

AREA, SEASON AND YEAR EFFECTS UPON  
THE VITAMIN A AND MINERAL  
ELEMENT STATUS OF  
BEEF COWS IN  
MANITOBA

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A Thesis  
Presented to  
the Faculty of Graduate Studies and Research  
The University of Manitoba

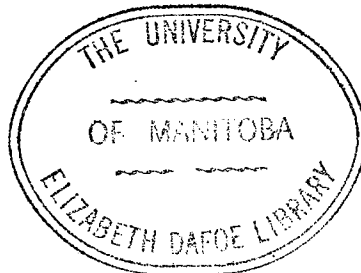
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In Partial Fulfillment  
of the Requirements for the Degree  
Master of Science

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by  
Glen Marshall Findlay

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## ABSTRACT

### AREA, SEASON AND YEAR EFFECTS UPON THE VITAMIN A AND MINERAL ELEMENT STATUS OF BEEF COWS IN MANITOBA

by

GLEN MARSHALL FINDLAY

Liver and plasma samples were collected from beef cows in two areas of Manitoba over a two-year period, from July 1962 to May 1964. Samples were collected in three seasons of the year (spring, summer and fall) from seven herds in Southwestern Manitoba and five herds in the Ste. Rose area of Manitoba. Test animals consisted of eight cows per herd in the Southwestern area and ten cows per herd in the Ste. Rose area.

The effects of area, season and year upon liver levels of copper, cobalt, molybdenum, vitamin A and carotene and the plasma levels of calcium, magnesium, inorganic phosphorus, vitamin A and carotene were determined.

Liver copper levels increased during summer and decreased through the winter period for both areas during the study. The maximum liver molybdenum level occurred in the fall of each year, whereas the minimum value was detected during early summer for both areas. Liver cobalt levels decreased during summer and increased during winter throughout the study.

Liver vitamin A levels showed a trend towards increased storage during late summer followed by a decrease during winter and early summer. Neither liver carotene, plasma vitamin A, nor plasma carotene level were correlated to the liver vitamin A level during the study.

The established trend in plasma calcium level was for an increase during summer followed by a decrease during winter in both areas. Plasma inorganic phosphorus levels were very similar for the two areas throughout the study period. The late summer period was characterized by a decrease in plasma inorganic phosphorus level, whereas the winter and early summer periods showed increases. The plasma magnesium levels were very similar for the two areas. However, definite season and year trends were not established.

Under conditions existing during this study the established plasma and liver nutrient levels can be considered as being within the normal range for breeding cows, with exception of one herd in the Ste. Rose area. This herd was considered to be copper deficient.

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## INTRODUCTION

There is little information available on the nutritional status of beef cows under Manitoba farming conditions. Commercial beef herds are usually maintained entirely or almost entirely on locally produced forages. Because of the possible effects of soil type, climate and forage utilization practices, on the quantities of various nutrients available to the animals, data obtained in surveys from other areas of North America are not necessarily relevant to conditions in Manitoba.

Information on the nutritional status of Manitoba beef cows could be beneficial from the standpoint of increasing the economy of beef cattle production. For instance in some present husbandry conditions dietary deficiency of some specific nutrients, which may be limiting production, could be readily corrected by use of supplements. On the other hand, it may be that current supplementation practices are not warranted.

In order to determine the adequacy of typical rations fed to beef cows in Manitoba, the Department of Animal Science, University of Manitoba initiated a study in July 1962 on the nutritional status of beef cows in Manitoba, in respect to vitamin A and certain mineral elements.

Two geographical areas wherein beef cattle production is a major industry were selected. Farms were then selected within these areas, which were reasonably typical but nevertheless represented a considerable range both in management practices and in types of forage used for the cow herd. To reduce the possible atypical effects of climate in a single year,



## REVIEW OF LITERATURE

### Copper

The importance of copper in animal nutrition became apparent with the discovery of diseases, in grazing cattle and sheep, that responded to copper therapy. Neal et al. (1931) observed a condition in cattle on sandy soils in Florida which he suggested was copper deficiency. Bennett and Chapman (1937) found that a demyelinating disease of lambs in Western Australia, named neonatal enzootic ataxia, was a manifestation of inadequate copper intake. These pioneer investigations have been followed by the demonstration of extensive copper deficiency areas in different parts of the world.

Copper within the body is widely distributed. In two adult sheep with extremely high liver copper stores, Dick (1954) found the distribution to be 72 - 79% in the liver, 8 - 12% in the muscles, 9% in the skin and wool, and about 2% in the skeleton. Cunningham (1931) reported the copper content in organs of different species (Table A).

TABLE A

Copper Content of Organs of Different Animals<sup>a</sup>

| ANIMAL           | LIVER | HEART | LUNGS | SPLEEN | KIDNEY | PANCREAS |
|------------------|-------|-------|-------|--------|--------|----------|
| Human - adult    | 24.9  |       |       | 5.2    | 17.5   | 4.3      |
| Bovine - adult   | 77.0  | 15.6  | 5.3   | 2.9    | 19.7   | 3.8      |
| Bovine - newborn | 470.0 | 14.8  | 4.9   | 4.8    | 15.7   | 8.5      |
| Bovine - fetus   | 262.8 | 10.4  | 3.6   | 5.4    | 8.5    |          |
| Sheep - adult    | 236.6 | 17.9  | 9.6   | 5.0    | 17.8   | 7.7      |
| Horse - adult    | 14.8  | 17.6  | 6.8   | 3.2    | 28.9   |          |
| Pig - adult      | 41.3  | 14.9  | 5.3   | 6.0    | 21.1   |          |

<sup>a</sup> Measured in ppm dry weight

Dick (1954) found that the amount of copper stored in the liver by sheep over a six-month period was 4.5 - 5% of the total intake. The liver is the main storage organ of the body for copper and can provide a reasonably reliable index of the copper status of animals (Underwood, 1962). The liver copper levels in table B were published by Cunningham (1946) (cited by Underwood, 1962) for the ovine and bovine.

TABLE B

The Influence of Animal Species, Age, and Copper Intake  
on the Concentration of Copper in the Liver

| SPECIES | AGE AND TREATMENT         | COPPER CONCENTRATION <sup>a</sup> |
|---------|---------------------------|-----------------------------------|
| Sheep   | newborn; normal           | 168 (74-430)                      |
| Sheep   | newborn; copper-deficient | 13 (4-34)                         |
| Sheep   | mature; normal diet       | 599 (186-1374)                    |
| Sheep   | mature; copper-deficient  | 27 (7-106)                        |
| Cattle  | newborn; normal           | 381 (143-655)                     |
| Cattle  | newborn; copper-deficient | 55 (8-109)                        |
| Cattle  | mature; normal diet       | 200 (23-409)                      |
| Cattle  | mature; copper-deficient  | 11.5 (2.9-32)                     |

<sup>a</sup> Measured in ppm dry weight

Liver copper levels reported by subsequent workers are given in table C.

Ralston et al. (1961) determined the effect of location and season on the copper status of Washington State beef cattle and found a significant season effect. In general, the liver copper levels increased during the pasture growing season and then decreased throughout the winter (table D).

TABLE CLiver Copper Levels of Ovine and Bovine

| SPECIES | COPPER<br>CONCENTRATION    | REFERENCE                    |
|---------|----------------------------|------------------------------|
| Bovine  | 50 - 100 <sup>a</sup>      | Dick (1950)                  |
| Bovine  | 302 (165-578) <sup>a</sup> | Gessert <u>et al.</u> (1952) |
| Bovine  | 49 (6-191) <sup>a</sup>    | McNaught (1948)              |
| Ovine   | 305 (123-584) <sup>b</sup> | Beck (1956)                  |

<sup>a</sup> Measured in ppm dry weight

<sup>b</sup> Measured in ppm wet tissue

TABLE DLiver Copper Levels of Washington State Beef Cattle <sup>a</sup>

| YEAR    | NUMBER OF<br>LOCATIONS | SEASON |        |        |        |
|---------|------------------------|--------|--------|--------|--------|
|         |                        | FALL   | WINTER | SPRING | SUMMER |
| 1956-57 | 9                      | 65     | 52     | 40     | 56     |
| 1957-58 | 11                     | 51     | 21     | 83     | 205    |

<sup>a</sup> Measured in ppm dry weight

Dick (1954) and Beck (1956) found that the concentration of copper in the liver varied directly with intake within the range of 3 - 20 mg. per day for cattle and sheep, but not for other species. Beck (1956) obtained a fairly high correlation between copper levels of pasture forages and liver copper levels of animals consuming these forages. Combined with this intake-storage relationship cattle have a greater ability to regulate their liver copper

storage than do sheep, when excessively high intakes are administered. Chapman et al. (1962) increased the average liver copper level of steers from 186 to 616 ppm wet tissue by feeding 12 gm. copper sulfate per day in a gelatin capsule for 16 months. This method of copper administration had no deleterious effects on the steers. However, when the same copper level was administered as a water drench the steers died within 65 days, and the average liver copper level increased from 107 - 2895 ppm wet tissue.

Dempsey et al. (1958) reported that a restriction of dietary copper caused a prompt decrease in serum copper and a somewhat slower and less extensive depletion of liver copper in adult rats. On the other hand, a high copper intake increased the concentration of copper in both serum and liver. The increase was more rapid in serum than in liver during the first 20 days; thereafter it was greater in liver than in serum.

Blood copper levels in cattle have been reported by Gessert et al. (1952) and Beck (1941) to range between 62 - 166 and 70 - 170 mcg. per 100 ml. blood, respectively. Subsequently, Beck (1956) reported the copper level of sheep blood to be 102 mcg. per 100 ml. and cows' blood to be 100 mcg. per 100 ml.

Cox and Mueller (1937) reported cows' milk to contain 0.6 mg. copper per liter, with higher levels occurring in colostrum. Elvehjem et al. (1929) reported that the addition of copper to cow rations did not increase the milk level of copper in cows already adequately supplied with this element. However, as noted by Beck (1941) (cited by Underwood, 1962) cows grazing copper deficient pastures had low levels of copper in their milk.

Robertson (1961) determined the copper content of forages obtained

from the Interlake region of Manitoba (table E). The difference between years in the forage copper level was not significant, but the difference between copper content of forage cut in summer and that cut in fall was significantly different.

TABLE E  
Copper Content of Manitoba Interlake Forages<sup>a</sup>

| YEAR   | AVERAGE | RANGE       |
|--------|---------|-------------|
| 1959   | 4.29    | 1.67 - 6.87 |
| 1960   |         |             |
| Summer | 5.45    | 2.56 - 7.93 |
| Fall   | 3.93    | 2.01 - 5.62 |

<sup>a</sup> Measured in ppm dry weight

Russel and Duncan (1956) reported copper deficiency in cattle grazing pastures containing less than 5 ppm (dry weight) of copper. Beck (1962) classified pastures with 3 - 6 ppm (dry weight) of copper as marginal since copper deficiency developed in cattle and sheep grazing Western Australian pastures with less than 3 ppm copper but not with those containing more than 6 ppm copper. Bredon (1964) studied forages from Uganda, East Africa, and found a copper content of 2.8 - 16.0 ppm with no indication of copper deficiency.

A wide variety of clinical disorders have been associated with a dietary deficiency of copper and some have responded to copper therapy. They include anemia, depressed growth, bone disorders, depigmentation of hair or wool, abnormal wool growth, neonatal ataxia, and gastrointestinal disturbances (Underwood, 1962).

Copper deficiency has been alleviated in Eastern Ontario cattle by feeding 2 gm. copper sulfate per head daily to mature cows, or by ad libitum feeding a mineral mixture containing 1 gm. copper sulfate per ounce to calves (Arthur et al., 1959). This level of supplementation resulted in liver copper levels of 66 ppm in the cows and 80 ppm (wet tissue) in the calves.

Dick and Bull (1945) showed experimentally that the storage of copper in the livers of sheep and cattle could be significantly reduced by an increase in dietary molybdenum intake. The problem has received considerable attention since this discovery and the present knowledge is summarized by Underwood (1962), as follows: For a particular intake of molybdenum, the limitation of liver storage is proportional to the sulfate content of the diet and that only in the presence of adequate sulfate, from dietary or endogenous sources, is this limitation by molybdenum exerted. In addition, (Miller and Engel, 1960) the degree of molybdenosis developed in an animal on any particular molybdenum intake depends upon the dietary intake of copper, sulfate, and protein, with a possible dependence upon the dietary intake of zinc, manganese, and calcium. A problem encountering this interrelationship has been reported in the British Columbia interior by Miltimore et al. (1964). Cattle grazing pastures containing 10.2 ppm copper exhibited profuse scouring. They found that by injecting copper into these cattle weight gains were increased by 0.45 to 0.52 pounds per day, but the scouring still persisted. The molybdenum content of forages causing scouring was 9.7 ppm, compared to 2.2 ppm for non-scouring forages. Thus it was suggested that the high level of molybdenum in these forages was the agent causing scouring, and not the level of copper.

## Molybdenum

Molybdenosis has been observed in several areas of the world including England, California, Nevada, New Zealand, and Manitoba. Attention was first directed to the significance of molybdenum in animals by its association with a disease of grazing cattle, known as "teart", occurring in parts of England. Ferguson et al. (1938) showed that the drastic diarrhea which characterizes this disease was due to ingestion of excessive amounts of molybdenum from the herbage of the affected areas, and could be controlled by treatment of the cattle with large amounts of copper. In a subsequent report by Ferguson et al. (1943) molybdenosis was further characterized by profuse scouring, rapid loss of condition, and development of harsh starring discolored coats. These workers found that the feeding of copper sulfate at the very high rate of 2 gm. per day to cows and 1 gm. per day to young stock quickly controlled the scouring. These findings are not in accord with those of Miltimore et al. (1964). They were unable to stop the scouring by copper administration. This difference in response to copper therapy may be due to the fact that Miltimore et al. (1964) injected the copper whereas Ferguson et al. (1943) gave it orally.

Pastures causing molybdenosis contain 20 - 100 ppm molybdenum on a dry basis as compared to 3 - 5 ppm for non-toxic pastures (Lewis, 1943). Molybdenosis of farm animals in England (Nutrition Reviews, 1962) occurred in areas where the feed contained 6 - 36 ppm of molybdenum. Sheep and cattle were most susceptible to the toxicity, while horses and pigs were most tolerant, and rats, rabbits, guinea pigs and poultry showed intermediate resistance. Musche and Schöberl (1961) reported molybdenosis in grazing cattle

on pastures with 19.2 ppm molybdenum as compared with normal pasture values of 0.47 - 0.68 ppm dry weight. Cunningham (1953) found evidence of molybdenum toxicity on certain farms in the Swan River valley of Manitoba. Molybdenum was found in the toxic forages at levels up to 25.6 ppm and the molybdenum content was found to vary closely with the severity of the toxicity. Forage samples collected from other areas of the province (Brandon and Winnipeg), where molybdenosis had not been observed, contained 0.5 - 1.9 ppm molybdenum. The disease was corrected by the administration of copper sulfate in the form of a drench or salt lick.

Data published by Higgins et al. (1956), and presented in table F, show that molybdenum is not highly concentrated in any particular organ or tissue, although the liver and kidneys contain consistently higher concentrations than do other body organs.

TABLE F

Typical Mo Concentrations in Animal Organs<sup>a</sup>

| SPECIES     | LIVER | KIDNEY | SPLEEN | LUNG | BRAIN | MUSCLE |
|-------------|-------|--------|--------|------|-------|--------|
| Adult Rat:  |       |        |        |      |       |        |
| normal diet | 1.8   | 1.0    | 0.52   | 0.37 | 0.24  | 0.06   |
| Chicken:    |       |        |        |      |       |        |
| normal diet | 3.6   | 4.4    |        |      |       | 0.14   |

<sup>a</sup> Values given in ppm dry weight

Examination of tissue levels of molybdenum in sheep showed that 50 to 75% of the total body molybdenum was located in the skeleton and only about 2% in the liver (Dick, 1956). However, considerable emphasis has been put on



liver levels since they can be influenced by diet. On normal diets the molybdenum level in the liver was of the same order in several species of animals; namely, 2 - 4 ppm dry weight (Higgins et al., 1956). Moore (1958) determined the molybdenum content of 125 samples of adult horse liver. In 109 of the samples it ranged between 3 - 19 ppm, but in five samples the concentrations were 42, 47.6, 54.8, 76.4, and 84.6 ppm. Ralston et al. (1961) in a Washington State survey of beef cows obtained liver molybdenum levels of 2.0 - 9.1 ppm dry weight with no toxicity. They observed a highly significant seasonal variation, which increased during the forage growing season and decreased during winter.

Higgins et al. (1956), Cunningham et al. (1959) and Davies et al. (1960) demonstrated that tissue levels of molybdenum can be increased or decreased, especially in the liver, kidney, bones, and skin, by raising or lowering dietary molybdenum intake. Underwood (1962) relates data of Davis (1950), who increased the molybdenum concentration in rat liver from 1 - 2 to 11 - 12 ppm by raising the molybdenum content of the diet from 1 to 30 ppm. Underwood (1962) reported data of Cunningham (1950) showing that the normal blood level of molybdenum (6 mcg. per 100 ml.) can be readily raised by molybdenum supplementation in the diet. Young cattle and breeding ewes fed diets containing 30 ppm of molybdenum had blood molybdenum levels of 60 - 80 mcg. per 100 ml. and 240 - 340 mcg. per 100 ml., respectively.

Investigations by Dick and Bull (1945) showed unusually high concentrations of molybdenum in livers of cattle suffering from molybdenosis. Cox et al. (1960) fed daily intakes of 200 or 400 ppm molybdenum to calves. This resulted in liver molybdenum levels of 42.4 and 41.7 ppm dry weight,

respectively, which was considerably higher than the 10.8 ppm in calves receiving a diet containing no molybdenum. Calves receiving the two levels of molybdenum showed the molybdenosis syndrome of growth depression, severe diarrhea and emaciation. These workers suggested that a point of molybdenum saturation was reached in the liver of calves receiving a diet of 200 ppm or less of molybdenum. Brinkman et al. (1961) found that feeding up to 400 ppm molybdenum per day severely limited rat growth and increased the liver levels of both molybdenum and copper. Cook et al. (1963) induced molybdenosis in cattle on pasture by giving daily intakes of 0, 68, and 136 mg. molybdenum per 100 pounds body weight. At 100 days cattle on the highest intake exhibited diarrhea, achromatrichia and loss of condition. In addition the cattle fed 68 and 136 mg. had higher plasma (0.028 vs. 0.65 and 0.78 ppm) and liver (0.74 vs. 2.27 and 1.78 ppm) molybdenum levels than control animals.

Lesperance and Bohman (1962) stated that plasma molybdenum is the best immediate criterion of excess molybdenum intake, but liver molybdenum is more indicative of toxicity over a long period of time.

### Cobalt

The first conclusive evidence that cobalt is a dietary essential came in 1935 (Underwood, 1962). This was a result of research by Marston in Southern Australia and Underwood and Filmer in Western Australia into the cause of certain naturally occurring debilitating diseases of sheep and cattle, known locally as "coast disease" and "wasting disease". This discovery gave a great stimulus to studies on the significance of cobalt in ruminant nutrition and led to the delineation of cobalt deficient areas in many parts of the world.

Comer (1948), Braude et al. (1949) and Rothery et al. (1953) studied the distribution of cobalt in the tissues of mice, rats, rabbits, pigs, sheep and cattle following oral or parenteral administration of radiocobalt. They concluded that cobalt was taken up by all tissues with the highest concentrations occurring in the liver and kidneys. Cobalt levels found in sheep tissues are presented in table G (Underwood, 1962). The cobalt levels determined in ovine and bovine livers by several workers are summarized in table H.

TABLE G  
Cobalt Concentrations in Sheep Tissues<sup>a</sup>

| CONDITION        | TISSUES |        |        |       |          |
|------------------|---------|--------|--------|-------|----------|
|                  | LIVER   | SPLIEN | KIDNEY | HEART | PANCREAS |
| Healthy          | 0.15    | 0.09   | 0.25   | 0.06  | 0.11     |
| Cobalt-deficient | 0.02    | 0.03   | 0.05   | 0.01  | 0.02     |

<sup>a</sup> Measured in ppm dry weight

TABLE H  
Cobalt Concentrations in Ovine and Bovine Livers

| SPECIES AND CONDITION    | COBALT CONCENTRATION <sup>a</sup> | REFERENCE                    |
|--------------------------|-----------------------------------|------------------------------|
| Sheep; cobalt-deficient  | 0.06                              | Underwood and Harvey (1938)  |
| Sheep; healthy           | 0.28                              | Underwood and Harvey (1938)  |
| Sheep; "coasty"          | 0.09                              | Marston <u>et al.</u> (1948) |
| Sheep; healthy           | 0.34                              | Marston <u>et al.</u> (1948) |
| Cattle                   | 0.32 (0.11 - 1.00)                | Gessert <u>et al.</u> (1952) |
| Cattle                   | 0.24 (0.12 - 0.40)                | McNaught (1948)              |
| Cattle; cobalt-deficient | 0.06                              | Correa (1957)                |
| Cattle; healthy          | 0.20                              | Correa (1957)                |

<sup>a</sup> Measured in ppm dry weight

Ralston *et al.* (1961) in a survey of Washington State cattle determined the liver cobalt levels for nine herds in 1956-57 (table J). No deficiency symptoms were noted by visual appraisal.

TABLE J

Seasonal Cobalt Levels in Beef Liver<sup>a</sup>

| FALL | SEASON |        | AVERAGE |
|------|--------|--------|---------|
|      | SPRING | SUMMER |         |
| 2.8  | 2.6    | 0.4    | 1.9     |

<sup>a</sup> Measured in ppm dry weight

The concentration of cobalt in the liver of sheep and cattle varies little with age but is sufficiently responsive to changes in dietary cobalt intake to be a valuable aid in diagnosing cobalt deficiency in the field (Underwood, 1962). From an extensive New Zealand study McNaught (1948) suggested that cobalt levels of 0.04 - 0.06 ppm, or less, on a dry basis in the livers of sheep and cattle indicate cobalt deficiency, and that 0.08 - 0.12 ppm, or more, indicate a satisfactory cobalt status.

