STUDIES ON OVINE UROLITHIASIS

Summary of a thesis presented for the Degree of Doctor of Philosophy of the University of Glasgow by A. David Weaver, Dr. med. vet., B.Sc., F.R.C.V.S., in November 1970.

Ovine urolithiasis is alleged to be associated with intensive feeding of concentrates, a type of husbandry which has similarities to methods of rearing male sheep in Scotland, and which may become increasingly common in intensive feeding systems in ruminants in Britain.

Part I of the thesis reviews the literature, in particular the predisposing factors, urine composition, and renal pathology.

In Part II various surveys revealed that obstructive urolithiasis was uncommon in Britain except in Scotland, where the incidence varied from 1-10% principally among housed male sheep, and where, since cases were often fatal, the economic loss was significant.

In determination of the liability of ovine urinary pH to change under different conditions of storage, samples kept in screw-topped containers to exclude air underwent no significant pH change in 24 hours.

In catheterised samples taken at monthly intervals for seven years, from healthy Cheviot and Half-bred ewes, a seasonal variation was shown in urinary pH, which, except in a few summer months, was acidic, a finding contrary to most textbook statements. Urinary pH was significantly correlated ($p < 0.01$) with urinary potassium concentration.

Studies on four housed Blackface rams (half-brothers) showed that their urinary pH was generally alkaline. Urinary mineral concentrations, especially magnesium, were higher than in grazing ewes. The urinary phosphorus concentration of one tup was significantly ($p < 0.01$) less than in the others.

Feeding experiments were carried out on 172 Blackface sheep to determine the incidence of clinical (obstructive) and subclinical urolithiasis (presence of calculi). Seven animals developed a fatal obstruction with calculi, a further three had non-fatal obstruction, and 92 sheep had renal calculi.

No calculi developed in six male lambs fed a mixture grossly inadequate in calcium (0.7 g daily) and adequate in phosphorus (3.81 g), for 120 days. Two clinical cases of obstruction by calculi developed and four others had renal calculi when ten castrated lambs were fed a high calcium

(2.90%) and phosphorus (1.70%) concentrate mixture with roots and wheat straw. Kidneys of obstructed sheep demonstrated hydronephrosis and pyelonephritis. Other sheep showed early foci of pyelonephritis.

Experiment 2 employed a feed composition known in the United States to be calculogenic but, despite a subsequent increase of the potassium acid phosphate concentration in the mixture from 2.14 to 4.28%, no animal developed obstruction. Eight of 30 sheep had calculi at slaughter.

On a relatively normal and narrow range of Ca and P intakes, differing quantities of concentrates and roots had no effect on the development of urolithiasis. Nineteen of 42 sheep (45%) contained calculi, including three fatalities. Liveweight increase was unrelated to the presence of renal calculi.

The calcium intake was then varied in 42 castrated lambs (1.51 g to 25.60 g daily), while the phosphorus intake was low (1.9 - 2.4 g). While 71% of the animals had calculi, the incidence was unrelated to calcium intake.

The previous experiment was repeated with ram lambs, and initially twelve were killed after 135 days of hay feeding alone, seven of which contained renal calculi. After 96 days of concentrate feeding, renal calculi were found in 22/27 sheep, including two animals with fatal urinary obstruction.

No significant differences in plasma mineral values were demonstrable between calculus-containing and other sheep. The pathological features of obstruction were similar to those in the natural disease. Microcalculi were demonstrated in renal collecting tubules.

Pyelonephritis was common in obstructive urolithiasis and was considered sometimes to result from ascending infection from a urethrotomy wound, and sometimes from blood-borne infection. Glomerulonephritis and chronic interstitial nephritis were rare. Intracytoplasmic PAS-positive globules occurred in the proximal convoluted tubules of sheep with renal calculi.

On biochemical analysis, ovine urinary calculi consisted of triple phosphate, together with some calcium phosphate and in two cases with calcium oxalate and carbonate. X-ray crystallography showed that some calculi were amorphous in structure while others were struvite (magnesium ammonium phosphate) or newberyite (magnesium hydrophosphate-trihydrate).

The results are discussed in Part III. As struvite is an important crystalline component in ovine calculi in Scotland, urinary pH is likely to be a significant factor in its production. From the results of these experiments it appears unlikely that extreme Ca and P intakes, high K_2HPO_4, or a high root intake are significant in producing an increased incidence of calculi in indoor sheep. Since animals developed calculi on hay feeding alone, it appears that factors such as climate, genetic profile, or elements such as K and Na may explain certain discrepancies in these results in Scottish sheep.

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P A R T I

INTRODUCTION

Urolithiasis is defined as calculus (urolith or stone) formation in the urinary tract, which comprises the paired kidneys and ureters, the bladder and urethra. The words calculus, urolith and stone are used synonymously in this text.

This study was stimulated by recognition of the existence of ovine urolithiasis in Britain and in particular in Scotland. Scotland has a considerably larger sheep population than England and Wales so that sheep diseases here have assumed a greater importance. Urolithiasis has been the cause of sporadic deaths for many years without any detailed knowledge of the predisposing factors leading to the onset of disease. Despite the fact that its occurrence in man has been known for several thousand years, and despite much experimental work in animals, the aetiology of the disease in man and animals has not yet been elucidated.

Recent work in ruminants in the United States has been increasingly directed at the biochemical nature of the organic matrix of calculi as a possible key to the aetiology but the results are inconclusive. Feeding experiments in sheep and cattle, designed to study particular factors such as the mineral balance in urolithiasis have led to contradictory results and conclusions. Meanwhile, similarities between the "feedlot" system in the Western United States and forms of indoor husbandry of both breeding and fattening ruminants under development in Britain have suggested that investigations into the aetiology of ovine urolithiasis should be undertaken in Scotland.

REVIEW OF THE LITERATURE

1. DISTRIBUTION OF OVINE UROLITHIASIS

Though the occurrence of urolithiasis in all domesticated British animals has been acknowledged (Dick, 1869; Youatt, 1893; Fleming, 1902), only isolated case reports in the last century have referred to sheep (Read, 1840; Gutteridge, 1840; Dickens, 1842; Tindal, 1842; Morton, 1844; Spooner, 1844). In one example, fatalities were recorded in 2 yearling Down rams with urethral calculi. In one of the recorded cases the animal was being prepared for the Cambridgeshire Agricultural Show and Gutteridge (1840) commented on "the direful effects of the forcing system." A century later, in Scotland, Nairn (1939) observed obstruction due to calculi in young rams being intensively fed for show and sale purposes and stated that this was a frequent cause of loss to breeders of rams for stud.

Outside the British Isles, all major sheep-producing countries have recognised urolithiasis - Australia (Cunningham and Cunningham, 1938; Underwood, 1941; Beveridge, 1942; Bennetts, 1950; Belscher, 1957; Sutherland, 1958), New Zealand (Easterfield, Rigg, Askew and Bruce, 1930), South

Africa (Steyn and Reinach, 1939; Belonje, 1965a) and the United States (Pontius, Carr and Doyle, 1930; Beesom, Pence and Holm, 1943; Elam, Ham and Schneider, 1957; Marsh, 1965).

In Australian sheep the incidence of urolithiasis is often high, and the disease is of economic importance (Gardiner, Nairn and Meyer, 1966; McIntosh, 1966). Losses may reach 2% in some areas (Gardiner, 1965). In the United States only eight of forty-five "prominent sheepmen," replying to a circular on the incidence of ovine urolithiasis stated that they did not know of its occurrence (Pontius et al., 1930). Later writers also recorded ovine urolithiasis as one of the most troublesome diseases in the Western States (Hawkins, 1965) and the incidence of obstructive urolithiasis may reach 10% in some flocks (Johnson, Palmer and Nelson, 1940). A study of 3 years' records indicated an incidence of 7% in intact males in Kansas (Oehme and Tillman, 1965).

The economic loss due to ovine urolithiasis has only been estimated in the United States of America where it is about 567,000 dollars per annum or 0.2% of the value of the annual sheep production (F.A.O. Yearbook, 1962).

Investigators in several European countries, apart from Britain, have also recorded the condition among sheep, for example, France (Moussu, 1923; Godard, 1948; Cottereau, Froget, Magat, Jaillardon and Monard, 1966), Germany (Oppermann, 1950; Bollwahn, 1961), and Eastern Europe (Petrichev, 1964; Egoshin, Ryskulov and Vidomskii, 1967), but no incidence figures have been published.

2. PREDISPOSING FACTORS

Many factors have been allegedly implicated in urolithiasis of animals and of man. Such factors include hypovitaminosis A, urinary stasis and infection, urinary pH, urinary colloid balance, mineral balance (calcium, phosphorus, calcium : phosphorus ratio, magnesium, potassium), hormonal disturbances, climatic factors, and miscellaneous factors such as early castration, specific feeds and the pelleting of compounded foodstuffs.

The early literature on factors predisposing to urolithiasis in sheep has been reviewed by Pontius et al. (1930), Newsom (1938) and Beveridge (1942).

(a) Vitamin A

Osborne and Mendel (1917) and later Higgins (1951) noted that phosphatic calculi developed in rats on a vitamin A deficient diet, while bladder calculi disappeared when vitamin A (Fujimaki, 1927) or cod liver oil (Higgins, 1935) was again included in the feed. Avitaminosis A was also considered the cause of the disease by others without specific evidence being produced (Van Leersum, 1928; McCarrison, 1931; Milks, 1935; Oppenheimer and Pollock, 1937; Flocks, 1944).

Newsom (1938) doubted whether avitaminosis A played a part in ruminant urolithiasis, and a similar view has been repeatedly expressed (Johnson et al., 1940; Beesom et al., 1943; Black, Ellis, Jones and Keating, 1947; Evereth, Bolin, Goldby and Ford, 1948; Lindley, Tayson, Ham and Schneider, 1953; Swingle and Marsh, 1953, 1956; Elam, Ham and Dyer, 1959; Shirley, Easley, Ammerman, Kirk, Palmer and Cunha, 1964). Yet in later work (Newsom, Tobiska and Osland, 1943) Newsom contended that a low vitamin A intake or its poor utilisation led to urolithiasis in sheep. Urolithiasis was produced in castrated male goats fed a vitamin A free mixture (Schmidt, 1941) though controls were not used and the mineral analysis of the diet was not stated. Australian lambs which were given a low vitamin A feed made satisfactory growth for one year, after which anorexia and muscular incoordination developed with sudden death soon afterwards. Post mortem examination revealed little or no abnormality in any system (Peirce, 1945).

Widespread keratinisation of renal epithelium developed in male goats on an almost carotene-free diet, with degenerated cells containing calcified granules in the renal tubules (Dutt, Majamdar and Kehar, 1959). Crystalline material was observed around desquamated human renal epithelial cells (Randall, 1937). Keratinisation developed in rats after 10 weeks on a vitamin A deficient diet (Higgins, 1951).

It is well recognised that vitamin A is directly concerned with healthy epithelium and it has been suggested that avitaminosis A leads to an apparent hyperplasia, metaplasia and finally atrophy of the renal pelvis and urethral mucosa and the release of mucosal fragments which may act as nuclei for calculus formation (Steiner, Zuger and Kramer, 1939).

(b) Urinary Stasis

In man, urinary stasis has been claimed as a significant and predisposing factor in urolithiasis in bedridden patients, or in relatively immobile patients who are chronically lame (Latimer, 1948). Fish et al. (1948) reported that 10 - 18% of bedridden patients developed urinary calculi. However, immobility due to orthopaedic conditions may also involve a disturbed calcium metabolism (Barr, Bulger and Dixon, 1929; Whedon and Shore, 1957). Lithiasis also appears frequently in patients with fractures of limbs (Heuberger, 1962).

Restricted exercise has been suggested as a predisposing factor for urinary stasis in sheep (Nairn, 1939), while Jubb and Kennedy (1963) refer to urinary stasis in association with ascending infection from the urethra.

No experimental work has been reported on the relationship of urinary stasis to urolithiasis in domestic animals.

(c) Urinary Infection

Bacterial infection of the urinary tract is claimed as a predisposing cause of lithiasis (Flocks, 1944), the role being twofold: firstly, the bacteria present in urine may themselves form the nuclei of calculi together with various cellular inflammatory products (Milks, 1935; Laughlin, 1944) or, secondly urinary pH may be altered through the presence of urea-splitting organisms (Counsellor and Priestley, 1955; Keyser, 1935; Hager and Magath, 1928; Flocks, 1944; Randall, 1951). However, Randall (1951) warned that urinary infection might itself be secondary to calculus formation, pointing out the large number of sterile cases of stones. Recent work showed that a group of germ-free rats had a higher incidence (35/70) of bladder stones, principally calcium oxalate and citrate, than non-germ-free rats (0/207) of the same strain (Gustafsson and Norman, 1962).

Infection is closely related to renal damage. On the other hand, infection may lead to the production of renal damage. Pyelonephritis in man is reported to be a complication of about 40% of stone cases (Biggs, Norfleet and Garvey, 1955). About 48% of repeat stone-formers have infection or hydronephrosis as an associated disorder. Proteus bacteria introduced into the bladder of rats as a retrograde infection produced severe renal parenchymal damage and an 85% incidence of renal uroliths (magnesium ammonium phosphate) in 3 months (Cotran, 1964). Bacterium coli on the other hand, either produced a short-lived infection of the renal pelvis (Cotran, 1964), or even inhibited calculus growth on a foreign body implanted in the bladder (Miller, Chapman, Seibutis and Vermeulen, 1956).

(d) Urinary pH

The urinary reaction has long been considered one of the more important factors in urolithiasis. In man (King, 1967) it is possible to predict fairly accurately the type of calculus from the urinary reaction, for example :

pH	Calculus
Under 5.0	Uric acid
5.0 - 6.5	Oxalate
Over 7.0	Phosphate

These values correspond well with the known solubilities of the relative salts (Prien, 1955; Elliott, Todd and Lewis, 1961).

Struvite (or triple phosphate) is reported as the only solid phase in urine above pH 8 (Elliott, Quaide, Sharp and Lewis, 1958) and struvite solubility is determined almost entirely by pH, the concentration of Mg++, NH_4 and inorganic PO_4 and the ionic strength (Elliott, Sharp and Lewis, 1959). Clinically, urinary acidification is alleged to be effective in the prevention of struvite stones (Fleisch, 1965).

Ruminant urine is stated normally to be alkaline in reaction (Beveridge, 1942; Dukes, 1955). Acidification should theoretically prevent phosphate calculus formation (Beveridge, 1942).

Alkaline urine has been claimed to be more conducive than acidic urine to calculi formation (Newsom, 1938; Crookshank, Keating, Burnett, Jones and Davis, 1960) but Udall and Chen-Chow (1963) found phosphatic calculi in lambs excreting urine of either acidic or alkaline pH. Markedly alkaline rations containing K_2CO_3 produced more calculi in sheep than less alkaline rations (Elan, Schneider and Ham, 1956), but no differences were found when a highly significant difference in urinary pH was ensured by dietary supplementation with phosphoric acid (H_3PO_4) or potassium bicarbonate (K_2CO_3). The urinary pH of feedlot steers forming phosphate stones is alkaline but no different from those not forming stones (Frank, Meinershagen, Baron, Scrivner and Keith, 1961).

Changes of urinary pH occur under different conditions. Reduction of feed intake, whether following climatic stress or in disease such as ketosis tends to lead to production of a more acidic urine (Cornelius and Kaneko, 1963). Urinary pH likewise falls in oliguria in man (Best and Taylor, 1955; Nutbourne and De Wardener, 1960; Frank et al., 1961). While man commonly experiences an alkaline post-prandial urine, turbid with phosphates, emphasising that crystalluria is not necessarily associated with stone formation (King, 1967), the opposite reaction occurs following the feeding of penned sheep, where Stacy and Brook (1964) recorded a fall from pH 7 - 8 to pH 5 - 6, at which magnesium ammonium phosphate will be soluble (Prien, 1955).

(e) Urinary Colloids and Matrix

Urine is a complex and supersatured solution in which the solubility is maintained by colloids (Lichtwitz, 1944 : quoted by Butt and Hauser, 1952). While one school of thought still believes that crystalline precipitation is the primary process (Vermeulen and co-workers, 1950, 1958, 1965, 1967), the hypothesis of the active role of matrix in stone formation goes back millenia to Hippocrates and Galen.

The concentration of urinary colloids can be artificially increased by the injection of hyaluronidase and its compounds (Butt and Hauser, 1952). Hyaluronidase prevents the formation or recurrence of urinary calculi in man, perhaps by acting as a potent dispersing or peptizing agent (Butt, 1956; Butt, Hauser and Seifter, 1952; Butt, Seifter and Hauser, 1952).

Puntriano (1954) concluded that hyaluronidase would act similarly in cattle and sheep, and suggested injections or implantations in animals liable to develop urolithiasis. Wethers given a calculus-predisposing feed and implanted with hyaluronidase showed neither colloid-crystalloid imbalance, nor evidence of urinary calculi, when compared with non-implanted controls (Puntriano, 1955), but later work by Crookshank et al. (1960) using injections of hyaluronidase was unconvincing. Hyaluronidase may be beneficial when combined with surgery in cases of urinary obstruction in rams (Bollwahn, 1961).

An organic matrix essentially similar for all types of calculi (King and Boyce, 1957) was found in a large series of renal calculi (Boyce, Pool, Meschan and King, 1958). The molecular orientation and aggregation involves a carbohydrate-protein conjugate, uromucoid, produced by transitional epithelium (Boyce, Garvey and Norfleet, 1957). The mechanism of orientation of particles is unknown but is ultimately associated with the binding of Ca^{++}, PO_4''' and CH' ions, which is claimed as the initial phase in all calculus formation, crystal deposition being a secondary phenomenon (Boyce et al., 1958).

X-ray diffraction studies of calculus material from sheep fed concentrates revealed principally magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$ or struvite) with amorphous material suggestive of carbohydrate-protein conjugates (Cornelius, Moulton and McGowan, 1959). Microcalculi were visible at the time of onset of partial or complete obstruction following feeding of a high phosphorus mixture (Cornelius et al., 1959). Proteins were reported to be similar to those found in man (Boyce et al., 1955), and the largest quantitative components were the smaller molecular proteins, α-globulins and albumin (Cornelius, Bishop, Berger and Pangborn, 1961). The α-globulin fraction is known to contain conjugated proteins similar to those of the neutral glycoprotein matrix of ovine calculus material (Cornelius and Bishop, 1961).

In steers the urinary mucoprotein concentration was found to be inversely proportional to the level of minerals or ash in the ration. The mucopolysaccharide fraction of the mucoproteins was directly related to the concentrate level, and it was hypothesised that this correlation might be a result of an increased body turnover induced by a faster growth rate (Udall, Deem and Maag, 1958).

Prolonged overfeeding of cattle with protein led to no renal lesion except for hypertrophy (Schilling, 1962).

Despite the suggested relationship between mucoprotein concentration and feedstuffs, it is still uncertain where mucoproteins originate, but recent evidence (Packett and Coburn, 1965; Boyce, 1966) suggests that it is in the uriniferous tubules, rather than serum or the transitional epithelium of the bladder.

While this organic matrix is invariably found in urinary calculi, it has long been and still is considered by some (Verneulen, Lyon and Fried, 1965) that the matrix may have a secondary role and be more an adventitious inclusion, and that stone formation is essentially a complex crystalline process. The favoured urinary supersaturation theory has recently been examined and supported with reference to the physico-chemical relation between the ions of calcium and inorganic phosphate (McGregor, Nordin and Robertson, 1965) and this aspect leads to an examination of mineral balance in relation to urolithiasis.

(f) Mineral Balance

(i) Calcium. In man hypercalciuria has been generally accepted as a major factor in renal stone formation (Burt, 1956; Hodgkinson and Pyrah, 1958) and, depending on the criteria adopted, 16 - 66% of all stone-formers are hypercalciuric, using 24 - hour excretion figures (Cottet, 1961; Cottet, Vittu and Cararelli, 1962). On the other hand, only about 24% of idiopathic hypercalciurics are recurrent stone-formers. Both an increased intestinal absorption of calcium (Jaworski, Brown, Fedoruk and Seitz, 1963) and an extrarenal, perhaps hormonal, induced reduction of tubular reabsorption (Edwards and Hodgkinson, 1965) have been claimed to be responsible, and important evidence suggesting that persons with idiopathic hypercalciuria absorb a greater proportion of dietary calcium than normal has been put forward by Peacock, Hodgkinson and Nordin (1967).

Hyperparathyroidism, one of the features of which is hypercalcemia, is known to produce renal calculi in man (Albright, 1936; Randall, 1937; Albright and Reifenstein, 1948), but 20% of one series of patients with hyperparathyroidism did not produce calculi (Hellström, 1955). Calcific foci are common in the normal human renal parenchyma (Lichtenstern, 1926; Anderson and McDonald, 1946; Pyrah, 1958), presumably the result of phagocytic ingestion of reabsorbed calcium (Boyce, 1967), and this process interferes with the intrinsic activity of the cell (Zweifach, 1959). This condition has not received attention as a possible factor in domestic animals.

Both Hager and Mcgath (1928) and Evereth and Miller (1939) claimed that a high calcium intake was responsible for ovine urolithiasis, the latter also incriminating a high magnesium intake.

(ii) Phosphorus. It has been suggested that the amounts of calcium and phosphorus excreted in urine, together with the urinary pH, are the most important group of factors involved in the formation of urinary concrements (Butt, 1956). The fact that the acidic radical of ovine calculi in Britain is inorganic phosphate (Lonsdale, 1965) makes the experimental work involving the element in ruminants of special interest.

Certain parameters differ in lambs which form calculi on a grain sorghum feed, compared with non-calculus-forming lambs on the same mixture; phosphorus and magnesium levels were high in both urine and blood of both groups, but phosphorus was significantly higher in the calculus group (Packett and Hauschild, 1964).

Veterinary aspects of the significance of phosphorus are discussed under "Experimental Production" (p. 33).

(iii) Calcium : Phosphorus Ratio. Absorption of calcium and phosphorus in sheep appears to be independent of the Ca : P ratio (Lueker and Lofgren, 1961), and is directly related to the quantity supplied, and, in the case of phosphorus, the form of supply. While a high Ca : P ratio reduces the incidence of urolithiasis (Robbins, Kunkel and Crookshank, 1965), the same workers found calculi were still produced on feeding a calcium and phosphorus supplement as bone meal. A significant difference was seen in one high phosphorus experiment, when a group given 0.31% Ca developed more calculi than a group given 0.58% Ca (Bushman, Emerick and

Embry, 1965a). Thus the Ca : P ratio may be a critical factor (Coburn and Packett, 1962; Bushman et al., 1965a, 1965b; King, 1967).

(iv) Magnesium. In laboratory animals the formation of magnesium ammonium phosphate calculi in rats was reduced by lowering the urinary magnesium or urinary phosphorus concentration (Vermeulen et al., 1950). A magnesium - deficient diet has led to renal calcification in rats (Watchorn and McCance, 1937), but the process can be forestalled by prior parathyroidectomy (Heaton and Anderson, 1965). Large quantities of magnesium carbonate given to rats induced similar calculi, and development was prevented by additional calcium carbonate (Watchorn, 1952).

A higher serum magnesium has sometimes been reported in sheep dying from urolithiasis (Kunkel, Whitaker, Packett and Crookshank, 1961) and was thought to be associated with a high magnesium intake (Evereth and Miller, 1939). Lambs on a high (1.5%) magnesium intake had a threefold increase in blood magnesium but developed no calculi (Johnson et al., 1940), though other animals from the same farm developed calculi on a high magnesium, low phosporus intake and had normal blood magnesium values. In two further experiments a high magnesium intake (as magnesium carbonate) failed to produce a significant increase of urolithiasis compared with controls (Beeson et al., 1943; Lindley et al., 1953).

