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The Influence of Rations low in certain Minerals on the Composition of the Blood and Milk of Cows, and on the Blood of their Progeny.*

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INTRODUCTION.

DURING the last three decades many prominent investigators have given much time and attention to problems (ever increasing in complexity) of mineral requirements of animal life and production, as well as the occurrence of minerals in nature.

The results of these researches have shown that many countries suffer acutely from some mineral deficiency or other, which, if not artificially corrected, seriously hampers the productivity of range stock in such parts. The most widely occurring deficiency known at present is one of phosphorus. A deficiency of this element in the herbage being readily reflected in the inorganic phosphorus content of the blood. A ready method is provided for detecting the deficiency, prior to the appearance in the animals of clinical symptoms of aphosphorosis, as shown by Malan, Green and du Toit (1928).

Nutrition workers are finding it necessary to give more attention to the changes that may be brought about in the blood-stream as a result of variations in the feed of animals. Changes in body-weight and size are not accurate indications of the efficiency of rations: It is essential, if progress is to be made, that such superficial methods be supported by the fundamental study of the blood constituents.

The present investigation proposes, therefore, to show whether the mineral content of the blood of cows and their progeny can be influenced by the feeding to the cows of rations low in certain minerals.

Since there exists no great certainty regarding the normal mineral content of cow's blood in this country, it became necessary to make a large number of blood mineral determinations on cows receiving rations adequate in respect of all mineral constituents.

The animals and the facilities necessary for determining calcium, phosphorus, magnesium, potassium, sodium, and chlorine in the blood of non-pregnant, pregnant, and lactating cows, as well as those for determining the elements in the milk, were provided at Onderstepoort in an experiment on, " The Minimum Mineral Requirements of Cattle," by du Toit, Malan, and Groenewald (1934). This work aims primarily at measuring general growth by weight and clinical condition, as well as reproduction of cows fed rations " constituted " to be extremely low in one of the above minerals.

In regard to the influence of diet upon its constituents, more attention has been paid to milk than to the blood as may be gathered from the very complete review of the older literature by Forbes and Keith (1914). In the present work also there was opportunity of observing the effect of certain rations either deficient in or containing minimal amounts of certain mineral elements, upon some milk constituents.

Schutte (1929) has drawn attention to the generally low solids-not-fat content of South African milks. With this in mind attention was given to milk protein, total ash, and sugar, as well as to fat and solids-not-fat determinations. It was judged that close observations of the minerals in blood and milk might not only throw light on the total solids of milk, a question of rising importance in the cheese industry, but might also augment our knowledge of the physiology of milk secretion, at present very incomplete.

In order that the data might be as complete as possible, blood samples taken from twelve calves within the first twenty-four hours of life were analysed for calcium, inorganic phosphorus, magnesium, potassium, sodium, and chlorine. Although the basis of the mineral intake of the cows varied, no changes could be detected in the level of the above mineral constituents in the daily blood samples of the calves during the first week of life. For the first week each calf received the milk from its mother. After a week all the calves were regarded as normal and fed on the mixed milk of the herd.

Lately much stress has been laid on the proper proportions, ratios, and concentrations of minerals in rations as embodied in Marek's (1932) idea of " alkali-alkalizität " and " Erdalkali-alkalizität ". This author attributes rickets mainly to an abnormal " Erdalkali-alkalizität ", which means that the $CaO + MgO - P_2O_5$ expressed in milligram equivalents per 100 grams dry matter of the ration, is either above or below the optimum when it registers between 20 and 25. It must be clearly understood at the outset that, although the condition in regard to the " Erdalkali-alkalizität " of the ration will be given, the primary object in planning rations low in certain minerals was to note what effects such rations had on the animal, regardless of the " Erdalkali-alkalizität " content. It was believed that an abnormal " Erdalkali-alkalizität " would have assisted in proving the object of this work by aiding the depletion of minerals in the system.

Mineral ratios as well as metabolism studies are, therefore, considered beyond the scope of this thesis. Ratios of normally occurring constituents in the milk and blood will, however, be presented. In comparing these figures an endeavour is made to ascertain the influence one mineral exerts on another in the blood stream.

It was desired to test the suitability of certain formulae for calculating protein and ash from the butter-fat percentages of milk, and sugar from the chlorine content of milk, because simplified analytic procedure, accumulated in a readily available form so as to be easily applied to the detection of mineral deficiencies may greatly facilitate the studies of these intricate problems. The necessity of such compiled methods is the more keenly felt under the conditions experienced in South Africa, where telluric factors give rise to extreme variations and shortages of protein and phosphorus as shown recently by du Toit and co-workers (1932). Another factor that should not be lost sight of is the increased productive requirements of our domesticated animals. The dairy cow of the present produces at least four times the quantity of milk secreted by her ancestors and necessary for the offspring. It is surprising that our veld animals withstand, as well as they do, the adverse forcing of increased productivity in the absence of a compensating feed supplement. But a process of natural adaptation takes place. Kelley (1932) describes very clearly the tendency for European breeds to deteriorate to the level of the indigenous stock when allowed to herd for themselves on the range.

REVIEW OF THE LITERATURE.

A complete review of the literature, relevant to the subject under consideration, would occupy a lengthy volume. Sufficient references have, therefore, been selected to indicate the general trend of our present knowledge. The work of previous investigators which bears more particularly on the various phases of this investigation may be grouped under four general headings:

I. The Influence of Diet on the Mineral Constituents of the Blood.

Under the general term "mineral constituents of the blood" are included the metallic elements, calcium, sodium, potassium, and magnesium, and the non-metallic elements, phosphorus and chlorine. As a rule these mineral elements tend to remain at a fairly constant level in the blood. A fact well illustrated by the finding of Eckles, Sjollema and Kaag (1932) that phosphorus, calcium, and magnesium were lowered in the blood of dairy cows only four days previous to parturition and became normal again soon after calving. Gowen and Tobey (1931) give an interesting account of the result of starving eight good milking cows in order to observe changes in the composition of the milk. Numerous curves are given illustrating the milk and blood constituents determined. Although no changes could be detected in the blood and milk calcium and the milk phosphorus, it is regrettable that these investigators did not determine the inorganic phosphorus content of the blood before stating that: "All the changes in the milk composition during starvation can be directly related to the simultaneous changes in the blood". These authors have, however, shown that inanition increased fat, total solids, and, to a slight extent, protein. There was a pronounced decrease in lactose and total milk. The decrease in lactose corresponded to a decrease in the dextrose content of the blood, supporting the conclusion that the lactose of milk has as its percussor the dextrose of the blood.

The alkalinity or acidity of the diet has long been held to have important influences on mineral assimilation. Barone and Bonanno (1932) state that an acid diet produces an increase in blood calcium and an alkaline diet a decrease in the blood potassium. Theoretically this finding is in keeping with an older German school, but has not been confirmed in many of the later investigations. It is, therefore, a field of research awaiting investigation. There is at present no doubt, however, that the inorganic blood phosphorus can be readily varied or limited by the intake as shown by Malan, Green and du Toit (1928), although there is apparently a wide daily range of normal variation of inorganic blood phosphorus, which Henderson and Landingham (1932) give as 0·46 mgm., while the normal variation for twelve days was found to be as much as 2·34 mgm. Palmer and Eckles (1927) found the inorganic blood phosphorus of dry cows receiving rations containing 0·08 per cent. P and 0·36 per cent. Ca, to be abnormally low, while the calcium of the blood remained constant.

