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The Assimilation of Calcium and Phosphorus by the Growing Bovine.

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I. INTRODUCTION.

SINCE the classical studies of Lehmann (1858), in which he demonstrated the absolute necessity of minerals in a ration, the various aspects of mineral metabolism have developed into a complex nutritional problem. Of all the minerals essential for growth and well-being of the organism, calcium and phosphorus have received the most attention. These two minerals, calculated as their oxides, comprise 95 per cent. of the total mineral content of the skeleton.

Either an imbalance under certain conditions or a deficiency of these minerals, which may be found in many food-stuffs, will greatly impair normal growth and development.

The natural pastures in most subtropical climates where the rainfall is unevenly distributed tend to contain insufficient phosphorus for the normal growth in animals, especially at certain times of the year when young actively growing pasture is not available. Under such conditions phosphorus deficiency is usually an acute problem in animal industry. Although a straight calcium deficiency is less likely to occur in animals kept on pasture (for the calcium content of pasture does not vary appreciably with its growth), it may be introduced in stables, where animals are fed on high concentrates, which are low in calcium. Such conditions are known to occur where the deficiency is so acute as to produce osteodystrophic diseases as for instance osteodystrophia fibrosa in horses.

Theiler *et al* (1924, 1927), Henrici (1928, 1930) and du Toit *et al* [1932, 1935, 1935 (*a*)] have established the fact that the pastures of the Union of South Africa are generally low in phosphorus. Orr (1929) showed that the position was equally true for some of the pastures of most countries. It is by no means uncommon for the grazing animal to have in its feed a $CaO : P_2O_5$ ratio of 8 : 1 during winter. Indeed a considerably wider ratio sometimes exists. Hart, Guilbert and Goss (1932) give variations of ratios from unity to 39 : 1.

Under the above grazing conditions of abnormal ratios, animals may show anorexia (Huffman *et al*, 1933), perverted appetite (Green, 1925), soft and porotic bones (Becker *et al* 1933), unthriftiness (Sheehy and Senior, 1930), bent leg (Elliot and Crighton, 1926) and abnormal growth and development (Eckles and Becker, 1926, and

Theiler *et al*, 1927). In order to compensate for the preponderence of calcium over phosphorus, or the deficiency of phosphorus, supplementary feeding of the latter element, in the form of phosphates, is usually resorted to, with markedly beneficial results.

Several authors have expressed regret that data pertaining to the actual mineral requirements for growth are so limited. This lack of information may partly be ascribed to the fact that the availability of minerals differ in various feeding stuffs. The form in which the elements under consideration occurs in the ration or mineral supplement, the Ca : P ratio and the vitamin D content may greatly effect their absorption and utilization.

In order to throw further light on the calcium and phosphorus requirements of growing bovines, metabolism experiments over a long period, were undertaken. The animals were divided into different groups which were fed rations (*a*) supplemented with phosphates, (*b*) at different levels of mineral intake, and (*c*) at different Ca : P ratios.

II. LITERATURE.

A. Review Articles.

As a result of extensive research throughout the scientific world, there are now available voluminous data on the problems associated with mineral metabolism, and a detailed survey of the literature will not be undertaken here. Several exceedingly well compiled reviews have been published on calcium and phosphorus metabolism, a considerable portion of them being devoted to the deficiency diseases associated with these two elements.

Forbes and Keith (1914) published extensive work on the phosphorus compounds in animal nutrition. These authors described in detail the different forms in which phosphorus exists in organic and biological materials, the major portion of the work being devoted to the absorption, excretion, metabolism and requirements of phosphorus or its compounds in health and disease. It must however be pointed out that nearly all the work discussed therein belongs to the " pre-vitamin era ". Much of the work cited on laboratory animals is partly invalidated by that fact.

In his monograph " Minerals in Pasture " Orr (1929) gave valuable data with regard to the mineral content of pastures and pointed out the deficiencies, to which grazing animals are subject. In certain parts of South and East Africa, Australia, Norway and the United States of America the pastures were so deficient in calcium or phosphorus or both, that they were incapable of supporting normal growth of stock.

Crichton (1930) in a discussion of the mineral requirements of dairy cattle deplores the absence of information regarding the actual requirements of calcium and phosphorus for growing bovines. The author considered that the information relating to the subject was not immediately applicable in practice, because the work had been accomplished under conditions which had no parallel in practice.

A very extensive review of the factors associated with rickets in human beings and animals, was given by Goldblatt (1931). The subject was discussed from clinical, pathological, historical, radiological and chemical viewpoints, by far the greater portion of the work being devoted to the rôles played by calcium, phosphorus and vitamins in the etiology of rickets. The difference in chemical composition of the skeleton and body fluids of normal and rachitic animals and human beings was described, and the factors which affects the calcium and phosphorus utilisation in the ration, as well as the rôle of artificial and solar irradiation, causing normal or abnormal calcification, were discussed.

This review is probably the most complete published in recent years on rickets; over 2,700 references are quoted.

In their recent publications, Marek and Wellmann (1931, 1932) gave considerable data of their own experiments on dogs and pigs, as well as of the literature on calcium and phosphorus metabolism in a discussion of their conception of the causes of osteodystrophic diseases in animals. They argued that the mineral value of the ration is signified by the alkali-alkalizität, i.e. $(K_2O + Na_2O) - (Cl + SO_3)$ and erdalkali-alkalizität, i.e. $(CaO + MgO) - P_2O_5$ when these values are expressed in milligram equivalents per 100 gm. dry matter.

Sherman (1932, 1935) discussed in detail the mineral requirements of human beings, and laid great stress on the necessity of a calcium sufficient diet for health and growth. He pointed out that this most important element is usually deficient in the ordinary human diet.

In his review, Shohl (1933) aimed at bringing up to date from 1930, the literature associated with the many phases of calcium metabolism. Attention was given mainly to the forms of calcium in the blood, the metabolism of calcium and phosphorus and the factors influencing bone calcification. The author considered that magnesium metabolism is intimately related to that of calcium and phosphorus.

Theiler and Green (1932) discussed in detail the effect of a phosphorus deficiency in the feed of cattle and sheep grazing on natural pastures. The history, cause and effects of Lamsiekte in South Africa were given and compared with deficiency diseases in other parts of the world.

B. Calcium and Phosphorus Requirements.

Weiske (1873) concluded from metabolism experiments that 5 to 6 months old calves required 12·0 gm. Ca and 9·5 gm. P daily. The body of a calf was found to contain 1·2 per cent. Ca and ·67 per cent. P by Lawes and Gilbert (1883). From these analyses Armsby (1917) computed that for the first 12 months calves should daily retain 15·4 gm. Ca and 8·1 gm. P.

Kellner (1912) argued that a bovine weighing 350 Kgm. contained 1·57 per cent. Ca and ·88 per cent. P. If the constituents in the body at birth were disregarded, the animal would require 15 gm. Ca and 8·3 gm. P per day; the food should contain 2 to 3 times as much of each mineral, as only 33 to 50 per cent. of the minerals in the ration was utilised.

As shown by Starling (1920), there was a close relationship between the amounts of minerals present in the milk of the mother and the ash of the young for each species. From this Crichton (1930) argued, that for every 1 lb. of protein in cows milk, there was ·8 oz. CaO. According to Wolff-Lehmann's tables a bovine of 500 lb. required 1 lb. protein per day. It should, therefore, assimilate ·8 oz. CaO per day.

Wellmann (1932) considered that 100 Kgm. live weight required daily for maintenance 3·6 gm. Ca, about 2 gm. P, and 2·5 gm. P per 100 gm. gain in body weight. In the case of plant foods, three times as much calcium and twice as much phosphorus as that required, must be taken for production and growth. Archibald and Bennett (1935) expressed their results in a slightly different way. They found that dairy heifers grew normally during the first year on a ration which supplied 1·8 gm. P and 3·8 gm. Ca daily per 100 lb. live weight, the amounts decreasing as the animals grew older.

Theiler *et al* [1920, 1927 (*a*)] estimated that owing to the deficiency of phosphorus in the pasture the intake of ranching cattle in Bechuanaland during the dry season was only 6-11 gm. P_2O_5 per 1,000 lb. live weight. This dificiency of phosphorus caused severe osteophagia. They estimated that 27 gm. P_2O_5 per 1,000 lb. live weight was the minimum phosphorus requirement for the prevention of osteophagia in mature cattle. Of this 27 gm. P_2O_5, 20 gm. was supplied by a supplement of 3 oz. of bone meal.

These authors (1924) found that in other districts a supplement of ½-¾ lb. bonemeal per week was sufficient for the prevention of osteophagia in grazing animals, but expressed the opinion that this amount was probably insufficient for optimum growth and condition.

Theiler, Green and du Toit (1927) under stall-fed conditions produced "styfsiekte" in growing heifers by feeding a fanko hay ration containing 6·9 gm. CaO and 5·1 gm. P_2O_5. They expressed the opinion that this disease "styfsiekte" occurring naturally in South Africa, in cattle grazing on phosphorus deficient pastures, was probably identical with osteomalacia. Theiler later (1931) by making an extensive histological study of the bones of the animals used in the above experiment, showed that the condition of aphosphorosis known as Styfsiekte and produced artificially in growing heifers, was osteomalacia in the mature and was referred to as rickets in the growing animal.

These authors found that an intake of 4·8 gm. P was insufficient for growing heifers, although half of it was supplied in the form of bonemeal, actual signs of a deficiency not becoming evident until after calving. A supplement of 3 oz. of bonemeal bringing the total daily intake of Ca to 37 gm. CaO and of P to 28 gm. P_2O_5 was found to supply the mineral needs of the heifers used in their experiment. Du Toit and Green (1930) concluded that a daily supplement of approximately 18 gm. P_2O_5 given in the form of bonemeal to growing bovines kept on phosphorus deficient pasture in the Bechuanaland area was probably in excess of their requirement for optimal growth, but that a daily supplement of 6 gm. P_2O_5 given as bonemeal was definitely below it .

Watkins (1933) found that a ration supplying 18 months old growing steers with 11·6 gm. Ca and 8·5 gm. P per day was sufficient with regard to these minerals. A slightly higher intake of phosphorus (10·8-12·3 gm.) was favoured by Lamb *et al* (1934) for growth and pregnancy in bovines from 18-30 months of age.

It would appear from the data quoted that young bovines will grow normally on a ration supplying about 20 gm. P_2O_5 and 20 gm. CaO per day. A considerable portion of this amount should however be in an available form such as in mineral supplements or in good quality hay.

C. Assimilation of Calcium and Phosphorus.

Maximum utilization of the food constituents is always aimed at in order to supply adequately the demands for growth, production and reproduction. Several factors impair the utilization of minerals even though the latter exist in an easily assimilable form.

The small intestine is the zone of absorption but the minerals absorbed are not necessarily retained, as a fair amount, especially calcium, is belived to be excreted into the large intestine, and to pass out with the faeces.

Meigs, Blatherwick and Cary (1919) expressed the opinion that the assimilation of calcium and phosphorus was facilitated by feeding these minerals alternatively, but no definite experiments have since been carried out to prove the validity of this theory.

McGowan (1933) maintained that for optimal utilization of Ca and P after absorption, it is essential that these minerals should be supplied in the food uncombined with one another and that they should be absorbed, as far as possible in this condition.

Intestinal absorption was proved by Miller, Yates and Jones (1926) to be partly controlled by the needs of the animal. In accordance with the above, Hodgson and Knott (1932) ascribed the poor utilization of minerals by dairy heifers, fed on artificially dried herbage, to the fact that when the animals were full-grown, their demands were very small.

Forbes *et al* (1922), Turner *et al* (1927, 1931), Haag *et al* (1929), Hayden, Monroe and Crawford (1930), Hart, Kline and Humphrey (1932) found negative mineral balances for high producing cows. In some of these cases more than 90 per cent. of the total mineral intake was excreted in the faeces and urine, the 10 per cent. left to supply the minerals contained in the milk being inadequate; the animal was forced to draw on her skeletal reserves. Hart *et al* (1933) concluded that the animal was unable to increase its assimilation of minerals even when the mineral intake was considerable.

Mitchell *et al* (1931) found, with pregnant gilts, an average daily retention of 4·38 gm. Ca and 1·32 gm. P, being 31·5 per cent. and 16 per cent. of the intakes respectively.

Cows given 58 gm. Ca and 51 gm. P by Turner, Kane and Hale (1933) utilized about 31 per cent of each element. The ration consisted of Timothy hay, a grain mixture and a calcium supplement. Hart *et al* (1933) found with a high producing Ayrshire cow fed on a ration of alfalfa, grain and corn silage, a retention of almost 44 per cent. of the Ca intake.

According to Meigs *et al* (1934) cows kept for several months on an inadequate calcium ration, may utilize about 50 per cent. of the calcium content of the ration.

Forbes *et al* [1921 (*b*)] found that pigs fed on a grain ration containing a calcium salt retained 50-70 per cent. of the calcium, while Spiers and Sherman (1936) with rats and Morgen *et al* (1933) with dogs, found a retention of 90 to 93 per cent of the calcium intake. However as far as the author is aware, such high values have not been reported for sheep, pigs or cattle.

It must be pointed out, however, that that percentage utilization or retention means very little, as such factors as demand of the animal, the total intake, the form in which the minerals are given and the vitamin D content of the ration or solar irradiation, effect the retention. Obviously the percentage retention based on a high intake of Ca and P may be low and hence gives a poor idea of the utilization of these minerals. In the same way poorer utilization or a lower intake may show an appreciably higher percentage retention.

D.—Factors Effecting the Utilization of Calcium and Phosphorus.

Considerable work has been done to determine the relative availability of the calcium and phosphorus of different feeding stuffs and of mineral supplements, but the results obtained are by no means conclusive.

Kramer, Potter and Gillum (1930), Fincke and Sherman (1936) and Kohman and Sauborn (1935) with rats and Hart *et al* (1927, 1930, 1931) with bovines determined the availability of the calcium of various foods.

Hesse and Barndt (1933) concluded that the absorption of calcium from the calcium salts tested (viz. calcium carbonate, di-calcium phosphate, tri-calcium phosphate, calcium gluconate) is entirely independant of the solubility of these salts.

Attention has furthermore been given to the availability of the phosphorus in different phosphatic supplements, especially di-calcium phosphate and bonemeal. A better utilisation of calcium and phosphorus was obtained by Bloom (1932) by feeding di-calcium phosphate than by feeding tri-calcium phosphate or bonemeal. Similarly, du Toit and Green (1930) concluded from supplementary feeding experiments with cattle, that taking weight for weight, di-calcium phosphate was three time more effective than bone meal. Di-calcium phosphate contains 42 per cent. P_2O_5 and bonemeal 24 per cent. P_2O_5. Later work by Malan and du Toit (1932) enabled them to place the supplements in the followign order of availability of the phosphorus.

(1) Sodium phosphate.

(2) Precipitated calcium phosphate.

(3) Bonemeal and degelatinised bone flour.

The conclusions of these authors were based on the weight increase of the animals.

The calcium and phosphorus of food such as cereals containing inositol phosphorus are poorly utilized. Booth, Henry and Kon (1935) and Bruce and Callow (1934) maintained that oatmeal contains mostly phytin phosphorus (inositol hexaphosphate) which they consider to be non-available to animals. According to Bleyer and Fisher (1931) and Forbes and Irving (1931) the Ca-Mg salt of inositol hexaphosphoric acid was well utilized by rats. The work of McCance and Widdowson (1935) also showed a partial utilization of the phytin ingested by human beings.

Steenbock *et al* (1930) observed that " equalization of the phosphorus content of cereal rations did not make them equally effective in bone formation ". No correlation was, however, found by Harris and Bunker (1935) between the phytin phosphorus content and the rachitogenic properties of the diet.

Later Templin and Steenbock (1933) found better calcification by feeding immature yellow dent field maize than when mature maize of the same variety and grown under identical conditions was fed. In a subsequent paper by these authors [1933 (*a*)] autolysed germinated maize was shown to possess definite antirachitic properties, when compared with mature maize.

Further work by Lowe and Steenbock (1936) clarified the position considerably. They showed that (a) the phytin phosphorus present in the rachitogenic diet was poorly available when compared with the phosphorus of phosphoric acid and sodium glycerophosphate and (*b*) treatment of maize with HCl improved its antirachitic properties in proportion to the extent that the phytin was hydrolyzed.

The anti-rachitic factor, Vitamin D, apparently regulates the absorption of calcium and phosphorus from the intestine and the deposition of minerals in the osseous tissue, to form bone.

A deficiency of vitamin D, affects the utilization of calcium and phosphorus detrimentally. Morgan Garrison and Hills (1933) maintained that vitamin D apparently prevents re-excretion of absorbed calcium into the intestine. The vitamin causes deposition of calcium to form bone rather than an increase of original absorption.

Rupel, Bohstedt and Hart (1933) and Bechel *et al* (1933) showed that calves require vitamin D in some form. Further work with calves by Wallis, Palmer and Gullickson (1935) and Duncan and Huffman (1936) indicated that the addition of vitamin D to a ration deficient in this constituent, but adequate in calcium and phosphorus, greatly increased the mineral retention. Negative mineral balances thereby became positive.

The animals used in the experiments reported in this publication were exposed to direct sunlight daily for several hours in their exercising paddocks. Hence abundant vitamin D was supplied in this way in addition to that contained in the feed.

It is generally accepted that the utilization of Ca and P is affected by the ratio in which these elements are present in the diet and from extensive investigations of this problem, it is generally conceded that a ratio of approximately 2 Ca to 1 P in the diet favours the best utilization of these elements. Theiler, Green and du Toit (1927) expressed the view that the current emphasis on Ca : P ratio was probably exaggerated especially when these elements were present in abundance in the ration of animals. At lower levels of intake the best utilization of these two minerals is an advantage to the animal and hence a correct ratio becomes more important; but here again it would seem that the level of intake of Ca or P or both becomes equally if not more important. For instance Brown, Shohl *et al* (1932) and Shohl (1932, 1936) stated that the Ca : P ratio inadequately defined the rachitogenic properties of a diet. Rickets could be produced in rats fed on diets low in phosphorus no matter what the ratio of Ca : P was. Theiler, du Toit and Malan (1937) have expressed the view that in the production of osteodystrophic diseases by limiting the intake of Ca or P or both, the ratio was decidedly of secondary importance. The rapidity with which the condition developed bore a direct relationship to the ratio, a normal ratio producing the condition by no means as rapidly as a wide ratio does, but the difference in the severity of the conditions produced ultimately not being always apparent.

It is doubted whether the ratio of dietary Ca to P is important to the health of animals under conditions of feeding which are usually considered normal except perhaps in the case of horses and pigs where the nature of the ration is such that it contains relatively high P and low Ca. Kintner and Holt (1932) believe that the ratio of 1 CaO to 2·5 P_2O_5 is dangerous to the health of horses and it is feared that this ratio is often present in their rations.. The wide ratio is effective in producing disease, but it must be remembered that the Ca is low in addition and it is freely admitted that the ratio will effect animals detrimentally under such conditions.

E.—Blood Analysis.

Robinson and Huffman (1926) found that the blood of normal mature cattle contained from 7·7 to 14·7 mg. of Ca per 100 ml. Allcroft and Green (1934) and Haag and Jones (1935), however, found values between the narrower limits of 8·6 to 11·6 mg Ca per

100 ml. about 90 per cent. of their values falling between 9 and 11 mg. It has been found by Kintner and Holt (1932) while investigating equine osteomalacia, Fitch *et al* (1933) and Groenewald (1935) with cattle, in experiments over a long period, Jones and Robson (1932) with rats and Fraser (1932) with sheep, that the serum calcium remained normal with diets deficient in calcium but with an adequate amount of vitamin D. When, however, a deficiency of both calcium and vitamin D existed in the ration, Auchinachie and Emslie (1932) and Fraser *et al* (1934) working with sheep, Bethke *et al* (1923) and Jones and Cohen (1936) with rats, found a lowered blood calcium. An addition of cod-liver oil (vitamin D) restored the serum calcium to the normal value.

With calves on an adequate mineral ration but lacking in vitamin D, Rupel, Bohstedt and Hart (1933), Huffman and Duncan [1935 (*a*)] found a lowering of the serum calcium, decreasing in some cases to 6 mgm. Ca per 100 ml. blood. The addition of cod-liver oil, however, promptly brought the concentration of this element in the blood back to normal.

Huffman and Robinson (1926), Palmer and Eckles (1927), Henderson and Weakly (1930), Malan, du Toit and Green (1928) showed that the phosphorus content of the ration of bovines had a marked influence on the inorganic phosphorus content of the blood. Such low values as 1·0 mgm. P per 100 ml. blood were obtained by Malan, du Toit and Green (1928) for mature cattle on phosphorus deficient grazing. Phosphorus sufficiency in the diet of mature bovines was reflected as an increase in the inorganic P content of the blood. Values above 3·5 mgm. per 100 ml. blood were regarded as normal for mature bovines.