The average cobalt content of normal cows' milk has been reported by Ellis and Thompson (1945) to be 0.6 mcg. per liter, and by Archibald (1947) as 0.5 mcg. per liter. Archibald (1947) was able to increase the cobalt concentration of normal cows' milk by supplementing ordinary rations with liberal amounts of cobalt. Supplementing the cow, and presumably the ewe, with cobalt could, therefore, be an effective means of raising the cobalt intake of nursing animals in cobalt deficient areas. That cobalt readily passes the placental barrier, was demonstrated by Thompson and Ellis (1947). These workers observed

increases of up to 50% in the liver and kidney cobalt levels of newborn calves, when the dams had received a prepartum dietary cobalt supplement for 21 - 120 days. McNaught (1948) showed that cobalt, unlike copper and iron, does not normally accumulate in the fetal liver. On the other hand O'Halloran (1961) showed that the concentration of cobalt in the liver of newborn lambs and calves is reduced below normal levels when the dam has received a cobalt-deficient diet. Therefore, information regarding the fetal liver content of cobalt is still controversial.

Cobalt functions in animal tissues as an integral part of the vitamin B<sub>12</sub> molecule (Smith et al., 1951). Underwood (1962) has summarized the recent knowledge in this field. Under conditions of cobalt sufficiency the majority of liver cobalt exists in the form of vitamin B<sub>12</sub>. Cobalt deficiency arises in the ruminant only when liver stores of vitamin B<sub>12</sub> are depleted. Therefore, cobalt deficiency is basically a vitamin B<sub>12</sub> deficiency. This is brought about by the inability of rumen microorganisms, in the presence of inadequate dietary cobalt, to synthesize sufficient quantities of this vitamin to meet the needs of the host animal. The cobalt deficiency symptoms are loss of appetite, loss of body weight, weakness, rapid wasting and severe anemia culminating in death.

Cobalt deficiency occurred in animals grazing pastures containing less than 1 ppm of cobalt in the dry matter of the forage (Russel and Duncan, 1956). Bredon (1964) published cobalt levels for Uganda forages of 0.04 - 0.63 ppm, and stated that a seasonal cobalt deficiency occurred in some areas of this country. Bentley et al. (1951) found hay to contain 0.042 (0.02 - 0.08) ppm and silage 0.09 (0.03 - 0.16) ppm of cobalt, and concluded that cobalt is a critical element in Northwestern Wisconsin. Robertson (1961)

determined the cobalt content of forages from the Interlake area of Manitoba (table K), and recommended cobalt supplementation for cattle and sheep in this area. He observed no significant difference between the year averages of 1957 and 1959, but in 1960 the cobalt content was significantly lower than in the two earlier years.

TABLE K  
Cobalt Content of Interlake Forages<sup>a</sup>

| YEAR | COBALT  |               |
|------|---------|---------------|
|      | AVERAGE | RANGE         |
| 1957 | 0.094   | 0.020 - 0.176 |
| 1959 | 0.097   | 0.040 - 0.139 |
| 1960 | 0.064   | 0.022 - 0.137 |

<sup>a</sup> Measured in ppm dry weight

Ray et al. (1948), Becker et al. (1949), and Marston and Lee (1949) showed that liver cobalt levels could be increased to 10 times normal levels by cobalt injection. However, relatively small quantities of these stores were available for vitamin B<sub>12</sub> synthesis in the rumen. Oral dosing of cobalt-deficient lambs with cobalt increases liver vitamin B<sub>12</sub> to normal levels, but the proportionate increase in liver cobalt is greater (Underwood, 1962). This suggests that rumen microorganisms are able to convert only a limited amount of dietary cobalt to vitamin B<sub>12</sub>. Supplementation should, therefore, consist of a uniform intake over an extended period of time rather than a single large dose. The work of Skerman et al. (1959) suggests that this also applies to cattle.

Ely et al. (1948), Becker et al. (1951), and Dunn et al. (1952) found a very wide margin of safety between the quantities of cobalt necessary to fulfill the nutritional requirements of sheep and cattle and the toxic limits of the element. These workers found that levels 100 times those normally supplied were tolerated by sheep and cattle.

#### Vitamin A and Carotene

Vitamin A, often ingested as its precursor carotene, has long been recognized as a dietary essential for beef cattle. The relative importance of vitamin A in beef cattle nutrition was demonstrated by Ensminger et al. (1955). These workers made a survey of American cattlemen and found that vitamin A deficiency accounted for 26% of all nutritionally sick cattle in the United States.

Guilbert and Hart (1934) in studies with beef cattle showed that the liver was the principal storage area for vitamin A. Further, Maynard and Loosli (1956) state that 67 to 93% of body vitamin A storage is in the liver and measurement of these stores is a useful technique in studies of vitamin A status. The liver vitamin A reserves of cows grazing green pastures may be quite variable. Baker et al. (1954) observed a range of 62 - 462 mcg. vitamin A per gm. dry liver in beef cows during a November sampling period.

Liver tissue has a remarkable capacity to store vitamin A (Shirley et al., 1962). Liver vitamin A levels are directly related to vitamin A intake. Hale et al. (1961) found that 40,000 I.U. of vitamin A per day were required to maintain initial stores of 96 mcg. per gm. fresh liver; however, an intake of 2,500,000 I.U. per day increased the store to 5,000 mcg. per gm. fresh liver. Perry et al. (1962) fed steers daily vitamin A intakes of 10,000;

20,000; 30,000; 40,000 and 50,000 I.U. and observed final liver vitamin A levels at 256 days of 7.0; 22.0; 48.5; 64.1 and 120.3 I.U. per gm. fresh liver, respectively. The corresponding blood vitamin A levels were 36.6; 45.0; 53.3; 52.0 and 59.1 mcg. per 100 ml. blood plasma. The plasma carotene levels were fairly consistent around 35 mcg. per 100 ml. on all levels of vitamin A intake. Guilbert and Hart (1934) and Wheeler et al. (1957) both concluded that pregnant range cows accumulate large stores of vitamin A when grazing summer range. This store will supply their vitamin A needs during winter even when very low levels of carotene or vitamin A are available in their wintering rations. Liver vitamin A levels of both carotene supplemented and non-supplemented cows decreased during late gestation and lactation (Wheeler et al., 1957 and Baker et al., 1954). However, after these cows were released to green range forage in the spring their hepatic reserves of vitamin A began to increase. The peak plasma carotene and vitamin A and liver carotene values were reached in June, but the peak in liver vitamin A level was not reached until August (table L).

TABLE L

Average Levels for "Normal" Cows in Northern Great Basin <sup>1</sup>

|                               | MONTH <sup>2</sup> |      |           |       |
|-------------------------------|--------------------|------|-----------|-------|
|                               | MAY                | JULY | SEPTEMBER | MARCH |
| Plasma Carotene <sup>a</sup>  | 3.0                | 9.1  | 1.0       | 3.0   |
| Liver Carotene <sup>b</sup>   | 4.5                | 7.0  | 3.5       | 2.8   |
| Plasma Vitamin A <sup>a</sup> | 0.25               | 0.59 | 0.33      | 0.32  |
| Liver Vitamin A <sup>b</sup>  | 50                 | 120  | 65        | 64    |

<sup>1</sup> Wheeler et al. (1957)

<sup>2</sup> Representative months selected from the established yearly pattern

<sup>a</sup> Measured in mcg. per ml.

<sup>b</sup> Measured in mcg. per gm. fresh liver



Pope et al. (1961) studied the effects of low carotene intake for 41 months on reproduction and tissue vitamin A levels (table M) in young beef cows. Each cow conceived and produced normal calves during each of the first two years, but all aborted at 6 - 7 months of the third pregnancy (at 42 months depletion).

TABLE M  
Depletion of Vitamin A and Carotene Reserves on Low  
Carotene Intake

| MONTHS OF DEPLETION | LIVER VITAMIN A <sup>a</sup> | PLASMA CAROTENE <sup>b</sup> | PLASMA VITAMIN A <sup>b</sup> |
|---------------------|------------------------------|------------------------------|-------------------------------|
| 0                   | 360                          | 60                           | 22                            |
| 12                  | 15                           | 30                           | 34                            |
| 24                  | 5                            | 10                           | 17                            |
| 36                  | 5                            | 22                           | 17                            |
| 42                  | 5                            | 8                            | 9                             |

<sup>a</sup> Measured in mcg. per gm. dry matter

<sup>b</sup> Measured in mcg. per 100 ml.

Ralston and Dyer (1960) in their survey of Washington State observed no symptoms of vitamin A deficiency. In addition, the seasonal means of liver samples taken from cows in the fall and summer were significantly higher in vitamin A than were those taken in the winter and spring, with little difference between the latter two seasons (table N). In contrast, liver carotenoid levels were significantly different for each season. Plasma carotenoids and vitamin A samples taken in the spring and summer were significantly higher than those taken in the fall and winter. Furthermore, fall samples were significantly higher than samples taken during the winter.

TABLE N  
Average Plasma and Liver, Vitamin A and  
Carotenoids of Washington State Beef Cows

|                          |                      | SUMMER | FALL | WINTER | SPRING |
|--------------------------|----------------------|--------|------|--------|--------|
| <b>LIVER</b>             |                      |        |      |        |        |
| Carotenoids <sup>a</sup> | 1956-57 <sup>d</sup> | 16.3   | 13.6 | 10.0   | 19.8   |
|                          | 1957-58 <sup>e</sup> | 9.9    | 15.0 | 15.5   | 11.8   |
| Vitamin A <sup>a</sup>   | 1956-57              | 156    | 177  | 138    | 133    |
|                          | 1957-58              | 358    | 287  | 257    | 261    |
| <b>PLASMA</b>            |                      |        |      |        |        |
| Carotenoids <sup>b</sup> | 1956-57              | 8.28   | 5.65 | 3.82   | 7.39   |
|                          | 1957-58              | 5.03   | 9.03 | 13.56  | 8.27   |
| Vitamin A <sup>c</sup>   | 1956-57              | 55.0   | 32.1 | 40.5   | 53.6   |
|                          | 1957-58              | 40.9   | 31.0 | 32.4   | 39.9   |

<sup>a</sup> Measured in mcg. per gm. fresh tissue

<sup>b</sup> Measured in mcg. per ml.

<sup>c</sup> Measured in mcg. per 100 ml.

<sup>d</sup> Average of nine locations

<sup>e</sup> Average of 11 locations

Marsh and Swingle (1960) determined the carotene and vitamin A levels of blood plasma from Hereford cattle at the United States Range Livestock Experiment Station at Miles City, Montana from May 1948 - June 1953. Plasma carotene showed an enormous seasonal variation. The concentration of carotene reached an average value between 500 and 1,000 mcg. per 100 ml. during each growing season (May to July), and fell to values below 50 mcg. per 100 ml. each winter. An October peak level was observed during one year due to an unusual

September rainfall and growth of green feed. Plasma vitamin A levels rose and fell seasonally with the carotene level, but over a much narrower range. The growing season maxima were between 30 and 41 mcg. per 100 ml. and winter minima between 18 and 25 mcg. per 100 ml.

Many workers have attempted to determine the relationships between blood and hepatic levels of vitamin A and carotene. If the levels are sufficiently correlated, those levels most easily obtained could be used to predict the levels less readily determined (Diven et al., 1960). These workers found that hepatic vitamin A was positively related to both hepatic and plasma carotenoids; however, there was no relationship to plasma vitamin A. Ralston and Dyer (1960) and Hale et al. (1961), similarly, found no relationship between the hepatic and plasma levels of vitamin A. However, Wheeler et al. (1957) did obtain a significant correlation between plasma and hepatic vitamin A levels. Correlation coefficients were, however, so small that any known variable had little predictive value (Wheeler et al., 1957; Diven et al., 1960).

Braun and Carle (1943) and Eaton et al. (1949) showed that extremely high intakes of carotene or vitamin A are necessary before substantial placental transfer occurred. Baker et al. (1954) observed that liver stores of vitamin A and carotene of newborn calves did not appear to be related to carotene supplementation of their dams during gestation or to the dam's liver stores at parturition. The dam's liver vitamin A level decreased during the first three months of lactation regardless of whether or not carotene was fed during this period. However, when carotene was fed the milk level of vitamin A increased, and the calves' liver stores of vitamin A at three months of age were 14.3 mcg. as compared to 2.5 mcg. per gm. dry matter for the calves of non-supplemented

cows. Roberts and Dyer (1959) found, that over a 170 day period, gestating heifers receiving 30 mg. carotene per day decreased their liver vitamin A level from 76 to 37 mcg. per gm. fresh liver. On the other hand, heifers receiving carotene-free rations decreased their liver vitamin A level from 76 to 19 mcg. per gm. fresh liver. All heifers produced calves which were small but otherwise normal at birth, with the exception of two heifers out of 20 which produced dead calves on the carotene-free ration. Liver vitamin A stores were low (7.5 to 3.1 mcg. per gm. fresh liver) in the calves at birth. The higher levels were noted in calves from dams receiving carotene as compared to those receiving no carotene. At 56 days of age there was a marked but variable increase in the liver vitamin A level of all calves.

Van Arsdell (1950), Roberts and Dyer (1959), and Pope et al. (1961) found that milk levels of vitamin A and carotene decreased with increasing time after parturition, regardless of carotene intake. However, the milk produced by dams receiving carotene was higher in both vitamin A and carotene than milk produced by dams receiving low or carotene-free diets.

Ralston and Dyer (1960) concluded from their ranch survey that supplementation of Washington cattle with vitamin A on a general basis seems ill-advised, but during extreme drought and adverse weather it may become necessary. Since vitamin A is not efficiently transported across the placental membranes, a period of 30 - 60 days prepartum and 30 - 60 postpartum supplementation may be advisable, especially when the quality of feed is poor. Wheeler et al. (1957) stated that vitamin A deficiency may be expected in young calves that are nursing their dams and are not permitted to consume at least small quantities of carotene containing feeds. Such conditions are, however, unusual.

The length of time required to produce vitamin A deficiency in cattle depends upon reserves, production requirements and level of intake (Guilbert and Hart, 1934). Wheeler et al. (1957) observed night blindness in cows kept on a very low carotene intake for one year. Hepatic reserves of carotene and vitamin A at the end of this period were 0.42 and 0.82 mcg. per gm. fresh liver, respectively.

Vitamin A deficiency has been reported in Alberta cattle (O'Donoghue, 1955). It was observed in young weak calves that suffered from diarrhea and sometimes secondary infections. Also, it appeared in older animals with damage to the peripheral nervous system which gave rise to incoordination. Finally, vitamin A deficiency caused yearling beef animals, without incoordination, to become blind through injury to the optic nerve.

#### Calcium and Phosphorus

These two mineral elements will be discussed together because, according to Maynard and Loosli (1956), they occur in the body, for the most part, in combination with each other. An inadequate supply of either in the diet can limit the nutritive value of both. Approximately 99% of the calcium and 80% of the phosphorus in the body are present in bones and teeth. The remaining small percentages are found throughout soft tissues of the body.

Whole blood and plasma levels of calcium and phosphorus have received a lot of attention in recent years. Blood cells are almost or entirely devoid of calcium, but the serum in normal animals contains 9 to 12 mg. per 100 ml. in most species (Maynard and Loosli, 1956). These authors cite whole blood as containing 35 to 40 mg. total phosphorus per 100 ml., most of which is in the organic form. This organic phosphorus is in continuous exchange with the inorganic phosphorus of plasma. In normal animals the

plasma inorganic phosphorus level ranges from 4 to 9 mg. per 100 ml. (Maynard and Loosli, 1956).

Numerous studies have been conducted to determine the average blood plasma levels of calcium and phosphorus in beef cattle. These studies are briefly summarized in table P.

TABLE P  
Calcium and Phosphorus in Blood Plasma of Beef Cows

| CATTLE TYPE             | CALCIUM<br>(mg./100 ml.) | INORGANIC<br>PHOSPHORUS<br>(mg./100 ml.) | REFERENCE                      |
|-------------------------|--------------------------|--|--------------------------------|
| Range beef<br>cattle    | 9.3 - 10.3               | 4.4 - 6.8                                | Bohman <u>et al.</u> (1961)    |
| Range cows              | 9.9 - 11.3               | 4.3 - 7.5                                | Savage and Heller (1947)       |
| Beef cows               | 10.6 - 12.8              | 1.4 - 4.9                                | Knox (1941)                    |
| Breeding<br>cows        |                          | 2.11 - 5.37                              | Watkins and Knox (1948)        |
| Beef cattle             | 10.8                     | 4.6                                      | Davis <u>et al.</u> (1958)     |
| Range beef<br>cows      | 12.0                     | 5.0                                      | Ralston <u>et al.</u> (1961)   |
| Beef cattle             |                          | 5.9                                      | Robinson and Huffman<br>(1926) |
| Aged cows               | 9.5                      | 4.9                                      | Payne <u>et al.</u> (1946)     |
| Two year<br>old heifers | 9.1                      | 5.1                                      | Payne <u>et al.</u> (1946)     |
| Herd bulls              | 13.0                     | 4.8                                      | Payne <u>et al.</u> (1946)     |
| Beef cattle             | 8.8 - 11.0               | 7.0 - 7.5                                | Ginsburg (1963)                |

Marsh and Swingle (1960) reported results of a five-year study on blood constituents of range cows at the United States Range Livestock Experiment Station at Miles City, Montana. The annual average plasma inorganic phosphorus levels of 1 to 6 year old female stock were 5.42, 4.39, 3.50, 3.97

and 3.68 mg. per 100 ml. The overall five-year average was 4.27, and the average for all cows during the three years of calf production was 3.72 mg. per 100 ml. The range of individual samples was from 0.7 to 9.8 mg. per 100 ml. plasma. The seasonal variation in plasma inorganic phosphorus, in general, paralleled the precipitation curve. The levels were high in May, June and July, dropping in late summer and fall to winter lows, which were at least 25% below the average for the year. The annual average plasma calcium levels of these cattle were 10.08, 10.04, 10.64, 9.73 and 9.11 mg. per 100 ml. The five-year average was 9.94 mg. and the average for the last three years was 9.80 mg. per 100 ml. The minimum and maximum values for individual samples were 6.6 and 14.7 mg. per 100 ml., respectively. The plasma calcium fluctuations were much less extensive than those of plasma inorganic phosphorus. However, there was no consistent pattern of seasonal variation in plasma calcium, nor relation to the plasma inorganic phosphorus level. The cows showed no clinical symptoms of phosphorus or calcium deficiency and produced normal calves each year.

Bohman et al. (1961) found a slight seasonal trend in plasma calcium of beef calves with maximum values occurring in the period June - August and minimum values in March. Thus, the effect of season upon plasma calcium level has not been clearly established. However, Bekemier et al. (1961) worked with dogs housed indoors under controlled conditions for one year. The mean value obtained for serum calcium was 11.06 mg. per 100 ml., with highest values in the periods from February to March and September to November and lowest values in the periods from December to January and June to August. However, the departures from the yearly mean averaged only about 0.25 mg. per 100 ml. These workers found the serum inorganic phosphorus

level to be 5.19 mg. per 100 ml. with no regular seasonal variation.

The Ca:P ratio in the ration is important for efficient assimilation of dietary calcium and phosphorus. Maynard and Loosli (1956) define the desirable ratio as one lying between 2:1 and 1:2, with adequate nutrition being possible outside these limits. Dowe et al. (1957) fed Ca:P ratios of 1.34:1.0, 4.34:1.0, 9.10:1.0 and 13.70:1.0 in growing rations of beef calves. The average rate of gain of the animals decreased as the Ca:P ratio became wider. These workers concluded that the critical Ca:P ratio lies between 4.3:1.0 and 9.10:1.0.

The effect of calcium and phosphorus intake on the blood level of these mineral elements has been investigated by many workers. The present viewpoint can be ascertained from a brief chronological summary. Greaves et al. (1934) found a close correlation between phosphorus intake and the inorganic phosphorus level of serum. Knox (1941) found this correlation to be 0.61. Watkins and Knox (1945) working with New Mexico grasses and range cows found that the plasma inorganic phosphorus level of non-supplemented cows was 2.16 mg. per 100 ml. when phosphorus intake was 6.9 gm. per day. The same ration after phosphorus supplementation to give intakes of 7.40 and 8.45 gm. per day, gave plasma inorganic phosphorus levels of 3.00 and 3.24 mg. per 100 ml., respectively. Watkins and Knox (1948) were able to raise plasma inorganic phosphorus levels by 0.32 mg. per 100 ml. by feeding cows a phosphorus supplement throughout the year as compared to feeding the supplement only during the winter months. Lewis et al. (1951) reported that plasma inorganic phosphorus levels were related to phosphorus intake, but plasma calcium levels showed fluctuations, which were not affected by calcium intake or related to the plasma inorganic phosphorus



level. Dowe et al. (1957) fed rations containing wide Ca:P ratios to beef calves and found that the excess calcium intake was not reflected by a change in the plasma calcium level, nor did it have any effect on the plasma inorganic phosphorus level. Wise et al. (1961) changed calves from a high phosphorus ration to a ration containing 0.11% phosphorus, and observed a decline in serum inorganic phosphorus from 8.5 to 4.7 mg. per 100 ml. over an eight-week period. These calves, and calves in a similar second experiment, showed a marked decline in serum phosphorus level during the first 14 days which was followed by a slower but steady decline. Bohman et al. (1961) gave a phosphorus supplement to calves on semidesert range and found that the rate of weight gain was increased, especially in the summer. This phosphorus supplementation increased the level of plasma phosphorus immediately, and the cattle which were supplemented had higher levels each month throughout the year. These workers also gave dietary calcium supplements but observed no effect on the plasma calcium level.

Marsh and Swingle (1960) concluded that the average plasma calcium (9.8 mg. per 100 ml.) and inorganic phosphorus (3.72 mg. per 100 ml.) levels, which they established, could be considered as within the normal range for cattle in the Northern Great Basin of the United States. Ralston et al. (1961) concluded from their Washington State survey of beef cows that in the Pacific Northwest cattle would be more apt to suffer from an imbalance rather than a deficiency of calcium per se.

