STUDIES IN MINERAL METABOLISM

Being a Thesis

submitted by

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Towards the Degree of
Master of Agricultural Science
at Massey Agricultural College
University of New Zealand

1960

ACKNOWLEDGEMENT

The writer wishes to express his sincere thanks and appreciation to Dr. J.C.Hawk, Dr.W.A.McGillivray and Dr.C.R.Barnicoat for their invaluable advice and guidance in the preparation of this Thesis.

In addition the writer is particularly indebted to Professor A.L.Rae and other members of the staff of the College and the D.S.I.R., and to fellow students, who made the animals available and helped in the collection of data; to the Librarian, Miss Mary G.Campbell, and her staff, for the use of Library facilities and for making available references on interloan; to the Dairy Research Institute for photographic work.

Thanks are due to Mr.A.C.Glenday, Statistician, D.S.I.R., Palmerston North, on his unfailing courtesy at all times in the preparation of the statistical data.

Finally, special gratitude is proffered to Mrs.H.Neita of Jamaica for her patience in typing this manuscript.

C O N T E N T S.

GENERAL INTRODUCTION

In 1956, a collaborative project was undertaken by the Plant Chemistry Laboratory and Grasslands Division, D.S.I.R., and the Sheep Husbandry Department, Massey Agricultural College.

The main objective of this project were as follows:-

(a) To examine the possibility that over a long period, ingestion of short-rotation reygrass and certified white clover might lead to iodine deficiency or goitregenic problems in sheep.

(b) To study the chemical composition of rapidly growing ryegrass from the following two points of view:-

(i) To attempt to identify the nutritional causes which give rise to ill-thrift in sheep when grazing on autumn ryegrass and to compare this with their behaviour on rapidly growing spring ryegrass.

(ii) In the event of an outbreak of facial eczema occuring, to study broadly the relationship which this might bear to the chemical composition of the pasture at the time.

The Quarterly Meeting of this group, on March 7, 1958, recorded that for young sheep bled September 4, 1957, the calcium level of the blood was lower in the perennial ryegrass treatments

than in the short rotation ryegrass treatments. The paucity of data on seasonal variations and other differences in the blood level status of calcium, phosphorus and magnesium in the grazing sheep, and under local conditions, is well known.

The present studies were therefore undertaken to observe whether such findings were indicative of the onset of deficiency diseases and malnutrition as a result of mineral insufficiency.

Franklin (1948) found that dietary levels of calcium and phosphorus were reflected in the values for blood serum with respect to these elements in sheep fed vitamin D. These animals, however, were not maintained under normal grazing conditions.

Sobel and his co-workers (1945 a,b; 1948), worked with rats and denoted variations in blood calcium and phosphorus despite the feeding of adequate vitamin D. This gave support to the findings in sheep by Franklin (1948).

The experiments completed in the present studies involved determinations of blood constituents of grazing sheep, and nutritional investigations with rats. From this work it was hoped to obtain a lead as to the factors which influence the differences in calcium, phosphorus and magnesium in the blood of grazing animals. The additional studies with rats sought to correlate the findings of Franklin (1948) and Sobel and his co-workers (1945 a,b; 1948).

Sheep, both on the hill-country and lowlands, are exposed to the widest range of dietary deficiencies during the annual cycle of seasonal changes.

The intakes of calcium and phosphorus, and the ratios of calcium to phosphorus, of foods ingested by sheep on perennial ryegrass and white clover pastures must differ widely. The teeth of sheep on the former(improved) pastures wear rapidly whereas those of sheep on poor hill pastures are often almost unworn(Barnicoat, 1957). As the difference in mineral composition between the two types of pasture is pronounced, it seemed justifiable to investigate the availability of the minerals from such pastures.

The system of blood analysis replaced herbage analysis as a measure of the degree of mineral deficiencies, and thus indicate the inability of herbage to meet physiological needs. Blood values are possibly not dependable as indicators of adequacy of intake or body status of mineral reserves. This is so because minerals such as calcium, phosphorus and magnesium are supplied in the animal body from both bones and the diet, and mingle freely in the bloodstream.

A REVIEW OF LITERATURE

The Calcium and Phosphorus of Blood and Bone

Mineral elements rank with vitamins in their importance to animal nutrition, and among these elements Calcium and Phosphorus are outstanding.

Numerous metabolic disturbances were early associated with deficiency of the minerals, and striking proof of these have been obtained through studies of the inorganic Calcium and Phosphorus in the blood e.g. in various forms of tetany and rickets.

The insights thus gained from studies of Calcium and Phosphorus in the blood were all the more important because blood levels are related to absorption, retention and excretion.

Kramer and Howland(1932) showed interest in the factors which affect the Calcium and Phosphorus concentrations in the blood serum. They reported their observations, made in 1922, which forced them to conclude that dietary Calcium and Phosphorus levels, the ratio of these components, and the amount of vitamin D preformed or produced by irradiation, were the main factors which determined the Calcium and Phosphorus levels in serum. Vitamin D supplied in fish oils, vegetable oils, and butterfat or through ultraviolet radiation was seen to have a balancing effect on the Calcium and Phosphorus concentration of serum. In 1932 these workers provided further evidence of the stabilising role of this vitamin. They analysed the sera of a large number of rats and from this they showed that with inadequate amounts of vitamin D, dietary levels of Calcium and Phosphorus affected serum levels. An antagonism was discerned where an increase in dietary Calcium increased the serum Calcium and depressed the serum Phosphorus.

The opposite effect was obtained when dietary Phosphorus was increased.

TABLE 1 *

	Groups	Sampled in NOVEMBER (Autumn)	Sampled in JANUARY (Winter)	Sampled in MARCH (Spring)	Sampled in JUNE (Summer)
Serum Ca in mg.per cent	Indoor (1Va)	11.45 (10.2-12.75)	12.40 (10.8-13.30)	9.40 (8.70-10.40)	10.70 (9.30-11.80)
	Outdoor (1Vb)	11.45 (10.2-12.75)	10.50 (10.20-10.8)	9.65 (9.20-10.45)	10.20 (9.10-10.75)
Blood in-organic P in mg.per cent	Indoor (1Va)	5.11 (4.39-6.40)	4.98 (4.23-5.72)	6.28 (5.79-6.75)	7.16 (5.63-8.68)
	Outdoor (1Vb)	5.11 (4.39-6.40)	4.89 -	6.72 (5.40-8.13)	7.0 (5.90-9.60)

*Adapted from Auchinachie & Fraser(1932)

Some very interesting findings were made by Auchinachie and Fraser(1932)at the Rowett Research Institute. They fed sheep on a basal diet deficient in calcium but containing excess phosphorus,and supplied supplemental calcium in the form of lime. The vitamin D source was added as cod liver oil. Table 1 is an adaptation of their studies on blood contents of calcium and phosphorus of two groups of sheep,samples being taken in the months of January,March and June. The figures for November as included,are pre-experimental ones taken at random from twenty six of the sheep before they were grouped. They were included as indication of the normal standards. The two groups adapted here were fed the basal diet, plus added lime,plus cod liver oil,and the only difference in treatment was that Group(1Va)was kept indoor and Group(1Vb) kept outdoor.

Compared to the other groups from which dietary lime or cod liver oil were excluded, the above groups emphasised the regulatory role of vitamin D on calcium and phosphorus metabolism. These also showed that with adequate vitamin D different conditions of environment did not affect blood levels to a detrimental extent. The antagonism described by Kramer and Howland(1932) was recognised here, and was referred to as an inverse relationship.

These findings, among others, suggested the existence of integrated biological and physico-chemical factors which preserve physiological equilibrium of serum calcium and phosphorus.

Bethke, Kick and Wilder(1932)agreed with Kramer and Howland, and in their study on rickets, Brown, Shohl, Chapman, Rose and Saurwein(1932)also maintained that in the absence of vitamin D, dietary contents of phosphorus were reflected in sera levels. A range of 10-11 mg. per 100 ml. serum was found for all serum calcium levels by Brown et al.(1932).

In their work on calcium and phosphorus retention in cattle Palmer, Eckles and Schutte(1928)referred to the striking ability of the blood system to maintain a nearly constant level of inorganic calcium in the face of severe negative calcium balances.

Analyses of bone ash show that approximately 50% of the ash is calcium, and it is stated that in adult cows over 98% of body calcium and 87% of body phosphorus are in bones. The purported apatite structure of bones is said to constitute a nucleus of $Ca_2(PO_4)_2$ upon which is adsorbed principally calcium carbonate, but in addition $CaHPO_4$ and $Ca(OH)_2$.

The mineral composition of bone is nearly but not quite constant and nowadays the lability of the skeleton is as frequently emphasised as was its stability in the past.

Bethke,Steenbock and Nelson(1923-24)found,with the phosphorus content of the diet constant,that on increasing the calcium and the fat-soluble vitamin of the diet simultaneously,the calcium content of rat bone increased up to a certain point only, beyond which addition of either or both had no further effect. Lability of calcium in bone was investigated by Bauer,Aub and Albright(1929) when they showed that the administration of the para-thyroid hormone led to diminution of the bone trabeculae of rabbits. A low calcium diet produced a similar effect in adult cats while a high calcium diet caused a storage of calcium in the form of deposits in trabeculae. The studies of Bauer et al(1929)thus proved that the trabeculae serves as a source of calcium,a readily available reserve supply,while calcium in the bone cortex served an apparently structural role. A negative calcium balance decreased the trabeculae and a positive balance increased them,as associated with low and high calcium diets respectively.

Stability in the bone shafts,so important in maintaining the body structure,is imposed upon when the trabeculae are unable to satisfy the demand for calcium during extreme need so calcium is drawn from the shafts(cortex)and rickets and osteomalacia results.

These enquiries were useful pointers to the importance of bone as the normal reserve called upon to provide calcium and phosphorus needed for lactation,and egg production, among other demands,through the process of resorption.

Different bones react differently to resorption and Duckworth(1953)chemically examined individual bones of sheep after pregnancy and lactation on diets low in calcium,and found resorption much more advanced in the vertebrae,ribs,and skull than in bones of the extremities(metacarpals and metatarsals).

Numerous workers support this differential response,and while Lund and Armstrong(1942) reported that periodontal bone is much affected in calcium and vitamin D deficiency, Wentworth, Smith and Gardner(1940)showed differing responses among bones of hormone treated animals.

Relationships between the Calcium and Phosphorus composition Of Blood and Bone.

A theory now held,is that bones regulate the supply of calcium and phosphorus to the body fluids,since with insufficient mineral matter available the maintenance of physiological balance in the blood gets first priority to rigidity of the skeleton - the latter being sacrificed. Some experimental evidence indicates the uncertainty of a direct relationship between changes in blood composition and bone changes.Duckworth and Hill(1953)cited the work of Riddle,Rauch and Smith(1944) who used pigeons and found that medullary bone formation was involved with a rise in serum calcium. This they declared was in contrast with the finding of Bloom,Bloom and McClean(1941) that sera level could be raised without increase in medullary bone. A study of the effect of ovarian hormones on the retention of calcium and phosphorus in pullets was done by Common,Bolton and Rutledge(1948)in a series of experiments,and it was significant that hypercalcaemia of the oestrogenised bird is not of necessity casually related to hyperossification. High serum calcium levels were effected with dosages of oestrogen without causing any increase of calcium retention by the growing pullet.

Common(1948) and his associates obtained contrary results where medullary bone formation occured without high serum calcium.

It is therefore interesting that Sobel, Rockenmacher and Kramer (1945a) reported dietary calcium and phosphorus as influencing both bone and blood serum. This stemmed from experimental evidence of an apparent relationship between the carbonate content of bone and the composition of serum, and Table 2 is an adaptation from this work.

TABLE 2 *

DIETARY GROUP	Ca % in diet	P % in diet	Blood Serum			Fat-Free Bone		
			Ca in mg. per cent	P in mg. per cent	CO_2 in vol. per cent	Ca %	CO_3 %	$\frac{CO_3}{Ca}$
A + vitamin D (Basal) (Low Ca - Low P)	0.03	0.32	8.7	8.9	54.7	11.8	1.8	10.2
B + vitamin D (Basal + 3% $CaCO_3$) (High Ca - Low P)	1.13	0.317	13.3	6.8	57.1	17.0	3.53	13.9
C + vitamin D (Basal + 2.75% anhydrous Na_2HPO_4) (Low Ca- High P)	0.029	0.905	8.8	9.3	52.6	12.5	1.85	9.9

* Sobel et al. (1945a)

The claim was that a high Carbonate to Calcium ratio in bone was found on high calcium-low phosphorus diets, while a low

calcium diet with either sub-optimum or large amounts of phosphorus lowered the carbonate to calcium ratio in bone.

Changes in bone carbonate to calcium ratio were said to be related to serum phosphorus to carbon dioxide ratio and the final link was that dietary calcium to phosphorus ratios and levels influenced serum Carbon dioxide to phosphorus ratios.

Sobel et al(1945b) supported their previous work in other experiments. Here an almost direct relationship between bone phosphorus to carbonate ratio and serum phosphorus to carbon dioxide ratio was stated. Throughout these experiments the more extreme the dietary calcium: phosphorus, the more sharply occuring were changes in serum composition. Vitamin D was noted to prevent these changes by preventing a fall in serum calcium or phosphorus. The theory here is that the final composition of bone depends on that of the liquid phase in contact with it, and dietary calcium and phosphorus is directly related to this.

Further verification of the above finding was offered by Sobel and Hanock(1948)when they related blood serum to the composition of the upper incisor of the white rat, hence to the diet.

Studies with sheep by Franklin(1948)tend to give support to the findings of Sobel et al with rats, that dietary levels of calcium and phosphorus are reflected in blood serum levels of these elements, in animals fed vitamin D. Briefly the results were:-

(a) Calcium deficient rations with a wide calcium to phosphorus ratio readily induced a severe fall in serum calcium.

(b) Addition of suitable calcium supplements prevented depression of serum calcium and restored hypocalcaemic sheep to normal.

(c) Fasting had a temporary depressing effect on serum calcium.

(d) Exercise may further harm hypocalcaemic sheep and cause more reduction in sera levels.

(e) Indoor housing detracted from ultraviolet irradiation effect,and with inadequate dietary vitamin D, normal serum calcium levels could not be maintained even with high dietary intake of calcium and phosphorus. Vitamin D administration,or exposure to sunlight,remedied these subnormal values.

(f) Serum magnesium and inorganic phosphorus values were inconsistent and so were excluded from these papers.

That bone mineral was more readily mobilised to maintain the level of serum calcium,but less readily to maintain that of phosphorus,was very much in evidence. T is further complicated the question of blood and bone relationships,so the advent of the radio-active isotope and the more precise quantitative data made available greatly enhanced the cause of mineral metabolism studies.

Manly and Baker(1939)noted the rapid deposition of labelled blood phosphorus,especially in the spongy parts of rat bones. A rise in radioactive phosphorus was not accompanied by a rise in total blood phosphorus,and the epiphyses showed a greater metabolic turnover than the diaphyses in the long bones. Radioactive phosphorus was less readily removed from the diaphyses. The ever-growing incisors in rats were seen to take up large amounts of phosphorus, and indications were that the bone reservoir provided much of this phosphorus.

Another study by Manly,Hodge and Manly(1940)emphasised the accretion of radioactive phosphorus in the stable portion of bone as the whole bone increases. The ratio of radioactive phosphorus to normal phosphorus in blood and labile bone showed a similar fall at the same time.

CALCIUM AND PHOSPHORUS REQUIREMENTS.

The mineral requirements of animals for growth and the maintenance of health is a basis of nutritional studies, and much work has been done in attempts to complete the relationship of levels of intake, absorption and retention, and excretion.

Early workers analysed the whole bodies of animals, assuming that the calcium and phosphorus content of the body at different stages of development would indicate animal requirements e.g. Lawes and Gilbert (1859; 1883) cited by Duncan (1958), Sherman and MacLeod (1925). With large animals e.g. the ruminants, this proved a costly and formidable endeavour in the face of supplying very crude and inadequate quantitative data, so very few of such analyses were done.

Studies of the whole skeleton and individual bones have helped to establish the fundamental functions of bones with respect to mineral metabolism. Workers in this field include Young (1936), Josland (1937), Underwood (1940), who used total ash to estimate mineralisation; Weidman and Rogers (1950)who compared the ash content of cortical and cancellous bone in the rabbit, rat and the cat; Ellenberger et al. (1950) and their analyses of the skeleton of cattle, as reported by Duncan (1958); and finally Ellinger, Duckworth, Dalgarno and Quencuille (1952), where it was shown that female rats on a diet deficient in calcium lost an average of 32% of the total skeletal ash.

However, a more direct method of assessing animal requirements was found in the balance study, in which the intake of calcium and phosphorus could be compared with the outgo from the animals' body and the difference assumed as the amount retained by the animal.

(a) BALANCE STUDIES.

The balance study method of correlating intake with retention was introduced to work with cattle by Forbes, French and Letonoff (1929), but the balances and intakes did not show adequate relationships over a period of six months, so longer investigations were recommended.

Lindsey,Archibald and Nelson(1931)undertook such an investigation using dairy heifers over a period of three years and they showed the following:-

(a) The total calcium retention was greater in heifers fed the higher calcium containing diets.

(b) The percentage retention showed that the heifers fed the lower intake used this more efficiently.

(c) An apparent limitation of phosphorus retention by the low calcium intake was explained as a natural corollary of the roughly 2:1 ratio of calcium: phosphorus in the skeleton,but Forbes et al(1929) and Otto(1938)do not support this.

A similar experiment by Archibald and Bennett(1935) made manifest the trend that:-

(a) as with calcium,the lower intake of phosphorus was the more efficiently used.

(b) After three years there was no group difference in growth or reproduction performance.

(c) The intake of phosphorus was without significant effect upon the retention of calcium.

Several workers confirmed the above results, and among these were Fairbanks and Mitchell(1936)with their study of calcium retention in young rats. They showed that a low calcium content in the body,caused by subsistence on a poor calcium diet,leads to high retention of calcium when liberally fed. Another conclusion was that growing animals had no maintenance requirements for calcium,the calcium being used in growth and calcification processes. Outhouse,Kinsman,Sheldon,Twomey,Smith and Mitchell(1939) found the same with pre-school children.

Interesting work was done along this line by Rottensten(1938),and he concluded that the degree of calcium "saturation" of the tissues affected the efficiency of calcium utilisation when calcium is fed at levels below the requirement for maximum retention. Duncan(1958)suggested the same theory when she attempted to explain the adaptation to low intake found in steers by Otto(1938).

Very few metabolic studies have been done on the smaller ruminants, but the statistical studies of Axelsson and Erikson, reported by Duncan (1958), and those of Wright (1955), concur with Otto(1938) and others, when they showed that the amount of dietary calcium and phosphorus,and not the dietary calcium to phosphorus ratio,was positively correlated with absorption and retention.

Current estimates of the calcium needs of human adults are based entirely upon balance estimates. On the basis of available balance data 800 mg. per day was chosen as the amount required to provide equilibrium in the average adult. Hegsted,Moscoso and Collazos(1952),however,found that the calcium required for maintenance of balance is simply a reflection of previous dietary intake. He found that Peruvians on low intakes(100-200 mg/day) maintained balance. Work with rats was later shown to support this by Henry and Kon(1953),as they described the relationship between calcium retention and body stores of calcium; so Gershoff et al(1958) finally supplied further verification with dogs.

It is appropriate now to mention excretion findings as depicted by Rottensten(1938). Rats on a low calcium intake excreted only very small amounts of faecal calcium,while those on a high intake lost a large percentage through this medium. A similar relationship held for urinary excretion of calcium.

On the contrary it was observed that on the low calcium dict the phosphorus was excreted mainly in the urine, and on the high calcium diet mainly in the faeces.

Generally, therefore, the greater efficiency of utilisation by animals on lowered intakes, stressed by previous workers, is more clear. Greater storage of calcium and phosphorus by animals on higher dietary levels is placed beyond any doubt.

(b) RADIOACTIVE ISOTOPE STUDIES.