Kramer and Holland (1923) with chickens and Rupel, Bohstedt and Hart (1933) with calves, showed that with an adequate mineral diet deficient in vitamin D the inorganic blood phosphorus dropped but was restored to normal when cod-liver oil was given.

The determination of inorganic phosphorus in the blood of animals provides an excellent way of diagnosing phosphorus deficiency in stock kept under conditions of vitamin D deficiency. In South Africa this method has been employed to collect information on the extent of phosphorus deficiency in stock kept on natural pastures.

According to Kay (1929-1930) a markedly raised concentration of plasma phosphatase in human bones was confined almost specifically to certain generalized diseases of the bone, increased values more than 20 times the normal being found.

Bodansky and Jaffe (1931, 1932) working with rats and guinea pigs found the plasma phosphatase to be affected by fasting and a deficient calcium intake.

A vitamin D deficiency, with adequate mineral intake, was found by Auchinachie and Emslie (1933) to increase the phosphatase content of the blood of sheep three to four times. These authors expressed the opinion that the concentration of plasma phosphatase gave much earlier indications of disordered calcium and phosphorus metabolism than either serum calcium, blood inorganic phosphorus, general state of health or body weight. In agreement with the abovementioned workers, Bodansky and Jaffe (1934) considered plasma phosphatase values to be better criteria for the severity and progress of rickets than those of the serum calcium and phosphorus.

Totally unexpected plasma phosphatase values were found by Palmer *et al* (1935). The calcium intake, with or without vitamin D, had no effect on the plasma phosphatase of cows. The bone analysis indicated that there was no disturbed metabolism.

It is possible that the determination of plasma phosphatase is not equally valuable for diagnosing poor calcification or even abnormal Ca P metabolism in all species of animals.

F. Bone Analysis.

Apart from the primary functions of the skeleton to serve as a framework for the body, it plays a very important role, namely, as a reservoir for the important minerals calcium and phosphorus. From this mineral reservoir calcium and phosphorus are withdrawn during periods of mineral stress. The bone is resorbed as a whole decreasing in weight and density and increasing in porosity, and the bone becomes osteoporotic, unless complicated by osteomalacia, when uncalcified osteoid tissue is deposited. The result is a decreased ash content with or without an increase in oganic matter, thereby rendering the bones less rigid.

Histological examinations of bone sections give a clear picture of the condition of the animal with regard to calcium and phosphorus but as such a study falls outside the scope of this paper and is fully discussed by Theiler *et al* (1937), only the physical and chemical analysis of bone will be discussed.

Forbes and his co-workers [1921 (*a*), (*b*), (*c*), (*d*)] made an extensive study of the causes of bone fragility in brood sows. Marked differences were shown in the different groups for the ash per c.c. bone (volume of bone taken), the breaking strengths of the bones and the live weight of the animals.

Rottensten and Maynard (1934) found that the average dry-fat-free femurs of rats on a low level of mineral intake, contained 59·6 per cent ash as against 64 per cent for the bones of animals receiving an adequate mineral supply. The average ash content of the dry-fat-free cannon bones of calves on a concentrated ration, was found to be 56·8 per cent by Mead and Regan (1931). The addition of calcium to this ration brought the ash content for the same bones of other calves to 64·5 per cent. Similar figures are given by Kintner and Holt (1932) for the metacarpal ash content of horses.

Bethke, Edgington and Kick (1933), Rupel, Bohstedt and Hart (1933) and Shohl (1936) concluded that the ash content of bone affords an excellent index of the degree of calcification. The figures for the breaking strength of bones by Becker, Neal and Shealy (1933-1934) were striking. Heavy producing cows were kept for several lactation periods on calcium deficient rations. Cow No. 59, after eleven consecutive lactations, fractured her pelvic arch in three places. The average breaking strength of her femurs and humeri over a six inch span, was 335 lb. per square inch as against over 3000 lb. per square inch for the same bones of animals receiving supplements of bone meal.

Hogan (1932) and Aubel, Hughes and Lienhardt (1936) found with pigs that the level of mineral intake had hardly any effect upon the diameter of the bone, but remarkably influenced the thickness of its wall.

Blum (1931), Holmes and Pigott (1931) and Hartman and Meigs (1931) showed that the Ca : P ratio of the bones of animals can not be altered by varying the mineral content of the ration. In contradiction with the above, Yeager and Winters (1935) concluded that there was some indication that the Ca : P ratio in the bones depended to some extent at least on the amounts of calcium and phosphorus present in the diet. This ratio, with animals fed at varying levels of mineral intake, was found by Brooke, Smith and Smith (1934) to vary from 2·3 : 1·0 to 2·1 : 1·0.

III. GENERAL DESCRIPTION OF INVESTIGATION.

A. Object.

The following investigations were undertaken with the object of studying—

(1) the availability of the phosphorus contained in different commercial phosphates which are used extensively as stock feed in the Union of South Africa;

(2) the effect of different levels of mineral intake upon the utilization of the calcium and phosphorus, when growing bovines are fed the same basal ration with a constant calcium to phosphorus ratio of 2 : 1;

(3) the effect of varying the calcium to phosphorus ratio of the ration upon the utilization of these minerals.

In the experiment to be reported on, the retention of these minerals was studied on young bovines growing normally and on those reared under adverse conditions which are often encountered in practical stock farming in the Union of South Africa.

B. Procedure.

The animals used for these experiments were young bovines which were periodically placed in concrete floored stalls, so constructed that the urine could be run off into receptacles sunk into the floor immediately after being voided. The faeces were collected by an attendant who was on duty day and night. As a result of this arrangement no intermixing of faeces and urine took place. The stalls were large enough to allow the animals to move freely and to lie down whenever they wished.

All the urine and the faeces voided by an animal during each week were gathered separately. To each collection formalin was added in order to avoid putrefaction and to render the samples less objectionable to work with. The moisture content of the faeces was determined while Ca and P were determined on both urine and faeces.

The basal ration contained a sufficient quantity of poor quality hay to provide roughage. The ingestion of calcium and phosphorus was thus kept as low as possible. Fanko (rolled maize endosperm) or samp (unrolled maize endosperm) both of which are very low in minerals, comprised the major portion of the ration and provided most of the energy and some of the protein. Meat meal or blood meal was added to increase the quantity and quality of the proteins, green feed or silage being included to avoid a possible deficiency of vitamins A and C. Full particulars of the animals and the rations will be given at the commencement of each experiment.

Twice daily each animal was fed a weighed quantity of feed. Tap water, of known calcium content, was provided, the total imbibed by each animal measured and recorded during the periods when faeces and urine were collected. Prior to the feeding of the experimental ration, the animals were kept in the stalls and fed the basal ration in order to accustom them to the conditions under which the experiment was to be conducted. If the animal did not finish the feed supplied, the remaining portion was weighed and recorded. An endeavour was subsequently made to regulate the quantity supplied in such a manner that nothing or very little remained over. When food was left over by an animal during a metabolism trial, its calcium and its phosphorus contents were determined and deducted from the intake.

The mineral content of each constituent of the basal ration was determined from time to time. This procedure was deemed necessary since considerable variations were obtained in the mineral content of different consignments of food.

The minerals intended for each animal were weighed out and added to the rations as required.

During the periods of rest when faeces and urine were not collected, the animals were allowed to exercise in a paddock during the day, where they had free access to water and sunlight. The paddock had a concrete floor to prevent earth eating.

The animals were weighed and bled monthly, these operations being carried out early in the morning before feeding and watering.

C. Methods of Analysis.

The technique adopted was that outlined by Malan and van der Lingen (1931) for taking blood samples and for determining inorganic blood phosphorus and blood calcium.

The calcium and phosphorus contents of the feeds and the excreta were determined by the method given by the same authors for the analysis of grasses. Benedict and Theis (1924), Fiske and Subbarrow (1925) and Roe and Kahn (1926-1929) have contributed to the standardization of these methods. A modification of the method of Clark and Collip (1925) for the determination of calcium in the feeds and excreta was introduced since it was found to be more applicable. The calcium and the phosphorus contents of the bone ash were determined by the standard macro-volumetric methods described by Treadwell and Hall (1930).

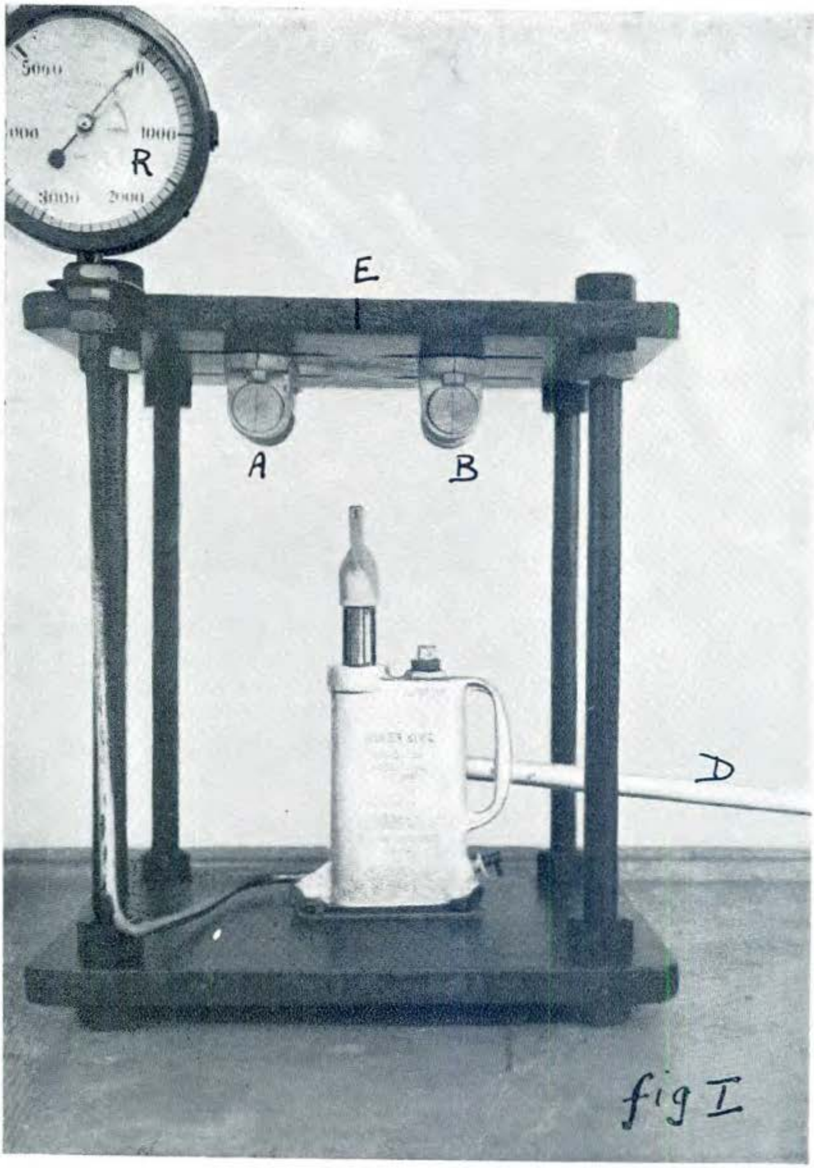

Fig. 1.

A and B are two adjustable rollers which can be set at any required distance apart. AE must always be equal to EB.

The animals were destroyed at the end of each experiment for the purpose of collecting the bones for chemical and histological examinations. All the meat was quickly and carefully removed from the bones without undue exposure of the latter to prevent loss of moisture.

Immediately after the bone had been cleaned, it was weighed and its measurements taken. The volume was obtained by immersion into water contained in graduated glass cylinders or specially marked glass jars, the vessels used depending upon the size of the bones. After removal from the water, the bone was dried by rubbing with a cloth. The breaking strength was then determined over a six inch span. The apparatus used for this purpose was a small scale model of a steel testing machine (Fig. 1).

Fig. 2.

In order to determine the breaking strength of the bone its centre is placed on the point C and by applying pressure by means of the lever D the bone is forced against the points A and B. The breaking pressure of the bone is registered on the pressure gauge R as pounds per square inch. The position in which the different bones were placed is indicated in Fig. 2.

The bone was then broken up with the aid of a chissel and hammer into small pieces about an inch long.

These fragments of bone and marrow were then collected in a receptacle and placed in a hot air oven at 105° C. for 48 hours in order to determine the water content by loss of weight. The fat was poured off and the pieces of bone collected in a linen bag and placed in a fat extractor, where the remaining fat was extracted with ether. After removal from the extractor the bones were dried in a hot air oven in order to obtain the dry-fat-free weight. The pieces of bone were then reduced to bonemeal in a bone shredder and an electric mill for the determination of ash, calcium and phosphorus.

IV. EXPERIMENT I.

THE AVAILABILITY OF THE PHOSPHORUS CONTAINED IN DIFFERENT PHOSPHATIC SUPPLEMENTS.

Extensive feeding of phosphorus to stock has resulted in the marketing of a number of phosphatic products. Bonemeal has been greatly favoured by stock farmers as a supplementary feed, it being the first to be recommended and easily procurable. The different brands of this product vary in colour from a dirty brown to a clear creamy white, depending on the treatment during the process of manufacture. For this reason it was considered advisable to investigate the assimilability of the phosphorus contained in the various bone meals as well as that of other phosphatic products commonly used.

The commercial phosphatic supplements generally used may be classed under three main types. Di-sodium phosphate and mono-ammonium phosphate were used as examples of water soluble phosphates which can be administered in solution in the drinking water of stock. Precipitated calcium phosphate which is mainly di-calcium phosphate is also extensively used. It is prepared as a by-product in the manufacture of gelatine from bones or is obtained from rock phosphate. This product is probably the cheapest for the supplementary feeding of stock. Bonemeal is used more extensively than any other phosphate and was therefore included in the experiment.

Experiment I (a).

Two half grade Friesland bovines were used in this experiment. At the commencement of the work their ages and weights were as follows:—

No.	*Age.*	*Initial weight.*
5151	15 months................	490 lb.
5164	17 months................	630 lb.

The basal ration with its calcium and phosphorus contents is tabulated below.

Feed.	Amount.	% P.	P gm.	% Ca.	Ca gm.
Hay	1·32 Kg.	0·048	1·634	0·1320	1·742
Fanko	3·00 Kg.	0·0393	1·180	0·0071	0·213
Meatmeal	·32 Kg.	0·247	0·560	0·043	0·099
Water	± 10 litres	—	—	—	0·583
Sodium chloride	15 gm.	—	—	—	—
Total intake	—	—	2·38 gm. P.	—	2·64 gm. Ca.

The mineral intake varied slightly with the mineral contents of the ration and the water supplied. Analyses of these were made form time to time.

TABLE 1.

Time-table for the Feeding of Supplements and the Collection of Faeces and Urine.

Week No.	Supplement.	Remarks.
1	0	Rest.
2	0	Collection of excreta.
3	A. Di-sodium phosphate + Calcium Carbonate....	Rest.
4	3·5 gm. P + 9·6 gm. Ca....	Collection.
5	3·5 gm. P + 9·6 gm. Ca....	Collection.
6	0	Rest.
7	0	Collection.
8	B. V. Bonemeal + calcium carbonate....	Rest.
9	3·5 gm. P + 10 gm. Ca....	Collection.
10	3·5 gm. P + 10 gm. Ca....	Collection.
11	0	Rest.
12	0	Collection.
13	0 (change of hay)....	Rest.
14	0	Collection.
15	C. H. Bonemeal + calcium carbonate....	Rest.
16	2·79 gm. P + 7·42 gm. Ca....	Collection.
17	2·79 gm. P + 7·42 gm. Ca....	Collection.
18	0	Rest.
19	0	Collection.
20	D. Mono-ammonium phosphate + Calcium carbonate	Rest.
21	2·88 gm. P + 7·42 gm. Ca....	Collection.
22	2·88 gm. P + 7·42 gm. Ca....	Collection.
23	0	Rest.
24	0	Collection.
25	E. Di-calcium phosphate + Calcium carbonate....	Rest.
26	2·97 gm. P + 7·57 gm. Ca....	Collection.
27	2·97 gm. P + 7·57 gm. Ca....	Collection.
28	0	Rest.
29	0	Collection.

The supplements tested out in this experiment were (A) Di-sodium phosphate, (B) V. Bonemeal, (C) H. Bonemeal, (D) Mono-ammonium phosphate and (E) Di-calcium phosphate.

Calcium carbonate was added to each supplement in order to keep the calcium-phosphorus ratio of the mineral intake constant.

Both animals were treated in exactly the same manner. They were fed on the same ration and supplements at the same time according to Table I.

The results of the balance trials are given in Tables II to III.

Table II.

Mineral Balance (daily average).

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 3·5 gm. P + 9·6 gm. Ca.

B = V. Bonemeal + $CaCO_3$ i.e. 3·5 gm. P + 10 gn. Ca.

C = H. Bonemeal + $CaCO_3$ i.e. 2·79 gm. P + 7·4 gm. Ca.

D = Mono-ammon. phosphate + $CaCO_3$ i.e. 2·88 gm. P + 7·42 gm. Ca.

E = Di-calcium phosphate + $CaCO_3$ i.e. 2·97 gm. P + 7·75 gn.. Ca.

Bovine 5151.

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
1	O	2·38	2·63	2·81	0·31	4·71	1·74	−0·74	−3·28
2	O	2·38	2·65	3·26	0·24	4·81	1·79	−1·12	−3·95
3	A	5·88	12·23	2·98	0·23	6·53	0·51	2·67	5·19
4	A	5·88	12·26	3·02	0·21	7·85	0·47	2·65	3·94
5	O	2·38	2·61	3·39	0·15	3·64	1·51	−1·16	−2·54
6	B	5·93	12·66	3·59	0·25	8·03	0·54	2·09	4·09
7	B	5·93	12·66	3·33	0·19	8·18	0·46	2·41	4·02
8	O	2·77	2·70	3·05	0·25	4·41	1·36	−0·53	−3·07
9	O	3·67	4·42	3·23	0·23	4·20	1·47	0·21	−1·25
10	C	6·46	11·92	3·46	0·21	6·51	0·45	2·79	4·96
11	C	6·46	11·95	3·58	0·20	5·96	0·45	2·68	5·54
12	O	3·67	4·49	2·98	0·22	3·86	1·66	0·47	−1·03
13	D	6·55	11·85	3·15	0·24	4·96	0·44	3·16	6·45
14	D	6·55	11·83	3·29	0·31	5·58	0·41	2·95	5·84
15	O	3·67	4·42	3·43	0·23	4·46	1·84	0·01	−1·88
16	E	6·64	11·99	3·45	0·27	5·93	0·46	2·92	5·60
17	E	6·64	11·99	3·75	0·30	5·88	0·41	2·59	5·70
18	O	3·67	4·42	2·91	0·23	3·64	1·74	0·53	−0·96

TABLE III.

Mineral Balance (daily average).

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 3·5 gm. P + 9·6 gm Ca.

B = V. Bonemeal + $CaCO_3$ i.e. 3·5 gm. P + 10 gm. Ca.

C = H. Bonemeal + $CaCO_3$ i.e. 2·79 gm. P + 7·4 gm. Ca.

D = Mono-ammon. phosphate + $CaCO_3$ i.e. 2·88 gm. P + 7·42 gm. Ca.

E = Di-calcium phosphate + $CaCO_3$ i.e. 2·97 gm. P + 7·5 gm. Ca.

Bovine 5164.

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
1	O	2·41	2·63	3·10	0·24	4·98	2·07	−0·93	−4·42
2	O	2·41	2·59	3·23	0·25	4·88	1·95	−1·07	−4·16
3	A	5·91	12·23	3·33	0·19	5·83	0·61	2·39	5·78
4	A	5·91	12·22	3·53	0·17	7·51	0·30	2·21	4·41
5	O	2·41	2·61	3·22	0·17	2·77	1·22	−0·98	−1·35
6	B	5·96	12·66	3·76	0·19	7·23	0·39	2·01	5·04
7	B	5·96	12·64	3·87	0·19	8·09	0·30	1·90	4·25
8	O	2·80	2·70	3·37	0·22	4·18	1·06	−0·79	−2·51
9	O	3·74	4·42	2·89	0·21	4·71	2·08	0·64	−2·3
10	C	6·53	11·92	3·40	0·23	5·38	0·41	2·99	6·13
11	C	6·53	11·90	3·65	0·24	6·35	0·44	2·64	5·11
12	O	3·74	4·49	3·20	0·23	3·94	1·74	0·31	−1·19
13	D	6·62	11·85	4·06	0·24	6·10	0·43	2·32	5·32
14	D	6·62	11·85	3·36	0·24	5·62	0·43	3·02	5·80
15	O	3·74	4·40	3·29	0·23	4·36	1·82	0·22	−2·74
16	E	6·71	11·90	3·26	0·26	6·28	0·56	3·19	5·15
17	E	6·71	11·98	3·72	0·26	6·05	0·53	2·63	5·40
18	O	3·74	4·42	3·00	0·20	3·73	1·53	0·54	−0·84

Discussion.

Some difficulty was experienced in determining a satisfactory basis for comparing the availability of the phosphorus contained in the different phosphates.