Henderson and Weakley (1930) and Huffman et al. (1933) (cited by Dowe et al., 1957) reported that a low concentration of inorganic phosphorus in the blood plasma is an indication of a dietary phosphorus deficiency. Davis (1959) reported that a plasma inorganic phosphorus level of 5 - 7 mg. per 100 ml. was normal for young cattle, but that a lower value was almost

conclusively diagnostic of a deficient situation.

### Magnesium

Though present in the body in a much smaller amount, magnesium is closely associated with calcium and phosphorus, both in its distribution and metabolism (Maynard and Loosli, 1956). Approximately 70% of the body supply is in the skeleton, the remainder being found widely distributed in the various fluids and other soft tissues. Depending on the species and individual the blood serum level of magnesium is 2 to 5 mg. per 100 ml. (Maynard and Loosli, 1956).

Marsh and Swingle (1960) determined the plasma magnesium content of range cattle over a five-year period and obtained annual averages of 1.52, 1.42, 1.76, 1.56 and 1.46 mg. per 100 ml. when the female stock were 1 to 5 years of age. The five-year average was 1.55 mg. per 100 ml. and the average for the last three years, while producing calves, was 1.59 mg. per 100 ml. The minimum and maximum values for individual cows were 0.0 and 3.2 mg. per 100 ml., respectively. In each of the five years the low point occurred in one of the spring months; April, May or June. The low magnesium levels occurred at the time of year when the hypomagnesaemic disease known as "grass tetany" characteristically occurs. However, these workers did not observe this condition in any of the experimental cattle. The authors concluded that the plasma magnesium values they obtained can be considered normal for range cattle in the Northern Great Basin area. If a magnesium deficiency did not occur in cows with 0.0 mg. of magnesium per 100 ml. plasma there must have been an error in their chemical analysis.

Further studies are lacking on the plasma magnesium levels that can be expected in normal range cows. However, studies on the effect of various

levels of magnesium intake on plasma magnesium concentrations have been reported. Mayo et al. (1959) in determining the magnesium requirement of the pig found that serum magnesium levels were reduced in pigs showing symptoms of magnesium deficiency. Smith (1959a) injected calves receiving a low magnesium diet with 1 gm. magnesium per day and increased plasma magnesium from 1.65 to 3.59 mg. per 100 ml. at one hour post-injection, and to 2.16 mg. per 100 ml. at 24 hours post-injection. The relationships between magnesium intake and plasma level that this worker demonstrated are shown in table R.

TABLE R

Plasma Mg. Level as Affected by Mg. Intake

| Mg. INTAKE<br>(gm./day) | PLASMA Mg. LEVEL<br>(mg./100 ml.) |
|-------------------------|-----------------------------------|
| 1.16                    | 1.33                              |
| 3.10                    | 1.88                              |
| 0.055                   | 0.95                              |
| 1.07                    | 0.92                              |

Storry and Rook (1963) removed a supplement of 5 gm. magnesium oxide from the diet of two dry Friesen cows and observed a drop in serum magnesium from 2.7 to 2.0 mg. per 100 ml. after two days, and to 1.0 and 1.5 mg. per 100 ml. after 14 days. In contrast, Brôchart and Larvor (1961) maintained two pairs of monozygous twin heifers in stalls for a years' study. No close relationship between intake and serum magnesium level was found, but there appeared to be some seasonal variation with the highest values in spring and the lowest in autumn.

Smith (1961) and Parr (1957) found that in milk-fed calves the fall in plasma magnesium was accompanied by a fall in plasma calcium. However, both the plasma magnesium and calcium levels could be restored to normal by magnesium supplementation. Parr (1957) reduced the serum magnesium and calcium levels to 0.4 and 6.0 mg. per 100 ml., respectively, with the milk diet and then restored both levels to normal in 48 hours, by supplementing with 8 gm. magnesium per day. Smith (1961), in giving the magnesium supplement, raised the plasma magnesium level from 1.35 to 2.34 mg. per 100 ml. and the plasma calcium level from 8.5 to 10.8 mg. per 100 ml.

Parr (1957) reported that the magnesium content of muscle, liver, and kidney from hypomagnesaemic calves was not significantly different from that of calves with normal serum magnesium levels. There was, however, a considerable reduction in bone magnesium. They also observed that hypocalcaemia and hypomagnesaemia are usually concomitant conditions in calves.

Smith (1959b) observed that normal bone is saturated with magnesium. He concluded that under normal conditions, when excess magnesium is entering the blood, the bone plays little or no part in regulating the plasma magnesium concentration. The control is effected by changes in urinary excretion. However, under conditions of subadequate magnesium intake, bone magnesium is liberated into the soft tissues and in hypomagnesaemia an interrelationship exists between bone and plasma magnesium. The plasma is not maintained at a normal concentration, and under this condition it drops in direct relation to the drop in bone magnesium. When calves were placed on a low magnesium diet the initial plasma magnesium fall was rapid, and then decreased much more slowly after reaching a level of about 0.7 mg. per 100 ml. The depletion of bone magnesium appeared to occur at a constant rate. Drops in

plasma magnesium concentration to 1.6 and 0.7 mg. per 100 ml. were associated with bone-ash magnesium values of 0.60 to 0.67% and 0.40 to 0.48%, respectively. Shortly before death of the calves bone magnesium values of 0.2 - 0.3% (about 60 - 70% depletion) were observed in association with plasma magnesium levels of about 0.3 to 0.5 mg. per 100 ml. Repeated injections of magnesium, following depletion, eventually restored both bone (9 - 16 days) and plasma (5 days) to their normal levels.

#### Sampling Techniques

Chapman et al. (1963) evaluated the liver biopsy technique for mineral nutrition studies with beef cattle. They describe the technique as an easy, simple operation with the main prerequisite being adequate means of immobilizing the animal. An examination of the livers, following biopsy, showed that no abscesses were present. All biopsy samples obtained by the technique consistently came from a very small area of the liver. These workers analysed samples taken from various sites in the liver for copper and molybdenum (table S). The site of biopsy did not significantly effect the level of molybdenum or copper (second study), nor was there a significant level x biopsy site interaction.

In direct contrast with the above data are those of Cassidy and Eva (1958). They obtained 30 liver subsamples of 40 gm. each from each of four pigs. Samples were taken so that all four lobes of each liver were represented. Chemical analysis revealed variations in the copper concentrations of samples from any one animal, which were considered sufficient to invalidate analysis from a single liver biopsy sample.

Beck (1956) states that liver copper values are subject to errors from two sources:

TABLE S

Average Cu and Mo Content of Liver Biopsy Samples

|                                  | SITE OF BIOPSY |      |      |      | AVERAGE |
|----------------------------------|----------------|------|------|------|---------|
|                                  | A              | B    | C    | D    |         |
| Cu - first study <sup>a</sup>    | 234            | 248  | 258  | 223  | 241     |
| Cu - second study <sup>a b</sup> | 567            | 632  | 609  | 586  | 598     |
| Mo <sup>a c</sup>                | 31.1           | 31.1 | 31.5 | 30.5 | 31.7    |

<sup>a</sup> Measured in ppm dry weight

<sup>b</sup> Each value is the average of six levels of Cu SO<sub>4</sub> intake

<sup>c</sup> Each value is the average of three levels of Mo intake

(1) An uneven distribution of copper in the liver. Duplicate samples from the same liver may vary up to 20%, although the difference is usually less.

(2) The presence of variable amounts of fat in the liver acts as a diluent and lowers liver copper concentrations (usually of rare occurrence).

Under normal conditions of cobalt sufficiency, most liver cobalt exists in the form of vitamin B<sub>12</sub> (Underwood, 1962). Thus the liver cobalt levels determined by biopsy sample should give a reasonably reliable indication of the cobalt status of the animal. However, more accurate measures would be the cobalt concentration in rumen ingesta (Phillipson and Mitchell, 1952) or vitamin B<sub>12</sub> concentration in the liver (Andrews *et al.*, 1958).

Anderson *et al.* (1962) measured the variation in hepatic vitamin A content of four sampling sites in 96 beef livers containing 2 - 5375 mcg.

vitamin A per gm. fresh tissue. Some significant differences in concentration were noted between sites. However, in some groups with the widest confidence interval about group means there were no significant site differences. This finding indicates that differences in site sampling, although measurable, do not have an appreciable influence upon the validity of the biopsy sample as an estimate of hepatic vitamin A levels. These workers found that the liver sample has a confidence interval, not greater than  $\pm 21.5\%$  if the vitamin A level is in the range 30 - 160 mcg. per gm. liver tissue and, alternately, not greater than  $\pm 62.0\%$  if the level is outside this range, when expressed as a percent of the observed value.

Pope et al. (1961), in an attempt to gain information on homogeneity of liver stores, obtained four livers from freshly slaughtered cattle. They took duplicate samples from six locations in various parts of the liver which included each lobe. The resulting data are presented in table T. The standard error appeared to increase with liver vitamin A concentration whereas the coefficient of variation implied the opposite trend. To these workers it appeared that the location from which biopsy samples were taken (dorsal lobe) was as representative as any other site. Furthermore, biopsy samples taken by subsequent biopsies came from a very small area of the liver, which makes relative comparisons quite valid.

Varnell and Erwin (1959), concluded, from adrenalin injection studies in sheep and cattle, that vitamin A and carotene levels in the liver and blood should not be influenced by the variation in excitability at sampling time.

Wise et al. (1961), in an attempt to determine the availability of phosphorus from various dietary sources for calves, found that, among the

response criteria used, serum inorganic phosphorus was one of the most sensitive and reliable. One distinct advantage of this criterion was that treatment differences were exhibited early and were persistent.

TABLE T

Average Vitamin A in Four Livers with Standard Error of Estimates

| LIVER | VITAMIN A<br>(mcg. per gm. wet tissue) | STANDARD ERROR | COEFFICIENT<br>OF VARIATION |
|-------|--|----------------|-----------------------------|
| A     | 14.3                                   | 0.72           | 12.3                        |
| B     | 8.2                                    | 0.53           | 15.7                        |
| C     | 66.3                                   | 2.53           | 9.4                         |
| D     | 12.2                                   | 1.04           | 20.9                        |

Payne *et al.* (1946) stated that plasma calcium levels may not reflect the true calcium metabolism picture, due to the withdrawal of calcium from the bones and soft tissues in order to maintain a normal plasma calcium level. Hence, the calcium level of blood plasma, when used as an index of the existing calcium picture, must be interpreted with caution.

Smith (1959b) showed that bone plays little or no part in the control of plasma magnesium under conditions of magnesium excess, but on magnesium deficient diets the concentration of plasma magnesium was influenced directly by the concentration of bone magnesium. This author concluded, therefore, that plasma magnesium is only an approximate guide to the magnesium status of individual animals.



## EXPERIMENTAL PROCEDURE

### General Procedure

Beef cows from 12 Manitoba herds were used in this study. The herds were localized in two main geographical areas, with seven herds in the Southwestern area of the Province and five herds in the Ste. Rose area. A map showing the two areas of the Province is given in Appendix I. The herds were selected within each area by Representatives of the local Agricultural District and Livestock Branch of the Manitoba Department of Agriculture and Conservation. The selected herds were ones which appeared to be normal and representative of the cow-calf type operations in these areas. Of the farmers asked to cooperate in the survey, only those willing to submit their herds for study during two consecutive years were used. No attempt was made to sample any particular breed of beef cow and, as a result, the herds selected consisted of a mixture of the three main beef breeds present in Manitoba (six Hereford, one Angus, and five Shorthorn X Hereford).

No control was exercised over the 12 different herds in regard to husbandry or feeding regime, with one exception. Throughout the first year of the study, the liver copper level of herd 12 was very low and the calves were scouring and generally unthrifty. The farmer was encouraged to feed a copper supplement during the winter of 1963-64 with the result that he gave the cows a mineral mixture containing 2% copper sulfate ad libitum. The remaining farmers were asked to continue their previous management practices throughout the tenure of the study. This resulted in different types of forages and different amounts of vitamin A and/or mineral supplements, if any, being fed. The major forage fed in the Ste. Rose area was Interlake

hay (Robertson, 1961) and in the Southwestern area the forage was approximately 50% native and 50% tame. All 12 farmers obtained their forages locally and the cattle grazed local pastures. The mineral supplements fed (ad libitum) were commercial mixtures which varied in composition. The vitamin A was either mixed with the minerals or dispersed in the animal's daily water supply. Very little grain, if any, was fed to the cows during the winter months. All herds were given cobalt-iodized salt (free choice) the year round.

As a general rule all farmers' bred their cows in June, July or August and weaned the calves in the fall.

A questionnaire (Appendix III Table IV), covering management practices, was answered by each farmer at the outset of the survey. A visual appraisal was made of each herd by the author during the two year survey period for management practices and herd condition, and scored as fair, good, or very good (Appendix III Table V).

#### Sample Collection

Liver and plasma samples were collected from each herd within the two areas, three times per year for two consecutive years (herds 4, 7 and 8 were not sampled in summer, 1963). The study began in July 1962 and concluded in May 1964 when the sixth and final collection was made. The yearly collections were made (1) in mid-summer at the height of the pasture season when the cows had calves at foot (July, with the Ste. Rose summer 1963 collection in August); (2) in late fall after the animals came off pasture and the calves were weaned (October or November) and (3) in early spring which was after parturition but before the cows went out to pasture (May).

### Sampling Procedure

At the first collection time young cows (3 - 5 years of age) were randomly selected within each herd, and the same test animals were sampled at each subsequent collection time. The study was initiated with the sampling of eight beef cows per herd in the Southwestern area and ten beef cows per herd in the Ste. Rose area. However, due to uncontrollable circumstances at the times of sample collection, it was sometimes inexpedient to sample all test animals within each herd (e.g. cows within 60 days of parturition were sampled for blood and not for liver).

Sampling, at each collection time, consisted of obtaining a liver sample by the liver aspiration biopsy technique of Erwin et al. (1956) and a heparinized blood sample by the jugular puncture technique, while the animals were restrained in a portable squeeze chute. Following sample collection the cows were given a 10 ml. injection of antibiotic (2 million I.U. Penicillin G Procaine and 2.5 gm. Dihydrostreptomycin sulfate) and the biopsy wound (about one inch long) was covered by a fly repellent powder or paste.

All equipment used to obtain the samples was scrupulously washed with detergent and rinsed with deionized water, on location, to prevent trace metal contamination. All surgical instruments were sterilized in a portable boiling water sterilizer immediately before use.

The liver samples, upon collection, were freed of blood by washing with physiological saline, blotted dry with filter paper, placed in sample vials and immediately frozen on dry ice. The heparinized blood (200 units heparin sodium U.S.P. per 40 ml. blood) was centrifuged at 2,000 rpm for 20 minutes in a portable centrifuge on location and the plasma decanted into vials and immediately frozen on dry ice. Both the liver and plasma samples

were maintained in a frozen state until analysed in the laboratory.

Representative samples of the forages that had been fed to the cows during the two winter seasons were collected by the farmers and sent to the laboratory for chemical analysis.

### Chemical Analysis

The hepatic tissues were analysed for vitamin A, carotene, copper, cobalt and molybdenum. Blood plasma was analysed for total calcium, total magnesium, inorganic phosphorus, vitamin A and carotene.

Individual liver samples were subjected to vitamin A and carotene analyses according to the method of Gallup and Hoefler (1946). However, after these analyses there was not enough hepatic tissue remaining for determination of copper, cobalt, and molybdenum on an individual liver basis. Therefore, the remaining liver tissue, within each herd, was pooled and as many 5 gm. subsamples as possible (one to three) were used for determination of the trace mineral elements. The 5 gm. subsamples were digested in micro-Kjeldahl flasks by a wet oxidation procedure described by Sandell (1959), which involved the use of nitric and sulfuric acids. The resulting clear digests were diluted to volume with deionized water in 25 ml. volumetric flasks. Ten ml. was removed from the diluted digest and used for determination of molybdenum according to the method described by Sandell (1959). The remaining 15 ml. of digest was used for copper and cobalt determinations by the A.O.A.C. (1960) procedure.

Total calcium and magnesium were determined on 1 ml. of plasma according to the method described by Walser (1960). The plasma was deproteinated and the supernatant used for the above determinations by EDTA titration. Inorganic phosphorus was determined on 1 ml. of plasma according

to the method described by Fister (1950). Vitamin A and carotene were determined in the plasma by the method described by Kimble (1939).

The forage samples were analysed for ether extract, crude fiber, crude protein, calcium, phosphorus and carotene according to methods of the A.O.A.C. (1960). No trace mineral analyses were done on the forages due to the great variability in forage species within samples.

### Statistical Analysis

For statistical analysis herd averages were used as individual observations in a 2 x 3 x 2 factorial design in which the year, season and area effects were considered as being fixed. Due to the occurrence of disproportionate subclass numbers, the method of unweighted means was used in conjunction with the analysis of variance described by Snedecor (1956). For the purpose of this study a treatment is defined as the herd averages within an area, a season and a year. Thus the data for each nutrient are subdivided into 12 treatments. Comparisons among significantly different treatment means were made with Duncan's new multiple-range test as described by Steel and Torrie (1960). To obtain an estimate of the variation within a treatment, standard errors were calculated for each treatment mean (Steel and Torrie, 1960).

## RESULTS AND DISCUSSION

The answers to the questionnaires (Appendix III Table IV) indicated that all herds were normal, with the possible exception of herd 12. This herd had previously encountered a scouring problem mainly in young calves, and to a lesser extent in cows during the winter months. The herd scores (Appendix III Table V) were classified as six, very good; four, good; and two, fair for management practices and six, very good; five, good; and one, fair for herd condition.

Throughout the study all test cows appeared to be in a reasonably good state of health. Most cows lost considerable weight through the winter, a situation which is quite common in Manitoba beef herds.

A total of 106 cows were each biopsied six times throughout the study without any marked adverse effects being shown by the animals. However, there were three notable exceptions. Two cows aborted in the fourth month of gestation, about two weeks after the November, 1962 samples were taken. The cooperators attributed this to the biopsy, but there is no concrete evidence to support their claim. Another test cow was very near death when the May 1963 samples were taken. It was destroyed and post-mortem examination revealed that the animal had a very severe kidney infection. Since there was no infection in the area of the liver it was concluded that the liver biopsy taken six months previous (November 1962) was not the cause of the animal's condition.

Results of the forage analysis (Appendix IV Table VI) revealed that the dry matter, ether extract, crude fiber and crude protein levels were quite similar to values reported by Robertson (1961) and Morrison (1958).

Individual herd averages for liver and plasma levels of the nutrients considered are presented in Appendix II Tables I, II and III.

### Copper

The liver copper levels for area, season and year are presented numerically in Table I and graphically in Figure 1. Liver copper levels increased from July (summer) to November (fall) and then decreased during the winter feeding period to a level in May (spring) quite similar to that observed during the previous summer (Figure 1). A similar trend was also observed during the second year, which indicates a certain degree of regularity in seasonal trends between years.

Mean squares presented in Table II show that only the season ( $P < .05$ ) and area ( $P < .01$ ) sources of variation had a significant effect upon the liver copper levels. Comparison of season means (Table III) indicated that the mean liver copper level in the fall was significantly ( $P < .01$ ) higher than those in the spring and summer. These results agree with those of Ralston *et al.* (1961), who found a significant ( $P < .01$ ) seasonal variation in liver copper level with an increase through the pasture growing season and a decrease throughout the winter.

TABLE III

Duncan's Comparison of Season Means  
for Liver Copper

| LEVEL OF<br>SIGNIFICANCE * | SEASON |        |       |
|----------------------------|--------|--------|-------|
|                            | SPRING | SUMMER | FALL  |
| 1%                         | 6.68   | 6.93   | 12.38 |

\* All means significantly different at 5% level.