Robinson and Huffman (1926) give the following average analyses for more than a hundred normal beef animals: Inorganic phosphorus 5·87, chlorine 329, calcium 11·0, potassium 27·3, and magnesium 2·16 mgm. per 100 c.c. in blood. These authors are of the opinion that an increased intake of calcium phosphate produced a slight increase in the inorganic blood phosphorus. Whelan (1925) found that following intravenous injections of chlorides, the chlorine in the blood of dogs showed no significant quantitative change. Magnesium, calcium, sodium and potassium showed slight rises following injections of these elements, but were soon back to their normal levels.

Fitch, Boyd and Eckles (1932), failed to cause a lowered blood calcium by feeding to dairy cows rations low in calcium. Similarly du Toit, Malan and Groenewald (1931) were unable to produce a lowered calcium content of the blood of a group of sheep whose daily calcium intake in the ration was only 0·84 gm. There is no doubt, however, that there is a sudden drop in blood calcium content during parturition. Godden and Allcroft (1932) recorded this condition in dairy cows and came to the conclusion that the blood calcium and inorganic blood phosphorus levels were lowered, the normal level again being reached four days after calving.

Hart, Steenbock, and Klein (1932) could not increase the level of blood and milk calcium or inorganic phosphorus by the administration of vitamin D. A drop in the blood calcium level at the onset of parturition is also reported by Wilson and Hart (1932), who further point out that milk fever is always associated with a low blood calcium content. Mirvish (1930) agrees that the calcium level is very constant in blood and varies only under special circumstances or when certain substances are injected. He shows that blood calcium is lowered by the injection of oatmeal extract, but that it reaches its normal level within 48 hours after the injection. The effect is, therefore, of a transient nature.

Morris and Morris (1930) conclude that, " in children the amount of volatile chlorine may be altered by the addition of various substances to the blood, but that for each blood there is a certain maximum and minimum beyond which the content does not pass ". It is possible that the blood chlorine may be regulated by the osmotic pressure within the blood stream, interchange taking place between the lactose of the mammary gland or even the dextrose of the blood and the blood chlorine.

The actual state or condition of the animal organism should be taken into consideration when determining its ability to absorb or make use of mineral supplements. Miller, Yates, Jones, and Brandt (1926) have proved conclusively that alimentary absorption is controlled to a certain extent by the needs of the animal. The percentage of calcium utilized by the system was greater at the height of lactation. It would naturally follow, that the greater utilisation of certain minerals, such as inorganic phosphorus, for milk production, would result in these minerals attaining lower levels in the blood stream during the lactation periods.

It is evident from the literature cited that most attention has in the past been given to the rôle of calcium and phosphorus in the animal organism. It is to be deplored that so little seems to be known about chlorine, potassium and sodium in relation to nutrition.

All investigators seem to agree that a low phosphorus intake is readily reflected in a lowered inorganic phosphorus content of the blood stream. The majority of experiments have shown that the blood calcium content has remained unchanged by variations in the level of calcium intake. This is true even under conditions of inanition. There are some investigators, however, who report slight changes in blood calcium level under certain experimental and clinical conditions. It is possible, for instance, that an abnormal " Erdalkalialkalizität " would be capable of bringing about a change in the calcium level of the blood. That calcium is lowered in the blood stream at the onset of parturition and during the occurrence of milk fever is generally conceded.

A perusal of the literature leaves no doubt that a great deal of uncertainty exists regarding the behaviour of magnesium, chlorine, potassium, and sodium in the blood stream. The evidence indicates that the chlorine content of the blood is not easily affected by lowering the chlorine intake in the diet. The behaviour of the other three elements in the blood stream remains obscure.

II. The Influence of Diet on the Mineral and Proximate Constituents of Milk.

In regard to selective breeding of dairy stock, it is interesting to note that Anderson and Langmack (1924), in a statistical compilation found the average protein content of milk to be increasing in Danish dairies. In this connection van Slyke (1923) states that the influence of breed is largely the cause of variations in milk in different countries. The breed of the cow greatly influences the solids content of the milk as shown by several investigators: Tocher (1925), Eckles (1909), and Judkins (1918). These authors also show that there is a gradual increase in solids-not-fat and in fat with age of cows.

That there is a definite tendency for milk to test low in both fat and solids-not-fat during the hot summer months, is shown by Eckles (1909), Hills (1894), and Cranfield (1927). Attention should, however, be drawn to the recent work of Bartlett, Golding, and Wagstaff (1932) who conclude that variations between summer and winter solids-not-fat percentages are due to a difference of lactometer readings and not to milk composition, an argument difficult to follow, especially in view of the fact that Richmond (1920) clearly states that long droughts in summer affect both the quantity and quality of the milk. It is, of course, well known that a smaller quantity of milk will yield a higher percentage of butter-fat than a larger quantity of milk from the same cow.

Van Slyke (1920) found that drought influenced the composition of milk only in regard to the casein content as shown in table I.

TABLE I.

Influence of Drought on Casein in Milk.

Month.	Conditions.	Pounds Casein in 100-lb. Milk.
June	Drought	2·33
July	Drought	2·20
August	Drought	2·26
September	Normal	2·47
October	Normal	2·69

It is seen that there occurred a slight drop in the casein content of the milk during the months of drought. Jordan, Jenter, and Fuller (1901) fed two cows variable amounts of protein and carbohydrates and showed an increase in both total solids and fat throughout the experimental period. One cow was, however, at the beginning of her lactation period, whereas the lactation period of the other cow was well advanced—a difference which, together with the short experimental period, does not justify significant conclusions.

Perkins (1932) who incidentally gives an extensive and able review of the literature, states on the basis of analysis of several hundreds of milk samples that " very little or no effect of the level of protein feeding was shown on the character of the milk ". Theiler, Green and du Toit (1927) arrived at the conclusion that a deficiency in phosphorus in the ration was not reflected in the percentage composition of the milk. Porcher (1932) is certain that a high chlorine content of milk is associated with a low lactose content, so that a low lactose content may cause a low solids-not-fat content of the milk. Cranfield (1927) records a low lime content in summer milk, which he ascribes to the lowered solids-not-fat content during that period.

Among the early workers von Wendt (1909) may be considered among the foremost who attempted to bring about variations in milk composition by the administration of certain salts. His extensive tables prove that he was not able to cause such changes. Fingerling (1914) concluded that neither the activity of the mammary glans, nor the composition of the milk was altered by feeding inorganic phosphorus compounds.

Overman (1932) found that various kinds of proteins from different sources affected both the chemical and the physical nature of the milk fats. But the recording of his results is unsatisfactory. That a deficient intake leads to a decrease in volume of milk rather than to a decrease in any of the milk constituents was shown by Bruwer (1931), who proved that the lactose and chlorine varied inversely to maintain the constancy of osmotic pressure, and that a high fat content was associated with a high protein content. Becker, Eckles, and Palmer (1927), fed dairy cows on rations low in calcium and found that, although calcium was a limiting factor in milk production, the milk calcium was not lowered. Neuman (1894) fed 100 gm. $Ca_3(PO_4)_2$ to milk cows with the result that the calcium and phosphorus in the milk slightly increased. Unfortunately the duration of his experiments (only 3-4 weeks) was far too short.