The absorption of calcium contained in the various feeds cannot be directly determined by metabolic balances as the difference between dietary calcium intake and faecal calcium loss. The stool contains calcium secreted along with the digestive juices into the gastrointestinal tract(ENDOGENOUS FAECAL CALCIUM) in addition to the calcium of the food which passes unabsorbed (EXOGENOUS).

Hevesy et al(1939)were the first to use the isotope dilution technique for the estimation of endogenous faecal phosphorus in man, followed by Visek, Monroe, Swanson and Comar (1953)who used it to measure endogenous faecal calcium in cattle, and phosphorus studies in cows by Kleiber, Smith, Ralston and Black(1951). Radioisotope procedures have thus made it possible to separate some of the various aspects of calcium and phosphorus metabolism, and the quantitative estimations of endogenous faecal calcium and phosphorus are important for the determination of true digestibility i.e. efficiency of calcium and phosphorus absorption. In addition, balance data proved an invaluable adjunct for the interpretation of the behaviour of radioactive calcium and radioactive phosphorus in the animal's body.

Investigations concerned with the physiological behaviour of calcium and phosphorus in growing rats maintained on various dietary regimes, by Hansard and Plumlee (1954), indicated the following:-

(a) Current calcium intake was of less influence upon endogenous losses than was the calcium status of the animal at the time of measurement (Hansard et al. 1951). The rats on the lower calcium intake excreted a greater percentage of calcium from their body stores but those on the higher intake excreted the greater quantity of metabolically derived calcium. This supported the earlier work of Kleiber et al. (1951), where radioactive phosphorus was used. Beside increasing the value of the work of Rottensten (1938), the results here also substantiated the latter, among other reports, by showing that body stores of the element affected the relative absorption of calcium from the tract and excretion into the tract.

(b) The efficiency of utilisation decreased with increased dietary calcium intake. Maintenance requirements for calcium varied with the nutritional status of the animals; and this requirement was closely associated with the animal's ability to adapt its metabolic losses to its mineral intake level, which is in agreement with the work of Hegsted et al. (1952).

(c) Rats with low calcium body stores increased their absorption and retention of orally administered radioactive calcium, but retained less radioactive phosphorus. This showed that inadequate calcium affected the absorption and retention of phosphorus, an

aspect previously recognised by Lindsey et al(1931) with heifers. Evidence is evinced as to the separate metabolic behaviours of absorbed calcium and phosphorus, and the subsequent re-excretion of radioactive phosphorus by way of the kidneys,here,relates to the excretion studies of Rottensten (1938).

(d) The soft tissues are maintained by the bone minerals, practically independently of dietary calcium and phosphorus,as was previously observed by Mitchell and Curzon (1939),Hansard et al.(1950,1951),cited by these workers.

(e) The intake of phosphorus was also without effect upon calcium utilisation in rats,which confirmed the earlier work of Archibald and Bennett(1935).

FACTORS AFFECTING CALCIUM AND PHOSPHORUS REQUIREMENTS.

(1) AGE AND GROWTH.

Knowledge of the trend of the calcium and phosphorus contents of the body is fundamental to an assessment of requirements, as the animal grows and develops,and allied to this is the modification that age imposes upon requirements after growth and development have been completed.

Forbes and Keith (1914),reported by Duncan(1958), subscribed to the skeletal changes involved in the statement: "The changes in the appearance of bones as an effect of age are so characteristic that anyone who is acquainted with beef recognises at once the soft vascular bones of young cattle and the white ,flinty bones of old animals".

A study of the calcium content of normal rats at different ages was done by Sherman and Macleod (1925),and they showed the very rapid increase in body calcium that occured during normal growth and development.

Analyses of calves by Hogan and Nierman (1927),of growing beef cattle by Moulton et al(1922), and of foetal to adult stages in cows dealt with by Ellenberger et al(1936;1950),as reported by Duncan(1958), and studies of Manly et al.(1940), provided excellent support for this fact in relation to calcium and phosphorus.

It is natural that the skeleton should reflect most of this rapid increase in body calcium and phosphorus,since this is commensurate with its function as the principal body stores.

Skeletal accretion involves a process of progressive calcification as the animal grows and develops,and numerous experimenters have shown that the rate of calcium absorption decreases with this process e.g. Fairbanks and Mitchell(1936), Rottensten (1938), Henry and Kon (1947). Growth and development is completed when the adult stage is reached,and here this progressive calcification is curtailed and excretion balances the intake.

Requirements over the pre-adult stages thus supersede the maintenance requirements of the fully developed animal,and work on rats and children by Mitchell and Curzon(1939),as reported by Holmes(1945),and Outhouse et al(1939),which emphasise this,also tend to give way to Fairbanks and Mitchell(1936) who concluded that in growing animals there is no requirement for maintenance and the calcium in the diet is used solely for growth purposes.

With advancing age,there is increased katabolism which leads to loss of bone salts(see e.g. McCay,Crowell and Maynard (1935),Henry and Kon,1947),and intake must now be geared to meet this and other endogenous losses of calcium and phosphorus to keep the animal in equilibrium. Endogenous loss thus denotes maintenance requirement,and the finding that no retention occured in adults,unless previously on a poor calcium intake(see Henry and Kon,1947, and 1953; Gershoff et al,1958) enunciates this. The significance of the previous dietary intake,already referred to,

is now quite obvious,and its role,in designating the ultimate maintenance requirement of an animal,emphasises the very variable aspects in formulating adequate intake for animals.

This argument is further aided by the fact that in young animals efficiency of absorption of calcium at any one age depends on the degree of saturation of the body - retention being better when the stores are low(Fairbanks and Mitchell,1936; Rottensten,1938; Outhouse et al,1939).

Finally,simultaneous chemical and radioistope balance and other studies by Hansard et al,(1951,1954,1957)and Hansard and Crowder(1957),showed that the calcium absorption,retention and excretion were greatest in young animals,decreased rapidly to the age of sexual maturity and more slowly to maturity and old age.

The daily endogenous faecal losses of calcium and the maintenance requirements increased slightly to maturity and became markedly so in the aged animal. It was noted that phosphorus had a constant relationship to calcium at all ages,as had Henry and Kon(1953).

(2) PREGNANCY AND LACTATION.

The female of the species has the responsibility of supplying nutrients to the young IN UTERO,so it was natural that Sherman and Macleod(1925)should find that the body calcium content of reproducing female rats was lower on a percentage basis than in females that had not raised young.

Insufficient calcium intake led to withdrawal of calcium from maternal skeleton to meet foetal requirements in pregnant sows studied by Evans(1929),and in supporting this,Bodansky and Duff(1941) stated that pregnancy alone did not impose a severe drain on the maternal skeleton(see also Boelter and Greenberg,1943; Ellinger et al,1952).

Species differences were also inferred regarding the degree of severity possible, since skeletal drain was greater in the sheep where the foetus is a more serious competitor for meagre calcium supplies, than in rats.

It is therefore generally agreed that lactation creates the more severe strain as to calcium and phosphorus demands on the maternal organism. In her review Duncan(1958) emphasised the definite nature of this by showing that negative calcium and phosphorus balances were found in well-fed dairy cows during early lactation, by prominent workers (and in rats - by Goss and Schmidt, 1930; Warnock and Duckworth, 1944). Lactation caused very severe bone mineral loss in rats reproduced on a very low calcium diet (Boelter and Greenberg, 1943) and severe resorption was evident on an apparently normal diet, due to lactation (Ellinger et al, 1952). As lactation progressed, however, a positive balance was regained and previous bone mineral losses were made good in the later stages of lactation, during the next gestation, and especially in the dry period (Ellinger et al. 1952; Benzie, Boyne, Dalgarno, Duckworth, Hill and Walker, 1956; Huffman et al. 1930a, b; Forbes, 1935, Ellenberger et al. 1931, 1932, 1936, - reported by Duncan, 1958).

Forbes et al (1935) considered that the calcium of bone was more available for milk formation than that of the diet and hence doubted the use of supplementary calcium for lactation purposes. This is in disagreement with Huffman et al. (1930) who showed a more efficient calcium utilisation during heavy milking, and the tendency for high-producing cows to utilise dietary calcium more than low-producing cows (see also Ellenberger et al, 1931; 1932, cited by Visek et al. 1952).

The balance of evidence favours the work of Hoffman et al.(1930),as maintained by Visek,Barnes and Loosli(1952), Luick,Boda and Kleiber(1957),and Duncan(1958),and the dietary requirements of calcium and phosphorus are positively related to the cycles of pregnancy and lactation animals undergo.

(3) EFFECT OF CONTENT AND AVAILABILITY IN PASTURES AND SUPPLEMENTARY FEEDS.

The value of a feed as a source of calcium and phosphorus depends not only upon its content of these minerals, but also on the amount that the animal can extract and retain for its own use. Armstrong,Thomas and Armstrong(1957)studied the availability of calcium in Perennial ryegrass,Cocksfoot and Timothy fed to rats,and stated that availability was greater in Timothy and Perennial ryegrass than in Cocksfoot. High faecal calcium values were associated with the lower availability found in Cocksfoot,an aspect which reaffirmed earlier findings of Armstrong,Thomas and Horner(1953)and Armstrong and Thomas(1952), beside the fact that legumes and herbs showed superior calcium availability as compared to the grasses. There were indications that fibre content and oxalic acid content deterred ease of availability, yet the general conclusion was that the total content of available calcium would be adequate enough to prevent deficiency when any species of the nine grassland plants they evaluated is fed to an animal.

Coop,Darling and Anderson(1953)found that although minimum levels of calcium and phosphorus are sometimes reached in some hill-country pastures,the annual concentrations of these minerals are probably adequate.

Animal intake will thus vary with seasonal fluctuations

in pasture content of calcium and phosphorus (Sears et al. 1953), as this governs the ultimate satisfaction of their requirements. Such a variation is authenticated in the statement of Barnicoat (1957) that the intakes of calcium and phosphorus by grazing sheep in New Zealand show wide differences. Kemp and 'T Hart (1957) and Dijkhoorn and 'T Hart (1957) have shown that increase in temperature from 10° to 20°C increased the cation content of pasture plants, and proposed a possible correlation with the incidence of grass tetany in dairy cows. However, much more research is required along similar lines to clarify such relationships.

The climatic influences are able to produce changes in the chemical composition of herbage through fluctuations in the ratio of grass to clover and the seasonal changes in leaf levels (Sears et al. 1953).

Hansard, Crowder and Lyke (1957) noted slightly greater efficiency of utilisation of inorganic sources of calcium than from alfalfa, lespedeza or orchard grass hay, among cattle. Long, Tillman, Nelson, Gallup and Davis (1957) did not find any differences in the phosphorus available to beef cattle from steamed bone flour, Curacao Island phosphate and dicalcium phosphate, used as mineral supplements. These efforts are supplemented by Tillman, Brethour and Hansard (1959) when their results suggested that phosphorus requirements for body weight gain and feed response (in Hereford steers) was greater than for bone growth or maintenance of plasma inorganic phosphorus level. Feed consumption was related to the phosphorus content of rations and they proffered this as a criterion of phosphorus functions.

Notwithstanding the fact that pasture content of calcium and phosphorus appears to be adequate for the needs of the animal, deficiency has been found to occur(Ewer and Bartrum,1948).

Factors suggested as the cause of inavailability in such circumstances include the lack of vitamin D (Fitch,1943),and the carotenoid which functioned as an anti-calcifying agent(Grant,1953). Again,a wide variety of plant foodstuff consumed by farm animals have much of their phosphorus organically bound in a hexaphosphoric acid ester of inositol called phytic acid. It occurs as salts of calcium,magnesium,potassium,etc., the complex being referred to as phytin.

Phosphorus in such form is poorly utilised by non-ruminants (Lowe and Steenbock,1936;McCance and Widdowson,1935;Krieger,Bunkfeldt and Steenbock,1940;Gillis,Norris and Heuser,1949,1953;Harris,1955; Gillis et al.1957),but seems to be utilised by ruminants(Reid,Franklin and Hallsworth,1947)through a process of hydrolysis in the rumen. Tillman and Brethour(1958)verified the latter aspect with their isotopic work on wether sheep,and also provided evidence of the retarding effect of excess dietary fat on the utilisation of dietary calcium.

Non-ruminants afforded some early examples of the depressing effect of aluminium on the availability of phosphorus(Cox,Dodds,Wigman and Murphy,1931;Street,1942),and a reduction in phosphorus absorption was suggested(MacKenzie,1931). Another element, zinc,was purported to decrease phosphorus assimilation (Sadasivan,1952)and calcium utilisation(Tucker and Salmon,1955;Hoekstra et al.1956). Thompson, Hansard and Bell(1959)therefore studied the effects of dietary aluminium and zinc upon the physiological availability of calcium and

availability of calcium and phosphorus in the rations for lambs, using radio-calcium and radio-phosphorus. They found that aluminium did not impair the retention of calcium or phosphorus in the ruminant, and thus justified its use as an alleviator for fluorine poisoning in such animals. Dietary zinc, on the other hand, was seen to decrease the retention of phosphorus and the gastrointestinal absorption of calcium.

(4) VITAMIN D AND THE PARATHYROIDS.

Reference has already been made to the stabilising role that vitamin D maintains on serum calcium and phosphorus, and its use in the remedy of hypocalcaemia. Nicolaysen (1937) presented indications that the vitamin permitted a greater absorption of calcium from the intestine of rats, which in turn caused increased absorption of phosphorus. An associated decrease in parathyroid activity was also alleged.

The conversion of organic phosphorus to inorganic phosphorus in bones, cited by Cohn and Greenberg (1939), is aided by the view of Greenberg (1945), that vitamin D exerts a direct effect on the mineralisation of bone in rachitic animals. Of course, the literature is abundant regarding the use of vitamin D in the prevention and cure of rickets (McCollum et al. 1921; Steenbock and Black, 1924; Otto, 1938; Fitch and Ewer, 1944; Andrews and Cunningham, 1945; Smith, 1957), and it is now quite clear that the vitamin effects control over the calcium metabolism of blood and bone, with strong pointers to a less direct control over the allied metabolism of phosphorus (Smith and Spector, 1940; Nut. Revs., 1958).

Pasture feeding animals derive their vitamin D from the precursor ergosterol ingested in feeds, the latter being converted by the action of ultraviolet radiation of the sunlight (Barnicoat, 1957).

Fluctuations in herbage content of the precursor, due to seasonal variations of pasture composition, and the availability of sunlight, will thus govern the amount of vitamin D the animals obtain (Franklin, 1948). The anti-calcifying factor referred to by Grant (1953) implied an anti-vitamin D effect, and McGillivray (1952) pointed to the dependence of the efficient utilisation of fat-soluble vitamins on the anti-oxidant properties of vitamin E.

The parathyroids also effect control on calcium metabolism (Greenwald, 1926; Selye, 1932; Campbell and Turner, 1942) and hyperparathyroidism promote a high serum calcium and dissolution of this element from the bones (Bodansky and Jaffe, 1931; Greenberg and MacKey, 1932). Greenwald (1926) observed that phosphate retention always followed parathyroidectomy, in addition to the reduced levels in serum calcium noted by other workers.

The glands do not seem to control the absorption of calcium and phosphorus as does vitamin D, but the increased urinary excretion of phosphorus in animals treated with parathyroid extract is supported by the radio-isotope study of Tweedy and Campbell (1944).

It is doubtful as to the relationship between vitamin D and the parathyroids, and there is no evidence to refute this since, for example, vitamin D cures rickets while the parathyroids extracts do not. Therefore, in spite of their common relation to calcium and phosphorus metabolism, they

both show essentially dissimilar actions.

The mechanism of the chemical activities of vitamin D and the parathyroid hormones still require elucidation.

(5) THE ROLE OF MAGNESIUM.

Magnesium is closely allied to calcium although it does not replace it, and the magnesium content of bones is said to range from 0.5 - 0.7 per cent(Morgulis, 1931).

Studies on bones have suggested an inverse relationship with calcium(Hammett, 1921) and removal of the parathyroids have been seen to increase the magnesium content of bone ash. Duckworth, Godden and Warnock(1940) discovered that severe magnesium deficiency in the young rat promoted the release of more bone magnesium to the soft tissues, especially in a calcium deficient diet(Duckworth and Godden, 1941).

Restoration of dietary magnesium levels reflected a relatively slow skeletal acquisition(Duckworth and Godden, 1943), so it is quite questionable as to the lability of skeletal stores in the adult animal.

Low serum magnesium has been reported in conditions of rickets and grass tetany in cows (Sjollema, 1932; Duncan, Huffman and Robinson, 1935), and high magnesium intakes caused slight changes in the serum and body content(Cunningham, 1933) - contrary to Huffman et al, (1941), and others. Yet, the factors which control variations in the blood level remain unknown.

It is to be expected that with the rather fragmentary knowledge of magnesium metabolism available, very little is known regarding ruminant requirements of this element. Huffman, Conley, Lightfoot and Duncan (1941) found that growing calves utilised magnesium in natural feeds much better than as magnesium salts, and concluded that hypomagnesaemia in calves cannot be directly correlated to low dietary magnesium since such a relationship does not always hold. It is also of interest that Allcroft (1947) reported the tendency for hypocalcaemia to be associated with hypomagnesaemia in cows, but Smith (1957) did not support this when the administration of vitamin D restored only the hypocalcaemia to normal.

Balch, Head, Line, Rook and Rowland (1956) carried out balance trials with milking cows, and found that the availability of magnesium in young spring grass varied between 9 - 28%, and frequently fell below the more adequate levels provided by stall rations during winter feeding.

The fact that spring herbage contained a lower magnesium content of the dry matter than winter rations, was further maintained by Rook and Balch (1958). Spring herbage induced a progressive development of hypomagnesaemia in dairy cows, following the change from winter feed. Urinary magnesium excretion fell rapidly at the same time, while serum magnesium showed some decrease, not falling below 1.8 mg. per 100 ml. of serum except in cases where urine magnesium fell to zero or to a very low level. The magnesium content of the herbage was unaffected by the stage of growth.

A renal threshold for magnesium, of the order of 2.0 mg. per 100 ml. of blood serum, was suggested (Rook, Balch and Line, 1958), and the relationship between blood serum level of magnesium and the excretion of magnesium was thus pointed out. These workers also observed that with sufficient magnesium in the diet the body retention of the element appeared to be independent of dietary supply of available magnesium, but was dependent on the rates of body retention of calcium and nitrogen.

Rook and Balch (1958) emphasised the difficulty of establishing the extent of variations in the availability of herbage magnesium, although they proffered the indication that many swards possess a lower availability than is typical of winter rations. The factors which cause the lower availability of herbage magnesium remain unknown.

From the balance of evidence it is clearly demonstrated that magnesium and calcium operate very differently in the body, and there are no interrelationships yet shown which depict a positive or negative affect of magnesium on the calcium and phosphorus requirements of farm animals.

The normal herbage intakes quite probably satisfy animal requirements of magnesium for their physiological needs, except in the case of insufficient feed being available or of deficient pastures on magnesium deficient soils.

SUMMARY.

From a summary of this review, there is no doubt that with the absence of vitamin D, the dietary levels of calcium and phosphorus are reflected in the blood contents

of these elements. Again the stabilising role of vitamin D in relation to these elements has been shown.

Evidence of changes in blood levels due to dietary fluctuations, in animals fed adequate vitamin D, seems in doubt, since most findings do not point to this. Furthermore, with adequate vitamin D animals on a low dietary intake of calcium and phosphorus appeared to mobilise bone reserves in order to maintain blood levels.

Blood levels of calcium and phosphorus do not indicate the direction in which these minerals are flowing, and high blood levels could indicate the flow of calcium and phosphorus from bones to faeces and urine, or from intestines to bones. Low serum levels could also show rapid deposition in bones, rather than dietary deficiency.

Using blood levels as an index of the calcium, phosphorus and magnesium status of the animal, with other things equal, is therefore quite questionable. This is made more obvious from the numerous factors which affect the animal requirements of calcium and phosphorus, especially in grazing animals under natural conditions.

PART 1

THE AVAILABILITY OF Ca, P, and Mg, FROM HIGH-YIELDING PASTURES, AS INDICATED BY BLOOD STUDIES ON GRAZING SHEEP.

SECTION 1

EXPERIMENTAL

(a) Introduction of Sheep Experiment.