TABLE I

AVERAGE LIVER LEVELS OF COPPER, MOLYBDENUM AND COBALT  
(ppm WET TISSUE)

|            | SUMMER         |               | FALL          |               | SPRING        |               |
|------------|----------------|---------------|---------------|---------------|---------------|---------------|
|            | 1962           | 1963          | 1962          | 1963          | 1963          | 1964          |
| SOUTHWEST  |                |               |               |               |               |               |
| COPPER     | 3.82 ± 0.869 * | 4.14 ± 1.414  | 8.22 ± 1.269  | 8.71 ± 2.361  | 3.11 ± 1.111  | 3.76 ± 0.956  |
| MOLYBDENUM | 1.44 ± 0.486   | 0.81 ± 0.367  | 2.09 ± 0.812  | 1.22 ± 0.050  | 1.01 ± 0.113  | 1.41 ± 0.067  |
| COBALT     | 0.301 ± 0.065  | 0.091 ± 0.028 | 0.125 ± 0.037 | 0.046 ± 0.014 | 0.187 ± 0.050 | 0.056 ± 0.014 |
| STE. ROSE  |                |               |               |               |               |               |
| COPPER     | 9.65 ± 3.725   | 10.09 ± 4.637 | 16.90 ± 6.632 | 15.69 ± 4.541 | 10.02 ± 3.837 | 9.81 ± 1.945  |
| MOLYBDENUM | 1.56 ± 0.112   | 0.79 ± 0.067  | 2.02 ± 0.232  | 1.07 ± 0.051  | 1.12 ± 0.137  | 1.13 ± 0.128  |
| COBALT     | 0.164 ± 0.070  | 0.126 ± 0.026 | 0.088 ± 0.014 | 0.057 ± 0.034 | 0.186 ± 0.040 | 0.035 ± 0.010 |

\* STANDARD ERROR



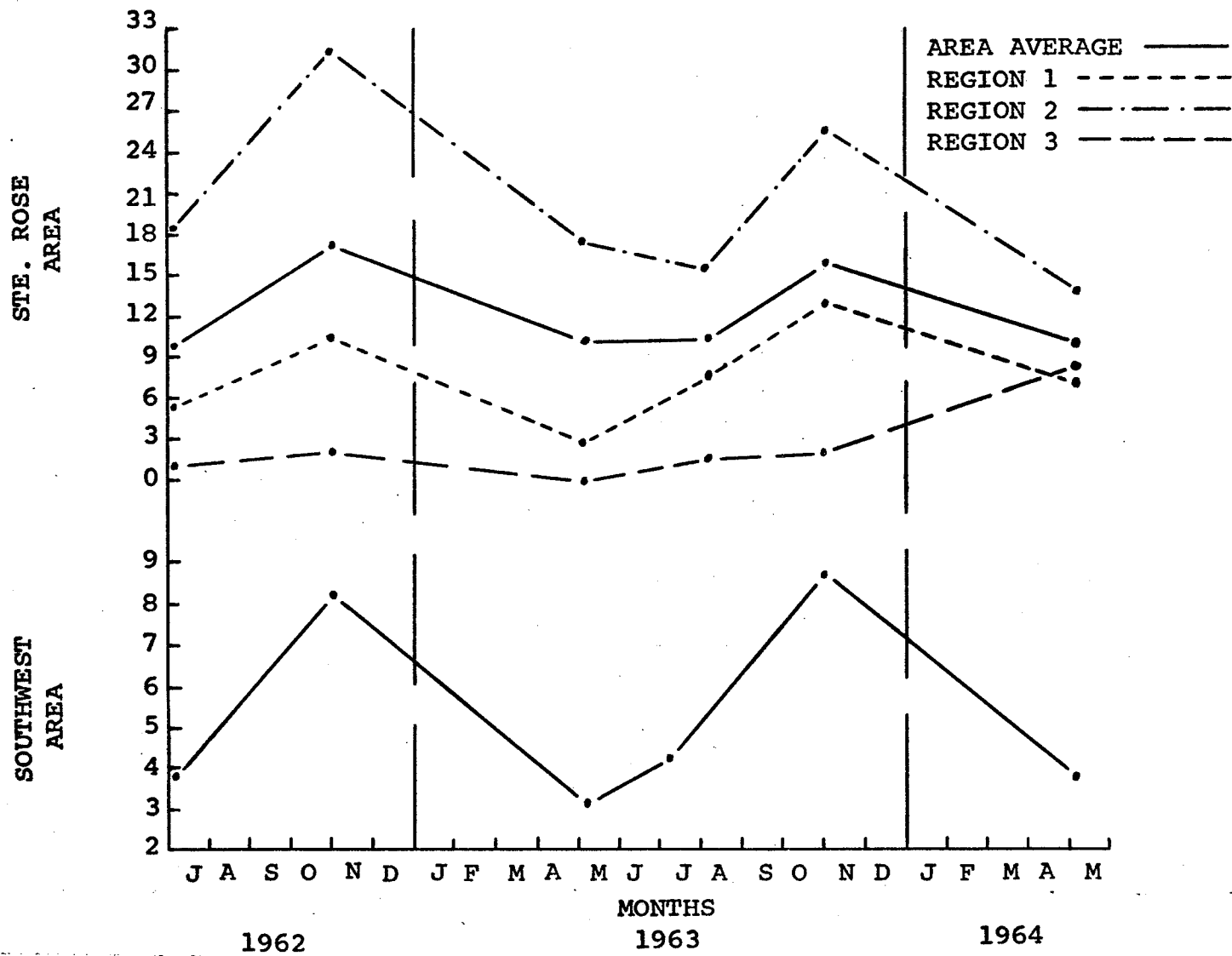


FIGURE 1. AVERAGE LIVER COPPER LEVELS  
(ppm wet tissue)

TABLE II

MEAN SQUARES FOR LIVER COPPER, MOLYBDENUM, AND COBALT

| SOURCE     | d.f. | LIVER<br>COPPER | LIVER<br>MOLYBDENUM | LIVER<br>COBALT |
|------------|------|-----------------|---------------------|-----------------|
| YEAR (Y)   | 1    | 0.0192          | 0.658 *             | 0.03413 **      |
| SEASON (S) | 2    | 41.5777 *       | 0.260               | 0.00848 *       |
| Y x S      | 2    | 0.1651          | 0.351               | 0.00208         |
| AREA (A)   | 1    | 136.0133 **     | 0.007               | 0.00188         |
| Y x A      | 1    | 0.4962          | 0.031               | 0.00333         |
| S x A      | 2    | 0.9891          | 0.008               | 0.00051         |
| Y x S x A  | 2    | 0.1939          | 0.007               | 0.00237         |
| ERROR      | 57   | 8.4183          | 0.1369              | 0.001699        |

\*  $P < .05$ \*\*  $P < .01$

The most marked increase in liver copper level might be expected during the period from May to July, when the cows are grazing young immature grasses, rather than during the late summer period August to November, when the grazing season is usually past its peak. Possible reasons for the substantial increase in liver copper level during late summer are:

- (1) The cows reached a peak milk production in May or June, followed by a steady decline throughout the remainder of the summer. This would represent a greater copper loss via the milk during early summer than in late summer. The end result would be greater liver copper storage during late summer than in early summer.
- (2) The early summer period was only two months, while the July to November period was four months; thereby, allowing the cows more time to build up a liver copper store in late summer.
- (3) The copper content of the early summer forages may be lower than that of late summer forages. However, this is the reverse of what Robertson (1961) found in Interlake forages. Another possibility could be that the predominating forage species in late summer has a higher copper content than that of early summer.

The average liver copper level in the Ste. Rose area was significantly ( $P < .01$ ) higher than that of the Southwestern area. Dick (1954), Beck (1956) and Dempsey et al. (1958) found a fairly good correlation between copper content of pasture and liver copper levels of sheep and cattle. Therefore, one might suggest that the Interlake grasses consumed during the year by herds 8, 9, 10 and 11 (herd 12 was fed tame forages during most of the year) in the Ste. Rose area were, on the average, higher in copper content than the grasses consumed by cows in the Southwestern area.

The standard errors of treatment means (Table I) show that variation among herds within treatments in the Southwestern area (ranging between 0.869 and 2.361) was considerably less than that in the Ste. Rose area (ranging between 1.945 and 6.632) for liver copper levels. Further examination of the herd averages (Appendix II Table I) suggested that the five herds in the Ste. Rose area (8, 9, 10, 11 and 12) could be subdivided into three geographical regions with regard to liver copper level. Region 1 consisted of herds 8 and 9 to the north of Ste. Rose, region 2 herds 10 and 11 to the east of Ste. Rose and region 3 herd 12 to the south of Ste. Rose along the foothills of Riding Mountain National Park.

The liver copper levels in the Ste. Rose area as affected by region, season and year are presented numerically in Table IV and graphically in Figure 1. For statistical analysis the region 3, spring 1964 copper level (Table IV) was taken as zero rather than 8.08 since copper supplementation was responsible for the higher than normal value. The mean squares (Table V) show that only the region source of variation significantly ( $P < .01$ ) affected liver copper levels. The seasons approached significance at the 5% level of probability (the level at which they are significant when considering all 12 herds in Table II) with the lack of significance being due to the small number of herds (1 or 2) and the variation between herd averages within regions.

The liver copper levels were consistent within regions for all seasons during the two years, with region 3 lowest, region 1 second and region 2 the highest. Comparison of region means (Table VI) revealed that they are significantly different at the 5% level of probability, and only the difference between means of regions 1 and 3 is non-significant at the 1% level. These relatively large and consistent region differences in liver copper level

TABLE IV

AVERAGE COPPER LEVEL IN STE. ROSE REGIONS  
(ppm WET TISSUE)

| REGION | SUMMER |       | FALL  |       | SPRING |        |
|--------|--------|-------|-------|-------|--------|--------|
|        | 1962   | 1963  | 1962  | 1963  | 1963   | 1964   |
| 1      | 5.52   | 8.56  | 10.14 | 12.99 | 2.86   | 7.04   |
| 2      | 18.07  | 15.19 | 31.17 | 25.29 | 17.19  | 13.45  |
| 3      | 1.09   | 1.52  | 1.88  | 1.90  | 0.0    | 8.08 * |

\* VALUE TAKEN AS ZERO FOR THE STATISTICAL ANALYSIS

TABLE V.  
MEAN SQUARES FOR LIVER COPPER IN  
STE. ROSE REGIONS

| SOURCE     | d.f. | LIVER<br>COPPER |
|------------|------|-----------------|
| YEAR (Y)   | 1    | 0.218           |
| SEASON (S) | 2    | 84.441          |
| Y x S      | 2    | 0.691           |
| REGION (R) | 2    | 555.912 **      |
| Y x R      | 2    | 21.379          |
| S x R      | 4    | 20.640          |
| Y x S x R  | 4    | 0.395           |
| ERROR      | 11   | 23.908          |

\*\*  $P < .01$

suggest that the copper content of the forages vary considerably among these regions. These data suggest that the forages in region 2 are higher in copper than those in regions 1 and 3. The herd in region 3 is located along the foothills of Riding Mountain National Park, and water draining off of the mountain towards Lake Manitoba could cause considerable leaching of the soil. The result could be a decrease in the copper content of forages grown on these soils.

TABLE VI

Duncan's Comparison of Region Means for  
Liver Copper Levels in Ste. Rose Area

| LEVEL OF<br>SIGNIFICANCE * | REGION |      |       |
|----------------------------|--------|------|-------|
|                            | 3      | 1    | 2     |
| 1%                         | 1.07   | 7.85 | 20.06 |

\* All means significantly different at 5% level.

Another possible explanation for the observed difference in liver copper level among regions may be the copper, molybdenum, sulfate interrelationship (Underwood, 1962). The ability of molybdenum to reduce copper retention is said to be dependent upon the sulfate content of the diet. The liver molybdenum levels, vide infra, were very similar among the three regions in the Ste. Rose area, but it is possible that the dietary inorganic sulfate levels were different. Well water analysis for total sulfate, conducted in 1960, gave the following average values (ppm) with the number of analysis in brackets; region 3 - 2,069.2 (5), region 2 - 473.1 (10) and region 1 - 544.2 (20). From these analyses one could postulate that dietary molybdenum and sulfate were interacting with copper to reduce liver copper stores in region 3.

Studies on the copper, molybdenum and sulfate content of feed and water are needed to clarify the picture.

During the 1963-64 winter, cooperator 12 (region 3) was encouraged to feed supplemental copper to his cows. The suggestion was made because the cows and calves exhibited profuse scouring (a copper deficiency symptom) and low liver copper levels were detected in the cows during the first five sampling periods. A mineral mix containing 2% copper sulfate was fed free choice, and this resulted in a considerable average increase in liver copper level (from 1.90 ppm in fall 1963 to 8.08 ppm in spring 1964). However, the increase in liver level varied from 1.72 to 16.05 ppm in individual cows, suggesting that the amount of mineral consumed free choice varied considerably among cows. The added copper did not prevent the diarrhea; however, it did decrease the number of young calf deaths in the spring, and an improvement in herd condition was noticed. This situation appears similar to that reported by Miltimore et al. (1964), who were unable to prevent scouring by copper injections but were able to increase weight gain by 0.5 pounds per day in grazing cattle. However, these workers suggested that the high level of molybdenum in these forages was the agent causing scouring.

The range in liver copper levels throughout the study period (herds 1 to 11) was 0.87 to 39.35 ppm wet tissue. Comparisons with data published by Cunningham (1946) (cited by Underwood, 1962) and Dick and Bull (1945) indicated that many of these liver copper levels would be considered as being in the deficient range. However, no copper deficiency symptoms were noted in any of these herds.



### Molybdenum

Average liver molybdenum levels as affected by area, season and year are shown numerically in Table I and graphically in Figure 2. Statistical analysis (Table II) indicated a significant ( $P < .05$ ) difference between years in liver molybdenum level. The mean squares for season and year x season interaction both approached significance ( $P > .05$ ).

As shown in Figure 2, the liver molybdenum levels were very similar for the two areas throughout the study. There appears to have been a trend in the first year towards increased liver levels during late summer (July to November) followed by a decrease during the winter (November to May) and subsequent early summer period (May to July). The trend was similar during late summer of the second year but was reversed during winter when a slight increase was observed. This variation among seasons between years is the primary reason for the year x season interaction approaching statistical significance and along with variation among herds within treatments is responsible for the season effect being non-significant ( $P > .05$ ).

The decrease in liver molybdenum level during the early summer period (May to June) is difficult to explain. It may have been due to a drain of molybdenum from the cow's body via the milk. Since the cow's peak milk production for the year occurs during early summer, the decrease in molybdenum loss via the milk during late summer could have been responsible for the build up of liver molybdenum stores during this latter period. Also, there is the possibility that forages growing during the early summer period were low in molybdenum; i.e. so low that the liver store was used to meet the metabolic demand during early summer.

The summer and fall liver molybdenum levels of the first year were

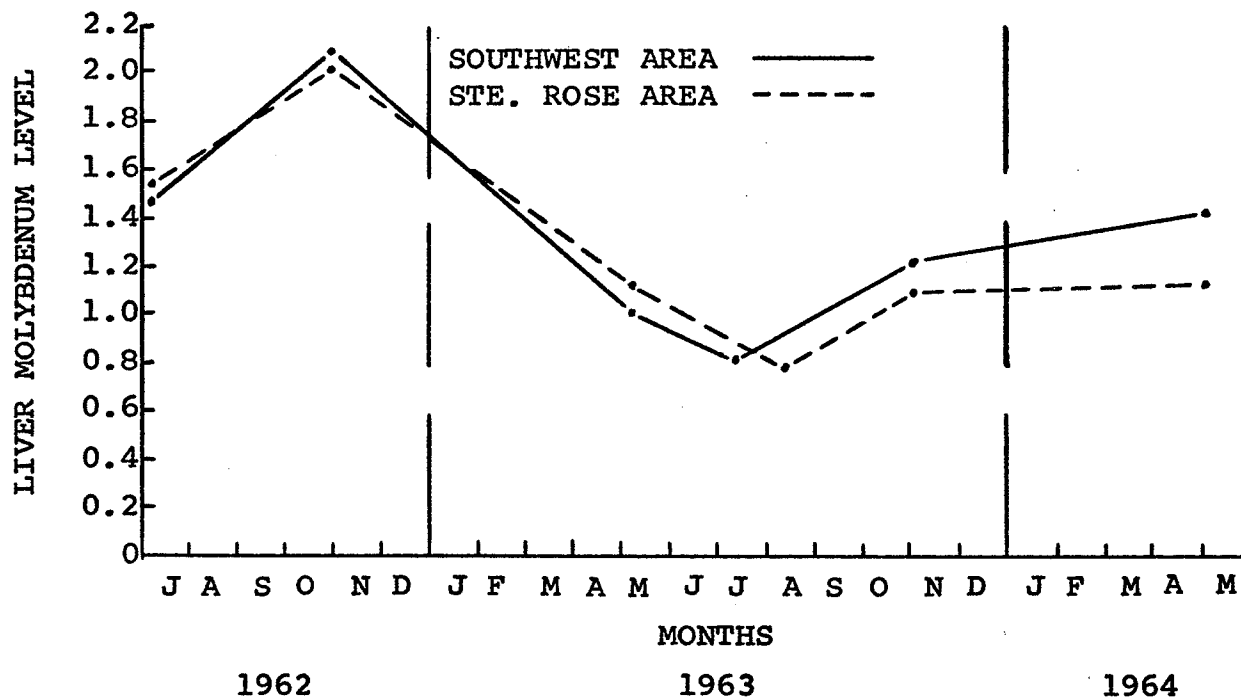


FIGURE 2. AVERAGE LIVER MOLYBDENUM LEVELS  
(ppm wet tissue)

considerably higher than those of the second year, while the spring levels were fairly similar for the two years. The mean squares (Table II) indicate that the liver molybdenum level in the first year was significantly ( $P < .05$ ) higher than that in the second year. Higgins et al. (1956), Cunningham et al. (1959) and Davies et al. (1960) have demonstrated that liver molybdenum levels are directly related to dietary molybdenum intake. Therefore, in the present study molybdenum intake must have been greater during the first year than during the second year. Since the trends during each year were very similar for the two areas, it might be surmised that the forage molybdenum levels were affected by some climatic condition such as rainfall or temperature. However, mean daily temperature and precipitation readings (Monthly Record) recorded at various stations throughout the two areas indicated no differences between the two years of the study (Appendix V Tables VII and VIII). Another possible explanation for lower liver molybdenum levels during the second year could be higher dietary sulfate levels, particularly during the summer. This would increase molybdenum excretion and thereby decrease molybdenum storage.

The liver molybdenum levels reported by Ralston et al. (1961) for beef cows showed trends similar to those reported here. These workers found a highly significant seasonal variation, with liver molybdenum stores increasing throughout the forage growing season and decreasing through the winter months. They also observed a marked difference between years. However, in their study liver samples for the second year came from different cattle herds than those for the first year. Therefore, a direct comparison between years cannot be made.

The highest liver molybdenum level was 6.24 ppm with the remainder of the herd averages ranging between 0.20 and 3.88 ppm wet tissue throughout

the study. This range corresponds to 0.66 - 12.9 ppm dry weight, assuming that liver tissue is 30% dry matter. None of the molybdenosis symptoms reported in cattle of the Swan River area (Cunningham, 1953) were noted in any of the cattle even though some of the liver levels were considerably higher than the normal values of 2 - 4 ppm dry weight reported by Higgins et al. (1956). The higher values are, however, lower than the 42.4 ppm dry weight reported by Cox et al. (1960) in the liver of cattle in which molybdenosis had been induced.

### Cobalt

The effects of area, season and year upon liver cobalt levels are presented numerically in Table I and graphically in Figure 3. The mean squares presented in Table II show that only the year ( $P < .01$ ) and season ( $P < .05$ ) sources of variation had significant effects upon liver cobalt levels.

The trends in liver cobalt were somewhat similar (Figure 3) for both areas throughout the study period. The liver cobalt level at each season of the second year was lower than that for the corresponding season of the first year. This resulted in significantly ( $P < .01$ ) lower cobalt levels throughout the second year. The seasonal trends established over the two-year period consisted of a decrease in liver cobalt levels throughout the entire summer period (May to November) followed by an increase during the winter feeding period of the first year with little or no change occurring during the second winter. The summer and fall means were significantly ( $P < .01$ ) different (Table VII), which means that the minimum values obtained for each year during the fall were significantly lower than the values obtained during the summer.

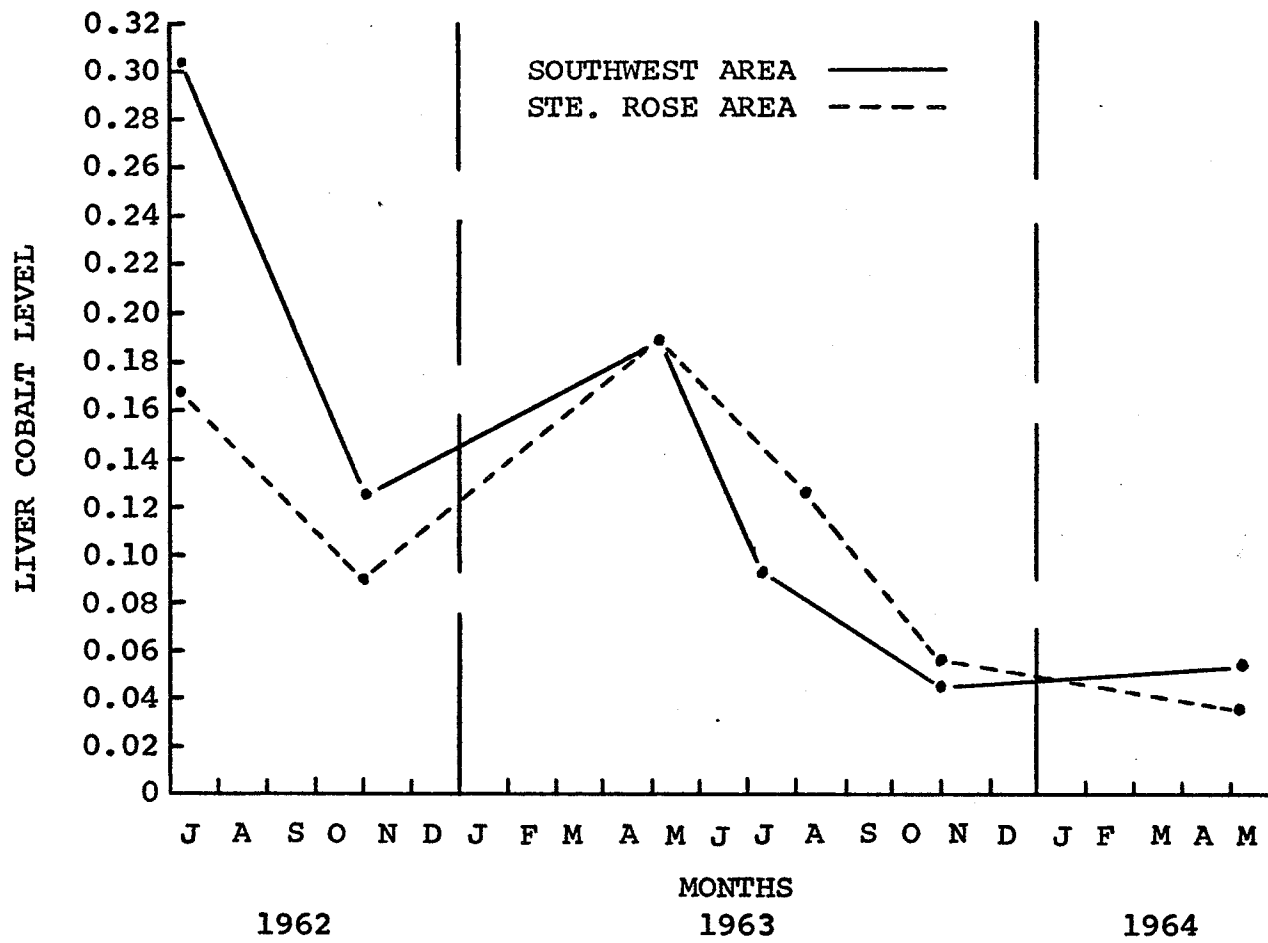


FIGURE 3. AVERAGE LIVER COBALT LEVELS (ppm wet tissue)

TABLE VII

Duncan's Comparison of Season Means  
For Liver Cobalt

| LEVEL OF<br>SIGNIFICANCE | SEASON |        |        |
|--------------------------|--------|--------|--------|
|                          | FALL   | SPRING | SUMMER |
| 5%                       | 0.079  | 0.116  | 0.171  |
| 1%                       |        |        |        |

Cobalt concentrations in the liver are sufficiently responsive to dietary cobalt to be a valuable aid in diagnosing cobalt deficiency in the field (Thompson and Ellis, 1947; Underwood, 1962). Therefore, it appears that the lower liver cobalt level during the second year was due to a lower level of cobalt intake throughout that year. Dietary cobalt comes from two main sources, the forage and salt. The amount of salt consumed was considerable in some herds but very low in others. This would result in considerable variation among herds in the percentage of dietary cobalt coming from the two main sources; but it might be surmised that the greatest percentage of dietary cobalt came from the forages. If this were true, then the differences between years in liver cobalt level would indicate a difference between years in the forage cobalt level, which corroborates the work of Robertson (1961). This worker found a significant difference between years in the cobalt content of Interlake forages and attributed this to possible differences in climatic conditions, namely, rainfall.