Hart McCallum and Humphrey (1909) state that "variations within wide limits in the form and quantity of supply of potassium, magnesium, or phosphorus did not influence the percentage of these elements in the milk". The validity of their observations would have been considerably strengthened had these authors employed more than one cow. Reed and Huffman (1930) are of the opinion that the importance of mineral requirements for dairy cattle is greatly exaggerated. The feeding of extremely low calcium diets made no difference to the milk production.

In summarising our present knowledge of the influence of diet on the mineral and proximate constituents of milk, it is obvious that, although factors are given as reasons for certain types of changes in milk composition, such as, for example, increased protein in certain countries or higher solids in certain breeds. It is nevertheless believed that protein is slightly lowered during severe droughts because of a lowered milk casein. During the hot summer months, when succulent feed abounds, milk is lower in fat and solids-not-fat percentages, because quantitative production is increased.

Lactose and chlorine vary inversely to maintain a constancy of osmotic pressure, enabling milk chlorine figures to be used in calculating the lactose content of the milk. Some difference of opinion exists as to whether milk may be influenced in regard to its calcium and phosphorus content. With reference to the magnesium, potassium, and sodium content of milk less data are available, and it would appear that no definite conclusions have been reached as to the effect of the minerals in the diet upon the composition of the milk in regard to these constituents.

Total milk production has been found to be very easily influenced by feeding.

III. Physiological Relationship between Milk and Blood Minerals.

In attempts to throw light upon the subject of milk secretion, comparisons have often been drawn between the quantities of certain minerals present in the blood stream and in the milk. It would, therefore, be profitable to devote some attention to the rather obscure question of milk secretion in a trial where definite figures are likely to be derived for blood and milk minerals respectively. The available evidence on the passage of blood minerals into the milk, is purely of a circumstantial nature, as clearly emphasised by Rogers (1928) who gives a complete bibliography on the question of milk secretion. Van der Laan (1916) found that the osmotic pressure of milk remained the same as that of the blood. The osmotic pressure of milk is almost entirely due to the lactose and soluble salts such as NaCl, so that there is a reciprocal relation between lactose and the soluble salts in milk.

Interesting work has recently been done by Blackwood and Stirling (1932), who show that in lactating and non-lactating cows, the inorganic phosphorus content of mammary blood is higher than that of jugular blood and approximately equal to that of arterial blood. It is suggested that this is due to the assimilation of inorganic phosphorus from the blood by the tissues drained by the jugular vein and to the absence of absorption of inorganic phosphorus by the mammary gland. Brenner (1933) states that the high inorganic phosphorus content of the mammary gland tissue is evidence of autolytic breakdown of organic phosphorus compounds. The protein-free extract decreases as the phosphate increases. There is thus a suggestion that a certain osmotic pressure must be maintained in the mammary gland for normal composition of the milk.

That lactalbumin and caseinogen are specific products of the mammary gland was shown by Crowther and Raistrick (1916) who thereby created the concept of dissociation of blood constituents and their reorganisation into totally new products in the milk. Rimington (1927) has very carefully shown how phosphorus is carried in milk by illustrating the structural make-up of the amino-acid phosphorus carriers of milk. Meigs, Blatherwick, and Cary (1919) however, have drawn the conclusions that phosphorus is concerned in the secretion of fat and that a large amount of excess phosphorus is returned into the blood stream in inorganic form, because the mammary blood phosphorus content is higher than that of the jugular vein.

It is obvious, therefore, that a vast amount of speculation exists in regard to the interchange of mineral substances from the blood stream to the milk and such speculation may be expected to continue in the future. Recent work suggests, however, that the osmotic pressure of milk remains the same as that of blood, and that this condition is almost entirely due to lactose and soluble chlorine salts. The inorganic phosphorus content of mammary blood has been found to be higher than that of jugular blood and equal to that of arterial blood, suggesting of course the absence of inorganic phosphorus absorption by the mammary gland. It is suggested, however, that phosphorus is carried by the proteins and fats of milk and that the excess phosphorus is carried away by the mammary vein in the form of inorganic phosphorus.

IV. Studies on Calf Blood Minerals.

It is generally accepted that the greatest influence on the offspring of the state of nutrition of the dam is one of a possible shortage of milk. The milk remains normal in the minerals under consideration, so that the calf blood may be regarded as a normal physiological study.

Godden and Allcroft (1932) found that calves at birth showed higher blood calcium and inorganic phosphorus than their dams, but that the chlorine content of the calf blood was lower. Bogert (1923) found that a child at birth registered 9·1 mgm. Ca. per 100 c.c. blood, when the Ca. of the maternal blood was 10·9 mgm. Robinson and Huffman (1926) report higher calcium in new born calves blood than in that of the dams. These authors find that after the first week the blood calcium of the calves gradually decreases. Green and Macaskill (1928), studying three cows and their calves, found that the potassium of calf blood was notably higher than that of the cow, and gradually assumed the adult level over a period of approximately ten weeks. The inorganic phosphorus of calf blood was also higher than that of the dam.

In regard to calcium, magnesium, sodium and chlorine, no characteristic differences were noted between the blood of a cow and that of her calf. It should be noted, however, that Theiler, Green and du Toit (1927) give figures indicating that a 24-hour old calf showed higher in blood potassium, magnesium, calcium, and inorganic phosphorus, and lower in sodium than an adult animal. Although the differences are in most cases very slight, they are plain enough to stimulate further search for facts.

It is evident then that the majority of investigators find that the calcium, inorganic phosphorus, and potassium content of the blood of the young is higher than that of adults. This is especially true in the case of potassium and inorganic phosphorus. Magnesium seems to be only very slightly higher in the blood of young at birth, while sodium is reported in one case to have been slightly lower at birth than that found in the blood of the adult at the time. It is, therefore, an open question whether there is any difference in the magnesium, sodium, and chlorine content of the blood of calves at birth or at any subsequent time.

OBJECTS OF THE PRESENT INVESTIGATION.

(1) To study the influence of a very low intake of certain minerals on the mineral content of the blood of dairy cows.

(2) To determine the influence these low mineral intakes, exercise on milk production, as well as milk composition.

(3) To determine the calcium, inorganic phosphorus, magnesium, chlorine, sodium, and potassium content of calf blood twenty-four hours after birth and at subsequent intervals, in order to compile a record of the behaviour of the above elements in the blood of calves from birth to the age of three months.

As a lowered phosphorus intake has recently been shown definitely to be reflected in a lowered inorganic phosphorus content of the blood, it was thought desirable to acquire accurate information as to the manner in which elements such as calcium, magnesium, potassium, sodium, and chlorine would behave in the blood stream if reduced to a minimum in the ration.

Even if any given element could not be influenced by increasing or lowering it in the blood stream, and is compared favourably with the same element in the blood of a control animal, the collected data would still be valuable in enabling standards to be set up for the normal mineral content of the blood of the cows.

Should the milk show no change in the quantity of any of its mineral constituents under such conditions of experimentally lowered mineral intakes, yet useful data would have been collected upon which to formulate the normal mineral content of South African milk.

The protein, sugar, total ash, solids-not-fat, and fat percentages of all the milk are recorded in the hope of throwing some light upon the notably low milk solids-not-fat content of the milk of South African veld herds.

PLAN OF INVESTIGATION.