Although the minerals of a compound have to be made available during digestion, i.e. be present in a form that would pass through the intestinal wall, in order to be retained, they may nevertheless not be retained although highly available, as in the case of an animal in equilibrium on a high level of mineral intake. In such a case it cannot be assumed that the Ca and P are not absorbed, as the minerals absorbed in any one part, do not show in the balance experiment, although they have been in circulation. There is no method of distinguishing, in the faeces between the amount of minerals not absorbed and that re-excreted by the intestines after absorption.

In this study the proportion of Ca and P retained has been accepted as the measure of the availability of the product in which the calcium and phosphorus were present. Such a procedure is justified on the basis that the Ca and P contained in the supplements fed were well below the requirement of the animals. In other words, conditions were created for the retention of as large a proportion as possible of the minerals fed, which obviously could serve as an indication or even measure of the availability of the Ca and P present in the products given.

When the animals were fed the basal ration, they showed negative mineral balances. (The phosphate balances became slightly positive when the mineral content of the basal ration increased.)

The daily basal ration contained 2·38 gm. P and was increased to 5·88 during the third and fourth week by the addition of 3·5 gm. P_2O_5 as di-sodium phosphate. The negative P balance became definitely positive, 2·66 gm. P being retained. If the assumption is made that no more P of the basal ration was retained during the period of feeding Na_2HPO_4 than when only the basal ration was given it is at once evident that an astonishingly large proportion of the P of the supplement was retained, viz., $\frac{2 \cdot 66 + \cdot 93^{(1)}}{3 \cdot 5} \times 100 = 108 \cdot 6$ per cent. in the case of animal No. 5151 and $\frac{2 \cdot 3 + 1}{3 \cdot 5} \times 100 = 94 \cdot 6$ per cent in cases of No. 5164. It would seem, therefore, that during the period of feeding Na_2HPO_4 either (1) some of the P of the basal ration was retained and that a balance on the basal ration, during the period immediately before the supplement was given, is not always a correct indication of the availability of P in the basal ration during a subsequent period of P feeding or (2) that all the P of the water soluble supplement fed under the conditions of the experiment was retained, in which case the P of the supplement should be regarded as 100 per cent. available.

This argument applies in a general way during the periods when the first two supplements were being fed. After that period the P balances on the basal ration were decidedly less negative or even slightly positive suggesting some utilization of the P of the basal ration or at least that the P present in the basal ration was sufficient to keep the animals in equilibrium with regard to P.

It must be noted that the increase in the amount of basal ration fed, resulted in an increase of P ingested from 2·41 to 3·74 gm. This quantity was apparently sufficient to keep the animals in equilibrium with regard to P as reference to the tables indicates.

Incidentally it may be mentioned that the total retention of P during the periods of supplying the phosphate supplement, shows little difference in spite of a smaller quantity of P being supplied in the supplement, a fact which again suggests 100 per cent. utilization of the supplementary P or increased utilization of the P present in the basal ration compared with that during the period of no supplementary feeding.

In regard to the retention of Ca as presented in Tables 2 and 3 it is surprising that the negative calcium balances were so low when the basal ration was the only source of Ca. Furthermore, relatively large quantities of Ca were retained during the period of supplementary feeding. So much so that it appears practically certain that

[1] $\frac{\left(\text{Average daily balance for 2 weeks of supplementary feeding}\right) - \left(\text{Average for week(s) prior to and week after supplementary feeding}\right)}{\text{Supplement}}$

some Ca of the basal ration was utilized while the supplement was given, unless one assumes 100 per cent. utilization of the supplementary Ca. For instance in case of animal No. 5164 during the 10th and 11th trial the percentage retention of supplementary Ca, if only this fraction could be retained was $\frac{5 \cdot 45 + 1 \cdot 78}{7 \cdot 42} \times 100 = 97 \cdot 5$. This is an unlikely result and suggests that the Ca balance during the feeding of the basal ration only, did not necessarily apply to the period of supplementary feeding. Nevertheless, in order to arrive at a basis of comparing the percentage retentions on the availabilities of P and Ca in the different supplements, it is assumed that the P and Ca balances during the periods when only the basal ration is fed do apply during the period immediately following that when the supplement is fed.

For six weeks prior to the commencement of the experiment the animals received a ration very low in calcium and phosphorus. Weeks 1 and 2 reported in the balance tables, were carried out during the 7th and 8th weeks respectively during which the mineral deficient ration was fed; a depletion of minerals from the skeleton was shown.

Young growing bovines approximately 16 months old whose skeletons required considerable amounts of minerals to grow to maturity were used. The total mineral intake did not exceed the amount considered to be the optimal requirement for this type of animal but was well below it. The supplement was only fed for 3 out of 5 weeks, the animals being on a very low mineral intake for the remaining two weeks; thus reducing the average intake considerably. Furthermore the fact that such high retention figures were actually recorded, show that the positive balances probably do not represent the maximum the animal was capable of storing at the time.

TABLE IV.

Supplementary Phosphorus Retained.

	*	A.	B.	C.	D.	E.	*Mean.*
5151	1st week............	1·089	0·840	0·878	1·014	0·892	0·963
	2nd week..........	*1·083*	*0·931*	*0·839*	*0·941*	*0·781*	*0·915*
	Mean..............	1·086	0·886	0·859	0·978	0·837	0·929
5164	1st week............	0·971	0·829	0·867	0·712	0·946	0·865
	2nd week..........	*0·920*	*0·797*	*0·774*	*0·955*	*0·758*	*0·841*
	Mean..............	0·946	0·813	0·821	0·834	0·852	0·853
Mean....	1st week............	1·030	0·835	0·873	0·863	0·919	0·904
	2nd week..........	*1·002*	*0·864*	*0·807*	*0·948*	*0·770*	*0·878*
	Mean..............	1·016	0·849	0·840	0·906	0·844	0·891

* The 1st and 2nd week denotes the 2nd and 3rd week respectively of supplementary feeding, i.e. the two weeks during which a mineral balance was determined on the supplemented ration.

The basal ration of the two animals was supplemented successively with five different compounds of phosphorus. As previously stated, each supplement was given continuously for three weeks, during two of which the determinations were made. Between the different periods of supplementary feeding periods of no-supplement existed.

It is possible, from the data collected, to determine the difference in the retention of the phosphorus between—

(*a*) the different supplements;

(*b*) the two animals;

(*c*) the two weeks (i.e. after the animals had received the supplement for one week or for two weeks respectively).

It was found from an analysis of the animal variance that there was only an indication of a slight variation in the respective retentions of phosphorus by the two animals, an indication which is not borne out by a difference in the retention of Ca between the two animals. The conclusion is not justified therefore, that any real difference existed between the two animals with regard to their respective retentions of Ca and P.

In order to study the relative retentions of phosphorus supplied by the different supplements, the following table must be referred to:

TABLE IV (*a*).

Mean Proportion of Phosphorus retained.

A. *Di-Sod. phosphate.*	B. *V. Bonemeal.*	C. *H. Bonemeal.*	D. *Mono-Am. Phosphate.*	E. *Di-Cal. phosphate.*
1·016	0·849	0·840	0·900	0·844
		Mean percentage of phosphorus retained.		
101·6	84·9	84·0	90·6	84·4

When the differences between the two means were compared by the t-tests (Fisher, 1935), it was found that A appeared to be greater than B, C and E, while all the other differences were insignificant. The relative decreasing order was :—

A, D, B, E and C.

The means for the two animals agreed very closely, being 0·878 for bovine 5164.

From Table IV (*a*) it will be seen that the phosphorus retention figures are exceedingly high, ranging from 100 per cent. for bonemeal and dicalcium phosphate. These high retention figures indicate that the optimal phosphorus requirement of the animals was not exceeded when these supplements were fed. The retention figures could therefore be used as a measure of the availability of the phosphorus in these supplements. The phosphorus of sodium phosphate may be considered as totally available whereas that of mono-ammonium phosphate, the two bonemeals and di-calcium phosphate is from 90-84 per cent.

It is remarkable that such high proportions of the phosphorus in the phosphatic supplements fed, were retained by the animals, as a matter of fact the differences in P retention among the supplements are insignificant compared with the high values obtained for the retentions in the case of each supplement. This observation is of great practical significance because it suggests that utilized P supplements when given below optimal quantities are significantly better than is generally accepted. In the experiment under discussion, it would have been interesting to compare the values obtained with those at higher levels of intake of P.

The retentions of Ca are compared in table V below.

TABLE V.

Supplementary Calcium retained.

		A.	B.	C.	D.	E.	*Mean.*
B. 5151..	1st week...........	0·879	0·691	0·822	1·066	0·927	0·877
	2nd week..........	*0·749*	*0·684*	*0·898*	*0·984*	*0·941*	*0·851*
	Mean..............	0·814	0·688	0·860	1·025	0·934	0·864
B. 5164..	1st week...........	0·890	0·699	1·066	0·982	0·914	0·910
	2nd week..........	*0·747*	*0·620*	*0·929*	*1·047*	*0·947*	*0·858*
	Mean..............	0·819	0·660	0·998	1·015	0·931	0·884
Mean.....	1st week...........	0·885	0·695	0·944	1·024	0·921	0·894
	2nd week..........	*0·748*	*0·652*	*0·914*	*1·016*	*0·944*	*0·855*
	Mean..............	0·816	0·674	0·939	1·020	0·932	0·875

TABLE V (*a*).

Mean Proportion of Calcium retained.

A.	B.	C.	D.	E.
Di-Sod. phosphate.	*B. Bonemeal.*	*H. Bonemeal.*	*Mono-Ammon. Phosphate.*	*Di-Calcium phosphate.*
0·816	—0·674	0·939	1·020	0·932
		Mean percentage of Calcium retained.		
81·6	67·4	93·9	102·0	93·2

When the separate means for the different forms of supplement are compared (by the t-tests referred to above) the retention of calcium for the mono-ammonium phosphate, di-calcium phosphate and H. Bonemeal periods was significantly greater than for that of the di-sodium phosphate and V. Bonemeal periods.

The calcium retention when the different phosphate supplements (plus calcium carbonate, where necessary) were fed, varied from 100 per cent. for mono-ammonium phosphate to 67·4 per cent. for V. Bonemeal.

There is no correlation between the amount of phosphorus and the amount of calcium retained from period to period but the proportion of Ca retained was remarkably high in all the animals.

Experiment 1 (b).

In view of the results of the preceeding experiment it was decided to conduct similar investigations with two other animals in a similar manner. One from each of the three main types of phosphates, namely (A) di-sodium phosphate, (B) di-calcium phosphate and (C) bonemeal was selected for the purpose of the test.

Animals.

Two Young Half-grade Herefords.

No.	*Initial Age.*	*Initial Weight.*
5429	12 Months..................................	510 lb.
5436	12 Months..................................	475 lb.

Ration.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp......................	1·50 Kg.	·037	·56	·015	·23
Hay......................	·25 Kg.	·039	·10	·240	·60
Bloodmeal..................	·10 Kg.	·109	·11	·136	·14
Greenfeed..................	·50 Kg.	·047	·24	·128	·64
Salt Mixture A..............	25 gm.	—	—	—	—
Water......................	10 litres	—	—	—	·53
TOTAL INTAKE....			1·01		2·14

The quantities given above were fed at the commencement of the experiment but were increased from time to time until finally the animals received the following ration.

3·0 Kg. Samp.

·5 Kg. Hay.

·5 Kg. Green feed.

·2 Kg. Blood meal.

25 gm. Salt mixture A.*

The final ration contained about 2·0 gm. P and about 2·8 gm. Ca.

The animals were kept for five weeks on the basal ration before faeces and urine were collected to determine the mineral balance on the basal ration.

The experimental animals were kept under the same conditions as those already discussed while the same general routine and technique applied. Each supplement was fed on three occasions in the course of the experiment.

The result of the mineral balances are given in Tables VI and VII.

* NOTE.—Salt mixture A: $NaHCO_3$ — 100 gm., $NaCl$ — 60 gm., K_2SO_4 — 60 gm., $FeSO_4$ — 2·5 gm., $Mg(OH)_2$ — 2·75 gm.

Table VI.

Mineral Balance (daily average).

Bovine 5429.

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 4·58 gm. P + 11·27 gm. Ca.
B = Di-calcium phos. + $CaCO_3$ i.e. 4·37 gm. P + 11·27 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 4·59 gm. P + 11·28 gm. Ca.

Trial I—

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
1	O	1·01	2·17	1·49	0·03	2·35	1·27	−0·51	−1·45
2	O	1·01	2·14	1·29	0·04	2·73	1·47	−0·32	−2·06
3	A	5·59	13·40	1·75	0·06	6·62	0·68	3·78	6·09
4	A	5·59	13·38	1·32	0·07	10·22	0·68	4·20	2·48
5	O	1·01	2·21	1·64	0·05	3·34	1·18	−0·68	−2·31
6	O	1·29	2·66	1·49	0·05	3·59	1·52	−0·25	−2·45
7	B	5·66	13·86	1·69	0·04	9·53	0·65	3·93	3·68
8	B	5·66	13·87	2·05	0·04	9·34	0·54	3·57	3·99
9	O	1·29	2·66	2·39	0·08	2·68	1·44	−1·18	−1·46
10	C	5·88	13·94	2·54	0·08	8·50	0·55	3·26	4·89
11	C	5·88	13·95	3·40	0·05	8·99	0·66	2·43	4·30
12	O	1·29	2·64	2·33	0·05	2·73	1·27	−1·09	−1·36

Trial II—

13	O	1·71	2·64	2·17	0·03	2·79	1·80	−0·49	−1·95
14	A	6·27	13·96	2·23	0·09	5·11	0·71	3·95	8·14
15	A	6·27	13·90	2·44	0·08	5·55	0·77	3·74	7·58
16	O	1·71	2·66	2·01	0·05	2·03	2·24	−0·35	−1·61
17	B	5·94	13·45	1·89	0·03	5·38	0·64	4·02	7·43
18	B	5·94	13·43	2·13	0·05	7·66	0·57	3·76	5·20
19	O	1·71	2·67	2·13	0·04	2·48	1·47	−0·46	−1·28
20	C	6·24	13·44	1·89	0·05	5·28	0·73	4·30	7·43
21	C	6·24	13·43	2·23	0·04	7·69	0·63	3·97	5·11
22	O	1·71	2·66	2·36	0·05	1·80	1·38	−0·68	−0·52

Trial III—

23	O	1·71	2·63	2·55	0·04	1·91	1·44	−0·88	−0·72
24	A	6·26	13·37	2·15	0·03	4·28	0·34	4·08	8·75
25	A	6·26	13·36	2·26	0·04	6·30	0·64	3·96	6·42
26	O	1·65	2·66	2·59	0·04	4·28	1·22	−0·98	−2·84
27	B	5·86	13·47	2·98	0·04	7·66	0·69	2·84	5·12
28	B	5·86	13·49	3·06	0·04	6·55	0·74	2·76	6·20
29	O	1·65	2·64	2·37	0·07	3·14	1·16	−0·79	−1·66
30	C	6·13	13·44	2·42	0·08	6·15	0·93	3·63	6·36
31	C	6·13	13·40	2·79	0·05	7·28	0·66	3·29	5·46
32	O	1·65	2·63	2·56	0·04	3·09	1·97	−0·95	−2·43

Table VII.

Mineral Balance (daily average).

Bovine 5436.

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 4·58 gm. P + 11·27 gm. Ca.
B = Di-calcium phos. + $CaCO_3$ i.e. 4·37 gm. P + 11·27 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 4·5 gm. P + 11·28 gm. Ca.

Trial I—

Week No.	Supplement.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
1	O	1·01	2·14	1·42	0·03	2·11	1·31	−0·44	−0·28
2	O	1·01	2·15	1·53	0·05	2·26	1·11	−0·57	−1·22
3	A	5·59	13·46	1·26	0·07	7·50	0·50	4·26	5·46
4	A	5·59	13·45	1·39	0·07	8·36	0·89	4·13	4·30
5	O	1·01	2·19	1·64	0·03	2·48	1·78	−0·66	−2·07
6	O	1·29	2·64	2·12	0·05	3·03	1·52	−0·88	−1·91
7	B	5·66	13·86	2·37	0·05	8·18	0·71	3·24	4·97
8	B	5·66	13·85	2·48	0·04	9·96	0·69	3·14	3·20
9	O	1·29	2·66	2·35	0·04	3·82	1·73	−1·10	−2·89
10	C	5·88	13·93	2·51	0·03	7·06	0·60	3·34	6·29
11	C	5·88	13·95	2·62	0·04	8·92	0·84	3·22	4·19
12	O	1·29	2·66	2·32	0·05	3·01	1·56	−1·08	−1·91

Trial II—

13	O	1·71	2·64	1·97	0·04	3·08	1·50	−0·30	1·94
14	A	6·26	13·88	2·12	0·04	4·93	0·57	4·10	8·38
15	A	6·26	13·93	2·44	0·04	4·74	0·44	3·78	8·75
16	O	1·71	2·65	2·02	0·03	2·29	1·71	−0·34	−1·35
17	B	5·93	13·47	1·91	0·03	7·06	0·50	3·99	5·81
18	B	5·93	13·44	2·05	0·04	6·56	0·30	3·84	6·58
19	O	1·71	2·68	1·97	0·03	2·61	0·90	−0·29	−0·83
20	C	6·24	13·44	1·87	0·04	2·56	0·37	4·33	6·51
21	C	6·24	13·41	1·91	0·05	6·08	0·30	4·28	7·03
22	O	1·71	2·64	1·35	0·03	1·98	1·54	0·33	−0·88

Trial III—

23	O	1·71	2·65	1·73	0·03	2·07	1·54	−0·05	−0·96
24	A	6·26	13·39	1·90	0·04	7·38	0·47	4·32	5·54
25	A	6·26	13·38	1·89	0·03	7·14	0·45	4·34	5·79
26	O	1·65	2·65	2·65	0·04	4·54	0·89	−1·04	−2·78
27	B	5·86	13·44	2·57	0·04	7·66	0·71	3·25	5·07
28	B	5·96	13·45	2·50	0·04	6·66	0·50	3·32	6·29
29	O	1·65	2·63	2·32	0·03	3·18	0·74	−0·70	−1·29
30	C	6·13	13·44	2·25	0·04	8·78	0·34	2·84	4·32
31	C	6·13	13·42	2·03	0·03	6·22	0·42	4·07	6·80
32	O	1·65	2·66	2·19	0·03	4·36	0·49	−0·57	−2·19

TABLE VIII.

(a) Phosphorus.

Supplementary Phosphorus retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial I.........	1st week.........	0·924	1·066	1·000	1·064	0·963	1·014	0·863	0·965	0·914	0·954	0·998	0·976
	2nd week........	*1·026*	*1·037*	*1·032*	*0·982*	*0·941*	*0·962*	*0·669*	*0·939*	*0·804*	*0·892*	*0·972*	*0·932*
	Mean............	0·980	1·052	1·016	1·023	0·952	0·988	0·766	0·952	0·859	0·923	0·985	0·954
Trial II........	1st week.........	0·960	0·971	0·966	1·050	1·019	1·035	1·070	1·024	1·047	1·023	1·005	1·016
	2nd week........	*0·916*	*0·901*	*0·909*	*0·988*	*0·983*	*0·986*	*0·998*	*1·013*	*1·006*	*0·997*	*0·966*	*0·967*
	Mean............	0·938	0·936	0·938	1·019	1·001	1·010	1·034	1·019	1·026	1·010	0·984	0·991
Trial III.......	1st week.........	1·106	1·070	1·088	0·818	0·979	0·899	1·004	0·777	0·891	0·976	0·942	0·959
	2nd week........	*1·079*	*1·075*	*1·077*	*0·800*	*0·995*	*0·898*	*0·929*	*1·051·*	*0·990*	*0·936*	*1·040*	*0·988*
	Mean............	1·093	1·073	1·083	0·809	0·987	0·899	0·967	0·914	0·940	0·956	0·991	0·974
Mean...........	1st week.........	1·000	1·036	1·018	0·977	0·987	0·982	0·979	0·922	0·951	0·985	0·982	0·984
	2nd week........	*1·007*	*1·004*	*1·006*	*0·923·*	*0·973*	*0·948·*	*0·865*	*1·001*	*0·933*	*0·932*	*0·963*	*0·963*
	Mean............	1·004	1·020	1·012	0·950	0·980	0·965	0·922	0·962	0·942	0·959	0·988	0·974

TABLE IX.