It seems peculiar that the body store of cobalt, or any nutrient, would decrease during the grazing season and then increase during the winter.

Robertson (1961) found that the cobalt content of Interlake forages was not influenced by stage of maturity. This being the case it is hard to visualize that the cows had a greater cobalt intake, via the forage, during the winter than during the summer. Therefore, the increased cobalt intake during the winter, which presumably took place, could have come from an increase in consumption of the cobalt-iodized salt, to which all animals had free access during the year. During winter the cows were confined in much smaller areas than in summer, which would keep them in closer contact with the salt source (loose mix or block) and possibly increase consumption. An alternative possibility could be that the cows had a lower cobalt requirement during winter than during the summer.

McNaught (1948) in New Zealand and Correa (1957) in Brazil reported that the liver cobalt levels of cobalt deficient cattle range between 0.04 and 0.06 ppm dry weight (which corresponds to 0.012 - 0.018 ppm wet tissue). In cattle with a satisfactory cobalt status they observed liver levels of 0.08 to 0.20 ppm dry weight (0.024 - 0.060 ppm wet tissue). Further, Ralston et al. (1961) reported a mean liver cobalt level of 1.9 ppm dry weight (0.570 ppm wet tissue) for a one year study of normal cattle in Washington State. They did not observe any incidence of cobalt deficiency. Therefore, it appears that considerable confusion exists with regard to minimal liver cobalt levels and manifestation of cobalt deficiency. Since in the present study no visual symptoms of cobalt deficiency were observed, it is concluded that the herd averages for liver cobalt (ranging between 0.002 and 0.530 ppm wet tissue) were normal for herds under the existing conditions of these two areas from 1962 to 1964. However, the lower liver cobalt levels are considered as marginal. Therefore, the recommendation of Robertson (1961), to supplement

cattle fed on Interlake forages with cobalt, should possibly be extended to cattle consuming all forages in the two areas investigated, and more probably to all cattle in Manitoba.

It is possible that some of the unthrifty and inappetent cattle on Manitoba farms are suffering from cobalt deficiency. This condition is difficult to detect from malnutrition by visual appraisal alone. However, it can be verified by observing the immediate growth response and increase in appetite and thrift following oral cobalt administration.

This study has demonstrated a tendency for liver cobalt levels to decrease during the summer months. Since the spring 1964 liver cobalt levels were quite low it is possible that some of the cows in these two areas could have exhibited cobalt deficiency if the trend continued during the summer of 1964.

#### Vitamin A and Carotene

Plasma and liver levels of carotene and vitamin A will be considered concomitantly. Average plasma and liver, carotene and vitamin A levels as affected by area, season and year are presented numerically in Table VIII and graphically in Figures 4 and 5.

The mean squares calculated by the analysis of variance (Table IX) for liver vitamin A levels do not attach statistical significance to any of the sources of variation at the 5% level of probability. However, both the season and year sources of variation are approaching significance. The high standard errors attached to the treatment means of liver vitamin A (Table VIII) indicate considerable variation among herd averages within treatments. This is responsible for the high error mean square for liver vitamin A (Table IX), which alternately is responsible for the non-significant ( $P > .05$ ) difference



TABLE VIII  
MEAN VITAMIN A AND CAROTENE LEVELS OF LIVER  
AND PLASMA

|                               | SUMMER         |              | FALL         |              | SPRING       |              |
|-------------------------------|----------------|--------------|--------------|--------------|--------------|--------------|
|                               | 1962           | 1963         | 1962         | 1963         | 1963         | 1964         |
| <b>SOUTHWEST</b>              |                |              |              |              |              |              |
| LIVER VITAMIN A <sup>a</sup>  | 158.8 ± 11.2 * | 141.9 ± 18.4 | 191.2 ± 18.2 | 167.8 ± 11.5 | 160.5 ± 20.6 | 157.5 ± 28.1 |
| LIVER CAROTENE <sup>a</sup>   | 13.43 ± 0.79   | 7.02 ± 0.66  | 8.15 ± 0.63  | 9.06 ± 0.66  | 5.23 ± 0.44  | 11.01 ± 1.01 |
| PLASMA VITAMIN A <sup>b</sup> | 59.56 ± 3.93   | 26.13 ± 3.53 | 42.54 ± 3.22 | 22.40 ± 1.73 | 36.34 ± 2.77 | 28.50 ± 1.98 |
| PLASMA CAROTENE <sup>c</sup>  | 8.06 ± 0.46    | 6.73 ± 1.14  | 6.69 ± 1.00  | 7.90 ± 1.17  | 3.47 ± 0.52  | 5.75 ± 0.79  |
| <b>STE. ROSE</b>              |                |              |              |              |              |              |
| LIVER VITAMIN A <sup>a</sup>  | 156.9 ± 16.9   | 141.3 ± 22.7 | 184.0 ± 16.8 | 172.5 ± 19.2 | 168.8 ± 28.8 | 154.8 ± 16.4 |
| LIVER CAROTENE <sup>a</sup>   | 8.87 ± 0.73    | 6.92 ± 0.59  | 6.73 ± 0.58  | 7.24 ± 0.77  | 7.23 ± 0.89  | 4.49 ± 0.52  |
| PLASMA VITAMIN A <sup>b</sup> | 54.74 ± 6.44   | 20.62 ± 1.75 | 36.69 ± 2.75 | 20.30 ± 1.27 | 27.74 ± 2.65 | 39.04 ± 2.48 |
| PLASMA CAROTENE <sup>c</sup>  | 8.65 ± 1.05    | 9.70 ± 1.92  | 8.07 ± 1.22  | 7.94 ± 1.01  | 8.21 ± 0.88  | 1.74 ± 0.33  |

\* STANDARD ERROR

<sup>a</sup> mcg. per gm. wet weight

<sup>b</sup> mcg. per 100 ml.

<sup>c</sup> mcg. per ml.

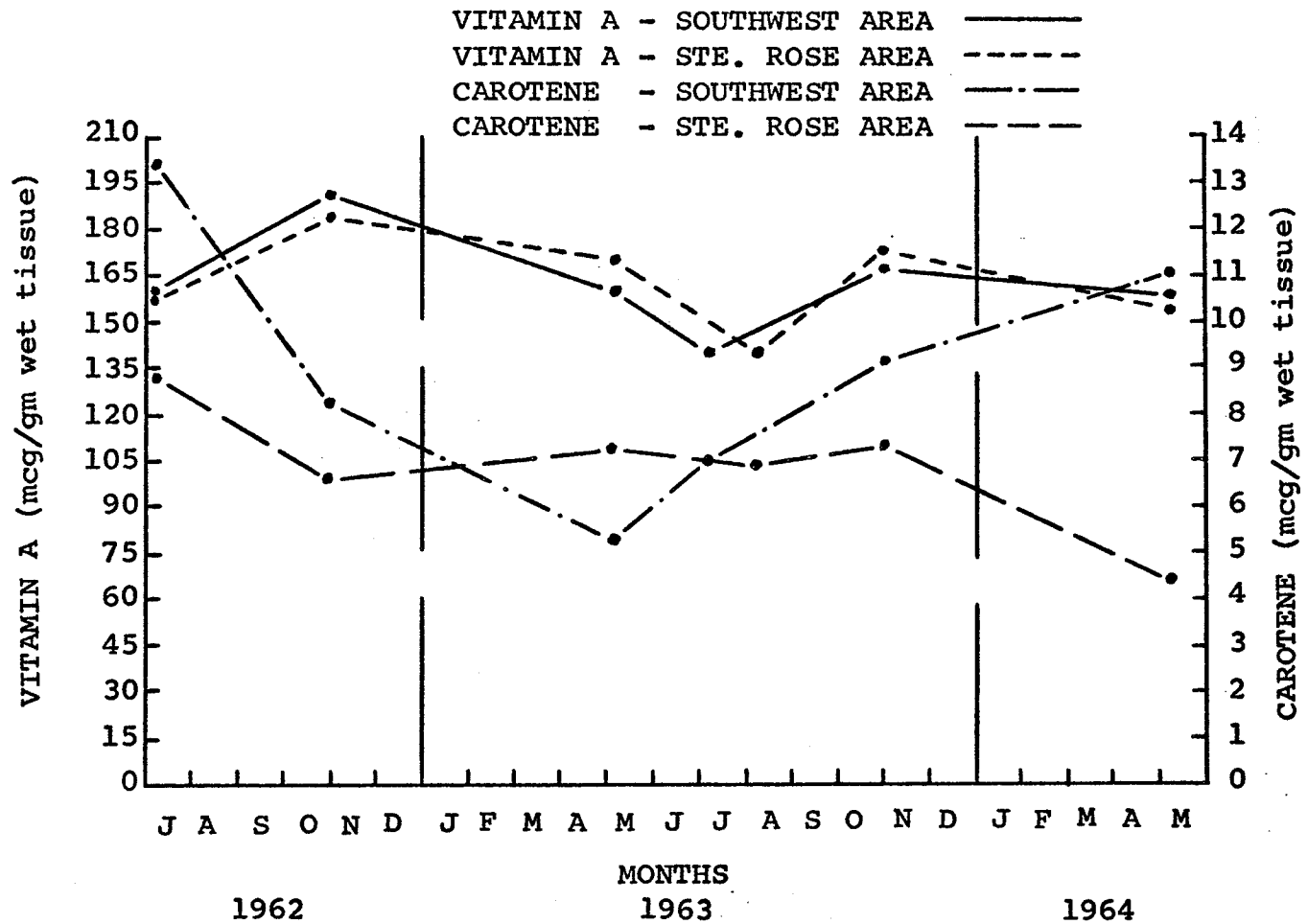


FIGURE 4. AVERAGE VITAMIN A AND CAROTENE  
 LEVELS IN THE LIVER

between year means and among season means. These data corroborate the results of Baker et al. (1954), who reported that vitamin A reserves of cows grazing green pasture may be quite variable. Although the differences among season means and between years are non-significant ( $P > .05$ ) for liver vitamin A levels, the seasonal trends over the two years indicated by Figure 4 were still meaningful. This is indicated by the following observations, which were obtained from the individual herd averages (Appendix II Table II):

- (1) During the summer to fall period 11 of the 12 herds increased during the first year and all nine herds increased during the second year.
- (2) Decreases occurred in seven out of 12 herds during the first year and eight out of 12 herds during the second year in the fall to spring period.
- (3) In the early summer period (May 1963 - July 1963) seven of the nine herds decreased.
- (4) The herd average of the first year was greater than that of the second year in six out of nine herds in the summer, 10 out of 12 herds in the fall and seven out of 12 herds in the spring.

Liver vitamin A levels are closely correlated with carotene and/or vitamin A intake (Hale et al., 1961; Pope et al., 1961). The variation in liver vitamin A levels observed in the present study among herds within treatments was probably due to variable carotene intakes. The carotene content of growing forages, cured hays and silage are greatly affected by temperature and rainfall (Ralston and Dyer, 1960). Furthermore, the harvesting procedure used to make cured hay and silage affects the carotene content of the resulting

TABLE IX

MEAN SQUARES FOR LIVER AND PLASMA VITAMIN A  
AND CAROTENE

| SOURCE     | d.f. | LIVER<br>VITAMIN A | LIVER<br>CAROTENE | PLASMA<br>VITAMIN A | PLASMA<br>CAROTENE |
|------------|------|--------------------|-------------------|---------------------|--------------------|
| YEAR (Y)   | 1    | 592.207            | 1.268             | 843.698 **          | 0.958              |
| SEASON (S) | 2    | 868.711            | 4.356 **          | 103.766 **          | 13.844 **          |
| Y x S      | 2    | 23.346             | 9.510 **          | 316.828 **          | 1.871              |
| AREA (A)   | 1    | 0.036              | 12.855 **         | 22.249              | 2.717              |
| Y x A      | 1    | 0.434              | 1.657             | 41.071              | 4.953 *            |
| S x A      | 2    | 5.530              | 0.153             | 10.585              | 0.545              |
| Y x S x A  | 2    | 32.782             | 10.752 **         | 27.075              | 8.027 **           |
| ERROR      | 57   | 409.107            | 0.5577            | 10.308              | 0.968              |

\* P&lt;.05

\*\* P&lt;.01

winter forage. The standard errors of the vitamin A treatment means were higher for liver samples collected in the spring than for those collected in summer or fall. Since the vitamin A levels in the spring liver samples were a reflection of carotene and/or vitamin A intake during the winter, these data suggest that there was considerable variation in carotene content of hay consumed during this period. Mean daily temperature and precipitation readings taken at various points throughout the two areas (Monthly Record) indicated no differences between areas or between years (Appendix V Tables VII and VIII). Therefore, the differences between herds in the carotene content of the winter forages were probably due to differences in the technique of making winter forage. This variation in carotene content could alternatively be due to differences in forage species consumed on summer pasture versus winter hay.

The seasonal trends for liver vitamin A levels (Figure 4) were quite similar for the two areas investigated. An average increase of 29.13 mcg. per gm. wet tissue occurred during late summer (July to November) with the resulting fall values being the yearly maxima. The subsequent winter and early summer periods (November to July) were characterized by a steady decline in liver vitamin A levels to the yearly minima in mid-summer (July), which was 45.95 mcg. below the maximum of the previous year. The liver vitamin A levels (Figure 4) for each season during the second year were all lower than those for corresponding seasons of the first year. This would indicate that carotene intake was greater during the first year than during the second year of the study. Also, it was greater during the late summer periods than during the winter and early summer periods. These results are similar to those of Ralston and Dyer (1960). They found that fall and summer liver samples were significantly higher in vitamin A than those taken in winter and spring with

yearly averages of 151 and 293 mcg. per gm. wet tissue.

The decrease in liver vitamin A level from May to July was unexpected because all test animals had been grazing green grass during this period, which would be high in carotene content according to Pope et al. (1961). However, the secretion of vitamin A and carotene in milk would be of considerable magnitude during this period of peak milk production (Van Arsdell et al., 1950; Roberts and Dyer, 1959). It is conceivable that the carotene intake was not sufficient to meet the lactating cow's requirement, and resulted in mobilization of vitamin A from the liver to aid the cow in meeting its requirement. By mid-summer both milk production and vitamin A and carotene content of the milk decreased sufficiently to allow a build up of vitamin A liver stores during late summer.

The intention of this study was not to influence the farmer in his feeding practices, and whether or not he fed supplemental vitamin A to his cows during the winter was up to his managerial discretion. Three herds (numbers 6, 10 and 11) received supplemental vitamin A during the winter of the first year and two herds (numbers 1 and 2) during the second winter. In May 1963 herds 6 and 11 had increased liver vitamin A levels by 17.31 and 27.30 mcg. per gm., respectively, and herd 10 had decreased by 8.90 mcg. per gm. from the respective levels in fall 1962. In May 1964 herds 1 and 2 had decreases of 20.50 and 17.46 mcg. per gm., respectively, from the fall 1963 levels. These liver vitamin A decreases in three of the five supplemented herds were not too different from the overall decreases of 22.9 mcg. per gm. in the first winter and 14.0 mcg. per gm. in the second winter. This indicates that vitamin A supplementation was of little or no value in helping the cows maintain their liver vitamin A levels over the winter months. However, it must be kept in mind that the vitamin A supplementation consisted of only about 20,000 I.U. per

animal per day, and given over a six-week period in late winter. This level of supplementation is not extremely high and one would not necessarily expect it to increase liver vitamin A levels.

Silage is considered by many people as a good method of putting up forage without too much carotene loss. Cooperator 3 fed grass silage and cooperator 7 fed corn silage during both winters. The fall and spring liver vitamin A levels for herd 3 were 266.90 and 267.07 mcg. per gm. in the first year followed by 210.81 and 241.79 mcg. per gm. in the second year. Corresponding values for herd 7 were 244.30 and 106.22 mcg. per gm. in the first year followed by 178.17 and 41.35 mcg. per gm. in the second year. Nitrate in corn silage has been implicated in oxidative destruction of carotene (Pope et al., 1961). Therefore, nitrate analyses were done on both the corn and grass silages in an attempt to establish a reason for the large decrease in liver vitamin A over the winter months in herd 7. No nitrate was detected in either of the forage samples. To further confuse the issue carotene analysis revealed a carotene content in grass silage of 2.0 mg. per pound and in corn silage of 3.0 mg. per pound wet weight (Appendix IV Table VI). Herd 7 was the only Aberdeen Angus herd tested in the study. Thus, one might speculate that this breed of cattle is less efficient in converting carotene to vitamin A than the Hereford and Shorthorn cattle of herd 3. However, Pope et al. (1961) presented evidence contrary to this suggestion, in that, vitamin A status of beef cows does not vary among these beef breeds on similar treatments. This leaves the suggestion that something, at present not identified, was in the ration of herd-7 cows which reduced the availability of carotene in the corn silage.

The liver vitamin A season averages over the two-year period were consistently highest for herd 11 and lowest for herd 9, with the exception of

the spring period when herd 7 was lowest. The carotene content of the forages fed by these two cooperators were quite similar (3.2 and 3.7 mg. per pound for 9 and 11, respectively) during the second winter (Appendix IV Table VI). It is not likely that herd 11 had a higher carotene intake because the cows consumed more forage, since both farms scored good or very good in management practices and herd condition (Appendix III Table V). Since Interlake hay was the only forage fed to both herds 9 and 11 it may be conjectured that the differences in liver vitamin A between these herds may have been due to some difference in management or environment not noticed by the author at the times of sample collection.

No vitamin A deficiency symptoms were noted in any of the test cows or their calves during the study. Furthermore, considerable increases in liver vitamin A levels were observed in some herds during winter, which suggests that vitamin A supplementation is not required by breeding cows maintained under conditions similar to those of the present study.

The mean squares for liver carotene (Table IX) indicate that the sources of variation, season, area, Y x S and Y x S x A are significant ( $P < .01$ ). The liver carotene levels of herds in the Southwestern area were significantly ( $P < .01$ ) higher than those of the Ste. Rose area during the study period. This seems peculiar when one recalls the similarity in liver vitamin A levels for the two areas. However, these results are in agreement with Ralston and Dyer (1960) who found no significant correlation between liver carotene and liver vitamin A levels. Comparison of the liver carotene season means (Table X) reveals that the summer mean was significantly higher than the spring ( $P < .01$ ) and fall ( $P < .05$ ) means when averaged over the two areas for the two years. The carotene content of native grasses is at its peak during the period May



to July (Pope *et al.*, 1961). Therefore, the yearly peak in liver carotene coincided with the suggested peak level of carotene in the forage.

TABLE X

Duncan's Comparison of Season Means  
for Liver Carotene

| LEVEL OF<br>SIGNIFICANCE | SEASON |      |        |
|--------------------------|--------|------|--------|
|                          | SPRING | FALL | SUMMER |
| 5%                       | 6.99   | 7.80 | 9.06   |
| 1%                       |        |      |        |

The main effects are complicated by a significant ( $P < .01$ ) Y x S interaction, which implies that the effect of season upon the liver carotene level varied with the year when averaged over the two areas. The effect of this interaction is evident in Figure 4. Further analysis of the interaction indicated a non-significant ( $P > .05$ ) difference between years when averaged over the two areas in fall; however, the second year was significantly ( $P < .05$ ) higher than the first year in spring, and the first year was significantly ( $P < .01$ ) higher than the second year in summer. Since the year effect was opposite in spring and summer, they tended to cancel the effect of each other and resulted in a non-significant ( $P > .05$ ) main effect for years.

The liver carotene picture is further confused by a significant ( $P < .01$ ) three-way interaction (Y x S x A), which implies that the Y x S interaction varied with the area. However, regardless of the complexity existing in the effects of area, season and year upon liver carotene levels, herd averages were fairly consistent within treatments. This is indicated by

the standard errors of the treatment means (Table VIII).

The analysis of variance (Table IX) indicate that the sources of variation, year, season and Y x S are significant ( $P < .01$ ) for plasma vitamin A. The seasonal trends of plasma vitamin A were quite similar for the two areas (Figure 5). The plasma vitamin A levels in the first year were significantly ( $P < .01$ ) higher than those in the second year when averaged over the two areas. Plasma vitamin A levels are related to carotene intake (Wheeler et al., 1958; Pope et al., 1961). Therefore, one can conclude that the carotene intake of the animals was greater during the first year than during the second year. This tends to confirm the earlier observation, although non-significant ( $P > .05$ ), that liver vitamin A levels at each season of the second year were lower than those of the corresponding seasons of the first year. As has previously been suggested, the difference between carotene intake of the two years was not due to rainfall or temperature, because mean daily temperature and rainfall recordings taken at stations throughout the two areas revealed no differences between years or between areas (Appendix V Tables VII and VIII). Further analysis of the significant ( $P < .05$ ) difference between season means indicate that the average summer value for plasma vitamin A was significantly ( $P < .01$ ) higher than the average fall and spring values (Table XI). Therefore, the annual maximum in plasma vitamin A level occurred at the time of year when carotene content of forage was probably highest.