Yearling grade Friesland heifers weighing approximately 500 lb. each were used. The twenty-one heifers that were available were specially bought at Bloemfontein on 26.11.29 for the purpose of the study of the minimum mineral requirements of cattle.

These animals were very uniform in weight and conformation. They were railed to Onderstepoort, immunised against redwater and gallsickness, and, with the exception of one animal that constituted an experimental group by itself, grouped into pairs.

They were numbered as follows: D.O.B. Nos. 3641, 3648, 3659, 3643, 3655, 3640, 3650, 3642, 3649, 3651, 3646, 3658, 3653, 3656, 3652, 3645, 3639, 3675, 3673, 3672 and 3677.

These heifers were housed in separate boxes of an open shed during the night and were allowed out in a concrete floored paddock during the day. Concrete floors were put down in order to prevent earth-eating. Nose bags were applied every morning except on Sundays, during the last three months of pregnancy, and the animals exercised by being driven for a distance of about a mile. The health of the animals was daily inspected by veterinary officers on the station.

In order to exercise the proper control over the mineral intake, especially where it is desired to create certain mineral deficiencies, it becomes essential not only to know the detailed analyses of the feed given, but to limit the mineral intake by reducing the roughage to a minimum.

In order to accustom the animals to rations supplying little bulk, they were on 11.5.30 put on the following preliminary ration: 3½ pounds of lucerne hay, 5 pounds of maize, 5 pounds of fanko, 20 grams of blood meal, and 4 ounces of bone meal each daily until conception, when the basal ration, to be described below, was given.

In considering the basal ration it may be stated here that the ideal method which suggests itself for studying the rôle of the inorganic elements individually and collectively in nutrition, and especially their effects on the blood mineral elements, would be to observe the effect of synthetic rations in which the content of every ingredient could be regulated at will, both as to quantity and quality. The basal ration made use of in this study was based on the findings of Theiler, Green and du Toit (1927) and according to these authors complied fully with all the physiological needs of the animals. Their ration was of as low a mineral content as it was possible to obtain without giving the animals a synthetic ration. All the minerals could, therefore, easily be supplemented by adding them to the daily ration.

The initial basal ration given daily to each animal as soon as she had been served was as follows: 3·5 pounds of veld hay, 5 pounds of crushed yellow maize, 5 pounds of fanko or maize endosperm, and 20 grams of blood meal.

The hay was given to each animal early in the morning and the concentrate mixture in the afternoon. The mineral mixture supplement was always previously weighed into paper bags and then simply added to the concentrate grain mixture each day.

The original plan was to feed ten pounds of fanko alone, but due to the high cost of this product, 5 pounds of mealies had to be substituted, except in the groups on a low phosphorus ration. The phosphorus content of 5 pounds of maize was too high to bring about a phosphorus deficiency in the latter animals; consequently they received 10 pounds of fanko. The basal ration was found to allow admirable growth provided it was supplemented by the necessary minerals. The percentage protein and the mineral contents of the feeds used were as follows:—

TABLE 2.

The Protein and Mineral Contents of the Feeds used (%).

	CaO.	MgO.	K_2O.	Na_2O.	P_2O_5.	Cl.	SO_3.	Protein.
Hay.........	0·35	0·25	1·20	0·80	0·13	0·25	0·40	4·8
Maize.......	0·03	0·24	0·36	0·16	0·50	0·08	0·16	9·0
Fanko.......	0·01	0·05	0·14	0·02	0·09	0·03	0·14	8·0
Blood meal..	0·20	0·03	0·40	1·60	0·60	1·00	0·80	64·8

The above ration contains the following approximate amounts of minerals and protein in grams per animal per day: $CaO = 6 \cdot 8$, $MgO = 11 \cdot 1$, $K_2O = 29 \cdot 3$, $Na_2O = 5 \cdot 7$, $P_2O_5 = 15 \cdot 4$, $Cl = 6 \cdot 7$, $SO_3 = 12 \cdot 9$, and protein = 472. Linton (1931) gives the following as being the protein requirement of a 1,000 pound cow giving 3 per cent. milk: maintenance + 1 gallon, 1·148 pounds (496 grams). Orr (1932) gives the standard requirements of heifers weighing 500 pounds as 450 grams protein. Blood meal was added to improve the " quality " of the protein, as lysine and tryptophane would compensate the deficiency in zein.

The ration contained sufficient protein for growing heifers weighing approximately 700 pounds, which was the weight at service. Since Theiler, Green and du Toit (1927) after feeding similar rations stated " that exogenous requirements of cattle for vitamins A, B and C are so low that they are covered by a few pounds of poor quality roughage ", it was considered unnecessary to upset the mineral content of the ration by giving substances that would doubly insure these factors. Water was always available in troughs.

All the heifers were served and the experiment was actually in full progress in September, 1930, when each heifer received the experimental ration supplemented by the mineral mixture, which was, of course, purposely low in the element to be studied. The quantities for each group are given below.

With the exception of one cow all the animals calved in May and June, 1931. In order to obtain equality of factors as far as possible, all animals were dried off after having milked for the period it took the poorest individuals to cease lactating spontaneously, which in this case was exactly 90 days. They were then placed with the same Red Poll bull for a second service in December, 1931. In February, 1932, 5 pounds of maize ensilage was added to the daily basal ration of each animal because many calves were born weak and abnormal. Considerable trouble was also experienced from retained placentae. These conditions were general for the herd and not confined to any particular group.

The percentage composition of maize ensilage is $CaO = 0 \cdot 10$, $MgO = 0 \cdot 07$, $K_2O = 0 \cdot 3$, $Na_2O = 0 \cdot 01$, $P_2O_5 = 0 \cdot 1$, $Cl = 0 \cdot 06$, $SO_3 = 0 \cdot 1$ and protein = 1·6. Hence the total daily intake per animal in grams was now $CaO = 8 \cdot 8$, $MgO = 12 \cdot 5$, $K_2O = 35 \cdot 3$, $Na_2O = 5 \cdot 9$, $P_2O_5 = 17 \cdot 4$, $Cl = 8 \cdot 0$, $SO_3 = 14 \cdot 9$ and protein = 510 grams. The cows now averaged about 900 pounds in weight showing that they were still receiving an adequate supply of protein for maintenance and growth. This quantity of protein had, however, proved insufficient for maintenance during lactation as could very easily be seen by the severe decrease in weight of all the cows. At the beginning of the second lactation period the deficiency was compensated for by adding to the ration 1·5 pounds of meat meal per animal.

The calves born after the addition of the ensilage were normal. The percentage composition of meat meal is as follows: $CaO = 1{\cdot}1$, $MgO = 0{\cdot}04$, $K_2O = 0{\cdot}4$, $Na_2O = 1{\cdot}4$, $P_2O_5 = 1{\cdot}4$, $Cl = 0{\cdot}85$, $SO_3 = 0{\cdot}7$, and protein $= 80{\cdot}0$. This brought the total daily mineral intake in grams per animal in the basal ration to: $CaO = 15{\cdot}1$, $MgO = 12{\cdot}8$, $K_2O = 38{\cdot}0$, $Na_2O = 15{\cdot}5$, $P_2O_5 = 27{\cdot}0$, $Cl = 13{\cdot}8$, $SO_3 = 19{\cdot}5$, and protein $= 1{,}026$ grams. The protein had, therefore, been raised to meet the requirements of a cow producing two gallons of 3 per cent. milk per day.