The investigation was undertaken with the purpose of determining the levels of blood calcium, phosphorus and magnesium of sheep used in the collaborative project being done by the Plant Chemistry Laboratory and Grasslands Division, D.S.I.R., and the Sheep Husbandry Department, Massey College.

TABLE 3.

Pasture Treatments	Mean value Serum Ca in mg. per cent	Range	No. of Animals.
Perennial ryegrass	8.3	5.8 - 10.0	15
Short-rotation ryegrass	9.1	6.8 - 11.6	17
P. ryegrass and White Clover	6.9	5.8 - 8.0	7
S.R. ryegrass and White Clover	9.8	9.0 - 10.9	6

Table 3 is adapted from a report on the results of the project, and indicates that the calcium level of the blood was lower on the perennial ryegrass treatments than on the short-rotation ryegrass. The body weight gains of the animals were in a similar order.

In the present experiment it was therefore intended to denote the possible effect that different pasture herbage and associated climatic factors could have on the blood levels of calcium, phosphorus and magnesium throughout the year and especially during the late-winter-early-spring period when the above findings were made.

(b) Plan of Experiment.

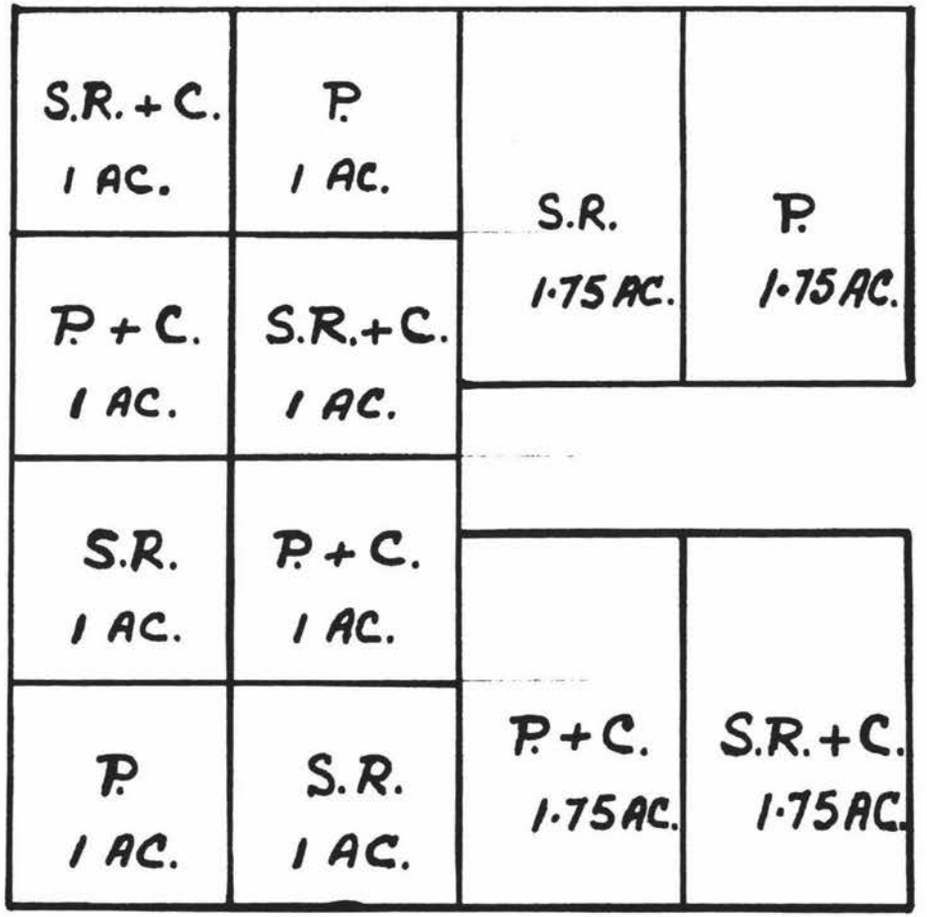
The experimental plots used were those involved in the collaborative project and located on the sheep farm at Massey College. The four pasture treatments were as follows:-

(i) Perennial ryegrass(P.)
(ii) Short-rotation ryegrass (S.R.)
(iii) Perennial ryegrass and White Clover (P.+ C.)
(iv) Short rotation ryegrass and White Clover(S.R.+ C.).

Romney sheep of similar age and sex were the animals used, and they were allocated to the various treatments by a process of randomisation.

Eight animals were selected at random from each treatment for blood sampling, and the first sampling was done on 4/12/58,from ewes put on these treatments on 10/5/57. These animals completed their period of experimentation in the collaborative project and were replaced by a flock of ewe lambs,introduced on 15/1/59. The lambs were sampled 16/1/59, and the subsequent aim was to follow the blood levels of these animals throughout the year. The only treatments made available were (i), (ii) and (iv). However,an outbreak of facial eczema occurred on the perennial pastures and all animals were immediately slaughtered. A few samples were taken from the treatments (i) and (iv) on 24/3/59 to detect a possible variation from the normal.

Ewe hoggets replaced the slaughtered lambs and were put on the plots on 16/5/59. These were sampled on 12/6/59 and 24/9/59,and with a sudden outbreak of rickets on treatment(ii) another was done on 14/10/59, and analysed for calcium only.



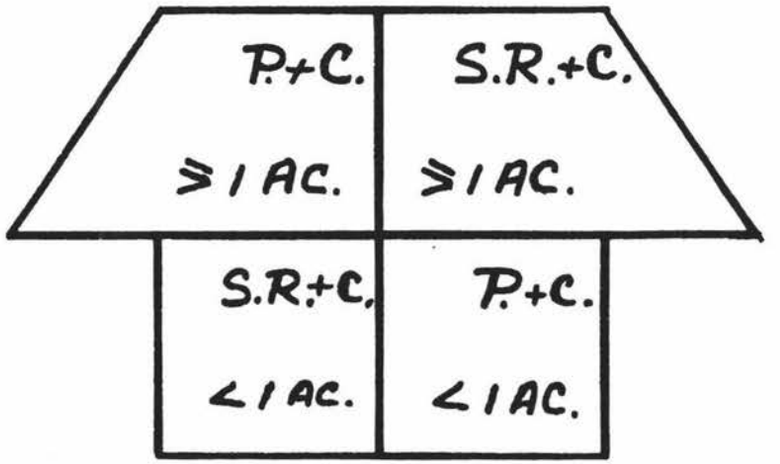


FIG. 1 :– THE LAY-OUT OF THE FOUR PASTURE TREATMENTS.

Perennial ryegrass (P.)
Short-rotation ryegrass (S.R.)
Short-rotation + White Clover (S.R.+C.)
Perennial + White Clover (P.+C.)

Herbage samples were taken during the experiment, and these were collected at random by cutting at "sheep-grazed" height. These were analysed for calcium and phosphorus. Observations were made on the conditions of the pastures as to their adequacy throughout the year, and gains in individual body weight were also followed.

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(c) Notes on Animal Management.

The animals were set-stocked in groups on the different treatment blocks. Whenever the pasture growth seemed excessive other sheep were brought in to maintain adequate control. These were taken off as soon as this was achieved.

The experimental paddocks were not supplied with lime during the experiment. Animals were ear-tagged to ensure their identity, and thus make it possible to use the same individuals of a group in successive samplings. Fig. 1 gives a layout of the different treatments on the three paddocks involved.

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(d) Methods.

(i) The Collection of Blood.

Blood samples were collected by puncturing the jugular vein with a hypodermic needle and allowing the blood to flow into test tubes. Test tubes to which had been added an aliquot of oxalate were used to collect blood for the estimation of inorganic

phosphorus. These were prepared by spreading two drops of saturated ammonium oxalate around the bottom of the test tube, drying this in an oven, and finally breaking up the oxalate with a fine rod. This quantity of ammonium oxalate served as anticoagulant for the 5 ml. of blood usually taken. Immediately after the sample was drawn it was mixed with the oxalate.

For the estimation of serum calcium and magnesium 10 ml. of blood were taken from each animal.

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(ii) The Determination of Blood inorganic phosphorus.

1) The Preparation of Samples for analysis:-

2 ml. of the oxalated blood, collected as above, were pipetted into a test tube containing 6 ml. of 13.3% trichloracetic acid. The pipette was once again filled with 2 ml. distilled water and the water slowly drained into the test tube. Trichloracetic acid was used to precipitate the blood proteins. After vigorous shaking the mixture was allowed to stand for a few minutes, and thenfiltered.

(2) The Estimation of Blood inorganic Phosphorus:-

Duplicate determinations of phosphorus were made on the filtrate by the method of Fiske and Subbarow(1925).

(iii) The Determination of Serum Calcium.

(1) The Preparation of Samples for analysis:-

The clotted blood was broken up into as fine a consistency as possible and then centrifuged for four minutes to separate the serum.

(2) The Estimation of Serum Calcium:-

These were done after the method of Clark and Collip(1925). Duplicated samples were analysed.

.....

(iv) The Determination of Serum Magnesium:-

(1) The Preparation of Samples for analysis:-

The supernatant fluid from the estimation of serum calcium was poured off into a dry test tube and put aside for the estimation of serum magnesium.

(2) The Estimation of Serum Magnesium:-

Duplicate estimations were done by the method used by Godden and Duckworth(1935).

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(v) The Determination of Calcium and Phosphorus in Herbage Samples.

(1) The Preparation of Samples for analysis:-

The samples of grass taken were weighed, then dried at 70°C in a ventilated oven for 24 hours, cooled, and finally grounded to a fineness equivalent to passage

through a sieve of 1 m.m. holes. The grounded material was stored in air-tight containers before chemical analysis.

Immediately before analysis, 2 g.samples were held at 100°C for 16 hours and after cooling were weighed. The difference from the original weight represented the hygroscopic moisture content,and was expressed as a percentage. Samples were later ashed, ashing being completed in a muffle furnace at about 600°C for 3 - 4 hours. The silica dishes containing the ash were cooled in a dessiscator,later weighed, and the ash weight expressed as a percentage.

10 ml. distilled water and 10 ml. 2N HCl were added to the ash in the silica dish and the contents heated on a boiling water-bath for twenty minutes. After filtration through a Whatman No.40 paper into a 100 ml. standard flask,the ash precipitate and paper were thoroughly washed with hot water,cooled to room temperature and made up to the volume with distilled water.

(2) The Estimation of Calcium:-

A 20 ml. aliquot of the ash extract was transferred to a 250 ml. beaker, and approximately 150 ml. distilled water and 2 drops of methyl red were added. The content of the beaker was first made alkaline with 25% NH_4OH, then acid with 10% acetic acid, and brought to boiling over a Bunsen and 10 ml. of boiling saturated ammonium oxalate added. During precipitation of the calcium oxalate the beaker was left on a hot plate maintained at 40°C for one hour.

Final precipitation took place over night. The next day the precipitate was filtered through a Whatman No.40 filter paper, and washed free of soluble oxalate with hot distilled water and hot 2% NH_4OH. Washing was continued until no precipitate occured with a dilute solution of silver nitrate and nitric acid. Precipitate and filter paper were transferred back to the original beaker and approximately 200 ml. distilled water and 10 ml. of (1: 1) H_2SO_4 added. Titration was finally done using 0.1N $KMnO_4$, the end-point being determined at 55-60° C.

$$1 \text{ ml. } 0.1N\ KMnO_4 = 0.0028 \text{ g. CaO.}$$
$$= 0.0028 \times \frac{40}{56} \text{ g. Ca.}$$

(3) The Estimation of Phosphate:-

A Gooch crucible was prepared by forming a thin mat of asbestos over a small circle of Whatman No.1 filter paper, and sucking this as dry as possible by strong suction on a water-pump. Washing with acetone followed and then another process of sucking dry at the pump for two to three minutes. The crucible was put in a dessicator and left over-night, then weighed the next day.

10 ml. aliquot of the ash extract was transferred to a 100 ml. beaker, 5 ml. concentrated HNO_3 added, and the contents evaporated on a steam-bath. To this was added 30 ml. of an acid reagent (50 ml. conc. H_2SO_4 - Sp. Gr. 1.84, 350 ml. conc. HNO_3 - Sp.Gr. 1.42, 760 ml. distilled water) and the content brought to incipient boiling over a water bath. After cooling for one minute 30 ml. of molybdate reagent (Lorenz method - see below) was added while

stirring. A further two to three minutes was allowed before more stirring was done, and the beaker was then set aside over-night. Next day the precipitate was filtered through the Gooch crucible, 2% ammonium nitrate being used to remove adhering precipitate from the sides of the beaker. The precipitate was washed three times with acetone, and the crucible transferred to a dessicator over-night, and weighed next day.

The precipitate contains 3.295% P_2O_5.

P_2O_5 content x $\frac{62}{142}$ = P content.

Molybdate Reagent (Lorenz Method)

100 g. pure dry $(NH_4)_2SO_4$ were dissolved in 1 litre HNO_3 (791 ml. conc. made up to 1 litre). Next 300 g. pure dry ammonium molybdate were dissolved in hot water, cooled, made up to 1 litre, and thoroughly mixed. The latter solution was poured in a thin stream with constant agitation into the solution of ammonium sulphate, and then allowed to stand for two days. The reagent was then filtered through hardened filter paper, and stored for use.

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SECTION 2

RESULTS

This project was complicated by the fact that it was not possible to confirm any treatment variations initially. It was only toward the end that conditions necessary for blood differences appeared. This later effect pointed to irregularities in natural conditions as possible factors which promote fluctuations in the blood calcium and phosphorus of the grazing animal. There was a strong suggestion that adequate synthesis of vitamin D was important to the maintenance of normal blood levels of calcium in the grazing sheep.

Tables 3,4, and 5 show the results obtained from the blood analyses of grazing sheep, while Table 6 presents the measurements of average body weights for these animals. These results are expressed as the mean or average for each treatment, and the ranges of values are given in brackets. All results were subjected to statistical analysis, and summaries of the analyses of variance, and means and standard errors (S.Es) are given in Tables 7 - 11.

Tables 12(a) and 12(b) show values of the calcium and phosphorus composition of herbage procured from some of the experimental treatments used, and from the Manawatu as a whole.

TABLE 3

VALUES FOR BLOOD INORGANIC P, GIVEN IN mg. PER CENT

(Mean values are given, range in brackets)

TREATMENTS	Ewes	Lambs		Ewe Hoggets		
	Sampled 4/12/58	Sampled 16/1/59	Sampled 24/3/59	Sampled 12/6/59	Sampled 24/9/59	
. P.	6.2 (5.2-7.8)	9.1 (8.1-10.2)	7.3 (7.0-8.3)	5.2 (3.7-6.0)	4.6 (3.4-5.5)	
. S.R.	5.8 (4.8-6.5)	8.8 (7.4-10.2)	-	4.7 (3.0-5.7)	4.6 (3.2-7.9)	
P + C.	5.1 (3.3-7.7)	-	-	5.1 (4.4-5.9)	5.3 (3.7-7.3)	
S.R. + C.	5.6 (3.5-7.5)	8.3 (7.4-9.6)	6.8 (5.3-7.8)	4.8 (3.6-5.5)	5.3 (4.0-6.4)	

TABLE 4

VALUES FOR SERUM CA, GIVEN IN mg. PER CENT

(Mean values are given, with range in brackets)

TREATMENTS	Ewes	Lambs		Ewe Hoggets		
	Sampled 4/12/58	Sampled 16/1/59	Sampled 24/3/59	Sampled 12/6/59	Sampled 24/9/59	Sampled 14/10/59
P.	11.1 (10.7-12.1)	12.4 (11.7-14.2)	10.0 (9.6-10.8)	10.1 (9.5-10.9)	8.7 (7.7-9.6)	8.7 (6.6-11.3
S.R.	10.9 (9.7-11.7)	12.4 (11.2-13.2)	-	9.8 (9.3-10.9)	8.4 (7.4-10.1)	8.8 (6.3-10.3)
P + C.	10.8 10.0-11.5)	-	-	9.6 (9.3-10.1)	8.7 (7.1-10.5)	9.3 (7.7-10.3)
S.R. + C.	10.9 (10.0-11.9)	12.5 (11.4-13.2)	10.3 (9.7-10.7)	9.7 (9.3-10.1)	9.6 (8.6-10.4)	10.1 (9.2-10.8)

TABLE 5

VALUES FOR SERUM Mg, GIVEN IN mg.PER CENT

(Mean values are given, with range in brackets)

TREATMENTS.	Ewes Sampled 4/12/58	Lambs Sampled 16/1/59	Lambs Sampled 24/3/59	Ewe Hoggets Sampled 12/6/59	Ewe Hoggets Sampled 24/9/59
P	2.5 (2.2-2.9)	2.8 (2.6-3.0)	2.4 (2.3-2.8)	2.4 (2.3-2.8)	2.5 (2.1-2.6)
S.R.	2.6 (2.4-2.8)	2.7 (2.3-3.1)	-	2.7 (2.5-2.9)	2.4 (1.9-2.7)
P + C.	2.6 (2.1-3.1)	-	-	2.7 (2.3-3.1)	2.5 (2.1-2.7)
S.R.+ C.	2.6 (2.2-3.0)	2.9 (2.4-3.2)	2.5 (2.3-2.8)	2.4 (2.2-2.7)	2.5 (2.4-2.7)

TABLE 6

AVERAGE AND RANGE OF BODY WEIGHTS ON THE DIFFERENT TREATMENTS

TREATMENTS.	Ewes sampled 4/12/58		Ewe Hoggets first sampled 12/6/59	
	First weighed 19/2/58 (lbs)	Last weighed 15/12/58(lbs)	First weighed 12/6/59 (lbs)	Last weighed 24/9/59 (lbs)
P.	93.2 (83 - 105)	97.2 (69 - 115)	73.5 (58 - 78)	83.4 (59 - 97)
S.R.	99.4 (81 - 113)	115 (97 - 138)	68.1 (62 - 75)	79.6 (60 - 101)
P + C.	85.5 (80 - 94)	119.7 (106 - 134)	72.1 (61 - 92)	80 (70 - 102)
S.R. + C.	135 (116 - 146)	136 (104 - 159)	78 (69 - 90)	95.4 (87 - 117)

TABLE 7

SUMMARY OF THE ANALYSES OF VARIANCE OF (a)BLOOD INORGANIC P, (b)SERUM Ca, (c)SERUM Mg, AND (d) GAINS IN BODY WEIGHTS, FOR EWES SAMPLED 4/12/58.

Source of Variance	d.f.	Mean Squares and Results of F Tests. (a) P	(b) Ca	(c) Mg.	(d) Weight Gain 19/2 to 15/12/58
Treatments	3				
Grass	1	0.038 N.S.	0.008 N.S.	0.008 N.S.	1262.53 **
Clover	1	3.45 N.S.	0.16 N.S.	0.0028 N.S.	830.28 *
Interaction	1	1.402 N.S.	0.195 N.S.	0.025 N.S.	3220.03 **
Error	28	1.93	0.309	0.0667	138.83
Total	31				
MEANS AND S.Es					
P.	7	6.2	11.1	2.5	4.00
S.R.	"	5.8	10.9	2.6	11.50
P. + C.	"	5.1	10.8	2.6	34.25
S.R.+C.	"	5.6	10.9	2.6	1.63
S.E. ±		±0.49	±0.19	±0.09	±4.16
COMBINED MEANS					
P.+(P.+C.)		5.6	11.0	2.6	19.12
S.R.+(S.R.+C)		5.7	10.9	2.6	6.56
S.E. ±		±0.34	±0.13	±0.06	±2.7
P. +S.R.		6.0	11.0	2.6	7.75
(P.+C)+(S.R.+C)		5.4	10.9	2.6	17.94
S.E. ±		±0.34	±0.13	±0.06	±2.7

N.S. = Not Significant
** = Significant at 1% level.
* = Significant at 5% level.

