

Rickets in Sheep

1. The Experimental Production of Rickets in Young Sheep

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Rickets as a widespread disease of children has been recognized since the seventeenth century, and although the therapeutic efficacy of cod-liver oil was recognized even in those days, the precise aetiology of this disorder was not fully determined until about 20 years ago, since it awaited the discovery of the decisive role played by vitamin D in osteogenesis and in phosphorus and calcium metabolism.

In these circumstances it is not surprising that though the existence of widespread osteodystrophic disease in ruminants had been described in parts of Europe (Marek & Wellmann, 1931) and particularly South Africa (Hutcheson, 1895), it was not until Theiler's studies (Theiler, Green & Du Toit, 1924; Theiler, 1934) demonstrated the primary part played by phosphorus that progress was made. Not long afterwards, Du Toit, Malan & Rossouw (1930) and Du Toit, Malan & Groenewald (1931, 1932) were able to show that rickets in lambs and osteoporosis of older sheep ('Lamsiekte') in South Africa were similarly due to phosphorus deficiency.

In Australia, Henry (1912) had demonstrated the value of phosphorus supplements for cattle in New South Wales, and Richardson, Trumble & Shapter (1931) showed the relationship between the low phosphorus content of pastures and occurrence of much ill health in ruminants. It was found, however, that the response to phosphate supplements was often disappointing. The explanation was advanced by Marston (1934) that there was an associated lack of protein in pasture from the phosphorus-deficient areas, sometimes also accompanied by a trace-element deficiency.

In New Zealand the same earlier confusion occurred, as is shown by the accounts of Reakes (1910), Aston (1930) and Wright & Taylor (1931). Though some osteodystrophic lesions encountered were almost certainly due to a suboptimal intake of phosphorus and calcium, much of the unthriftiness of certain sheep-farming areas has since been shown to be due primarily to cobalt deficiency.

The lack of association in South Africa and Australia of rickets or osteomalacia in the ruminant with insufficient vitamin D is hardly surprising in view of the almost universal practice of keeping animals out of doors all their lives, combined with the fact that the latitude of most of the sheep-carrying areas in both countries is such that the incidence of the sun's rays would, even in winter, not be less than 35°. This was claimed by Tisdall & Brown (1929) to be the critical angle below which the anti-

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rachitic effect of solar radiation rapidly diminishes. The South Island of New Zealand and much of Great Britain, however, do not receive sufficient ultraviolet radiation during the mid-winter months for young children, and the importance of vitamin D in the aetiology of rickets in hoggets in New Zealand has come to be recognized. At the same time, rainfall in both countries is more evenly spread, climate more temperate and pasture growth much less subject to drought with its attendant drop in mineral and protein content. The analyses of pasture made by Woodman & Evans (1930) in England and by Elliot, Orr, Wood & Cruickshank (1926) in Scotland show this last point clearly enough.

Under these conditions, various osteodystrophic diseases of sheep have been rather loosely described as 'rickets'. Elliot, Orr, Wood & Crichton (1926), in reporting experiments carried out in Scotland between 1921 and 1924 in which they observed the effect of adding calcium and cod-liver oil to the diet of young sheep, claimed that a rachitic disease, 'bent-leg', occurs spontaneously amongst sheep and is of widespread distribution. Auchinachie & Fraser (1932) were able to produce 'bent-leg' experimentally on a diet deficient in calcium and relatively high in phosphorus (Ca:P ratio = 1:13). Vitamin D alone, given as 10 ml. cod-liver oil daily, was as fully protective as extra calcium. W. L. Stewart (1933) described a disease of sheep prevalent at that time in lambs in Northumberland and Yorkshire and locally called 'cripples', although other names, including rickets, have been given to it. The disorder occurs in lambs from 6 days to 2 months old, with the greatest incidence in animals 1-2 weeks old. The pathological picture appears to be one of deficient osteogenesis, since the bone trabeculas are very slender, the corticalis narrow and osteoblasts few. The evidence from chemical studies of the blood (Shearer & Stewart, 1931) and field experimental work (Piercy, 1934*a, b*) all indicated that this was not true rickets. Similarly, a bone disease of older sheep in Yorkshire, described by Bowes (1932) and known locally as 'cappie' or 'double scap' because of the thinning of the frontal bones, does not appear to have shown the typical manifestation of rickets. Investigations by Bosworth & Stewart (1932-3) led to the conclusion that the bone lesions were due to arrested osteogenesis associated with porosis.

It had been suggested by Leslie (1935) that the lameness often seen in a proportion of lambs being overwintered in the South Island of New Zealand might be caused by rickets, but it was Fitch (1943) who confirmed the existence of true rickets from a pathological examination of material from a typical case of winter lameness in a hogget in Canterbury, New Zealand. Field trials followed on areas where the disease had been reported to occur to a varying extent each winter, and this led to the discovery that different winter fodder-crops varied in their rachitogenicity, but that young green oats, widely grown for grazing purposes in both New Zealand and Australia, were most actively rickets-producing (Fitch & Ewer, 1944). More rigidly controlled experiments were conducted over the next few years (Ewer & Bartrum, 1948) which clearly established the efficacy of single massive vitamin D dosage both prophylactically and therapeutically and also that the occurrence of rickets in untreated hoggets was always associated with low blood values for inorganic phosphorus but normal values for serum calcium.

Before studying the relationship between phosphorus and vitamin D in the growing sheep, it was necessary to produce rickets experimentally. This communication describes the production of rickets in lambs and the effect of supplementing a rachitogenic diet with vitamin D₂ and of altering the Ca:P ratio.