Supplementary Calcium retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial I..........	1st week..........	0·735	0·631	0·683	0·504	0·654	0·579	0·559	0·769	0·664	0·599	0·685	0·642
	2nd week..........	*0·414*	*0·519*	*0·467*	*0·531*	*0·497*	*0·514*	*0·506*	*0·584*	*0·545*	*0·484*	*0·533*	*0·509*
	Mean..............	0·575	0·575	0·575	0·518	0·576	0·547	0·533	0·676	0·605	0·542	0·609	0·575
Trial II..........	1st week..........	0·880	0·890	0·885	0·821	0·638	0·730	0·771	0·682	0·730	0·824	0·737	0·782
	2nd week..........	*0·831*	*0·834*	*0·833*	*0·615*	*0·709*	*0·662*	*0·556*	*0·731*	*0·644*	*0·667*	*0·758*	*0·713*
	Mean..............	0·856	0·862	0·859	0·718	0·674	0·696	0·663	0·707	0·721	0·740	0·747	0·748
Trial III..........	1st week..........	0·978	0·686	0·832	0·681	0·657	0·669	0·778	0·561	0·670	0·812	0·635	0·724
	2nd week..........	*0·762*	*0·709*	*0·736*	*0·781*	*0·770*	*0·776*	*0·694*	*0·791*	*0·743*	*0·746*	*0·757*	*0·751*
	Mean..............	0·870	0·698	0·784	0·731	0·714	0·723	0·736	0·676	0·706	0·779	0·696	0·738
Mean..............	1st week..........	0·864	0·736	0·800	0·669	0·650	0·650	0·703	0·671	0·687	0·745	0·686	0·716
	2nd week..........	*0·669*	*0·687*	*0·678*	*0·842*	*0·659*	*0·651*	*0·585*	*0·702*	*0·643*	*0·632*	*0·683*	*0·658*
	Mean..............	0·767	0·712	0·739	0·656	0·654	0·655	0·644	0·686	0·664	0·689	0·684	0·687

Discussion.

The conduction of this experiment was identical with the previous one, except that the treatments and observations were repeated in three consecutive trials, making the data collected considerably more reliable and conclusive.

A statistical analysis of the results indicates that the retention of the phosphorus of di-sodium phosphate appears to be greater than that of V. Bonemeal while the retention of the phosphorus present in di-calcium phosphate is not significantly different from that of either di-sodium phosphate or that of V. Bonemeal.

TABLE VIII (*a*).

Mean Proportion of Phosphorus retained.

A.	B.	C.
Di-sodium phosphate.	*Di-calc. phosphate.*	*V. Bonemeal.*
1·012	0·965	0·942
	Percentage retention.	
100	96·5	94·2

TABLE IX (*a*).

Mean Proportion of Calcium retained.

A.	B.	C.
Di-sodium phosphate.	*Di-calc. phosphate.*	*V. Bonemeal.*
0·739	0·655	0·665
	Percentage retention.	
73·9	65·5	66·5

When the means for both animals and the three trials are taken the retention in the three supplements showed small variations as will be seen from the above table.

Remarkably high phosphorus retention figures were obtained for all three supplements.

As in the previous experiment the phosphorus of di-sodium phosphate was apparently completely retained.

Periods of maximum retention of phosphorus were not necessarily periods of maximum retention of calcium.

Both animals were consistently on a negative phosphorus and calcium balance during the periods when only the basal ration was given. Apparently therefore the available P and Ca of the basal ration was definitely below the maintenance requirements of the animals, while there is always the probability that what little P there was in the basal ration viz. 1·01 gm. was not necessarily in an easily available form; this view is strengthened by the high proportion of P voided in the faeces.

The consistent loss of Ca and P from the body during the periods when no supplement was fed i.e. when active resorption of skeleton took place, reveals that favourable conditions existed for maximum absorption of Ca and P during the periods when supplement was supplied, as the animal, starved for Ca and P, is obviously in a better position to retain these minerals than when the body is not in dire need of them.

The practical significance of this observation is important for it suggests that the greater the need of the animal for minerals the more economical use is made of the supply. In other words completely satisfying the mineral need of animals need not necessarily be, and probably is not, the most profitable way of feeding minerals under practical conditions. Furthermore, this observation throws light on the observation frequently made viz. that the stockman who feeds approximately half the phosphate required by his stock kept on P deficient pasture obtains well over that proportion of profit obtained by satisfying the total requirements. If a daily supplement of 3 oz. bonemeal per head produces a gain of 150 lb. in weight over 12 months then 1·5 oz. will produce well over 75 lb. (du Toit and Green, 1930).

The extraordinary high retention of P contained in the supplements given, was highly favoured by the low level of P intake and it would appear that whatever the digestive system converted into an absorbable form was absorbed and utilized; in the case of the water soluble sodium phosphate all was apparently absorbed and retained and even of the comparatively insoluble di-calcium phosphate and bonemeal very little was not digested and utilized by the animal. There was a tendency for the faecal P to increase during the periods of feeding bonemeal and di-calcium phosphate, again suggesting that the slight difference in retention between these two supplements and di-sodium phosphate was merely incomplete conversion of the former two substances into soluble and absorbable entities. It would be interesting to determine whether the difference in retention remains almost neglible at higher levels of supplementary intake.

Experiment I (c).

It was clear from Experiment I (*b*) that there was no difference in the efficiency of the calcium and phosphorus retention of the two animals when they were kept on the same level of mineral intake. Therefore in order to determine the retention of the phosphorus at different levels, the supplement for one animal was doubled.

A further trial, using the same animals as in Experiment I (*b*) was conducted in exactly the same way, as in the former three trials, to determine whether the levels of intake of the supplements affected the retention of P and Ca significantly. It is realized that the value of the results would be considerably reduced by placing only one animal on each of the two levels of intake but as other suitable animals were not available at the time and as these two animals showed no significant difference in utilizing P in the previous experiment, it was hoped that the results might produce indication, possible, of what could be expected.

A period of eight weeks elapsed from the end of the previous experiment and the commencement of this one. The animals were, however, kept under the same routine of feeding. At the end of the previous experiment the animals received for three weeks the same supplement of phosphorus in the form of V. Bonemeal as during the trials. For two weeks no supplement was fed, followed by three weeks of V. Bonemeal supplement. The animals were then fed the basal ration only for two weeks, during the latter of which faeces and urine were collected, this being the first week recorded in this experiment.

Bovine 5429 received the same amount of supplement as in the previous three trials and bovine 5436 was given twice that amount of supplement.

The mineral balances are recorded in Tables XXIV to XXVII.

TABLE X.

Mineral Balance (daily average).

Bovine 5429.

Supplement A = Na_2HPO_4 + $CaCO_3$ ie.. 4·54 gm. P + 11·27 gm. Ca.
B = $CaHPO_4$ + $CaCO_3$ i.e. 4·42 gm. P + 10·72 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 4·47 gm. P + 11·40 gm. Ca.

Trial IV—

Week No.	Supple-ment.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
33	O	2·05	2·80	2·35	·05	2·43	1·26	−0·35	−0·89
34	A	6·60	13·03	2·54	·05	4·78	0·31	4·01	7·94
35	A	6·59	13·17	2·64	·05	7·34	0·32	3·90	5·51
36	O	2·02	2·78	2·63	·04	1·50	2·09	−0·65	−0·81
37	B	6·45	13·46	2·77	·04	6·50	0·37	3·64	6·59
38	B	6·45	13·54	2·73	·04	5·60	0·32	3·68	7·62
39	O	2·04	2·72	2·58	·04	1·25	1·52	−0·58	−0·05
40	C	6·50	14·30	2·69	·04	8·12	0·53	3·77	5·65
41	C	6·52	14·16	3·24	·05	5·02	0·26	3·23	8·88
42	O	2·04	2·78	2·56	·05	1·50	1·12	−0·57	0·16

TABLE XI.

Mineral Balance (daily average).

Bovine 5436.

Supplement A = Na_2HPO_4 + $CaCO_3$ i.e. 9·08 gm. P + 22·54 gm. Ca.
B = $CaHPO_4$ + $CaCO_3$ i.e. 8·84 gm. P + 21·44 gm. Ca.
C = V. Bonemeal + $CaCO_3$ i.e. 8·94 gm. P + 22·80 gm. Ca.

Trial IV—

Week No.	Supple-ment.	Intake.		Outgo gm. P.		Outgo gm. Ca.		Balance.	
		gm. P.	gm. Ca.	Faeces.	Urine.	Faeces.	Urine.	gm. P.	gm. Ca.
33	O	2·05	2·74	2·55	·05	2·41	1·08	−0·55	−0·75
34	A	11·14	25·32	3·80	·05	13·70	·40	7·29	11·22
35	A	11·13	25·34	4·40	·05	14·72	·37	6·64	10·25
36	O	2·02	2·72	2·77	·04	1·97	·79	−0·79	−0·04
37	B	10·85	24·34	4·60	·04	13·92	·37	6·21	10·05
38	B	10·85	24·32	3·90	·05	14·19	·34	6·90	9·79
39	O	2·04	2·76	3·17	·04	1·92	·59	−1·17	−0·25
40	C	10·97	25·58	4·24	·04	14·62	·36	6·69	10·60
41	C	10·97	25·62	4·68	·04	16·15	·34	6·25	9·13
42	O	2·04	2·90	2·72	·05	2·26	·94	−0·73	−0·30

TABLE XII.

Supplementary Phosphorus retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial IV........	1st week.........	0·993	0·877	0·935	0·968	0·813	0·891	0·973	0·855	0·914	0·978	0·848	0·913
	2nd week........	*0·969*	*0·805*	*0·887*	*0·977*	*0·891*	*0·934*	*0·852*	*0·805*	*0·829*	*0·933*	*0·834*	*0·883*
	Mean............	0·981	0·841	0·911	0·973	0·852	0·913	0·913	0·830	0·872	0·956	0·841	0·898

TABLE XIII.

Supplementary Calcium retained.

		A.			B.			C.			Mean.		
		B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.	B. 5429.	B. 5436.	Mean.
Trial IV........	1st week.........	0·780	0·516	0·648	0·655	0·476	0·566	0·486	0·477	0·482	0·640	0·490	0·565
	2nd week........	*0·764*	*0·472*	*0·618*	*0·751*	*0·464*	*0·608*	*0·769*	*0·413*	*0·591*	*0·761*	*0·450*	*0·606*
	Mean............	0·772	0·494	0·633	0·703	0·470	0·587	0·628	0·445	0·536	0·701	0·470	0·585

Discussion.

The mean proportion of P and Ca retained by the two animals are presented in Table XXVIII and a summary of the data in Table XIV. Animal No. 5429 received 4·5 gm. P and 11·15 gm. Ca and No. 5436 8·9 gm. P and 22·3 gm. Ca in their supplement. Di-sodium phosphate, di-calcium phosphate and V. bonemeal were fed durings periods A, B, and C respectively.

TABLE XIV.

Bovine No.	Supplement.		A.		B.		C.	
	P.	Ca.	P.	Ca.	P.	Ca.	P.	Ca.
5429........	4·5	11·1	98·1	71·2	97·3	70·3	91·3	62·8
5436........	8·9	22·3	84·1	94·4	85·2	47·0	83·0	44·5

A glance at Table XIV reveals a remarkably high retention of Ca and P by both animals. Furthermore there was no significant difference between the retention of P or Ca for either animal when the supplement was given as di-sodium phosphate or calcium phosphate or V. bonemeal. It would appear, therefore, that these animals were able at both the levels of intake to " digest ", absorb and retain these two minerals equally well whether they were supplied as the water soluble di-sodium phosphate or the relatively insoluble di-calcium phosphate or V. bonemeal. This result is remarkable and tends to strengthen the inference made in the discussion of the last experiment viz. that the phosphorus contained in di-sodium phosphate, di-calcium phosphate and in V. bonemeal could be regarded for practical purposes as being made equally available to the animal body during digestion.

The retention of both Ca and P was reduced at the high levels of intake; P decreased from approximately 95 per cent. retention to 84 per cent. and Ca from about 70 to 45 per cent. There is the possibility of course that the higher level of mineral intake exceeded the mineral requirement of the animals and that the reduction in retention was due to the elimination of the P and Ca present in excess of the requirement. In that case appreciable differences in the availability of P or Ca in these supplements fed might be completely masked by the excess present, unless, of course, the product was so poorly available that the P or Ca provided during absorption did not satisfy the requirement of the animal. The supplements fed showed only slight differences at the lower level of intake and indicated therefore that the P and Ca they contained were approximately equally available to the animals at that level. Furthermore, the higher level at which both P and Ca were fed is certainly not less than that accepted for the P and Ca requirement for the type of animal used, and one is forced to the conclusion, that the reduction in percentage retention at the higher level of intake is mainly due to the elimination of the Ca and P present in excess of the quantities required by the animals, and not necessarily to the inavailability of these minerals in the products fed. As a matter of fact a true picture of the availability of Ca and P present in different products cannot be obtained when the latter is given at levels that are in excess of the requirements of the experimental animals.

Summarizing the results obtained in the experiments on the availability of P in di-sodium phosphate, di-calcium phosphate and bonemeal one is struck by the remarkably high retention of P at the lower level of intake of P viz. 4·5 gm. Over 90 per cent. P was retained in the animal body, while even when the intake of P was doubled, which probably slightly exceeded the P requirements of the animals more than 80 per cent. of the P ingested was retained. Sodium phosphate appeared to possess a slight advantage over the other phosphates in that in two experiments slightly more of its P was retained than of that given as di-calcium phosphate and as bonemeal; in the third experiment where only one animal was used no difference existed between the quantities of P retained when di-sodium phosphate or di-calcium phosphate was fed.

V. EXPERIMENT II.

THE UTILIZATION OF CALCIUM AND PHOSPHORUS BY BOVINES ON RATIONS CONTAINING DIFFERENT LEVELS OF MINERAL INTAKE WITH A CONSTANT CALCIUM TO PHOSPHORUS RATIO.

Animals:—Ten half-grade Friesland steers of the following ages and weights were used in this experiment.

At commencement of experiment: 24.6.33.

Group.	Bovine No.	Age months.	Weight.
			lb.
I..	4712	17	821
	3478	18	800
II..	3480	18	812
	3465	18	767
III..	3464	18	798
	3467	18	818
IV..	3454	18	756
	3456	18	836
	Introduced into Expt. 9/7/34.		
V..	5430	12	450
	5431	12	400

The animals were divided into five groups of two animals each. Group V was introduced into the experiment 12½ months after the commencement.

Ration of Groups I-IV.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp........................	3·5 Kg.	·039	1·37	·071	·25
Hay........................	1·2 Kg.	·105	1·26	·297	3·56
Meatmeal....................	·24 Kg.	·808	1·94	·820	1·97
Water........................	± 10 litres	—	—	—	·50
Sodium chloride..............	15 gm.	—	—	—	—
Total mineral intake.........	—	4·57 gm. P	and 6·38	gm. Ca.	

Three months after the commencement of the experiment ·5 Kg. green feed was added to the ration. Towards the end of the experiment, i.e. from the 22nd month onwards the basal ration was increased to 4·5 Kg. samp, 2 Kg. hay and meatmeal was substituted for blood meal. The total mineral intake was not affected by these changes.

The four groups of animals were given the basal ration for six weeks, the mineral balance being determined on the ration during the last two weeks. The supplements were then given continuously until the end of the experiment.

The following Table shows the amount of calcium and phosphorus which was added as di-calcium phosphate and calcium carbonate to the basal ration of each group.

TABLE XV.

Calcium and phosphorus added to basal ration.

Group.	Basal Ration.		Added.		Total Intake.		Total Intake.
	Ca gm.	P gm.	Ca gm.	P gm.	Ca gm.	P gm.	Ca : P ratio.
I................	6·32	4·51	9·14	3·49	15·46	8·00	1·93 : 1
II................	6·32	4·51	5·21	1·28	11·53	5·85	1·97 : 1
III................	6·32	4·56	18·10	1·28	24·42	5·85	4·18 : 1
IV................	6·32	4·54	2·38	—	8·70	4·54	1·92 : 1
V................	2·14	1·01	—	—	2·14	1·01	2·12 : 1

From the above Table it will be seen that the Ca : P ratio of groups I, II, IV and V was about 2·0 : 1, whereas for group III, the ratio was 4 :1, the level of phosphorus intake being the same as that of group II.

At a later date group V was included in the experiment in order to determine to what extent bovines could utilize the calcium and phosphorus of a ration containing a lower mineral content than that supplied to group IV.

Ration of Group V.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp........................	1·50 Kg.	·037	·56	·015	·23
Hay.........................	·25 Kg.	·039	·10	·240	·60
Blood meal..................	·10 Kg.	·109	·11	·136	·14
Green feed..................	·50 Kg.	·047	·24	·128	·64
Water.......................	± 10 litres	—	—	—	—
Salt mixture A..............	15 gm.	—	—	—	—
Total mineral intake.........	—	—	1·01 gm.	P and 2·14	gm. Ca.

The ration of group V was the same as that given to bovines 5429 and 5436 in Experiment I (*b*). As in the latter case, the samp, hay and blood meal was finally doubled. It thus became:—

3·0 Kg. Samp.

0·5 Kg. Hay.

0·2 Kg. Blood meal.

0·5 Kg. Green feed.

15 gm. Salt mixture A.

The Ca : P ratio under the above conditions showed a slight alteration, but it was decided not to add minerals to the ration in order to bring the ratio nearer to 2·0 : 1.

In both animals of each group the mineral balances were determined (commencing with group V at the 15th month of the experiment) for one week monthly, the calcium and inorganic phosphorus content of the blood being determined at monthly intervals. In the case of group V the plasma phosphatase was also determined at the above intervals. The body weights and detailed results of the balance trials are given in the appendix in Tables XXIV to XXXIV which have been summarized in Table XIX. The P and Ca of the blood and bone analyses are given in Tables XVI, XVII and XX respectively.

TABLE XVI.

Inorganic Blood Phosphorus in mg. per 100 c.c. blood.

Month of Expt.	Bovines.									
	4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
4	7·6	6·9	7·0	7·2	7·2	6·9	6·6	6·0	—	—
5	7·3	7·1	7·2	7·0	6·5	6·4	6·5	6·2	—	—
6	6·4	7·4	6·4	6·9	7·0	6·2	6·0	5·2	—	—
7	7·2	8·0	6·6	5·8	6·4	5·8	5·2	4·3	—	—
8	6·8	6·8	5·8	5·4	5·9	5·6	4·6	3·9	—	—
9	5·8	7·2	5·2	5·0	4·8	5·0	4·3	3·3	—	—
10	6·2	6·6	5·6	5·2	5·0	4·2	—	3·6	—	—
11	5·8	7·2	4·6	4·9	3·6	3·5	—	3·2	—	—
12	5·4	7·6	4·8	—	3·9	3·7	—	3·0	—	—
13	5·2	7·7	4·4	—	3·4	3·4	—	2·7	—	—
14	5·3	—	3·0	—	2·8	2·6	—	2·5	—	—
15	6·1	—	3·5	—	3·2	2·9	—	2·9	7·0	4·4
16	6·2	—	3·7	—	3·3	3·4	—	2·6	6·4	5·0
17	6·2	—	3·9	—	3·4	3·1	—	2·7	5·5	4·0
18	6·2	—	4·7	—	3·5	2·7	—	2·8	4·4	3·2
19	6·1	—	4·6	—	4·0	—	—	2·9	4·8	3·6
20	5·7	—	4·8	—	3·6	—	—	2·8	4·7	3·5
21	6·0	—	5·1	—	3·4	—	—	2·8	3·5	2·3
22	5·7	—	4·7	—	3·6	—	—	2·9	3·4	3·0
23	5·6	—	4·3	—	3·4	—	—	2·7	3·2	2·6
24	5·3	—	4·0	—	3·5	—	—	2·9	3·3	2·6
25	5·2	—	3·9	—	3·4	—	—	2·8	3·1	1·8
26	5·0	—	3·5	—	3·3	—	—	2·9	2·9	1·6

TABLE XVII.

Blood Calcium in mg. per 100 c.c. blood.

Month of Expt.	Bovines.									
	4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
4	10·2	10·6	10·0	9·9	10·2	9·8	10·4	1·0	—	—
5	10·0	10·2	9·8	10·0	9·8	10·2	9·6	8·9	—	—
6	9·6	10·0	8·9	10·1	10·4	10·0	8·9	9·2	—	—
7	10·4	10·6	10·6	11·2	11·2	9·3	10·0	10·8	—	—
8	10·2	9·9	10·2	10·6	10·8	9·4	9·4	10·4	—	—
9	9·8	10·4	10·0	10·4	10·4	9·5	10·2	10·0	—	—
10	10·0	10·0	10·1	10·7	10·3	9·8	—	9·6	—	—
11	9·4	10·6	9·8	10·5	10·7	9·7	—	9·2	—	—
12	10·6	9·8	10·0	—	10·1	10·0	—	9·0	—	—
13	10·4	10·0	9·6	—	10·0	9·6	—	8·0	—	—
14	9·4	—	9·5	—	10·6	9·5	—	10·4	—	—
15	9·8	—	10·0	—	10·7	10·3	—	11·2	10·8	10·5
16	8·9	—	10·2	—	10·4	11·2	—	10·6	10·4	10·7
17	10·6	—	10·1	—	8·9	10·8	—	10·4	10·6	10·2
18	10·2	—	10·4	—	9·6	11·0	—	8·4	10·4	10·5
19	10·0	—	10·6	—	10·4	—	—	9·4	10·2	10·0
20	10·0	—	10·3	—	8·6	—	—	10·6	10·2	10·1
21	10·2	—	9·7	—	10·4	—	—	8·6	10·9	10·6
22	9·8	—	10·6	—	10·2	—	—	10·4	8·9	9·6
23	9·6	—	9·5	—	10·9	—	—	10·2	10·0	11·0
24	10·1	—	10·0	—	10·0	—	—	10·0	8·0	8·9
25	9·8	—	10·2	—	9·9	—	—	8·4	8·6	8·9
26	8·9	—	9·1	—	9·6	—	—	9·0	9·1	8·4

TABLE XVIII.