TABLE 8

MEANS AND STANDARD ERRORS OF (a) BLOOD INORGANIC P, SERUM Ca, AND (c) SERUM Mg, FOR LAMBS SAMPLED 16/1/59 AND 24/3/59

TREATMENTS	No.	Means and S.Es (a) P.	(b) Ca.	(c) Mg.	
P.	6	9.1 ± 0.3	12.4 ± 0.36	2.8 ± 0.06)	16/1/59
S.R.	10	8.8 ± 0.27	12.4 ± 0.6	2.7 ± 0.07)	
S.R.+C.	8	8.3 ± 0.3	12.5 ± 0.6	2.9 ± 0.03)	
S.E. ±				)	
P.	6	7.3 ± 0.67	10.0 ± 0.6	2.4 ± 0.08)	24/3/59
S.R. +C.	8	6.8 ± 0.34	10.3 ± 0.13	2.5 ± 0.07)	

TABLE 9

SUMMARY OF THE ANALYSES OF VARIANCE OF(a)BLOOD INORGANIC P, (b) SERUM Ca, AND (c) SERUM Mg, FOR EWE HOGGETS SAMPLED 12/6/59.

Source of Variation	d.f.	(a) P.	(b) Ca.	(c) Mg.
Treatments	3			
Grass	1	1.051 N.S.	0.180 N.S.	0.011 N.S.
Clover	1	0.005 N.S.	0.605 N.S.	0.0012 N.S.
Interaction	1	0.02 N.S.	0.5 N.S.	0.55 **
Error	27	0.53	0.334	0.037
Total	30			
MEANS AND S.Es.				
P.	7	5.2	10.1	2.4
S.R.	"	4.7	9.8	2.7
P.+ C.	"	5.1	9.6	2.7
S.R.+C.	6	4.8	9.7	2.4
S.E. ±		±0.25	±0.2	±0.021
COMBINED MEANS				
P.+(P.+C.)		5.2	9.9	2.6
S.R.+(S.R.+C.)		4.8	9.8	2.6
S.E.±		±0.18	±0.14	±0.015
P.+S.R.		4.9	10.0	2.6
(P.+C)+(S.R.+C)		4.9	9.7	2.6
S.E.±		±0.18	±0.14	±0.015

N.S. = Not Significant.
** = Significant to 1% level.

TABLE 10

SUMMARY OF THE ANALYSES OF VARIANCE OF (a) BLOOD INORGANIC P, (b) SERUM Ca, (c) SERUM Mg, AND (d) GAINS IN BODY WEIGHTS, FOR EWE HOGGETS SAMPLED ON 2 /9/59

Source of Variance	Degrees of Freedom, Mean Squares and Results of F Tests. (a) P d.f.	M.S.	F.	(b) Ca. d.f.	M.S.	F.	(c) Mg. d.f.	M.S.	F.	Weight Gain 12/6 to (d) 24/9/59 d.f.	M.S.	F.
Treatments	3			3			3			3		
Grass	1	0.0003	N.S.	1	0.69	N.S.	1	0.008	N.S.	1	253.12	N.S.
Clover	1	3.99	N.S.	1	2.82	*	1	0.09	N.S.	1	32.00	N.S.
Interaction	1	0.0028	N.S.	1	2.94	*	1	0.008	N.S.	1	128.00	N.S.
Error	24	1.62		27	0.51		27	0.039		28	71.42	
TOTAL	27			30			30			31		
MEANS AND S.Es												
P.	6	4.6		6	8.7		6	2.5		7	9.8	
S.R.	"	4.6		7	8.4		7	2.4		"	11.5	
P. + C.	"	5.3		"	8.7		"	2.5		"	8.0	
S.R.+ C.	"	5.3		"	9.6		"	2.5		"	17.5	
S.E. ±		± 0.45			± 0.25			± 0.07			± 2.9	
COMBINED MEANS												
P.+(P. + C)		4.9			8.7			2.5			8.9	
S.R.+(S.R. +C)		4.9			9.0			2.5			14.5	
S.E. ±		± 0.33			± 0.17			± 0.05			± 1.71	
P. + S.R.		4.6			8.6			2.5			10.7	
(P.+C) + (S.R.+C)		5.3			9.2			2.5			12.7	
S.E. ±		± 0.33			± 0.17			± 0.05			± 1.71	

N.S. = Not Significant
* = Significant at the 5% level.

TABLE 11

SUMMARY OF THE ANALYSES OF VARIANCE OF SERUM Ca, FOR THE EWE HOGGETS SAMPLED 14/10/59.

Source of Variation	d.f.	Mean Squares and Results of F.Tests. Ca	
Treatments	3	2.34 N.S.	
Error	28	1.28	
MEANS AND S.Es.			
P.	6	8.7 ± 0.43	
S.R.	13	8.8 ± 0.3	
P. + C.	5	9.3 ± 0.46	
S.R. + C.	4	10.1 ± 0.56	
S.E. ±			
COMBINED MEANS.			
P. + (P.+C.)		9.0 ± 0.3	
S.R. +(S.R.+ C.)		9.7 ± 0.37	
S.E. ±			
P.+ S.R.		8.8 ± 0.25	
(P.+C) + (S.R.+C)		9.7 ± 0.37	
S.E. ±			

N.S. = Not Significant.

TABLE 12a

Ca AND P. COMPOSITION OF HERBAGE AT DIFFERENT STAGES OF GROWTH.

(Per Cent of Dry Matter).

Season Sampled	Herbage Species	Hygroscope Moisture	Ash	Ca	P	Ca: P
Mid-Spring	Short-Rotation Ryegrass	5.60	9.50	0.611	0.442	1.38
Mid-Summer	-do-	9.40	11.25	0.548	0.322	1.70
-DO-	-do-	9.70	8.40	0.450	0.255	1.76
Mid-Autumn	-do-	6.50	10.10	0.55	0.441	1.25
Mid-Spring	Perennial Ryegrass	5.50	8.70	0.495	0.331	1.50
Mid-Autumn	-do-	7.05	12.80	0.470	0.421	1.12
-do-	-do-	8.00	12.50	0.415	0.360	1.12
Early Summer	-do-	9.10	8.68	0.611	0.460	1.33

TABLE 12(b)*

Herbage Species	Ash	Ca	P	Ca:P
Perennial ryegrass	11.0	0.40	0.46	0.9
-do-	10.1	0.90	0.39	2.3
White Clover	8.9	0.99	0.40	2.5
-do-	-	1.00	0.39	2.5

* Adapted from Barnicoat (1957)

SECTION 3

D I S C U S S I O N

(a) Blood Inorganic Phosphorus.

No significant differences were denoted between the treatments for all the animals sampled (Tables 7 - 10). The variations between the animals in any treatment were greater than the variations between treatments. Lambs had higher levels of blood inorganic phosphorus than ewes, an aspect compatible with the higher mineral turnover of the young growing animal. This reduction in levels of blood inorganic phosphorus, with increasing age, was indicated by the values shown in Tables 3 and 8. Facial eczema did not affect blood values among the affected lambs on the Perennial plots, and the changes noted among the latter animals also occured on the short rotation ryegrass and white clover plots. It seems worthwhile to add that similar changes were seen for serum calcium and magnesium, and for the same reasons.

Although there are definite variations in the phosphorus content of pastures, as denoted in Table(12a), and observed by Sears et al.(1953), the indications are that pastures levels of phosphorus do not fall below the accepted minimum adequate requirements of 0.18 - 0.24% of Dry Matter(D.M.) for non-pregnant ewes (Barnicoat, 1957).

The grazing animals, under the conditions of this experiment, appeared to maintain a suitable balance with the environmental factors which control the availability of phosphorus. From this evidence, it is concluded that there were no irregularities in the mechanisms which preserve

physiological equilibrium of blood inorganic phosphorus among the animals, throughout the periods of sampling.

(b) Serum Calcium.

There were no significant differences between treatments for serum calcium values taken at the early sampling periods. However, as shown in Table 10, the ewe hoggets sampled on 24/9/59, gave evidence of higher values among the animals kept on short-rotation ryegrass and white clover. This difference was significant at the 5% level. The presence of white clover produced no significant effects on the values for serum calcium when the clover is associated with perennial ryegrass, but the addition of clover to short rotation ryegrass maintained a significant level of serum calcium.

A general fall in the levels of serum calcium became apparent with the advent of the late winter-early spring flush in pasture growth, among the ewe hoggets, and this trend was parallel to that previously demonstrated by the collaborative project.

The outbreak of rickets among animals pastured on the experimental plots, enforced another sampling on 14/10/59. Mean values for serum calcium were indicative of a rising trend, and this followed the general fall (24/9/59) mentioned above. The most severe cases of rickets were found on the plots containing short-rotation ryegrass, and so interest was at first centred on this treatment. Two animals from this treatment showed advanced rachitic symptoms, including severe muscular incoordination of the limbs. X-ray and histological studies of the bones of these animals by Wallaceville workers verified

the rachitic conditions. The serum calcium values for both animals were 8.6 and 8.7 mg.per 100 ml. of serum, while the values for blood inorganic phosphorus and serum magnesium remained quite normal. These values for serum calcium were approximately equal to the mean value for the group. X-ray studies also showed that rickets of a much less severe nature occured on the other treatments,among some animals. Non-rachitic animals from the seriously affected group had values of serum calcium which ranged from 6.3 - 10.3 mg. per 100 ml. The hoggets kept on perennial ryegrass showed a range of 6.6 -11.3 mg. per 100 ml.,but were very much less rachitic. One animal,for example, had a serum value of 7.8 mg. on 24/9/59, and the value increased to 9.5 mg. on 14/10/59, despite the presence of severe rickets.

An important point thus shown was that the severely rachitic animals were not the ones with the lowest levels of serum calcium. This depicted a marked variation between individual animals as to the minimum level of serum calcium required for susceptibility to the disease.

The general fall denoted above,could be explained on the basis that the change was due to a reduction in mineral turnover with increasing age (Hansard et al.1951,1954,1957; Hansard and Crowder,1957). This view is not supported by the recovery that followed the general fall,and loses more ground where animals on the short-rotation ryegrass and white clover plots did not exhibit this seemingly reduced mineral turnover, although their levels of serum calcium rose with the general

recovery afterwards.

A reduction in mineral turnover with increasing age did not appear to account for the fall, and other factors were therefore entitled to consideration.

Assuming that the calcium composition of the diet is directly related to that of the blood (Sobel and Hanok, 1948), the general fall, as above, was indicative of the following causes:-

(i) a reduced intake of dietary calcium due to inadequate pasture content on all treatments other than short-rotation ryegrass and white clover.

(ii) A decrease in total intake due to unpalatability, scarcity of herbage, or any such factors, on the other pasture treatments.

(iii) Decreased availability of dietary calcium because of an imbalance of minerals, either through calcium and phosphorus, or through the action of a "trace" element, or magnesium.

This assumption apart, and with adequate quantities of available calcium in the pasture, the likely explanation for the significant difference and the general fall, is an upset of the vitamin D mechanism which stabilises the blood level of calcium, and controls the absorption of calcium from the intestine. Vitamin D exerts a direct affect on the mineralisation of bone in rachitic animals (Greenberg, 1945), and inadequate amounts of this vitamin, in addition to decreasing levels of pasture intake on the plots, could also account for the outbreak of rickets among the animals.

Observations made on pasture production showed that during winter and spring the short-rotation ryegrass yielded more herbage than the perennial ryegrass. Short-rotation ryegrass responded much more readily to the late winter-early spring period of quick growth.
During the summer and autumn perennial ryegrass appeared to be the better producer. The short-rotation ryegrass and white clover plots surpassed the perennial ryegrass and white clover ones in herbage production over the winter to spring period, and with the advent of summer there came a tendency for the clover to dominate the grass species. Perennial ryegrass and white clover showed a greater capacity for the production of herbage over summer and autumn, and there was not the same tendency for the plots to be clover dominant.

In spring, animals were inclined to graze the short-rotation plots more closely than the perennial ryegrass plots, and this suggested a distinct preference for this the more palatable herbage. Despite the close grazing noticed, it could not be said that the pasture herbage was quantitatively inadequate to the point of inducing incipient malnutrition, because reasonable quantities of herbage could be obtained from one of the short-rotation plots.
Close grazing of the short-rotation and white clover treatments was not in evidence because the growth of clover during this period maintained a more adequate level of pasture herbage. Clover was readily grazed at all times by the animals on these treatments.

These observations are inclined to nullify the possibility of a reduction in pasture intake as being the direct cause of the downward trend in serum calcium. In addition, these animals showed a general increase in body weights, and this does not reflect a serious scarcity of edible herbage. The question of unpalatable herbage, determined by the choice of herbage grazed by the animals on the different treatments, was peculiar only to perennial ryegrass when compared to the other species. This trend took place over the late-spring and summer period of growth, and as the herbage became more and more mature. This could not produce significant differences in the dietary intakes of the animals during late winter and early spring. Sears et al. (1953) have pointed out that changes in the chemical composition of herbage throughout the year are the results of both the ratio of grass to clover, and of the seasonal changes in leaf levels. They showed that with the transition from winter to summer, clover contributes an increasing amount of the calcium found in the total herbage. Greater levels of calcium were discerned in clovers (1.0 - 1.6% D.M.) than in grasses (0.4 - 0.6% D.M.), a superiority also reported by Armstrong and Thomas (1952) from English work. Low calcium levels occurred in grasses and clovers during winter and spring.

It is to be emphasised, however, that although seasonal fluctuations bring about variations in herbage content of calcium, these contents have not been observed to fall below the accepted standards for minimum requirements (0.2 - 0.33% D.M.) for non-pregnant ewes (Barnicoat, 1957). The balance of evidence does not support the theory of inadequate contents

of calcium in the pasture herbage as the main reason for the general decline in serum calcium,and for the significant difference found.

The possibility of an imbalance in the pasture content of calcium and phosphorus producing increased non-availability of dietary calcium, is not supported by the finding that among farm animals the intake, absorption,retention and loss of calcium and phosphorus are neither based on the approximately 2:1 ratio found in bones,nor on any other dietary ratio of calcium and phosphorus (Otto,1938;Wright,1955; Duncan,1958). This experiment,and many others,did not demonstrate such an imbalance in grazing animals,and it is improbable that such a causative factor led to the variations in serum calcium. Work with cattle showed that magnesium depressed the retention of calcium and caused losses of calcium from body tissues when the phosphorus content of the ration fed was low (Palmer, Eckles and Schutte,1928), but no such antagonism has been seen in this and other experiments.

Variations in the availability of herbage calcium have not been experimentally related to any deficiencies or nutritional imbalances of trace elements (i.e. iodine, copper, molybdenum, cobalt,iron,fluorine and others). This does not exclude the possibilities of such effects, and research work in this direction might prove useful. However, it seems unlikely that the significant difference found on the plots containing short-rotation ryegrass and white clover, and the general fall denoted on all treatments, could be due to trace elements.

Armstrong et al.(1957)indicated that fibre content and oxalic acid content deterred ease of calcium availability in the grasses studied. No analysis for oxalic acid content

was done in the present experiment,and so the finding cannot be confirmed. Under the conditions of this experiment the fibre content of the herbage was not believed to be high enough,during the early stages of growth which occur at late winter to early spring,in the Manawatu,to induce the differences and trends observed in serum calcium. The retarding effects of a high fibre content in pasture herbage are more synonymous with advanced maturity over summer and early autumn.

The general fall in serum calcium values was observed before the outbreak of rickets. This depicted a related trend,hence any reasons which explain the former should in fact account for the incidence of the latter. It is evident that the possible non-availability of herbage calcium was not a principal factor in the incidence of rickets,because the serum calcium values in seriously affected animals were approximately equal to the mean value for all animals on the same treatment. Adequate levels of available calcium were therefore afforded the hoggets on the short-rotation plots although this may have been on the threshold level which borders inadequacy on some plots. Furthermore,this evidence points to adequate intake and absorption of dietary calcium on all other treatments.

With the assumption that the calcium composition of the diet is directly related to that of the blood,there are now suggestions that a number of factors may have contributed to the trends observed. The calcium content of grasses and clovers are at a seasonal minimum during winter and spring (Sears,1953). In addition, the possibility of existing factors, yet indefinite,which deter the availability of herbage calcium at this time will not be denied. A variable herbage intake is known to occur among pasture grazing sheep (Barnicoat,1957).

All these factors could collectively contribute to a low dietary intake of calcium, and therefore promote the fall observed in blood calcium levels.

It seems that during the onset of the general fall in blood calcium values, the hoggets on the short-rotation ryegrass and white clover maintained more constant serum calcium levels because they fed on the more palatable short-rotation ryegrass, which grew quicker during the flush period of growth. This was aided by the increased fertility provided by the presence of clover, and these animals also fed on the clover which contained a higher calcium content than the grasses. The significant difference denoted in Table 10, is explained on this basis. In contrast, the animals on the pure grass plots would therefore lose the benefits which accrued from the presence of clover, especially on the more palatable, but more closely grazed, short-rotation ryegrass. The sheep on the plots which contained perennial ryegrass and white clover, grazed on the less palatable perennial ryegrass, which grew more slowly in the spring flush, and on clover which was retarded in its productivity by the more dominant ryegrass. The result in the latter case was the production of a loss of fertility of the ryegrass, an aspect which does not enhance quicker spring growth. These animals, and those grazed on the pure grasses, did not therefore find it possible to maintain constant levels of serum calcium at a time when pasture contents were at the lowest. The reasons afforded here have not elucidated the incidental occurence of rickets, especially because the rachitic animals did not show the lowest levels of serum calcium. A review of the literature offers doubts that the

calcium composition of the diet is directly related to that of the blood when adequate vitamin D is also fed. Again,Ewer and Bartrum (1948) showed that hoggets developed rickets on Italian ryegrass which contained levels of calcium and phosphorus much above the minimum requirements. The administration of vitamin D supplements in the diet protected unaffected animals from the disease. Vitamin D insufficiency due to inadequate irradiation during the winter was not considered the primary cause of rickets, by these workers, and the inference was that some specific principle interfered with phosphorus metabolism. In the light of the postulate of Grant (1953),inhibition of the function of vitamin D may have been the retarding principle.

The evidence,at this stage,suggests that some of the reasons given for the general fall and the significant difference, in the preceding paragraph,were important ones,with the substantiation that these effects on serum calcium values were aided by the decreased functioning of vitamin D. This decrease in function was thought to be due to lowered synthesis of the vitamin, an aspect which is compatible with a reduction in solar radiation during winter,in the Manawatu, and which tends to lessen the stabilising effect that the vitamin exerts on calcium in the blood.

For the duration of the experiment,cloudy,wet and cold weather predominated over the period lasting from late July to early September, with few clear and sunny days. This seemed to enhance the effects of the reduction in sunshine hours during the winter.

There are no estimations of the amount of vitamin D that sheep obtain from the effects of irradiation in the Manawatu, and it is possible that with other deficiencies present a reduction

in vitamin D synthesis could help to produce the results observed.

The close grazing of herbage on the short-rotation plots showed a lower than usual intake of feed,hence a dimunition in the calcium intakes from such plots at a time when the contents of grass were lowest. One of the plots, however,contained ample levels of herbage but animals also showed serious rachitic infection. T is suggested the influences of factors which cause non-availability either through an anti-vitamin effect, or through retardation of the extraction of calcium from the ingested herbage.

Few anti-vitamin D agents have been brought forward,and that proffered by Grant (1953) require confirmation in more investigations. The efficient utilisation of vitamin A is dependent on the anti-oxidant properties of vitamin E (McGillivray,1952), and a similar effect is possible for other fat-soluble vitamins. Efficient utilisation of vitamin D would however be maintained on the plots which contain the pure grasses because the deficiency of vitamin E is peculiar to clover-rich pastures. This deficiency could not produce the differences demonstrated during the spring flush on plots that were not regarded as clover-rich.

A high dietary intake of calcium and phosphorus failed to maintain normal levels of serum calcium and blood inorganic phosphorus in sheep housed indoors (Franklin,1948), and the low values were quickly corrected by the administration of a vitamin D supplement. Animals which were similarly fed but were also exposed to abundant sunlight maintained normal blood levels of both minerals. The work of Franklin(1948),emphasises the importance of vitamin D synthesis to the pasture grazing

sheep, and supports the present claim that inadequate formation of this vitamin, and not the non-availability of dietary calcium, was the main cause of the falling trend and the subsequent outbreak of rickets. Latterly, rachitic animals did not possess the lowest levels of serum calcium, and this imposes doubts on the suggested retardation of the extraction of calcium from the ingested herbage.