The reported increased urinary Mg excretion may be associated with a reduced tubular reabsorption, which has been found in renal disease, which in turn has followed administration of sodium, calcium and ammonium salts (Rook and Storry, 1962).

A reciprocal relationship has been claimed between the urinary excretion of magnesium and phosphorus in wether lambs, in which an 80% calculus incidence on a daily mixture supplying 6g Ca, 5g P and 4.4g Mg was associated with a lower urinary magnesium and higher urinary phosphorus than mineral mixtures producing a significantly lower incidence of calculi (Robbins et al., 1965). Recently, it has been suggested that this relationship may have been mediated by magnesium interfering with phosphorus absorption, rather than by any effect at the kidney level (Hjerpe, 1968).

(v) Potassium. Artificial supplement of foodstuffs has been practised with 2.5% dipotassium hydrogen phosphate to induce urolithiasis (Tayson, Lindley, Schneider and Ham, 1951; Lindley et al., 1953; Cornelius et al., 1959), in mixtures with a ratio of 1 part roughage : 4 parts concentrate. In another experiment the highest incidence resulted from both potassium and phosphate supplementation rather than either radical alone (Elam et al., 1956).

Addition of 1% potassium chloride, however, to a known calculus-provoking mixture reduced the incidence from 38% to 10% compared with reduction to 25% with additional sodium chloride (H. R. Crookshank, 1963 : unpublished observation quoted by Robbins et al., 1965).

(g) Hormonal Factors

(i) Oestrogens. Implantation of diethylstilboestrol has been used to attain an increased weight gain and feed efficiency in sheep, including Blackface wether lambs (Armstrong and Cameron, 1959; 1961; Preston, Greenhalgh and McLeod, 1959). Unfortunately, under some conditions, sheep implanted with or fed stilboestrol may have a higher incidence of urolithiasis (Jordan, 1959; Bell, Smith and Erhart, 1954; Wilkinson, O'Mary, Wilson, Bray, Pope and

Casida, 1955; Marsh, 1961). Losses amounted to 20% in one experimental feeding trial using a 30 mg. stilboestrol implant (Udall and Jensen, 1958). However, experiments involving different calcium and phosphorus intakes together with 2 levels of stilboestrol dosage (a single 30 mg. implant or 2 mg. per os daily) showed no significant differences in urolithiasis incidence (Emerick and Embry, 1964).

Urinary calculi have also been associated with the grazing of sheep on oestrogenic subterranean clover (Gardiner et al., 1966).

Administration of stilboestrol results in an increased size of secondary sex organs, especially the bulbo-urethral glands and seminal vesicles, due to interstitial cell hyperplasia and some epithelial metaplasia (Herrick and Vatne, 1961; Marsh, 1961). Sloughed epithelial material appears to be increased (Herrick and Vatne, 1961) and may cause blockage (Marsh, 1961) while the structural changes are likely to reduce the pelvic urethral diameter.

(ii) Castration. The time of castration was found to have an effect on urethral diameter of calves, wherein the early castrated animal has a narrower urethra than the later castrated calf (Marsh and Safford, 1957). This factor was thought to explain the lower incidence of the disease in bulls and late castrated steers. No significant difference was found in the urethral diameters of steers with or without bladder calculi, while the kidneys were not examined (Frank et al., 1961). No comparable information is available for sheep.

The terminal portion of the urethra (processus urethrae or vermiform appendage) is a fine worm-like structure, about 3 to 4 cm. long (Sisson, 1938). The vermiform appendage at birth is adherent to the prepuce. Detachment from the prepuce is dependent on activity of the male sex hormones. Detachment was complete in well grown Merino ram lambs at about 5 months, but was delayed under conditions of poor nutrition (Johnstone, 1948). Belonje (1965b) suggested that the arrested development of a normal unadhesed appendage, occurring in adult wethers as opposed to rams, was significant in arresting the passage of urinary calculi.

(iii) Parathyroid. The parathyroid gland has been discussed briefly under calcium (p. 16).

(h) Climatic Factors

In some countries such as South Africa (Steyn and Reinach, 1939), the United States (Newsom, 1938) and Australia (Bennetts, 1950), ovine urolithiasis occurs most frequently in arid and semi-arid areas with a hot dry climate, just as the human disease is commoner in similar tropical or subtropical areas such as India (McCarrison, 1931), Thailand (Passmore, 1953; Halstead, 1961) and the Middle East (Guersel, 1936).

In more temperate climates, cases are frequently reported to follow sudden falls in temperature or severe snowstorms in winter (Newsom, 1938; Udall, 1959a) or associated with a known sudden restriction of water (Morton, 1844; Newsom, 1938) or food intake. The increased urinary concentration in hot climates or following water restriction is thought likely to increase the risk of concretion formation and, when urine flow returns to normal, the movement of calculi from kidneys or bladder to cause obstruction (Udall et al., 1958).

Urinary pH decreases in man when the urinary output falls (Best and Taylor, 1955; Nutbourne and De Wardener, 1960; Frank et al., 1963), and the same is true of penned sheep (Stacy and Brook, 1964). Swingle and Marsh (1956), however, found that calves with a limited water intake excreted less urine of a slightly higher pH. Under temperate conditions (Scotland), certain Blackface rams have a significantly different urinary volume from other similar rams (Evans, 1957; Blaxter, Graham and Wainman, 1959 : quoted by Schmidt-Nielsen, 1964), associated with an inherited difference in the potassium composition of red cells.

The renal response of sheep to sodium chloride (Potter, 1961, 1963) in the prevention of urolithiasis (Elam et al., 1956) merits discussion since it was thought first to be associated purely with a diuretic action, increasing the fluid intake and causing simple urinary dilution (Udall, 1959b). It was later considered to involve a specific ion-binding effect of the chloride ions which may be a displacement of magnesium and phosphorus from centres of nucleation of the matrix (Udall, Seger and Chen-Chow, 1965). A significant reduction of urolithiasis occurred in lambs receiving 4% or 7% sodium chloride in pelleted rations compared with 1% sodium chloride (Udall and Chen-Chow, 1963). No deleterious effect was seen in sheep following prolonged administration of sodium chloride in water at levels up to 1.3%

(Potter, 1963) or in feed up to 10% (Udall, 1959b).

(i) Miscellaneous Foodstuffs

Numerous specific foodstuffs have been claimed to produce urolithiasis in sheep, but experimental work is lacking in most cases. Some examples are listed below :

a) Roots, e.g. turnips (Williams, 1884), mangolds (Youatt, 1893; Michael, 1910; Linton, 1927; Newsom, 1943; Pontius *et al.*, 1930), sugar beet (Michael, 1910) or sugar beet pulp (Gough, 1942; Lindley *et al.*, 1953; Tayson, 1961), beet molasses (Evvard, Culbertson and Wallace, 1923).

b) Leaves of roots, e.g. mangolds and sugar beet (Harris and Alexander, 1953; Garner, 1961) which contain high quantities of oxalates.

c) Clover (Read, 1840; Pope, 1963).

d) Grain, e.g. cottonseed cake or meal (Newsom, 1938; Harris and Alexander, 1953), milo (Black *et al.*, 1947; Jones, Keating, Ellis and Crookshank, 1962; Helmy and Fouad, 1964), wheat and grain (Newsom, 1938; Helmy and Fouad, 1964).

Pelleting of a mixed foodstuff, which was known to be calculogenic to lambs, resulted in an increased but inexplicable incidence of urolithiasis (Crookshank, 1962; Crookshank, Packett and Kunkel, 1965). Calculogenicity was inversely related to pellet size (Crookshank, 1962).

3. COMPOSITION OF OVINE URINE

(a) Normal

Some recent figures relating to the composition of ovine urine are summarised in Table 1.

The average daily urinary volume varies with the age and size of the subject, the environmental temperature and humidity, as well as the type of food (Ketz, 1960).

The mean daily urinary volume is stated to range from 0.5 to 2 litres with an average value of 1 litre (Dukes, 1955; Scheunert-Trautmann, 1965). Values obtained in feeding experiments (Table 1) indicate a still wider range in which values between 0.20 and 4.48 litres were reported (Robbins *et al.*, 1965; Udall, 1962; Stacey and Brook, 1964; Newsom *et al.*, 1943). In contrast to other animals, the proportion of water eliminated by the kidneys (approximately

Reference.	No. of measure-ments.	Form of Diet.	Type of Animal.	Vol.(ml.)	Ca. (	Mg. mg%	PO_4)	pH.	Other recordings.	Remarks.
Udall (1962)	8	Pelleted	wether	1710	-	-	-	-	protein 216.4 mg/day	alfalfa, beet pulp, milo, cottonseed.
" "	"	"	"	2620	-	-	-	-	protein 374.0 mg/day	alfalfa 76 days later.
Tayson (1961)	216	mixture	ram	847	2.62	0.64	15.90	7.3	-	alfalfa hay + wheat straw, beet pulp, linseed, oats & Wheat
Crookshank, Packett & Kunkel (1965)	25	pelleted	wether	-	14	79	2	-	Potassium 1218 mg%	alfalfa hay, sorghum grain & molasses.
Bushman, Emerick and Embry (1965)	19	mixture	wether	-	1.2	54.8	3.4	8.74	-	maize, soya bean, meal & silage (0.31% Ca, 0.25% P.)
Robbins, Kunkel & Crookshank (1965)	32	mixture	wether (1 or 15)*	-	(69^o	362^o	10^o)	-	-	alfalfa & sorghum grain.
Packett & Hauschild (1964)	?	mixture	wether	709	-	-	18.1	-	-	alfalfa, sorghum grain, corn, cotton seed, molasses.
Cornelius, Moulton and McGowan (1959)	?	pelleted	wether	977	-	-	-	-	-	wheat straw, beet pulp & linseed meal, oats, wheat.
Lindley, Tayson, Ham & Schneider (1953)	36	mixture	ram	745	2.13	0.65	6.61	7.13	-	wheat straw, beet pulp, linseed meal, oats & wheat.
Kolb (1967)	?	?	?	500-2000	3	-	18	-	-	-
Pontius, Carr & Boyle (1931)	6	-	various	312	-	-	-	9.0	-	alfalfa hay
	6	mixture	"	207	-	-	-	7.16	-	grain & corn silage
	6	mixture	"	2310	-	-	-	8.62	-	mangels & corn stover
Packett, Lineberger & Jackson (1968)	20	pelleted	wether	776	3.4	62.8	67.4	-	Potassium 824 mg%.	alfalfa, grain, cottonseed, molasses.

* selected as having lowest incidence of calculi and no mineral supplementation.

o quantity per day not per 100 ml.

[redacted]

one third) is low (Anaud and Parker, 1966).

As an example of the effect of temperature and the availability of water, Australian sheep in winter excreted 1.34 ± 0.65 litres daily, compared with 0.73 ± 0.39 litres in the hot summer (Schmidt-Nielsen, 1964); while MacFarlane, Morris and Howard (1956), quoted by the same author, found that on deprivation of water, the urinary volume dropped from 1.68 litres to 0.13 litres on day 5.

That variations in feed cause differences in urinary volume is shown in Table 1, in early work by Pontius et al. (1930).

The table shows that little calcium, magnesium or phosphate is excreted in normal ovine urine. Figures from the control groups of other feeding experiments confirm these figures (Crookshank et al., 1965; Kolb, 1962).

As in man, urinary magnesium excretion varies more than calcium or phosphorus (Scheunert-Trautmann, 1965). Urinary Mg excretion has been claimed to occur over the threshold of about 2 mg% in blood plasma.

A limited amount of work supports the textbook statement that ovine urine (as herbivorous urine) is normally alkaline in reaction (Dukes, 1955; Marek and Mocsy, 1958;

Cornelius and Kaneko, 1963; Coles, 1967). Pontius *et al.* (1930) reported a mean pH value of 9.0 (range 9.0 - 9.2) on alfalfa hay, a range of 7.5 - 8.5 on rape pasture and 7.6 on blue grass pasture. Johnson *et al.* (1940) found the pH varied from 7.4 to 8.7 with a mean of 8.1. More recently, Kinne, MacFarlane and Butz-Olsen (1961) have reported figures of 8.2 - 8.6 on lucerne, and Stacy and Brook (1964) a mean of 7.6 (range 6.97 - 8.40) on experimental feeding of lucerne and chaff.

Some workers have found acidic urine in normal sheep. Witzigmann (1940) and Christoph (1965) stated the range to be 5.3 - 8.7, mean 5.9, in their series. About 40% of 158 samples tested by Habisreitinger (1961) were amphoteric or acidic.

Stacy and Brook (1964) found the pH dropped after feeding to 5.1 - 6.65. Other conditions in which acidic urine may be found include prolonged hunger, small intestinal catarrh or febrile conditions (Marek and Mocsy, 1958; Cornelius and Kaneko, 1963).

As for other constituents, protein is not normally present in urine (Habisreitinger, 1961) but may occur following various diseases and is transiently seen following violent exercise. Some cases of positive proteinuria are possibly attributable to protein originating from sites other than kidneys, for example the bladder, or the uterus or vagina in females. Nevertheless, levels of protein as high as 1.5g% have been recorded in sheep which have been healthy (Stewart, 1929).

Normal ovine urine contains no glucose (Healy, Bullard and Spears, 1928; Kachmann, 1937; Baker, Reid and Owen, 1928; Cornelius and Kaneko, 1963). Likewise ketones are absent (Healy et al., 1928; Stewart, 1936) but intense ketonuria may develop in advanced pregnancy (Oppermann, 1950; Cornelius and Kaneko, 1963). Haemoglobinuria and haematuria are unusual in normal sheep, but may be occasionally present on high concentrate feeding (Stewart, 1936; Leblanc and Savigne, 1899).

(b) Composition of Ovine Urine in Urolithiasis

The following changes in composition of urine from sheep with urolithiasis have been reported :

1. Increased concentration and daily excretion of P (Cornelius et al., 1959; Packett and Hauschild, 1964);

2. Increased concentration of P, and decreased concentration of Mg (Crookshank and Robbins, 1962);

3. Increased excretion of P, reduced excretion of Mg (Robbins et al., 1965);

4. Increased "sedimentation" of P and Mg (Jean-Blain et al., 1968);

5. Decreased concentration of K (Crookshank et al., 1965);

6. Increased alkalinity (Hoar, Emerick and Embry, 1968);

7. Presence of "microcalculi" (Cornelius et al., 1959).

4. EXPERIMENTAL PRODUCTION OF OVINE UROLITHIASIS

Water Deprivation

Glenn, Hamilton and Tucker (1956) included a low water intake as compared with *ad lib.* water in a multi-factorial experiment, and claimed an increased incidence of clinical obstruction on a limited water intake. Conversely, addition of NaCl in the feed to levels of 4 - 10% of the concentrate intake, has reduced the incidence of uroliths and of clinical disease (Elam *et al.*, 1957; Udall, 1959a).

Experimental Feeding

Production of the disease has generally been by the feeding of allegedly "calculogenic" mixtures.

Rations based on a high ratio (4 : 1 or more) of concentrate to roughage have provoked urolithiasis experimentally (Udall *et al.*, 1958). Mixtures containing over 50% of corn or milo (Udall, 1959b; Packett and Hauschild, 1964) were claimed to be calculogenic. Other foodstuffs used in producing calculogenic mixtures included other high grain mixtures of wheat, oats, linseed meal, beet pulp and uncut straw prepared as a pelleted feed, to which 2.4 - 2.5% K_2HPO_4 had been added (Elam et al., 1959). In most instances the calculogenicity was attributed primarily to the high K and P contents of these mixtures.

Other work, involving high P intakes without high K, has also led to a high incidence of uroliths (Emerick and Embry, 1962, 1963; Bushman et al., 1965a; Crookshank, 1967). Low P intakes, when combined with high Ca in a high concentrate feedstuff were also found to be calculogenic in steers (Udall et al., 1958). When different phosphate salts were compared as mineral supplements, no calculi formed with calcium phosphate, while the incidence of affected sheep was 60-70% for disodium phosphate, monosodium phosphate or sodium tripolyphosphate. Increase of the calcium intake in the last-named group to 0.58% reduced but did not prevent urinary calculi (Bushman et al., 1965a). A failure of mixtures, found elsewhere to be calculogenic, has been briefly reported (Reynolds and Lindahl, 1968). In this instance, mixtures used as "calculogenic" by Crookshank (personal communication, 1966) and by Bushman et al.(1965a) did not result in calculus production in other sheep. Newsom (1938) fed excessive calcium carbonate with cane fodder to produce urolithiasis in sheep. Both Evereth and Miller (1939) and Haag and Miller (1928) claimed high calcium intakes were responsible for urolithiasis in sheep, the first-named workers also incriminating a high magnesium intake.

Lindley *et al.* (1957) concluded, in a comparative feeding trial involving different mineral supplements, that neither calcium carbonate nor magnesium carbonate were causative factors in ovine urolithiasis.

Other methods used in attempts to reproduce disease have been stilboestrol implantation (Udall and Jensen, 1958), which led to urinary blockage in a significant minority of castrated males compared with unimplanted controls, and the pelleting of concentrate mixture (Crookshank *et al.*, 1965) compared with meal mixtures.

No surgical methods, such as ureteral ligation or bladder implants, have been used in sheep.

5. RENAL PATHOLOGY IN OVINE UROLITHIASIS

Attention has been paid to the character of calculi recovered from ovine kidneys, and to the histochemistry of normal and diseased kidneys. The suggestion that primary renal injury and inflammation, with the production of organic debris, is the origin of the nidus on which it is claimed the calculus material forms, has led to study of renal ultrastructure. Attention has been drawn away from the grosser renal changes which are found in urolithiasis. Comparative data is lacking in man, since recent textbooks often do not mention the renal pathology of urolithiasis, except to describe calculi (Strauss and Welt, 1963; Heptinstall, 1966).

Inflammation may extend up from an acute haemorrhagic urethritis, following urethral obstruction, to involve bladder and kidneys (Jubb and Kennedy, 1963). The cystitis is haemorrhagic, acute or chronic (Davis, Scott, Crookshank and Spjut, 1969). Hydronephrosis is not a prominent feature with urethral calculi, since urethral or bladder rupture soon terminates the condition. When hydronephrosis does develop, it follows a course in the sheep similar to that in other species. If inflammatory changes are the original cause of the obstruction, then pyelonephritis is superimposed. (Jubb and Kennedy prefer to reserve the term pyelonephritis to denote an ascending urogenous infection from the lower urinary tract). Focal pyelonephritis may not be accompanied by evidence of bacteria in renal tissue (Davis et al., 1969), and may represent chemical or mechanical irritation.

Involvement of the pelvis and medulla is always greater than of the cortex. Jubb and Kennedy refer to suppurative nephritis of haematogenous origin as embolic suppurative nephritis but do not refer further to sheep.

Pathological details of renal changes in natural cases of urolithiasis are limited. Schmidt (1941) described enlargement and mottling of the kidneys, with much tissue destruction. One autopsy report of a sheep which died following bladder rupture recorded somewhat enlarged kidneys, large haemorrhages beneath the pelvic epithelium, petechiation of the cortex and medulla and parenchymatous degeneration (Pontius et al., 1930). In this case, as in others (for example Cornelius et al., 1959), no calculi were found in the kidneys though many were located in the bladder and urethra.

Lindley et al. (1953) produced phosphatic urolithiasis experimentally and found the kidneys were usually greatly dilated with many grey areas in the cortex, extending in streaks to the pelvis. No histology was performed.

A recent account of renal pathology in ovine urolithiasis in Russia has been given by Egoshin et al. (1967) who found hydronephrosis and, in the uncomplicated form of the disease, an interstitial nephritis, the intensity of which varied whether or not sand or stones were present in the renal tubules. There was proliferation of connective tissue which replaced the glomeruli and tubular casts, foci of lymphoid cells and tubular distension. Bowman's membrane was increased in thickness up to 10 times, with corresponding atrophy of the glomeruli, and cortical vascular dilatation. Complicated cases showed similar lesions but in addition necrotic areas, sometimes large, with gram-positive and gram-negative cocci at their periphery. The necrotic foci generally had a fibrous capsule.

The renal pelvis contained blood clots, protein aggregates including hyaline, cell casts, mineral salts and cocci, and the pelvic epithelium showed a catarrhal inflammatory reaction.

The lesions of hydronephrosis and interstitial nephritis resulting from urolithiasis were thought by the Russian workers to be part of a generalised metabolic disease.

American workers have claimed the earliest demonstrable lesion is the precipitation of "calculary" materials in the medulla. This material was both intratubular and interstitial and was a complex of calcium-magnesium phosphate salts (Davis et al., 1969).

Histochemical study of the kidneys in phosphatic urolithiasis ("calculosis") (Cornelius, 1963) has shown PAS-positive material in the cytoplasm of proximal and distal convoluted tubules of certain nephrons, also in the brush borders of the proximal convoluted tubules, collecting tubule cells and transitional epithelium of the pelvis and bladder. Increased PAS-positive material was also detected in renal casts, but the origin of this material in sheep is not clear (Cornelius, 1963).

6. COMPOSITION OF OVINE URINARY CALCULI

Calculi of sheep have been found to vary in composition under different types of feeding and in different countries. Most reports give a phosphate-type calculus when a concentrated feedstuff has been fed (Johnson et al., 1940; Cornelius et al., 1959; Cornelius, 1963; Robbins et al., 1965; Belonje, 1965; Jean-Blair et al., 1968).

One quantitative analysis of ruminant feedlot calculi was: phosphate 75%, magnesium 20%, calcium 3%, remainder 2% (Shirley et al., 1964). X-ray diffraction studies of the phosphate form has revealed principally magnesium ammonium phosphate ($Mg.NH_4PO_4.6H_2O$), with some amorphous material (Cornelius et al., 1959).

Silicate calculi are the dominant form where a high silica hay or grass is consumed (Beesom et al., 1943; Bennetts, 1950; Gardiner, 1965).

Other forms of calculi recorded from sheep are as follows: carbonate (Hobday, 1922; Sutherland, 1958; Cottereau et al., 1966; Gardiner, 1965; Gardiner et al., 1966), purine (Nordstoga, 1962), and xanthine (Easterfield et al., 1930; 1931; Grünberg, 1964b).

This thesis attempts to investigate the distribution and incidence of the disease in Britain, to produce the disease experimentally by dietary means, and records the gross and microscopic pathology of affected sheep. The normal composition of ovine urine is investigated as a preliminary step towards urine analysis in urolithiasis.

PART II

EXPERIMENTAL SECTION

1. MATERIALS AND METHODS

A. DISTRIBUTION AND INCIDENCE OF OVINE UROLITHIASIS

Information was obtained from 5 sources:

1. Abattoir data
2. Clinical records
3. Insurance Companies
4. Veterinary practices in England, Wales and Scotland
5. Pedigree sheep farmers.

The method of collection of data from each of these sources is given below.

1. Abattoir Data

From existing records of 1961 and 1962, figures were obtained for the total number of sheep passing through the Glasgow Corporation abattoir, and total condemnation for uraemia. Uraemia was assessed by meat inspectors on odour of the carcass. Condemnation of the kidneys alone was not recorded.

2. Clinical Records

Records for sheep submitted from Scottish farms to the

Glasgow and Edinburgh University Veterinary Faculties were available. These animals were diagnosed as cases of urolithiasis on clinical and/or _post mortem_ examination. In the Glasgow series the total number of sheep received in the Surgery Department was also available. Data was obtained from the owner of all urolithiasis cases as to the number of affected animals, the total number of sheep at risk and the type of husbandry.

3. Insurance Companies

Three branches of two livestock insurance companies made available their records of causes of death in insured sheep. All sheep were male. The cause of death was determined by veterinary examination in all cases.