The significant year and season effects upon plasma vitamin A level are complicated by a significant ( $P < .01$ ) Y x S interaction. Analysis of this two-way interaction reveals that the difference between years in plasma vitamin A level, when averaged over the two areas, was non-significant ( $P > .05$ ) for spring. However, the first year was significantly ( $P < .01$ ) higher than the

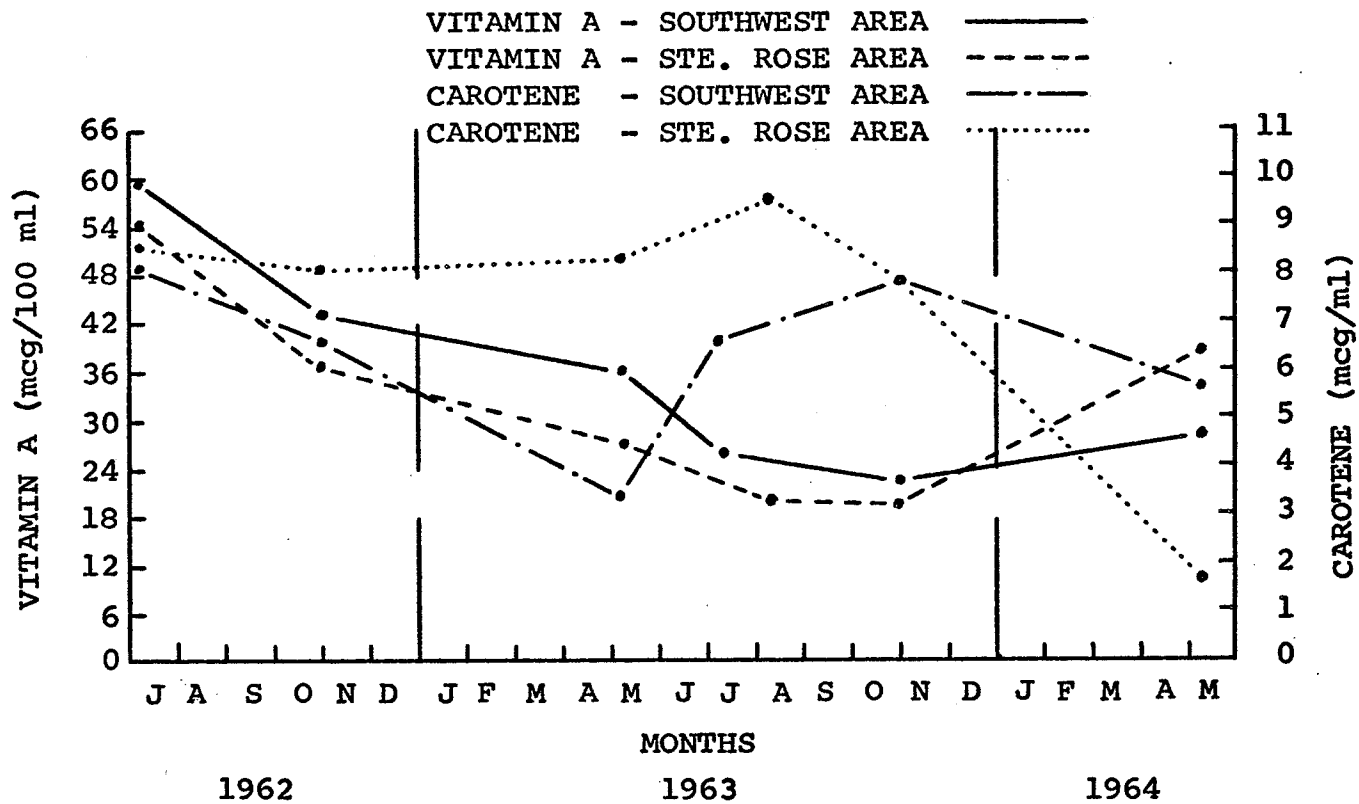


FIGURE 5. AVERAGE VITAMIN A AND CAROTENE LEVELS IN THE PLASMA

second year in summer and fall. The interaction, then, restricts the significant effect of the first year over the second year to the summer and fall seasons. Thus, it might be suggested that carotene intake was greater during the summer of 1962 than during any other period of the study. This is also reflected in the liver vitamin A values (Table VIII), because those obtained for both areas in fall 1962 were higher than any other treatment mean obtained during the study period.

TABLE XI  
Duncan's Comparison of Season Means  
for Plasma Vitamin A

| LEVEL OF<br>SIGNIFICANCE * | SEASON |        |        |
|----------------------------|--------|--------|--------|
|                            | FALL   | SPRING | SUMMER |
| 1%                         | 30.48  | 32.91  | 40.26  |

\* All means significantly different at 5% level.

The standard errors of the treatment means (Table VIII) for plasma vitamin A indicate a fairly constant degree of variation among herds within treatments.

The mean squares for plasma carotene (Table IX) indicate that the sources of variation, season, Y x A and Y x S x A are significant ( $P < .05$ ). The significant difference between seasons, upon further analysis, reveals that the spring mean was significantly ( $P < .01$ ) lower than the summer and fall means when averaged over the two areas for two years (Table XII). This indicates that the plasma carotene level was highest during the period of greatest carotene intake (summer), and corroborates work of Pope et al. (1961), who

reported that the yearly pattern of plasma carotene corresponded directly with the carotene content of native grasses.

TABLE XII  
Duncan's Comparison of Season Means  
for Plasma Carotene

| LEVEL OF<br>SIGNIFICANCE * | SEASON |      |        |
|----------------------------|--------|------|--------|
|                            | SPRING | FALL | SUMMER |
| 1%                         | 4.79   | 7.65 | 8.29   |

\* All means significantly different at 5% level.

The considerable variation in plasma carotene between areas and between years (Figure 5) is exemplified by a significant ( $P < .05$ ) Y x A interaction. Analysis of this two-way interaction over the three seasons revealed no difference between the two years in the Southwest area. However, the difference was significant ( $P < .05$ ) in the Ste. Rose area where mean plasma carotene levels were higher during the first than during the second year. This inconsistency of plasma carotene levels is further exemplified in the significant ( $P < .01$ ) Y x S x A interaction which indicates that the Y x A interaction varied with season.

The lower standard errors for plasma carotene treatment means (Table VIII) in the spring indicate a less variable carotene and/or vitamin A intake among herds during the winter than during the summer. This is, however, in direct opposition to the conclusion drawn from standard errors of the liver vitamin A treatment means. Nevertheless these results are in agreement with Ralston and Dyer (1960) and Pope et al. (1961), who obtained a non-significant

correlation between plasma carotene level and liver vitamin A level.

The value of plasma vitamin A and carotene levels in estimating the vitamin A status of animals has been questioned by many workers. Some have obtained significant correlations between liver vitamin A level and the vitamin A and/or carotene level of the plasma, while others have not. Due to the limited conversion of endogenous carotene to vitamin A within the animal's body (Maynard and Loosli, 1956), the level of liver carotene, which is usually found to be unrelated to liver vitamin A level, is of very doubtful significance.

To determine the degree of relationship that liver vitamin A possesses with each of liver carotene, plasma vitamin A and plasma carotene the appropriate correlation coefficients were calculated over the two-year study period, and they are 0.015, 0.132 and 0.067, respectively. None were statistically significant at the 5% level of probability, which corroborates the work of Ralston and Dyer (1960).

#### Calcium and Phosphorus

Total plasma calcium and plasma inorganic phosphorus will be discussed concomitantly. Table XIII presents numerically and Figure 6 presents graphically the effects of area, season and year upon the plasma levels of calcium and inorganic phosphorus.

For plasma calcium, the mean squares (Table XIV) attach statistical significance to the season, area, Y x S and S x A sources of variation. The trends in plasma calcium are fairly similar for the two years. The average plasma calcium level for the Ste. Rose area was significantly higher than that of the Southwestern area when averaged over the three seasons for the two years. Further statistical analysis of the significant season effect (Table XV) indicates

TABLE XIII

MEAN PLASMA LEVELS OF CALCIUM, PHOSPHORUS,  
AND MAGNESIUM  
(mg. per 100 ml.)

|            | SUMMER        |              | FALL         |             | SPRING      |              |
|------------|---------------|--------------|--------------|-------------|-------------|--------------|
|            | 1962          | 1963         | 1962         | 1963        | 1963        | 1964         |
| SOUTHWEST  |               |              |              |             |             |              |
| CALCIUM    | 9.87 ± 0.16 * | 9.56 ± 0.29  | 10.34 ± 0.23 | 9.80 ± 0.11 | 9.37 ± 0.12 | 9.78 ± 0.18  |
| PHOSPHORUS | 5.67 ± 0.29   | 5.13 ± 0.39  | 4.10 ± 0.27  | 4.01 ± 0.21 | 4.72 ± 0.42 | 4.59 ± 0.41  |
| MAGNESIUM  | 1.88 ± 0.19   | 2.07 ± 0.29  | 2.76 ± 0.13  | 1.86 ± 0.07 | 2.14 ± 0.10 | 1.70 ± 0.12  |
| STE. ROSE  |               |              |              |             |             |              |
| CALCIUM    | 10.78 ± 0.17  | 10.31 ± 0.36 | 10.02 ± 0.11 | 9.99 ± 0.14 | 9.24 ± 0.26 | 10.37 ± 0.16 |
| PHOSPHORUS | 5.02 ± 0.60   | 5.55 ± 0.17  | 3.84 ± 0.30  | 3.93 ± 0.44 | 4.50 ± 0.59 | 4.45 ± 0.32  |
| MAGNESIUM  | 1.69 ± 0.19   | 2.01 ± 0.25  | 3.22 ± 0.13  | 2.10 ± 0.11 | 2.40 ± 0.24 | 1.75 ± 0.23  |

\* STANDARD ERROR

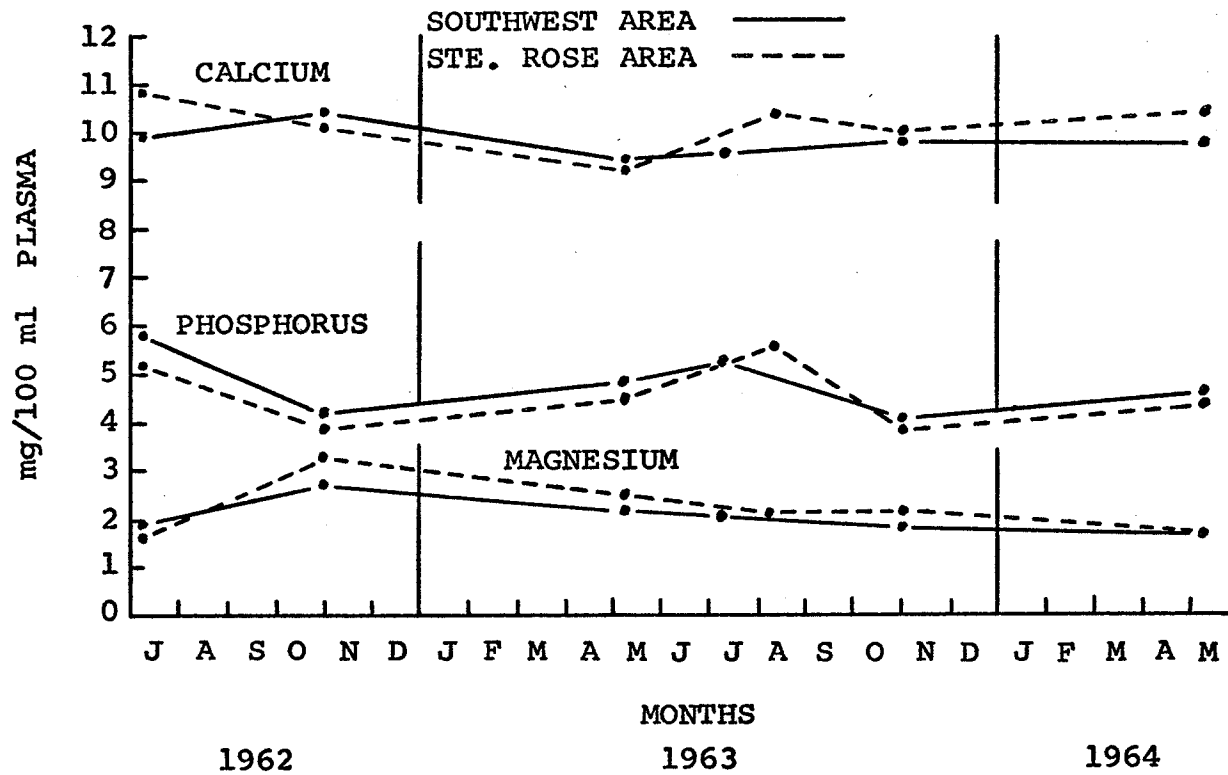


FIGURE 6. PLASMA LEVELS OF CALCIUM, PHOSPHORUS AND MAGNESIUM



TABLE XIV

MEAN SQUARES FOR PLASMA LEVELS OF CALCIUM,  
PHOSPHORUS, AND MAGNESIUM

| SOURCE     | d.f. | CALCIUM  | PHOSPHORUS | MAGNESIUM |
|------------|------|----------|------------|-----------|
| YEAR (Y)   | 1    | 0.003    | 0.003      | 0.563 **  |
| SEASON (S) | 2    | 0.216 ** | 1.895 **   | 0.382 **  |
| Y x S      | 2    | 0.412 ** | 0.003      | 0.410 **  |
| AREA (A)   | 1    | 0.330 ** | 0.072      | 0.048     |
| Y x A      | 1    | 0.096    | 0.148      | 0.008     |
| S x A      | 2    | 0.208 ** | 0.001      | 0.057     |
| Y x S x A  | 2    | 0.053    | 0.074      | 0.001     |
| ERROR      | 57   | 0.0381   | 0.152      | 0.029     |

\*\*  $P < .01$

that the spring mean for plasma calcium was significantly lower than the summer ( $P < .01$ ) and fall ( $P < .05$ ) means when averaged over the two areas for the study period.

TABLE XV  
Duncan's Comparison of Season Means  
for Plasma Calcium

| LEVEL OF SIGNIFICANCE | SEASON |       |        |
|-----------------------|--------|-------|--------|
|                       | SPRING | FALL  | SUMMER |
| 5%                    | 9.69   | 10.04 | 10.13  |
| 1%                    |        |       |        |

The significant ( $P < .01$ ) S x A and Y x S interactions (Table XIV) tend to confound the main effects of year, season and area upon plasma calcium level. Further statistical analysis of these interactions indicate that for S x A there was a non-significant ( $P > .05$ ) difference between the plasma calcium levels of the two areas when averaged over the two years in spring and fall. However, the Ste. Rose area was significantly ( $P < .01$ ) higher than the Southwestern area in summer. For the Y x S interaction the difference between years in mean plasma calcium level was non-significant ( $P > .05$ ) for fall and summer when averaged over the two areas. However, the second year was significantly ( $P < .01$ ) higher than the first year in spring.

In brief, the main effects upon plasma calcium level were:

- (1) A significant ( $P < .01$ ) area effect which was restricted (by S x A interaction) to the summer season, wherein, the mean for the Ste. Rose area was greater than that of the Southwestern area.

- (2) The main year effect was non-significant ( $P > .05$ ). However, the Y x S interaction reveals that the mean for the second year was significantly ( $P < .01$ ) higher than that for the first year in the spring season.
- (3) The mean spring level was significantly ( $P < .05$ ) lower than the summer and fall means when averaged over the two areas for the two years.

The slight seasonal trends corroborate work done by Bohman et al. (1961), who obtained a minimum plasma calcium level of 9.3 mg. per 100 ml. in March and a maximum of 10.3 mg. per 100 ml. in the June - August period with calves. Contradictory to this is work done by Marsh and Swingle (1960), who did not obtain a seasonal variation pattern during a five-year study with range cows.

Calcium intake does not affect the plasma calcium level directly unless it is very low for a long period of time (Dowe et al., 1957; Bohman et al., 1961). Therefore, differences obtained in the present study cannot be attributed to intake. Due to the presence of significant interactions, that tend to confound the main effects, it is possible that the differences in plasma calcium level resulted from variations in the environment, which could include mineral interrelationships in the diet.

Standard errors of the treatment means for plasma calcium (Table XIII) indicate a small variation among herd averages within treatments. Differences among herd averages throughout the study period were relatively small, being within a range of 8.45 - 11.41 mg. per 100 ml. These values are similar to those obtained by Marsh and Swingle (1960), who reported a range of 8.20 - 11.60 mg. per 100 ml. for plasma calcium over a five-year study

with range cows. Similarly, Payne et al. (1946) obtained a mean value of 9.52 mg. per 100 ml. for plasma calcium in range cows. All the values are within what is called a normal range.

Results of the statistical analysis (Table XIV) indicate that only the season source of variation significantly ( $P < .01$ ) affected the plasma inorganic phosphorus level. The seasonal trends in plasma inorganic phosphorus level were quite similar for the two areas (Figure 6). Statistical comparison of the season means (Table XVI) reveal that the differences among all seasons were significant ( $P < .05$ ), and only the difference between spring and fall means was non-significant at the 1% level of probability. Wise et al. (1961) showed that plasma inorganic phosphorus levels were directly related to phosphorus intake. Therefore, phosphorus intake of the cows in this study must have been greater during the winter and early summer periods when plasma phosphorus levels increased, than in the late summer period (July to November) when a significant decrease occurred. To get an increase in the plasma inorganic phosphorus level during early summer the phosphorus intake must have been substantially higher than that of late summer, because this is the period of peak milk production. Therefore, one might suggest that grasses growing in early summer had either a higher phosphorus content, or that the phosphorus of these forages was more available to the animal. Early summer could simultaneously be characterized by a higher than average dry matter consumption because forages growing during this period are quite succulent. Since grains are relatively high in phosphorus, one might attribute the winter period increase in plasma inorganic phosphorus to them. However, their overall contribution to the rations of these cows was very small since eight of the 12 cooperators fed no grain.

TABLE XVI

Duncan's Comparison of Season Means  
for Plasma Inorganic Phosphorus

| LEVEL OF<br>SIGNIFICANCE * | SEASON |        |        |
|----------------------------|--------|--------|--------|
|                            | FALL   | SPRING | SUMMER |
| 1%                         | 3.97   | 4.57   | 5.34   |

\* All means significantly different at 5% level.

Soils in the Ste. Rose area have been criticized as being phosphorus deficient. This has enticed many farmers to describe cattle suffering from apparent malnutrition as being phosphorus deficient, rather than possibly suffering from a lack of energy. The forages grown in the Interlake area were lower in phosphorus than forages grown in the Southwestern area (Appendix IV Table VI), which could indicate lower phosphorus levels in the Interlake soils. However, the herd averages (Appendix II Table III) for plasma inorganic phosphorus did not reflect this difference. Therefore, phosphorus intake of cows in the Ste. Rose area was sufficient to meet their requirement, and the higher intake of cows in the Southwestern area appears to have been in excess of their requirement.

Low fertility has been a problem in many herds of the Ste. Rose area. Phosphorus supplementation of phosphorus deficient pastures has been shown to increase calf crop by as much as 30% (Maynard and Loosli, 1956). Since there was no difference between the area averages for plasma inorganic phosphorus in this study, low fertility in the Ste. Rose area can not be attributed to a phosphorus deficiency in the cows. Furthermore, the annual maximum in plasma

inorganic phosphorus level occurred during the breeding season (June - August). A more logical explanation for the low fertility can be obtained from the questionnaire (Appendix III Table IV), which revealed that some of the Ste. Rose farmers used one bull for 40 - 60 cows. Furthermore, the pastures in this area are large with considerable woodland. It is suggested that the low fertility was simply due to a bull's inability to service all the cows during the breeding season.

The standard errors of the treatment means for plasma inorganic phosphorus (Table XIII) are fairly consistent among treatments. Thus, variation among herd averages within treatments is quite consistent. The range in herd averages was 2.44 - 6.53 mg. per 100 ml. during the study period. These values are similar to those of Marsh and Swingle (1960), who obtained a three-year average of 3.72 mg. inorganic phosphorus per 100 ml. in range cows, and Watkins and Knox (1948) who reported a range of 2.11 - 5.37 mg. inorganic phosphorus per 100 ml. in breeding cows. The values obtained in the present study can be considered normal for cows maintained under the existing conditions of these two areas, because deficiency symptoms were not observed and the values agree with those published by other workers where phosphorus deficiency was not observed.

The calcium and phosphorus levels in the forages (Appendix IV Table VI) are in fairly close agreement with values presented for similar forages by Morrison (1958). The majority of Ca:P ratios (Appendix IV Table VI) ranged from 1.4:1.0 to 8.2:1.0 with one ratio at 30.0:1.0 in the forage samples. Dowe et al. (1957) stated that the critical Ca:P ratio may exist between 4.3:1.0 and 9.1:1.0. Thus the ratios obtained in the present study are within a range which should not adversely affect the metabolism of either element,

with the possible exception of one ratio (30.0:1.0). However, it must be remembered that these ratios were for the forages only, and any supplemental feeding of grain or minerals would alter the Ca:P ratio of the ration which was consumed by the cows.

### Magnesium

The area trends in plasma magnesium were quite similar for the three seasons in each of the two years (Table XIII and Figure 6). Analysis of variance (Table XIV) indicates a significant ( $P < .01$ ) difference between years, among seasons and in Y x S interaction for plasma magnesium levels. The average plasma magnesium level of all cows during the first year was significantly ( $P < .01$ ) higher than that of the same cows during the second year. Further analysis of the significant season effect (Table XVII) indicate that the average plasma magnesium level in the fall was significantly ( $P < .01$ ) higher than that of summer and spring. Examination of Figure 6 reveals that the high fall value was due primarily to the large contribution by the two area means for plasma magnesium in the first year. This variation in season effect with year is indicated statistically by the significant ( $P < .01$ ) Y x S interaction (Table XIV). This interaction, upon further analysis, revealed that the significant ( $P < .01$ ) difference between years was restricted to the fall and spring seasons when averaged over the two areas.