Plasma Phosphatase in Bodansky Units.

Bovine.	Month of Experiment.				
	22.	23.	24.	25.	26.
5430......................	5·2	4·8	6·9	6·8	8·0
5431......................	6·8	8·2	8·0	10·9	11·3

TABLE XIX.

Mean Daily Mineral Intake and Retention in gm.

(Summary of Tables XXIV to XXXII.)

Group.	Animal.	Phosphorus.		Time on experimental ration.	Calcium.	
		Intake.	Retention.		Intake.	Retention.
I...	4712	8·34	4·42	3rd–25th month expt.	16·36	6·36
	3478	8·59	4·57	3rd–13th month expt.	16·35	7·19
II...	3480	5·83	2·47	3rd–25th month expt.	12·09	3·67
	3465	6·42	2·14	3rd–12th month expt.	12·62	4·13
III...	3464	5·48	2·03	3rd–25th month expt.	24·09	5·09
	3467	5·79	2·18	3rd–18th month expt.	24·42	4·23
IV...	3454	4·80	1·20	3rd–10th month expt.	8·96	2·49
	3456	3·64	1·07	3rd–25th month expt.	7·94	1·78
V...	5430	1·68	−0·11	15th–26th month expt.	2·70	−0·54
	5431	1·68	−0·05	15th–26th month expt.	2·70	−0·35

Discussion.

From weeks Nos. 1 and 2, in the mineral balance tables it will be seen, that the animals of groups I to IV while receiving the basal ration (without supplements) reacted uniformly with regard to the retention of calcium and phosphorus. They were on a negative calcium balance, while the phosphorus balance varied from slightly negative to slightly positive.

TABLE XX.

Bone Analysis.

Bone.	Group.	Bovine.	Green Weight.	Green Bone.				Breaking Strength per sq. in. 6 in. span.	Dry fat free bone.			Percentage Ash of green weight.
				Percentage Water.	Percentage Fat.	Percentage Dry fat-free.	Sp. Gravity.		Percentage Ash.	Percentage Ca.	Percentage P.	
Femur...........	I	4712	2,470	13·1	35·2	51·7	1·240	2,900	65·5	38·7	18·2	33·8
	II	3480	2,555	17·0	38·4	44·6	1·245	1,800	61·7	38·9	17·7	27·5
	III	3464	2,374	16·4	40·8	42·8	1·174	1,550	61·5	39·5	17·8	26·3
	VI	3456	2,385	21·4	41·3	37·3	1·150	3,120	57·0	39·7	17·2	21·3
	V	5431	1,205	14·7	44·6	40·7	1·205	1,600	58·9	38·6	17·1	24·1
Humerus........	I	4712	2,075	13·1	38·6	48·3	1·200	1,500	64·9	39·1	18·1	31·3
	II	3480	1,876	17·3	44·2	38·5	1·225	1,350	61·9	39·7	17·4	23·3
	III	3464	1,916	17·5	42·5	40·0	1·230	2,200	61·6	39·6	17·5	24·6
	IV	3456	1,840	23·0	40·5	36·5	1·150	2,010	55·0	39·4	17·0	20·1
	V	5431	867	17·0	43·8	39·2	1·120	1,400	58·7	38·0	17·2	23·0
Metacarpus......	I	4712	598	11·3	22·9	65·8	1·410	3,740	67·1	39·7	18·0	44·1
	II	3480	505	17·6	25·7	56·7	1·345	2,695	65·2	38·6	17·7	37·0
	III	3464	562	18·2	24·3	57·5	1·400	2,850	65·9	38·7	17·7	37·9
	IV	3456	520	20·7	26·5	52·8	1·300	1,680	59·1	39·8	17·9	31·4
	V	5431	282	17·0	28·4	54·6	1·310	1,250	61·9	39·1	17·8	33·8
Sixth Right Rib..	I	4712	347	27·10	19·0	53·90	1·422	—	62·0	—	—	33·3
	II	3480	307	30·35	19·92	49·73	1·296	—	57·3	—	—	28·6
	III	3464	268	27·49	19·28	53·23	1·340	—	58·6	—	—	31·3
	IV	3456	251	35·46	23·11	41·43	1·141	—	56·8	—	—	3·5
	V	5431	138	27·81	26·54	45·65	1·212	—	52·3	—	—	23·9

Group 1, Bovines 4712 *and* 3478.

The average daily mineral intake of this group was 8·47 gm. P and 16·36 gm. Ca.

The monthly weights of the animals of group I (Table XXIV), indicate that the basal ration permitted neither maximum growth nor the putting on of extra fat. The animals ate greedily, readily finishing their ration and licking their troughs. If they had been given food *ad lib.* they would certainly have eaten much more and gained considerably in weight. The food intake was limited by the group showing the lowest consumption.

During the first twelve months of the experiment the average gain in weight was just over 200 lb. At this stage bovine 3478 died of acute haemorrhagic gastro-enteritis. Bovine 4712 gained 250 lb. from the 14th to the 26th month of the experiment.

The Ca : P ratio of the ration may be considered as optimal for growing cattle. The phosphorus contained in the ration was well utilized, the average daily retention being 4·5 gm. P or 53 per cent. of the total intake, while 6·7 gm. Ca, that is, 41·5 per cent. of the calcium was retained.

The inorganic phosphorus and the calcium content of the blood remained within normal limits while the blood calcium values are of no significance in this experiment. The inorganic phosphorus values suggest sufficiency of P in the ration. It must, however, be pointed out that over 40 per cent. of the total phosphorus of the ration was given in inorganic form which was highly available.

From Table XX it is seen that for each of the four bones examined, the green weight and the percentage of dry-fat-free bone were greater, while the percentages of water and fat were less in the bones of group I than in those of any other group. As the specific gravity values of the bone fluctuate no definite conclusions can be drawn from them.

With the type of machine used, it was exceedingly difficult to keep the femurs and humeri in the required position while determining their breaking strength and the values obtained cannot be regarded as presenting a true indication of the breaking strength of these bones. The metacarpus whose shape prevents undue slipping was found to be most suitable for this work.

The metacarpus of the animal of group I registered 3,740 lb. per square inch over a six inch span, this figure being considerably higher than that for the same bone in any of the other groups.

The proportion of ash in the dry-fat-free bone shows its highest values for the bones of group I, the percentage of ash of the metacarpus, rib, humerus and femur being 67·1, 62·0, 64·9 and 65·5 respectively.

The calcium and phosphorus content of the ash, of the different bones of the animals representing the different groups remained practically constant, the Ca : P ratio of the ash oscillating very closely round 2·2 : 1.

The most striking variation was obtained when the percentage was calculated on the green weight of the bones. This value was considerably higher for the bones of group I. Of the metacarpus almost half and of the femur, humerus and rib one-third of the green bone is ash.

Group II, Bovines 3480 *and* 3465.

The average daily mineral intake was 6·13 gm. P and 12·35 gm. Ca.

The animals of this group reacted disappointingly. At times they lost appetite, leaving some of the basal ration. During the 13th month of the experiment Bovine 3465 became suddenly sick and died of renal calculi. During the 6th month of the experiment Bovine 3480 became sick and did not recover completely before the 11th month. At that time there was no steer of the same age available on the station to replace it, and there was no alternative but to continue the experiment with only this animal in group V.

It decreased to its initial weight during the time it was sick. After recovery from the 11th month onwards it consumed its full ration satisfactorily and for the last twelve months of the experiment it gained as much in weight as the animal in group I for the same period.

The average daily retention of calcium and phosphorus for the total experimental period was 3·9 gm. and 2·3 gm. respectively, being 31·8 per cent. of the calcium and 37·5 per cent. of the phosphorus intake.

The inorganic blood phosphorus values leave no doubt that the phosphorus intake and, consequently, the retention under these conditions were below the optimal requirement of the animals. The blood calcium values remained normal, being in the vicinity of 11 mgm. per 100 c.c. blood.

The weights of the green bones of the animals in this group compared favourably with those in group I. The percentages of water and of fat in the bones were definitely higher, while the percentages of dry-fat-free bone and of ash were lower than those of group I. The breaking strength of the metacarpus registered more than a thousand pounds less, and the percentages of ash were about 6 per cent. less, than in the previous group.

Group III, Bovines 3464 *and* 3467.

The average daily mineral intake of this group was 24·26 gm. Ca and 5·64 gm. P, with a Ca : P ratio of 4·18 : 1 which was double that of any other group.

During the fourth month after commencement of the experiment these animals suffered from loss of appetite to such an extent that Bovine 3464 after eight months weighed almost the same as at the beginning. During the second half of the experimental period the appetite improved and the animal gained in weight. Bovine 3467 behaved slightly better, but it unfortunately died of anaplasmosis after 18 months in the experiment.

The average daily retention was 2·1 gm. P and 4·6 gm. Ca, being 37·2 per cent. of the phosphorus and 19·2 per cent. of the calcium of the ration. Both these animals showed about the same retention of phosphorus as the previous group, but a slightly higher retention of calcium.

The inorganic blood phosphorus values of about 3·5 mgm. per 100 c.c. were below normal, indicating that a daily retention of 2·1 gm. P was insufficient for growing bovines.

The fact that the inorganic blood phosphorus values of this group were slightly lower than those of group III may indicate that the increased addition of calcium carbonate to the rations of group III had a detrimental effect upon phosphorus metabolism.

There is hardly any difference in the chemical analysis of the bones of the animals of group II and group III in spite of the more favourable ratio of Ca : P in the former group. The breaking strength of the metacarpus of group III was slightly higher than that of group II.

Group IV, Bovines 3454 *and* 3456.

The daily ration of this group contained 8·5 gm. Ca and 4·2 gm. P.

Bovine 3454 ate very well without showing any signs of loss of appetite. It gained 127 lb. during the first eight months, this being equal to the weight increase of the animals in group I.

The average daily retention of Bovine 3454 was 2·49 gm. Ca and 1·20 gm. P, this being 27·8 per cent. and 25·0 per cent. of the total intake respectively. The Ca : P ratio of the minerals retained was 2·08 : 1. Unfortunately this animal had to be destroyed in the 10th month of the experiment, on account of a fracture of the femur. Its inorganic blood phosphorus at this time showed signs of a phosphorus deficiency in the ration.

Bovine 3456, the surviving animal, went off feed almost at the beginning of the experiment and could not be induced to consume the full ration. Therefore the samp of the ration was reduced to 2·5 Kg. and the hay to 0·7 Kg., giving an average daily intake of 3·64 gm. P and 7·94 gm. Ca. The average daily retention was 1·14 gm. P and 2·14 gm. Ca, or 27 per cent. and 25 per cent. of the total intake respectively. The Ca : P ratio of the minerals retained was 1·88 : 1. Owing to the reduced ration, this animal gained only 50 lb. in weight for the entire experimental period.

The inorganic phosphorus of the blood soon dropped below 3 mgm. per 100 c.c. and remained at this level. The calcium content of the blood was not influenced by the low level of mineral intake but remained normal.

The green weights of the bones were on the average slightly less than those of the other groups. Calcification, however, was very incomplete, more so than in the three previous groups. For each of the four bones examined in this group, the percentage of water and fat was high, while that of the dry-fat-free bone was low, the specific gravity also being low. The metacarpal break registered 1,680 lb.

per square inch, being about 1,000 lb. lower than that of group II, and about 2,000 lb. lower than that of group I. The percentage of ash in the green femur, humerus and rib was just over 20, as against 30 per cent, for that of group I. The percentage ash of the green bones of the last-mentioned group ,was 1½ times that of group IV.

Group V. Bovines 5430 *and* 5431.

Average daily mineral intake: 1·68 gm. P and 2·7 gm. Ca.

These two animals were given a basal ration as low as possible in Ca and P. The total daily P and Ca present was only about 1·6 gm. of each. Hence it is not surprising that those quantities were insufficient for maintenance as reference to Table XXXII will indicate.

These animals were in negative calcium and phosphorus balances, and only after about seven months did their balances gradually become slightly positive. The average mineral balance for the entire period, however, was negative for both animals. The inorganic blood phosphorus showed that these animals were suffering from severe aphosphorosis, the blood phosphatase values in Table XVIII further indicate a disturbed calcification of the bones.

Although the animals consumed the whole ration and appeared to be in good health, they gained only about 200 lb. each, during the 13 months in which they were in the experiment. It is, however, doubtful whether they would have consumed more of this mineral ration, if the quantity had been increased.

At the end of the experiment one animal, viz, bovine 5431, was destroyed, and bones collected for analysis. The green bones weighed considerably less than those of the previous groups, which could not be regarded as suitable controls on account of the remarkable differences in age between group 5 and the others.

Except for the femur the specific gravity of the bones was very low.

The breaking strength of the metacarpus registered only 1,250 lb. per square inch. The percentage of dry fat-free bone, its percentage ash and the percentage ash of the green bone were low.

General.

Before the animals were placed in the experiment, and fed the basal ration, which was deficient in both calcium and phosphorus, they had adequate minerals to supply the tissues with their normal requirements. With the change to the mineral deficient rations, a state of negative calcium and phosphorus balance ensued, the mineral intake sinking below the level of excretion, resulting in a direct loss to the tissues.

The addition of supplements to the basal ration resulted in a moderate retention of the minerals in group I. As the levels of mineral intake were decreased below the optimal requirements (groups II to V) the total retention decreased, those animals at the lowest level of mineral intake (group V) being in negative balances.

Less efficient retention of the minerals was observed in Experiment I (C) at the higher than at the optimal level of mineral intake. In this experiment the efficiency of retention of the mineral decreased as the mineral intake was diminished below the optimum. In both experiments the maximum percentage retention was found at the optimal level of mineral intake.

During the periods when only the basal ration was fed and in the group at the lowest level of phosphorus intake, over 50 per cent. of the total calcium consumed might be excreted in the urine. It was noted in group V (deficient Ca and P) that as the calcium balance eventually became positive the calcium content of the urine decreased. The urine always contained minimal amounts of phosphorus, irrespective of the level of intake.

As the level of mineral intake was lowered from group I to group V the inorganic phosphorus of the blood decreased. The values of the animals of group I alone may be considered as normal, whereas those of bovine 5431 (group V), finally decreasing to the low level of 1·6 mgm. per 100 c.c. blood indicate that this animal was suffering from severe aphosphorosis.

The blood calcium values remained normal irrespective of the level of mineral intake.

The animals of group I, fed adequate minerals, consumed their full rations, eating greedily, but as the level of mineral intake was lowered loss of appetite became significant in groups II to IV.

In group V, fed a reduced ration and in experiment for only 13 months, symptoms of anoxeria were less pronounced.

The results obtained from the bone studies show that the level of mineral intake had a marked effect upon the breaking strength of the metacarpus, the percentage of dry-fat-free bone, its ash content and the percentage of ash in the green bone. It is apparent that the calcium and phosphorus content of the ration may serve as limiting factors in bone calcification, gravities, and the breaking strengths of the femurs and humeri.

Bovine 5431 of group V being younger and weighing less, the green weights and measurements of its bones were, as expected, smaller than those of the other groups.

The findings of the histological examinations of the bones are discussed in a paper by Theiler *et al* (1937).

From a critical study of the results obtained in this experiment it would appear that the animals in group I, receiving daily 8·5 gm. P and 16·4 gm. Ca received an adequate supply of these two minerals. A reduction in P intake to approximately 6 gm. whether the Ca be reduced proportionately or not, caused relatively poorer utilization of P; whereas 4·5 gm. P was retained when 8·4 gm. was given, approximately 2·3 gm. was retained when about 5·8 gm. was supplied. The ratio of Ca to P was apparently without significant effect on the retention of P. Group IV received no supplementary P. Approximately 4 gm. was present in the basal ration, of which about half was retained. A reduction of P in group V to 1·68 gm. brought about a continual loss of P from the body. It is remarkable that growth could have taken place at all under these conditions.

The percentage ash of the bones affords an excellent index of the degree of calcification. This figure varied from 44·1 per cent. for the metacarpus of the animal receiving adequate Ca and P to 31·4 per cent. for that in the mineral deficient group. The breaking strength of the metacarpus affords confirmatory evidence for the conclusions based on ash determinations.

Increasing demands for minerals by the animals fed mineral deficient rations may diminish losses by more economical re-utilization of endogenous minerals and hence reduce excretion in the later as compared with the earlier stages of the experiment and so abolish a negative mineral balance.

VI. EXPERIMENT III.

Animals.—For this experiment twelve young Friesland heifers were specially selected for uniformity of age and conformation. They were divided into six groups of two each, as follows:—

Commencement of Experiment: October, 1933.

Group.	Bovine.	Age, months.	Weight.
			lb.
I................................	5154	16	640
	5163	15	600
II................................	5155	16	580
	5157	16	570
III................................	5158	16	570
	5159	16	620
IV................................	5160	16	650
	5161	15½	600
V................................	5153	16	670
	5167	11½	510
VI................................	5147	16½	580
	5149	16½	570

Basal Ration.

At the commencement of the experiment these animals were given a basal ration consisting of:—

3·0 Kg. Fanko (maize endosperm).

·5 Kg. Hay.

·5 Kg. Green feed.

·25 Kg. Meat meal.

25 gm. Salt mixture A.

Rain water *ad lib.*

This ration supplied 4 gm. P and 4·5 gm. Ca. Six weeks after the commencement of the experiment two consecutive mineral balances were determined, being weeks Nos. 1 and 2 in the mineral balance tables.

After these mineral balances had been determined the rations of the animals were supplemented with di-sodium phosphate and calcium carbonate according to the following table:—

TABLE XXI.

Group.	Bovine No.	Basal Ration.		Supplement.		Total Intake.		Ca : P ratio of total intake.
		Ca gm.	P gm.	Ca gm.	P gm.	Ca gm.	P gm.	
I......	5154	4·52	4·02	18·4	7·0	22·92	11·02	2·08 : 1
	5163	4·52	4·02	18·4	7·0	22·92	11·02	2·08 : 1
II......	5155	4·52	4·02	18·4	—	22·92	4·02	5·72 : 1
	5157	4·52	4·02	18·4	—	22·92	4·02	5·72 : 1
III......	5158	4·52	4·02	40·0	—	44·52	4·02	11·1 : 1
	5159	4·52	4·02	40·0	—	44·52	4·02	11·1 : 1
IV......	5160	4·52	4·02	—	—	4·52	4·02	1·12 : 1
	5161	4·52	4·02	—	—	4·52	4·02	1·12 : 1
V......	5153	4·52	4·02	40·0	7·0	44·52	11·02	4·04 : 1
	5167	4·52	4·02	40·0	7·0	44·52	11·02	4·04 : 1
VI......	5147	4·52	4·02	—	7·0	4·52	11·02	0·41 : 1
	5149	4·52	4·02	—	7·0	4·52	11·02	0·41 : 1

From the beginning of the 8th month the basal ration was increased and modified as follows:—

TABLE XXII.

Basal Ration.

Feed.	Amount.	Percentage P.	P gm.	Percentage Ca.	Ca gm.
Samp......................	4·0 Kg.	·059	2·36	·007	·29
Hay......................	·5 Kg.	·105	·525	·286	1·43
Green feed..................	·5 Kg.	·0385	·192	·070	·35
Meat Meal..................	·15 Kg.	·81	1·21	·971	1·46
Tap water..................	± 10 litres	—	—	·005	·50
Salt Mixture A..............	25 gm.	—	—	—	—
Total Intake................	—	—	4·29 gm. P.	—	4·03 gm Ca.

The animals were given this basal ration for the rest of the experimental period. Variations in water consumption and in the analyses of the feeds caused slight variations in the mineral intake of the animals.

From the 9th month onwards, i.e. seven months after minerals had been added to the rations, mineral balance trials were carried out regularly for a period of one week every month, until the animals were killed for the purpose of collecting the bones for chemical and histological studies. The mineral balance of only one animal in each group is recorded, although frequently the mineral balance of both animals in any particular group was determined. These analyses are not reported as they were mainly used as a check.

The animals of groups II and III could not be induced to consume the full ration, those of group III being worse in this respect During the first 14 months of the experiment it was often necessary to reduce the ration of group II to 2 Kg. samp instead of 4 Kg. for a week or two. As soon as the animals consumed the 2 Kg. samp ration satisfactorily, they were again given 4 Kg. From the 15th month onwards, however, it became necessary to feed 3 Kg. samp daily.

Eight months after the commencement of the experiment the samp in the ration of group III was reduced to 2 Kg. A month later the total basal ration was given for three weeks, when the samp had again to be reduced to 2 Kg. at which level it remained until the end of the experiment.