EXPERIMENTAL

Sheep

In an effort to reduce the variability to a minimum, twenty Welsh lambs of similar age and breeding were selected from a mob of ninety and brought to the Department's farm at Cambridge. They had been born in the latter part of April 1947 and arrived at the beginning of December of the same year and appeared to be in thriving condition. Samples of faeces were taken three times during the following 3 weeks and examined for fluke eggs or parasitic strongylid infestation. No fluke eggs were found and there was only a light nematode infestation (average egg count of 200/g.). However, in view of the interference with mineral metabolism that may occur through chronic worm infestation (Stewart & Shearer, 1932-3; Franklin, Gordon & Macgregor, 1946), anthelmintic treatment was given in the form of a dose of 15 g. phenothiazine on 27 December. On that day the lambs were brought inside and henceforth kept penned indoors with the windows distempered, to be certain that the lambs were not exposed to ultraviolet radiation.

For various reasons, amongst which was the difficulty of getting the sheep to take the experimental diet and of procuring some of its ingredients, the main experimental period did not start until 27 February 1948. Over the pre-experimental period weights were recorded at 3-weekly intervals and blood was analysed for haemoglobin, calcium and phosphorus. The sheep were weighed on 28 January. They were brought fully on to the experimental diet on 27 February and, following weighing on that day, were randomized into five groups of four sheep. The treatment of each group is shown in Table 1.

All sheep were weighed and radiographed, and blood samples were taken at 3-weekly or monthly intervals throughout the experiment. Each group was given its daily ration at 9 a.m. after any residues had been collected and weighed.

Diet

The main requirements appeared to be that the basal diet should be low in phosphorus, relatively high in calcium, contain sufficient protein of high biological value to meet the hoggets' potential growth needs, have enough vitamin A and a minimum quantity of vitamin D. The mixture that was designed to fulfil these needs and formed the daily basal diet given to all the sheep was: dried sugar-beet pulp 600 g., oat-straw chaff 80 g., blood meal 80 g., urea 5 g., molasses 150 g., calcium carbonate 14 g., salt 6 g.

Sufficient sugar-beet pulp and oat-straw chaff were bought to last the entire experiment. The quantities of both these ingredients were twice adjusted during the 1st month to bring the residues to a minimum before they were established at the above

Table 1. *Treatment of experimental sheep*

| Group no. | Sheep no. | Treatment |
|-----------|-----------|--|
| 1 | 4 | Low-P, basal diet only |
| | 13 | |
| | 16 | |
| | 20 | |
| 2 | 7 | High-P, basal diet with 15 g. $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ daily |
| | 9 | |
| | 11 | |
| | 14 | |
| 3 | 1 | Low-P* (as group 1) with vitamin D_2 25 mg. calciferol on 27 Feb. 1948; 23 mg. calciferol on 28 May 1948 |
| | 6 | |
| | 15 | |
| | 19 | |
| 4 | 2 | Low-P (as group 1) with vitamin D_2 later (sheep nos. 17 and 18 given 25 mg. calciferol on 6 August 1948) |
| | 3 | |
| | 17 | |
| | 18 | |
| 5 | 5 | High-P (as group 2) with vitamin D_2 later (sheep nos. 8 and 10 given 25 mg. calciferol on 6 August 1948) |
| | 8 | |
| | 10 | |
| | 12 | |

* The sheep in group 3 were given their first dose of calciferol (equivalent to 1,000,000 i.u. vitamin D_2) dissolved in 10 ml. arachis oil by mouth.

figure. The molasses was mixed with sufficient water (1.5 l.) to moisten the beet pulp and to enable the blood meal to be thoroughly mixed in.

Protein. The complete diet contained 1.21 % nitrogen and, since most of this was from the blood meal, of which the protein may be assumed to contain 16 % nitrogen, the crude protein content of the diet would be 7.6 %.

Phosphorus. The diet was found to contain 0.08 % P, and subsequent measurement of the actual daily intake of P of the low-P sheep expressed as an average figure over the 229 days of the main experimental feeding period was 0.3 g. daily. This is a lower intake than that maintained by the sheep on the low-P diets of Du Toit *et al.* (1932) or Martin & Pierce (1934). It may in fact be considered to represent about one-seventh of the optimum P intake of sheep of this age if the daily requirement of 2.2 g. P suggested by Beeson, Johnson, Bolin & Hickman (1944) be accepted.

Each sheep on the high-P diet was given 15 g. sodium monohydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) daily, equivalent to 1.20 % P in the diet, as a solution in 50 ml. warm water, usually administered on alternate days, except when any of these sheep were being used in a balance trial, when each was dosed daily. From 8 October 1948, the daily supplement was reduced to 7 g./sheep which was equivalent to 0.55 % P in the diet.

Calcium. The basal diet was designed to supply a high intake of Ca. This was done in order to have a wide Ca:P ratio, which has been recognized as being necessary for the establishment of rickets in rats, although Theiler (1934) stated that the production of rickets in sheep is dependent mainly on the amount of P in the diet rather than upon the relative amounts of P, Ca or magnesium. In addition, it was desired to avoid

complication with any of the osteodystrophies of sheep that have been shown to be associated with low serum-Ca values. The condition known as 'bent-leg' (Elliot, Orr, Wood & Crichton, 1926) has already been mentioned. Franklin (1934-5) has shown that a diet low in Ca could cause pathological changes in the bones of sheep which Innes (1934-5) suggested might be regarded as 'rickets'. Moreover, Duckworth, Godden & Thomson (1943), in their study of the development of rickets in sheep where a diet containing 1.6 g. Ca and 1.9 g. P was given, maintained that the most constant diagnostic sign of the onset of the disease was a fall in the serum Ca below 6 mg./100 ml.