4. Veterinary Practices in England, Wales and Scotland

The Veterinary Clinical Observation Unit conducts surveys of the incidence of diseases and effectiveness of treatment using questionnaires which are sent to co-operating veterinary practices. The urolithiasis survey was conducted in 52 veterinary practices with sheep work. The only selection was the deliberate exclusion of small animal practices. This survey was conducted in 1965 and was designed to reflect the general incidence of urolithiasis, not the incidence in 1965. Practices were given the option of describing the incidence as "common", "not rare", and "rare", and these terms were not defined more precisely.

In 1968 a further survey was conducted by the Unit, in which the number of cases of ovine urolithiasis was reported for the previous 12 months. The breeds were divided into Blackface and other, and the practices were asked to give the approximate number of male sheep at risk.

5. Pedigree Sheep Farmers

In 1962 a pilot questionnaire was sent to 150 Scottish farmers whose names appeared in the annual Year Book (1961) of the Border Leicester Sheepbreeders Association. Every second name in the Scottish region was selected.

At the same time a similar pilot questionnaire was sent to every twentieth member of the Scottish Blackface Breeders Association resident in Scotland (Fig. 1).

The farmer was asked to state the number of ram lambs bred in the previous 5 years and the number of cases of urolithiasis, giving the fatalities separately. He was also asked to give the number of unconfirmed cases. Further information was sought on treatment, type of feeding and husbandry and the conditions thought by the owner to predispose to urolithiasis in his last recorded case.

In 1965 the Blackface survey was repeated and 500 life members of the Breed Association were circulated and requested to give data covering the previous 3 years (i.e. 1962-1965). These names were selected from the 1965 Year Book, excluding members resident outside Scotland, and each fifth name was chosen.

August 1962.

Dear

You as a pedigree sheep breeder may now be preparing ram lambs for the autumn shows and sales. I am investigating one of the diseases which may affect such lambs and would like your help.

Urolithiasis, or urinary calculi ("gravel", "sand", "stone in the bladder") actually affects both man and animals. The most serious losses in Scotland are in ram lambs which suddenly cannot pass urine due to a blockage. Straining follows which if unrelieved causes eventual death from bladder rupture. Much is still unknown about the causes of the condition and in an attempt to learn more about it, this investigation has been started. Experimental production of the disease is necessary to learn how it may best be prevented. No other work on this line is being done in Britain at present and any help you can give me would be much appreciated. The eventual results should be of direct benefit to you.

Would you now answer the questions below, tear off and return in the enclosed envelope? Your reply will, of course, be treated as confidential.

Your help may be a means to the solution of this problem. Thank you!

Yours sincerely

A.D. WEAVER.

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1. Approximate number of ram lambs (for stud) bred in last 5 years .... lambs.

2. Known cases of urolithiasis (defined above)
   in these 5 years .... cases.
   of which .... lambs died.

3. Suspected (unconfirmed) cases .... cases

4. The successful treatment in the last known recovered case was:-
   - (a) surgery yes .... no ....
   - (b) injection yes .... no ....
   - (c) change of diet yes .... no ....
   - (d) none yes .... no ....

5. Ration fed to last affected lamb:-

   Bulk was:

   Concentrates were:

6. Husbandry of last affected lamb at time of disease
   - (a) housed exclusively
   - (b) partly at grass, partly housed.
   - (c) at grass exclusively.

7. Indicate conditions you think important in production of this disease.
   - (a) lack of water .... yes/no
   - (b) lack of exercise .... yes/no
   - (c) excessive concentrates (which?) .. yes/no
   - (d) excessive roots .... yes/no
   - (e) bad quality feed .... yes/no

Fig. 1 : Questionnaire sent to sheep breeders.

B. COMPOSITION OF OVINE URINE

Experiments were performed on urine from normal sheep as follows:

1. Estimations on stored urine;
2. Normal sheep at slaughterhouse;
3. Normal sheep at pasture;
4. Normal housed male sheep.

1. Estimations on Stored Urine

These samples were collected from slaughterhouse animals or ewes at pasture. Eighty samples were examined.

The methods of preservation of urine to determine the extent of possible changes in pH were as follows:

The effect of preservation at room temperature (20°C) was compared with refrigeration (7°C). Likewise, preservation in a screw-topped universal bottle of a sample excluding air was compared with a smaller sample (11 ml) in which about half the volume of the bottle was occupied by air, and also with a sample in an open 50 ml. beaker. Recordings were made at 24, 48, 96 and 168 hours. All samples were collected under liquid paraffin which formed a thin seal on the surface (approximately 1 ml).

2. Normal Sheep at Slaughterhouse

Two groups of 20 sheep were selected at random. Group 1 comprised 9 12-15 months old Blackface cross-bred castrated lambs and 11 15-36 months old Blackface or Half-bred female sheep. Ages were estimated on dentition. Group 2 comprised 14 5-6 months old Suffolk cross-bred wether lambs and 6 2 years old Blackface ewes.

Both groups of sheep were from various farms 50-150 miles from the slaughterhouse and had been at grass for at least 3 months prior to slaughter. Group 1 was rested 24-48 hours before slaughter, Group 2 for 3-24 hours and given free access to hay and water.

Samples of urine were taken by direct bladder puncture with a sterile 20 ml. glass syringe and 2-inch 16 B.W.G. needle about 5-10 minutes following stunning, bleeding out and dressing of the carcass. Sheep with less than 20 ml. bladder urine were rejected. Samples were put into sterile universal bottles under 2 ml. liquid paraffin, transferred to vacuum flasks with ice in the base and examined in the laboratory within 2 hours. pH was measured at 20°C using an E.I.L. pH meter, model 23A. The measurements were made in duplicate and the mean value recorded.

3. Normal Sheep at Pasture

The flock consisted of 32 Half-bred ewes (1961-1966) and 28 Cheviot ewes (1966-1968), weight 45-55 kg., of the Veterinary Field Station, grazing a series of ley pastures.

The flock was collected in a pen and 20 ewes in succession were restrained and catheterised for samples of bladder urine. The procedure was as follows: each ewe was restrained in a standing position with the left flank against a wall by one assistant. A second man lifted the tail and prevented lateral movement of the hindquarters. The operator, behind the ewe, separated the vulval lips with the first and second fingers of the right hand to expose the ventrally located opening of the external urethral orifice. With the right hand a sterile Nielsen catheter was introduced by direct vision into the bladder. Urine flowed spontaneously from the free end of the catheter and a midstream sample was collected under liquid paraffin and preserved as previously described. Any samples contaminated with blood or faeces, or of a volume less than 15 ml. were discarded. The first 5 ewes were re-sampled at the end of the period. Recording of pH was made within 2 hours. Other estimations were completed in 2 days.

Urine calcium and magnesium were estimated by a modification of Friedmann and Rubin (1955) and inorganic phosphate by a modification of Fiske and Subbarow's method (1925). Urine protein was determined using the method of King and Haslewood (1936). Bilirubin and acetone were estimated using impregnated reagent strips (Ames Company, Slough). Urinary potassium was estimated by a photometer (E.E.L.) (Varley, 1964).

4. Normal Housed Male Sheep

Four male Blackface tups, aged about 18 months, weighing 66-75 kg., were used. The sheep were half-brothers, having the same sire. The sheep had been housed on the farm for 8 months and fed a hay and concentrate ration in a semi-open yard. Feeding consisted of 700 g concentrate cubes and 1500 g mixed meadow hay and free access to water. All food was supplied twice a day at 7.30 a.m. and 4 p.m.

The animals were weighed and transferred to metabolism cages in a byre as in later experiments. The period of acclimatisation was 14 days. Urine and faeces were collected as elsewhere detailed (p. 56). Five millilitres of a saturated solution of thymol was placed in the urine collection jugs to hinder bacterial decomposition.

The water intake and urine output were measured. Aliquot samples of urine (25 ml) were taken daily for analysis after thorough mixing of the daily urine volume.

C. EXPERIMENTAL PRODUCTION OF UROLITHIASIS

1. Experimental Animals

Entire or castrated male Blackface sheep were used in all experiments. Ages ranged from 5 - 9 months. Weights varied from 18-34 kg. (40-75 lb.). Prior to purchase, animals had been reared on various Scottish hill farms and had been at grass all their lives, as far as could be ascertained, except in one experiment. All animals were examined clinically for general health at purchase, and all appeared normal.

(a) Preliminary Prophylaxis : All animals were given prophylactic vaccination against clostridial (type D) infection ("Pulpy Kidney") on admission, and again 2-3 weeks later. Sheep were given injections of a compound vitamin A and D preparation ("Vetrivite" - Crookes Laboratories) at monthly intervals. The quantity injected was estimated to supply the total requirements of these vitamins (1.5 ml supplied 250,000 i.u. vitamin A and 50,000 i.u. vitamin D_3 per millilitre) as recommended by the National Research Council (1957).

A preliminary control period at grass (season permitting) or housed and fed on hay and water extended over a minimum period of 4 weeks in all experiments. Animals were weighed on admission, and at regular intervals thereafter, on a cage spring balance, and weights were recorded to the nearest 0.5 kg.

In the course of these experiments, apart from death due to urolithiasis, sporadic casualties were encountered from pasteurella pneumonia, unthriftiness and possible clostridial infection.

(b) Feeding Methods : The concentrate was fed either as a simple mixture or later as a pelleted ($^3/8$" or $^3/16$" pellet) concentrate ration, made up commercially (British Oil and Cake Mills, Renfrew, Scotland) and its composition varied in the different experiments.

Hay feeding was local meadow or timothy hay. Roots were local turnips, fed cleaned and sliced, or sugar beet.

(c) Water : Water was the main supply from Glasgow Corporation Loch Katrine reservoir. Analysis at the laboratory on one occasion revealed the following mineral values :

Ca	:	0.05 mg%
Mg	:	0.04 mg%
PO_4	:	0.05 mg%

The City Water Department confirmed these very low values and the intake of these minerals in the water has therefore been ignored in all the experiments.

2. Method of Management

(a) Housing : Animals were housed in 4 pens, each measuring 12 feet x 16 feet, in a large barn with a high roof. Adjacent to one pen was a door, 12 feet x 8 feet, facing west, which was kept open to maintain good ventilation. Sheep were bedded on barley straw or sawdust. Hay was fed from 8-feet commercial hayracks against one wall, and concentrates from galvanised iron troughs with sufficient space for all animals to feed simultaneously. Water was freely available in all experiments from glazed earthenware containers and was renewed every second day.

(b) Exercise : Exercise was only permitted in the pens, except for removal to an adjacent passage for sampling procedures.

(c) General Examination : Sheep were examined daily for general health and any sign of inappetance or straining which might be indicative of urinary obstruction.

3. Collection and Preservation of Samples

(a) Blood : Blood samples were taken at weekly or fortnightly intervals at the same time of day in all experiments by direct venepuncture of the jugular vein after clipping of the wool and distension of the vein by digital pressure. Samples of 10 ml. whole blood were collected into heparinised tubes.

(b) Metabolism Cage Studies : Studies were made using a series of 5 metabolic cages made of galvanised zinc ("Handyangle", Glasgow) and each fitted with a steel wire mesh (1 inch) floor beneath which was a galvanised collecting tray and funnel. The external dimensions were 4' 6" x 6' x 2' 6"; the internal dimensions 4' x 2' 6" x 2' 6". At the front was a galvanised bowl subdivided for water and concentrates. Hay was supplied from a galvanised zinc rack. The equipment is illustrated in Figs. 2 and 3.

Urine was collected in a 5-litre polythene narrow-necked jug under thymol (10 ml. of a saturated solution per collection). Urine passed into the jug through a coarse filter designed to retain faeces particles and consisting of an 8-inch diameter polythene funnel with filter paper or a plug of glass wool (Fig. 4).

Faeces were collected in specially manufactured moulded faeces bags (Avon Rubber Company, Bradford-on-Avon, Wiltshire), held by a leather harness and quick release clips.

Hay was weighed to the nearest 250 g and concentrates to the nearest 100 g on a spring balance, and water consumption was recorded by a one-litre measuring funnel.

All animals had a period of initial acclimatisation lasting 4-8 days in metabolism cages.

Twenty-four hour samples of urine and faeces were obtained for 3-6 day periods in different experiments. A 30 ml. aliquot of each 24-hour urine sample was stored in a rubber-sealed screw-topped glass universal bottle. All urine estimations were carried out at once.

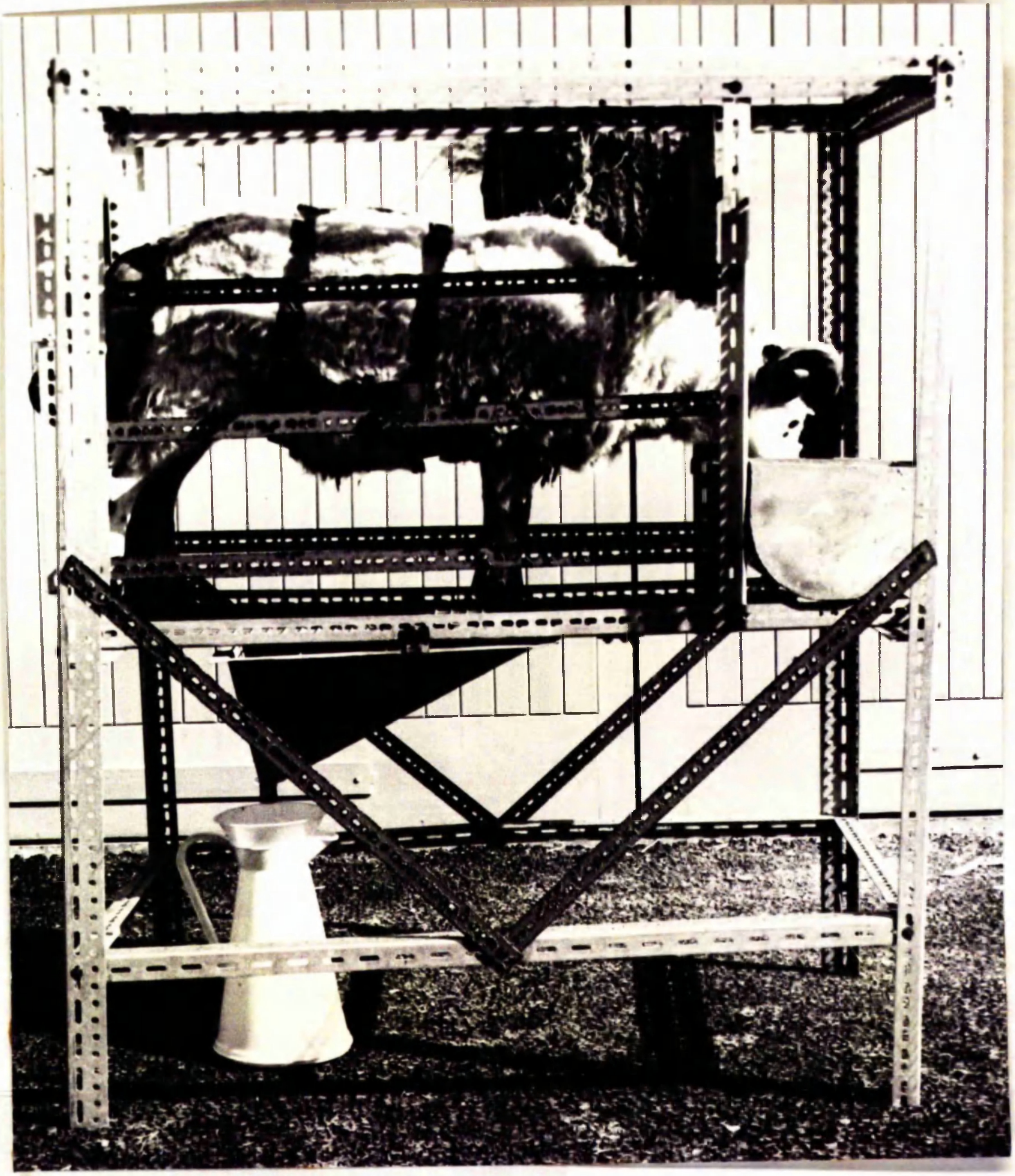


FIG. 2. SIDE VIEW OF METABOLISM CAGE CONTAINING WETHER. HARNESS AND FAECES BAG ARE VISIBLE, AS IS THE URINE COLLECTION DEVICE

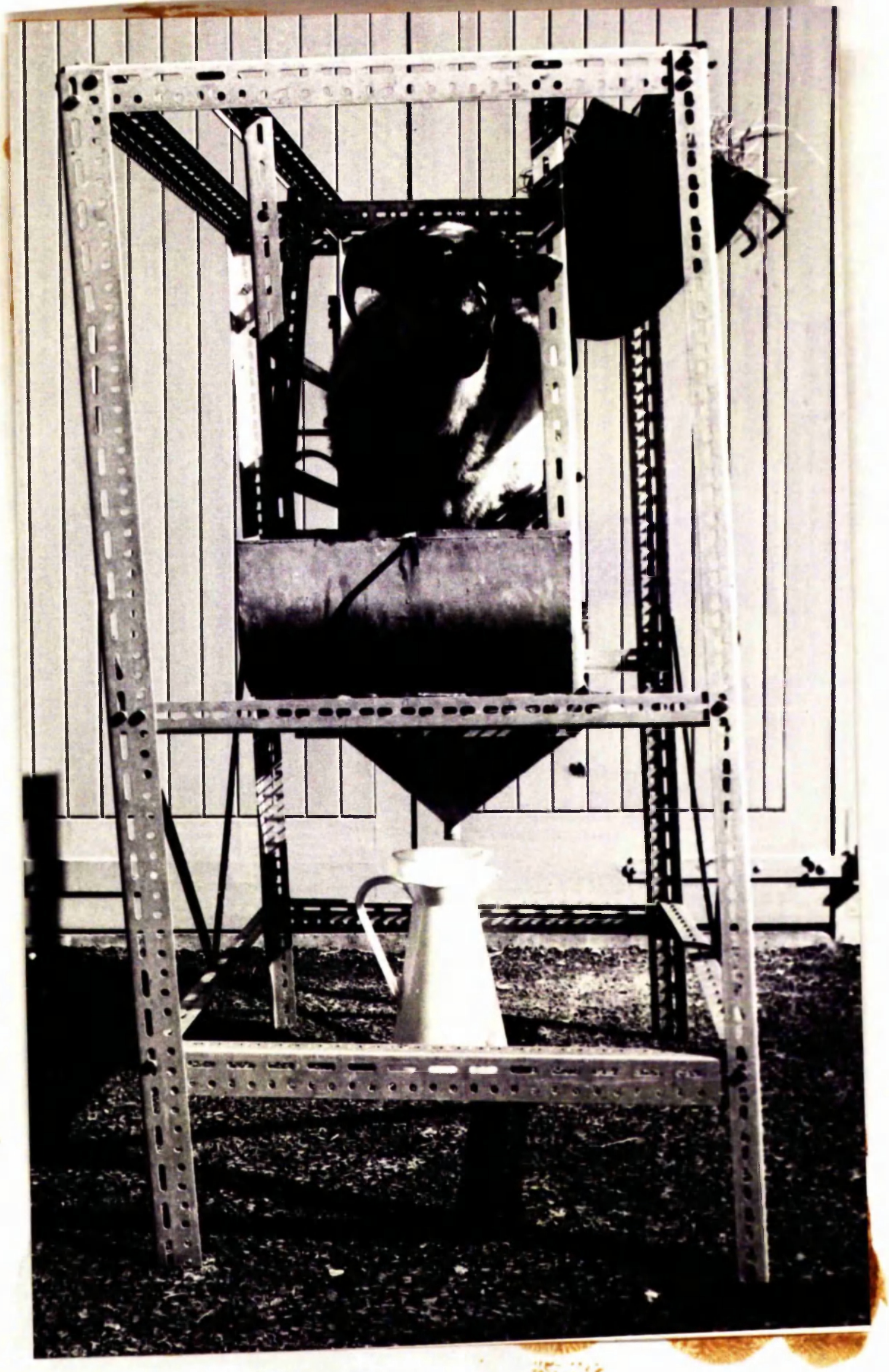


FIG. 3. FRONT VIEW OF METABOLISM CAGE

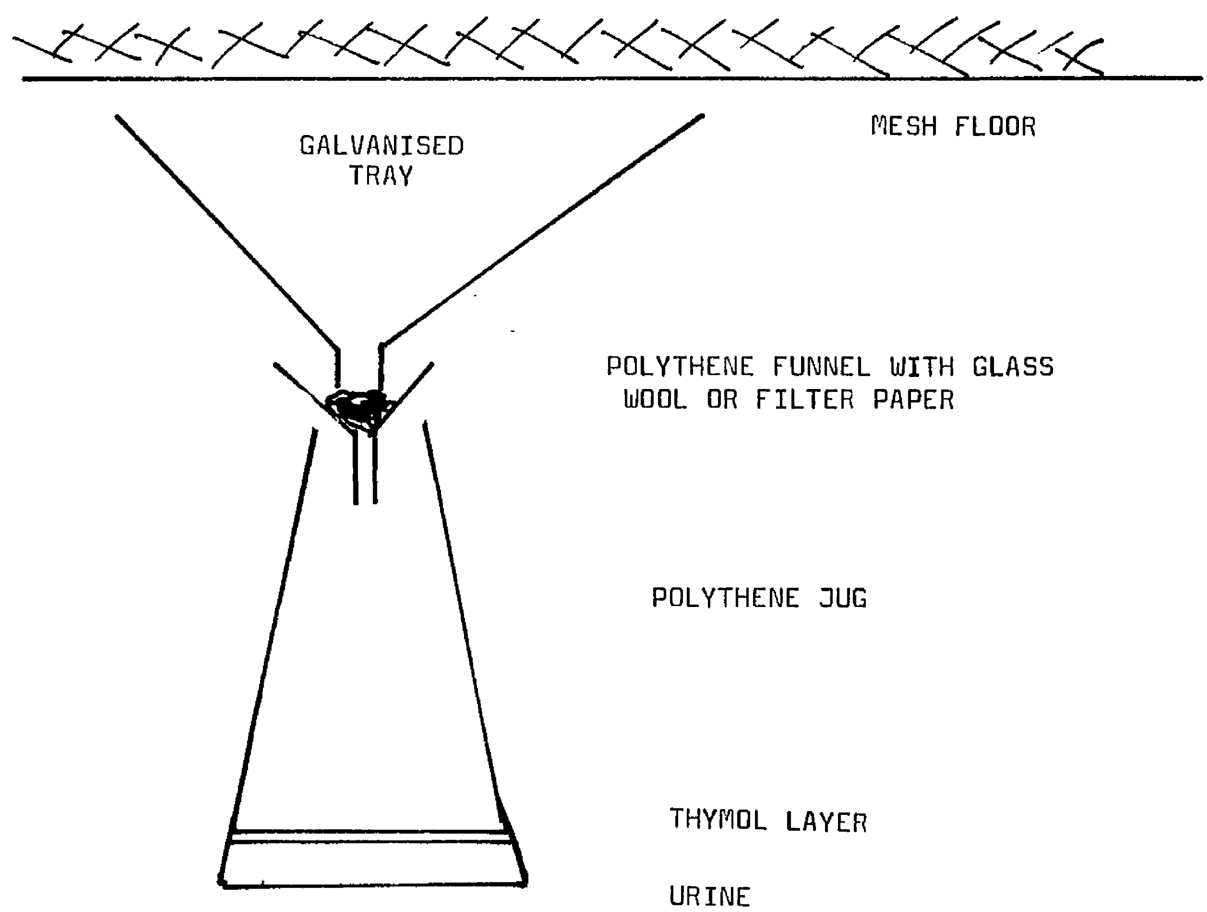


FIG. 4 DIAGRAM SHOWING METHOD OF URINE COLLECTION IN METABOLISM CAGE

4. Analytical Procedures for Blood and Urine

Blood samples were centrifuged at once and estimation of plasma calcium and magnesium was by the method of Friedmann and Rubin (1955), and inorganic phosphate by the method of Fiske and Subbarow (1925). Details are given in the appendix (p. 280). Urea was measured by the urease Nesslerisation method (Varley, 1964).