The variable increases in body weights among individual animals reflected differences in the levels of herbage intake on any one treatment. These differences in intake could be critical among plots where other deficiency factors were at work. The combination of factors which produced the trends observed, and which resulted in the degrees of rickets that occurred, appeared to include the effects of a variable and lowered intake of herbage calcium. A strong view is that some of these factors could have reduced the total amount of calcium available, to a level below the optimum required by individual animals to counteract the imbalance created by an already diminishing availability of vitamin D. Individual variations in the minimum levels of serum calcium required to induce susceptibility to rickets have been shown, and this connotes other factors rather than the non-availability of dietary calcium as the ultimate cause of the disease. It appears that but for this imbalance in vitamin D function, the total amount of available calcium would have been adequate on the most severely affected treatments despite the critical state of other factors. The animals on the other treatments showed the retarding effects due to this imbalance in the vitamin but were able to offset the incidence of very severe

and advanced rickets because their dietary intakes of available calcium were enough to counteract this imbalance. Animals on other pastures in the area did not show rickets, probably because the concentration of sheep was less per acre and the pasture herbage contained more plant species. This could afford higher dietary intakes of available calcium to maintain a more healthy mineral status.

The explanations given are strengthened by the fact that when the mineral intake is sub-optimal, the demand for vitamin D is increased (Ewer & Bartrum, 1948). The converse relationship has been shown (Auchinachie & Fraser, 1932; Kramer & Howland, 1932), but to a lesser extent owing to the influence that vitamin D exerts on the absorption of calcium from the intestine.

In conclusion, it is suggested that given adequate levels of vitamin D throughout the year, pasture grazing animals will maintain normal serum calcium values under Manawatu conditions. These values will then incline to constancy despite the lowering of the herbage intake and the calcium content of the pasture herbage at certain seasons, and although the suspected detrimental effects of fibre content, oxalic acid, vitamin E deficiency, and others, continue to exert their influences.

(c) Serum Magnesium.

Duncan, Lightfoot and Huffman (1938) found little change in the mean magnesium content of blood plasma with increasing age among normally reared calves, but there was considerable individual variation. The work of Smith (1957) supported the fall in plasma magnesium with the advance of age in calves. The present experiment shows a similar trend with the lambs, and a greater variation among individuals than

between the treatments for serum magnesium with all animals.

In general, there were no significant differences between the treatments at most of the samplings, but the ewe hoggets showed a highly significant difference on 12/6/59. With perennial ryegrass the presence of clover raised the serum magnesium level, but with short-rotation ryegrass the presence of clover reduced the level of serum magnesium. This could mean that the magnesium level of White clover is normally between those of perennial ryegrass and short-rotation ryegrass. No analyses were done for the magnesium content of herbage, but Thomas, Thompson, Oyenuga and Armstrong (1952) examined eight grasses and four legumes growing under English conditions and found the magnesium levels to be greater in legumes than in grasses.

The animals were put on the treatments on 16/5/59, and doubts therefore arise as to the rapid and particular effect denoted for the clover, especially from the fact that no significant differences were seen on 24/9/59. It is furthermore remarkable that the differences were indicated only over the autumn when perennial ryegrass yielded more herbage than short-rotation, and when the ill-effects of clover dominance would have affected the short-rotation ryegrass and white clover plots rather than the perennial and white clover ones.

Rook and Balch (1958) emphasised the difficulty of establishing the extent of variations in the availability of herbage magnesium, and no direct relationship has yet been shown between herbage magnesium and the level

of blood serum. Spring herbage was found to promote progressive hypomagnesaemia in dairy cows by Rook et al,(1958),but the magnesium content of herbage did not vary with the stage of growth. With the latter finding as a basis,and the fact that the pastures of New Zealand are overall more productive than those of European and North American countries,this experiment helps to demonstrate that the magnesium content of New Zealand pastures are enough to satisfy the nutritional requirements of sheep.

The highly significant differences found could simply have been variations between the groups when they were randomised to the separate treatments, and which were still present on 12/6/59,but were reduced by 24/9/59. This is supported by the small difference between the significant results(2.4: 2.7 mg. per 100 ml.of serum), and in particular,by the greater difference found among individuals within any one treatment.

Under Manawatu conditions,the greatest demands for herbage magnesium by sheep occur during lactation. This coincides with the spring flush in pasture growth, and because no deficiencies have been reported in these pastures, it can be assumed that the demands of the animals are satisfied. It is possible that deficiencies sometimes occur due to heavy lactation demands,and with insufficient herbage intake immediately after parturition,but the extra needs are probably met by bone reserves (Blaxter & McGill,1956) over the short period involved.

(d) The Gains in Body Weight.

The only significant differences shown in body weight gains during this experiment were those among the ewes

sampled on 4/12/58. Values obtained from a report on the collaborative project showed that the differences in mean body weights between the animals on the four treatments ranged from 62.3 - 63.1 lb. at the start, on 10/5/57. At the last weighing on 15/12/58, the animals on the perennial ryegrass plots made the largest gains in body weights, i.e. for the period 19/2/58 to 15/12/58, followed by those on the short-rotation ryegrass plots. The gains on the other treatments were very much less in comparison, as depicted by values shown in Table 7.

Highly significant effects were denoted for the interaction of perennial ryegrass and white clover, and, as a result, for the difference between grasses. The effect of clover was significant at the 5% level, hence there was every indication that the plots containing perennial ryegrass and clover contributed most to gains in body weights over the summer. This latter aspect is commensurate with the higher summer productivity of perennial ryegrass and white clover already referred to. These plots are not prone to clover dominance, and with the transition from early spring to early autumn, the clover contributes to an increasing amount of the total pasture herbage.

The difference in mean weight gains between animals on the pure grasses were not significant although the animals on short-rotation showed more gain. This may indicate the greater proneness to maturity of the perennial ryegrass with the passage of summer, and the earlier tendency to go to seedhead. A decline in nutritive value comes with

FIG. 2.

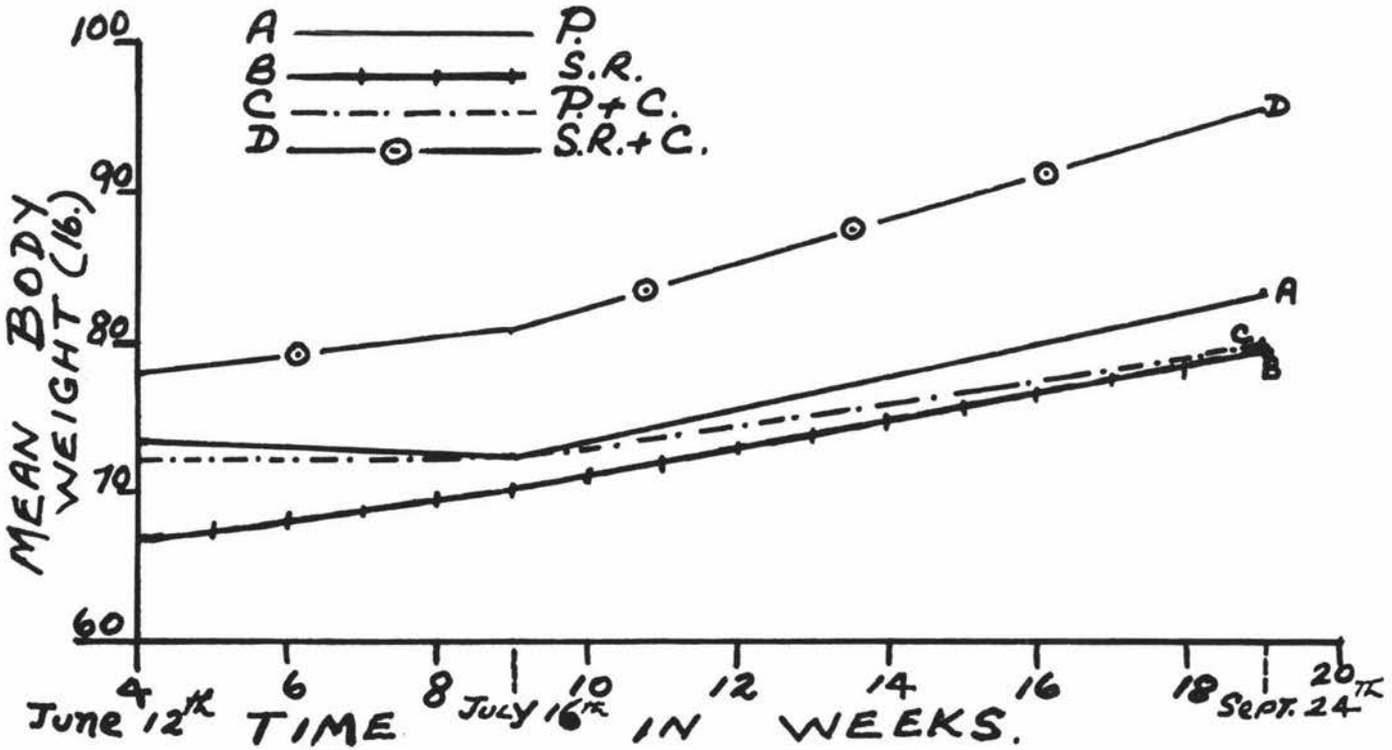
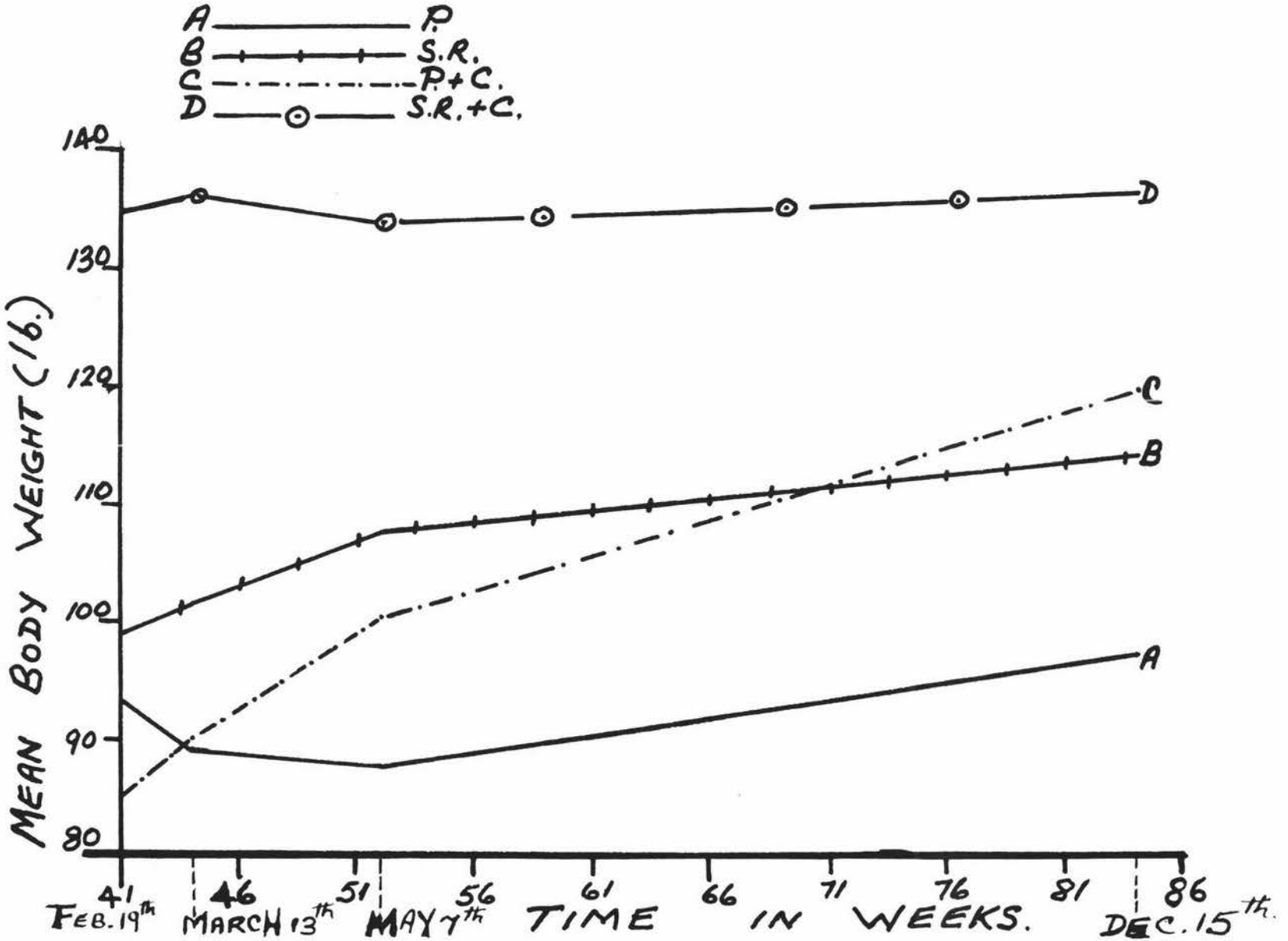

A P.
B S.R.
C P.+C.
D S.R.+C.
MEAN BODY WEIGHT (16.)
100
90
80
70
60
4
6
8
10
12
14
16
18
20
June 12th
July 16th
Sept. 24th
TIME IN WEEKS.
A
B
C
D

FIG. 3.


A — P.
B — S.R.
C — P+C.
D — S.R.+C.
140
130
120
110
100
90
80
MEAN BODY WEIGHT (lb.)
41
46
51
56
61
66
71
76
81
86
FEB. 19th
MARCH 13th
MAY 7th
TIME IN WEEKS.
DEC. 15th
A
B
C
D

increased maturity of pasture herbage.

The lowest gains in body weights were shown by animals on the short-rotation and clover plots, and the reason offered is the tendency for clover dominance over the summer. Clover would therefore have contributed an increasing amount of the total herbage, to the detriment of the grass and the total quantity of herbage on the plots. An interesting view is that this clover dominance reduces the availability of adequate quantities of vitamin E to the animals (McGillivray, 1952) as summer advances. This results in poor utilisation of the fat-soluble vitamins, including vitamins A and D, which are important contributors to increasing growth in animals.

For the period 10/5/57 to 15/12/58 the animals on the clover treatments have demonstrated the greater increases in body weights.

Although there were no significant differences for the ewe hoggets sampled on 24/9/59, the indications were that the animals on both short-rotation treatments showed superior gains in body weights. This agrees with the greater productivity of the short-rotation ryegrass treatments during winter and early spring. Figs. 2 and 3 illustrate the trends of the mean body weights on the four treatments for ewes and ewe hoggets.

PART 11

BLOOD STUDIES BASED ON FEEDING TRIALS WITH RATS.

SECTION 1

EXPERIMENTAL

(a) Introduction of Experiments with Rats.

The experiments with young Wistar rats were carried out in order to support the work done with sheep. Differences observed in sheep, in the collaborative project, may be due to several factors.

The use of blood values as an index of the mineral status of the animal has already been questioned. Work with rats therefore undertook to establish the validity of the experimental results from sheep, based on blood data.

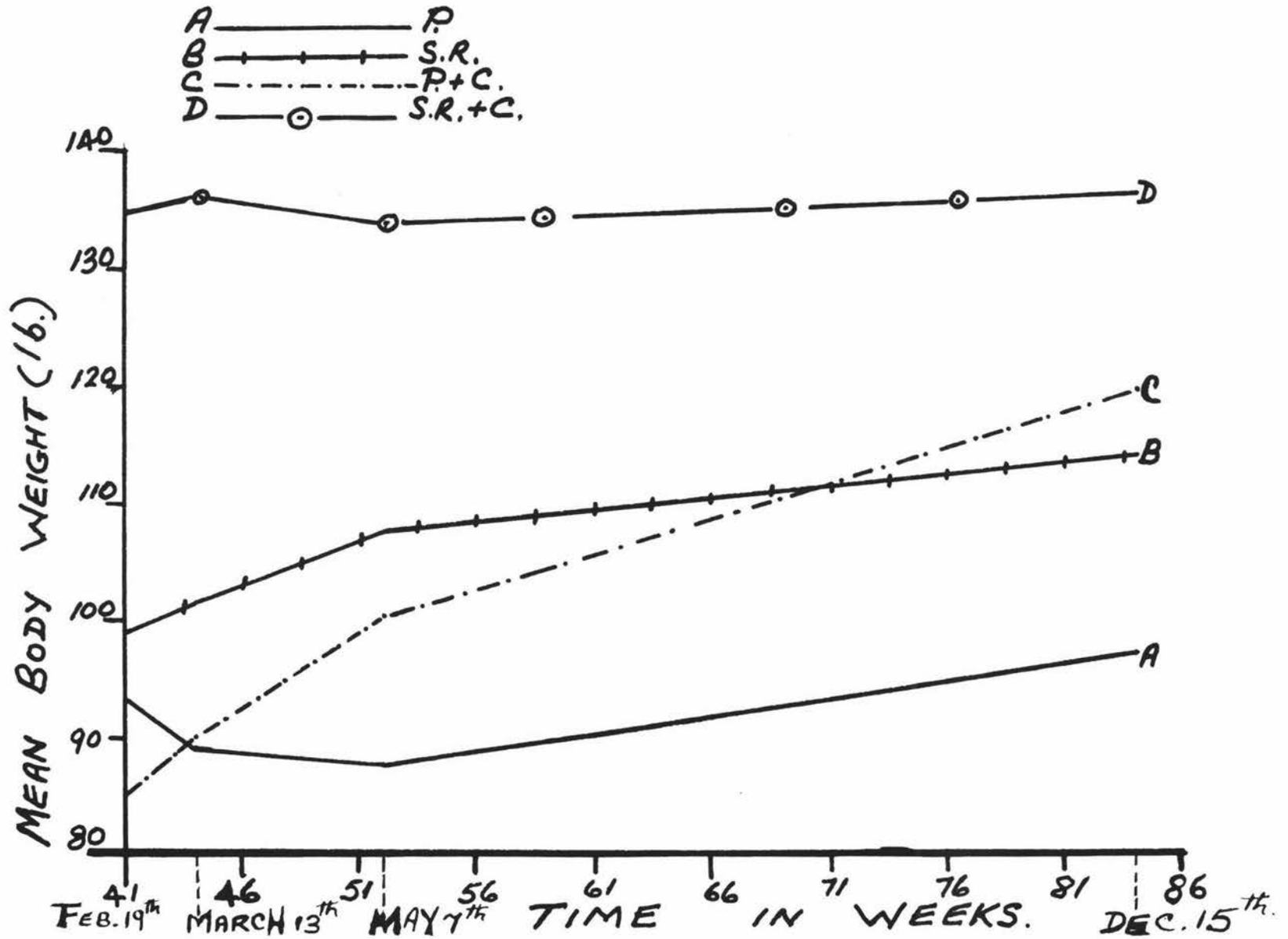
Evidence that changes in blood levels of calcium and phosphorus vary directly with fluctuations in dietary levels, in animals fed adequate vitamin D, is shown by Sobel and his co-workers (1945 a, b; 1948). Their findings have received very little support by other workers. The present experiments with rats aimed to contribute to the controversy surrounding these findings.

Again, there were no measurements of the intakes and utilisation of the calcium and phosphorus in herbage grazed by the sheep. It was therefore considered necessary to investigate the availability of these minerals from two different types of pastures grazed by sheep. Data on blood values and mineral intake and utilisation in rats should indicate allied relationships.

Levels of serum magnesium and the calcium and phosphorus contents of femurs of groups of rats were included as being associated with the above interests.

It is well known that rats thrive with difficulty on a grass diet, and so grass ash was used by way of addition

FIG. 3.


A — P.
B — S.R.
C — P.+C.
D — S.R.+C.
MEAN BODY WEIGHT (lb.)
140
130
120
110
100
90
80
D
C
B
A
41
46
51
56
61
66
71
76
81
86
FEB. 19th
MARCH 13th
MAY 7th
TIME IN WEEKS.
DEC. 15th.

to a synthetic diet in two experiments. Grounded grass was fed in one experiment, and the animals were sacrificed as soon as they began to lose thrift.