The mineral elements had been lowered in the rations to the lowest level yet attained for cows, and lower than it was thought possible for animals such as producing cows to be able to live on for more than a year without deleterious effects on health.

The Ca and P requirements of a 1,000 pound cow giving 3 per cent. milk are given by Crichton (1930) as:—

	Ca.	*P.*
Maintenance + 1 gallon	59·4 gm.	32·5 gm.
,, + 2 ,,	86·3 ,,	55·0 ,,
,, + 3 ,,	113·2 ,,	77·5 ,,
,, + 4 ,,	140·1 ,,	100·0 ,,
,, + 5 ,,	167·0 ,,	122·5 ,,

Kellner (1926) is, however, of the opinion that these requirements are:—

	Ca.	*P.*
Maintenance + 1 gallon	41·28 gm.	17·0 gm.
,, + 2 ,,	49·9 ,,	24·0 ,,
,, + 3 ,,	58·6 ,,	31·0 ,,
,, + 4 ,,	67·3 ,,	38·0 ,,
,, + 5 ,,	76·0 ,,	45·0 ,,

Kellner's figures are considerably lower than those given by Crichton which goes to emphasize the uncertainty of our knowledge of the mineral requirements of which calcium and phosphorus are the best known elements.

A logical basis upon which to base the daily mineral requirements is twelve pounds of good quality English hay, the quantity it is assumed animals of this type normally consume on pasture. With mineral supplement the total ingested by the animal would equal the amount obtained by the quantitative analyses of 12 pounds of English hay. The desired element could, therefore, easily be deleted when making the other elements up to what was taken to be the optimum.

These inorganic mineral substances were made up into paper packages for the various groups as follows:—

Group.	D.O.B.	Mineral Mixture.
Low calcium and low phosphorus............	3641 3648	25 grams NaCl. 15 ,, Mg $(OH)_2$. 75 ,, KCl.
Low phosphorus.........................	3659	80 ,, $CaCO_3$. 15 ,, Mg $(OH)_2$. 25 ,, NaCl. 75 ,, KCl.
Low calcium.............................	3643 3655	70 ,, K_2HPO_4. 15 ,, Mg $(OH)_2$. 25 ,, NaCl. 57 ,, KCl.
Low calcium and low magnesium............	3640 3650	70 ,, K_2HPO_4. 25 ,, NaCl. 57 ,, KCl.
All minerals low except calcium and phosphorus	3642 3649	75 ,, $CaHPO_4$. 25 ,, $CaCO_3$.
Low sodium and low chlorine...............	3651 3646	80 ,, CaCO. 75 ,, K_2HPO_4. 15 ,, Mg $(OH)_2$.
Low chlorine.............................	3658 3675	80 ,, $CaCO_3$. 75 ,, K_2HPO_4. 15 ,, $Mg(OH)_2$. 40 ,, sodium citrate.
Low sodium..............................	3653 3672	65 ,, $CaCl_2$. 75 ,, $CaHPO_4$. 15 ,, Mg $(OH)_2$. 65 ,, KCl.
Low potassium...........................	3673 3656	76 ,, $CaCl_2$. 75 ,, $CaHPO_4$. 15 ,, Mg $(OH)_2$. 25 ,, NaCl.
All minerals sufficient + K.I...............	3673 3656	75 ,, $CaHPO_4$. 25 ,, $CaCO_3$. 15 ,, Mg $(OH)_2$. 25 ,, NaCl. 75 ,, KCl.
All minerals sufficient (control)..............	3645 3639	75 ,, $CaHPO_4$. 25 ,, $CaCO_3$. 15 ,, Mg $(OH)_2$. 25 ,, NaCl. 75 ,, KCl.

In order to view the experiment in its complete setting, as well as to see at a glance what the total daily mineral intake in the ration plus that in the supplement is, table 3 is given:—

TABLE 3.

Daily Intake of Minerals in Grams per Head in Basal Ration plus Mineral Supplements.

Group.	D.O.B. No.	Period.	CaO.	MgO.	K_2O.	Na_2O.	P_2O_5.	Cl.	SO_3.
Low Ca and P	3641	19.9.30–17.2.32	6·8	21·4	76·3	18·9	15·4	57·4	12·9
	3648	19.2.32–17.5.32	6·3	16·3	72·3	16·7	8·4	56·2	12·9
		18.5.32– 8.8.32	8·3	17·3	78·3	16·9	8·4	57·5	14·9
		Last 3 months	14·8	18·0	81·0	27·5	17·4	63·3	19·5
Low P......	3659	19.9.30–17.2.32	51·6	21·4	76·3	18·9	15·4	57·4	12·9
		18.2.32–17.5.32	51·1	16·4	72·3	15·8	6·4	56·2	12·9
		18.5.32– 8.8.32	53·1	17·8	80·3	16·0	8·4	57·5	14·9
		Last 3 months	59·1	20·1	83·0	25·6	18·0	65·3	19·5
Low Ca.....	3643	19.9.30–17.5.32	6·8	21·4	101·3	18·9	45·1	49·8	12·9
	3655	18.5.32– 8.8.32	8·8	22·8	107·3	19·1	46·8	50·2	14·9
		Last 3 months	15·1	23·1	110·0	28·7	56·7	56·0	19·5
Low Ca and Mg.	3640	17.9.30–17.5.32	6·8	11·1	101·3	18·9	45·1	48·9	12·9
	3650	18.5.32– 8.8.32	8·8	12·5	107·3	19·1	46·8	50·2	14·9
		Last 3 months	15·1	12·8	110·0	28·7	56·7	56·0	19·5
All mineral deficiency except P. and Ca.	3642	19.9.30–17.5.32	47·1	11·1	29·3	5·7	45·1	6·7	12·9
	2649	18.5.32– 8.8.32	49·1	12·5	35·3	5·9	46·8	8·0	14·9
		Last 3 months	55·4	12·8	38·0	15·5	56·7	13·8	19·5
Low Na and Cl.	3651	19.9.30–17.5.32	56·1	21·4	75·0	5·7	45·1	6·7	12·9
	3646	18.5.32– 8.8.32	53·6	22·8	80·0	5·9	46·8	8·0	14·9
		Last 3 months	59·9	23·1	80·0	15·5	56·7	13·8	19·5
Low Cl.....	3658	19.9.30–17.5.32	51·6	21·4	76·3	18·9	45·1	6·7	12·9
	3675	18.5.32– 8.8.32	53·6	22·8	80·0	19·1	46·8	8·0	14·9
		Last 3 months	59·9	23·1	80·0	28·7	56·7	13·8	19·5
Low Na.....	3653	19.9.30–17.5.32	51·6	21·4	76·3	5·7	45·1	57·4	12·9
	3672	18.5.32– 8.8.32	53·6	22·8	82·3	5·9	46·8	58·7	14·9
		Last 3 months	59·9	23·1	80·0	15·5	56·7	64·5	19·5
Low K......	3673	19.9.30–17.5.32	51·6	21·4	29·3	18·9	45·1	57·4	12·9
	3656	18.6.32– 8.8.32	53·6	22·8	35·3	19·1	46·8	46·8	14·9
		Last 3 months	59·6	23·1	38·0	28·7	56·7	53·8	19·5
All mineral sufficiency plus K.I...	3677	19.9.30–17.5.32	47·1	21·4	76·3	18·9	45·1	57·4	12·9
	3652	18.5.32– 8.8.32	49·1	22·8	82·3	19·1	46·8	57·4	14·9
		Last 3 months	55·4	23·1	85·0	28·7	56·7	64·5	19·5
All mineral sufficiency	3645	19.9.30–17.5.32	47·1	21·4	76·3	18·9	45·1	57·4	12·9
	3639	18.5.32– 8.8.32	49·1	22·8	82·3	19·1	46·8	58·7	14·9
		Last 3 months	55·4	23·1	85·0	28·7	56·7	64·5	19·5

It will be noted that more changes have been made in the rations of the first two groups in Table 3 than in the case of the other groups. This, it should be remembered, was due to the stubstitution of 10 pounds of fanko only, on 19.2.32 for the 5 lb. of crushed mealies and 5 lb. of fanko that had previously been given to these animals.