Urine calcium and magnesium were estimated by a modification of the method of Friedmann and Rubin (1955) and phosphorus by a modification of Fiske and Subbarow's (1925) method. Urinary pH was recorded using an E.I.L. model 23A direct reading instrument. Urinary protein was estimated by the method of King and Haslewood (1936).

5. Procedure at Slaughter

All animals seen to be straining and to have partial or complete urinary obstruction were slaughtered forthwith. Surviving sheep were killed in an adjacent slaughterhouse. Blood samples were taken and final weights were also recorded on the last day.

Since it had previously been observed at a slaughterhouse that stunned animals frequently urinated, precautions were taken to ensure collection of any urine and calculi voided at this time.

The entire urinary tract was removed. Sterile collection of urine was made by direct bladder puncture before dissection of the tract from the carcass or after removal from the animal. Urine samples were preserved under liquid paraffin and swabs of urine were sent for aerobic and anaerobic bacteriological examination.

6. Examination of Urinary Tract

The tract, preserved in a plastic bag, was examined within 2 hours of slaughter. Later identification of the left and right kidneys was made by observation of the peritoneal surface of the periureteral fat and by tracting the retrograde course of the ureters. The perirenal and periureteral fat was then removed and the renal cortical surface was examined for any abnormalities of the capsule or underlying tissue. The kidneys were sectioned at the renal-ureteric junction, whereby renal and ureteric urine was allowed to drip on to a stainless steel dish. Weighing of the isolated kidney was then performed, recorded to the nearest 0.5 g.

A longitudinal section was made through each kidney and the major and minor calyces opened with scissors. The size, number and position of calculi, which were then collected by washing each kidney separately under a fine stream of water over a stainless steel bowel, were recorded.

In some experiments, classification was done as follows:

Number of Calculi:

Few (0-6)	+
Moderate (7-20)	++
Numerous (>20)	+++

Size of Calculi:

<1 mm	+
1-2 mm	++
>2 mm	+++

The ureters were opened along their length to detect any calculus material and to examine the mucosa; the bladder was opened near the vertex, which was held upwards, and the contents likewise emptied into a bowl. The mucosal surface was examined for abnormalities.

The distal part of the penis was first examined. The glans was extruded from the prepuce, and the vermiform appendage was examined visually and manually by palpation of the organ between forefinger and thumb for the detection of any minute particles in the lumen. In one experiment the degree of separation of the appendage from the prepuce was recorded (in mm). The urethra was then opened along its dorsal surface from the bladder neck to the ischial arch, and from this point distally was opened ventrally. At a point about 5 cm. behind the glans penis, a blunt hypodermic needle (no. 17 B.W.G.) attached to a 10 ml. syringe filled with saline was inserted distally into the urethra to ascertain whether urine flowed out through the tip of the vermiform appendage. Finally, dissection was continued down to the distal urethra.

7. Histology

Sections approximately 2 x 1 x 1 cm. were taken for histological examination of:

(1) Both kidneys of all animals; additional sections were taken where there was evidence of gross calculi, in which case the affected area, including cortex, medulla and the papillary zone, was selected.

(2) Ureter; random sections were taken from each series. In addition, sections were taken from any ureter which showed abnormality such as oedema or dilatation or obvious obstruction by a calculus.

(3) Bladder; samples from each series (fundus) and any abnormal tissue.

(4) Urethra; in selected cases sections were taken from pelvic urethra; penis at sigmoid flexure; penis at/or posterior to glans penis; appendage.

Specimens were placed in 10% formalin for fixation and were then processed, embedded in paraffin wax and sectioned at 7 μ. The stains employed were haematoxylin and eosin, von Kossa and Periodic Acid Schiff (PAH), (Lilly, 1954). Details are given in the Appendix.

D. RENAL PATHOLOGY

Gross and microscopic pathology was performed on all sheep which died in the experiments, as well as on 20 clinical cases. The methods are given in the previous section.

E. COMPOSITION OF CALCULI

Material from 20 cases of naturally occurring urolithiasis was analysed. The material was recovered at post mortem from kidneys (15), bladder (3) and urethra (2 animals). All animals had shown clinical signs of urolithiasis. Analytical methods were routine qualitative biochemical tests (Varley, 1964). Since each test required a reasonable amount of calculus material, it was often only possible to test for a limited number of radicals.

In addition material from 10 further cases underwent x-ray crystallography in the laboratory of Professor K. Lonsdale, London. Ten specimens were examined:

Experiment 3 : 2 cases (23988/11 and 23977/26)
Experiment 4 : 3 cases (25849/2, 13 and 25)
Clinical cases : 4 cases (25233, 25286, 25388, 26311)
One experimental worm-free lamb (P 927).

All the specimens were sampled by removing part of the stone and either taking an x-ray diffraction photograph of the untreated sample on a Unicam photogoniometer, or by powdering a larger quantity and fastening the powder with Durofix to the specimen holder of a Nonius powder camera to achieve a bigger resolution (Lonsdale, 1965).

2. R E S U L T S

A. DISTRIBUTION AND INCIDENCE

(i) Abattoir Data. About half a million sheep pass through Glasgow Corporation abattoir annually. In 1961 and 1962 (533,713 and 535,879), there were respectively 3 and 6 condemnations for uraemia. Figures for later years were similar.

Uraemia results from extensive loss of renal function due to pyelonephritis, chronic interstitial nephritis, hydronephrosis, and other causes. The presence of renal urolithiasis *per se* would not result in renal dysfunction and the development of uraemia. Unilateral involvement of a kidney in hydronephrosis can result from ureteral or renal pelvic obstruction, but information from the slaughterhouse, though not recorded, suggested that hydronephrosis was uncommon and all meat inspectors described renal calculi as rare in their experience, as judged on the internal examination of condemned kidneys.

For comparison purposes, condemnations of bovine kidneys, which were individually recorded, indicated an overall kidney condemnation rate of 0.51% in the years 1960-1962. In cows, the rate was 1.3% and was chiefly due to cystic kidneys, nephritis and abscesses. Hydronephrosis and renal calculi were also rare in cattle.

Abbattoir data did not appear to be a useful way of assessing the incidence of ovine urolithiasis due to the difficulty in determining the presence of calculi in the kidneys. Examination of 500 ovine bladders after slaughter failed to reveal calculi but this absence did not necessarily imply an absence of calculi from the kidneys of the same animals.

It was thought that clinical records might be a suitable means of investigating the distribution and incidence.

(2) Clinical Records. In the period 1961-1966, 35 sheep affected with obstructive urolithiasis, confirmed on clinical and/or *post mortem* examination, were received in this Hospital. These animals originated from 30 different farms in different parts of Scotland, predominantly the West. The breed incidence of these 35 cases was: Blackface, 30; Border Leicester, 4; Suffolk, 1. Their ages were: 6 months, 1 case; 6-12 months, 20 cases; 1-2 years, 8 cases; 2-3 years, 5 cases; the remaining animal was 5 years old.

Thirty-three of the 35 sheep were entire males and 2 were castrated males.

The mortality rate in these clinical cases was high. Nine animals returned to further breeding. Table 2 shows the incidence related to the type of management.

Table 2 : Incidence of Obstructive Ovine Urolithiasis on 30 Farms related to the Type of Management.

	Entirely Housed	Partly Housed	Outdoors	Total
Number of farms	16	2	12	30
Number of known affected animals	45	5	16	66
Total of animals at risk	743	50	262	1055
Percentage of animals affected	6.06%	10.0%	6.1%	6.2%

The Surgery Department of the Edinburgh University Veterinary Faculty had records of 15 cases of ovine urolithiasis in 12 years. All cases were in entire males, and 11 animals were aged 6 months to 1 year. Most cases occurred in the Blackface breed, and 6 cases recovered. The method of management and the incidence on affected farms was not recorded.

Information of this type is limited in value since it is highly selective. Animals sent to veterinary hospitals tend to be valuable or potentially valuable, and this, allied to the naturally greater number of younger, as opposed to older rams, may explain the greater incidence in young subjects.

The high proportion of the Blackface breed (41 out of 50 sheep) is interesting, but the Blackface is the dominant breed in Scotland, in particular in the west and north, where it forms about 90% of the sheep population.

The type of management was examined since it has been stated (Nairn, 1939) that close confinement and lack of exercise may be predisposing factors. Though many animals were affected on farms from which the 35 Glasgow clinical cases were obtained, the proportion of animals affected was similar under contrasting management systems, outdoor husbandry or housed stock. This suggests that the type of management is unimportant.

The similarity of the Edinburgh figures suggests that the problem is not a purely local one. The smaller number of cases in the east can be attributed partly to a smaller sheep population and partly to a less intense investigation of this particular problem.

The third source of information was the records of insurance companies.

(3) Insurance Companies: Over a period of 3 or 6 years, 3 branches of insurance companies found a limited number of claims due to urolithiasis. The details are given in Table 3.

Table 3 : Number of Claims for Urolithiasis in Insurance Company Records

	Claims All Causes	Claims for Urolithiasis	Period (years)
Company A	150	0	3
Company B, Branch 1	250	2	6
Company B, Branch 2	212	12	6

Claims formed about 10% of all sheep insured. The majority of claims were for accidental injury (fractures, drowning). In one branch of the second company (Table 3), urolithiasis claims represented about 6% of all claims. In this branch the majority of the insured sheep were animals about 6 months old, which were insured immediately after purchase at an autumn ram sale. The diagnosis of urolithiasis was dependent on an adequate post mortem examination by the owner's veterinary surgeon and in some cases the carcass had obviously, from the certificate, undergone considerable autolysis. Cases of 'ruptured bladder' and 'ruptured urethra' were assumed to denote urinary tract obstruction due to calculi, even though calculi were not stated to have been found. Other cases stated to be 'pneumonia' may have been a secondary pneumonia superimposed on an undiagnosed urolithiasis.

The knowledge that veterinary surgeons are in a position to give some information on disease incidence, when conditions are not recorded officially by the Ministry of Agriculture, led to the fourth approach.

(4) Veterinary Practices in England, Wales and Scotland: Forty-nine of 52 practices, circulated by the Veterinary Clinical Observation Unit in 1965, replied and the figures, which refer to the incidence of ovine urolithiasis in different practices, are given in Table 4.

Table 4 : Frequency of Incidence of Ovine Urolithiasis in Veterinary Practices in England, Wales and Scotland. (Report of Veterinary Clinical Observation Unit, 1965)

	"Common"	"Not Rare"	"Rare"
Scotland	7	7	6
England	0	6	39
Wales	0	1	4

(The difference in frequency between Scotland and England/Wales is statistically significant ($Chi^2 = 23.04$, 2 degrees of freedom, $p \ll .001$)).

The figures show that the condition was never "common" in English or Welsh practices. About a third of the Scottish practices claimed ovine urolithiasis was "common", and a similar proportion "not rare". Statistical analysis of this data shows that the difference in frequency between Scotland and combined England and Wales was significant ($p < .001$).

No figures were obtainable for the actual number of cases seen and it must be expected that the interpretation of the terminology of frequency differed from practice to practice. Since the numbers of animals at risk in a practice are not known, it is hard to conceive a method of analysis which would give the true incidence.

In 1968, a further attempt was made to obtain factual data from practices. Fifty-one useful returns were obtained from 60 circulated practices. The frequency is expressed, as in the previous table, in respect of practices. On this occasion a simple separation was made into practices with and without ovine urolithiasis cases in the previous 12 months (Table 5).

Addition of the individual practice figures in respect of breed showed that 9 of a total of 76 Blackface rams at risk were affected and 2 died, while 12 of about 200 rams of other breeds were affected and 7 sheep died. It was evident from this questionnaire that there was a poor return from Scotland.

Table 5 : Occurrence of Urolithiasis in Practices in England, Wales and Scotland.

(Veterinary Clinical Observation Unit, 1968)

	Positive	Negative	Total
Scotland	2	3	5
England	3	40	43
Wales	0	3	3

(The difference in frequency between Scotland and England/Wales is not significant).

The reason for this is uncertain but it does not necessarily indicate a low incidence, indeed the amount of work necessary to complete the questionnaire may have led to this failure. A number of practices in areas of high sheep density reported no cases of ovine urolithiasis for this, and several preceding years. The decreasing value of individual sheep has meant that flock problems have received more attention in recent years.

Finally, tracing back to sources of possible information, pedigree sheep farmers were investigated in terms of sheep losses due to urolithiasis.

(5) Pedigree Sheep Farmers. Seventy-one of 150 farmers (47%) replied to the Border Leicester questionnaire, and 11 (15%) had experienced urolithiasis in the previous 5 years. The incidence of disease was 1-10% of animals at risk and mortality was 60-100% of affected sheep.

The return from the Blackface breeders was 54 of 140 circulars (38%) and the incidence was 50%, since 27 farmers reported cases in the previous 5 years. Morbidity and mortality were similar to the Border Leicester breed.

In the more ambitious 1965 survey, 89 replies were received (17.5% return), of which 23 were discarded due to lack of details.

Thirty-two per cent (21/66) of the breeders had experienced urolithiasis in the previous 3 years among ram lambs. In Table 6, the affected flocks have been divided into large (more than 100 ram lambs reared) and small (less than 100 ram lambs). The incidence of disease was 1-12% as shown in Table 6. Mortality varied from 30-100%.

Table 6 : Incidence of Clinical Urolithiasis among Breeders of Blackface Ram Lambs (1965 Survey).

		At Risk	Number Affected	%
Large flocks	Flock 1	245	25	10
(≥100 lambs reared)	Flock 2	300	3	1
Small flocks	Flock 3	40	5	12
(≤100 lambs reared)	Flock 4	50	1	2

Flocks 1 and 3 were those with the highest incidence over the 3-year period. Flocks 2 and 4 had the lowest incidence of positive cases. The range of incidence was similar in large and small flocks.

The returns showed that 54/145 sheep with urolithiasis died or were slaughtered, representing a mortality rate of 37%. Expressed as a percentage of male animals at risk, mortality was 1.4%.

The method of husbandry on farms where urolithiasis had occurred was then compared with farms with 'healthy' animals in respect of urolithiasis. This information is summarised in Table 7.

Table 7 : Type of Husbandry on Farms with Urolithiasis 1962-1965 compared with Farms without Urolithiasis (Blackface Breeders).

	Farms with Urolithiasis	Farms without Urolithiasis	Total
Housed exclusively	14	8	22
Pasture during day : Housed at night	6	17	23
Pastured exclusively	1	20	21
Totals	21	45	66

(χ^2 = 11.3 at 2 d.f. $P < .005$. Animals housed exclusively have a significantly higher incidence of affected animals compared with those part-housed or at pasture).

Animals which were on farms where the practice was to house the ram lambs were significantly more liable to be affected with urolithiasis than animals kept at pasture continuously. This type of indoor management is not only a different method of rearing, but almost always involves a different type of feeding. At grass, most animals received hay supplementation in winter with limited concentrates. Indoor animals were fed limited roughage and a high concentrate intake.

As in the series of clinical cases previously detailed, all animals with urolithiasis had, regardless of their housing, been receiving concentrates, either a compounded high protein cake, or homegrown foodstuffs, or both.

Eleven cases of suspected but unconfirmed urolithiasis were recorded. This figure is small, compared with the high figure (145) for confirmed cases, and suggests that the clinical signs are specific and that the farmer finds diagnosis to be easy.

The major limitation of this breeder survey is the poor return of information. This weakness leads to the possibility that farmers with interest in and experience of urolithiasis would be more likely to complete the questionnaire giving rise to an inaccurately high incidence rate. That the incidence is sporadic and variable has been confirmed by scientists in several Scottish research departments where housed sheep have been kept for experimental purposes (West of Scotland Agricultural College; Rowett Research Institute, Aberdeen; Departments of Animal Husbandry and Experimental Veterinary Medicine, Glasgow University Veterinary School).

DISCUSSION

Five sources of information were used to attempt an assessment of the incidence of ovine urolithiasis in Britain, and specifically in Scotland. All sources have obvious disadvantages, and any information obtained under such circumstances must be carefully assessed.

The abattoir study showed that sheep sent to one large Scottish abattoir rarely had severe renal disease. Under normal conditions of meat inspection the kidneys are not opened but are examined for abnormalities of shape, consistency and colour. The perirenal fat masks much of the surface. Many kidneys may have contained calculi in the renal pelvis, but this complaint is rarely heard from butchers, and limited personal observation of kidneys from outdoor, extensively reared sheep confirms this opinion.

The selection of animals sent for treatment in an animal hospital is dependent on several factors such as the value of the animal, the severity of disease, distance from the hospital and advice from the veterinary practitioner. Doubtless the 35 cases of urolithiasis referred to Glasgow

University Veterinary Hospital tended to be more valuable than usual and were predominantly from the West of Scotland. It is clear that younger sheep were more often sent for treatment than older rams, and also that, a reflection of the local breed distribution, Blackface sheep formed the majority. This observation of age and breed incidence was confirmed in the records of insurance companies where the only branch where the incidence was of note was in a company which specialised in the insurance of young male sheep immediately following the autumn sales. Again, the majority of animals sold at such sales are Blackface sheep about 6 months old. Both clinical and insurance records indicated that cases were more common in the winter and spring months than in the summer and autumn, and animals exposed at ram sales in Scotland in September would therefore be liable to have a critical period of maximum risk commencing within 2 months.

Records obtained by the Veterinary Clinical Observation Unit have been valuable in defining the regional distribution of various diseases not subject to control by legislation. The 2 surveys on ovine urolithiasis emphasise the variable annual incidence, since examination of the figures shows that in the second survey the incidence in Scotland was not significantly different from England. This interpretation is largely due to a poor return response from Scottish practices. In the second survey, which required estimation of animals at risk and affected, the proportion of Blackface sheep with urolithiasis is twice that of other breeds, but no significance can be attached to these figures since total numbers are too small.

A random survey of sheep breeders presents, theoretically, the best method of defining disease incidence when the condition is well recognised and characteristic, as in urolithiasis. The method is time-consuming and invariably a poor percentage return is anticipated. The introduction to the survey circular explained the purpose of the enquiry and expressed the hope that useful information would filter back to the individual breeder. Returns of 47%, 38% and 17.5% are poor, but, bearing in mind the limitations, the incidence of affected farms over a 5 and 3 year period appears to be about 50% and 30%, with a mortality rate of about 1% of animals at risk. In severe instances, the incidence may be about 10%. The mortality was generally acknowledged to be high, and housed animals were more frequently affected.

These surveys, though their value was limited, served to illustrate problems of the estimation of disease incidence in Britain, and emphasise the need for a uniform system of causes of death as recently started by the Veterinary Investigation Service of the Ministry of Agriculture. The low, and above all, the variable incidence of urolithiasis made impracticable the biochemical, nutritional and pathological study of the disease on known affected farms, and it was therefore decided that, after a study of normal ovine urine, attempts would be made to produce the disease experimentally.

B. COMPOSITION OF OVINE URINE

1. Estimations on Stored Urine. The results on pH changes in stored urine are summarised in Table 8.

Table 8 : Changes in pH undergone by Urine under Different Conditions of Storage.

Temperature	Condition of Storage	Interval	pH Change + or - **
20°C	Enclosed bottle	24 hours	-0.07) -0.06) slaughterhouse
"	" "	96 hours	+0.01
"	" "	24 hours 7 days	-0.05) +0.47) catheter
"	Beaker	24 hours	+0.81 catheter
"	"	96 hours	+0.46 slaughterhouse
"	"	7 days	+2.10 catheter
7°C	Enclosed bottle*	24 hours	+0.60)
"	" "	96 hours	+0.53)
"	" "	24 hours	-0.09) catheter
"	" "	96 hours	-0.05)
"	Beaker	24 hours	+0.91)
"	"	96 hours	+0.91)

* These samples were of 10-12 ml. urine in a universal bottle, remaining space being occupied by air.

** + = increased alkalinity; - = increased acidity.

The direction of pH change was usually towards an increased alkalinity, but the results show clearly that the change is insignificant over 24 hours with a sample preserved in an enclosed bottle, and that preservation of such samples in a refrigerator does not alter the extent of change. On the other hand, preservation in an open beaker leads to considerable pH changes (up to 1 pH unit in 24 hours), and even exposure to the air in a half-filled but closed universal bottle makes the sample unreliable for a delayed measurement of pH.

Measurement of pH of urine in full universal bottles made at intervals of 2 hours for 8 hours showed no significant changes.

The implication of these findings for methods of collection in metabolism cages will be considered later.

The estimation of urinary pH should normally be done as soon as possible after collection, but these results indicate that a delayed estimation on a properly stored sample (closed universal bottle, full and covered by liquid paraffin seal) will give an accurate result.

2. Normal Sheep at Slaughterhouse. Group 1 had a mean pH recording of 6.07 ± 0.52, Group 2 of 6.2 ± 1.08 (mean and standard deviation). All values in Group 1, with one exception (7.45) were acidic, the lowest being 5.5. In

Group 2, the range was 5.4 - 8.2.

(3) Normal Sheep at Pasture (Fig 4A)

Urinary pH : Table 9 shows the mean urine pH for the individual months over a 7-year period. Alkaline urine is produced during a few spring and summer months, rising from lower and acidic values which prevailed for much of the autumn, winter and early spring. A similar pattern was shown in each year. There was no significant difference in pH of the first 5 ewes between their first and repeat samples on the same day.

Table 9 : Mean Urinary pH Values for 20 Ewes at Pasture over a 7-Year Period : February 1962/January 1969

	Mean pH ± S.D.
January	5.43 ± 0.39
February	5.64 ± 0.49
March	5.81 ± 0.82
April	6.68 ± 0.98
May	7.52 ± 0.41
June	7.42 ± 0.73
July	7.11 ± 0.75
August	6.83 ± 0.68
September	6.00 ± 0.84
October	6.50 ± 0.76
November	6.12 ± 0.80
December	5.78 ± 0.58

Fig. 5 also shows the urinary potassium concentration of the same samples, together with the urinary pH, based on monthly samples collected in the years 1967 and 1968. The urinary potassium is closely related to the urinary pH. ($p < 0.01$).