Working with cattle, Palmer, Cunningham and Eckles (1930) pointed to the marked day to day variation in blood inorganic phosphorus. They found that feeding causes blood phosphorus to rise in the first hour and then to return to normal three hours afterwards. Lactating goats were studied by Visek et al. (1952), and they showed the rapid removal of radioactive calcium from the blood subsequent to intravenous administration.

These endeavours depicted a very rapid removal of calcium and phosphorus from the blood for immediate use and storage. An experiment was designed to test this view.

(b) Plan of Experiments.

Experiment 1.

Young weaned rats were fed (ad lib.) a basal diet which contained 3.6% salt mixture (the details of dietary components are given later) over a pre-feeding period of two weeks. The animals were then randomised into four groups, so that the average body weights of the separate groups were approximately equal. The groups were fed separate experimental diets as follows:-

Group (i). (4 rats), received the diet containing 3.6% salt mixture, the control diet.

Group (ii), (4 rats), were maintained on a diet containing 4.5% salt mixture.

Group (iii), (5 rats), received the control diet, but with the calcium content raised to five times that of the control by the addition

of $CaCO_3$.

Group (iv), (5 rats),were fed the control diet,but with the phosphorus content raised to five times that of the control,by the addition of Na_2HPO_4.

The intake of each group was regulated to that of the lowest intake group,and the body weights were measured once weekly. This experiment lasted for seven weeks,the animals being allowed tap water, ad lib.,and a few drops of arachis oil containing vitamins D,E, and K once per week.

Experiment 2.

After a pre-feeding period,as above,these rats were similarly grouped, and fed as follows:-

Group 1, (5 rats), received the control diet containing 3.6% salt mixture.

Group 11, (4 rats), received the diet containing 4.5% salt mixture.

Group 111,(4 rats), were fed the control diet, but with perennial ryegrass ash added as 12.5% of the complete diet. The reason for using this proportion of ash was that the ryegrass contained 12.5% ash on a dry matter basis.

Group 1V,(4 rats),were fed the control diet,but with the ash from a mixed pasture added as 8% of the complete diet. The mixed pasture herbage contained 8% ash on a dry matter basis.

The other stages of this experiment were conducted in a similar manner to Experiment 1.

Experiment 3.

This experiment differed in design from the preceding two in that six groups of three animals each were used. THREE dietary regimes were maintained, with two groups of rats ascribed to each.

The animals were six weeks old at the start of the experiment. The level of salt mixture was raised to 5.4% during the two weeks allowed for as the pre-feeding period, because the animals began to lose thrift on the previous diet containing 4.5% salt mixture.

At the end of the pre-feeding period the rats were randomised into six groups, so that the average body weights of the separate groups were approximately equal. The experimental diets were fed as follows:-

Groups C_1 and C_{11}, were fed the diet containing 5.4% salt mixture, and this was used as the control.

Groups R_1 and R_{11}, received the control diet, but with perennial ryegrass ash added as 12.5% of the complete diet.

Groups M_1 and M_{11}, received the control diet, but with the ash from a mixed pasture added as 8% of the complete diet.

The intake of each group was regulated to that of the lowest intake group, and the body weights were measured once weekly. Tap water ad lib., and a few drops of arachis oil containing vitamins D, E, and K were added to the diet once per week. This experiment lasted for 10 weeks, and a 10 day mineral balance trial was done on these groups.

Experiment 4.

The experimental design used was similar to that in Experiment 3. The same control diet was also used. At the start of the experiment the animals were seven weeks old, and they were maintained on the diet containing 5.4% salt mixture for the pre-feeding period of ten days. Subsequent to randomisation into the respective groups, animals were fed the following dietary regimes:-

Groups (a),(A), received the diet containing 5.4% salt mixture, the control diet.

Groups (b),(B), were fed the control diet, but with grounded perennial ryegrass added as 33.3% of the complete diet(on a D.M. basis).

Groups (c),(C), obtained the control diet, but with grounded mixed pasture added as 33.3% of the complete diet(on a D.M. basis).

Animals were reported to thrive with difficulty on a grass diet and so the experiment was terminated after twenty four days as soon as the rats threatened to lose thrift.

Experiment 5.

This experiment was designed to indicate the hourly changes which occured in blood levels of calcium and phosphorus when rats kept on a diet at the 5.4% salt mixture level, were suddenly fed much higher dietary levels of Calcium (*Ca x 5) and phosphorus (* P x 5). Table 18 shows the design and results.

* Control Diet

(c) Animal Management.

Animals were kept in cages in the house specially-designed for small experimental animals, at Massey College. They were fed and watered daily, and the trays used for the collection of faeces and urine were emptied as often as possible to prevent atmospheric pollution. Adequate heating was provided in cold weather and the ventilation was suitable at all times.

The nature of the diets fed did not facilitate conversion to pellets so animals obtained their feed from small enamel dishes. Wastage due to spilling was toosmall to be of significance in these experiments.

The rats were obtained from pure strains bred at the Animal Research centres at Ruakura and Wallaceville. Those used in Experiments 1, 3, and 5 were all of the male sex. The distribution of the sexes in Experiment 2 were found to be as follows:-

Group 1 - 5 females.

Group 11 - 1 female + 3 males.

Group 111 - 1 female + 3 males.

Group 1V - 1 female + 3 males.

Experiment 4 showed the following distribution of the sexes:-

Group (a) - 3 males.

Group (A) - 2 males + 1 female.

Group (b) - 2 males + 1 female.

Group (B) - 3 males.

Group (c) - 2 males + 1 female.

Group (C) - 3 males.

It is worth pointing out that animals were requested as males, and that the distribution of females in Experiments 2 and 4 were purely the result of randomisation.

(d) Diets.

The basal diet and salt mixture were based on that used by Moore(1957) and Hansard et al. (1951), and subject to some modifications,are as follows:-

Cassein (commercial)	20 parts.
Sucrose	60 parts.
Arachis oil	15 parts.
Brewers yeast	10 parts.

Salt mixture 4, 5,and 6 parts for control diets containing 3.6%,4.5%,and 5.4% salt mixture,respectively.

Vitamins fed each rat per week.

E - dl- a-tocopheryl acetate ..... 2 mg.

K - 2-methyl-1-4-naphthoquinone...50 µg.

D - Calciferol.....1.5 µg.

Salt Mixture.

$Ca_3(PO_4)_2$	20 parts.
KCl	27.2 parts.
NaH_2PO_4 (anhydrous)	5 parts.
$MgSO_4$ (anhydrous)	8.7 parts.
NaCl	5.4 parts.
$FeCl_3 \cdot 6H_2O$	2.5 parts.
$CuSO_4 \cdot 5H_2O$	0.4 parts.
$ZnSO_4 \cdot 7H_2O$	0.5 parts.
$MnSO_4 \cdot 4H_2O$	0.2 parts.
$CoSO_4 \cdot 7H_2O$	0.0005 parts.
KI	0.1 parts.
NaF	0.05 parts
Total.....	70.0505 parts.

For the diet fed to Group (iii) in Experiment 1, 5.4 parts of $CaCO_3$ were added to the control diet and the sucrose content

reduced by a similar amount. For Group (iv), 6.6 parts of Na_2HPO_4 were added to the control diet, and the sucrose content reduced by a similar amount. The aim was to maintain the salt mixture in the same proportion in the diets. Careful mixing and grinding in a mortar were entailed in the preparation of these diets to ensure uniform distribution of all constituents, especially dietary micro-nutrients. Diets were stored in bottles.

Pasture Ash.

Both the perennial ryegrass ash and that from the mixed pasture were prepared from herbage cut at "sheep-grazed" height, by the method described above for the determination of calcium and phosphorus from herbage samples. Analysis of all herbage was done to denote the various plant species present. The grounded herbages fed in Experiment 4, were prepared for analysis by a similar method.

(e) Methods.

(i) The Collection of Blood and Bones.

At the end of each experiment the animals were anaesthetised with diethyl ether, and the blood removed from the heart with a hypodermic needle. Death ensued, and the femurs were removed and set aside for analysis, except in Experiments 4 and 5. The blood and bones from the rats in each group were pooled for chemical analyses.

(ii) The Determinations of Calcium, Phosphorus and Magnesium in the Blood.

These determinations were done by the methods used on the blood samples of sheep.

(iii) The Determinations of Calcium and Phosphorus in Casein, Yeast and Herbage.

The methods employed were those used to estimate

the calcium and phosphorus of herbage samples in the work with sheep.

(iv) The Determination of Calcium and Phosphorus in Bones.

(1) The Preparation of the Samples for analysis:-

After the femurs were removed from the rats, they were scraped clean and soaked for a few hours in three changes of alcohol. They were next extracted with ether and dried to a constant weight at 105°C. Cooling took place in a dessicator and the bones weighed immediately afterwards. The Fat-free Dry weight was thus obtained. Ashing was done by placing the silica dishes which contained the bones in a muffle furnace set at 600°C for 12 hours. The weight of ash was estimated and expressed as a percentage of the Fat-free Dry weight.

The ash extract was prepared by the method adopted for the preparation of the pasture ashes earlier, and the bone ashes were grounded in a glass mortar and removed by the 10 ml. of distilled water and 10 ml. 2N HCl used in the extraction procedure. The remainder of the preparation was similar to the above method.

(2) The Estimation of Calcium and Phosphorus in Bones:-

These estimations were done by the methods used in the ash extracts of herbage samples in the work on sheep.

(v) The Determination of Calcium and Phosphorus in Faeces and Urine.

The faecal and urinary collections from the mineral balance trial were stored in weighed bottles, and kept in a dry place.

Preparation for analysis and the estimations of calcium and phosphorus from this mixed excreta were made by the procedure employed for pasture herbage, in the work on sheep.

SECTION 2

RESULTS

Tables 13, 14,15,16a,16b,16c,17a,17b,17c, and 18 show the results obtained from these investigations with rats.

Figures 4 to 7 present mean body weights of various groups used in Experiments 1 to 4.

During the course of each experiment, it was attempted to equate the total amount of ration eaten by each animal irrespective of treatment, by regulating each group intake to that of the lowest intake group. This was not possible, however, in experiments 1 and 2, owing to the variable feed intakes encountered. The resultant effect was not considered large enough to alter significantly the levels of dietary calcium and phosphorus intended for the separate treatments.

Table 13

RESULTS FROM EXPERIMENT 1

Groups	No.of Animals at end of Expt.	Initial Aver. Body Weight (g.)	Final Aver. Body Weight (g.)	Total Ca Intake (g.)	Total P Intake (g.)	% Ca in Diet	% P in Diet	Ca:P in Diet	Blood Inorg. P in mg.per 100 ml.	Serum Ca in mg. per 100 ml.	Serum Mg.in mg.per 100 ml.	No.of Bones Analysed	Weight of Fat-free Dry Bones(g)	% Ash in Fat-free Bones	Mean Ca Content of Bones (mg)	Mean P Content of Bones (mg)
(i) Control Diet (3.6% Salt Mixture)	2	65	88	2.908	3.65	0.433	0.543	0.8	8.32	_*	_*	2	0.327	63.6	35.45	18.19
(ii) Control Diet (4.5% Salt Mixture	4	64	136	6.507	7.39	0.54	0.614	0.88	8.64	13.6	2.4	4	0.5935	64.4	27.0	17.6
(iii) Ca in Control Diet x 5	4	65	99	32.54	7.453	2.421	0.554	4.37	9.6	12.8	1.95	4	0.613	64.2	35.6	17.4
(iv) P in Control Diet x 5	5	68	115	6.47	29.9	0.442	1.876	0.24	9.92	10.4	2.44	4	0.591	67.0	36.1	17.98

* The number of surviving animals did not facilitate the collection of enough blood to permit a serum calcium estimation.

Table 14.

RESULTS FROM EXPERIMENT 2.

Groups	No.of Animals at end of Expt.	Initial Aver. Body Weight (g.)	Final Aver. Body Weight (g.)	Total Ca Intake (g.)	Total P Intake (g.)	% Ca in Diet	% P in Diet	Ca:P in Diet	Blood Inorganic P in mg. per 100 ml.	Serum Ca in mg.per 100 ml.	Serum Mg in mg.per 100 ml.	No.of Bones Analysed	Weight of Fat-free Bones (g.)	% Ash in Fat-free Bones	Mean Ca Content of Bones (mg.)	Mean P Content of Bones (mg.)
I Control Diet 3.6% Salt Mixture	5	99	100	8.4178	10.4925	0.442	0.5508	0.8	7.7	10.4	2.06	4	0.684	64.0	39.0	19.67
II 4.5% Salt Mixture	4	95	142	8.0634	9.1738	0.54	0.615	0.88	8.3	10.4	2.05	4	0.904	64.9	51.7	26.2
III Ryegrass Ash as 12.5% of Diet	4	94	83	6.432	7.7021	0.44	0.525	0.84	7.3	10.1	1.62	4	0.6575	60.7	35.2	18.09
IV Mixed Ash as 8% of Diet	4	93	88	6.1925	7.4193	0.44	0.524	0.84	8.8	11.2	1.81	4	0.648	63.4	36.0	18.11

Table 15

Average Ca and P Composition of Herbage Fed in Experiments 2 and 3 (Per cent Dry Matter).

Herbage	Hygroscopic Moisture	Ash	Ca	P	Ca:P
Perennial Ryegrass	8.0	12.50	0.415	0.36	1.15
Mixed Pasture	8.0	8.00	0.38	0.21	1.81

Species Composition of Herbage

Perennial Ryegrass		Mixed Pasture	
Species	Percentage (%)	Species	(%)
Perennial ryegrass	99.8	Perennial ryegrass	6
Poatrivialis	0.2	Timothy	2
		Brown top	66
		Sweet Vernal	6
		Yorkshire Fog	15
		White Clover	3
		Hawkbit, Plantago major	2
Total	100.0	Total	100.0

Table 16 (a)

RESULTS FROM EXPERIMENT 3.

Groups	No.of Animals at end of Expt.	Initial Aver. Body Weight (g.)	Final Aver. Body Weight (g.)	Total Ca Intake (g.)	Total P Intake (g.)	% Ca in Diet	% P in Diet	Ca:P in Diet	Blood Inorganic P in mg. per 100 ml.	Serum Ca in mg.per 100 ml.	Serum Mg.in mg.per 100 ml.	No.of Bones Analysed	Weight of Fat-free Dry Bones (g.)	% Ash in Fat-free Bones	Mean Ca Content of Bone (mg.)	Mean P Content of Bone (mg.)
C I Control Diet 5.4% Salt Mixture	3	172	175	10.24	11.017	0.619	0.666	0.93	7.4	13.5	3.4	3	0.8705	69.2	74.5	36.7
C II Control Diet 5.4% Salt Mixture	3	172	187	10.24	11.017	0.619	0.666	0.93	6.5	12.0	3.4	3	0.9535	68.6	80.5	39.8
R I Ryegrass Ash as 12.5% of Diet	3	172	157	9.82	10.385	0.593	0.627	0.95	6.7	12.0	2.7	3	0.8075	69.6	70.0	34.14
R II Ryegrass Ash as 12.5% of Diet	3	172	141	9.82	10.385	0.593	0.627	0.95	7.0	12.0	2.7	3	0.777	64.6	62.3	31.07
M I Mixed Ash as 8% of Diet	3	172	146	10.09	10.574	0.61	0.639	0.95	6.5	10.5	3.0	3	0.801	68.2	66.0	32.99
M II Mixed Ash as 8% of Diet	3	172	158	10.09	10.574	0.61	0.639	0.95	6.0	10.5	2.9	3	0.832	68.1	70.7	35.15

Table 16 (b)

A Mineral Balance Trial From Experiment 3.

Groups	Total Dietary Ca (g.)	Total Ca in Excreta (g.)	% Ca Retained	Total Dietary P (g.)	Total P in Excreta (g)	% P Retained
C I Control Diet (5.4% Salt Mixture)	1.572	1.027	34.67	1.67	0.9269	44.5
CII Control Diet (5.4% Salt Mixture)	1.572	0.673	56.47	1.67	0.8672	48.07
RI Rye.Ash as 12.5% of Diet	1.503	1.18	21.5	1.572	1.1524	36.38
RII Rye.Ash as 12.5% of Diet	1.503	1.297	13.72	1.572	1.3160	16.27
M I Mixed Ash as 8% of Diet	1.528	1.218	20.28	1.607	1.1010	31.49
M II Mixed Ash as 8% of Diet	1.528	1.216	20.40	1607	1.111	30.86

Table 16 (c)

Summary Of The Analyses of Variance of (a) Blood Inorganic P, (b) Serum Ca, (c) Serum Mg, From Experiment 3.

Source of Variation	d.f.	Mean Squares and Results of F Tests. (a) P.	(b) Ca.	(c) Mg.
Treatments	2	0.285 N.S.	2.6225 N.S.	0.25 **
Error	3	0.193	0.38	0.0033
Total	5			
MEANS AND S.Es.				
C I C II		6.95	12.75	3.4
R I R II		6.85	12.0	2.7
M I M II		6.25	10.5	2.95
S.E ±		± 0.31	± 0.43	± 0.04

N.S. = Not Significant
** = Significant at 1% level.

Table 17 (a)

RESULTS FROM EXPERIMENT 4.

Groups	No.of Animals at end of Expt.	Initial Aver. Body Weight (g.)	Final Aver. Body Weight (g)	Total Ca Intake (g.)	Total P Intake (g.)	% Ca in Diet	% P in Diet	Ca:P in Diet	Blood Inorganic P in mg. per 100 ml.	Serum Ca in mg.per 100 ml.	Serum Mg. in mg. per 100 ml.
(a) Control Diet (5.4% of Salt Mixture)	3	170	158	4.908	5.2147	0.639	0.679	0.94	6.6	10.4	2.75
(A) Control Diet (5.4% Salt Mixture)	3	169	183	4.908	5.2147	0.639	0.679	0.94	7.8	11.0	3.16
(b) Perennial Ryegrass as 33.3% of Diet(D.M.)	3	175	160	4.864	4.6301	0.633	0.603	1.05	6.4	11.9	3.63
(B) Perennial Ryegrass as 33.3% of Diet(D.M.)	3	171	170	4.864	4.6301	0.633	0.603	1.05	6.9	11.8	2.66
(c) Mixed Pasture as 33.3% of Diet(D.M.)	3	172	170	4.941	4.5251	0.643	0.589	1.09	5.9	12.4	3.33
(C) Mixed Pasture as 33.3% of Diet(D.M.)	3	169	148	4.941	4.5251	0.643	0.589	1.09	6.9	11.6	3.06

Table 17 (b)

Summary of The Analyses of Variance Of (a) Blood Inorganic P, (b) Serum Ca, (c) Serum Mg From Experiment 4.

Source of Variation	d.f.	Mean Squares and Results of F Tests. (a) P.	(b) Ca.	(c) Mg.
Treatments	2	0.36 N.S.	1.01 N.S.	0.031 N.S.
Error	3	1.33	0.17	0.196
Total	5			
MEANS AND S.Es.				
a A	)	7.2	10.7	2.95
b B	)	6.6	11.8	3.14
c C	)	6.4	12.0	3.19
S.E ±		± 0.81	± 0.29	± 0.33

N.S. = Not Significant.

Table 17 (c)

AVERAGE Ca AND P COMPOSITION OF HERBAGE FED IN EXPERIMENT 4 (% D.M.)

Herbage	Hygroscopic Moisture	Ash	Ca	P	Ca:P
Perennial ryegrass	9.10	8.68	0.622	0.451	1.38
Mixed Pasture	9.10	12.30	0.652	0.410	1.59

SPECIES COMPOSITION OF HERBAGE

Perennial Ryegrass		Mixed Pasture	
Species	%	Species	%
Perennial ryegrass	95	Perennial ryegrass	7
Poa trivialis	4	Cocksfoot	6
Hawkbit and Rumex	1	Timothy	2
		Poa trivialis	27
		Chewings fescue	5
		Brown top	11
		Yorkshire Fog	6
		Praire Grass	4
		White Clover	19
		Suckling Clover	2
		Hawkbit)	
		Plantago Major)	
		and other weeds)	11
Total	100.0	Total	100.0

Table 18

Experiment 5

THE TRENDS OF BLOOD Ca AND P AFTER ORAL ADMINISTRATION (mg per 100 ml.)