Unfortunately mishaps befel several of the animals during the experimental period, these will be noted in due course. It should be pointed out at this stage, however, that D.O.B. 3672 contracted tuberculosis, and is for this reason not considered in respect of weight changes. Her blood composition does not, however, show any differences as compared with that of her mate D.O.B. 3653, and was for this reason taken into consideration in this work. Similarly D.O.B. 3639 was injured at the time of her first calving, so that she became thin and generally unthrifty and was discarded from the experiment of du Toit, Malan and Groenewald (1934). These authors could afford the loss of one control animal in view of the fact that the gains made by the other control D.O.B. 3645 compared very favourably with those made by the control animals of Theiler, Green, and du Toit (1927). However, for purposes of blood and milk analytical work such as given here, there is no reason why she should not fill her proper place as a control animal, because there is no indication that the mineral constituents of her blood were in any way affected and comparison with those of D.O.B. 3645 was therefore not precluded.

EXPERIMENTAL METHODS.

All blood samples were drawn from the jugular vein at regular monthly intervals, except when a cow calved, in which case the blood and milk samples were collected, one, two, and three months after the date of calving. From June, 1930, to December, 1931, lithium citrate was used exclusively as an anti-coagulant for the blood in the determination of all the mineral elements. Salit (1932) found that lithium interfered with sodium determinations, and that a precipitate was formed in the trichloracetic acid solution. For this reason the blood samples for sodium determinations were drawn into separate bottles containing 10 per cent. solution of potassium oxalate as anti-coagulant. This procedure was followed from January, 1932, to December. 1932, and the following results taken haphazardly from blood samples are given in table 4:—

TABLE 4.

Sodium Determinations in Blood Samples.

Date bled.	Date analysed.	Results in mgm/100 c.c.	Date bled.	Date analysed.	Results in mgm/100 c.c.
8. 3.31	30.1.33	280, 284, 276, 293, 288	22.9.32	1.2.33	261, 243, 259, 293, 288
26.10.31	1.2.33	261, 276, 250, 289, 272	25.5.32	28.5.32	302, 280, 280, 266, 276
22. 4.31	25.4.31	304, 286, 260, 306, 291	22.7.32	23.7.32	284, 284, 260, 301, 300
21. 9.31	28.9.31	243, 261, 293, 280, 288	22.8.32	26.8.32	360, 320, 286, 292, 256
Averages.....		272, 252, 270, 292, 285			302, 282, 271, 288, 280

The differences occurring in the above table may be considered to be normal individual variations and prove that the anticoagulant has no marked effect on the sodium in blood precipitate. The figures, therefore, indicate that for practical purposes there is no necessity to have separate sample bottles when sodium is to be determined. Lithium citrate as a non-coagulant suits the purpose admirably. Attention should further be drawn to the fact that in two cases where lithium citrate had been used, more than a year had elapsed between the bleeding of the animals and the making of the sodium determinations. Yet even here there were no marked differences in the sodium values as compared with values obtained from samples analysed the day following bleeding.

The treatment of the blood sample may best be described by quoting Malan and van der Lingen (1931): "The blood is drawn from the jugular vein into bottles containing the anticoagulant, 1 c.c. of a 20 per cent. lithium citrate solution per 100 c.c. blood. 20 c.c. blood are then transferred in a pipette to a flask containing 80 c.c. trichloracetic solution (5 per cent.). The flask is closed with a rubber stopper and shaken vigorously to ensure the complete precipitation of the proteins. After 30 minutes the mass in the flask is filtered through Whatman No. 40 filter paper. The filtrate, called trichloracetic acid filtrate, is used for the determinations of the inorganic constituents in question". The methods used by these authors will be found convenient in doing blood analyses.

I. Blood Analysis.

(*a*) *Calcium*.—Calcium was precipitated as tricalcium phosphate and the phosphate estimated by the Benedict and Theis (1924) method for inorganic phosphorus, also according to Roe and Kahn (1928), Roe and Kahn (1926), Fiske and Subborrow (1929), and Kuttner and Cohen (1930), all of whom contributed towards the standardisation of the present colorimetric method.

(*b*) *Phosphorus*.—The method for arriving at the inorganic blood phosphorus content was based on the production of a colour by a phosphate in the presence of ammonium molybdate, sulphuric acid, and a reducing agent as described by Briggs (1924) and Green (1928).

(*c*) *Magnesium*.—The calcium was removed as calcium oxalate, the magnesium precipitated as magnesium phosphate, and the phosphate determined colorimetrically according to Kolthof (1927), Kramer and Tisdall (1921), and Briggs (1924).

(*d*) *Potassium*.—The potassium was precipitated from the blood filtrate as potassium cobaltinitrite which is insoluble in water and in weak acid (pH 5.7-7.0) and can be determined volumetrically against $KMnO_4$ [Kramer and Tisdall (1921) and Morgulis (1928)].

(*e*) *Sodium*.—The sodium was precipitated as uranyl-zinc-sodium acetate [$(UO_2)_3\ Zn\ Na\ (CH_3COO)_9\ 9\ H_2O$] which was dissolved in acetic acid and on addition of potassium ferrocyanide gave a red brown colour, the intensity of which was proportional to the amount of uranium present. This is in accordance with the method of Barrenscheen and Meissner (1927).

(*f*) *Chlorine*.—The chlorides in the blood were precipitated as AgCl, and excess $AgNO_3$ determined against KCNS in accordance with the method of Smirk (1928).

II. Milk Analysis.

Milk for analytical purposes should be fresh and cool. It was found necessary to use equal volumes of a 20 per cent. trichloracetic acid solution in order to ensure a prefectly clear filtrate after filtering the mass in the flask through Whatman No. 40 filter paper.

The procedure for determining all the mineral elements, except total phosphorus, was essentially the same as that employed for blood analysis. The concentrations found convenient for the determination of each element will be given:—

(*a*) *Total Phosphorus*.—1 c.c. of milk and 19 c.c. of water are shaken up well and 1 c.c. of this diluted milk placed in a silica crucible. 2 or 3 drops of a 10 per cent. ca-acetate solution are added and the crucible placed on a steam oven until the contents have evaporated to dryness. The residue is then heated to whiteness over an open flame, cooled, and treated with 10 c.c. 10 per cent. H_2SO_4 over a steam oven for a few minutes. The solution is now quantitatively filtered into a 100 c.c. cylinder and the precipitate washed until the solution is brought up to approximately 60 c.c. It is then neutralised with NH_4OH and 2 c.c. of Reagent B* added.