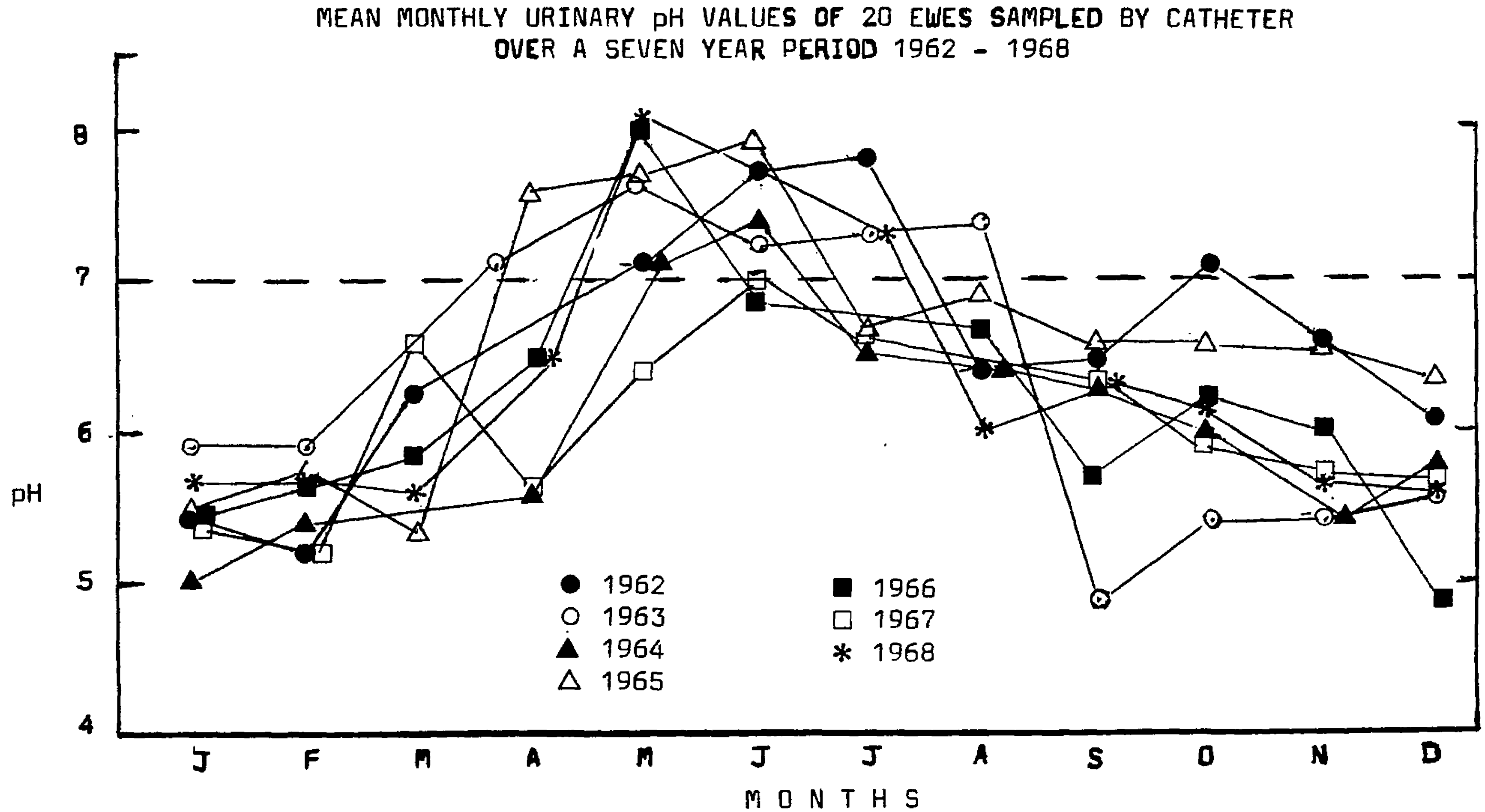
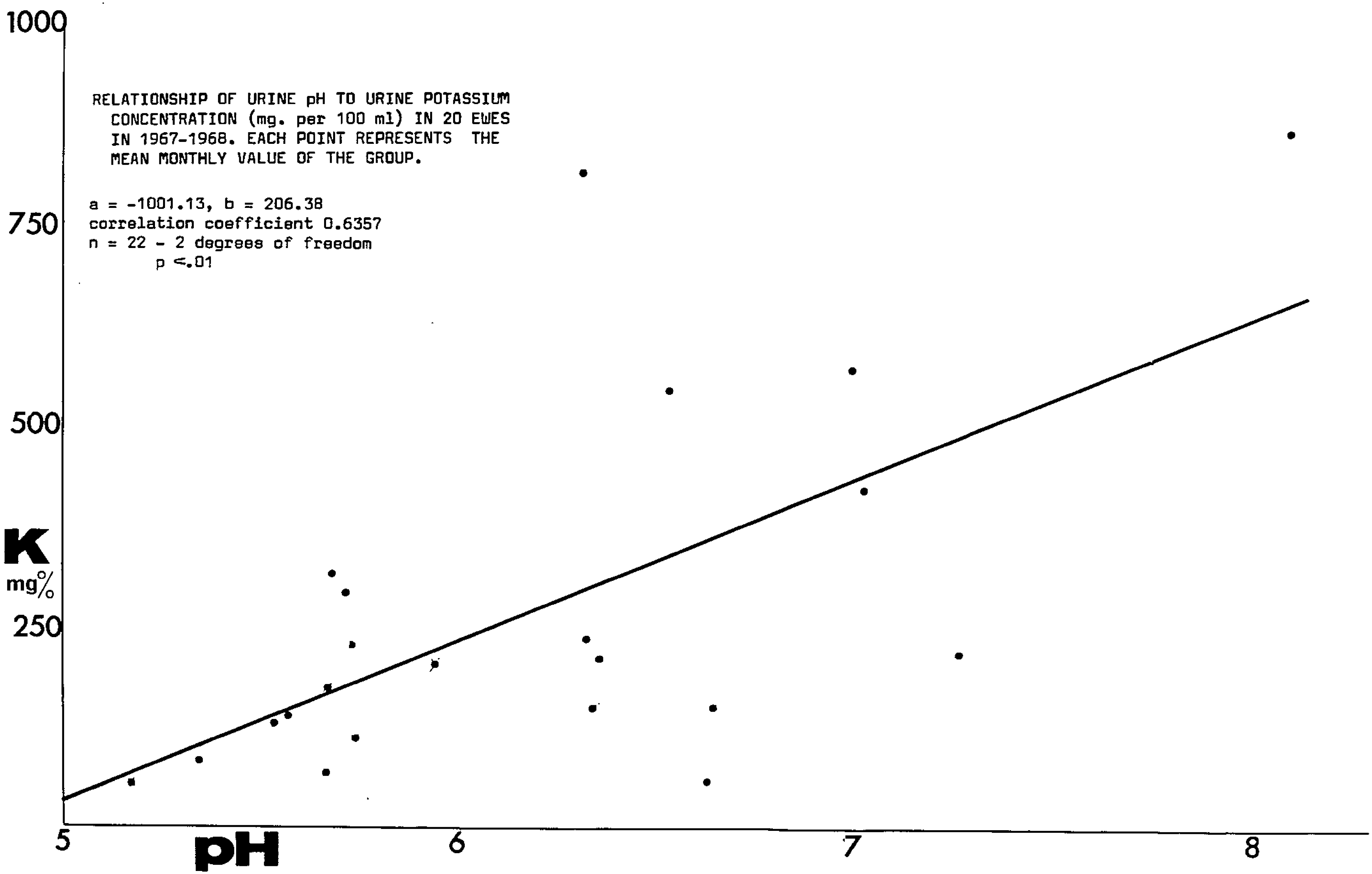


FIG 4A. MEAN URINARY pH VALUES OF 20 EWES SAMPLED BY CATHETER OVER A SEVEN YEAR PERIOD 1962 - 1968



RELATIONSHIP OF URINE pH TO URINE POTASSIUM CONCENTRATION (mg. per 100 ml) IN 20 EWES IN 1967-1968. EACH POINT REPRESENTS THE MEAN MONTHLY VALUE OF THE GROUP.

a = -1001.13, b = 206.38
correlation coefficient 0.6357
n = 22 - 2 degrees of freedom
p <.01

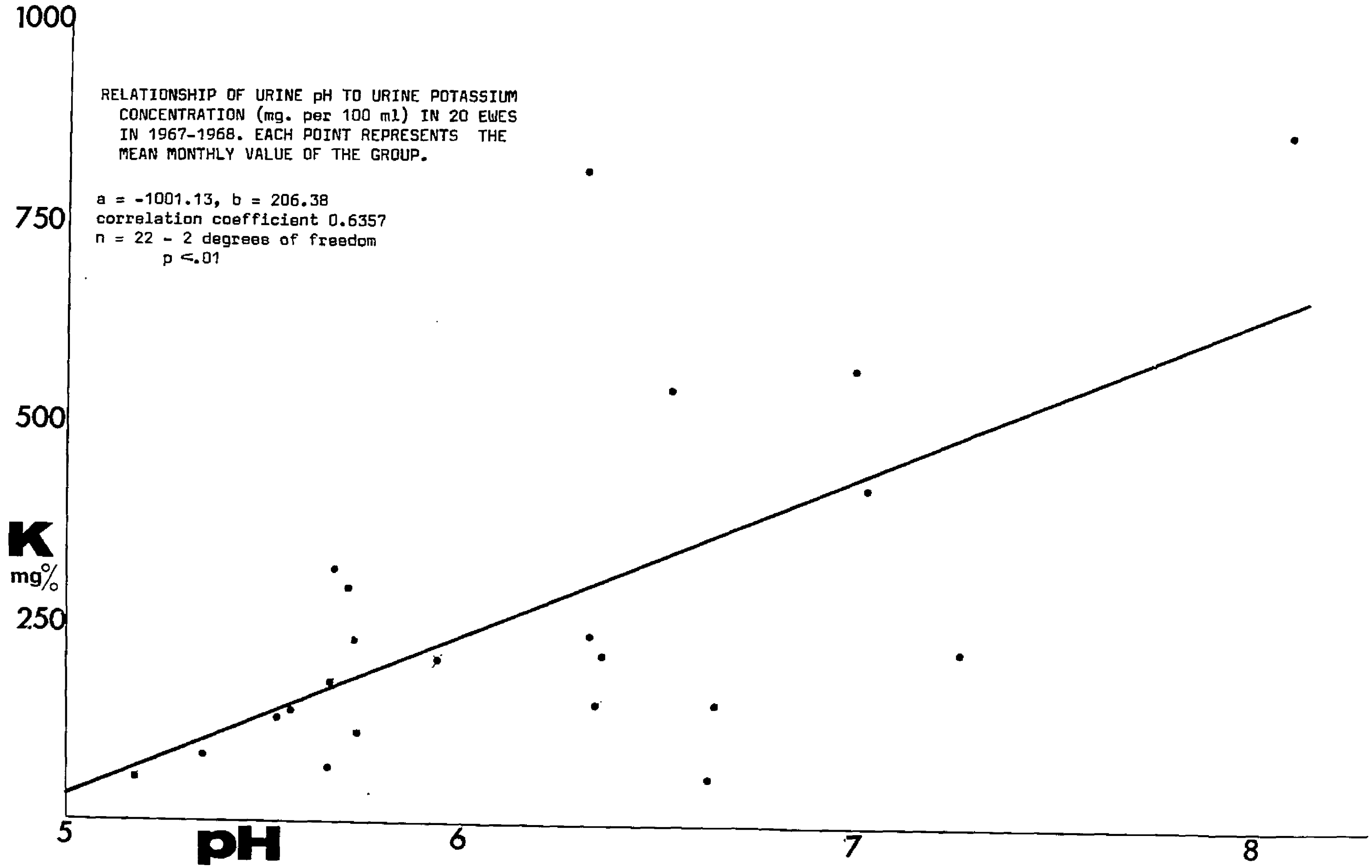


FIG. 5. RELATIONSHIP OF URINE pH TO URINE POTASSIUM CONCENTRATION IN 20 EWES SAMPLED AT MONTHLY INTERVALS IN 1967 and 1968

Urinary Calcium, Magnesium and Inorganic Phosphate : Table 10 gives the figures for these minerals over a 3-year period. All values were low, ranging from 0-14 mg%. There was no apparent seasonal incidence.

Table 10 : Calcium, Magnesium and Inorganic Phosphate in Urine of Grazing Sheep over Period January 1965 to December 1967 : Units are mg% : Figures are Mean Values.

	Calcium			Magnesium			Inorg. Phosphate		
	1965	1966	1967	1965	1966	1967	1965	1966	1967
January	2	0.1	2	4	0.3	2	3	4	10.5
February	4	0.3	2	4	3	1	4	2	3.5
March	5	4	4	11	2	2	6	0.5	0.1
April	2	6	6	5	9	2	0.7	0.5	0.1
May	2	3	5	2	10	0.7	0.5	0.5	0.1
June	5	0.6	7	14	1	3	0.5	0.6	0.1
July	2	0	14	2	0	8	0.3	0	0.3
August	3	2	0	0.5	0.5	0	0.5	0.2	0
September	3	5	3	1	2	1	1	0.1	0.2
October	3	3	4	4	3	7	0.5	0.5	0.5
November	2	2	0.6	2	1	0.9	2	1.5	0.6
December	5	1	0.5	1	1	1.3	7	7	4.4

Urinary Protein, Acetone and Bilirubin : Table 11 gives the number of cases in which the individual constituents were found in the 20 samples each month over a 3-year period. Both protein and acetone were found present sporadically, with a tendency for more positive cases in the more immediate pre - and post-partum months. Bilirubinuria was recorded 7 times in the 680 samples, and is therefore rare.

Table 11 : Protein and Acetone in Urine : Number of Positive Cases in 20 Sampled Ewes

	Protein			Acetone		
	1965	1966	1967	1965	1966	1967
January	1	0	0	0	0	0
February	0	1	1	9	3	0
March	13	5	0	7	6	0
April	4	8	0	1	3	0
May	1	11	0	0	0	1
June	2	1	2	0	0	1
July	4	0	0	0	0	0
August	0	0	0	0	0	0
September	1	0	0	1	0	0
October	5	0	2	0	0	0
November	2	1	0	0	0	0
December	6	0	0	0	0	0
Total	39	27	5	18	12	2

(4) Normal Housed Male Sheep. The analysis of the sheep feed was as follows (results on DM) in Table 12.

Table 12 : Composition of Feed of 4 Normal Housed Male Sheep (% Dry Matter)

	Concentrate	Meadow Hay
Calcium	0.84	0.54
Magnesium	0.28	0.22
Phosphorus	0.71	0.31
Crude protein	14.20	8.86

The mineral intakes daily of 700 g concentrates and 1500 g hay were therefore Ca 13.9 g; Mg 4.2 g; P 9.5 g. These intakes are well in excess of the minimum recommended intake (Agricultural Research Council, 1965).

Animals ate the proferred food well. Nevertheless, the tups lost some weight (4-13 kg) over the period of 1 month in the metabolic cages. The values for plasma calcium, magnesium and phosphorus remained throughout within the normal range (Ca 10.0-11.3 mg%, Mg 0.7-1.8 mg%, P 7.5-9.7 mg%).

Forty-eight urine samples were examined for pH. The mean and standard deviation was 8.2 $\pm$ 0.3. The lowest values were 6.4, 6.5 and 7.2. The remaining 45 values were in the range 8.01-8.75.

The water intake, urinary volume and the concentration and total daily urinary excretion of calcium, magnesium and phosphorus is given in Table 13.

Table 13 : Water Intake, Urinary Volume and Mineral Excretion of 4 Normal Male Blackface Sheep

	Sheep Number			
	1	2	3	4
Water intake (ml)	2407	2653	2761	2678
Urine volume (ml)	725	652	851	681
Urinary daily excretion (mg) : Phosphorus	21 $\pm$ 3	87 $\pm$ 15	102 $\pm$ 12	9 $\pm$ 4
: Calcium	145 $\pm$ 62	53 $\pm$ 32	68 $\pm$ 21	204 $\pm$ 51
: Magnesium	330 $\pm$ 79	273 $\pm$ 52	306 $\pm$ 78	258 $\pm$ 36
Urinary concentration (mg%): Phosphorus	3.4	13.2	11.9	1.3
: Calcium	20.0	8.0	7.9	30.0
: Magnesium	45.5	41.0	36.0	42.5

On statistical analysis the urinary phosphorus excretion of tup 4 was significantly less than the others ($p \leq 0.01$). There was also a significant reduction in tup 1 compared with tups 2 and 3 ($p \leq 0.02$). Principally due to wide daily fluctuations, the low urine calcium of tups 2 and 3 was statistically insignificant.

DISCUSSION

Preliminary pH estimations on urine samples were found to contradict many textbook statements of urinary pH of herbivores, and indicated a need for the subsequent examination of the sampling technique and method of preservation of specimens. The study on methods of preservation for pH estimation showed dramatic differences depending on the prolonged exposure of the specimen to the atmosphere. Samples obtained by a technique which is as far as possible aseptic and anaerobic, and kept in screw-topped bottles without an air trap, and having a liquid paraffin seal, give reliable pH values and show little change after periods up to 3 days. Therefore, long-term (i.e. 24 hours) collection of urine from animals (sheep) whereby the fluid is gathered in open vessels placed under the restrained subject in a metabolism cage, appear unlikely to yield reliable results.

The preliminary results of pH estimations showed that acidic urine samples were very frequently obtained from slaughterhouse material and from grazing ewes. The slaughterhouse sheep may well have experienced a starvation ketosis during transport and after arrival in the lairage where the food consumption would be expected to drop in unaccustomed surroundings. A reduction of food intake will lead to production of more acid urine (Cornelius and Kaneko, 1963). No such explanation can be found for the grazing ewes which had ample food available. As other results in this section show, grazing ewes in this flock rarely had ketosis as indicated by acetone in urine, and then it was usually confined to the winter and spring months.

Extensive examination of urine from grazing ewes showed that the reaction was moderately acid except in a few summer months. Since the sheep were maintained in reasonable condition, receiving protein supplementation before lambing, and since no cases of clinical pregnancy toxaemia were seen (a condition which develops following undernourishment in the pre-partum period (Blood and Henderson, 1963)), these pH findings must be considered to refer to normal subjects. A similar discrepancy in urinary pH has recently been reported by Hjelle (1969) who found most Norwegian sheep excreted acid urine.

Though dramatic falls in urinary pH have been reported soon after the consumption of concentrates (Stacy and Brook, 1964) or hay (Stacy, 1969), the form of feeding adopted by grazing sheep results in a more continuous intake of roughage unlikely to lead to intercompartmental shifts of water and sodium.

The significant ($p \leq 0.01$) correlation of urinary pH with urinary potassium led to an examination of the potassium intake, the normal requirement of which is 0.3-0.5% of the dry matter intake (Telle, Preston, Kintner and Pfander, 1964; Campbell and Roberts, 1965). Most feeds meet this demand adequately (Morrison, 1959) and since the herbage and concentrate figures in this case were about 1.5% and 0.39-0.78%, the supply is considered to have been adequate.

The organic acids in herbage are combined with potassium, and as organic acids are highest in the summer months (Martin, 1969) it is reasonable to predict that urinary pH would be alkaline at this time. Also, the summer herbage is of better quality and the grazing period is longer following the shorter period of darkness (Tribe, 1949; England, 1954).

Neither the sodium content of the urine, nor the non-protein nitrogen is liable to have contributed significantly to the urinary pH.

Confirmation of the possible basic reaction of ovine urine was obtained from these same ewes when they were confined for a feeding trial in January 1969. The reaction was pH 8.22 $\pm$ 0.21 which was significantly different ($p < .001$) from the previous month, when the sheep were outdoors (pH 5.66 $\pm$ 0.24), and any other winter month. It appears therefore that both the diet and environmental factors play a role in the determination of urinary pH.

Values for normal urinary mineral excretion in sheep are scanty, but as seen in Table 1 the figures indicate a low urinary excretion of phosphorus in the normal animal, and are confirmed in the present study in respect of Blackface rams indoors. It appears that individuals may have an unusually low urinary phosphorus excretion.

The urinary calcium excretion figures, though very variable, lie higher than those in the recent literature, and may be related to the high calcium intake. The calcium intake was higher than in 2 reports (Packett and Hauschild, 1964; Robbins et al., 1965) and though the phosphorus intake was likewise higher, the Ca : P ratio was also high, so permitting possibly a higher absorption of calcium, reflected in a higher plasma calcium than in the Packett and Hauschild series. Urinary magnesium values were highly variable as shown by the high standard deviations.

The values obtained in the grazing ewes are now compared with those of the indoor sheep quoted in the literature. If the total daily urinary volume is arbitrarily assessed to be 2 litres, then the following figures are the total daily urinary excretions for grazing ewes:

Calcium	2 - 120 mg.
Magnesium	10 - 300 mg.
Phosphorus	2 - 140 mg.

These values correspond well with the figures for indoor sheep already recorded (Table 1).

Urinary volume in male sheep lies well within the expected limits, as based on the veterinary literature, having regard to the fact that these experimental animals were mature and heavy rams.

The presence of proteinuria confirms the observation of Stewart (1929) and the origin of this material is uncertain. Since midstream samples were collected, it is unlikely to originate in the vagina. The absence of a cell deposit likewise rules out lesions such as acute cystitis where blood and epithelial cells would have been evident in urine samples. The quantity was normally small ($\leq$ 30 mg%), and never exceeded 100 mg%, and is considered insignificant.

Ketonuria can clearly occur in clinically healthy female sheep, and is not confined to the period around the time of parturition. It appears to be temporary since at some times of the year no cases were reported. Bilirubinuria is generally regarded as abnormal (Cornelius and Kaneko, 1963), and in the absence of haemoglobinuria may be due to mild transient liver damage, perhaps associated with fascioliasis.

With the exception, therefore, of the urinary pH, this investigation confirmed the findings of previous workers, and in conclusion the following figures are presented as the normal range for sheep:

pH	5 - 7.5 (grazing)
	6 - 8.5 (indoors)
Ca	$\leq$1 - 15 mg%; very low - 200 mg per 24 hours
Mg	$\leq$1 - 50 mg%; very low - 350 mg per 24 hours
P	$\leq$1 - 15 mg%; very low - 140 mg per 24 hours
Protein	$\leq$100 mg%
Acetone	Occasionally present
Haemoglobin	Absent
Bilirubin	Normally absent

C. EXPERIMENTAL PRODUCTION OF UROLITHIASIS

(i) Preliminary Experiment (Exp. P. - 20835)

The preliminary experiment was designed to determine whether a simple, low calcium, adequate phosphate diet was calculus-provoking. This mineral balance was stimulated by the fact that phosphate calculi appeared in sheep fed a normal or high phosphorus mixture, and the proportion of affected animals was greater when the calcium intake was low (Kunkel *et al.*, 1961; Emerick and Embry, 1962; Bushman et al., 1965a).

Material and Methods

Six male Blackface lambs (ref. 20835 - 20840) aged 5 months, weighing 20 kg, were given oat straw and meadow hay for one month indoors. For 120 days they were then fed a mixture of 50% flaked maize, 45% bran and 5% blood meal *ad lib*. Analysis on a dry matter basis was 0.021% Ca, 0.545% P, Ca : P ratio = 1 : 26.

The bulk portion of the feed was oat straw (Ca = 0.29%, P = 0.27%), which was also used as bedding, and was fed to appetite.

Blood samples were taken at weekly intervals. Anterior-posterior radiographs of the forelimbs were taken after 90 days' feeding to determine whether the animals were rachitic. The 6 sheep were slaughtered and examined post mortem after 120 days of experimental feeding.

Results

The intakes of the concentrate and of straw are shown in Table 14 with Ca and P consumption.

Table 14 : Estimated Daily Calcium and Phosphorus Intake during Period of Concentrate Feeding

	Consumption (dry matter)	% Ca	% P	Intake Ca	Intake P
Concentrate mixture	600 g	0.021	0.545	0.12	3.27
Oat straw	200 g	0.29	0.27	0.58	0.54
Total	800 g	-	-	0.70	3.81

No clinical cases of urolithiasis were seen during the feeding period, nor were urinary calculi recovered at slaughter. Histological examination of the kidneys revealed no abnormality.