An increased magnesium level of plasma is usually the result of increased magnesium absorption (Smith, 1959b). Therefore, the high plasma magnesium levels encountered during the study in fall and spring of the first year were probably due to higher than average magnesium absorption during late summer and winter of that year. This higher absorption can be attributed to either increased levels of magnesium in the forage or to mineral interrelationships in the digestive tract which promote magnesium absorption.

TABLE XVIIDuncan's Comparison of Season Means  
for Plasma Magnesium

| LEVEL OF<br>SIGNIFICANCE | SEASON |        |      |
|--------------------------|--------|--------|------|
|                          | SUMMER | SPRING | FALL |
| 5%                       | 1.91   | 2.00   | 2.49 |
| 1%                       |        |        |      |

The hypomagnesaemic disease known as "grass tetany" was not observed or reported in any of the test animals during the study. Smith (1959b) stated that plasma magnesium levels below 0.5 mg. per 100 ml. are indicative of a hypomagnesaemic state in calves. The range in herd averages of plasma magnesium during the study period was 0.99 - 3.58 mg. per 100 ml. with lowest values occurring in spring and summer. This is the period of year in which the highest incidence of "grass tetany" has been reported by other workers. The range in plasma magnesium level obtained in the present study is higher than that obtained by Marsh and Swingle (1960), who report 0.4 - 0.9 mg. per 100 ml. over a three-year study period without any incidence of "grass tetany".



## SUMMARY

Liver and plasma samples were collected from beef cows in two areas of Manitoba over a two-year period, from July 1962 to May 1964. Samples were collected in three seasons of the year (spring, summer and fall) from seven herds in Southwestern Manitoba and five herds in the Ste. Rose area of Manitoba. Test animals consisted of eight cows per herd in the Southwestern area and ten cows per herd in the Ste. Rose area.

The study has established the following information about beef cows in these two areas:

- (1) The liver biopsy technique did not have any adverse affect upon the cows.
- (2) An annual peak in liver copper level occurred in fall of each year. This mean value was significantly ( $P < .01$ ) higher than that obtained in spring and summer. Similar trends were obtained for each of the two years in liver copper level. The Ste. Rose mean value was significantly ( $P < .01$ ) higher than that of the Southwestern area. The three regions in the Ste. Rose area differed significantly ( $P < .05$ ) in liver copper level. The range in liver copper levels throughout the study period (herds 1 to 11) was 0.87 - 39.35 ppm wet tissue.
- (3) The liver molybdenum levels were very similar for the two areas throughout the study. The levels increased during late summer and decreased during winter and early summer. Levels during the first year were significantly higher than those for the second year of the study. The highest herd average obtained

mcg. per gm. wet liver.

- (7) Plasma vitamin A levels were quite similar for the two areas throughout the study period. The average level for the second year was significantly ( $P < .01$ ) lower than that for the first year. The annual peak in plasma vitamin A tended to coincide with the supposed peak forage carotene level for the year. However, the season trends tended to vary with the year. Herd averages ranged between 16.62 - 78.37 mcg. per 100 ml. during the study.
- (8) Definite trends in plasma carotene level were not established. However, the spring mean value appeared to be lower than the summer and fall means. The yearly trends varied considerably between the two areas. The herd average range was 0.81 - 15.49 mcg. per ml. throughout the study.
- (9) Liver vitamin A levels were not correlated with any of the following: liver carotene, plasma vitamin A, or plasma carotene.
- (10) A slight seasonal trend was detected in plasma calcium levels with the spring mean being significantly ( $P < .05$ ) lower than that of summer and fall. Trends were quite similar for the two years, whereas the mean level for Ste. Rose was significantly ( $P < .01$ ) higher than that for the Southwestern area. Herd averages throughout the study period were within the range 8.45 - 11.41 mg. per 100 ml.
- (11) Plasma inorganic phosphorus levels were quite similar for the two areas throughout the study. Trends were also quite similar

in the study was 6.24 ppm with all other herd averages ranging between 0.20 - 3.88 ppm wet tissue.

- (4) Liver cobalt levels were quite similar for the two areas throughout the study. The established trend was for a decrease throughout the summer period followed by an increase during the winter. The average liver cobalt level in the summer was significantly ( $P < .01$ ) higher than that obtained in the fall. Meanwhile the level during the second year was significantly ( $P < .01$ ) lower than the mean level of the first year. The liver cobalt herd averages ranged between 0.002 - 0.530 ppm wet tissue during the study.
- (5) The liver vitamin A levels were not significantly ( $P > .05$ ) affected by area, season, or year, which was primarily due to the high variation among herds within treatments. However, the average trend was towards increased storage during late summer followed by a decrease in the liver vitamin A store during winter. The range in herd averages during the study was 41.35 - 269.44 mcg. per gm. wet tissue.
- (6) The study was unable to establish definite trends in the liver carotene level. Nevertheless, the yearly peak in liver carotene tended to coincide with the supposed peak level of carotene in the forage. The average level for the Southwestern area was significantly ( $P < .01$ ) higher than that for the Ste. Rose area. The Y x S interaction revealed that the seasonal trends were not the same for the two years. The range in herd averages during the study was 3.30 - 15.49

during each of the two years. A definite seasonal pattern was established for plasma inorganic phosphorus levels. It was characterized by an increase during winter and early summer followed by a decrease during late summer. The range for herd averages was 2.44 - 6.53 mg. per 100 ml. during the study period.

- (12) A significant ( $P < .01$ ) Y x S interaction restricted the significant ( $P < .01$ ) difference between years and among seasons in plasma magnesium level to the fall and spring seasons wherein the first year was significantly higher than the second year. The plasma magnesium trends were very similar for the two areas during the study. The range in herd averages during the study period was 0.99 - 3.58 mg. per 100 ml.
- (13) Deficiency symptoms were not evident for any of the nutrients under consideration during this study with the exception of a possible copper deficiency in herd 12.

## CONCLUSIONS AND RECOMMENDATIONS

Since herds 1 to 11 did not show any copper deficiency symptoms, the liver copper levels of these herds were considered to be adequate under the existing conditions. For these herds the liver is an efficient storehouse of copper. The store, built up during summer under conditions of adequate intake, was sufficient to maintain a satisfactory copper status in the animals during the winter feeding period. However, the liver copper level of herd 12 was in the marginal range. It is recommended that cooperators 12 continue to feed his cows copper; at least in winter. Better methods of administration would, however, be:

- (1) In grain, so that each animal receives 1.0 to 2.0 gm.  $\text{Cu SO}_4$  per day.
- (2) Injections of about 150 mg. copper in the spring and again in the fall of each year.

Furthermore, it is recommended that the calves be given a copper drench or injection before they are one week of age because the milk of dams receiving a low copper intake would be correspondingly low in copper.

Molybdenum has received more attention regarding its toxic effect than its requirement as an essential nutrient. The liver molybdenum levels were apparently high enough to meet the metabolic requirements of the animals, but low enough so that molybdenosis did not occur. Therefore, the liver molybdenum range of 0.20 - 3.88 ppm wet tissue was considered normal for cows living under the existing conditions of these two Manitoba areas.

The majority of the liver cobalt levels were low. It is recommended that farmers in the two areas investigated, and more generally throughout

Manitoba, give their cattle a cobalt supplement the year round. It can be given in a trace mineral mix, which is probably the easiest method, or in the form of a dense slow releasing cobalt bullet given via the mouth, which is undoubtedly the best method of insuring an adequate intake.

The cows had relatively high liver vitamin A stores throughout the study period. Supplementation of cows in these two areas with vitamin A would not likely improve their performance during years of similar climatic conditions.

The plasma levels of calcium and inorganic phosphorus do not indicate a deficiency of either nutrient in beef cows of the two areas. From the wide Ca:P ratios present in the forage it appears that phosphorus supplementation would be more beneficial than calcium supplementation.

The values obtained for plasma magnesium indicate a satisfactory status in the animals investigated and that hypomagnesaemia is not a problem in beef cattle of these two areas.

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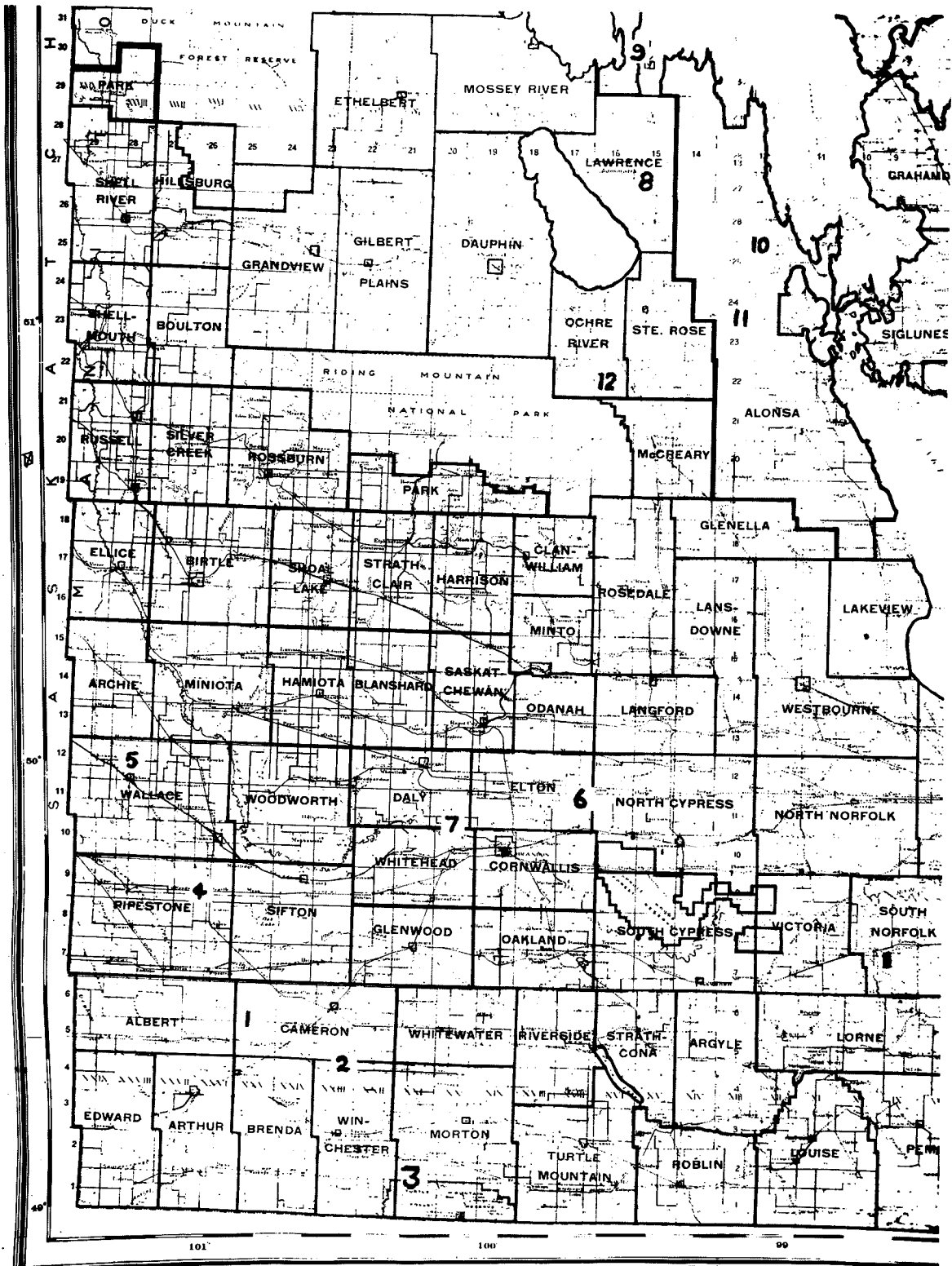
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APPENDIX I





MAP SHOWING THE SECTION OF MANITOBA WHEREIN THE TWO GEOGRAPHICAL AREAS INVESTIGATED IN THIS STUDY ARE LOCATED. THE SOUTHWEST AREA COMPRISES HERDS 1-7 AND THE STE. ROSE AREA HERDS 8-12

APPENDIX II

TABLE I

HERD AVERAGES FOR LIVER LEVELS OF COPPER, MOLYBDENUM AND COBALT

| HERD<br>NUMBER         | SUMMER |       | FALL  |       | SPRING |       |
|------------------------|--------|-------|-------|-------|--------|-------|
|                        | 1962   | 1963  | 1962  | 1963  | 1963   | 1964  |
| LIVER COPPER (ppm)     |        |       |       |       |        |       |
| 1                      | 2.38   | 2.46  | 8.01  | 8.61  | 3.15   | 3.40  |
| 2                      | 3.57   | 1.15  | 6.08  | 2.47  | 1.04   | 1.64  |
| 3                      | 4.24   | 5.53  | 8.96  | 14.60 | 2.38   | 4.81  |
| 4                      | 5.13   |       | 9.26  | 15.26 | 9.51   | 8.97  |
| 5                      | 8.09   | 9.01  | 10.54 | 14.99 | 2.26   | 2.73  |
| 6                      | 1.71   | 2.54  | 12.57 | 3.36  | 0.87   | 1.80  |
| 7                      | 1.59   |       | 2.13  | 1.68  | 2.58   | 2.98  |
| 8                      | 4.84   |       | 6.86  | 12.39 | 2.89   | 7.54  |
| 9                      | 6.20   | 8.56  | 13.42 | 13.58 | 2.82   | 6.53  |
| 10                     | 14.35  | 23.24 | 22.99 | 21.91 | 15.08  | 9.56  |
| 11                     | 21.78  | 7.03  | 39.35 | 28.66 | 19.29  | 17.34 |
| 12                     | 1.09   | 1.52  | 1.88  | 1.90  | 0.0    | 8.08  |
| LIVER MOLYBDENUM (ppm) |        |       |       |       |        |       |
| 1                      | 0.53   | 0.32  | 3.88  | 1.42  | 1.15   | 1.46  |
| 2                      | 0.72   | 0.95  | 1.17  | 1.20  | 1.11   | 1.20  |
| 3                      | 0.55   | 0.20  | 0.90  | 1.36  | 1.02   | 1.58  |
| 4                      | 1.27   |       | 6.24  | 1.24  | 1.30   | 1.67  |
| 5                      | 1.57   | 2.19  | 0.97  | 1.12  | 0.59   | 1.22  |
| 6                      | 4.22   | 0.40  | 0.81  | 1.05  | 0.60   | 1.32  |

TABLE I (continued)

| HERD<br>NUMBER     | SUMMER |       | FALL  |       | SPRING |       |
|--------------------|--------|-------|-------|-------|--------|-------|
|                    | 1962   | 1963  | 1962  | 1963  | 1963   | 1964  |
| 7                  | 1.25   |       | 0.65  | 1.15  | 1.29   | 1.44  |
| 8                  | 1.44   |       | 1.49  | 1.15  | 1.10   | 1.14  |
| 9                  | 1.34   | 0.75  | 2.21  | 0.99  | 0.82   | 0.69  |
| 10                 | 1.49   | 0.91  | 2.48  | 1.14  | 0.82   | 1.19  |
| 11                 | 1.99   | 0.61  | 1.44  | 1.15  | 1.33   | 1.15  |
| 12                 | 1.54   | 0.87  | 2.48  | 0.90  | 1.51   | 1.49  |
| LIVER COBALT (ppm) |        |       |       |       |        |       |
| 1                  | 0.210  | 0.049 | 0.033 | 0.044 | 0.270  | 0.015 |
| 2                  | 0.530  | 0.161 | 0.041 | 0.005 | 0.386  | 0.026 |
| 3                  | 0.500  | 0.020 | 0.280 | 0.016 | 0.296  | 0.030 |
| 4                  | 0.350  |       | 0.160 | 0.074 | 0.074  | 0.124 |
| 5                  | 0.310  | 0.157 | 0.012 | 0.015 | 0.131  | 0.050 |
| 6                  | 0.110  | 0.070 | 0.170 | 0.112 | 0.132  | 0.080 |
| 7                  | 0.100  |       | 0.180 | 0.056 | 0.020  | 0.066 |
| 8                  | 0.050  |       | 0.110 | 0.049 | 0.274  | 0.037 |
| 9                  | 0.050  | 0.052 | 0.047 | 0.030 | 0.232  | 0.030 |
| 10                 | 0.110  | 0.131 | 0.061 | 0.015 | 0.042  | 0.027 |
| 11                 | 0.410  | 0.177 | 0.100 | 0.002 | 0.174  | 0.010 |
| 12                 | 0.200  | 0.142 | 0.120 | 0.190 | 0.208  | 0.072 |

TABLE II

HERD AVERAGES FOR VITAMIN A AND CAROTENE LEVELS IN THE LIVER AND PLASMA

| HERD<br>NUMBER                 | SUMMER |        | FALL   |        | SPRING |        |
|--------------------------------|--------|--------|--------|--------|--------|--------|
|                                | 1962   | 1963   | 1962   | 1963   | 1963   | 1964   |
| LIVER VITAMIN A (mcg. PER gm.) |        |        |        |        |        |        |
| 1                              | 151.51 | 94.77  | 151.65 | 141.90 | 128.78 | 121.40 |
| 2                              | 121.81 | 114.36 | 139.37 | 120.07 | 145.97 | 102.61 |
| 3                              | 177.71 | 190.18 | 266.90 | 210.81 | 267.07 | 241.79 |
| 4                              | 133.61 |        | 166.87 | 157.08 | 168.61 | 242.43 |
| 5                              | 186.22 | 131.49 | 199.62 | 178.92 | 120.32 | 165.55 |
| 6                              | 140.02 | 178.84 | 169.36 | 187.43 | 186.67 | 187.30 |
| 7                              | 200.85 |        | 244.30 | 178.17 | 106.22 | 41.35  |
| 8                              | 161.32 |        | 185.74 | 199.92 | 154.40 | 173.39 |
| 9                              | 113.97 | 89.64  | 140.19 | 127.47 | 90.80  | 102.81 |
| 10                             | 146.28 | 131.65 | 165.92 | 136.44 | 157.02 | 135.13 |
| 11                             | 217.08 | 199.47 | 242.14 | 229.79 | 269.44 | 201.77 |
| 12                             | 145.62 | 144.48 | 185.96 | 169.00 | 172.50 | 161.05 |
| LIVER CAROTENE (mcg. PER gm.)  |        |        |        |        |        |        |
| 1                              | 15.24  | 5.12   | 5.74   | 8.49   | 3.86   | 7.54   |
| 2                              | 12.95  | 6.60   | 7.07   | 7.24   | 5.81   | 8.55   |
| 3                              | 12.83  | 6.50   | 8.68   | 6.93   | 6.53   | 15.04  |
| 4                              | 15.46  |        | 7.09   | 11.88  | 6.88   | 13.09  |
| 5                              | 9.89   | 9.04   | 8.25   | 9.24   | 4.68   | 11.90  |
| 6                              | 12.12  | 7.83   | 10.53  | 10.52  | 4.54   | 11.69  |

TABLE II (continued)

| HERD<br>NUMBER                      | SUMMER |       | FALL  |       | SPRING |       |
|-------------------------------------|--------|-------|-------|-------|--------|-------|
|                                     | 1962   | 1963  | 1962  | 1963  | 1963   | 1964  |
| 7                                   | 15.49  |       | 9.71  | 9.14  | 4.34   | 9.23  |
| 8                                   | 6.88   |       | 5.88  | 6.62  | 6.94   | 6.08  |
| 9                                   | 7.32   | 5.35  | 5.08  | 5.75  | 4.69   | 3.30  |
| 10                                  | 10.07  | 7.60  | 6.82  | 10.13 | 6.63   | 3.44  |
| 11                                  | 10.26  | 8.02  | 8.42  | 6.32  | 10.22  | 5.16  |
| 12                                  | 9.81   | 6.72  | 7.45  | 7.37  | 7.68   | 4.49  |
| PLASMA VITAMIN A (mcg. PER 100 ml.) |        |       |       |       |        |       |
| 1                                   | 62.27  | 35.11 | 51.84 | 23.27 | 33.26  | 30.25 |
| 2                                   | 51.03  | 26.55 | 40.14 | 30.54 | 40.05  | 29.77 |
| 3                                   | 64.70  | 17.89 | 31.61 | 22.07 | 39.86  | 28.12 |
| 4                                   | 65.80  |       | 45.42 | 25.22 | 30.68  | 37.37 |
| 5                                   | 73.83  | 18.45 | 47.80 | 18.18 | 26.60  | 24.07 |
| 6                                   | 56.80  | 32.63 | 49.96 | 20.53 | 48.93  | 20.72 |
| 7                                   | 42.50  |       | 31.04 | 17.00 | 35.03  | 29.23 |
| 8                                   | 50.82  |       | 36.53 | 20.42 | 25.50  | 45.43 |
| 9                                   | 42.67  | 22.60 | 35.59 | 18.83 | 35.13  | 31.94 |
| 10                                  | 44.52  | 16.62 | 28.39 | 19.07 | 25.32  | 43.48 |
| 11                                  | 78.37  | 18.93 | 37.27 | 18.05 | 20.40  | 35.59 |
| 12                                  | 57.31  | 24.34 | 45.66 | 25.13 | 32.33  | 38.75 |
| PLASMA CAROTENE (mcg. PER ml.)      |        |       |       |       |        |       |
| 1                                   | 9.37   | 4.98  | 3.60  | 5.48  | 2.33   | 4.10  |
| 2                                   | 7.19   | 5.35  | 4.68  | 3.99  | 3.63   | 5.45  |