Time After Feeding	No. of Animals	Dietary Ca and P Levels	
		P x 5	Ca x 5
1 hour	1	8.9	-
2 hours	3	8.8	12.0
6 hours	3	8.6	12.0
16 hours	2	-	10.5
20 hours	3	7.7	10.5

SECTION 3

DISCUSSION

(a) Phosphorus Metabolism.

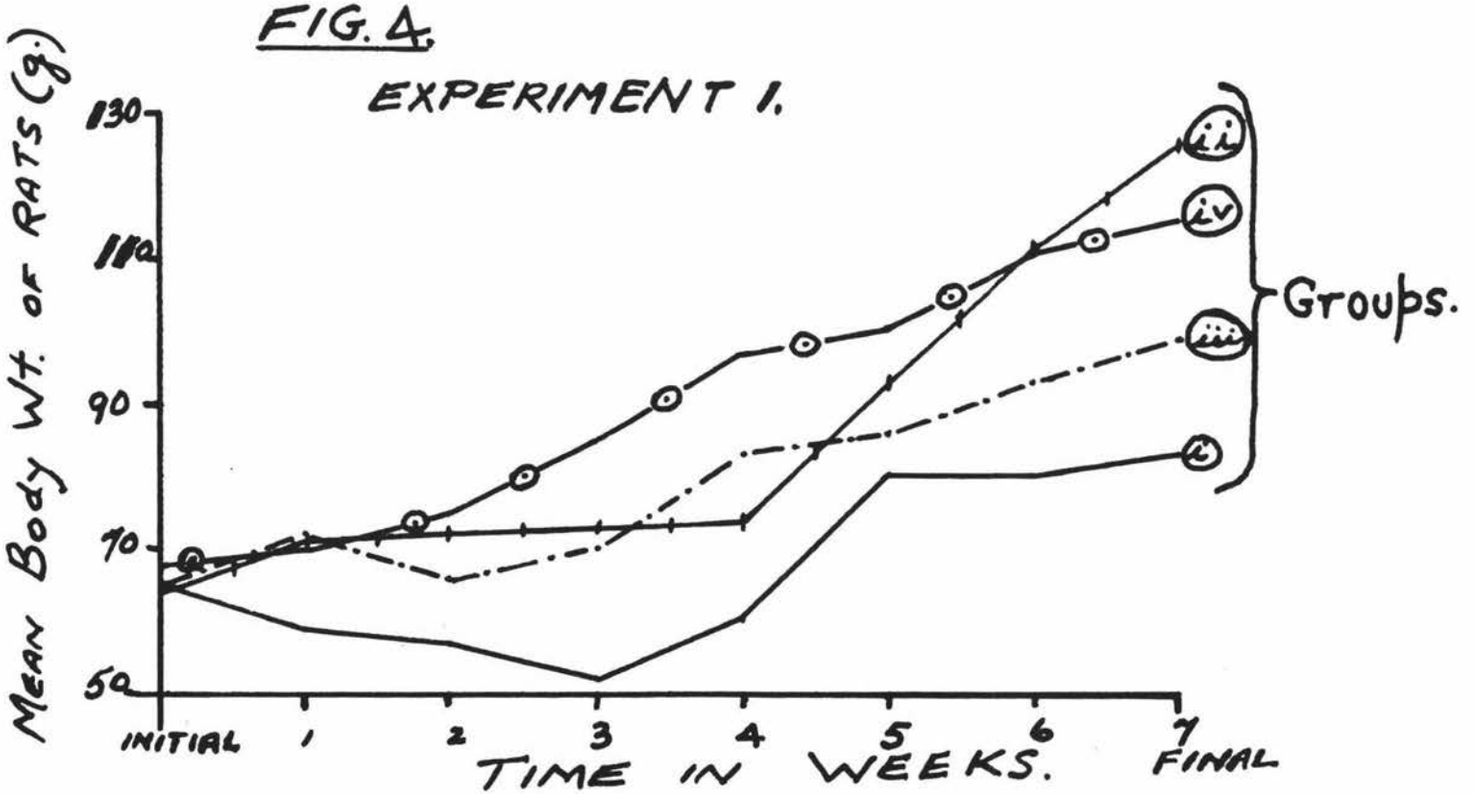
The results from Experiment 1 (Table 13) show that Group (iv) possessed a value for blood inorganic phosphorus that is not significantly different from that obtained for Group (iii), although the latter were fed dietary phosphorus equivalent to one fifth of the quantity fed to Group (iv). Groups (i) and (iii) have received equivalent intakes of phosphorus but show a comparatively greater difference in blood values.

Attempts were made to equate the total dietary intakes of the individual groups in all experiments, but the variable feed intakes between groups did not make this possible in this experiment. The result is that Group (ii) obtained a higher percentage of dietary phosphorus than Groups (i) and (iii), as originally planned, but the mean intakes of phosphorus for the three groups were equivalent.

Groups (i) and (iii) were unthrifty (Fig.4), and during the experiment two of the animals from Group (i) and one from Group (iii), died. Group (iii) fed voraciously and reflected a much larger dietary requirement than was allowed. The high excitability of this group was probably due to the very high level of calcium fed.

Rats in Group (ii) proved the most thrifty, and despite the equivalent intake of phosphorus, this group maintained a much lower level of blood inorganic phosphorus than Group (iii).

FIG. 4.

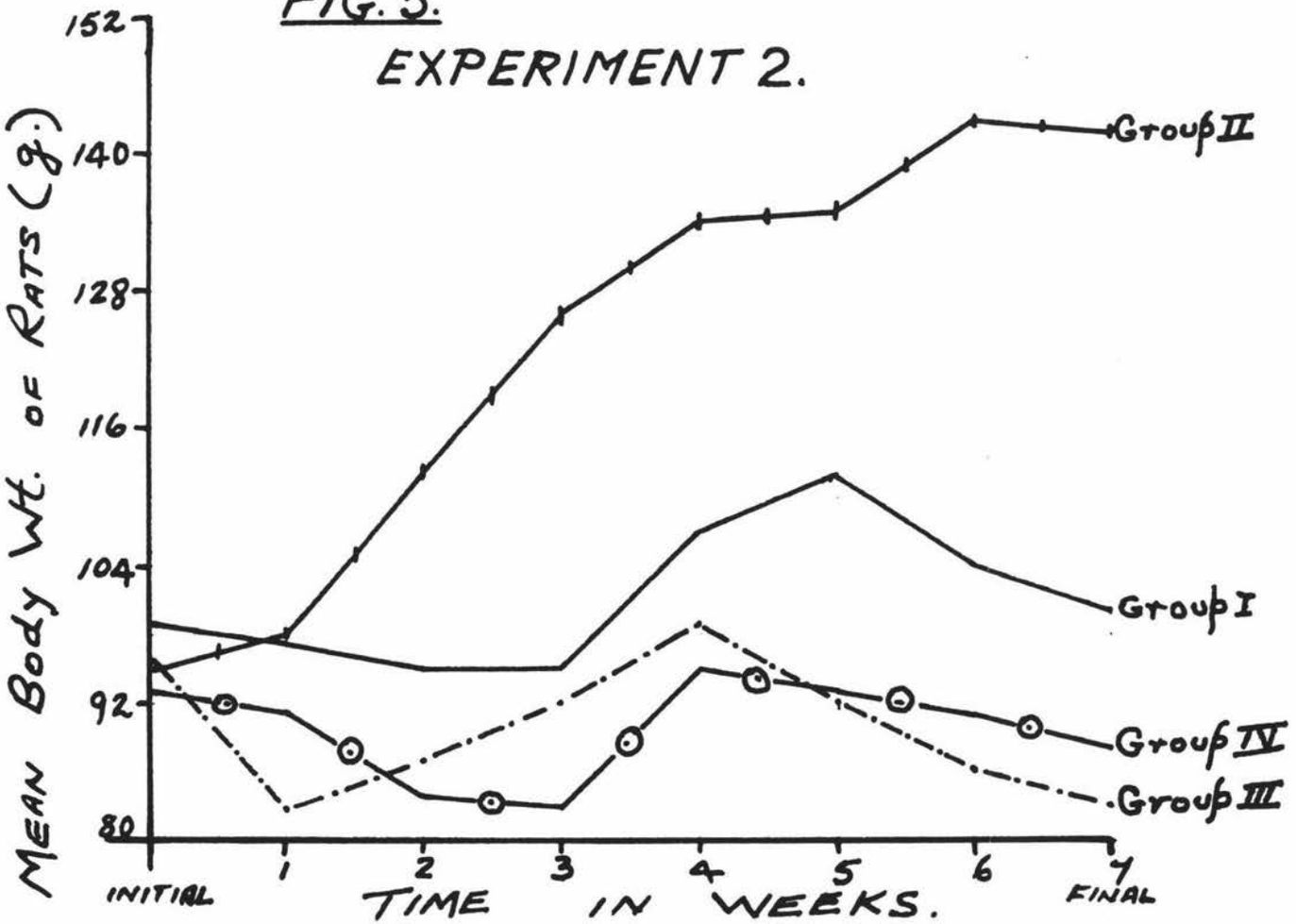

EXPERIMENT I.
MEAN BODY WT. OF RATS (g.)
130
110
90
70
50
INITIAL
1
2
3
4
5
6
7
FINAL
TIME IN WEEKS.
Groups.
ii
iv
iii
i

It can be assumed that the most thrifty group has obtained the most adequate percentages of dietary calcium and phosphorus. On this basis the percentage of dietary phosphorus fed to Group (i) was inadequate. This is so although equivalent quantities of phosphorus were fed to both Groups (i) and (ii). Percentages of dietary phosphorus are indicated to be more important to the thrift of the animal than the total amount fed over a given period. Excessive levels of dietary phosphorus would therefore fail to contribute proportionately to the thrift of the animals, over this period.

The marked difference in blood phosphorus values between Group (iv) and Group (ii) is not reflected in the phosphorus content of bones from both groups. A direct relationship has not been established between the phosphorus contents of the diets and the bones, and this queries the possible significance of the difference in blood values. Furthermore, a similar difference exists between Group (iii) and Group (i), although the percentages of dietary phosphorus are the same for both groups. The mean phosphorus contents of bones do not relate to the trends in blood inorganic phosphorus levels, for the latter groups.

Hansard and Plumlee (1954) demonstrated that inadequate intakes of calcium affected the absorption and retention of phosphorus. Adding this view to the assumption made, based on the thrift of Group (ii), it seems that the calcium intakes of Groups (i) and (iv) were inadequate and should retard the utilisation of dietary phosphorus. This could account for the absence of a higher value for blood inorganic phosphorus in Group (iv), and for the lowest value of 8.32 mg. per 100 ml. found in Group (i). It also appeared that the excessive calcium fed to group (iii) enhanced the value of 9.6 mg. shown for blood inorganic phosphorus.

FIG. 5.


EXPERIMENT 2.
MEAN Body Wt. OF RATS (g.)
152
140
128
116
104
92
80
Group II
Group I
Group IV
Group III
INITIAL
1
2
3
4
5
6
7
FINAL
TIME IN WEEKS.

These assumptions, however, do not relate the trends in bone phosphorus contents with the differences in blood inorganic phosphorus values. The evidence in this Experiment does not support the findings of Sobel and his co-workers (1945 a, b; 1948), which relates the phosphorus content of the diet, the blood and bone in animals fed adequate vitamin D.

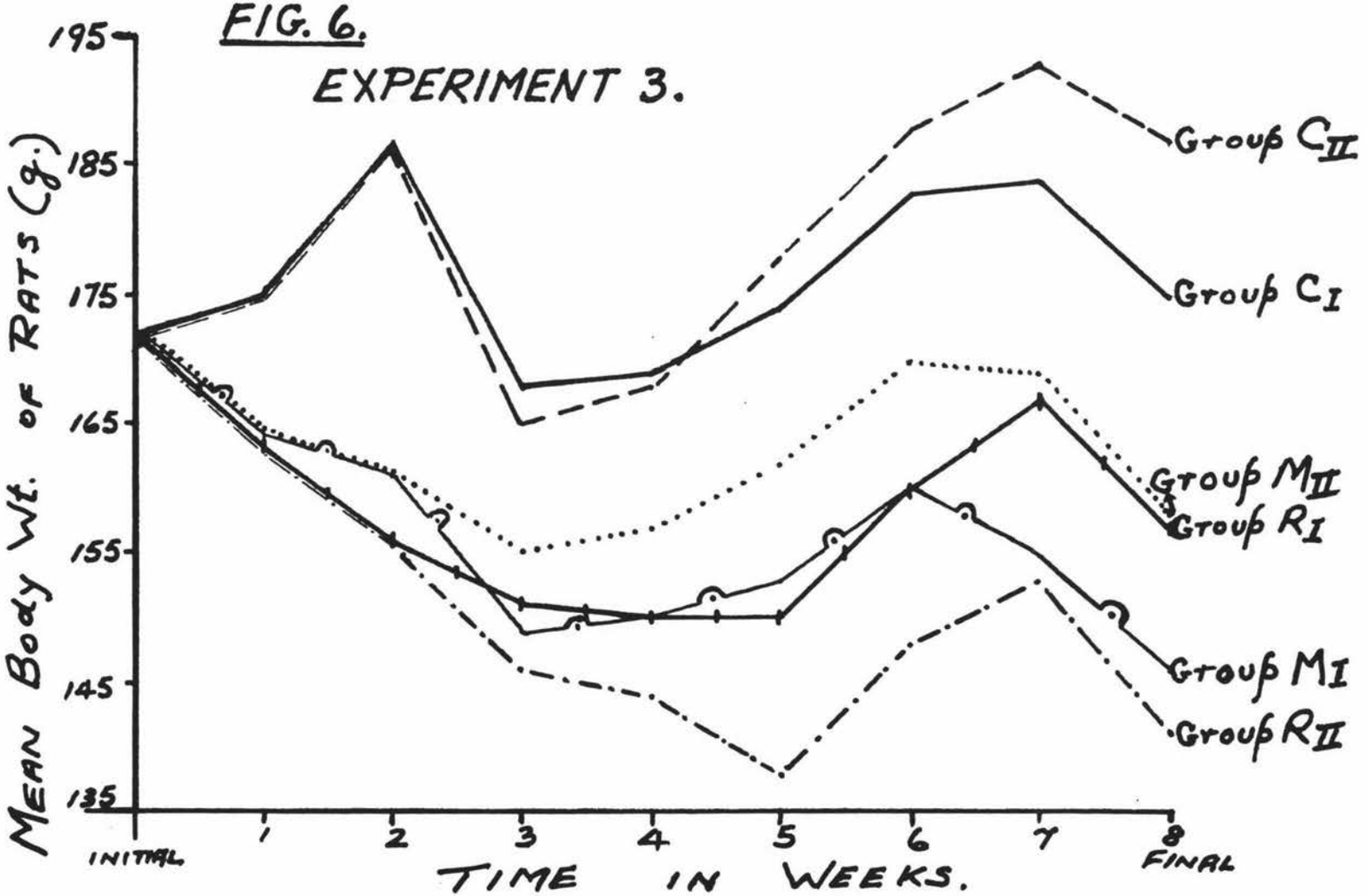
Table 14 contains the results from Experiment 2. As in the previous experiment the group fed the diet containing 4.5% salt mixture was the most thrifty, followed by the group fed 3.6% salt mixture (Fig.5). The two groups which received pasture ashes in the diets obtained the lower dietary calcium and phosphorus, showed the lesser thrift, but did not have a lower mean value for blood inorganic phosphorus than the other two groups.
The mean bone phosphorus content of Groups I and II were however greater than that of Groups III and IV. This shows that a greater quantity of available phosphorus was fed to the first two groups.

A comparison of Groups I and II showed that the higher percentages of phosphorus in the diet were associated with the more thrifty animals in Group II. The latter group displayed the higher contents of blood inorganic phosphorus and the mean phosphorus content of bones. Group IV, on the other hand, had the highest blood value although they received the lowest intake of phosphorus. The mean phosphorus content of bones is similar for Groups III and IV.

The thrift of the separate groups are not directly related to the levels of blood inorganic phosphorus, but to the phosphorus contents of the bones and the diets.

FIG. 6.

EXPERIMENT 3.



Dietary phosphorus did not directly affect the levels of blood inorganic phosphorus among the groups. Ash provided a greater proportion of dietary phosphorus in Group III than in Group IV, but a difference in availability is not reflected in the bone phosphorus contents. The mean phosphorus contents of bones were similar for both groups and indicate equal storage of dietary phosphorus, in spite of the differences in blood inorganic phosphorus.

In Experiment 3, the thrift of individual groups as represented by the gains in body weights (Fig.6) is again related to the phosphorus contents of the bones and the diets, but not to the blood values. The control groups were fed similar diet but showed different values for blood inorganic phosphorus.

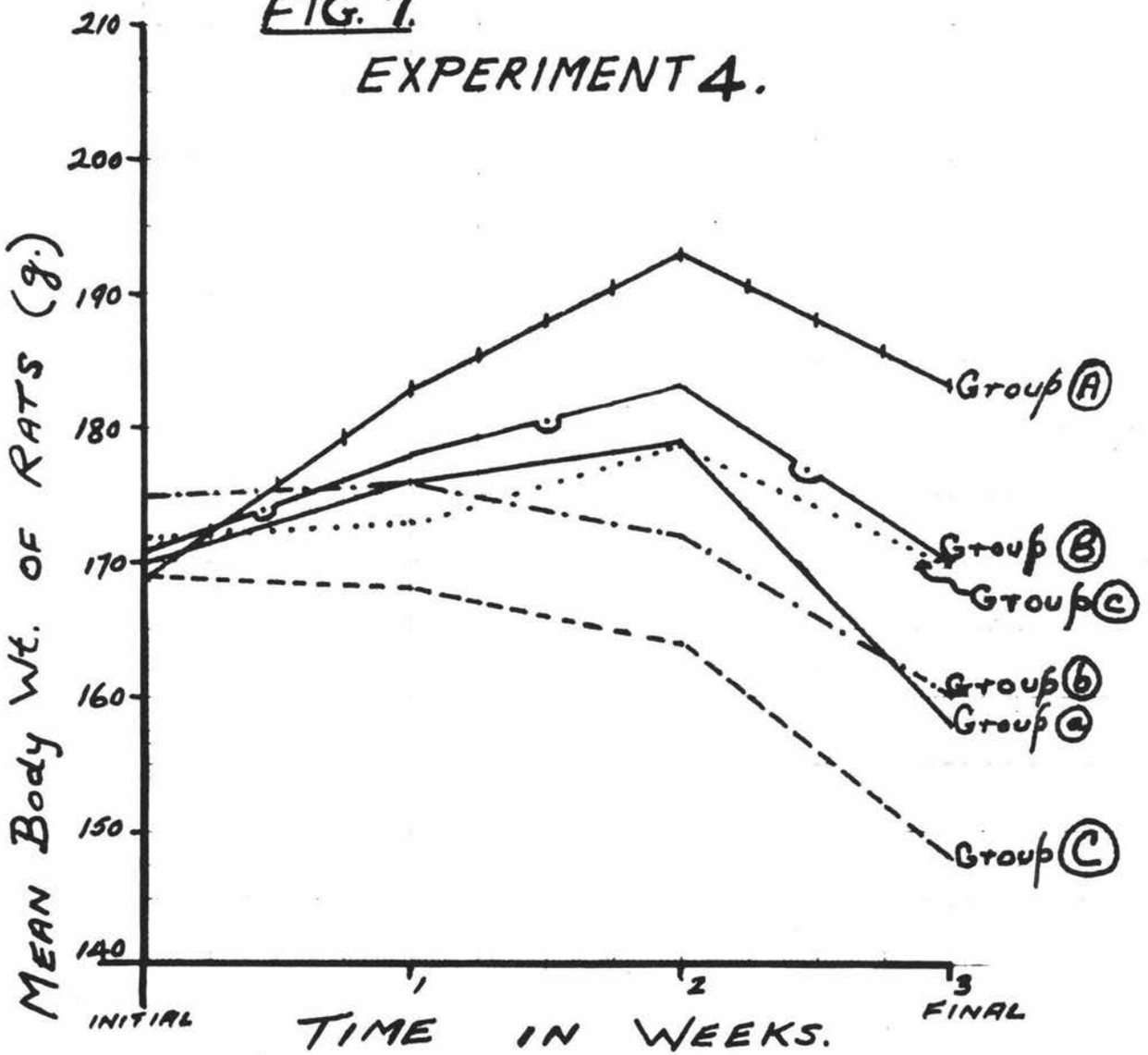
A noticeable feature is that the within treatment variations exceed that found between treatments, for blood values. An analysis of variance, summarised in Table 16c evinced no significant differences between treatments.