The basal diet in the present work was found to contain 1.24 % Ca but, for the above reasons, 14 g. commercial calcium carbonate were added to the daily ration of all the sheep. This brought the Ca content up to 2.37 % (by analysis). In October 1948, when the P supplement was halved, the calcium carbonate was similarly reduced.

Vitamins. It has been shown by Miller, Hart & Cole (1942) and by McElroy & Goss (1940*a, b*, 1941*a, b*) that sheep are able to synthesize in their rumen most, if not all, of the vitamin B complex they require. Similarly, it was made clear by the work of Thomas, La Grange & Culbertson (1942) that the requirements of vitamin E in sheep are met by the common feeds. Guilbert, Miller & Hughes (1937) indicated that the minimum carotene requirement was about 3 mg./100 kg. body-weight, with a recommended allowance of four times this amount.

It is in relation to vitamin A that our diet was probably low, since experimental work concerned with vitamin A deficiency in calves, undertaken at this period by other workers in the Department, in which the same oat-straw chaff was used, indicated that its carotene content was very low. It is, however, well known that sheep normally have a high liver-storage capacity for vitamin A, and Hart (1940-1) showed that efficiency of utilization increases with low intake. It can therefore be reasonably assumed that these experimental sheep had sufficient vitamin A to satisfy their needs for the first 6-8 months. In October the vitamin A content of the blood plasma of the remaining sheep was estimated at weekly intervals, and it appeared that although there was a very wide variation between individual sheep, the trend was fairly sharply downward. For example, between 22 November and 11 January the mean value fell from 86 to 42 i.u./100 ml. A carotene-concentrate solution was then given at the daily rate of 130 i.u. carotene/kg. live weight, and from early March until the end of the experiment this was replaced by vitamin A acetate given in arachis oil at a rate of 20 i.u./kg./day.

The vitamin D content of the diet was of particular interest, and an assay made by Dr W. F. J. Cuthbertson upon an ether extract of 8 kg. of the dried, ground ration as fed gave a value of 0.025 i.u./g. Andrews & Cunningham (1945) found that the vitamin D requirement of young sheep was 160 i.u./100 lb. live weight. From the average intake figures of the sheep receiving different treatments it was found that the diet was theoretically unable to supply the vitamin D requirement of even the high-P animals, and the low food intakes of the low-P sheep would have made this deficiency more serious.

Analytical methods

Blood. Blood samples were obtained from the jugular vein. P estimations were made on duplicate samples of whole blood within 2 hr., using Gomori's (1942) modification of the method of Fiske & Subbarow (1925). Alkaline phosphatase was estimated on the plasma by the method of King & Armstrong (1934) and Ca by the method of Kramer & Tisdall (1921).

Haemoglobin. During the early months of the experiment and until some time after rickets had developed, haemoglobin estimations were made by the alkaline-haematin method (King, 1947), using a photoelectric colorimeter.

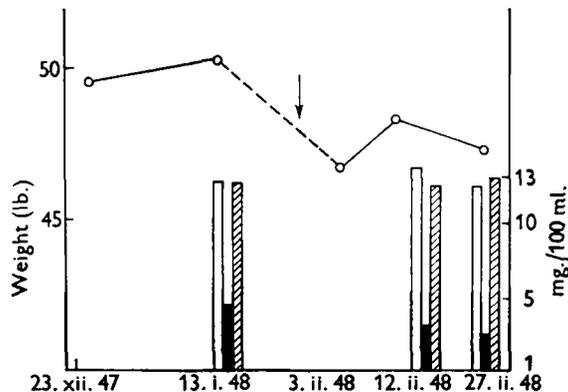


Fig. 1. Mean weights, haemoglobin, blood inorganic-P and serum-Ca values of sheep during the pre-experimental period. ○—○, weight; □, haemoglobin; ■, blood inorganic P; ▨, serum Ca; ↓, shorn.

Pathological examinations. The course of the bone changes was followed by making regular X-ray examinations of the distal epiphysis of the right radius of each sheep and by periodic biopsies of costochondral junctions performed upon representative sheep from the main treatment groups. This latter method was adopted because of the belief expressed by some workers (Follis, Jackson, Eliot & Park, 1943) that the only satisfactory way of diagnosing subclinical rickets is by microscopic examinations of rib junctions.

Tissue phosphatase. Samples of the main organs of rachitic and normal sheep were taken at slaughter, and use was made of Gomori's (1939, 1941) technique to study the distribution of alkaline phosphatase. These results and that of the microscopic examination of the rib junctions will be reported elsewhere.

RESULTS

Pre-experimental period. The effect of the pre-experimental period of feeding upon the weight, haemoglobin values, total serum Ca and blood inorganic P of all the sheep is indicated in Fig. 1, which shows the mean values.

Food consumption. The well-known effect of a low-P diet upon appetite, first noted by Theiler *et al.* (1924) in cattle and subsequently by Du Toit *et al.* (1930) in sheep, soon became apparent. The South African workers, both in the preliminary reports

cited and in their further experiments (Du Toit *et al.* 1932), found that this effect did not become obvious for several months. Martin & Pierce (1934) found that the chaff consumption of their low-P sheep rose almost as much as in their high-P sheep in the early period and began to fall steadily from the 4th month. Stewart (1934-5), although he did not measure the food consumption directly for all sheep, showed that the low-P and control sheep made similar gains over the first 3 months.