EXPERIMENT P. PLASMA MI

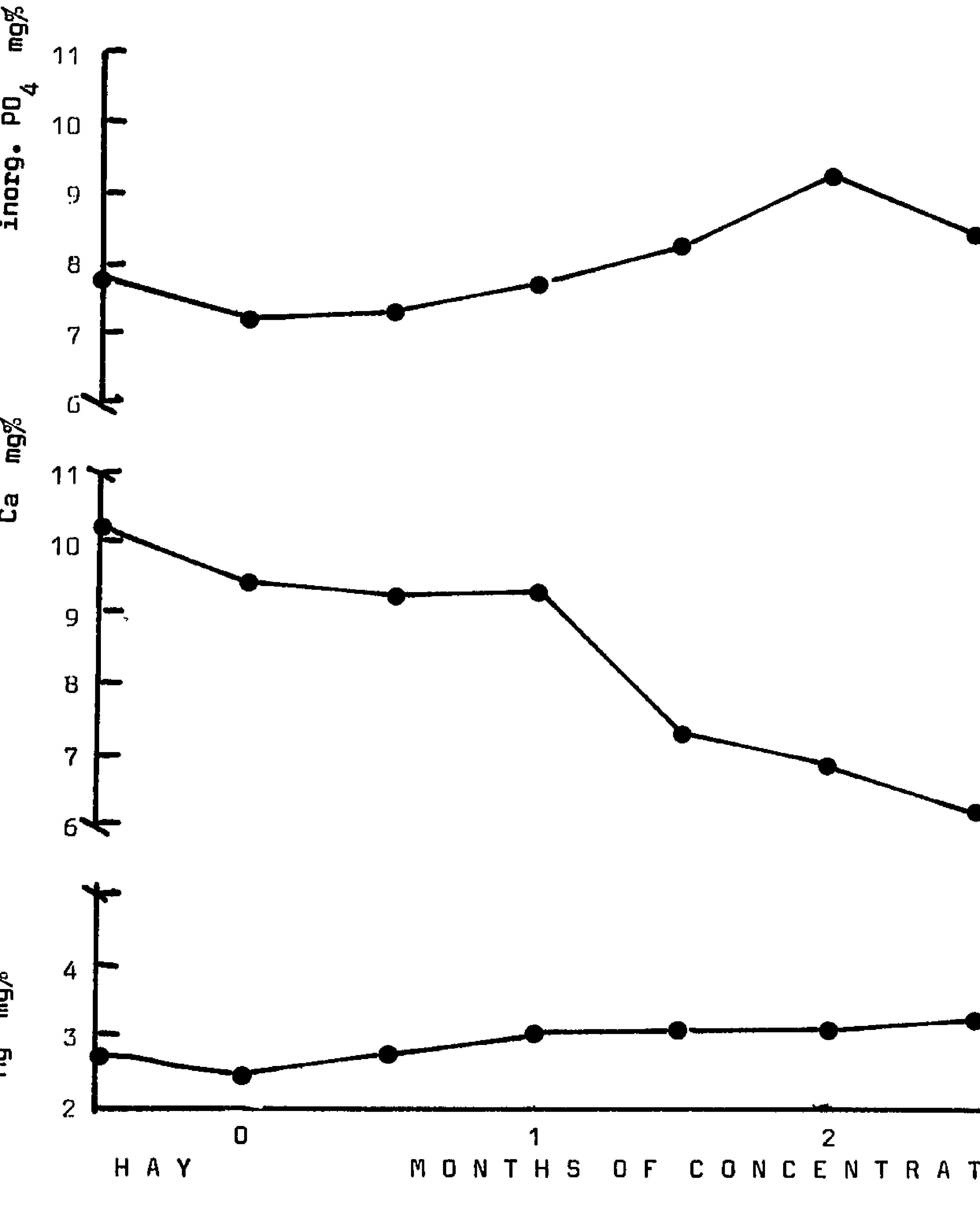


FIG. 6. PLASMA MINERAL LEVELS ON CONCENTRATES AND STRAW DIET GIVING LOW CALCIUM INTAKE (EXPERIMENT P)

The values for plasma calcium, magnesium and phosphorus are shown in Fig. 6. Plasma calcium fell steadily from a mean value of 9.7 mg% during the control period to between 5.5 and 6.5 mg% after 3 months, after which it increased to 9.3 mg%.

Plasma inorganic phosphate rose from 7.1 mg% in the control period to 11.8 mg% after 3 months and then fell to about 9 mg%.

Plasma magnesium assumed no particular pattern.

Discussion

The feed intake in this experiment was grossly deficient in calcium, while phosphorus satisfied requirements, since the current (1965) A.R.C. figures for calcium and phosphorus requirements for these sheep are respectively 3.1 g and 1.6 g, calculated for 20 kg and a daily weight gain of 100 g. Calcium intake was therefore about one quarter of the calculated requirement.

The drop of plasma calcium suggests a severe deficiency and it might have been expected that considerable demineralisation of bone would have occurred, yet clinical and radiological evidence of rickets was absent. Similar findings of the effects of a low Ca diet - a failure to develop rickets on a low Ca ration - have recently been reported by Suttle *et al.* (1968).

The unexpected result of the absence of any urolith formation is in direct contrast to the earlier findings of Kunkel *et al.* (1961), Emerick and Embry (1962), and Bushman *et al.* (1965a) who, feeding slightly less Ca deficient but adequate P mixtures, produced urolithiasis in a significant proportion of sheep, compared with controls which received an adequate Ca intake. These results are more fully discussed later.

(ii) Experiment 1

This experiment was designed to determine whether animals fed on roots and a high mineral mixture, without a gross intake of concentrates, were liable to develop into clinical cases of urolithiasis. Since numbers were deliberately limited in this pilot experiment, no controls were used.

Material and Methods

Ten healthy castrated Blackface lambs aged 10 months, and weighing about 23 kg, were obtained from a local market. The sheep were fed meadow hay for 30 days, and were then divided into 2 groups of 5 animals. For 130 days they were given sugar beet (Group 1) or mangolds to appetite (Group 2), and ate 150 g (dry matter), with wheat straw (200 g dry matter) and 450 g daily of a concentrate ration similar to that in preliminary experiment except for the inclusion of steamed bone flour 10%. This concentrate mixture had a crude protein content of 13.13%.

Analysis of this concentrate mixture, wheat straw and roots for Ca and P and calculated Ca : P ratio was as in Table 15.

Table 15 : Daily Intakes of Calcium and Phosphorus in Experiment 1

	Consumption (dry matter)	% Ca	% P	Ca : P Ratio	Intake Ca g	Intake P g
Concentrates	450 g	2.90	1.76	1.65:1	13.0	7.8
Wheat straw	200 g	0.20	0.10	2.00:1	0.4	0.2
					13.4	8.0
Sugar beet	150 g	0.41	0.17	2.41:1	0.6	0.2
Mangolds	150 g	0.23	0.12	1.91:1	0.3	0.2
Total intake	800 g	Sugar beet group			14.0	8.2
		Mangolds group			13.7	8.2

Analysis of the concentrate also showed the following figures (D.M.) : Mg 0.29%, K 0.70%, NaCl 0.02%.

All sheep were placed in rotation into metabolism cages for urine and faeces collection. An initial acclimatisation period of 10 days was followed by 2 collection periods of 3 days each.

Anaerobic urine samples were taken by bladder puncture after slaughter and bacteriological, pH and urinary mineral examinations were made.

Results

1. Incidence of Urolithiasis

The daily intake of calcium and phosphorus (Table 15) was 13.7 - 14.0 g Ca and 8.2 g P, with a Ca : P ratio of about 1.7 : 1. This represents over twice the calculated daily requirements of these animals.

Two animals died of obstructive urinary urolithiasis (21890, 21899) on the 52nd and 87th days of concentrate feeding. One animal was destroyed due to unthriftiness, 4 days before the end of the experiment. When the remaining 7 animals were killed, 4 contained renal calculi, as below :

Number of Animals		10
Number with Calculi		6
Distribution	: Bilateral	4
	: Unilateral	2
Number of Calculi*	: +++	1
	: ++	4
	: +	1
Size of Calculi*	: +++	2
	: ++	3
	: +	1

* see definitions on p. 60.

2. Plasma Levels of Calcium, Magnesium and Phosphorus (Fig. 7)

The plasma mineral levels showed significant changes in this experiment. There was a significant ($p \ll .01$) rise in phosphorus and magnesium after the introduction of the high mineral level concentrate followed by a fall and a secondary rise 8 weeks later, with no significant difference between the 2 groups. Plasma magnesium rose slowly thereafter. Plasma calcium fell significantly ($p \ll .01$) on introduction of concentrates, but subsequent changes were approximately parallel to the phosphate values. The only notable difference

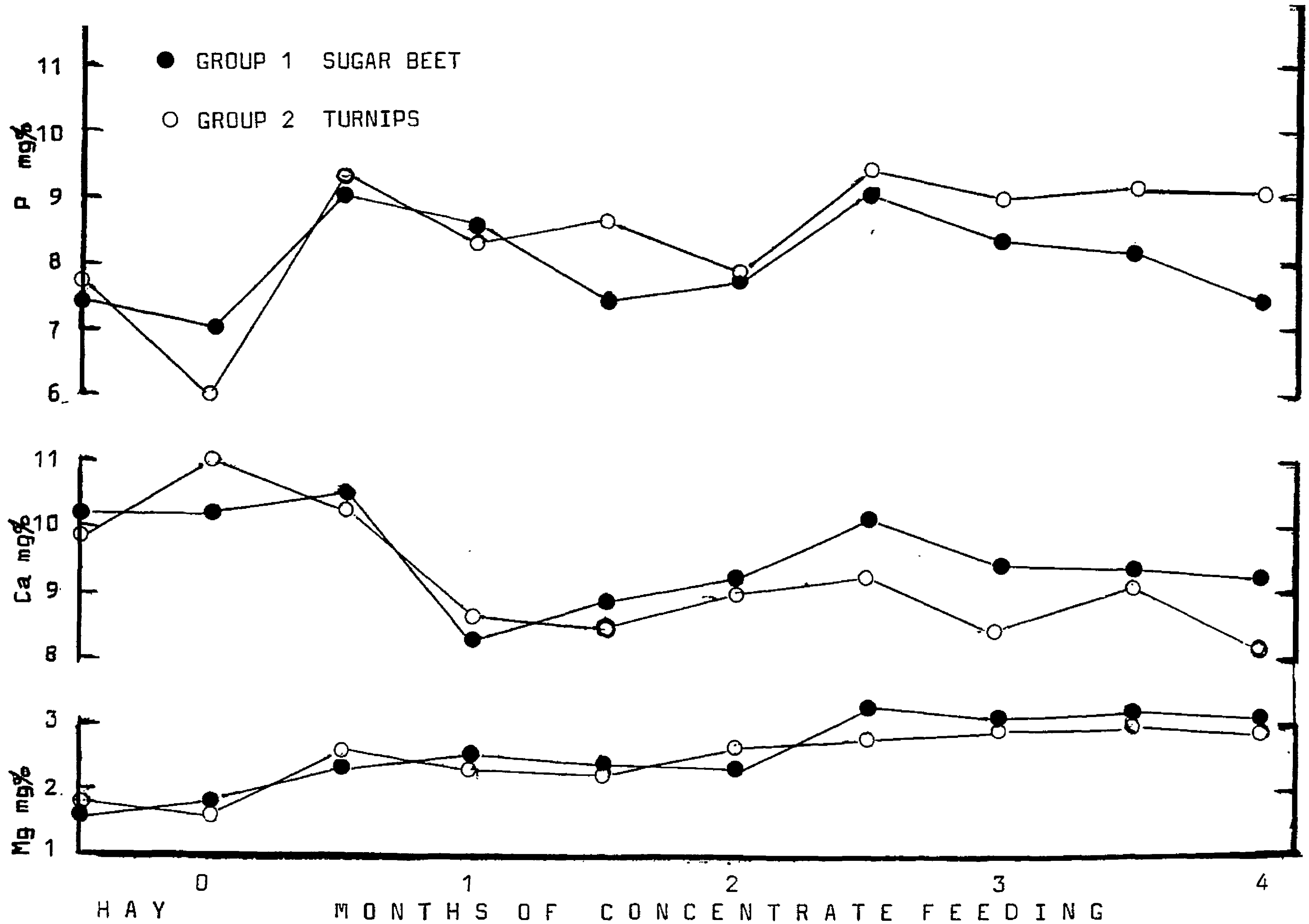


FIG. 7. PLASMA MINERAL LEVELS IN SHEEP RECEIVING CONCENTRATES, WHEAT STRAW, AND EITHER SUGAR BEET OR TURNIPS (MANGOLDS)

between the sugar beet and mangold groups was an increased terminal plasma phosphorus in the latter animals. The 2 obstructed sheep showed no difference during the last 3 pre-obstructive weeks in which they were compared with non-obstructed but otherwise similar sheep.

3. Urinary Phosphate Excretion and Urinary pH

All sheep except 21890 (which died prematurely) completed 2 periods of 3 days in the metabolism cage. The method used gave technically satisfactory 24-hour urine samples after initial acclimatisation.

Table 16 gives the values for the concentration and daily phosphorus excreted by the individuals expressed as a mean figure of the 2 periods of collection. The daily excretion ranged from 0.76 to 6.10 g P. (The first obstructed case (21890) was not recorded since it died before the sampling series commenced).

Table 16 : Concentration (mg%) of Urinary Phosphorus and and Total Daily Excretion of Phosphorus for 9 Lambs Figures are Mean for 3 Days

	mg%	Urinary Volume (ml)	Total Urinary Phosphorus (g)
Group 1 (Sugar Beet)			
91	173	500	0.86
92	170	450	0.76
93*	182	570	1.03
94	192	440	0.84
Group 2 (Mangolds)			
95	143	1835	2.62
96*	134	2250	3.01
97*	185	1700	3.14
98*	158	1200	1.89
99+	121	5043	6.10

+obstructive urolithiasis
*calculi present at slaughter

The highest urinary phosphorus excretion was in the second obstructed case. The 3 lambs with the lowest urinary phosphorus excretion were negative for calculi. The mean daily urinary phosphorus excretion was markedly higher in the mangold fed group but this was mainly due to a considerably greater urinary volume ($p < .05$). The urinary phosphorus concentrations showed little variation from animal to animal. The mean concentration was slightly but insignificantly higher in the sugar beet group.

Values for urinary pH taken from animals in the metabolism cages ranged from 7.5 to 9.0 in samples from a 24-hour collection period.

The results of urine examination after bladder puncture are shown in Table 17 and indicate in most cases a low urinary phosphorus, compared with values in the metabolism cage. Calcium values showed little variation while magnesium figures were very variable. The urinary pH was acidic in all cases at slaughter.

Table 17 : Urinary Phosphorus, Calcium and Magnesium Concentrations at Slaughter (Bladder Puncture) in mg%

	Phosphorus	Calcium	Magnesium
Group 1 (Sugar Beet)			
91	40	15.2	24.6
92	34	13.7	18.8
93	193	16.7	66.2
Group 2 (Mangolds)			
95	8.8	6.3	2.8
96	17.6	11.1	45.7
97	114	17.5	6.6
98	8.8	8.1	5.8

4. Weight Gain and Condition at Slaughter

Weight gain averaged about 500 g per week for the first half of the experiment and 750 g for the later period. One animal (21899) gained weight at twice this rate until urinary obstruction developed. Another animal (21894) very slowly lost weight for unknown reasons which were not clarified at *post mortem* examination.

5. Pathological Examination

(a) Obstructed Sheep

(i) 21890 : This sheep showed symptoms of acute urinary obstruction on April 22nd 1963 (day 52 of concentrate feeding) and was destroyed. *Post mortem* examination was performed at once. The animal weighed 40.5 kg and was in good condition.

Gross pathology showed that the bladder was ruptured for 1 cm. along the right anterolateral surface. There was congestion and haemorrhage over a wide area of the bladder epithelium. No calculi were found in the bladder. The abdomen contained 1200 ml blood-stained urine. There was no sign of macroscopic peritonitis.

The kidneys showed mild hydronephrosis and contained numerous small white or yellow flaky calculi. The size and weight of the kidneys were :

	Dimensions (cm)			Weight (g)
	Length	Width	Depth	
Right kidney	7.5	5.0	3.5	72
Left kidney	8.0	5.0	4.0	78

Macroscopically the kidneys otherwise appeared normal.

The ureters were normal. The glans penis showed gross discolouration, with deep congestion and sloughing of the surface epithelium. The vermiform appendage was stiff and was blocked at 2 distinct points: for a length of 8 mm from the urethral orifice proximally and also over 5 mm near the base. The appendage was ruptured between these points. When opened, the urethral lumen was solidly blocked with small calculi and tissue debris from the glans penis back to the upper section of the sigmoid flexure.

(ii) 21899: Symptoms of acute urinary obstruction were seen on May 28th 1963 (day 87) and the animal was destroyed and *post mortem* examination was made at once. The body-weight was 50 kg. The carcass was in good condition.

Gross pathology revealed that the bladder was intact and

and contracted, containing about 13 ml of blood-stained urine. The bladder mucosa was haemorrhagic over an area surrounding the bladder neck and the commencement of the urethra.

Both kidneys showed marked changes of hydronephrosis. The left kidney was surrounded by a massive subcapsular haematoma, with a deeper layer of gelatinous oedema directly covering the cortical surface. Small hard white gritty calculi were present in the pelvis and calyces. Many pale 1 - 3 mm diameter lesions were scattered throughout the cortex. The right kidney showed small capsular haemorrhages but no calculi were recovered. The pelvis was filled with a gelatinous material. Similar cortical lesions were seen in the right kidney.

Both ureters were grossly swollen, oedematous and congested, with an external diameter exceeding 1 cm compared with a normal value of 3 mm.

The glans penis and appendage appeared normal externally. Calculi were found in the glans, immediately distal to the origin of the appendage, and in the upper part of the sigmoid flexure. These calculi were of similar character to the renal calculi. The entire urethral mucosa was congested, having a deep red mottled appearance. When the bladder was squeezed urine could be forced through the unopened urethra with great difficulty.

(b) Non-obstructed Sheep

21981, 21892, 21895 : In 3 sheep no macroscopic renal calculi were present. The only macroscopic abnormality was a slight hyperaemia at the corticomedullary junction of one case (21895).

21893, 21896, 21897, 21898 : In 4 sheep renal calculi were present except for the occurrence of hard white sharp edged calculi measuring 0.5 - 4 mm in length. In one animal (21896) their colour was yellow-orange and semi-translucent. The maximum size was 4 mm x 3 mm.

Histopathology

(1) Obstructed Sheep : In obstructed sheep (e.g. 21899), the kidneys showed marked focal inflammation of the cortices, characterised by areas of mixed white cell reaction and tubular destruction. Some tubules contained polymorphs. The lesion was spread throughout the cortex but was especially prominent beneath the capsule. There was extensive tubular dilatation. Pyelitis was also seen, and adjacent collecting ducts contained

cellular casts. The medulla was relatively normal. The ureters showed a few scattered plasma cells and lymphocytes in small groups in the muscularis and an oedematous reaction was obvious. The bladder showed subepithelial haemorrhage and inflammation. The urethral changes were variable. Some areas appeared normal while others, especially distally, showed gross epithelial and subepithelial haemorrhage and extensive loss of the superficial layers of epithelium.

Pathological diagnosis : urolithiasis with pyelonephritis.

(2) Non-obstructed Sheep : (i) Without calculi : Where no calculi were visible in the kidneys no histological lesions were present in one animal. A few tubules contained a protein-like precipitate. Von Kossa staining was negative in all 3 cases. One animal (21891) showed pyelonephritis in the subcapsular region, with invasion by polymorphs and lymphocytes, and extensive tubular destruction. In one instance a deposit of amorphous material and cells was present in a renal calyx with a white cell reaction localised around the calyx, while the remainder of the kidney contained a few small isolated areas of interstitial mononuclear reaction and a few tubular casts (21892).

(ii) With calculi : Those animals in which renal calculi were found all showed a deposit of amorphous hyaline material and white cells in and around several calyces. In one instance (21898) a few similar lesions occurred in the cortex. An intratubular protein-like precipitate was found in some lambs. Early focal lesions of pyelonephritis was seen in the cortex, medulla and pelvic epithelium in one case (21897).

Discussion

This concentrate mixture is fairly typical of the feeding which some tup lambs in Scotland receive, in so far as the mineral intake is concerned. Care was taken that a normal (i.e. approximately 1%) level of sodium chloride was included in the mixture.

The occurrence of two typical cases of obstructive urolithiasis initially suggested that the type of feeding was satisfactory for production of the disease. Later, the fact that renal calculi were confined to only half the surviving animals after 4 months on experimental feeding indicated that this mixture was not suitable for use on a wider scale, as a consistent effect was not obtained.

Interpretation of the blood mineral changes is difficult, since marked depression of plasma calcium was not expected in view of the normal calcium : phosphorus ratio. The lack of individual variation in the 2 obstructed sheep, in respect of inorganic phosphate indicates that the uraemic syndrome must have developed rapidly, as phosphate retention, and a raised plasma phosphate, is characteristic of uraemia. On the other hand, the high urinary phosphate excretion of the one sampled obstructive case (75% of the intake was excreted in urine) is suggestive of a raised urinary excretion of this element, perhaps as a result of a partial failure in tubular reabsorption.

In addition, the excessive urinary volume of one obstructed sheep suggests a degree of renal damage, which was shown on post mortem examination 2 weeks later.

Apart from the case of fatal obstruction, all other sheep in the mangold group had an inexplicably high urinary volume compared with the sugar beet group. The absence of severe renal lesions ruled out the chance that renal function was impaired. Mangolds do not have any diuretic activity, and the water intake from this source of roots would not account for more than a small fraction of the difference between the groups. Notably, the larger urinary volume was not associated with any more efficient "washing out" of renal calculi.

The pH values of 24-hour urine samples reflect normal figures as quoted in the veterinary literature, but the known and relatively rapid change in pH as a result of bacterial decomposition makes the figures suspect, especially as known anaerobic samples taken at post mortem gave very different values, which may in turn have been subject to distortion as a result of the previous 24 hours starvation of the animals.

Pathological findings in the obstructed sheep revealed lesions resembling early acute pyelonephritis, which has been noted elsewhere in this work as an accompaniment of some natural cases of urolithiasis.

This experiment suggests that the route of infection to the kidneys is most likely to be haematogenous, rather than urogenous, since, firstly, there was no artificial interference with the urinary tract and secondly, the course of disease was so short as to make the period of urinary stasis correspondingly short. Unfortunately no bacteriological study of the renal lesions was made.

Weight gain on this diet was poor, so that in this respect the experiment did not reproduce the rapid growth experienced in natural conditions. Since the protein content was reasonable (13.13%) and the concentrates proved palatable, the drawback may have been the wheat straw component, but intake of up to 20% of the ration in other experiments (Elam et al., 1956; Lindley et al., 1953; Elam et al., 1957) have been not only well tolerated but have been the basis of the so-called "calculogenic mixtures." The next step in experimental feeding was therefore the adoption of a known "calculogenic mixture."

(iii) Experiment 2

The experiment involved use of a ration, composed of beet pulp, wheat straw, linseed meal and grain, with added potassium and phosphorus known to be calculogenic in the United States (Elam et al., 1956).

Material and Methods

Thirty Blackface ram lambs (18199/1-30), aged about 4 months, weight 17-26 kg were purchased in July 1961 from a local hill farm.

The sheep grazed an old permanent pasture for 35 days and were then divided at random into 3 groups of 10 lambs. Two groups were placed in indoor pens (previously described) while the third group (III) remained at grass. The indoor groups (I and II) were fed respectively mixtures B and A, detailed below, for 50 days.

The heaviest and lightest lambs of each group were then slaughtered and half of each indoor group was transferred to outdoor grazing for 21 days while the remaining indoor lambs continued to consume mixture B. After 21 days, mixture C was fed to 20 surviving lambs, composed of 8 lambs from Groups I and II and 4 lambs from Group III. The remaining 4 lambs stayed at grass as controls and received a hay supplement during the final month (December). All animals were slaughtered after a further 50 days.