TABLE II (continued)

| HERD<br>NUMBER | SUMMER |       | FALL  |       | SPRING |      |
|----------------|--------|-------|-------|-------|--------|------|
|                | 1962   | 1963  | 1962  | 1963  | 1963   | 1964 |
| 3              | 8.73   | 6.91  | 8.78  | 9.33  | 3.36   | 9.54 |
| 4              | 8.55   |       | 7.20  | 8.40  | 5.40   | 5.59 |
| 5              | 9.27   | 11.09 | 8.19  | 11.15 | 5.20   | 5.65 |
| 6              | 6.25   | 5.32  | 10.46 | 11.86 | 1.94   | 6.90 |
| 7              | 7.08   |       | 3.95  | 5.11  | 2.43   | 3.01 |
| 8              | 8.04   |       | 6.37  | 5.09  | 9.93   | 2.83 |
| 9              | 6.04   | 6.30  | 6.70  | 6.05  | 5.48   | 0.81 |
| 10             | 6.98   | 9.62  | 7.33  | 9.64  | 10.12  | 1.79 |
| 11             | 11.28  | 15.10 | 12.90 | 10.18 | 8.49   | 1.78 |
| 12             | 10.89  | 7.79  | 7.03  | 8.75  | 7.05   | 1.49 |

TABLE III

HERD AVERAGES FOR PLASMA LEVELS OF CALCIUM, INORGANIC PHOSPHORUS AND MAGNESIUM

| HERD<br>NUMBER                                | SUMMER |       | FALL  |       | SPRING |       |
|---|--------|-------|-------|-------|--------|-------|
|   | 1962   | 1963  | 1962  | 1963  | 1963   | 1964  |
| PLASMA CALCIUM (mg. PER 100 ml.)              |        |       |       |       |        |       |
| 1   | 9.52   | 9.37  | 10.21 | 9.99  | 9.70   | 9.85  |
| 2   | 9.46   | 9.18  | 9.18  | 10.03 | 9.17   | 9.99  |
| 3   | 9.78   | 10.66 | 11.20 | 9.96  | 9.23   | 9.55  |
| 4   | 10.28  |       | 10.25 | 9.43  | 8.96   | 9.76  |
| 5   | 9.57   | 9.54  | 10.56 | 10.10 | 9.56   | 9.49  |
| 6   | 10.62  | 9.03  | 10.63 | 9.65  | 9.78   | 10.64 |
| 7   | 9.84   |       | 10.32 | 9.43  | 9.21   | 9.16  |
| 8   | 11.41  |       | 10.28 | 10.19 | 10.05  | 10.53 |
| 9   | 10.67  | 11.11 | 9.68  | 9.95  | 8.45   | 10.37 |
| 10  | 10.80  | 9.35  | 10.04 | 10.30 | 9.25   | 9.75  |
| 11  | 10.68  | 10.43 | 9.94  | 9.50  | 9.39   | 10.51 |
| 12  | 10.36  | 10.33 | 10.18 | 10.03 | 9.04   | 10.70 |
| PLASMA INORGANIC PHOSPHORUS (mg. PER 100 ml.) |        |       |       |       |        |       |
| 1   | 5.82   | 4.39  | 3.48  | 4.03  | 2.44   | 3.64  |
| 2   | 6.04   | 6.27  | 4.29  | 4.41  | 4.41   | 5.61  |
| 3   | 6.21   | 5.80  | 4.22  | 3.72  | 4.71   | 3.94  |
| 4   | 6.53   |       | 5.05  | 3.50  | 5.39   | 4.14  |
| 5   | 4.60   | 4.83  | 4.29  | 3.72  | 5.81   | 3.43  |
| 6   | 5.90   | 4.34  | 4.50  | 3.62  | 4.80   | 6.26  |



TABLE III (continued)

| HERD<br>NUMBER                     | SUMMER |      | FALL |      | SPRING |      |
|------------------------------------|--------|------|------|------|--------|------|
|                                    | 1962   | 1963 | 1962 | 1963 | 1963   | 1964 |
| 7                                  | 4.62   |      | 2.85 | 5.06 | 5.47   | 5.12 |
| 8                                  | 4.10   |      | 3.27 | 3.41 | 3.88   | 5.12 |
| 9                                  | 6.07   | 5.25 | 3.42 | 2.88 | 5.14   | 4.45 |
| 10                                 | 3.20   | 5.28 | 3.42 | 3.83 | 3.02   | 3.25 |
| 11                                 | 5.37   | 5.74 | 4.27 | 5.51 | 6.42   | 4.66 |
| 12                                 | 6.37   | 5.91 | 4.82 | 4.01 | 4.06   | 4.76 |
| PLASMA MAGNESIUM (mg. PER 100 ml.) |        |      |      |      |        |      |
| 1                                  | 1.96   | 2.72 | 3.25 | 2.08 | 2.10   | 1.72 |
| 2                                  | 2.49   | 2.05 | 3.13 | 1.54 | 1.67   | 1.51 |
| 3                                  | 1.77   | 0.99 | 2.40 | 1.73 | 2.20   | 1.39 |
| 4                                  | 1.87   |      | 2.39 | 1.85 | 2.53   | 1.97 |
| 5                                  | 2.44   | 2.18 | 2.56 | 1.91 | 2.30   | 1.61 |
| 6                                  | 1.48   | 2.39 | 2.79 | 2.00 | 2.26   | 1.47 |
| 7                                  | 1.13   |      | 2.80 | 1.92 | 1.95   | 2.26 |
| 8                                  | 1.04   |      | 3.17 | 1.89 | 1.79   | 1.06 |
| 9                                  | 1.61   | 1.87 | 2.98 | 2.19 | 3.06   | 1.50 |
| 10                                 | 1.87   | 2.72 | 3.42 | 2.42 | 2.08   | 2.40 |
| 11                                 | 2.17   | 1.92 | 3.58 | 2.13 | 2.85   | 2.02 |
| 12                                 | 1.78   | 1.54 | 2.94 | 1.87 | 2.22   | 1.78 |

APPENDIX III

QUESTIONNAIRE

The questions refer primarily to the cow herd:

Name \_\_\_\_\_

Address \_\_\_\_\_

Range \_\_\_\_\_ Township \_\_\_\_\_ Section \_\_\_\_\_

Agricultural Representative \_\_\_\_\_

Local Veterinarian \_\_\_\_\_

What treatments and vaccinations is the cow herd given each year?  
Please be specific and give dates if possible. \_\_\_\_\_

When is castration usually done each year? \_\_\_\_\_

When is dehorning usually done each year? \_\_\_\_\_

Have you in recent years observed any problems in your herd which  
may possibly be related to mineral deficiencies, or excesses? Please give  
details if positive. \_\_\_\_\_

Has any farm or specific region in your area been suspected in  
recent years of being mineral deficient, because of problems which have  
arisen in the herd or herds? \_\_\_\_\_

Please give details if positive:

Approximate size of cow herd \_\_\_\_\_

Total number of animals over 6 months of age \_\_\_\_\_

Total number of calves per year \_\_\_\_\_

Number of infant calf deaths in 1961 \_\_\_\_\_ and 1960 \_\_\_\_\_

Breed of cow herd \_\_\_\_\_

Breed of bull or bulls \_\_\_\_\_ or do you use  
Artificial Insemination? \_\_\_\_\_

Type of cattle production usually practised \_\_\_\_\_

What is the usual calving period each spring? \_\_\_\_\_

PASTURE

Do your cows usually go to pasture before calving is finished?  
\_\_\_\_\_

Approximate date that the cow herd usually go to pasture each  
spring \_\_\_\_\_

Type of pasture grazed by the cow herd in Spring \_\_\_\_\_

Summer \_\_\_\_\_

Fall \_\_\_\_\_

Does your herd normally pasture within a five mile radius of the  
farmstead? \_\_\_\_\_

If not, how far? \_\_\_\_\_

and on what Township \_\_\_\_\_ Section \_\_\_\_\_

and Range \_\_\_\_\_

What is the general soil type of grazing area? \_\_\_\_\_

Do you feed a mineral supplement when the cows are on pasture?  
\_\_\_\_\_

If so, what? \_\_\_\_\_

and when \_\_\_\_\_

What is the water supply when on pasture? \_\_\_\_\_

When is the bull usually put out with the breeding herd? \_\_\_\_\_

or when do you start using A. I.? \_\_\_\_\_

When is the bull taken away from the breeding herd? \_\_\_\_\_

or when does your usual breeding season end with A. I.? \_\_\_\_\_

WINTER

Usual date at which cattle come off pasture for the winter?  
\_\_\_\_\_

Type of winter housing? \_\_\_\_\_

Type of winter feeding? \_\_\_\_\_ Hand fed or  
self-fed? \_\_\_\_\_

Type of forage or forages fed in the winter? \_\_\_\_\_

Amount of forage allowed per cow per winter? \_\_\_\_\_

Percentage native forage fed? \_\_\_\_\_

Percentage tame forage fed? \_\_\_\_\_

Sources of tame forage? Local or distant and percentage of each?  
\_\_\_\_\_

Sources of native forage? Local or distant and percentage of  
each? \_\_\_\_\_

What is the winter water supply? \_\_\_\_\_

Has it ever been analysed? \_\_\_\_\_

SUPPLEMENTARY FEEDING OF COW HERD

Grain mixture fed? \_\_\_\_\_

Amount fed? \_\_\_\_\_

Period during which it is fed? \_\_\_\_\_

Is a complete supplement fed? \_\_\_\_\_

Amount fed to cows? \_\_\_\_\_

Period during which it is fed? \_\_\_\_\_

Mineral mixtures fed? \_\_\_\_\_

Amount fed to cows? \_\_\_\_\_

Period during which it is fed? \_\_\_\_\_

Vitamins fed? \_\_\_\_\_

Amounts fed to cows? \_\_\_\_\_

Period during which it is fed? \_\_\_\_\_

HERD SCORE

| HERDS | MANAGEMENT<br>PRACTICES | HERD<br>CONDITION |
|-------|-------------------------|-------------------|
| 1     | Very Good               | Very Good         |
| 2     | Very Good               | Very Good         |
| 3     | Good                    | Very Good         |
| 4     | Good                    | Good              |
| 5     | Good                    | Very Good         |
| 6     | Very Good               | Good              |
| 7     | Very Good               | Very Good         |
| 8     | Fair                    | Good              |
| 9     | Good                    | Very Good         |
| 10    | Very Good               | Good              |
| 11    | Very Good               | Good              |
| 12    | Fair                    | Fair              |

APPENDIX IV



TABLE VI

PERCENTAGE DRY MATTER, ETHER EXTRACT, CRUDE FIBER, CRUDE PROTEIN, CALCIUM AND  
PHOSPHORUS IN THE FORAGES FED DURING THE TWO WINTERS, 1962-63 AND 1963-64,  
AND THE CAROTENE CONTENT OF THE 1963-64 FORAGES

| HERD<br>NUMBER | TYPE OF<br>FORAGE                           | DRY<br>MATTER | ETHER<br>EXTRACT | CRUDE<br>FIBER | CRUDE<br>PROTEIN | CALCIUM | PHOSPHORUS | Ca:P<br>RATIO | CAROTENE<br>mg. PER<br>POUND |
|----------------|---|---------------|------------------|----------------|------------------|---------|------------|---------------|------------------------------|
|                |   |               |                  | 1962-63        |                  |         |            |               |                              |
| 1              | 75% Native: 25% Alfalfa                     | 89.45         | 1.43             | 30.25          | 8.39             | 0.58    | 0.12       | 4.8:1.0       |                              |
|                | 40% Native: 60% Alfalfa                     | 87.67         | 1.84             | 27.66          | 10.04            | 0.37    | 0.13       | 2.8:1.0       |                              |
| 2              | Clover                                      | 87.88         | 0.72             | 39.09          | 9.63             | 0.53    | 0.11       | 4.8:1.0       |                              |
|                | Brome grass                                 | 87.31         | 1.20             | 31.11          | 8.86             | 0.39    | 0.10       | 3.9:1.0       |                              |
|                | 50% Brome: 10% Alfalfa:<br>40% Sweet clover | 88.71         | 1.50             | 32.71          | 8.88             | 0.57    | 0.14       | 4.1:1.0       |                              |
| 3              | Grass silage                                | 32.20         | 3.46             | 32.03          | 11.49            | 1.15    | 0.20       | 5.8:1.0       |                              |
|                | Native hay                                  | 88.59         | 1.72             | 30.19          | 6.54             | 0.56    | 0.11       | 5.1:1.0       |                              |
| 4              | 50% Alfalfa: 30% Wild<br>oats: 20% Brome    | 88.75         | 1.68             | 29.62          | 10.31            | 0.80    | 0.15       | 5.3:1.0       |                              |
|                | 90% Brome: 10% Alfalfa                      | 89.13         | 1.84             | 30.99          | 7.58             | 0.22    | 0.17       | 1.3:1.0       |                              |
| 5              | 50% Straw: 30% Green<br>wheat: 20% Brome    | 87.65         | 1.44             | 34.20          | 6.54             | 0.34    | 0.16       | 2.1:1.0       |                              |
|                | 50% Brome: 45% Green<br>oats: 5% Alfalfa    | 88.68         | 1.55             | 29.37          | 8.33             | 0.32    | 0.18       | 1.8:1.0       |                              |

TABLE VI (continued)

| HERD NUMBER | TYPE OF FORAGE                              | DRY MATTER | ETHER EXTRACT | CRUDE FIBER | CRUDE PROTEIN | CALCIUM | PHOSPHORUS | Ca:P RATIO | CAROTENE mg. PER POUND |
|-------------|---|------------|---------------|-------------|---------------|---------|------------|------------|------------------------|
| 6           | 90% Brome hay: 5%<br>Alfalfa: 5% Slough hay | 88.96      | 1.20          | 33.52       | 6.76          | 0.27    | 0.17       | 1.6:1.0    |                        |
| 7           | Corn silage                                 | 24.30      | 1.62          | 30.12       | 8.40          | 0.27    | 0.19       | 1.4:1.0    |                        |
|             | Alfalfa                                     | 91.48      | 1.25          | 35.06       | 10.34         | 0.98    | 0.15       | 6.5:1.0    |                        |
|             | 50% Brome: 50% Alfalfa                      | 91.50      | 1.39          | 33.38       | 8.98          | 0.69    | 0.12       | 5.8:1.0    |                        |
| 8           | Interlake hay                               | 89.25      | 1.92          | 29.32       | 8.23          | 0.38    | 0.086      | 4.4:1.0    |                        |
| 9           | Interlake hay                               | 88.84      | 2.22          | 29.11       | 7.41          | 0.44    | 0.10       | 4.4:1.0    |                        |
| 10          | 90% Interlake hay:                          |            |               |             |               |         |            |            |                        |
|             | 10% Bullrushes                              | 88.76      | 1.67          | 30.31       | 7.12          | 0.33    | 0.078      | 4.2:1.0    |                        |
|             | Interlake hay                               | 88.23      | 1.80          | 27.79       | 8.54          | 0.27    | 0.067      | 4.0:1.0    |                        |
| 11          | Interlake hay                               | 89.89      | 2.23          | 27.99       | 6.02          | 0.42    | 0.11       | 3.8:1.0    |                        |
|             | 33% Slough hay: 33%                         |            |               |             |               |         |            |            |                        |
|             | Timothy: 33% Fescue                         | 88.69      | 2.21          | 29.79       | 5.65          | 0.51    | 0.099      | 5.2:1.0    |                        |
| 12          | 95% Clover: 5% Brome                        | 88.68      | 1.39          | 34.51       | 9.34          | 0.89    | 0.19       | 4.7:1.0    |                        |
|             | 75% Native hay: 25%                         |            |               |             |               |         |            |            |                        |
|             | Alfalfa                                     | 88.98      | 1.59          | 34.98       | 8.49          | 0.49    | 0.19       | 2.6:1.0    |                        |
| 1963-64     |   |            |               |             |               |         |            |            |                        |
| 1           | 50% Native hay: 30%<br>Brome: 20% Alfalfa   | 91.88      | 2.22          | 30.80       | 7.24          | 0.46    | 0.12       | 3.8:1.0    | 2.8                    |

TABLE VI (continued)

| HERD NUMBER | TYPE OF FORAGE                              | DRY MATTER | ETHER EXTRACT | CRUDE FIBER | CRUDE PROTEIN | CALCIUM | PHOSPHORUS | Ca:P RATIO | CAROTENE mg. PER POUND |
|-------------|---|------------|---------------|-------------|---------------|---------|------------|------------|------------------------|
| 2           | 90% Brome: 10% Alfalfa                      | 92.46      | 2.05          | 27.77       | 12.63         | 0.79    | 0.28       | 2.8:1.0    | 10.8                   |
|             | 50% Brome: 50% Clover                       | 92.72      | 1.74          | 33.88       | 9.14          | 0.72    | 0.16       | 4.5:1.0    | 4.9                    |
|             | Native hay                                  | 91.39      | 2.06          | 31.59       | 7.20          | 0.34    | 0.16       | 2.1:1.0    | 2.6                    |
| 3           | Grass silage                                | 27.20      | 0.90          | 10.40       | 3.83          | 1.86    | 0.062      | 30.0:1.0   | 2.0                    |
| 4           | Brome hay                                   | 93.60      | 1.10          | 35.80       | 6.06          | 0.46    | 0.092      | 5.0:1.0    | 3.2                    |
|             | 75% Brome: 25% Alfalfa                      | 93.00      | 1.83          | 30.54       | 6.56          | 0.49    | 0.11       | 4.5:1.0    | 6.8                    |
| 5           | 50% Native hay: 50%<br>Oat straw            | 94.00      | 2.00          | 35.10       | 6.16          | 0.31    | 0.17       | 1.8:1.0    | 0                      |
| 6           | 90% Brome: 10% Native<br>hay                | 93.12      | 2.24          | 28.51       | 7.56          | 0.30    | 0.21       | 1.4:1.0    | 2.9                    |
| 7           | Corn silage                                 | 21.10      | 0.40          | 6.50        | 1.26          | 0.22    | 0.14       | 1.6:1.0    | 3.0                    |
| 8           | 50% Interlake hay:<br>50% Clover            | 94.20      | 1.60          | 28.60       | 9.57          | 0.90    | 0.11       | 8.2:1.0    | 2.3                    |
| 9           | Interlake hay                               | 92.44      | 1.96          | 30.81       | 8.30          | 0.39    | 0.15       | 2.6:1.0    | 3.2                    |
| 10          | Interlake hay                               | 91.50      | 2.10          | 31.20       | 7.16          | 0.34    | 0.097      | 3.5:1.0    | 12.2                   |
|             | 50% Interlake hay:<br>25% Clover: 25% Brome | 92.70      | 1.64          | 32.94       | 7.53          | 0.50    | 0.102      | 4.9:1.0    | 1.4                    |

TABLE VI (continued)

| HERD NUMBER | TYPE OF FORAGE                   | DRY MATTER | ETHER EXTRACT | CRUDE FIBER | CRUDE PROTEIN | CALCIUM | PHOSPHORUS | Ca:P RATIO | CAROTENE mg. PER POUND |
|-------------|----------------------------------|------------|---------------|-------------|---------------|---------|------------|------------|------------------------|
| 11          | 95% Interlake hay:<br>5% Alfalfa | 92.96      | 1.93          | 32.45       | 5.84          | 0.40    | 0.16       | 2.5:1.0    | 3.7                    |
| 12          | 50% Brome: 50% Alfalfa           | 89.90      | 2.21          | 31.66       | 8.97          | 0.90    | 0.14       | 6.4:1.0    | 3.1                    |

APPENDIX V

TABLE VII  
MONTHLY AVERAGES FOR MEAN DAILY TEMPERATURE  
 (degrees Fahrenheit)

| MONTH         | YEAR | AREA      |           |
|---------------|------|-----------|-----------|
|               |      | SOUTHWEST | STE. ROSE |
| JUNE - AUGUST | 1962 | 63.9      | 61.9      |
| SEPTEMBER     | 1962 | 52.1      | 50.5      |
| OCTOBER       | 1962 | 45.4      | 45.5      |
| NOVEMBER      | 1962 | 30.6      | 27.7      |
| DECEMBER      | 1962 | 11.5      | 7.1       |
| JANUARY       | 1963 | - 3.3     | - 6.4     |
| FEBRUARY      | 1963 | 5.3       | - 0.5     |
| MARCH         | 1963 | 25.2      | 17.7      |
| APRIL         | 1963 | 38.9      | 38.2      |
| MAY           | 1963 | 48.9      | 47.3      |
| JUNE - AUGUST | 1963 | 65.5      | 63.8      |
| SEPTEMBER     | 1963 | 57.8      | 54.8      |
| OCTOBER       | 1963 | 53.3      | 52.4      |
| NOVEMBER      | 1963 | 27.1      | 25.0      |
| DECEMBER      | 1963 | 3.5       | 4.8       |
| JANUARY       | 1964 | - 7.8     | - 4.5     |

TABLE VIII

TOTAL PRECIPITATION PER MONTH  
(inches)

| MONTH     | YEAR | AREA      |           |
|-----------|------|-----------|-----------|
|           |      | SOUTHWEST | STE. ROSE |
| JUNE      | 1962 | 2.08      | 1.52      |
| JULY      | 1962 | 2.11      | 2.57      |
| AUGUST    | 1962 | 5.93      | 5.50      |
| SEPTEMBER | 1962 | 0.36      | 0.59      |
| OCTOBER   | 1962 | 1.59      | 1.23      |
| NOVEMBER  | 1962 | 0.72      | 1.06      |
| DECEMBER  | 1962 | 0.80      | 0.94      |
| JANUARY   | 1963 | 0.37      | 0.40      |
| FEBRUARY  | 1963 | 0.86      | 1.22      |
| MARCH     | 1963 | 0.60      | 0.93      |
| APRIL     | 1963 | 1.67      | 3.27      |
| MAY       | 1963 | 2.49      | 2.40      |
| JUNE      | 1963 | 4.53      | 6.67      |
| JULY      | 1963 | 3.11      | 2.38      |
| AUGUST    | 1963 | 2.71      | 2.58      |
| SEPTEMBER | 1963 | 1.09      | 1.24      |
| OCTOBER   | 1963 | 0.24      | 0.17      |
| NOVEMBER  | 1963 | 0.33      | 0.90      |
| DECEMBER  | 1963 | 1.11      | 0.64      |
| JANUARY   | 1964 | 0.49      | 1.52      |