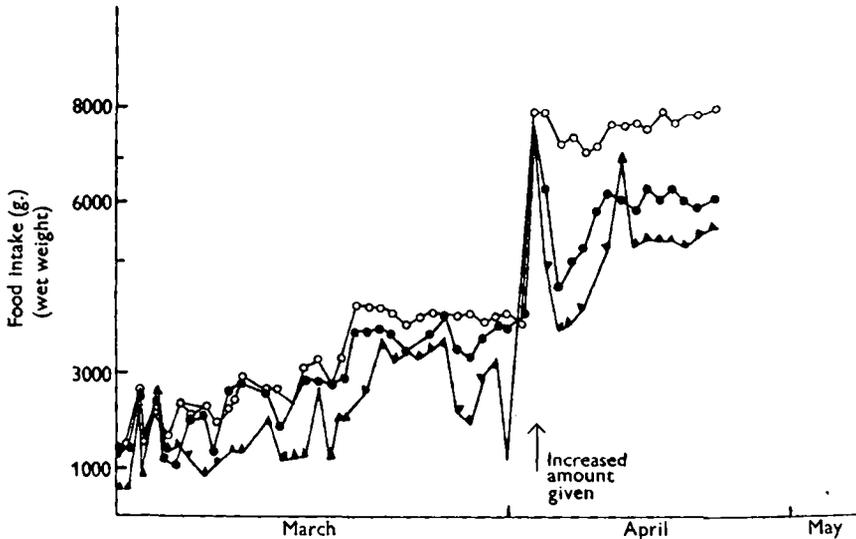


Fig. 2. Net food consumption of the sheep. ●—●, groups 1 and 4 (low-P, high-Ca); ○—○, groups 2 and 5 (high-P, high-Ca); ▲—▲, group 3 (low-P, high-Ca with vitamin D₃).

Within a week of the start of the present experiment the low-P lambs in groups 1, 3 and 4 were consuming less food than the high-P lambs in groups 2 and 5, and this difference became more marked from April onwards (5 weeks after the start of the experiment), when the water added to the beet was increased by 1 lb. This is seen in Fig. 2. The P supplement, in fact, enabled the mean daily food intake to be maintained at approximately 50% above that of the control sheep in group 3.

The addition of vitamin D in the form of two massive doses of calciferol (group 3) had no beneficial effect on food consumption. Actually the intake of this group was the lowest of all, but it is felt that this can hardly be ascribed to a specific effect of vitamin D, since the numbers in each group were small and the variation in the food consumption of individual sheep in this group, as revealed by balance trials, showed as wide a variation within each of the low-P groups as between the mean values of the groups.

Body-weight. The weight gains naturally reflected the variations in food intake. At the start of the experiment the mean weight of each group was within 1.4 lb. of the total mean weight and the maximum variation was ± 4.2 lb. Fig. 3 shows the weight gains for each sheep over the main experimental period.

Blood composition. The mean values for blood inorganic P soon reflected the

different levels of P intake, and changes also occurred in the serum-Ca levels. The group averages for both elements are shown in Fig. 4.

The tendency, already mentioned, of the blood inorganic P to fall during the pre-experimental period was reversed during the first 10 days of the trial. But after this

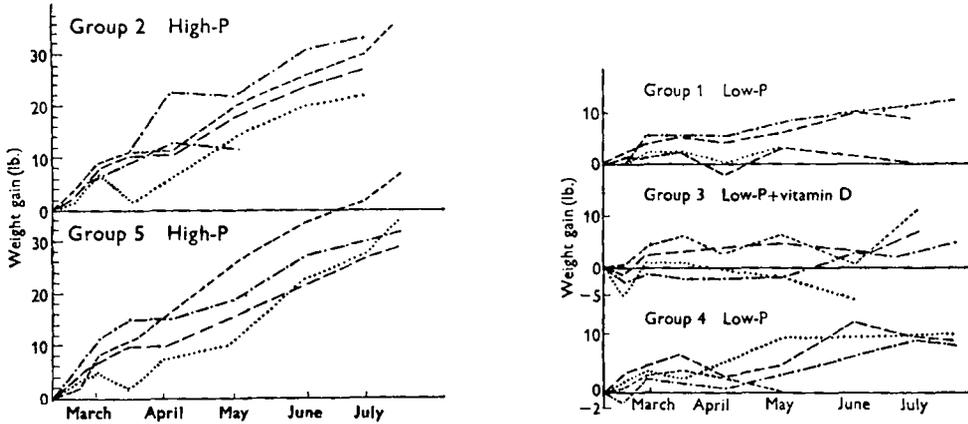


Fig. 3. Weight gains (lb.) of each sheep during the main experimental period.