Composition of different mixtures was as follows :

Basic Diet (Mixture A)

Wheat straw	24%
Beet pulp (without molasses)	24%
Cane sugar	4%
Wheat	14%
Oats	16%
Linseed meal	18%

Analysis of Mixture A

	%
Crude protein	13.0
Ca	0.92
P	0.34
Ca : P ratio = 2.7 : 1	
Mg	0.14
K	0.55
NaCl	0.50

Mixture B was composed of Mixture A with the addition of 48 lb. per ton (or 2.14%) potassium acid phosphate (K_2HPO_4). The composition was :

	%
Ca	1.10
P	0.72
Ca : P ratio = 1.5 : 1	
K	0.97

Mixture C was Mixture A supplemented with 96 lb. per ton (4.28%) potassium acid phosphate. Analysis was :

	%
Ca	1.27
P	1.20
Ca : P ratio = 1.05 : 1	
K	1.39

The vitamin A supplement was fed as shark-liver oil incorporated in an oatmeal carrier and was checked for vitamin A potency at monthly intervals. These rations were given *ad lib.* as a dry pelleted feed ("pencils" of 3/8ths of an inch diameter). No hay was fed.

Water and common salt (as lick, 93% NaCl) were available.

Indoor and outdoor sheep were both weighed and blood sampled at intervals of 1 and 4 weeks respectively. Estimations were made of plasma calcium, magnesium and phosphorus.

Additional procedures were undertaken as follows :

(a) Blood urea was determined 3 times at the start of concentrate feeding (20 samples), after 21 days of concentrate feeding (30 samples), and after 21 days concentrate feeding (6 samples).

(b) 24-hour urine and faeces samples were collected from lambs in metabolism cages and the mineral levels in urine were estimated. Urine pH was also determined.

(c) At slaughter of the first 6 lambs, specimens of midshaft left radius and ulna were taken for calcium estimation.

(d) At slaughter of the remaining lambs, a urine sample was collected by aseptic bladder puncture. Urine pH and urine phosphorus were estimated and a spun deposit was examined for the character of any deposit. A further sample of urine was sent for bacteriological examination following aerobic and anaerobic culture.

Results

1. Obstructive Urolithiasis and the Occurrence of Calculi

No symptoms of urinary obstruction were shown by any lambs throughout the experiment. Autopsy of 6 lambs at the conclusion of the first part of the experiment (50 days) showed the presence of renal calculi in 2 lambs, one from each of the 2 indoor pens. Lamb no. 2 showed several hundred fine white, semi-transparent, sharp-edged calculi in each kidney while Lamb no. 19 had a smaller number of similar calculi confined to the pelvis of the right kidney. Several calyces were involved in each lamb. Their form was characteristic of triple phosphate. Biochemical analysis revealed that the main radicals were phosphorus, magnesium and ammonium.

Autopsy of 24 lambs after 121 days revealed renal calculi in 6 lambs, all of which had been reared indoors and fed mixture C (Table 18). Analysis of calculi revealed that they were phosphate, and microscopic examination showed typical triple phosphate crystals again.

Table 18 : Calculated Daily Intakes of Calcium and Phosphorus and Incidence of Renal Calculi* in Lambs at Slaughter

	Mixture B	Mixture A	Mixture C	Controls
Ca (g)	7.5	9.0	17.7	—
P (g)	2.8	5.9	16.8	—
After 50 days	1/2	1/2	-	0/2
After 121 days	-	-	6/20	0/4

* Composition in 5 cases magnesium ammonium phosphate, in 3 cases amorphous.

2. Weight Gain and Food Consumption

There was no unthriftiness in those lambs in which calculi were found, as confirmed on liveweight records. The mean weights and standard deviation of 6 lambs with renal calculi before slaughter was 40.5 ± 3.9 kg, that of 14 lambs on the same mixture, but showing no lesion, was 38.3 ± 4.2 kg.

In the first 50 days, lambs on mixtures A and B consumed equal quantities of food. During the first month indoors (liveweight 27 kg) the average dry food intake was 820 g. During the second part of the experiment the figure rose to about 1400 g at 36 kg. Mean water intake in the last month was about 2.2 litres daily per head. Weight gains were more satisfactory in the first part of the experiment than when lambs were consuming mixture C. No digestive disturbance occurred during the experiment and all lambs ate the proffered mixtures readily.

Calculation of calcium and phosphorus intakes on above consumption is shown in Table 18.

Intake of magnesium was about 1.96 g daily in all sheep.

3. Pathological Examination

No calculi were found in any organ other than kidneys, all of which were rather wet.

The kidneys containing calculi varied in weight from 45 to 110 g, which is within the weight range of the normal kidneys 45 - 135 g. Kidney size was related to bodyweight.

One calculus-containing kidney (no. 4) showed mild hydronephrosis, and this animal was one of 2 sheep to have a ureteric abnormality. The left ureter was swollen and oedematous over a distance of 2 cm commencing 6 cm from the renal hilus. The ureteric diameter was 5 mm (normal about 3 mm). Histological examination confirmed oedema and a mild superficial inflammatory reaction. The second ureteric lesion (lamb no. 23), also in a renal calculus-positive lamb, showed similar oedema over a distance of 7 cm of the proximal portion of each ureter. These 2 cases contained by far the largest number of calculi. The bladders and urethrae of both animals contained no calculi.

Microscopic lesions were confined to the kidneys and ureters.

Some renal sections in which calculi were found showed a mild interstitial nephritis, but this lesion was not considered significant, since similar lesions were noted in sections from control lambs.

Eosinophilic-staining material was frequently observed in the collecting tubules of urolithiasis-affected sections, On staining with Von Kossa, this material appeared black and resembled the microcalculi described by Cornelius et al. (1959).

High-power examination of collecting tubules showed the presence of similar, thought to be Ca - or P - containing, material within the cells of the lining epithelium. Other sections showed these cells cast, shed off into the lumen of the collecting tubules, from which they apparently proceeded to the pelvis as a mass of cellular debris and mineral salt.

4. Bacteriological Examination

In every case, culture of urine samples at slaughter showed a profuse growth of a variety of organisms (Table 19), none of recognisable pathological significance. These organisms were probably all contaminants. The diphtheroids were shown not to be *Corynebacterium renale*. A similar range of organisms was recovered from renal swabs of the first 6 slaughtered lambs.

Table 19 : Variety of Organisms recovered from Kidney and Bladder of Lambs (2 Examples only)

Lamb No. 1

Bladder	Non-haemolytic streptococcus Coagulase-negative staphylococcus Sarcina Micrococci
Kidney	α-haemolytic streptococci Diphtheroids α-haemolytic micrococcus Gram-negative cocci

Lamb No. 4

Bladder	Coagulase-negative staphylococci Micrococci *B. coli*
Kidney	Diphtheroid Large gram-negative coccus Very pleomorphic gram-negative bacillus *B. coli*

Other organisms included *Staph. citreus*; *Staph. albus*; *Paracolon bacillus*; *Staph. saprophyticus*.

5. Biochemistry

(a) Blood Urea, Creatinine, Na and K : After 1 month on mixtures A and B, the blood urea figures were within the normal range (26 - 69 mg%).

Mixture B	42.1 ± 8.6 mg%
Mixture A	41.9 ± 9.2 mg%
Controls (grass)	51.0 ± 12.3 mg%

At slaughter of the first 6 lambs, blood urea was again estimated, together with creatinine, Na and K levels. No significant variations were found between the lambs, even as regards potassium in those receiving 2.14% K_2HPO_4 supplement (Table 20).

Table 20 : Plasma Sodium, Potassium and Creatinine in 6 Lambs at Slaughter

	Na meq/L	K meq/L	Creatinine mg%
Lamb No. 1) Mixture B	154	6.2	1.0
4)	151	5.6	0.8
Lamb No. 11) Mixture A	154	5.8	0.76
19)	-	-	1.1
Lamb No. 22) Controls	163	6.2	1.2
29)	151	5.4	0.8

(b) P, Mg and Ca Levels : The detection of any possible critical level of blood mineral constituents and the relationship to clinical urolithiasis could not be determined in the absence of obstructive cases, but values on the different mixtures showed some variations (Fig. 7A).

Within one week of starting mixture B, there was a marked rise in plasma phosphorus followed by a rapid fall. Later, a second rise persisted to give higher than normal values for the remaining feeding period. Lambs on mixture A demonstrated a slow rise in plasma phosphorus and almost reached the level of those lambs on mixture B. Control lambs at pasture showed a slow fall but values remained within the normal range.

Plasma Ca figures showed little variation throughout the experiment with no apparent depression demonstrable in lambs on the high P intake (mixture C).

The plasma Mg level showed considerable variations during the experiment in all groups, quite unrelated to the type of feeding :

		mg%
Mixture A	Range	2.2 - 3.4
Mixture B	"	1.9 - 3.8
Mixture C	"	1.0 - 3.9
Controls	"	1.8 - 2.9

(c) Urinary pH and Urinary Phosphorus : The mean urinary pH of mixture B lambs after 2 - 6 weeks indoors concentrate feeding was 8.5 ± 0.9, and the corresponding figure for mixture A was 8.4 ± 0.4. The difference is insignificant.

Lambs on mixture B excreted more urinary phosphorus than those on mixture A, and both groups excreted more than the controls. There was little increase in phosphorus concentration on mixture C compared with B, but the daily urine volume was increased. Since these observations relate to single random samples from different lambs each day, no statistical significance can be attached to the results.

(d) Skeletal Ca : The results of the percentage ash and percentage Ca in bone ash indicated no loss of calcium from skeletal reserves during the first part of the experiment.

Similar specimens taken from 4 controls and 8 lambs on mixture C at the end of 121 days again showed no evidence of loss of bone calcium.

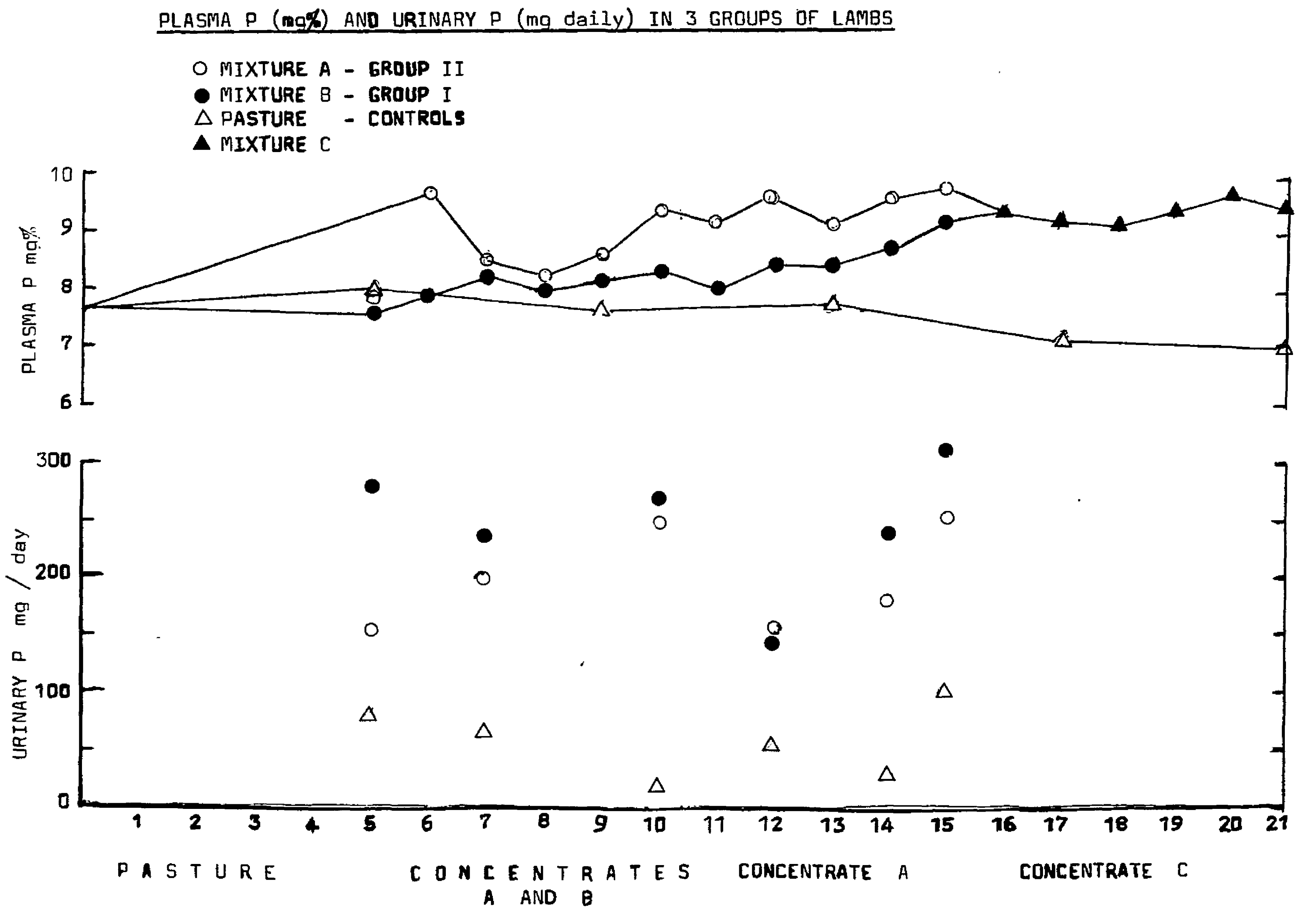


FIG. 7A. PLASMA PHOSPHORUS (mg%) AND URINARY PHOSPHORUS (mg DAILY) IN THREE GROUPS OF LAMBS (EXPT. 2).

Discussion

A notable advance in the study of ruminant urolithiasis was made in the development of a urolithiasis-provoking diet (Elam, Ham and Schneider, 1956) composed of beet pulp, wheat straw, linseed meal and grain with added potassium and phosphorus and which has been the basis of several experiments (Tayson et al., 1951; Elam et al., 1957; Cornelius et al., 1959; Elam et al., 1959; Tayson, 1961).

On a ration with the same ingredients as mixture B of the present experiment, other workers (Elam et al., 1956) have recorded that 4 out of 5 lambs became affected with urolithiasis. Nineteen out of 20 lambs on rations containing added phosphorus, potassium and beet pulp developed urinary calculi. The affected lambs usually showed clinical symptoms.

The diet in the present experiment differed only very slightly from that of the American workers, Elam et al. (1956) in having a slightly higher vitamin A content. The American vitamin level was considered adequate to avoid avitaminosis A. In the American work, wethers weighing about 35 kg (76 lb) were used while in the present investigation uncastrated sheep were preferred. When put on the experimental diets, the rams in this experiment averaged 25 kg. In the American experiment the wethers weighed 45 kg.

The value of using ram lambs lay in the closer realisation of practical conditions as experienced in Britain and the possibility of testing for service capabilities following operation for the relief of urolithiasis.

No reference has been found to any difference in urethral diameters of castrated and uncastrated ram lambs. Marshall and Safford (1957) showed that in calves there was a slightly greater diameter in the bull calf than in the castrated steer and suggested that deferred castration might lead to a lowered incidence of urolithiasis. In the present ram investigation any increase in urethral diameter as compared with that of castrated lambs is likely to be compensated by the greater maturity and presumably greater urethral development of the lambs used by Elam _et al._ (1956).

Comparison of the diets on chemical analysis revealed that the calcium content of the British basic ration was about twice as high as the corresponding Washington ration (that of lot 9) (Elam, 1961). The phosphorus contents agreed favourably. Elam considered mixture B to be calculus-provoking, assuming that the high calcium content did not tend to prevent calculus formation, in which case mixture C was likely to be successful. The Ca : P ratio of the Washington mixtures which were highly conducive to calculi formation was usually about 1 : 1.7, which is far different from the 1.5 : 1 and 1.05 : 1 in mixtures B and C. This is the only marked variation in the nutrition of the experimental animals. Housing and management were similar in both experiments. One could possibly query whether the length of experimental period, 121 days, was sufficient to enable calculus formation to take place, but in similar sheep experiments (Tayson et al., 1951; Elam et al., 1957) calculus formation was reported in times varying from 25 to 33 days. It is suggested that the Ca : P ratio is probably of greater importance than hitherto imagined when this type of diet is employed. Since this experiment was performed it has been demonstrated that moderately high Ca levels tend to reduce the calculogenicity of a high P mixture (Bushman et al., 1965a; Bushman, 1968). Also, the same "calculogenic" mixture which proved disappointing in this experiment has also proved non-calculogenic in other parts of the United States (Reynolds and Lindahl, 1968).

Of other information obtained in this experiment the form, but not the size or quantity, of the renal calculi appears to resemble that of the American workers (Lindley _et al._, 1953; Elam _et al._, 1956). While microcalculi (Cornelius, 1963) were observed in the kidneys of several lambs, the spun urinary deposit of lambs sampled during the experiment or at eventual autopsy failed invariably to show similar microcalculi. Biochemical analysis showed that the calculi were composed principally of magnesium ammonium phosphate, and this agrees with the findings of Elam _et al._ (1956).

The wet, flabby nature of the kidneys, in the absence of dilatation, is not suggestive of hydronephrosis and was a finding in all animals. Subsequent study involving ligation of the penile tip of normal lambs before slaughter (personal observation) has reproduced this type of appearance.

(iv) Experiment 3

The next experiment was designed to determine the effect of quantitative variations in the daily intake of concentrates and roots on the production of urinary calculi in sheep. In addition, the relationship of the weight and weight gain of the lambs to occurrence of renal calculi was studied. The quantity of urinary sediment excreted by lambs in the metabolism cage was studied in relation to the urinary phosphorus concentration, since this sandy material has been associated with urinary blockage in Scottish sheep (Nairn, 1939).

Material and Methods

Forty-two Blackface wether lambs (23988/1-42), aged about 5 months, weight 20-26 kg, were bought from a hill farm. These animals had never been housed.

Housing and general management were as in previous experiments except as below. A control period of hay feeding continued for one month before concentrates and roots were given.

Clostridial vaccine (Pulpy Kidney) and an anthelmintic (Promintic, I.C.I.) were injected twice in the first month following housing. Vitamins A and D_3 (Parentrovite, Crookes) were injected at monthly intervals throughout the experiment.

The sheep were divided into six groups occupying three pens, each of which was subdivided into two halves with common access to opposite sides of a hay rack. The six groups represented a 2 x 3 factorial system, with three levels of concentrate feeding and two levels of roots.

The six groups were enumerated as follows :

Low concentrate, low roots (LCLR)	Group 1
Low concentrate, high roots (LCHR)	Group 2
Medium concentrate, low roots (MCLR)	Group 3
Medium concentrate, high roots (MCHR)	Group 4
High concentrate, low roots (HCLR)	Group 5
High concentrate, high roots (HCHR)	Group 6

The terms low, medium and high concentrates denoted 300 g, 600 g and 900 g per day; turnips (cleaned and chopped) were fed at 500 g and 1000 g per day. Timothy hay was fed to appetite. Water was always available.

The concentrate mixture was prepared (British Oil and Cake Mills, Renfrew) in 3/16" pellets and consisted of linseed cake (20%), oats (30%), maize meal (30%) and flaked beans (20%). This mixture was selected primarily for palatability.

Weighing and blood sampling procedures were performed every 2 weeks as in previous experiments. Blood urea was also determined on 3 occasions: during control period and on days 94 and 108 of concentrate feeding. Each animal was put into a metabolism cage for 8 days, between days 56 and 84 and again between days 85 and 114 of experimental feeding. Total urine and faeces collection was made over the last 3 days during which water consumption was also recorded. Apart from estimation of urinary Ca, Mg and P, the total dried urinary sediment was measured and the results expressed as g%.

The surviving animals were slaughtered after 115 days' concentrate feeding. The slaughter procedure was as previously detailed.

Results

1. Mineral Intake

The analysis of the foodstuffs is shown in Table 21. The food intake was as theoretically expected, except that Groups 5 and 6 consumed 900 g concentrates per head daily. Groups 1 and 2 ate 600 g hay, 3 and 4 ate 450 g, and 5 and 6 ate 300 g daily. The calcium and phosphorus intakes are given in Table 21.

Table 21 : Analysis of Feedstuffs (% in dry matter) and Daily Intakes of Ca and P in Different Groups

	Concentrate	Hay	Roots
(Moisture	10	14	90)
Ca	0.80	0.23	0.40
P	0.49	0.17	0.33
Mg	0.19	0.03	0.07
Na	0.22	0.09	0.15
Cl	0.26	0.26	0.27
Group	**Ca**	**P**	**Ca : P Ratio**
1. (LCLR)	4.06	2.73	1.49 : 1
2. (LCHR)	4.26	2.90	1.48 : 1
3. (MCLR)	5.69	3.70	1.54 : 1
4. (MCHR)	6.25	4.03	1.55 : 1
5. (HCLR)	7.86	5.91	1.60 : 1
6. (HCHR)	8.06	5.14	1.57 : 1

2. Development of Obstructive Urolithiasis and Occurrence of Calculi

Three animals developed acute obstruction on day 80 (2 sheep) and day 89 and showed typical signs of urolithiasis and died (1) or had to be destroyed (2). These cases were from different groups : Group 6 (HCHR), Group 2 (LCHR) and Group 1 (LCLR).

A further lamb (from Group 5 - HCLR) was found dead after

27 days of concentrate feeding but pathological examination failed to reveal the cause of death. No calculi were detected in the urinary tract.

Nineteen of the 42 lambs developed urolithiasis (45%). Distribution was very similar among different levels of concentrate feeding (7 : 6 : 6) and root feeding (10 : 9). This is shown in Table 22. The severity, as judged by whether one or both kidneys was affected, did not differ significantly from one group to another, but bilateral involvement was seen in 13 of the 19 animals.

Table 22 : Incidence of Calculi in Different Groups (7 lambs per group)

Group	With Calculi	Bilateral	Unilateral	Number**			Size**		
				+++	++	+	+++	++	+
1. (LCLR)	3	2*	1	1*	1	1	0	2*	1
2. (LCHR)	4	3	1*	2	0	2*	2*	0	2
3. (MCLR)	4	2	2	0	2	2	1	2	1
4. (MCHR)	2	2	0	2	0	0	2	0	0
5. (HCLR)	3	2	1	1	2	0	1	1	1
6. (HCHR)	3	2	1*	2	1*	0	2*	0	2
Totals				8	6	5	8	5	7

* includes one fatality

** number and size categories +++, ++ and + are defined on page 60.

3. <u>Relationship of Lamb Weight to Incidence of Urinary Calculi and Weight Gain</u>

The average weight gain after 115 days' concentrate feeding was 8 kg. Weight gain was poor in the first half of concentrate feeding, when concentrate intake was relatively small, but was satisfactory in the second 8-week period, especially in the 2 groups (5 and 6) receiving large quantities of concentrates (Table 23).

Table 23 : Weight Gain in First and Second Halves of Experiment in Different Groups

Group	Weeks 1-8 Mean Weight Gain per lamb per week (kg)	Net Gain	Weeks 9-16 Mean Weight Gain per lamb per week (kg)	Net Gai
1. (LCLR) 2. (LCHR)	0.037) -0.072)	-0.018	0.309) 0.395)	+0.352
3. (MCLR) 4. (MCHR)	0.063) 0.205)	+0.134	0.680) 0.725)	+0.702
5. (HCLR) 6. (HCHR)	0.363) 0.047)	+0.205	0.955) 0.985)	+0.970

There was a tendency for calculus-containing lambs to be in the heavier half of the individual groups more than the lighter half (10 : 6). As a result of the exceptionally poor weight gain of 2 calculus-containing lambs, the mean final death weights were not significantly different for 16 calculus lambs compared with 22 non-calculus lambs (31 kg : 30.5 kg). There was therefore no indication that renal calculi occurred more frequently in the heavier type of sheep nor that, on the other hand, renal calculi lead to poor weight gain. The poor weight gain of 2 calculus lambs could not be related to a severe renal lesion.

4. Plasma Mineral Changes

Plasma phosphorus rose in all groups when compared with the control values (obtained after 28 days of hay feeding). At 3 months there was a significant ($p < 0.05$) increase in Groups 5 and 6 on high concentrate feeding. At 4 months all groups had a significantly higher plasma phosphorus than the control values ($p < 0.05$). There was no significant difference between the groups at 4 months.