Bovine 5159 of group III died in the 13th month and bovine 5161 of group IV in the 19th month of the experiment.

Bovine 5158, the surviving animal of group III, was in a very poor condition and consumed the decreased basal ration with difficulty. In the 18th month of the experiment 1·07 gm. P as di-sodium phosphate, was added to the basal ration, partly to save the animal and partly in order to supplement the phosphorus lost in the 2 Kg. samp by which the ration was reduced.

In group II ·53 gm. P in the form of di-sodium phosphate was added to the ration of bovines 5155 and 5157, this representing the amount present in 1 Kg. of samp, by which the basal ration was reduced.

The animals received these additions of phosphorus just after they had been bled in the 18th month, that being approximately fourteen days before the mineral balance trial had to be conducted. The supplementary phosphate feeding was discontinued after the bleeding at the beginning of the 21st month.

Detailed results of the balance trials are given in the appendix in Tables XL-LI, a summary of which appears in Table XXIII.

Tables XXXV-XXXIX representing the body weights and values for blood calcium appear in the appendix.

Table XXIII.

Daily Mean Mineral Intake and Retention.

Summary of Tables XL to LI.

Group.	Bovine.	Intake.		Mean Ca : P ratio of ration.	Balance.		Percentage retention.	
		Ca gm.	P gm.		Ca gm.	P gm.	Ca.	P.
I......	5154	22·91	10·49	2·18 : 1	6·69	6·58	42·3	62·6
II......	5157	22·61	3·44	6·57 : 1	4·38	·35	19·4	10·2
III......	5158	43·30	3·03	14·26 : 1	10·58	·68	24·4	22·4
IV......	5160	4·23	3·44	1·23 : 1	0·14	·76	—	22·0
V......	5153	45·00	10·68	4·21 : 1	14·76	5·31	32·8	49·8
VI......	5149	4·14	10·68	·39 : 1	1·12	4·29	27·0	40·2

Discussion.

The mineral balances as tabulated for the 1st and 2nd weeks, when the unsupplemented basal ration was fed indicate that all the animals behaved similarly. They were in slightly negative Ca and slightly positive P balances. Negligible quantities of phosphorus, but fair proportions of calcium were eliminated in the urine.

As already stated the mineral balance of only one animal in each group has been reported, the same animal being killed at the end of the experiment for the chemical and histological study of the bones.

Group I. Bovines 5154 *and* 5163.

The average daily mineral intake was 22·91 gm. Ca and 10·49 gm. P, of which bovine 5154 retained 9·69 gm. Ca and 6·58 gm. P, being 42·3 and 62·7 per cent. respectively.

Both animals grew normally and maintained an excellent condition throughout the experiment, the inorganic phosphorus, calcium and phosphatase contents of the blood remaining normal.

On the unsupplemented basal ration these animals already showed a slightly positive balance. Furthermore the phosphorus of di-sodium phosphate has been shown to be in the vicinity of 100 per cent. available, and as 7 gm. P in the form of di-sodium phosphate was added to the ration, 62 per cent. retention of phosphorus seems a likely value on the combined P intake.

Slightly less Ca than anticipated was retained, the ratio of Ca : P retained being 1·6 : 1 instead of the usually accepted 2 : 1 ratio. Although it is impossible to state from the balance trials that these animals received enough P for their requirements, their weight increase, inorganic P content of the blood as well as earlier work on P requirement do suggest that such was the case. The ratio in which the P when compared with Ca was present also favoured maximum absorption.

Each of the four bones analysed of bovine 5154 contained the lowest proportion of water and fat and the highest proportion of dry-fat-free bone. The ash content of the dry-fat-free bones suggest good calcification. The ash content of the green bones varied from 34·4 per cent. for the humerus to 44·4 per cent. for the metacarpus.

The metacarpus of Bovine 5154 registered a breaking pressure of 2280 lb. per square inch over a six inch span, which is higher than that obtained in all the other groups except group V.

Group II. Bovines 5155 *and* 5157.

Average daily mineral intake: 22·61 gm. Ca and 3·44 gm. P. While the calcium intake was kept at the same level as that of the previous group, viz. 22·9 gm. Ca per day, the phosphorus level was reduced to 3·44 gm. P per day. This P was contained entirely in the basal ration. Shortly after the commencement of the experiment, the animals of group II began losing their appetite and its was necessary to reduce the samp of the ration from 4 Kg. to 2 Kg. per day and eventually increase it again to 3 Kg.

During the first couple of months while these animals consumed their full rations, they gained normally in weight, but on the reduced rations they became thin and lost weight, finally weighing about 300 lb. less than those of group I.

In the 8th month of the experiment the exceedingly low inorganic phosphorus value of 1·7 mgm. per 100 c.c. blood was obtained, showing that the animals of group II were suffering from severe aphosphorosis. At the beginning of the 18th month the phosphatase content of the blood was 9·0 units (Bodansky) which is indicative of disturbed calcium-phosphorus metabolism. Commencing on the day after they had been bled for the determination of the above constituents, the animals of this group were given ·53 gm. P as di-sodium phosphate daily for a period of three months. The mineral balance was determined a fortnight after the addition of the phosphorus supplement.

The addition of ·53 gm. P to the ration of this group favourably influenced the inorganic phosphorus and phosphatase contents of the blood. When the supplementation was discontinued at the beginning of the 21st month, this beneficial effect was lost. The amount of phosphorus supplemented was, however, insufficient to relieve the phosphorus deficiency and restore the blood constituents to normal.

Bovine 5157 retained 4·38 gm. Ca and ·35 gm. P per day, being 19·4 per cent. and 10·2 per cent of the intakes respectively. The exceedingly low phosphorus content of the ration adversely affected the retention of the calcium, which dropped to less than half that of the previous group on the same level of calcium intake.

It seems remarkable that so little P was retained in the body over the comparatively long period of the experiment as reference to Table XLII in the appendix will indicate. What new bone was formed must have derived its Ca and P almost entirely by resorption from the skeleton and substituting inferior bone. The bone analysis bears out this contention.

The green bones of group II were considerably lighter in weight, the metacarpus and the rib being about ¾ of that of the corresponding bones of group I. The percentage of dry-fat-free bone was from about 10-13 per cent less and the specific gravities slightly less than those of group I. The metacarpus registered a breaking strength of 1,800 lb. per square inch as against 2280 lb. per square inch for the same bone of group I. The percentage ash in both the dry-fat-free bones and the green bones was low.

The ration did not supply sufficient phosphorus and only a negligible quantity of that present could be utilized, whereas a considerable amount of calcium was retained. The results of the bone analyses illustrated clearly the abnormal calcification. Although the ash content was very low its Ca : P ratio remained normal.

The Ca : P ratio of the ration was too high and the level of phosphorus intake too low to permit of sufficient minerals being retained in the correct proportion for normal bone calcification and general health of the animals.

Group III. Bovines 5158 *and* 5159.

Average daily mineral intake: 43·3 gm. Ca and 3·03 gm. P.

With the phosphorus content of the ration at the same deficient level and the calcium content double that of group II, the debility and loss of appetite of the animals were greatly aggravated. Shortly after the experimental ration had been given, these animals showed signs of severe anorexia; consequently the ration was reduced to 2 Kg. samp. Both animals showed stiffness and were in a very poor general condition. For the first couple of months, while on the basal ration, they gained in weight. In the third month prior to the feeding of the experimental ration, bovine 5158 weighed 630 lb. which was also, in the 22nd month its final weight, being 160 lb. less than the weight of bovines 5157 of the previous group.

The inorganic phosphorus and phosphatase contents of the blood of group III attained the same abnormally low value as that of the previous group. The addition of 1·07 gm. P in the form of di-sodium phosphate from the 18th to the 21st month, resulted in a higher inorganic phosphorus and a lower phosphatase content than were obtained by the addition ·53 gm. P to the ration of group II. These results suggest better calcification during that period in group III than in group II, which is confirmed by more favourable retention of P during that period by the former group than by the latter.

The 1·07 gm. P supplemented had no lasting effect, for when the supplement was discontinued, these blood constituents returned to their former values.

The average daily mineral retention of bovine 5158 was 10·58 gm. Ca and ·68 gm. P, being 24·4 per cent and 22·4 per cent. of the intake respectively. As in group II a high proportion of calcium was eliminated in the urine. In view of the fact that the Ca : P ratio of bone ash remained practically constant, the data obtained do not permit of explaining what happened to the relatively large amount of calcium which was retained by the animal. The animals of group III drank almost double the amount of water inbibed by the other groups.

The form in which the phosphorus appears in the ration is undoubtedly of great importance for, during the three months from the beginning of the 18th to the beginning of the 21st month, when 1·07 gm. P in the form of di-sodium phosphate was added to the ration, the average daily retention was 1·27 gm. P, whilst during the 21st month the retention was ·22 gm. P, indicating that the entire amount of phosphorus added must have been retained by the animal. The phosphorus intake was admittedly lower during the 21st month than during the three previous months, but higher than during the 17th month although the retention was the same.

The average daily retention of phosphorus for the entire period was slightly better in this group than in group II. The retention during the three months of supplementary phosphate feeding greatly contributed to the average retentions of groups II and III.

The green bones of Bovine 5158 weighed less than those of any of the other groups but the percentages of dry-fat-free bone and the specific gravities were slightly higher than those of Bovine 5157 of group II. The breaking strength of the metacarpus was 1,200 lb. per square inch, being the lowest figure obtained in the experiment. For each of the bones the percentage ash in the green bone was higher in this group than in group II, but considerably lower than in group I. This may be ascribed to the fact that group III retained slightly more phosphorus.

Bovine 5158 of group II would with little doubt have died before the end of the experiment if the supplements of phosphorus had not been given. This addition, though small, undoubtedly influenced the figures obtained in the analyses.

Group IV. *Bovines* 5160 *and* 5161.

Average daily mineral intake. 4·23 gm. Ca and 3·44 gm. P.

The rations of these animals were not supplemented with minerals; therefore both the calcium and the phosphorus were at the lowest levels. The Ca : P ratio of the ration was 1·2 : 1, which could probably still be regarded as normal.

Although these animals did at times leave some of their food over, they never showed loss of appetite to such an extent that it was necessary to reduce their rations for more than a week at a time. They gained in weight almost to the same extent as those of the normal group, viz. I. If, however, all the groups had been fed *ad lib*, it is doubtful whether this group would have consumed more whereas group I would certainly have eaten more and consequently have gained more in weight.

Bovine 5160 showed an average daily retention of ·76 gm. P being 22 per cent. of the intake. The calcium balance oscillated between slightly negative and slightly positive values.

It is surprising that although the animals retained small amounts of phosphorus, the calcium which was well absorbed was eliminated in the urine.

The inorganic blood phosphorus values were at the same low level as in the previous groups, decreasing finally to 1·4 mgm. per 100 c.c. blood. The phosphatase content reached a high figure of 16·2 units (Bodansky) per 100 c.c. blood. The final values for these blood constituents showed a more severe degree of mineral deficiency than those for groups II and III.

The blood calcium values were uneffected, remaining normal at almost 10 mgm. per 100 c.c. blood.

The green rib of group IV contained 25·3 per cent. ash as against 38·5 per cent. for that of group I. Therefore 100 gm. green rib of the latter group could lose 13·2 gm. i.e. 34·3 per cent. of its ash before attaining the same mineral content as that of group IV.

The dry-fat-free bone and percentage ash suggest poor calcification which is confirmed by the low breaking strength of the metacarpus.

Group V. Bovines 5153 *and* 5167.

Daily average mineral intake: 45 gm. Ca and 10·68 gm. P.

The calcium content of this ration was equal to that of group III but twice that of group I, while the phosphorus content was equal to that of group I. The Ca : P ratio was 4·21 : 1, being almost double that of the normal group.

The average daily retention of calcium was 14·76 gm. and of phosphorus 5·31 gm. being 32·8 per cent. and 49·8 per cent. of the intake respectively. When a comparison is made with group I, it appears that increased calcium had a disadvantageous effect upon the phosphorus retention while increased phosphorus had a beneficial effect upon the retention of calcium.

Although the Ca : P ratio of the ration of this group was slightly lower than that of group II, it was at a higher level of mineral intake, which allowed sufficient minerals to be retained for the purpose of proper bone formation, the excess being discarded in the excreta.

It is noteworthy that towards the end of the experiment, the phosphorus content of the urine of group V was about 20 times higher than normal, whereas negligible amounts of phosphorus were contained in the urine of group I which was on the same level of phosphorus intake. The calcium content of the urine of group V remained normal. It appears that this abnormal elimination of phosphorus in the urine is associated with the excess of calcium in the ration.

The inorganic phosphorus and the phosphatase content of the blood remained normal.

The breaking strength of the metacarpus indicates well calcified bone. As a matter of fact the value obtained over a six inch span was about 400 lb. higher than that of the metacarpus of the animal killed in group I, receiving enough Ca and P.

The values for the percentage dry-fat-free bones, their ash contents, and the ash contents of the green bones were slightly lower than those of group I.

These animals consumed their full rations throughout the experiment and gained normally in weight.

Group VI. *Bovines* 5147 *and* 5149.

Average daily mineral intake 4·14 gm. Ca and 10·68 gm. P.

The phosphorus content of the ration of group VI was equivalent to that of the normal group, being 10·68 gm. P while the calcium content was reduced to 4·14 gm. Ca, which was the same as that of group IV.

The average daily retention of 4·29 gm. P, i.e. 40·2 per cent. of the intake was lower than that retained by either group I or group V, which were on the same level of phosphorus intake but higher Ca. Bovine 5147 retained 1·12 gm. Ca per day, i.e. 27 per cent of the intake. A high proportion of phosphorus was eliminated in the urine. At times this figure was about equal to that excreted in the faeces and more than double that retained by the animal.

These animals ate well and gained normally in weight, the inorganic phosphorus, phosphatase and calcium contents of the blood remaining normal. There was no indication during life of a deficiency of either mineral.

The results of the bone analyses, however, showed signs of abnormal bone formation. The percentage dry-fat-free bone and ash content of the femur, humerus and metacarpus were only slightly lower than in those of groups I to V. The rib, however, showed more marked differences.

If the data obtained in this group be compared with those of the phosphorus deficient groups the conclusions appear justified that low phosphorus in the ration of stock when sufficient calcium is present is more detrimental than when low calcium with sufficient phosphorus is given.

A probable explanation for this finding is that their calcium requirement is lower than their phosphorus requirement.

General.

Three groups, namely, groups I, V and VI were given a ration containing the same amount of phosphorus but with the Ca : P ratios of about 2 : 1, 4 : 1 and 0·26 : 1 respectively. These represented the optimal, the high and the low ratios of this experiment.

Group I showed the highest percentage retention of both calcium and phosphorus. In group V there was a depression of the phosphorus retention and an increased calcium retention.

It would appear that the deficiency of calcium in group VI, receiving a phosphorus adequate ration, had a more detrimental effect upon the total retention of phosphorus, than excess of calcium as in group V.

Compared with group IV, where both minerals were deficient, the balance figures of group VI suggest that in digestion excess of phosphorus over calcium increases the solubility of the calcium compounds and renders absorption of calcium easier.

The feeding of phosphorus adequate rations containing sufficient and excess calcium (groups I and V respectively) resulted in a better calcium retention than when the rations were phosphorus deficient (groups II and III).

In the three phosphorus deficient groups, namely II, III and IV, the average daily phosphorus retentions of groups III and IV were about the same, that of group II being slightly less. But as the animals of groups II and III were given a phosphate supplement for a period of three months, they are not quite comparable with group IV. If the balance figures for the three months of added phosphate feeding be omitted from the calculations it is found that group IV retained slightly more phosphorus than either groups II or III, between which there was no difference.

From these experiments it can however not be claimed that a wide Ca : P ratio in a phosphorus deficient ration caused a depression of the phosphorus retention as the retention of group II was lower than that of group III.

The blood analyses indicate that in the groups receiving adequate phosphorus, the inorganic phosphorus and the phosphatase content remained normal. If the phosphatase content of the blood is affected by a deficiency of calcium, then the degree of calcium deficiency, as experienced by group VI, was not acute enough to affect a rise in the phosphatase content of the blood.

The blood calcium values remained normal in all the groups, irrespective of the Ca : P ratio or the mineral content of the ration. As the rations of these animals contained sufficient vitamin D such values were anticipated.

A deficiency of phosphorus in the ration resulted in a loss of appetite. This condition was slighty noticeable in group IV fed on a ration with a normal Ca : P ratio, but became more pronounced as the Ca : P ratio was increased, those animals (group III) fed on the ration with a Ca : P ratio of 14·3 : 1 eating the least. When adequate phosphorus was fed, with either a deficiency of calcium (group VI) or excess calcium (group V) the abnormal Ca : P ratio of the ration caused no noticeable signs of anorexia.

It would seem therefore that the loss of appetite is associated with abnormal Ca : P metabolism rather than with a specific factor such as a low P or low Ca or abnormal ratio, which obviously might be responsible for the abnormal metabolism as stated by Theiler, du Toit and Malan (1937).

The green weights and measurements of the bones examined in groups II, III, IV and VI fed on rations deficient in either or both calcium and phosphorus, were smaller than those of groups I and V whose rations were adequately supplied with both elements, but as the green weight is also dependent upon the size of the bone—an inherited factor—it is obviously not as true a criterion as percentage ash for instance.

The determination of the percentage ash in the green bones affords an excellent index of the degree of calcification. Either calcium or phosphorus whichever was retained in lesser quantity, limits the degree of calcification. Thus, for example, a diet adequate in calcium but poor in phosphorus leads to a state of minimal phosphorus retention, and in such a case, a superfluity of calcium will not help to raise the total percentage of ash in the bones above that determined by the lesser quantity of phosphorus retained.

From the data obtained the Ca : P ratio of the ration appears to be important for normal growth and development when the ration was deficient in either or both minerals, but was without appreciable influence in the case of one abnormal ratio where phosphorus was present in an adequate quantity (group V).

In a paper by Theiler, du Toit and Malan (1937) full details of weights, blood analyses, clinical symptoms, histological examination of the bones and the development of osteodystrophic diseases are given and discussed.

Summarizing the results of the experiment described one is led to conclude that 22·9 gm. Ca and 10·5 gm. P, of which respectively 9·69 and 6·58 gm. were retained provided sufficient Ca and P for normal growth.

The retention of P on the same level of intake was affected by the amount of Ca present as indicated in the following table.

Ca intake.	*P intake.*	*Ca retained.*	*P retained.*
22·9	10·49	9·7	6·6
45·0	10·49	14·7	5·3
4·14	10·49	1·1	4·3

The Ca retention is decreased or increased by respectively decreasing or increasing the amount of the Ca ingested. When the P intake was reduced to approximately 3 gm. the best retention of P was shown when the Ca intake was proportionately decreased.

It appears that the main factor which determines retention of Ca or P is the level of intake of the mineral in question apart from the ratio in which it is associated with the remaining mineral in the food.

SUMMARY.

1. The availability of the P in several commercial phosphates was determined in balance experiments with bovines. The availability was measured in terms of the retention of P. High

retention was favoured by giving the phosphates at a level, viz. 6 gm. P, well below that of the P requirement of the class of animal used. The ratio of Ca : P was kept constant for all phosphates at approximately 2:1.

2. Di-sodium phosphate appeared to be slightly more available at the given level of P intake than either di-calcium phosphate or bonemeal, between whose retentions there was no significant difference. Taking all the results obtained into consideration it is doubtful whether animals when fed phosphates well below their requirements will retain more P when it is given as di-sodium phosphate than as the relatively insoluble di-calcium phosphate or bonemeal. In the experiments reported the best retention of P in di-sodium phosphate was 100 per cent. and that of di-calcium phosphate and bonemeal 97 and 94 per cent. respectively.

3. The retention of Ca which was supplied as $CaCO_3$ when insufficient was present in the phosphatic supplement and the basal ration varied from 65-100 per cent. irrespective of the supplement fed.

4. The remarkably high percentages of P retained when the supplement was fed well below the requirements of the animals were reduced by about 10 per cent. at approximately the optimal level of P intake.

5. Long term metabolism experiments were conducted with growing bovines fed on rations at different levels of mineral intake but with a constant optimal Ca : P ratio. At the end of the experiment these animals were killed and the bones used for chemical and histological studies.

6. Rations containing 8·5 gm. P and 16·4 gm. Ca i.e. 19·5 gm. P_2O_5 and 23 gm. CaO were found to be adequate for growing steers, whereas 5·8 gm. P and 12 gm. Ca and less were insufficient. Inorganic blood phosphorus, mineral balances and bone analyses were taken as criteria.

7. A determination of the percentage ash in the green bones afforded an excellent index of the degree of calcification. A close correlation was noted between the above index and the breaking strength of the metacarpus for the animals of the same age.

8. Data are further recorded of long term metabolism experiments conducted with growing heifers fed a ration containing different levels of P and Ca and hence varying Ca : P ratios.

9. 23 gm. Ca and 10·5 gm. P permitting an average daily retention of about 9·7 gm. Ca and 6·6 gm. P were found to be adequate for growing heifers, the conclusions being based on the mineral balances, and blood and bone analyses. The histological findings of the bones of the animals used in these experiments are discussed in detail by Theiler *et al* (1937).