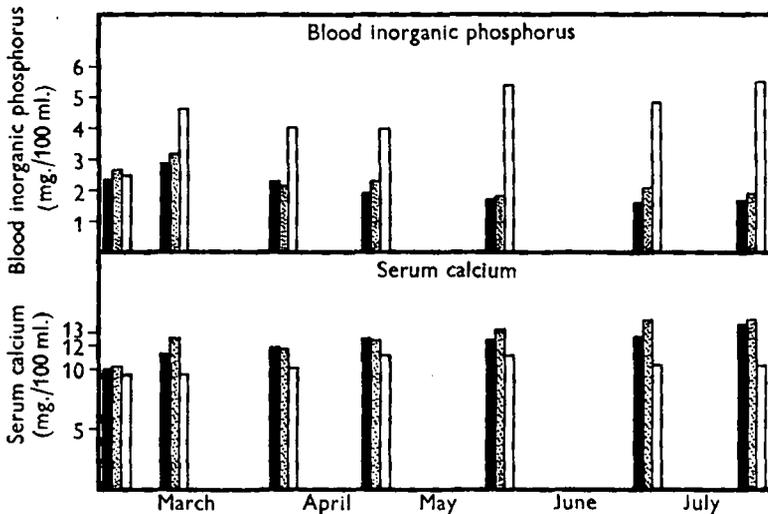


Fig. 4. Mean blood inorganic-P and serum-Ca values of sheep in main treatment groups. ■, groups 1 and 4 (low-P); ▨, group 3 (low-P with vitamin D); □, groups 2 and 5 (high-P).

a severe hypophosphataemia steadily developed in the low-P groups, until the mean group levels in July (6 months from the start) were 1.6 mg./100 ml. in group 1, and 1.7 mg./100 ml. in groups 3 and 4. In the two groups getting the high-P diet, normal levels were reached in the first 10 days and these were maintained throughout.

The effect of the low P intake associated with the high Ca content of the ration was to produce some degree of hypercalcaemia in most sheep in these groups. This

group, and the others were also beginning to show lesions. The antirachitic effect of the vitamin, however, can be clearly seen if the radiograph of florid rickets in sheep no. 4 in group 1 is contrasted with that of a representative (sheep no. 1) of the animals in group 3 (Pl. 1). Weight gains and blood-phosphate values were equally low in both animals. The mild rickets to be seen in most sheep in groups 2 and 5 when the experiment started disappeared as a result of the high P supplement. Normal bone growth was attained by the four sheep of group 5 within 3 months, but for some reason the sheep of group 2 were a little slower in giving a normal picture, although their growth rate was similar to that of group 5. One sheep (no. 11) never gave a completely normal radiograph. Its weight gains were satisfactory but its blood inorganic-P values were below those of the others in the group. In October it exhibited transitory haemoglobinuria associated with inappetence, and a few weeks after its apparent recovery it died suddenly from enterotoxaemia.

A further demonstration of the power of a large dose of vitamin D₂ to influence bone calcification was afforded by studying its effect in sheep already rachitic. Two sheep in group 4 (low-P) and two in group 5 (high-P) were given 25 mg. calciferol in 20 ml. arachis oil by mouth on 6 August. It later became necessary to use one sheep from each group in an experiment with isotopic ³²P but the other two, no. 10 (group 5) and no. 17 (group 4) were kept for further observation. No. 10, a normal sheep, made no obvious response to vitamin D unless its weight gain of 13 lb. during the following 132 days, contrasted with 10 lb. in sheep no. 5 in the same group, is to be regarded as significant. No. 17, a rachitic sheep, made a dramatic response (Pl. 1, 2). On 6 August, the day the vitamin was given, its radiograph was classified as showing moderately severe rickets. By 9 September increased calcification had already occurred to the extent of markedly reducing the intensity of the bone-lesion grading, and by 20 October the sheep was almost normal. It remained radiographically normal until the following February although its blood inorganic-P level was as low as 1.8 mg./100 ml. in December. The sheep also maintained its weight. From February until the last radiograph was taken on 14 July 1949, there was a progressive increase in the width of the uncalcified epiphyseal cartilage until the rachitic lesion was again of moderately severe intensity. Pl. 1, 2 illustrates some of these changes. During the period in which it was free from rickets (6 months or so), this sheep lost all sign of lameness and enlarged knee joints, and even on 1 June it was striking to compare the stiff, restricted gait of no. 4, which had received no vitamin D, with the free, fast walk of no. 17.

Clinical observations. The precise date at which lameness developed in any particular animal on the unsupplemented basal diet was difficult to ascertain with the sheep kept in small pens. A note was made, however, of an alteration in walk, the so-called 'propy gait', occurring in several animals in groups 1 and 4 by mid-May, about 2½ months from the start of the experiment. Certainly, by 14 August 1948, when all groups were turned out in a large open yard for a short period to be filmed, the development of rickets had progressed to the stage when not only were most of the lambs in these two groups showing the characteristic walk, but obvious bony enlargement of the distal ends of the radii had occurred in many of them. Pl. 1, 3 provides an illustration of the apparent enlargement of the carpus of no. 4 (group 1).

Vitamin D, when given to the sheep of group 3, prevented the appearance of any clinical sign of rickets, except in no. 6, which in August showed slight lameness, but no bone enlargement. All the sheep in groups 2 and 5 remained very lively throughout.