10 c.c. phosphorus standard (2 mgm. P_2O_5 per 100 c.c.) is treated similarly and 2 c.c. Reagent B added. Both cylinders are now made up to the 100 c.c. mark with water and 10 drops of freshly prepared stannous chloride solution added to each cylinder. After 10 minutes the colours are fully developed and may be read. The colours will last $\pm$ 30 minutes.

(*b*) *Inorganic Phosphorus*.—The method is the same as that for the determination of inorganic phosphorus in blood, except that the concentration is 5 c.c. of thrichloracetic milk filtrate (1:1) made up to 200 c.c. with water in a standard measuring flask and 10 c.c. of this dilution is used for the colorimetric reading.

(*c*) *Calcium*.—The method is the same as that used for blood calcium. 2·5 c.c. of the milk filtrate (1:1) is made up to 100 c.c. with water in a standard measuring flask, and 10 c.c. of this dilution is used for every calcium determination.

(*d*) *Magnesium*.—In the case of magnesium 1 c.c. of the milk filtrate (1:1) is made up to 10 c.c. with water in a centrifuge tube and the determination carried out as for blood magnesium.

(*e*) *Potassium*.—In determining potassium, 0·5 c.c. of the milk filtrate (1:1) is placed in a centrifuge tube, the total solution in the tube made up to 5 c.c. with water, and the procedure for blood analysis followed.

(*f*) *Sodium*.—For sodium, 10 c.c. milk filtrate (1:1) is made up to 25 c.c. with water. 1 c.c. of this solution is now treated as in the case of blood sodium determination.

(*g*) *Chlorine*.—For determining chlorine in milk it was found necessary to use 20 c.c. of the milk filtrate (1:1) for every determination. The procedure is the same as that described for the determination of chlorine in blood.

As pointed out previously, van Slyke suspected that conditions such as drought influenced the casein content of milk. When it was found that Kahlenberg (1913) had obtained good results from the formula $P = 0{\cdot}8977 + 0{\cdot}6393$ F, the correlation coefficient being $0{\cdot}7027 \pm 0{\cdot}0295$, this formula was used in the present work. It was, however, considered necessary to do a number of actual determinations of protein, although it is unfortunate that time did not permit the completion of many more.

(*h*) *Protein*.—The standard Gunning-Kjeldahl method for the determination of protein was used according to that given by the Association of Official Chemists (1920). For each determination 5 c.c. of milk was found to be sufficient.

* *Reagent B*.—10 grams ammonium molybdate dissolved in 100 c.c. of water, and mixed with 150 c.c. of sulphuric acid diluted with 150 c.c. of water.

The close relationship shown to exist between the chlorine and the sugar content of the blood, as well as between the chlorine content of milk and lactogen stimulated interest in the sugar content of milk in this investigation. Sugar forms a very large percentage of the milk constituents and would reasonably be expected to vary if milk composition is subject to variation. Only a limited number of milk samples could be determined chemically, the rest were done by the formula given by Sundberg (1931 which is: $L = 7 \cdot 07 - 18$ Cl.

(*i*) *Sugar.*—The lactogen was determined by Folin and Wu's (1920) modification of the Shaffer-Hartman sugar method. Since many constituents of ash were determined it would be a useful check to give total ash contents of the milk as well. The ash was calculated by the formula, $A = 0 \cdot 36 + 0 \cdot 11P$, given by Richmond (1920).

(*j*) *Ash.*—The method adopted for the accurate ash determination of milk was that described in Hawk and Bergheim (1927).

All butter-fat percentages were determined by carefully taking composite morning and evening samples of milk over a 48 hour period, and the Gerber tester was used in these fat determinations. The solids-not-fat readings were made by means of the Quevenne lactometer and the calculations worked according to Richmond's formula: $T = \cdot 25\ G + 1 \cdot 2\ F = \cdot 14$.

RESULTS.

In order to substantiate the tables giving the analytical results of the blood and milk determinations, the feed consumed and general condition of the animals will be reviewed briefly.

All the animals, except those of one group, consumed all their feed completely every day. It may, therefore, be taken that all the minerals given were ingested and that the animals apparently experienced no ill-effects due to these large quantities of inorganic substances. The faeces remained of a normal doughy consistence and brown in colour throughout the experimental period.

The animals receiving the ration low in all minerals except calcium and phosphorus (D.O.B. 3642 and 3649) became listless and thin and at times refused their feed. Taken over the whole period their " orts " averaged a pound a day.

The group receiving the ration low in calcium and phosphorus (D.O.B. 3641 and 3648) as well as the animal (D.O.B. 3659) on a low phosphorus intake soon showed signs of unthriftiness and later developed styfsiekte, eventually succumbing to a phosphorus deficiency. No other serious nutritional disturbances appeared in the remaining groups as judged by the condition of the animals. The animals in the low sodium and chlorine group (D.O.B. 3651 and 3646) as well as the group on low potassium intake (D.O.B. 3673 and 3656) periodically exhibited a drooling saliva flow accompanied by short respirations, a condition stated by Rupel, Bohstedt and Hart (1933) to be due to exhaustion and weakness.

Quarterly weight records are given in table 5.

TABLE 5.

Quarterly Weight Records in Pounds.

Group.	D.O.B.	June.	Sept.	Dec.	Mar.	June.	Sept.	Dec.	Mar.	June.	Sept.	Dec.	Comments.
Low Ca. and low P...	3641	700	820	875	975	920	790	825	976	1100	—	—	Died due to debility (styfsiekte).
	3648	591	760	800	900	855	780	805	910	948	1055	950	Killed in extremis (styfsiekte).
Low P............	3659	685	805	910	965	1026	765	818	940	1055	880	—	Died due to debility (styfsiekte).
Low Ca............	3643	595	800	875	941	860	835	880	964	1125	1060	1015	—
	3655	687	800	872	1020	940	915	942	1058	1175	960	980	—
Low Ca and Low Mg..	3640	585	795	865	900	897	1000	1052	1150	1252	1090	1075	No calf the first lactation.
	3650	680	850	935	1020	895	810	815	930	1126	1030	1025	—
All minerals low except Ca. and P.	3642	620	800	845	920	1030	670	737	880	1025	875	950	—
	3649	596	745	800	865	767	700	770	837	1028	850	900	—
Low Na. and low Cl..	3651	700	865	925	990	963	790	883	979	1082	1095	1285	No calf the second lactation.
	3646	606	776	855	946	862	830	845	—	—	—	—	Died of pneumonia.
Low Cl............	3658	715	840	935	1030	1090	815	853	1017	1036	1155	—	Killed on account of T.B.
	3675	612	775	825	920	820	795	817	915	1075	—	—	Died of acute peritonitis.
Low Na............	3672	615	780	810	880	734	670	697	840	—	—	—	Dismissed due to T.B.
	3653	650	840	902	960	868	670	750	907	1082	855	910	—
Low K............	3656	555	705	770	835	—	—	—	—	—	—	—	Died due to metritis.
	3673	570	800	850	930	875	700	720	825	988	1125	1255	—
All mineral sufficient + KI.	3677	715	880	903	985	910	825	870	980	1130	—	—	Dismissed on account of T.B.
	3652	585	750	840	900	872	790	830	958	1112	—	—	Died due to metritis.
All mineral sufficient...	3645	600	796	905	965	1070	940	950	1051	1150	1150	1105	—
	3639	635	775	858	970	830	775	822	940	1005	1030	—	Killed to use bones as control for 3641, 3648, and 3659.