Table 16b indicates that the control groups retained a larger proportion of dietary phosphorus than the other groups. This points to greater non-availability of dietary phosphorus among the ash-fed groups, and explains the higher phosphorus contents in bones from the controls. There is, however, a marked difference in the percentage of phosphorus retained between Group R_I and Group R_{II}, despite similar intakes, and the absence of a significant difference between the values for blood phosphorus.

The high variation within a treatment suggests the importance

FIG. 7.

EXPERIMENT 4.



of physiological variation between individual groups, irrespective of the levels of phosphorus intake.

Ash supplied a higher proportion of the total dietary phosphorus in Groups R_I and R_{II} than in Groups M_I and M_{II}. This could account for the lower mean retention associated with the former groups, but fails to explain the greater difference in retention within treatments. It is difficult, therefore, to establish a difference in phosphorus availability between the two pasture treatments.

There are indications that the differences in blood phosphorus values are a reflection of normal functional variations between the groups. Dietary phosphorus intakes are allied to bone phosphorus contents because of the storage function of bones. The blood serves to convey minerals from the site of absorption to other organs for use and storage, hence it maintains constant concentrations of the circulating minerals under normal conditions (Shohl, 1939). This view negates the easy upset of the state of phosphorus equilibrium suggested by the findings of Sobel et al. (1945 a, b).

Experiment 4 (Tables 17a, b) supports the view that blood values are independent of levels of phosphorus intake.

The animals used in Experiment 5 were fed a diet which contained 5.4% salt mixture for a period of ten weeks, starved for a day, and then fed calcium and phosphorus as designated in Table 18, for one hour. The basic assumption was that a homogeneous population of experimental rats, kept on the same diet would show similar values for blood calcium and phosphorus. On this basis the

results intimated that the sudden feeding of high levels of dietary phosphorus causes an immediate rise in blood values, followed by a decline back to normal within twenty four hours. This strengthens the view that absorbed phosphorus is rapidly removed from the blood for use and storage elsewhere (Palmer et al., 1930; Cohn and Greenberg, 1938).

The marked within treatment variation denoted in previous experiments limits the value of the suggestions in this experiment. Studies with radio-active isotopes have however verified the rapid removal of absorbed calcium and phosphorus from the blood (Cohn and Greenberg, 1938; Hansard, Comar, Plumlee, Hood and Hobbs, 1950; Visek et al., 1952).

(b) Calcium Metabolism.

It will be seen from Experiment 1 (Table 13) that the group fed the diet with a calcium content of 2.42%, had a lower value for serum calcium than the group given the diet which contained 0.54% calcium. The mean calcium content of bones from Groups (iii) and (iv) did not reflect the great difference in the dietary calcium fed. One suggestion is that the very high level of dietary phosphorus fed to Group (iv) promoted increased storage of the comparatively lower dietary calcium. Hansard and Plumlee (1954) did not find that the intake of phosphorus affected the utilisation of calcium in rats.

In order to account for the absence of greater storage of bone calcium in Group (iii), it appears that the high level of calcium fed was in excess of the threshold level beyond which bone accretion ceased and increasing storage was at a minimum.

It is well-known that the efficiency of utilisation decreased with increasing dietary intake of calcium. Hansard and Plumlee (1954) observed that animals on the higher intake excreted the greater quantity of metabolically derived calcium, and thus showed that body stores of the element affected the relative absorption of calcium from the intestines, and excretion into the intestines. Furthermore, in young animals the efficiency of absorption of calcium at any one age depended on the degree of saturation of the body (Fairbanks and Mitchell, 1936; Rottensten, 1938; Outhouse et al., (1939).

In the first four experiments, the percentages of calcium in the diets are seen to be related to the thrift of the animals, especially where the percentages of dietary calcium are parallel to the percentages of dietary phosphorus. The relationship does not appear where an excess of either calcium or phosphorus is fed. This could indicate the value of the calcium:phosphorus ratio in inducing adequate utilisation of both minerals, when the appropriate ratio, and dietary percentages of calcium and phosphorus have been attained.

Levels of dietary calcium are not directly related to the values for serum calcium from results shown in Tables 13,14,16(a) and 17(a). There is, however, a direct relationship between the gains in body weights, the fat-free bone weights, and the calcium content of bones in Experiments 2 and 3. The mean intakes of calcium are also directly related to the mean calcium content of bones in the latter two experiments.

Bone calcium contents, unlike blood values, are more reliable as indicators of the availability of dietary calcium to the growing animal. From Tables 14 and 16(a), the groups which received additional calcium in the form of ash had the lower contents of calcium in their bones. The group fed ryegrass ash obtained a greater proportion of dietary calcium in the form of ash, than the groups on mixed pasture ash. Mean values for the calcium content of bones, for both ash treatments, reflected the slightly lower calcium content of the bones of the ryegrass ash groups. The latter difference, however, could not be definitely ascribed to a difference in the availability of calcium from the ash of both types of pastures. A higher retention for the mixed ash groups, as denoted in Table 16(b), may indicate the higher proportion of calcium fed in synthetic form to this treatment.

Once again, the large within treatment variation found between groups on the same diet places doubt on the significance of the difference in mean retention between the pasture treatments.

The control animals, on the completely synthetic diets, received more available calcium than those fed pasture ash. This is due to the lower availability of calcium from the pasture ash, and the lower calcium content of the ash diets.
Serum calcium values do not relate to the differences shown in availability of dietary calcium. Differences in mineral availability do occur in the types of pastures to which sheep are exposed, but from the evidence, in these experiments, blood values do not normally show the fluctuations due to such differences.

Despite the suggestion that the intake of phosphorus enhanced the utilisation of dietary calcium in Group (iv), Table 13, the general evidence does not show that the dietary calcium : phosphorus ratio is distinctly correlated to the absorption and retention of calcium, or to serum calcium values.

(c) Magnesium Metabolism.

Throughout these experiments no estimations were made as to the magnesium intake of the animals. Data was collected for values of serum magnesium content in order to observe the nature of possible differences, and to note the relationship between calcium and magnesium in the blood.

The results shown in Table 13 point to the high level of dietary calcium fed to Group (iii)as a possible cause of the lowest value for serum magnesium. Any suggestion of an antagonism between both elements is however weakened,since subsequent results,as seen in Tables 14, 16a, and 17a do not support this. Analyses of variance showed highly significant differences between the treatments (Table 16c) in Experiment 3. The value of these significant results is lessened by the unusually small degree of experimental error found, and which contributes to the high significance denoted at the 1% level. This view is strengthened by the fact that the within treatment variations were greater than those found between treatments, (Table 17a) in Experiment 4.

Dietary intakes of calcium and serum calcium values have not been related to the values for serum magnesium in these experiments, and the general indications are that the variations discerned are largely due to normal physiological variations between groups of similar animals.
Rook and Balch (1958) pointed out the difficulty of establishing the extent of variations in the availability of herbage magnesium. The use of blood values as an indicator of variations in the availability of magnesium from pasture herbage are denoted to add to this difficulty in the present experiments.

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CONCLUSION

The use of blood values as an index of the availability of calcium, phosphorus and magnesium from pasture herbage affords inherent limitations. This appeared in Part 1 where such data was the sole source of information on the trends in mineral metabolism. The discussion on serum calcium has necessarily followed the pattern of an elimination process owing to the difficulties of pinpointing the main reasons for the significant trends discerned.

Numerous factors could have contributed to the trends denoted in the grazing animal. In addition, the absence of supporting data on the intake, retention and bone levels of these minerals enhanced these difficulties.

Estimations of the mineral content of pasture herbage were done to show that natural variations do occur. They were not done to denote a direct relationship with blood values.

In Part II, a more precise relationship was depicted in the discussion because of the several sources of data relating to the mineral turnover of the animals.

Briefly, the preceding studies showed the following:-

(1) That the blood is not a reliable index of the calcium, phosphorus and magnesium status of the body, not of the availability of these minerals from pasture herbage.

(2) That under normal conditions the calcium and phosphorus levels of the blood do not vary with fluctuations in dietary intakes of these minerals.

(3) That a continuous exposure to adequate solar radiation was compatible with the constant synthesis of vitamin D, and that this was essential to the maintenance of the normal serum calcium levels in the grazing sheep.

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APPENDIX I - IX

DATA FOR BLOOD VALU... AND BODY WEIGHTS

OF SHEEP USED IN PART 1.

APPENDIX I

Blood Values For Ca. P. and Mg From

Cows Sampled On 4/12/58(mg. per 100ml.)

Cow No.	Treatment	P.	Ca.	Mg.
53		7.8	11.1	2.32
57		6.4	10.3	2.36
28		6.7	10.7	2.8
72	P.	5.5	11.1	2.6
90		5.9	11.[illegible]	2.48
71		5.8	10.9	2.16
110		5.2	12.1	2.44
105		6.3	11.1	2.68
45		6.5	9.7	2.76
35		5.6	10.7	2.68
16		6.5	11.2	2.76
14	R.	5.9	10.8	2.48
19		5.5	10.3	2.64
112		6.4	11.7	.84
104		4.8	10.7	2.36
118		5.7	11.7	2.60
20		3.3	11.5	3.08
67		4.0	11.2	2.96
47		4.2	10.7	2.64
87		3.8	11.1	2.84
77	P. + C.	4.6	10.4	2.16
97		5.5	10.8	2.60
8		5.2	10.0	2.66
34		5.0	10.7	2.12
10		3.5	11.1	2.84
32		5.1	10.0	2.8
120		4.3	11.9	2.72
96		4.4	11.1	3.00
4	S.R. + C.	6.7	10.7	2.52
52		6.4	11.7	2.16
70		5.1	10.5	2.4
78		4.6	10.4	2.52

APPENDIX II

Blood Values For Ca, P and Mg. From Lambs Sampled On 16/1/59(mg.per 100 ml.)

Lamb No.	Treatment	P	Ca	Mg
539	Short Rotation Ryegrass (S.R.)	8.75	11.8	2.3
765		8.1	12.5	2.8
571		9.0	13.2	2.76
546		7.8	11.2	2.85
640		7.4	12.8	2.8
658		9.3	11.8	2.42
616		10.2	12.7	2.8
645		9.3	12.1	2.55
707		9.5	12.6	3.06
606		8.5	13.2	2.9
521	Perennial Ryegrass (P.)	10.1	11.9	2.72
553		9.2	12.2	3.00
729		8.4	12.7	2.85
714		8.1	11.7	2.85
579		10.2	14.2	2.72
657		8.9	12.0	2.63
661	Short Rotation and White Clover (S.R. + C)	8.3	13.1	2.85
778		8.4	11.4	2.85
506		7.5	12.7	2.42
563		9.6	12.7	2.68
761		7.4	13.2	3.21
618		8.1	12.2	3.21
634		8.5	12.0	3.0
581		8.75	12.7	3.2

APPENDIX III

Blood Values For Ca. P. and Mg. from

Lambs Sampled on 24/3/58(mg.per 100 ml.)

Lamb No	Treatment	P	Ca.	Mg
714		7.0	10.4	2.33
657		7.3	10.3	2.76
729		7.0	9.6	2.43
521	P	7.0	10.0	2.52
579		8.3	9.7	2.30
553		7.0	9.7	2.33
506		7.3	10.7	2.40
581		7.6	10.7	2.66
618	N.R. + C.	5.6	10.7	2.30
563		7.0	9.7	2.40
661		7.8	10.1	2.52
778		5.3	10.1	2.24
601		7.6	10.2	2.33
776		6.5	10.2	2.62

APPENDIX IV

Blood Values For Ca. P and Mg from Ewes Sampled On 12/6/52(mg. per 100 ml.)

Ewe No.	Treatment	P	Ca	Mg
39		5.6	10.9	2.6
30		3.7	10.7	2.3
103		5.3	10.4	2.3
152	P	5.6	10.1	2.8
41		5.2	9.8	2.3
147		5.5	10.1	2.5
86		4.3	9.8	2.3
82		6.0	9.4	2.5
62		5.7	10.6	2.8
39		3.7	9.6	2.5
64		5.3	9.3	2.7
97		5.0	10.9	2.5
32	S.R.	5.3	9.6	2.6
3		4.7	9.5	2.9
35		3.0	9.3	2.7
58		5.2	9.3	2.7
38		5.2	9.3	2.8
25		4.7	10.1	2.7
38		4.8	9.9	3.1
65		4.4	9.8	2.8
100	P. + C.	5.2	9.3	2.3
129		5.1	10.1	2.4
17		5.9	9.3	2.7
8		5.7	9.3	2.7
133		4.5	9.9	2.4
23		5.5	9.9	2.5
57		5.3	10.1	2.4
125	S.R. + C	4.5	9.3	2.2
130		5.1	9.3	2.7
47		5.2	10.1	2.3
83		3.6	9.6	2.3

APPENDIX V

Blood Values For Ca, P and Mg. From Ewes Sampled 24/9/59 (mg.per 100 ml.)

Ewe No	Treatment	P	Ca	Mg
99		5.2	9.6	2.57
30		4.6	8.3	2.64
103		4.4	9.6	2.6
152	P.	3.4	8.1	2.6
147		5.5	9.6	2.34
86		4.1	7.7	2.5
92		5.0	8.0	2.1
62		3.6	7.8	2.53
39		7.9	8.8	1.9
64		-	7.8	2.6
97	S.R.	4.2	10.1	2.57
32		3.2	8.4	2.34
3		4.3	7.7	2.42
35		3.3	9.1	2.71
58		5.	7.4	2.28
88		-	7.7	2.6
25		7.3	10.5	2.1
58		3.9	9.3	2.6
65	P. + C.	3.7	8.6	2.57
100		6.1	9.9	2.5
12		7.3	8.6	2.68
17		5.0	7.8	2.6
8		4.0	7.1	2.63
133		5.2	8.6	2.39
23		-	9.9	2.68
57		5.8	10.4	2.5
125		5.6	10.1	2.53
130	S.R. + C.	4.0	8.6	2.57
139		6.4	9.4	2.6
47		4.7	9.3	2.68
83		5.4	10.4	2.39

APPENDIX VI

Blood Values For Ca From Ewes

Sampled On 14/10/59 (mg.per 100 ml.)

Ewe No	Treatment	Ca
147		8.4
36		8.6
46	P.	8.4
50		7.9
73		11.3
103		9.9
63		6.6
143		8.5
80		9.3
97		8.9
71		8.1
33		9.5
35		9.8
39	S.R.	6.3
40		10.1
102		9.0
111		10.3
62		9.5
3		8.2
87		7.6
32		8.2
112		7.7
129		9.3
142		9.9
25	P. + C.	10.2
49		8.4
122		10.3
70		9.2
127		10.8
125	S.R. + C.	10.4
133		9.6
11		10.3

APPENDIX VII

Body Weights (lb.) And Time Of Weighing

Ewe Hoggets Sampled 12/6/59.

Ewe No.	Treatment	12/6/59	16/7/59	24/9/59
99	P.	77	78	89
30		75	70	84
103		75	73	97
152		58	59	59
41		78	66	74
147		78	78	90
86		70	75	79
82		77	80	95
62	S.R.	72	74	77
39		69	70	70
64		66	72	101
97		72	74	92
32		62	62	60
3		65	69	71
35		64	65	73
58		75	77	93
88	P. + C.	78	76	87
25		61	64	75
38		61	62	72
65		92	92	102
100		78	80	82
129		72	67	70
17		70	73	73
8		65	66	74
133	S.R. + C.	80	83	89
23		87	90	107
57		69	75	90
125		72	75	89
130		80	80	87
139		90	97	117
47		72	73	92
83		73	74	92

APPENDIX VIII

Body Weights (lb) And Time of Weighing Of Ewes Sampled 4/12/58.

Ewe No.	Treatment	19/2/58	13/3/58	7/5/58	15/12/58
105	P.	88	83	81	104
90		95	95	99	110
58		105	101	102	115
57		90	92	95	101
28		83	71	62	69
72		97	96	93	96
71		86	74	74	91
110		102	103	95	92
45	S.R.	81	81	87	97
35		113	119	125	99
16		103	106	112	138
14		106	110	116	133
19		97	101	109	114
112		98	98	106	109
104		103	106	108	122
118		94	93	98	105
20	P. + C.	85	90	100	112
67		90	96	105	119
47		94	86	102	131
87		82	90	96	109
77		80	88	103	115
97		82	88	99	106
8		83	90	98	132
34		88	93	101	134
10	S.R. + C.	138	138	137	151
32		145	150	152	123
120		120	121	115	104
96		131	132	126	133
4		138	133	139	150
52		145	149	142	157
70		116	118	110	115
78		146	149	150	159

APPENDIX IX

Mean Body Weights Of Sheep (lb.)

Ewe Hoggets Samples 12/6/59

Treatment	12/6/59	16/7/59	24/9/59
P.	73.5	72.4	83.4
S.R.	67.0	70.4	79.6
P. + C	72.2	72.5	80.0
S.R. + C.	78.0	30.8	95.4

Ewes Sampled 4/12/58

Treatment	19/2/58	13/3/58	7/5/58	15/12/58
P.	93.2	89.4	87.6	97.2
S.R.	99.4	101.7	107.6	114.2
P. + C.	85.5	90.1	100.5	119.7
S.R. + C.	135.0	136.2	134.0	136.5

APPENDIX X - XI

DATA FOR BONE VALUES AND BODY WEIGHTS,
ON RATS USED IN PART II

APPENDIX X

Ca and P contents of Right Femura

Experiment 1

Groups	No. of Bones	Total Bone Ca (g.)	Total Bone P. (g.)
(i)	2	0.0709	0.03639
(ii)	4	0.108	0.07049
(iii)	4	0.1425	0.0697
(iv)	5	0.1444	0.07193
Experiment 2			
I	5	0.156	0.07869
II	4	0.207	0.10473
III	4	0.141	0.07236
IV	4	0.144	0.07243
Experiment 3			
C I	3	0.2235	0.11019
C II	3	0.2415	0.11947
R I	3	0.21	0.10243
R II	3	0.137	0.09322
M I	3	0.198	0.09898
M II	3	0.2121	0.10545

APPENDIX XI

Mean Body Weights Of Rats (g.)

Experiment 1.

Groups	(25/5/59) Initial	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	(13/7/59) Final
(i)	65	59	57	52	60	80	80	83
(ii)	64	71	72	73	74	95	111	126
(iii)	65	72	66	70	83	86	93	99
(iv)	68	70	75	85	97	100	111	115

Experiment 2.

	(23/6/59) Initial	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	(11/8/59) Final
I	99	97	95	95	107	113	104	100
II	95	98	112	124	134	135	143	142
III	96	83	87	92	99	92	86	83
IV	93	91	84	83	95	93	91	88

Experiment 3.

Groups	(15/9/59) Initial	1st Wk.	2nd Wk.	3rd Wk.	4th Wk.	5th Wk.	6th Wk.	7th Wk.	(10/11/59) Final
C I	172	175	137	163	169	174	133	134	175
C II	172	175	137	165	163	173	133	193	157
R I	172	163	156	151	150	150	160	167	157
R II	172	163	156	146	144	138	143	153	141
M I	172	164	161	149	150	153	160	155	146
M II	172	164	161	155	157	162	170	169	158

Experiment 4.

Groups	(26/12/59) Initial	1st Wk.	2nd Wk.	(16/1/60) Final
(a)	170	176	179	158
(A)	169	183	193	183
(b)	175	176	172	160
(B)	171	178	183	170
(c)	172	173	179	170
(C)	169	168	164	148