Ca:P ratio. From 20 October 1948 until 7 July 1949 two sheep—nos. 5 and 10—which had previously been given the high-P, high-Ca ration and thus acted as normal controls, were put on to the basal ration without supplementation with either P or Ca, that is, they received a diet low in P but normal in Ca. Analysis showed that the diet contained 0.087 % P and 1.25 % Ca. The effect upon growth was pronounced. Though these two sheep had maintained their rate of growth up to October (264 days) at 0.14 and 0.15 lb./day, it fell to 0.086 and 0.062 lb./day during the 210 days following the removal of additional P and Ca from the ration. Even when allowance is made for the natural decline in the rate of gain in weight with increasing age, this sharp fall may be fairly ascribed to the reduction in appetite due to the low-P diet. On 20 October 1948 the blood inorganic-P values for the two sheep were 6.7 and 6.6 mg./100 ml. By 21 April they had gradually fallen to 2.9 and 2.1 mg./100 ml., respectively, and were maintained at about this level until July. Total serum calcium remained at a normal level. In spite of this considerable fall in blood phosphate and the maintenance of so low a level for several months, associated with the exhaustion of reserves of vitamin D and its very low content in the ration, no evidence of rickets was observed from clinical and radiographic examination. This, indeed, is similar to Stewart's (1934-5) finding, and may mean that though the very wide Ca:P ratio of my experimental diet (29.6:1) made the diet strongly rachitogenic for sheep, it lost most of its rachitogenicity when the ratio was reduced to 14:1 by removal of the Ca supplement, even though the low P content of 0.08 % was unaltered.

Deaths of sheep during experiment. Three sheep died during the course of the experiment, and a fourth was slaughtered following infection of a rib-junction biopsy wound. With the first two deaths, following post-mortem examination and subsequent bacteriological and serological tests, the cause of death was found to be enterotoxaemia due to *Clostridium welchii* type D. These two cases occurred close together, one at the end of a balance trial on 17 June 1948 in no. 3, a low-P, high-Ca sheep, and the other 10 days later, in no. 19, a sheep from group 1 also on low-P, high-Ca diet. Both sheep died suddenly without showing any premonitory signs, as indeed is usually the way with this disease. Homologous anti-serum was at once obtained and all sheep were given the appropriate dose. The third death (no. 11, a high-P, high-Ca sheep) occurred on 22 December 1948 and was due to pneumonia. This animal had on several occasions gone off its food for short periods and once had to be withdrawn from a balance trial for this reason. A ruminal cannula had been inserted under general anaesthesia on 18 December and the animal was a very long time in regaining consciousness. Apparently it was these conditions of shock and lowered resistance allied to a weakness of constitution that resulted in the rapid development of pneumonia.

DISCUSSION

The basal diet used in this study provided a lower intake of phosphorus (0.3 g. daily) than that used by other workers studying the phosphorus metabolism of sheep; the diet used by Martin & Pierce (1934) gave 0.6 g., that used by Stewart (1934-5) gave over 1.0 g., and that of Du Toit *et al.* (1930) gave 0.47 g. per sheep. The vitamin D reserves of the lambs in the present work were not known but they would certainly be reduced during the 2 months pre-experimental period. The ration itself contained no more than 0.025 i.u. of vitamin D and thus would not supply what is believed to be the daily requirement of any of the sheep, and the greatest deficiency would be in those on the low-P diet, whose total food intake was so low.

Though the above factors were no doubt important in helping to make the diet so strongly rachitogenic, the main cause appeared to be the wide Ca:P ratio of 29.6:1, effected by high supplementation with calcium carbonate. When this was omitted, bringing the Ca:P ratio to 14:1, rickets did not occur in two of the sheep in spite of hypophosphataemia lasting several months. If the mechanism of rickets production with a wide Ca:P ratio is through the precipitation of phosphorus as insoluble calcium phosphate from an excess of Ca ions, then it must be presumed that there was enough unprecipitated phosphate when the dietary ratio of Ca:P was 14:1 to supply the minimum needs of sheep aged 20 months. This indication of the dependence of ruminants upon the Ca:P ratio of the feed is rather in contrast to the earlier South African work which emphasized the importance of intake levels rather than of the Ca:P ratio. In fact Theiler (1934) went so far as to say that his experiments had shown that the Ca:P ratio found to be so important in the aetiology of rickets in rats did not apply to ruminants, whose requirements for Ca are much lower than for P.

Massive doses of vitamin D₂ were markedly antirachitic and it is suggested that this may result in part from an increased turnover of phosphate from soft tissue for use in mineralizing bones, and partly from a reduction in the rate at which phosphate is excreted which would tend to conserve the available dietary phosphate. A subsequent communication (Ewer, 1951) deals with these points more fully.

SUMMARY

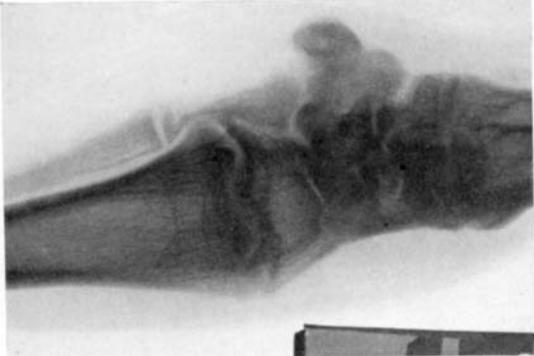
1. A study was made of the effect of phosphorus, calcium and vitamin D₂ on the aetiology of rickets experimentally produced in Welsh lambs.
2. A diet containing a minimum quantity of vitamin D (0.025 i.u./g.) and P (0.3 g.) daily but supplemented with Ca was found to be strongly rachitogenic.
3. Large doses of vitamin D₂ given orally (25 mg. calciferol in 10 ml. arachis oil) prevented the occurrence of rickets but had no effect upon growth. The protective action lasted approximately 2 months.
4. A similar dose of vitamin D₂ was effective in temporarily curing rickets in a sheep kept on the low-P, high-Ca diet.
5. The addition of disodium phosphate to bring the intake of P to 1.9 g./day prevented rickets and enabled the lambs to grow well.
6. Transferring two normal sheep from a high-P, high-Ca diet to the low-P diet