By studying the figures presented in table 5, it will be noticed that there was a decided drop in weight in all the animals during the first lactation period which was from June to September, 1931. There was not such a severe drop in weight during the second lactation, however, indicating that sufficient protein had been supplied. In fact some groups, for instance the " all minerals low except adequate calcium and phosphorus " ration actually gained in weight during this period.

It was decided to discontinue the work in December, 1932, because only ten animals survived, in fact only three groups were left intact and had suffered no loss.

I. The Mineral Content of the Blood.

In order to obviate repetition and unnecessarily long discussions, the groups will not be dealt with separately but rather reviewed collectively by means of tables giving the individual as well as the average figures for the determinations. Each table will incorporate only such groups as were considered important in the study of the particular element under consideration. For instance, no mention will be made of a group receiving a ration low in chlorine, when the figures are presented for the calcium contents of the blood.

(*a*) *Blood calcium.*—The figures representing the monthly calcium content of the blood in milligrams per hundred c.c. are given in table 6.

From the above table it is apparent that all the blood calcium values are remarkably constant and all variations may be considered as mere normal monthly fluctuations. As no group could be considered as lagging, or could for this reason not be directly comparable to the control or any other group, the averages of all the calcium figures were taken as indicative of true average blood calcium values for the non-lactating period, averaging 8·1 mgm. per 100 c.c., the lactating period averaging 8·3 mgm., and a general average of 8·2 mgm. per 100 c.c. of blood.

Thus it would seem that it is not possible to bring about any change in the blood calcium content of the cows, even when the calcium ingested is reduced to an absolute minimum as may be seen in the case of the low calcium and low phosphorus group (D.O.B. 3641 and 3648), the low calcium group (D.O.B. 3643 and 3655), and the low calciun low magnesium group (D.O.B. 3640 and 3650).

(*b*) *Inorganic blood phosphorus.*—The monthly inorganic blood phosphorus figures are given in table 7.

In comparing the individual as well as the group averages in table 7, it immediately becomes apparent that all the groups may not be collectively considered in general averages as was possible for the calcium figures in table 6. The inorganic blood phosphorus determinations show that there has been a decided lowering of the inorganic phosphorus content of the blood in the group receiving low calcium and low phosphorus (D.O.B. 3641 and 3648) and also in the case of the animal (3659) receiving a ration low in phosphorus. The average inorganic blood phosphorus contents of these groups are: non-lactating period 4·2; lactating period 2·3, and for the whole experimental period 3·4 mgm. per 100 c.c. of blood. It is no wonder, therefore, that these animals were unable to survive the strain of milk production during the second lactation period.

The figures for inorganic blood phosphorus in all the other groups compare very favourably with those obtained in the control group and are consequently given in general averages as follows: lactating period 6·2, non-lactating period 7·4, and for the whole experimental period 7·2 mgm. per 100 c.c. Here again there was a decided lowering of the inorganic phosphorus content of the blood due to the drain of milk production, although the ration was adequate in phosphorus content.

In studying the figures for the low phosphorus groups mentioned above, it will be noticed that there is a decided drop in the inorganic blood phosphorus from the third month of 1932. This phenomenon agrees with the substitution of 10 pounds of fanko in these groups for the 5 pounds of mealies and 5 pounds of fanko previously fed.

(*c*) *Magnesium in blood.*—The results obtained for the magnesium determinations of the blood are given in table 8.

It is evident that the figures indicating the monthly blood magnesium values, table 8, show but slight variations from month to month; all the average levels may be said to be on a par with those of the control group receiving sufficient or an optimum of all mineral substances in the ration and supplement. The average blood magnesium values may, therefore, be given as follows, for all the groups: lactating period 3·1, non-lactating period 2·6, and the whole experimental period 2·7 mgm. per 100 c.c. of blood. A tendency for the blood magnesium to be slightly raised during the lactation period, although present, was not significant enough to be of importance.

(*d*) *Potassium in blood.*—The blood potassium content was determined monthly and is given for the groups likely to be influenced by the potassium intake of the ration in table 9.

The blood potassium figures given for the monthly blood determinations in table 9 show a very noticeable range of potassium fluctuation from month to month in the blood of the same animal. As this condition obtains for all the animals, regardless of the potassium intake in the ration, it may be considered normal. As the average value for anyone group does not fall markedly below the average for any other group, or for the control group, the figures have all been averaged in order to establish collective averages for the cows, which are as follows: lactating period 50·2, non-lactating period 59·6, and for the whole experimental period 58·7 mgm. per 100 c.c. of bood.

Attention is further drawn to the remarkable high blood potassium figures exhibited throughout the whole experimental period by D.O.B. 3649, a cow receiving a ration low in all minerals excepting calcium and phosphorus. Her group mate D.O.B. 3642 had blood potassium values that were comparable with those of any other animal in the experiment.

(*e*) *Sodium in blood.*—Sodium determinations likewise varied slightly in the monthly blood samples from groups in which sodium or related elements played a role in the daily intake. These analyses are given in table 10.

The figures indicating the monthly blood sodium values of the animals in table 10 show that there are no noticeable differences in the average values of any group as compared with the values obtained for the control group. For this reason general averages are given as follows: lactating period 273, non-lactating period 303, and the value for the whole experimental period was 302 mgm. per 100 c.c. of blood. It is noticed from these averages that a marked blood sodium depression occurred during the lactating periods. This was not, however, any greater in the case of the group on a low sodium intake (D.O.B. 3672 and 3653), although the general sodium level of the blood does seem to be slightly lower in the groups on a low sodium diet than those animals receiving optimum amounts of sodium in the ration.

(*f*) *Chlorine in blood.*—The monthly blood chlorine determinations were made for the groups considered in tables 9 and 10 and are given in table 11.

The figures indicating the monthly blood chlorine values were likewise averaged in order to obtain figures such as given for the elements previously discussed. These average values were found to be: lactating period 310, non-lactating period 324, and for the whole experimental period 314 mgm. per 100 c.c. of blood. The blood chlorine level was lowest during the lactation period. This drop may not be regarded as significant, as it was well within normal chlorine variation in blood from month to month. There is, however, a tendency for the groups receiving a low chlorine ration to exhibit a blood chlorine level slightly lower than that of the control group. Whether this is of any significance will be more fully discussed later.

II. The Mineral Content of the Milk.

In presenting the results of monthly milk analyses recourse has been had to simple tables in order that the position may be seen at a glance. In order to obviate repetition the tables giving the mineral elements calcium, inorganic phosphorus, total phosphorus, magnesium, potassium, sodium, and chlorine in the milk will be considered collectively in presenting the results. A careful study of these tables will show that the lowering of certain mineral elements in the rations had no apparent effect upon the quantity of these elements in the milk.

The monthly mineral fluctuations in the milk which are more pronounced in the case of certain elements than in others, may be considered to fall within the limits of experimental error and are, therefore, considered to be normal. Attention should be drawn to the fact that the low protein intake during the first lactation period had no effect upon the mineral composition of the milk as may be seen in all these elements by comparisons of the levels of the various elements in the first and second lactation period respectively.

The tables giving the monthly milk analyses for the different mineral constituents are collectively presented in the following pages. All milk mineral elements remained unaffected by lowering such elements in the ration.