10. The quantity of Ca or P present in the ration limited the retention of the remaining element. Varying the intakes of the calcium and the phosphorus from the optimum resulted in less efficient retention of both elements.

11. The quantities of Ca and P present in the ration apparently determined more than any other definable factor the quantities of these minerals retained in the body. A high intake of Ca or P or both is associated with high retention of that constituent.

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APPENDIX.

TABLE XXIV.

EXPERIMENT II.

Group I: Phosphorus Balance (daily average).

Week No.	Bovine 4712. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.	Month of Expt.	Bovine 3478. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.
1	4·51	4·24	·028	0·24	2	4·51	4·44	·031	0·04
2	4·51	4·88	·027	−0·40	3	4·51	4·48	·024	0·01
3	8·00	3·80	·025	4·12	4	8·00	4·51	·027	3·46
4	8·00	5·07	·036	2·89	5	8·00	4·42	·031	3·55
5	8·83	4·25	·043	4·54	6	8·83	5·00	·043	3·79
6	8·83	4·33	·050	4·45	8	8·83	4·10	·053	4·68
7	8·67	4·46	·061	4·15	9	8·67	3·76	·052	4·86
8	8·67	3·47	·075	5·12	10	8·67	3·96	·038	4·67
9	8·87	4·08	·060	4·73	11	8·87	3·48	·048	5·34
10	8·87	3·88	·071	4·92	12	8·87	3·15	·051	5·67
11	8·54	3·06	·047	5·43	13	8·54	3·36	·050	5·13
12	8·07	4·37	·049	3·65	15	—	—	—	—
13	7·73	4·37	·081	3·28	16	—	—	—	—
14	7·73	3·40	·080	4·25	17	—	—	—	—
15	8·17	4·50	·007	3·66	18	—	—	—	—
16	8·17	3·37	·013	4·79	20	—	—	—	—
17	8·14	3·82	·008	4·31	21	—	—	—	—
18	8·30	3·16	·008	5·13	24	—	—	—	—
19	8·14	4·50	·008	3·63	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–11
	8·34			4·42		8·59			4·57

TABLE XXV.

EXPERIMENT II.

Group I: Calcium Balance (daily average).

Week No.	Bovine 4712. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.	Month of Expt.	Bovine 3478. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.
1	6·31	4·93	2·27	−0·89	2	6·30	3·62	3·12	−0·44
2	6·32	4·41	2·59	−0·68	3	6·36	3·23	2·71	0·42
3	15·47	7·48	1·24	6·75	4	15·40	6·12	1·04	8·24
4	15·49	8·00	1·71	5·78	5	15·52	6·40	0·98	8·14
5	17·44	7·21	1·43	8·80	6	17·48	8·42	1·22	7·84
6	17·49	9·07	1·74	6·68	8	17·51	8·10	1·66	7·75
7	17·48	9·07	1·48	6·93	9	17·51	7·43	1·44	8·64
8	16·07	8·21	1·34	6·52	10	16·17	8·14	1·23	6·80
9	15·87	7·93	1·46	6·48	11	15·86	7·64	1·64	6·58
10	15·88	8·14	1·35	6·39	12	15·80	9·00	1·28	5·52
11	15·84	8·07	1·59	6·18	13	15·88	8·93	1·76	5·19
12	15·84	8·13	1·03	6·68	15	—	—	—	—
13	16·11	6·46	1·75	7·90	16	—	—	—	—
14	15·78	8·50	1·66	5·62	17	—	—	—	—
15	16·46	9·07	1·71	5·68	18	—	—	—	—
16	16·31	8·74	1·74	5·83	20	—	—	—	—
17	16·88	11·88	1·00	4·00	21	—	—	—	—
18	16·88	9·00	1·14	6·74	24	—	—	—	—
19	16·88	10·42	1·36	5·10	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–11
	16·36			6·36		16·35			7·19

Table XXVI.

EXPERIMENT II.

Group II: Phosphorus Balance (daily average).

Week No.	Bovine 3480.				Month of Expt.	Bovine 3465.			
	Intake gm. P.	Outgo gm. P.		Balance gm. P.		Intake gm. P.	Outgo gm. P.		Balance gm. P.
		Faeces.	Urine.				Faeces.	Urine.	
1	4·57	4·25	·033	0·29	2	4·50	4·01	·033	0·46
2	4·57	4·46	·031	0·08	3	4·50	4·38	·038	0·08
3	5·85	4·48	·034	1·34	4	5·78	4·20	·040	1·54
4	5·85	4·09	·035	1·72	5	5·78	4·65	·033	1·10
5	—	—	—	—	6	6·61	4·37	·046	2·19
6	—	—	—	—	8	6·61	3·98	·043	2·59
7	—	—	—	—	9	6·63	4·06	·042	2·53
8	—	—	—	—	10	6·63	4·39	·045	2·19
9	5·77	2·90	·039	2·83	11	6·65	4·55	·051	2·05
10	5·77	3·57	·053	2·15	12	6·65	3·69	·046	2·91
11	5·77	3·19	·044	2·54	13	—	—	—	—
12	5·86	3·22	·040	2·60	15	—	—	—	—
13	5·51	3·54	·042	1·93	16	—	—	—	—
14	5·51	3·37	·041	2·10	17	—	—	—	—
15	5·97	2·99	·044	2·94	18	—	—	—	—
16	5·97	2·61	·026	3·33	20	—	—	—	—
17	5·94	3·41	·048	2·48	21	—	—	—	—
18	6·10	3·61	·048	2·44	24	—	—	—	—
19	5·94	4·13	·057	1·75	25	—	—	—	—
		Mean for	weeks	3–19			Mean for	weeks	3–19
	5·83			2·47		6·42			2·14

TABLE XXVII.

EXPERIMENT II.

Group II: Calcium Balance (daily average).

Week No.	Bovine 3480.				Month of Expt.	Bovine 3465.			
	Intake gm. Ca.	Outgo gm. Ca.		Balance gm. Ca.		Intake gm. Ca.	Outgo gm. Ca.		Balance gm. Ca.
		Faeces.	Urine.				Faeces.	Urine.	
1	6·29	4·48	2·64	−0·83	2	6·32	4·52	1·98	−0·18
2	6·32	5·07	2·14	−0·89	3	6·30	3·78	2·45	0·07
3	11·45	7·59	0·94	3·02	4	11·65	6·90	1·18	3·57
4	11·53	7·08	0·93	3·52	5	11·50	8·64	1·22	1·64
5	—	—	—	—	6	13·63	7·53	·91	5·09
6	—	—	—	—	8	13·52	7·28	·93	5·31
7	—	—	—	—	9	13·49	6·90	·89	5·70
8	—	—	—	—	10	13·51	7·95	·84	4·72
9	11·87	6·64	0·74	4·49	11	11·90	7·93	·90	3·07
10	11·90	8·28	1·33	2·29	12	11·87	7·05	·85	3·97
11	11·88	7·19	0·76	3·93	13	—	—	—	—
12	11·33	7·28	1·48	2·57	15	—	—	—	—
13	11·76	6·85	1·28	3·63	16	—	—	—	—
14	11·77	5·28	2·12	4·37	17	—	—	—	—
15	12·51	7·59	1·81	3·11	18	—	—	—	—
16	12·43	7·90	1·28	3·25	20	—	—	—	—
17	12·90	6·33	1·71	4·86	21	—	—	—	—
18	12·87	6·64	1·50	4·73	24	—	—	—	—
19	12·88	7·14	1·86	3·88	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–10
	12·09			3·67		12·62			4·13

Table XXVIII.

EXPERIMENT II.

Group III: Phosphorus Balance (daily average).

Week No.	Bovine 3464. Intake gm. P.	Bovine 3464. Outgo gm. P. Faeces.	Bovine 3464. Outgo gm. P. Urine.	Bovine 3464. Balance gm. P.	Month of Expt.	Bovine 3467. Intake gm. P.	Bovine 3467. Outgo gm. P. Faeces.	Bovine 3467. Outgo gm. P. Urine.	Bovine 3467. Balance gm. P.
1	4·56	3·72	·033	0·81	2	4·56	4·35	·029	0·18
2	4·56	4·33	·035	0·19	3	4·56	4·59	·034	−0·06
3	5·84	4·18	·037	1·62	4	5·84	4·11	·042	1·69
4	5·84	3·78	·031	2·03	5	5·84	4·33	·039	1·47
5	4·26	2·81	·036	1·41	6	5·79	3·20	·043	2·55
6	4·26	2·50	·037	1·72	8	5·79	3·37	·050	2·37
7	4·92	2·70	·045	2·17	9	5·79	3·75	·051	1·99
8	4·92	3·35	·053	1·52	10	5·79	3·21	·050	2·53
9	4·89	2·19	·049	2·64	11	5·68	2·67	·052	2·96
10	5·68	2·92	·050	2·71	12	5·68	3·41	·053	2·22
11	5·68	2·70	·050	2·93	13	5·68	2·62	·045	3·01
12	5·87	3·50	·034	2·34	15	5·87	3·94	·054	1·88
13	5·53	4·21	·045	1·27	16	5·53	4·62	·045	0·86
14	5·53	3·74	·031	1·76	17	5·97	3·96	·031	1·98
15	5·97	4·11	·048	1·81	18	5·97	3·04	·048	2·88
16	5·97	3·82	·052	2·10	20	—	—	—	—
17	5·94	4·27	·061	1·61	21	—	—	—	—
18	6·10	3·43	·092	2·58	24	—	—	—	—
19	5·94	3·52	·048	2·37	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–15
	5·48			2·03		5·79			2·18

TABLE XXIX.

EXPERIMENT II.

Group III: Calcium Balance (daily average).

Week No.	Bovine 3464.				Month of Expt.	Bovine 3467.			
	Intake gm. Ca.	Outgo gm. Ca.		Balance gm. Ca.		Intake gm. Ca.	Outgo gm. Ca.		Balance gm. Ca.
		Faeces.	Urine.				Faeces.	Urine.	
1	6·30	4·19	2·48	−0·37	2	6·28	4·62	1·98	−0·32
2	6·30	4·18	3·43	−0·31	3	6·34	4·47	2·20	−0·33
3	24·41	18·35	2·35	3·71	4	24·38	18·09	3·24	3·05
4	24·38	15·85	2·38	6·15	5	24·38	17·17	2·53	4·68
5	23·04	13·78	2·88	6·38	6	24·94	18·64	3·50	2·80
6	22·33	16·42	1·56	4·35	8	24·91	16·14	3·70	5·07
7	23·35	16·78	1·73	4·84	9	24·96	14·57	3·04	7·35
8	23·24	14·42	1·74	7·08	10	24·95	18·49	3·62	2·84
9	22·83	16·57	1·28	4·98	11	23·31	13·07	3·57	6·67
10	22·83	16·14	2·96	3·73	12	23·26	17·93	3·74	1·60
11	22·81	17·28	3·33	2·20	13	23·28	14·14	2·59	6·55
12	24·72	19·21	2·77	2·74	15	24·73	19·14	3·60	1·99
13	24·54	17·46	3·00	3·08	16	24·56	17·85	3·44	3·27
14	24·53	16·71	3·14	4·68	17	24·55	19·64	3·51	1·40
15	25·80	18·72	1·44	5·04	18	25·28	15·10	2·41	7·77
16	25·23	16·04	1·94	7·25	20	—	—	—	—
17	25·73	15·21	2·28	8·24	21	—	—	—	—
18	25·71	17·21	2·86	5·64	24	—	—	—	—
19	25·71	16·92	2·43	6·36	25	—	—	—	—
		Mean for weeks		3–19			Mean for weeks		3–15
	24·09			5·09		24·42			4·23

TABLE XXX.

EXPERIMENT II.

Group IV: Phosphorus Balance (daily average).

Week No.	Bovine 3454.				Month of Expt.	Bovine 3456.			
	Intake gm. P.	Outgo gm. P.		Balance gm. P.		Intake gm. P.	Outgo gm. P.		Balance gm. P.
		Faeces.	Urine.				Faeces.	Urine.	
1	4·54	4·03	·031	0·48	2	4·54	4·20	·031	0·31
2	4·54	3·83	·030	0·68	3	4·54	4·47	·027	0·04
3	4·54	4·24	·037	0·26	4	3·47	4·61	·028	−1·17
4	4·54	4·15	·031	0·36	5	3·79	4·37	·032	−0·61
5	5·37	3·58	·032	1·76	6	4·16	3·47	·033	0·66
6	4·38	2·39	·034	1·96	8	2·20	1·53	·033	0·64
7	5·15	3·45	·038	1·66	9	3·44	1·77	·036	1·63
8	—	—	—	—	10	2·73	1·75	·037	0·94
9	—	—	—	—	11	4·89	2·27	·038	2·58
10	—	—	—	—	12	4·85	2·32	·040	2·49
11	—	—	—	—	13	4·48	2·25	·032	2·20
12	—	—	—	—	15	4·23	2·19	·019	2·02
13	—	—	—	—	16	2·93	2·50	·055	0·37
14	—	—	—	—	17	2·56	1·96	·039	0·56
15	—	—	—	—	18	3·76	2·36	·031	1·37
16	—	—	—	—	20	3·28	2·16	·031	1·09
17	—	—	—	—	21	3·71	2·78	·052	0·88
18	—	—	—	—	24	3·87	2·26	·061	1·55
19	—	—	—	—	25	3·50	2·46	·048	0·99
		Mean for weeks		3–7			Mean for weeks		3–19
	4·80			1·20		3·64			1·07

TABLE XXXI.

EXPERIMENT II.

Group IV: Calcium Balance (daily average).

Week No.	Bovine 3454. Intake gm. Ca.	Bovine 3454. Outgo gm. Ca. Faeces.	Bovine 3454. Outgo gm. Ca. Urine.	Bovine 3454. Balance gm. Ca.	Month of Expt.	Bovine 3456. Intake gm. Ca.	Bovine 3456. Outgo gm. Ca. Faeces.	Bovine 3456. Outgo gm. Ca. Urine.	Bovine 3456. Balance gm. Ca.
1	6·30	4·53	2·64	−0·87	2	6·32	3·89	2·43	—
2	6·30	4·00	2·08	0·22	3	6·33	3·86	2·66	−0·19
3	8·69	5·01	1·90	1·78	4	6·30	5·46	2·23	−1·39
4	8·70	5·93	1·54	1·23	5	6·98	6·46	2·43	−1·91
5	10·67	4·53	1·54	4·60	6	8·54	4·53	2·28	1·73
6	8·38	3·61	2·24	2·53	8	7·67	2·21	2·43	3·03
7	8·37	3·64	2·41	2·32	9	8·66	3·15	2·64	2·87
8	—	—	—	—	10	8·31	3·75	2·53	2·03
9	—	—	—	—	11	7·99	3·52	2·53	1·94
10	—	—	—	—	12	8·73	3·38	2·88	2·47
11	—	—	—	—	13	8·71	3·53	2·53	2·65
12	—	—	—	—	15	7·95	3·25	2·07	2·63
13	—	—	—	—	16	6·23	3·93	2·06	0·24
14	—	—	—	—	17	3·72	1·34	2·24	0·14
15	—	—	—	—	18	9·00	3·51	4·26	1·23
16	—	—	—	—	20	7·55	3·02	2·32	2·21
17	—	—	—	—	21	9·37	4·86	1·36	3·15
18	—	—	—	—	24	9·34	3·88	1·50	3·96
19	—	—	—	—	25	9·97	5·31	1·43	3·23
		Mean for weeks		3–7			Mean for weeks		3–19
	8·96			2·49		7·94			1·78

Table XXXII.

EXPERIMENT II.

Group V: Phosphorus Balance (daily average).

Week No.	Bovine 5430. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.	Month of Expt.	Bovine 5431. Intake gm. P.	Outgo gm. P. Faeces.	Outgo gm. P. Urine.	Balance gm. P.
1	1·01	1·37	·018	−0·38	15	1·01	1·50	·022	−0·51
2	1·01	1·52	·026	−0·54	16	1·01	1·44	·031	−0·46
3	1·29	2·01	·031	−0·75	17	1·29	2·00	·031	−0·74
4	1·29	1·99	·035	−0·74	18	1·29	2·03	·026	−0·77
5	1·71	2·36	·035	−0·69	19	1·71	2·27	·039	−0·60
6	1·71	1·82	·026	−0·14	20	1·71	1·72	·035	−0·05
7	1·71	2·14	·018	−0·45	21	1·71	1·61	·035	0·06
8	1·71	1·82	·018	−0·13	22	1·71	1·39	·031	0·29
9	1·71	1·62	·044	0·05	23	1·71	1·44	·031	0·24
10	1·71	1·38	·044	0·29	24	1·71	1·75	·026	0·07
11	1·98	1·48	·031	0·47	25	1·98	1·47	·048	0·46
12	1·98	1·68	·038	0·26	26	1·98	1·35	·038	0·59
		Mean.					Mean.		
	1·68			—·11		1·68			—·05

Table XXXIII.

EXPERIMENT II.

Group V: Calcium Balance (daily average).

Week No.	Bovine 5430. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.	Month of Expt.	Bovine 5431. Intake gm. Ca.	Outgo gm. Ca. Faeces.	Outgo gm. Ca. Urine.	Balance gm. Ca.
1	2·14	3·05	0·80	−1·71	15	2·16	2·98	0·84	−1·66
2	2·15	2·47	1·03	−1·35	16	2·17	2·51	0·80	−1·14
3	2·17	2·44	1·87	−2·14	17	2·16	1·36	2·21	−1·41
4	2·18	1·47	2·68	−1·97	18	2·19	1·22	3·15	−2·18
5	2·67	1·85	2·30	−1·48	19	2·68	·85	3·00	−1·17
6	2·71	2·83	1·88	−2·00	20	2·70	·89	2·91	−1·10
7	2·81	2·22	1·43	−0·84	21	2·78	1·16	1·29	0·33
8	2·78	1·37	0·70	0·71	22	2·80	1·23	1·41	0·16
9	2·80	1·45	0·86	0·49	23	2·81	1·32	1·07	0·42
10	2·83	1·04	1·03	0·76	24	2·82	1·89	0·75	0·18
11	3·00	1·25	1·21	0·54	25	3·03	1·43	·87	0·73
12	3·02	1·36	1·09	0·57	26	3·01	1·52	·91	0·58
		Mean for	weeks	3–12			Mean for	weeks	3–12
	2·70			−0·54		2·70			−0·35

TABLE XXXIV.

EXPERIMENT II.

Weights of Animals in lb.

Date.	Month of Experiment.	Group I.		Group II.		Group III.		Group IV.		Group V.	
		4712.	3478.	3480.	3465.	3464.	3467.	3454.	3456.	5430.	5431.
1933.											
June	1	821	800	812	767	798	818	756	836	—	—
July	2	841	810	831	790	796	831	779	857	—	—
August	3	880	860	863	814	834	854	814	855	—	—
September	4	890	865	873	827	851	860	820	860	—	—
October	5	861	861	863	816	832	845	797	836	—	—
November	6	894	887	883	839	839	845	833	839	—	—
December	7	900	886	819	844	820	844	855	838	—	—
1934.											
January	8	906	914	824	850	815	871	863	848	—	—
February	9	934	934	850	865	805	865	883	855	—	—
								Died			
March	10	949	983	872	874	816	880	—	849	—	—
April	11	975	1,000	885	888	830	895	—	850	—	—
May	12	995	1,004	875	890	826	882	—	851	—	—
June	13	1,020	1,036	875	Died	853	890	—	875	—	—
July	14	1,014	Died	910	—	892	896	—	869	450	400
August	15	1,000	—	935	—	920	915	—	880	—	—
September	16	1,040	—	925	—	915	945	—	865	470	440
October	17	1,065	—	950	—	925	1,000	—	845	490	460
November	18	1,060	—	940	—	925	Died	—	820	510	500
December	19	—	—	—	—	—	—	—	—	500	508
1935.											
January	20	1,085	—	960	—	940	—	—	860	495	500
February	21	1,100	—	1,000	—	965	—	—	840	520	520
March	22	1,125	—	970	—	1,000	—	—	875	560	540
April	23	—	—	—	—	—	—	—	—	580	550
May	24	1,170	—	1,055	—	1,050	—	—	895	615	600
June	25	1,215	—	1,100	—	1,080	—	—	890	615	610
July	26	1,265	—	1,130	—	1,105	—	—	885	625	620

TABLE XXXV.

EXPERIMENT III.

Inorganic Blood Phosphorus in mgm. per 100 c.c. Blood.