Plate I

T. K. EWER. RICKETS IN SHEEP. I



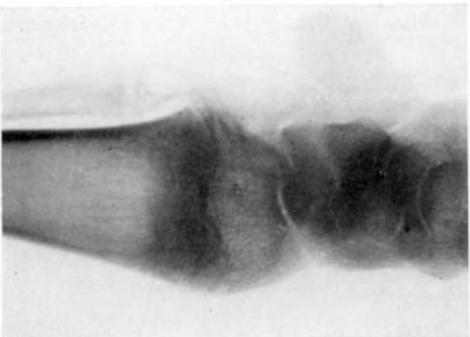
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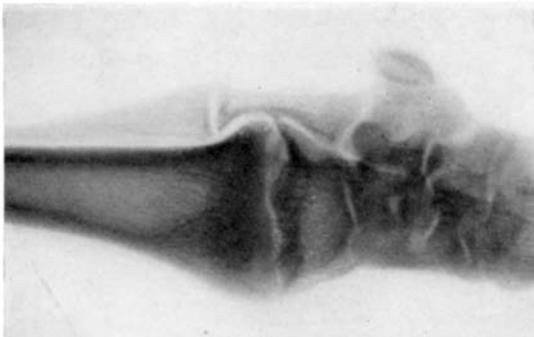
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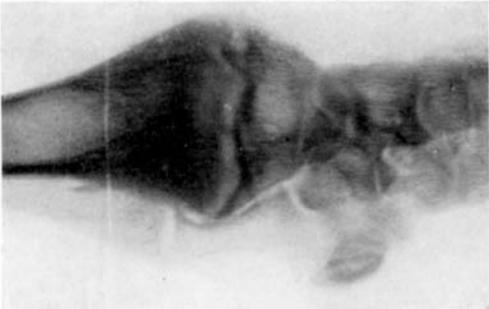
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a

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unsupplemented with Ca led to a fairly severe hypophosphataemia but no rachitic lesion.

7. The importance of a wide Ca:P ratio in the causation of rickets in sheep is emphasized. The low demand for vitamin D in sheep over 1½ years old when phosphorus intake is sufficiently high is also stressed.

The kindness of Miss E. Eden of the Dunn Nutritional Laboratory in undertaking the vitamin A analyses, and of Mr A. L. Bacharach and Dr W. F. J. Cuthbertson of Glaxo Laboratories Ltd. in undertaking the vitamin D assay, is most gratefully acknowledged.

EXPLANATION OF PLATE

1. Radiographs of sheep nos. 4 and 1. (a) Sheep no. 4 (basal diet only), active rickets. (b) Sheep no. 1 (basal diet with vitamin D), normal, 150 days after commencement of experiment.
2. Radiographs of sheep no. 17 (basal ration throughout, vitamin D given on 1 August 1948. (a) Rickets, 27 July 1948. (b) Normal, 9 September 1948. (c) Rickets again developing, 21 April 1949.
3. The swollen carpus of sheep no. 4 (basal diet), typical of well-developed rickets in lambs.

REFERENCES

- Andrews, E. D. & Cunningham, I. J. (1945). *N.Z. J. Sci. Tech.* **27**, sect. A, p. 223.
- Aston, B. (1930). *N.Z. J. Agric.* **41**, 10.
- Auchinachie, D. W. & Fraser, A. H. H. (1932). *J. agric. Sci.* **22**, 560.
- Beeson, W. M., Johnson, R. F., Bolin, D. W. & Hickman, C. W. (1944). *J. Anim. Sci.* **3**, 63.
- Bosworth, T. J. & Stewart, J. (1932-3). *Rep. Inst. Anim. Path. Univ. Camb.* no. 3, p. 33.
- Bowes, H. G. (1932). *Vet. J.* **88**, 209.
- Du Toit, P. J., Malan, A. I. & Groenewald, J. W. (1931). *Rep. vet. Res. S. Afr.* no. 17, p. 453.
- Du Toit, P. J., Malan, A. I. & Groenewald, J. W. (1932). *Rep. vet. Res. S. Afr.* no. 18, p. 611.
- Du Toit, P. J., Malan, A. I. & Rossouw, S. D. (1930). *Rep. vet. Res. S. Afr.* no. 16, p. 313.
- Duckworth, J., Godden, W. & Thomson, W. (1943). *J. agric. Sci.* **33**, 190.
- Elliot, W., Orr, J. B., Wood, T. B. & Crichton, A. (1926). *J. agric. Sci.* **16**, 65.
- Elliot, W., Orr, J. B., Wood, T. B. & Cruickshank, E. M. (1926). *J. agric. Sci.* **16**, 89.
- Ewer, T. K. (1951). *Brit. J. Nutrit.* **5**, 300.
- Ewer, T. K. & Bartrum, P. (1948). *Aust. vet. J.* **24**, 73.
- Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
- Fitch, L. W. N. (1943). *Aust. vet. J.* **19**, 2.
- Fitch, L. W. N. (1944). *Aust. vet. J.* **20**, 303.
- Fitch, L. W. N. & Ewer, T. K. (1944). *Aust. vet. J.* **20**, 220.
- Follis, R. H. Jr., Jackson, D., Eliot, M. M. & Park, E. A. (1943). *Amer. J. Dis. Child.* **66**, 1.
- Franklin, M. C. (1934-5). *Rep. Inst. Anim. Path. Univ. Camb.* no. 4, p. 111.
- Franklin, M. C., Gordon, H. M. & Macgregor, C. H. (1946). *J. Coun. sci. industr. Res. Aust.* **19**, 46.
- Fraser, A. H. H. (1932). *Biochem. J.* **26**, 2166.
- Gomori, G. (1939). *Proc. Soc. exp. Biol., N.Y.*, **42**, 23.
- Gomori, G. (1941). *J. cell. comp. Physiol.* **17**, 71.
- Gomori, G. (1942). *J. Lab. clin. Med.* **27**, 955.
- Guilbert, H. R., Miller, R. F. & Hughes, E. H. (1937). *J. Nutrit.* **13**, 543.
- Hart, G. H. (1940-1). *Nutr. Abstr. Rev.* **10**, 261.
- Henry, M. (1912). *Agric. Gaz., N.S.W.*, **23**, 885.
- Hutcheson, D. (1895). *Rep. vet. Surg. C. G. H.*
- Innes, J. R. M. (1934-5). *Rep. Inst. Anim. Path. Univ. Camb.* no. 4, p. 206.
- King, E. J. (1947). *Micro-Analysis in Medical Biochemistry*. London: J. and A. Churchill.
- King, J. & Armstrong, R. (1934). *Canad. med. Ass. J.* **31**, 376.
- Kramer, B. & Tisdall, F. F. (1921). *J. biol. Chem.* **47**, 475.
- Leslie, A. (1935). *Bull. Canterbury agric. Coll., N.Z.*, p. 8.
- Marek, J. & Wellmann, O. (1931). *Die Rachitis in ihren ätiologischen, biochemischen, pathogenetischen, pathologisch-anatomischen und klinischen Beziehungen: Pathologischer Teil*. Jena: Gustav Fischer.
- Marston, H. R. (1934). *Bull. Coun. sci. industr. Res. Aust.* no. 85.
- Martin, C. J. & Pierce, A. W. (1934). *Bull. Coun. sci. industr. Res. Aust.* no. 77, p. 44.
- McElroy, L. W. & Goss, H. (1940a). *J. Nutrit.* **20**, 527.

