Shorthorns	5.8	7.7	6.7	5.3	8.7	9.2	9.7	9.2	8.7
Santa Gertrudis	8.1	9.7	9.1	8.2	10.0	11.3	10.2	9.4	10.5
					Open Shed				
Brahmans	12.1	9.8	10.0	9.1	9.0	10.1	8.6	10.0	10.9
Shorthorns	8.4	8.5	8.1	8.6	8.3	10.4	9.0	11.3	11.8
Santa Gertrudis	8.4	9.3	9.6	9.1	10.7	13.4	10.6	11.3	14.3