Group No.	Bovine.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.
I........................	5154	5·4	5·0	5·2	5·0	4·6	5·9	6·5	—	6·1	5·9	7·7	5·8	7·5	5·2	6·7
	5163	5·1	4·9	4·4	4·6	5·1	5·5	4·5	—	5·2	6·7	5·1	4·5	5·3	4·7	4·8
II........................	5155	1·7	1·5	1·6	1·9	1·8	2·2	1·8	—	2·3	2·1	2·4	3·0	2·9	2·9	2·4
	5157	1·7	1·7	1·6	2·3	1·8	1·5	1·5	—	3·0	2·5	2·5	3·0	2·3	3·0	2·0
III........................	5158	1·9	2·2	2·0	1·8	1·7	3·4	2·4	—	3·0	2·6	2·4	4·6	4·1	4·3	2·4
	5159	1·6	1·5	1·9	2·3	2·0	1·6	1·4	—	Died	—	—	—	—	—	—
VI........................	5160	1·9	2·0	2·1	2·3	1·7	1·5	1·7	—	2·4	3·0	1·6	1·6	1·3	1·3	1·4
	5161	1·6	1·6	2·0	1·8	1·6	1·9	1·3	—	3·0	3·4	Died	—	—	—	—
V........................	5153	5·8	5·7	5·3	6·1	7·3	5·8	5·6	—	7·8	7·3	5·0	5·0	5·8	5·0	5·0
	5167	6·0	6·0	7·7	5·7	6·4	5·9	4·6	—	7·7	7·1	5·4	5·7	5·2	5·7	5·2
VI........................	5147	5·2	4·9	4·8	3·8	4·8	4·8	5·8	—	5·5	6·8	4·0	4·2	5·2	5·4	4·4
	5149	5·1	5·0	5·8	4·6	5·7	5·4	5·9	—	6·6	6·4	5·1	4·6	5·9	4·1	4·9

TABLE XXXVI.

EXPERIMENT III.

Blood Calcium given Monthly in mgm. per 100 c.c. Blood.

Group No.	Bovine No.	Month of Experiment.														
		8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.
I.	5154	10·6	9·8	11·0	10·3	10·2	9·9	10·3	—	10·0	10·1	8·5	11·2	9·5	8·7	8·7
	5163	11·5	9·8	10·0	11·1	10·8	10·5	10·2	—	10·5	11·0	10·0	8·2	9·2	8·6	9·8
II.	5155	11·6	10·6	10·6	10·2	10·6	11·3	10·5	—	10·0	11·1	10·0	9·8	8·9	9·8	9·8
	5157	11·2	9·8	11·0	10·5	10·5	11·5	11·1	—	10·0	11·3	9·8	9·3	8·7	9·4	9·6
III.	5158	11·8	10·0	10·6	11·1	10·0	11·0	10·3	—	11·0	11·1	9·6	8·9	8·4	9·4	10·0
	5159	10·7	9·6	9·6	11·2	10·8	11·4	10·1	—	—	—	—	—	—	—	—
IV.	5160	10·7	10·0	10·0	10·6	11·0	11·5	10·6	—	10·3	11·2	9·8	8·8	10·8	9·8	9·5
	5161	10·2	11·2	11·0	11·4	10·5	11·3	11·0	—	10·1	10·7	—	—	—	—	—
V.	5163	11·0	9·8	9·6	10·4	10·2	10·6	10·0	—	9·8	10·2	9·2	8·7	8·2	8·7	8·5
	5167	10·9	10·6	11·0	10·6	10·4	11·0	10·6	—	10·6	10·6	10·2	8·7	9·7	8·6	9·1
VI.	5147	10·0	10·4	9·8	10·5	10·8	10·6	10·6	—	10·1	10·8	10·3	10·0	9·1	8·5	9·8
	5149	10·8	10·2	10·2	10·1	10·0	10·3	10·5	—	10·0	10·4	9·8	8·4	8·7	8·4	8·6

Table XXXVII.

EXPERIMENT III.

Phosphatase Content of Blood in Bodansky Units.

Group.	Bovine No.	Month of Experiment.				
		18.	19.	20.	21.	22.
I.	5154	3·5	3·3	4·6	4·4	3·9
	5163	5·2	1·6	5·0	5·1	5·3
II.	5155	8·6	6·4	7·1	8·5	9·0
	5157	9·4	7·5	9·6	8·2	9·8
III.	5158	9·4	3·8	5·5	7·7	8·9
IV.	5160	13·9	9·9	16·2	11·7	12·1
	5161	8·5	—	—	—	—
V.	5153	5·3	3·0	4·1	5·4	4·9
	5167	3·6	2·8	4·4	3·9	3·8
VI.	5147	2·8	·5	5·2	4·0	5·2
	5149	4·9	2·5	4·2	4·1	2·6

Table XXXIX.

EXPERIMENT III.

Weights in lb.

Month of Expt.	I.		II.		III.		IV.		V.		VI.	
	5154	5163	5155	5157	5158	5159	5160	5161	5163	5167	5147	5149
1	640	600	580	570	570	620	650	600	670	510	580	573
2	685	600	600	625	600	655	630	540	715	515	625	605
3	655	630	630	630	630	685	760	605	720	550	640	585
4	675	670	640	635	625	700	700	625	775	575	650	600
5	635	710	660	669	625	740	715	690	780	570	670	625
6	735	730	690	710	660	740	755	695	800	580	720	670
7	765	740	705	710	650	745	765	700	800	620	700	685
8	780	790	735	720	665	770	825	745	855	670	760	725
9	840	800	740	750	650	760	825	760	885	715	800	760
10	850	805	715	750	600	745	840	770	905	700	755	755
11	890	855	710	765	600	725	865	800	925	750	770	780
12	915	840	710	735	575	725	890	825	950	785	805	805
13	930	980	700	770	560	Died	885	845	985	800	865	810
14	935	900	715	765	580	—	895	835	1,020	835	900	835
15	955	920	725	775	585	—	900	850	1,025	855	885	870
16	1,000	955	725	750	600	—	925	900	1,035	910	925	865
17	1,045	955	705	785	595	—	965	925	1,080	915	945	900
18	1,020	995	685	745	610	—	970	920	1,065	945	945	930
19	1,060	990	725	765	615	—	1,000	Died	1,100	960	965	940
20	1,075	1,070	705	780	625	—	1,010	—	1,100	980	960	920
21	1,100	1,050	740	770	620	—	1,040	—	1,160	1,010	1,000	1,000
22	1,100	1,035	735	790	630	—	1,020	—	1,155	1,030	1,020	985

TABLE XXXVIII.

EXPERIMENT III.

Bone Analysis Bovines.

Bovine.	Group.	Green Weight.	Green Bone.				Breaking Strength per sq. in. 6 in. span.	Dry fat-free bone.			Percentage Ash of Green weight.
			Percentage Water.	Percentage Fat.	Percentage Dry fat free.	Sp. gr.		Percentage Ash.	Percentage Ca.	Percentage P.	
					FEMUR.						
5154..........	I.......	2098	12·05	34·85	53·1	1·296	2850	66·5	38·7	17·7	35·4
5157..........	II.......	1794	15·84	43·62	40·54	1·142	2650	61·8	39·4	17·1	25·1
5158..........	III.......	1622	14·68	41·92	43·40	1·220	3020	63·1	39·4	17·3	27·4
5160..........	IV.......	1700	14·10	45·50	40·40	1·157	1990	60·2	38·6	17·0	24·3
5153..........	V.......	2288	12·68	37·52	4·980	1·271	3425	65·8	39·6	18·0	32·8
5149..........	VI.......	572	14·00	38·70	47·30	1·211	2990	64·7	38·4	17·4	30·6
					HUMERUS.						
5154..........	I.......	1617	9·20	39·20	51·60	1·274	3080	66·6	38·5	17·9	34·4
5157..........	II.......	1310	13·7	45·8	40·5	1·091	2500	61·0	40·7	17·9	24·7
5158..........	III.......	1239	14·40	43·60	42·00	1·181	2800	63·3	39·4	17·7	26·7
5160..........	IV.......	1343	15·10	45·50	39·4	1·148	2480	60·0	40·0	17·7	23·6
5153..........	V.......	1732	11·50	40·60	47·90	1·211	2700	66·0	39·2	17·8	32·6
5149..........	VI.......	1332	12·36	39·90	47·74	1·211	1950	65·0	39·2	17·7	31·1
					METACARPUS.						
5154..........	I.......	449	11·57	21·80	66·63	1·522	2280	66·6	38·5	17·7	44·4
5157..........	II.......	356	12·37	30·92	56·71	1·368	1800	65·8	38·4	17·7	37·6
5158..........	III.......	345	13·80	23·30	62·90	1·395	1200	65·5	38·7	17·6	41·2
5160..........	IV.......	352	13·50	32·2	54·3	1·375	1375	62·7	39·3	17·9	34·1
5153..........	V.......	443	11·75	23·75	64·50	1·477	2650	67·1	38·9	17·8	43·3
5149..........	VI.......	348	13·55	21·62	64·83	1·402	1725	66·16	38·6	17·6	43·1
					RIB.						
5154..........	I.......	305	22·9	16·8	60·3	1·525	—	63·6	—	—	38·5
5157..........	II.......	220	25·5	27·4	47·1	1·257	—	57·9	—	—	27·2
5158..........	III.......	187	28·8	23·2	48·6	1·390	—	59·6	—	—	29·0
5160..........	IV.......	262	31·2	22·5	46·3	1·312	—	54·6	—	—	25·3
5153..........	V.......	358	23·5	19·7	56·8	1·432	—	62·2	—	—	35·3
5149..........	VI.......	249	28·7	21·8	49·5	1·343	—	61·1	—	—	30·2

NOTE.—The bone measurements were the same as those reported in Experiment II.

TABLE XL.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group I. Bovine 5154.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	3·34	·02	0·57	3	Basal Ration.
2	4·02	3·02	·02	0·98	3	,, ,,
3	10·11	1·88	·03	8·20	10	Expt. Ration.
4	10·39	3·21	·05	7·13	11	,, ,,
5	10·39	4·53	·06	5·80	12	,, ,,
6	10·41	4·14	·05	5·22	12	,, ,,
7	10·42	4·02	·04	6·36	14	,, ,,
8	10·30	4·52	·07	5·71	16	,, ,,
9	10·30	4·21	·04	6·05	17	,, ,,
10	10·86	3·87	·08	6·91	18	,, ,,
11	10·92	2·60	·18	8·14	19	,, ,,
12	11·16	3·72	·08	7·36	20	,, ,,
13	11·08	5·25	·10	5·63	21	,, ,,
		Mean for weeks 3–13				
—	10·49	—	—	6·58	—	—

TABLE XLI.

Calcium Balance (daily average).

Group I. Bovine 5154.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·43	4·05	·62	−0·24	3	Basal Ration.
2	4·52	5·08	·53	−1·09	3	,, ,,
3	22·48	5·52	·20	16·76	10	Expt. Ration.
4	22·54	15·90	·21	6·43	11	,, ,,
5	22·51	13·65	·37	8·49	12	,, ,,
6	22·53	14·20	·38	7·95	13	,, ,,
7	22·69	14·50	·22	7·97	14	,, ,,
8	22·53	9·00	·50	13·03	16	,, ,,
9	22·47	16·00	·41	6·06	17	,, ,,
10	23·72	12·43	·11	11·18	18	,, ,,
11	23·68	13·60	·14	9·94	19	,, ,,
12	23·27	14·20	·06	9·01	20	,, ,,
13	23·54	13·60	·09	9·85	21	,, ,,
		Mean for weeks 3–13				
—	22·91	—	—	9·69	—	—

Table XLII.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group II. Bovine 5157.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	3·26	·02	0·65	3	Basal Ration.
2	4·02	3·30	·03	0·69	3	,, ,,
3	4·29	2·74	·04	1·51	10	Expt. Ration.
4	2·57	2·21	·06	·30	11	,, ,,
5	3·30	3·45	·06	−0·12	12	,, ,,
6	3·41	4·15	·06	−0·80	13	,, ,,
7	3·42	3·03	·11	·28	14	,, ,,
8	2·52	2·42	·11	− ·01	16	,, ,,
9	2·98	3·36	·06	−0·44	17	,, ,,
10	3·71	2·72	·11	·88	18	·53 gm. P supplemented.
11	3·76	2·32	·12	1·32	19	,, ,, ,,
12	3·96	3·19	·16	·61	20	,, ,, ,,
13	3·94	3·43	·24	·27	21	Expt. Ration.
		Mean for weeks 3–13				
—	3·44	—	—	·35	—	—

Table XLIII.

Calcium Balance (daily average).

Group II. Bovine 5157.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·42	4·53	0·52	−0·63	3	Basal Ration.
2	4·50	4·86	0·72	−1·08	3	,, ,,
3	22·64	6·00	5·20	11·44	10	Expt. Ration.
4	14·85	5·28	4·70	4·87	11	,, ,,
5	22·90	13·40	2·40	7·10	12	,, ,,
6	22·91	16·25	5·16	1·50	13	,, ,,
7	23·74	17·20	4·86	1·68	14	,, ,,
8	23·03	17·20	3·50	2·33	16	,, ,,
9	22·50	17·40	1·35	3·75	17	,, ,,
10	24·18	11·65	9·00	3·53	18	·53 gm. P supplemented.
11	24·10	13·50	6·08	4·52	19	,, ,, ,,
12	23·81	14·15	4·41	5·25	20	,, ,, ,,
13	24·08	15·10	6·76	2·22	21	Expt. Ration.
		Mean for weeks 3–13				
—	22·61	—	—	4·38	—	—

Table XLIV.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group III. Bovine 5158.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	3·62	·02	0·29	3	Basal Ration.
2	4·02	3·14	·03	0·85	3	,, ,,
3	3·11	1·66	·02	1·43	10	Expt. Ration.
4	3·39	2·45	·04	0·90	11	,, ,,
5	2·57	2·05	·04	0·48	12	,, ,,
6	2·58	2·07	·05	0·46	13	,, ,,
7	2·58	2·00	·08	0·50	14	,, ,,
8	2·52	3·15	·03	−0·66	16	,, ,,
9	2·52	2·12	·05	0·35	17	,, ,,
10	3·35	2·33	·04	0·98	18	1·07 gm. P. supplemented.
11	3·75	2·28	·04	1·43	19	,, ,, ,,
12	3·89	2·44	·06	1·39	20	,, ,, ,,
13	3·02	2·72	·08	0·22	21	Expt. Ration.
		Mean for weeks 3–13				
—	3·03	—	—	0·68	—	—

Table XLV.

Calcium Balance (daily average).

Group III. Bovine 5158.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·66	4·87	·46	−0·87	3	Basal Ration.
2	4·54	4·30	·54	−0·30	3	,, ,,
3	44·12	27·45	2·56	14·11	10	Expt. Ration.
4	44·20	26·40	3·24	14·56	11	,, ,,
5	44·03	27·70	4·18	12·15	12	,, ,,
6	44·00	26·70	4·83	12·47	13	,, ,,
7	44·52	27·20	6·32	11·00	14	,, ,,
8	43·80	29·50	1·12	13·18	16	,, ,,
9	43·24	33·55	1·65	8·04	17	,, ,,
10	40·82	30·30	5·59	4·93	18	1·07 gm. P. supplemented.
11	46·14	33·25	4·41	8·48	19	,, ,, ,,
12	46·09	31·90	3·72	10·47	20	,, ,, ,,
13	35·36	24·42	3·21	7·13	21	Expt. Ration.
		Mean for weeks 3–13				
—	43·30	—	—	10·58	—	—

Table XLVI.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group IV. Bovine 5160.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	3·21	·02	0·70	3	Basal Ration.
2	4·02	3·58	·02	0·42	3	,, ,,
3	3·11	1·66	·03	1·42	10	Expt. Ration.
4	3·39	2·89	·04	·46	11	,, ,,
5	3·39	1·96	·06	1·37	12	,, ,,
6	2·58	1·91	·06	·61	13	,, ,,
7	3·42	3·01	·04	·37	14	,, ,,
8	3·30	2·98	·06	·26	15	,, ,,
9	3·30	2·75	·10	·45	16	,, ,,
10	3·72	2·49	·07	1·16	17	,, ,,
11	3·78	2·56	·08	1·14	18	,, ,,
12	3·92	2·89	·19	·84	19	,, ,,
13	3·94	3·52	·17	·25	20	,, ,,
		Mean for weeks 3–13				
—	3·44	—	—	0·76	—	—

Table XLVII.

Calcium Balance (daily average).

Group IV. Bovine 5160.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·47	5·18	·63	−1·34	3	Basal Ration.
2	4·54	4·34	·72	−0·52	3	,, ,,
3	4·18	2·87	·41	0·90	10	Expt. Ration.
4	4·18	3·75	·82	−0·39	11	,, ,,
5	4·14	2·56	1·30	0·28	12	,, ,,
6	4·02	3·56	1·29	−0·83	13	,, ,,
7	4·31	3·35	1·26	−0·30	14	,, ,,
8	3·97	3·25	1·49	−0·77	15	,, ,,
9	4·40	3·25	2·10	−0·95	16	,, ,,
10	4·64	2·93	1·92	−0·21	17	,, ,,
11	4·57	3·05	1·35	0·17	18	,, ,,
12	3·91	3·24	·43	·24	19	,, ,,
13	4·20	3·61	·27	·32	20	,, ,,
		Mean for weeks 3–13				
—	4·23	—	—	−0·14	—	—

Table XLVIII.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group V. Bovine 5153.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	2·98	·02	0·93	3	Basal Ration.
2	4·02	3·60	·03	0·39	3	,, ,,
3	11·29	3·05	·04	8·20	10	Expt. Ration.
4	10·39	4·18	·52	5·69	11	,, ,,
5	10·39	2·62	·26	7·51	12	,, ,,
6	10·41	3·82	·16	6·43	13	,, ,,
7	10·42	3·52	·14	6·76	14	,, ,,
8	10·30	5·75	2·05	2·50	16	,, ,,
9	10·30	3·46	2·78	4·06	17	,, ,,
10	10·86	4·52	2·54	3·80	18	,, ,,
11	10·92	3·83	2·18	4·91	19	,, ,,
12	11·16	4·04	1·41	5·71	20	,, ,,
13	11·08	5·85	2·44	2·79	21	,, ,,
		Mean for weeks 3–13				
—	10·68	—	—	5·31	—	—

Table XLIX.

Calcium Balance (daily average).

Group V. Bovine 5153.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·40	4·53	·55	−0·68	3	Basal Ration.
2	4·50	4·30	·61	−0·41	3	,, ,,
3	44·17	22·50	·65	21·02	10	Expt. Ration.
4	44·09	26·30	·20	17·59	11	,, ,,
5	44·18	17·50	·19	26·49	12	,, ,,
6	44·17	27·00	·14	17·03	13	,, ,,
7	44·33	30·30	·65	13·38	14	,, ,,
8	44·37	35·30	·88	8·19	16	,, ,,
9	44·25	33·20	·40	1·65	17	,, ,,
10	46·53	36·16	·48	9·89	18	,, ,,
11	46·46	29·10	·41	16·95	19	,, ,,
12	46·15	35·90	·89	9·36	20	,, ,,
13	46·30	32·60	1·87	11·83	21	,, ,,
		Mean for weeks 3–13				
—	45·00	—	—	14·76	—	—

TABLE L.

EXPERIMENT III.

Phosphorus Balance (daily average).

Group VI. Bovine 5149.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	3·93	2·74	·03	1·16	3	Basal Ration.
2	4·02	3·54	·02	0·46	3	,, ,,
3	11·29	3·48	1·09	6·72	10	Expt. Ration.
4	10·39	4·14	2·80	3·45	11	,, ,,
5	10·39	3·32	1·70	5·37	12	,, ,,
6	10·41	3·94	·96	6·51	13	,, ,,
7	10·42	4·37	2·01	4·04	14	,, ,,
8	10·30	4·83	1·47	4·00	16	,, ,,
9	10·30	4·39	4·08	1·83	17	,, ,,
10	10·86	4·35	4·50	2·01	18	,, ,,
11	10·92	3·37	2·06	5·49	19	,, ,,
12	11·16	3·33	2·30	5·53	20	,, ,,
13	11·08	4·21	4·53	2·34	21	,, ,,
		Mean for weeks 3–13				
—	10·68	—	—	4·29	—	—

TABLE LI.

Calcium Balance (daily average).

Group VI. Bovine 5149.

Week No.	Intake gm.	Outgo gm.		Balance gm.	Month of Experiment.	
		Faeces.	Urine.			
1	4·43	4·27	·72	−0·56	3	Basal Ration.
2	4·51	4·89	·64	−1·02	3	,, ,,
3	4·21	2·85	·17	1·19	10	Expt. Ration.
4	4·11	2·06	·27	1·78	11	,, ,,
5	4·14	2·56	·12	1·46	12	,, ,,
6	4·15	2·82	·19	1·14	13	,, ,,
7	4·39	4·60	·53	−0·74	14	,, ,,
8	4·07	3·00	·26	1·81	16	,, ,,
9	3·93	3·34	·25	0·24	17	,, ,,
10	4·35	2·48	·12	1·75	18	,, ,,
11	4·34	2·73	·16	1·45	19	,, ,,
12	3·87	2·16	·24	1·47	20	,, ,,
13	3·96	2·76	·43	0·77	21	,, ,,
		Mean for weeks 3–13				
—	4·14	—	—	1·12	—	—