- McElroy, L. W. & Goss, H. (1940*b*). *J. Nutrit.* **20**, 541.
 McElroy, L. W. & Goss, H. (1941*a*). *J. Nutrit.* **21**, 163.
 McElroy, L. W. & Goss, H. (1941*b*). *J. Nutrit.* **21**, 405.
 Miller, R. F., Hart, G. H. & Cole, H. H. (1942). *Bull. Calif. agric. Exp. Sta.* no. 672, p. 31.
 Piercy, S. E. (1934*a*). *Vet. J.* **90**, 53.
 Piercy, S. E. (1934*b*). *Vet. J.* **90**, 223.
 Reakes, H. (1910). *Ann. Rep. Dep. Agric. N.Z.* no. 18.
 Richardson, A. E. V., Trumble, H. C. & Shapter, R. E. (1931). *Bull. Coun. sci. industr. Res. Aust.* no. 49.
 Shearer, G. D. & Stewart, J. (1931). *Rep. Inst. Anim. Path. Univ. Camb.* no. 2, p. 86.
 Stewart, J. (1934-5). *Rep. Inst. Anim. Path. Univ. Camb.* no. 4, p. 179.
 Stewart, J. & Shearer, G. D. (1932-3). *Rep. Inst. Anim. Path. Univ. Camb.* no. 3, p. 87.
 Stewart, W. L. (1933). *Vet. J.* **89**, 63.
 Theiler, A. (1934). *Vet. J.* **90**, 143.
 Theiler, A., Green, H. H. & Du Toit, P. J. (1924). *J. Dep. Agric. S. Afr.* **8**, 460.
 Thomas, B. H., La Grange, W. F. & Culbertson, C. C. (1942). *J. Anim. Sci.* **1**, 61.
 Tisdall, F. F. & Brown, A. (1929). *J. Amer. med. Ass.* **92**, 860.
 Woodman, H. E. & Evans, R. E. (1930). *J. agric. Sci.* **20**, 587.
 Wright, C. M. & Taylor, N. H. (1931). *N.Z. J. Sci. Tech.* **13**, sect. A, p. 57.

Rickets in Sheep

2. Measurement of Phosphorus Absorption

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A previous paper (Ewer, 1951) described the production of rickets in lambs by means of a diet low in vitamin D and phosphorus and high in calcium, and the clinical effect of supplementation with vitamin D and phosphorus. Quantitative measurement of the P retention by these sheep forms the subject of the present communication. The value of balance trials in helping to establish quantitatively the absorption of various constituents of an animal's diet is well established. In rickets in the rat, dog and man, Ca and P metabolism has been investigated in this way by a number of workers and, in a study of Ca metabolism in sheep, Franklin (1934-5) also made use of the method. Bethke, Kick & Wilder (1932) reviewed the earlier work of McCollum, Simmonds, Parsons, Shipley & Park (1920-1) and of Sherman & Pappenheimer (1920-1) and found that the optimum Ca:P ratio for the rat was between 1.0 and 2.0.

It has been shown that the digestibility of many of the major food constituents in a sheep's diet, including dry matter, can be affected by the addition of such things as crude fibre, readily available carbohydrate and nitrogenous compounds (Swift, Thacker, Black, Bratzler & James, 1947), and that the ash from lucerne hay can increase the digestibility of roughage from ground maize-cobs (Burroughs, Gerlaugh & Bethke, 1950). Observations were therefore made upon the dry-matter digestibility

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