Plasma calcium was initially 10.4 ± 0.9 mg%. At 3 months no significant changes had occurred but at 4 months there was a slight but significant increase ($p < 0.05$).

Plasma magnesium values fell slowly but the wide scatter of values at 3 months and at 4 months resulted in no statistical difference between values on concentrate feeding and on hay.

The plasma changes are shown in Fig. 8.

Plasma mineral levels in obstructed sheep are recorded in Table 24 for the 3 affected sheep and refer to samples taken respectively 1, 5 and 9 days before death. These values are compared with the mean of the remainder of the group of origin and also with the remainder when divided into calculus (C) and non-calculus cases (NC). Plasma phosphorus and magnesium are higher in the obstructed sheep.

Table 24 : Plasma Mineral Levels in Obstructed Sheep and in Remainder of Group

	P	Ca	Mg
Sheep 34 - Group 1 (LCLR)	10.0	10.1	2.35
Group 1 - mean	9.3	11.2	1.62
- mean C	8.6	11.6	1.8
- mean NC	9.6	11.0	1.5
Sheep 32 - Group 2 (LCHR)	14.2	9.4	2.15
Group 2 - mean	7.4	11.0	1.88
- mean C	6.9	11.0	1.92
- mean NC	7.9	11.0	1.84
Sheep 24 - Group 6 (HCHR)	11.3	12.0	2.54
Group 6 - mean	9.7	10.6	2.17
- mean C	10.3	10.3	1.9
- mean NC	9.3	10.9	2.2

C = calculus sheep in group

NC = non-calculus sheep in group

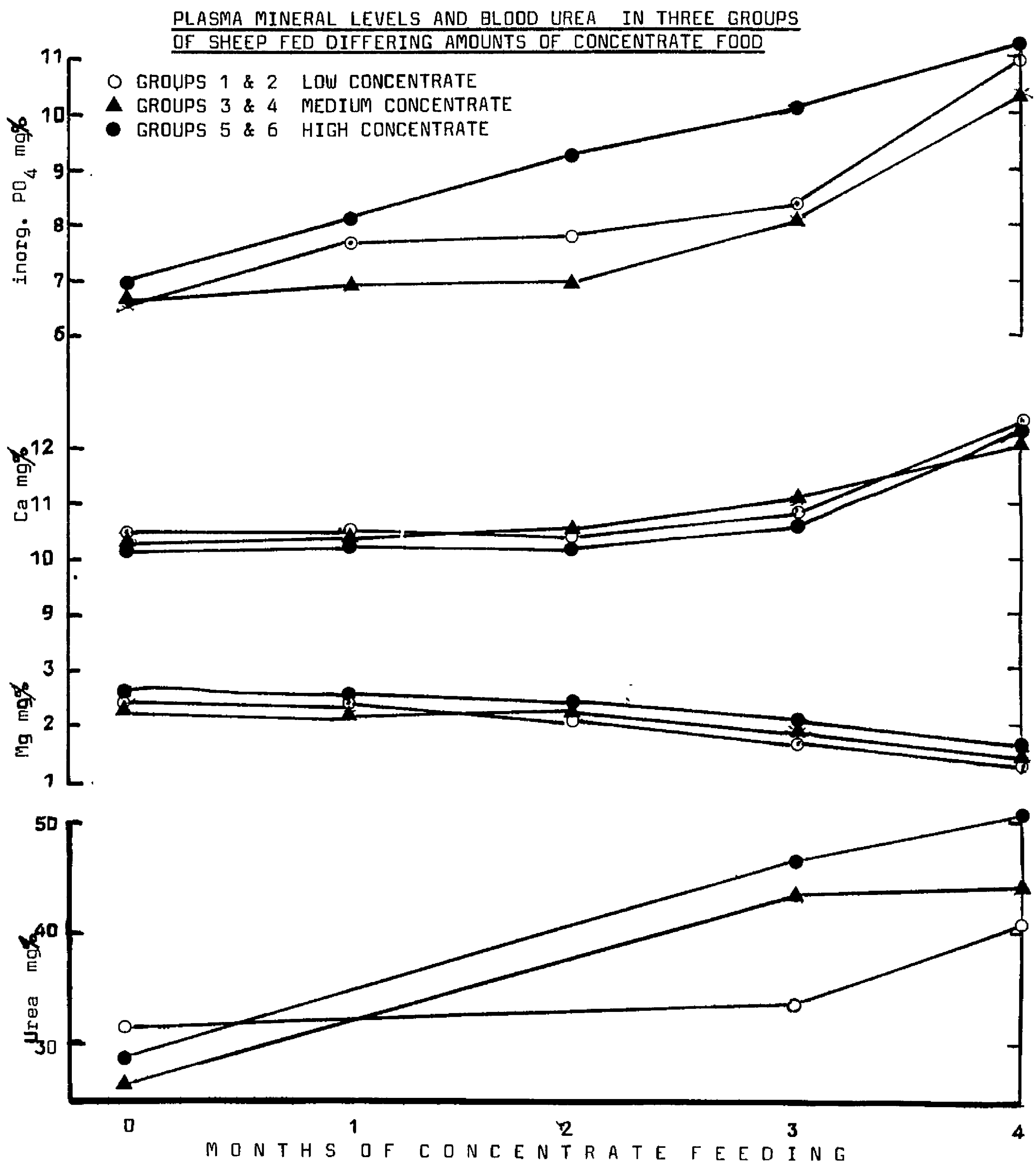


FIG. 8. PLASMA MINERAL LEVELS AND BLOOD UREA IN THREE GROUPS OF SHEEP FED DIFFERING AMOUNTS OF CONCENTRATE FOOD.

5. Water Intake and Urinary Volume

Table 25 shows the water intake and urinary volume expressed as the mean value of 3 successive days. There was a significant ($p < 0.02$) increase in water intake between Groups 1/2 and 3/4, 1/2 and 5/6, and a less significant ($p < 0.05$) increase between 3/4 and 5/6. The urinary volume increased only slightly in animals on higher levels of concentrate feeding.

Table 25 : Water Intake and Urinary Volume of Sheep (Mean Values of 3 Consecutive Days)

Groups	Water Intake ml/day	Urine Volume ml/day	Urine Volume / Water Intake
1. (LCLR) 2. (LCHR)	405 ± 127*	423 ± 97	104%
3. (MCLR) 4. (MCHR)	831 ± 146	539 ± 108	65%
5. (HCLR) 6. (HCHR)	1092 ± 102	551 ± 87	50%

* If one sheep is disregarded, which drank 30 ml in 3 days, this figure is increased to 463 ± 108.

The mean water intake of the "high root" groups was slightly but not significantly higher than the "low root" groups (796 ml cf. 759 ml). The urinary volume was also slightly but insignificantly greater in the "high root" groups (565 ml and 446 ml respectively).

If the figures for urinary volume are examined for differences between calculus and non-calculus animals, significant differences are seen in some groups. Calculus-containing animals on low concentrates excreted 295 ml $\pm$ 97, compared with 487 ml $\pm$ 84 ($p < 0.05$), and the same pattern applied to groups on medium concentrates. Though the results were insignificant in groups on high concentrates, the mean volume of urine was again lower in calculus sheep.

6. Blood Urea

All groups showed an increased blood urea 3 and 4 months after the start of concentrate feeding, when compared with the control period of hay feeding. This increase was most noticeable in the groups on the high concentrate intake (Fig. 8). Analysis of the figures in respect of calculus and non-calculus sheep showed no significant differences in blood urea.

7. Urinary Sediment

The quantity of sediment excreted by calculus-containing lambs (Table 26) expressed as g per 100 ml was greater than in non-calculus lambs, but could be attributed almost entirely to the lower urinary volume in calculus animals. The total daily weight of urinary sediment was very similar in both calculus and non-calculus lambs (mean values respectively 15.38 g and 14.7 g). The differences were not significant statistically.

Table 26 : Mean Quantity of Urinary Sediment in g% in Calculus and Non-Calculus Lambs

	Groups		
	1/2 LCLR LCHR	3/4 MCLR MCHR	5/6 HCLR HCHR
Calculus lambs	1.2	3.8	1.9
Non-calculus lambs	0.5	2.0	1.2

When the quantity of urinary sediment in gm per 100 ml urine was plotted on a graph against the urinary phosphorus concentration in mg per 100 ml, no correlation was obtained, indicating that the 2 factors appeared independent of one another. The differentiation of calculus (17 samples) and non-calculus lambs (20 samples) likewise gave no useful pattern. Examination of the sediment microscopically showed a large quantity of cellular debris but the major constituent appeared to be phosphate crystals of the typical triple phosphate shape.

8. Gross Pathology

The incidence and distribution of calculi has already been detailed.

The right and left kidneys were similar in size in most animals. The difference was 5 g or less in all cases except one (difference 13 g) in which bilateral calculi were found (No. 19). Thirteen animals showed slight congestion of the kidneys on section but this was considered normal.

Two animals (No. 19 and No. 23) showed unilateral ureteral dilatation, one case containing numerous calculi free in the ureteral lumen. Close examination showed the calculi were of 2 types, one white and semi-translucent, the other yellow brown. In Case 23, one ureter was dilated to about 8 mm. diameter (normal 3 mm.), about 4 cm. from the left renal hilus, over a distance of 2 cm. A very mild associated inflammatory reaction was seen at this site.

The bladder was normal in all animals, except one with a slight degree of haemorrhage over a few mucosal folds, and contained no calculi. The urethra was normal in all cases. In these lambs the appendage was adhesed along most of its length. In one animal the vermiform appendage was absent and appeared to have sloughed off. In 3 sheep, the maximum free length was 5 mm.

9. Clinical Histories of 3 Animals with Obstructive Urolithiasis (Nos. 23988/24, 32, 34)

These 3 wethers were all aged about 9-10 months at the time of obstruction. All cases occurred within 10 days.

Case 24 : Sheep noticed dull and anorexic on February 17, 1964, with pendulous abdomen. Temperature was normal, no tenesmus. Animal died the following day. Pathological features are given in Table 27.

Case 32 : Sheep was noticed to be dull on February 17 (i.e. as Case 24). Partial anorexia persisted until death. Paracentesis on February 18 revealed presence of abdominal urine. Penis could not be extended due to adhesion of appendage to prepuce. On using manual force to break down adhesion about 3 ml urine spurted through the urethral orifice. Blood urea was 209 mg% (February 19) and the animal was destroyed 2 days later as, when the animal was placed in a metabolism cage, it could be ascertained that urinary obstruction was complete.

Case 34 : Sheep was apparently normal when put into metabolism cage on February 26, but was dull the following day, though appetite was still good. On February 28, animal was dull, recumbent, markedly dyspnoeic, groaning, with severe tenesmus. The animal was destroyed at midday the same day.

Table 27 : Pathological Details of 3 Clinical Cases of Fatal Urinary Obstruction (23988/24, 32, 34)

Abdominal urine (ml)	4500	5000	Nil
Abdominal calculi	Yes	Yes	No
Site of urinary tract rupture	Bladder: right dorsal wall 1.5 x 0.5 cm	Bladder: right dorsal wall 0.5 x 0.2 cm	Urethral appendage base laterally 2 sites of 1 mm
Kidneys	Enlarged flabby No calculi	Mild congestion. No calculi	Swollen peripelvic oedema. Left cortex showed multiple haemorrhages. Bilateral calculi
Ureters	Normal	Normal	Congestion
Bladder	Moderate localised haemorrhagic cystitis	Severe haemorrhagic cystitis*	390 ml urine; severe generalised haemorrhagic cystitis
Urethra	Mild urethritis whole length	Mild urethritis extrapelvic urethra	Congestion and urethritis distally
Remarks	Calculi only recovered from abdomen	Single calculus obstructing base of appendage acute uraemic syndrome	Single calculus at tip of appendage; enlargement and congestion of all abdominal lymph nodes; Peyer's patches; hepatic haemorrhages; Intestinal mucosal petechiae; fibrinous pleurisy; acute uraemic syndrome.

* Illustrated in Plate 1

Discussion

This experiment showed that neither the levels of concentrate feeding nor the quantity of roots had a significant effect either on the incidence of renal calculi or obstructive urolithiasis. These findings are in disagreement with those of others (Michael, 1910; Newsom et al., 1943; Nairn, 1939; Pontius et al., 1930; Crookshank et al., 1967) but often few parameters were measured by these workers.

The course of clinical disease (obstructive) was short and the clinical signs were relatively non-specific. Only one animal was seen to strain. An interesting and common feature was the severity of the reaction in the bladder, in the absence of gross calculi to cause trauma to the mucosa. This reaction is attributed to the generalised reaction and uraemic condition. The degree of urethritis was less than that commonly seen in natural cases of urolithiasis. Some natural cases of urinary obstruction, with bladder rupture, fail to reveal the presence of urinary calculi anywhere in the urinary tract (Weaver, 1969) but it is incorrect to assume, without careful examination of the abdominal cavity, that urolithiasis is not the cause. Case 24 demonstrated that calculi may be recovered from the abdomen. It is assumed that these calculi were in the bladder until the time of rupture. The site of the uretero-vesicular orifice may also tend to promote the dispersal within the abdominal cavity of any calculi situated in the bladder neck, following rupture of the organ.

The 3 cases of typical obstructive urolithiasis occurred within a period of 10 days in the total 115 days of concentrate feeding, and all fell ill after a cold spell, suggesting the possibility of climatic stress as a predisposing factor (Newson, 1938; Udall, 1959b).

This experiment shows that calculi can readily develop in animals fed small quantities of concentrates and roots, and suggests that excessive emphasis may have been placed on the dangers of excessive feeding of these feedstuffs. In all groups (except Group 1) some calculi were of such size - 3 mm long - as to have blocked the vermiform appendage, had they passed out of the kidneys.

Concentrate feeding was also associated with an increase in blood urea, but the differences are not significant statistically, when compared with each other or with the control values on hay feeding. The greatest mean increase occurred in those groups fed the largest amount of protein, the expected result. The markedly better growth rates on high protein feeding are likewise as expected.

The plasma mineral changes were unremarkable. The dietary intake in respect of minerals was above the minimal estimated requirement in all groups. The significant increase in plasma phosphorus is attributed to the low values obtained during hay feeding, when the phosphorus balance was probably negative. There was no evidence of any "protective" effect of a high calcium intake (Crookshank et al., 1967).

The figures clearly indicate that, ignoring the root consumption, high concentrate feeding was associated with a significantly higher water intake per day, and this was true even when the figures were adjusted to millilitres of water per kg bodyweight. A comparison may be made with American figures for the normal water intake and urinary excretion of 66.8 and 27.1 ml/kg respectively (Anand and Parker, 1966) in this case equivalent to approximately 1675 and 675 ml. In this experiment, intake of water was much lower than the American normal values, while urine excretion was slightly lower. The proportion of the water intake excreted as urine was higher.

The quantity and character of the urinary sediment bore no relation to the intake of concentrates, but appeared to be larger in animals with renal calculi (mean values, statistically no significance). No explanation can be found for the lack of correlation between a high urinary phosphorus and a large quantity of urinary sediment which appeared to be composed principally of phosphate crystals. Cornelius et al. (1959) found the urinary sediment considerably greater in their animals fed a high concentrate mixture than when fed alfalfa hay. Jean-Blaïn et al. (1968) refer to the presence of urinary sediment as a clinical syndrome per se ("sedimentose"), and prefer this term to use of "urolithiasis", and their microphotographs of urinary deposit, as well as those of Rumanian workers (Adamesteanu et al., 1969) are very similar to the appearance of deposits in this experiment. Jean Blaïn et al. (1968) claim that the sediment is almost entirely mineral, with a minor cellular component, and find the material distributed widely throughout the urinary tract. In this experiment, material was localised to the kidneys, though the occasional evidence of ureteral oedema and the sloughing of the urethral appendage suggests that this material may have been partly excreted prior to the slaughter date and could have been encountered elsewhere in the lower urinary tract.

The variable colour of the renal calculi was not investigated by microanalysis or other techniques. The white semi-transparent calculi may contain relatively more crystalline material or may be bathed in less concentrated urine than the yellowish calculi.

The increase in blood urea values in this experiment reflect the increased protein intake and consequential increased nitrogenous breakdown. In no instance was the level pathologically increased, but it is known that a considerable volume of kidney substance must become non-functional before this change is reflected in blood urea levels. If calculi caused physical blockage of a kidney by occlusion of the pelvic ureteral opening, leading to hydronephrosis, the second kidney could, by itself, maintain normal renal function.

(v) Experiment 4

This experiment was designed to determine the effect of a variable calcium intake on the incidence of urolithiasis. The number of variable factors was reduced: calcium was fed at 3 levels in concentrate food, and while 3 groups received a similar daily intake of concentrate, a fourth group received a small quantity of the low calcium concentrate, and served as a control.

To avoid accidental production of an abnormally high "low Ca" mixture, test mixings were first undertaken. The ingredients were initially the same (linseed cake 20%, oats 30%, maize meal 30% and flaked beans 20%), but the Ca figure could not be brought below 0.40% on a dry matter basis. With the maize reduced to 10% and oats correspondingly increased, the figure was 0.33%. Finally, barley was substituted for linseed cake, with an anticipated loss of palatability, but giving a Ca value of 0.20%.

Materials and Methods

Forty-two Blackface wether lambs (25849/1-42), aged about 5 months, weight 18-28 kg, which had been at grass all their lives, were obtained from a hill farm.

The lambs were housed for an initial control period of 54 days, during which they received meadow hay to appetite. Bedding was sawdust.

Blood samples were taken after 3 weeks, and subsequently every 2 weeks, and plasma Ca, Mg and P were determined. Blood urea values were determined initially and at monthly intervals thereafter.

During the control period, 20 lambs were placed in metabolism cages. Three days' acclimatisation were followed by 3 days' collection of urine and faeces. At the same time, water intake was measured.

After 54 days, the sheep were re-weighed and divided into 4 groups of 11, 11, 10 and 10, of similar weight distribution (average 22 kg). The feeding of concentrates is as given in Table 29. The pelleted (3/16") mixture had a crude protein content of 12%. Analysis of the concentrate (4%moisture) and timothy hay (20% moisture) on a dry matter basis (%) is given in Table 28. Calcium supplementation was with ground limestone. Concentrate feeding was started at 250 g daily and increased slowly to the calculated 1000 g daily in Groups 1, 3 and 4, on day 40. Timothy hay was fed to appetite

(Group 1 : 200 g; Group 2 : 900 g; Group 3 : 200 g; Group 4 : 200 g), and there was always free access to fresh water.

Table 28 : Analysis of low Calcium Concentrate Feed and Hay (% Dry Matter Basis)

	Concentrate		Hay	
Ca	0.20		0.13	
P	0.31		0.13	
Mg	0.18		0.14	
Na	0.37)	0.79	0.05)	0.36
Cl	0.42)		0.31)	
K	2.4		2.1	

During concentrate feeding metabolic cage studies were made as in the control period. The animals were killed on day 94 of concentrate feeding. Post mortem examination was as in previous experiments.

A note was made of the degree of separation of the urethral appendage. The internal diameter of the appendage was measured in 20 sheep using low power magnification and again recorded after forced irrigation with water, compared with findings 86 days previously.

Anaerobic urine samples were collected from the bladder by direct puncture using oiled syringes and transferring the sample to a universal bottle, and kept under mineral oil.

Results

1. Mineral Intakes: The mean and maximum daily intake of Ca and P are given in Table 29.

Table 29 : Mean and Maximum Daily Intake of Ca and P (Hay and Concentrate)

	Mean (Day 0-94)			Maximum (Day 40-94)		
	Ca	P	Ca : P Ratio	Ca	P	Ca : P Ratio
Group 1 : 1000 g low Ca	1.52	2.42	0.6 : 1	2.26	3.26	0.7 : 1
Group 2 : 250 g low Ca	1.67	1.94	0.8 : 1	1.67	1.94	0.8 : 1
Group 3 : 1000g med.Ca	6.90	2.41	2.8 : 1	9.76	3.26	3.0 : 1
Group 4 : 1000g high Ca	25.60	2.25	11.3 : 1	39.26	3.26	12.0 : 1

2. Development of Obstructive Urolithiasis and Incidence of Calculi : No lambs showed signs of obstructive urolithiasis during the experiment. At slaughter the appendage was obstructed by calculi in 2 lambs, and in each the urethra had ruptured immediately behind this point. Calculi were found in 30 of the 42 animals and were yellow or brown-coloured when removed from the kidneys but were white when dry. The results of the distribution, size and numbers of calculi in different groups are

given in Table 30.

Table 30 : Distribution, Number and Size of Renal Calculi

	No.	Distribution		Total	Number**			Size**		
		Bilateral	Unilateral		+++	++	+	+++	++	+
Group 1 : 1000 g low Ca	11	3*	4	7	1	2*	4	2	2*	3
Group 2 : 250 g low Ca	11	7	1	8	3	5	0	1	4	3
Group 3 : 1000 g med. Ca	10	5*	2	7	2*	1	4	1	1	5*
Group 4 : 1000 g high Ca	10	3	5	8	1	4	3	2	4	2

* Indicates case of urethral obstruction in Groups 1 and 3

** Number and size categories +++, ++ and + are defined on page 60.

3. Relationship of Lamb Weight to Incidence of Calculi :

There was no relationship between lamb weight and urolithiasis (Table 31).

Table 31 : Comparison of Weights of Animals with Calculi and Animals without Calculi (kg)

	1	2	3	4
With calculi	26 (7)	22 (8)	27 (7)	25 (8)
Without calculi	27 (4)	23 (3)	26 (3)	22 (2)

( ) refers to numbers of animals on which figure is based

4. Plasma Mineral Changes : The initial plasma values were based on 3 samples at fortnightly intervals from each lamb, a total of 126 samples (mean and standard deviation):

	mg%
Phosphorus	7.0 ± 0.4
Calcium	11.5 ± 0.4
Magnesium	1.65 ± 0.29

Plasma phosphorus (Fig. 10) rose immediately on commencement of concentrate feeding and was highest in Group 1, where the mean figure for the last 2 weeks of the experiment was 12.4 mg%. In the other regions the rise was less pronounced and the difference between Groups 2, 3 and 4 compared with Group 1 was significant ($p < 0.05$). Significant differences between Groups 2 and 3, 3 and 4, 4 and 2 were not found. The final phosphorus figure for Group 4 was 8.4 mg%.

Plasma calcium, initially high in the control period, rose further at the first sampling after concentrate feeding to a mean 12.6 mg%, and remained near 11.5 - 13 mg% in the higher calcium groups throughout. However, in Groups 1 and 2, the figure fell only slightly, and at no time were there significant differences between groups. The mean of the different groups in the last month were (mg%):

Group 1 : 1000 g low Ca	10.6
Group 2 : 250 g low Ca	10.4
Group 3 : 1000 g med. Ca	11.45
Group 4 : 1000 high Ca	11.5

Plasma magnesium showed no significant changes in the experiment. Changes in plasma minerals are shown graphically in Fig. 10.

5. Blood Urea : The initial blood urea was 30.2 $\pm$ 6.7 mg% (range 22-43 mg%). The figure remained low in Group 2, while in the remainder increases varied. At the end of the experiment, there was a significant difference ($p < 0.02$) between Group 2 and Groups 1, 3 and 4 (Fig. 10).

6. Water Intake and Urinary Volume : Urinary output varied considerably on concentrate feeding but little during hay feeding (Table 32). The most marked drop in consumption was in Group 2 where the fall was to about 30% of the control level.

When the water intake during hay and concentrate feeding is compared, there is little variation. Groups 1 and 2 showed a slight increase while Groups 3 and 4 had a very slight decrease.

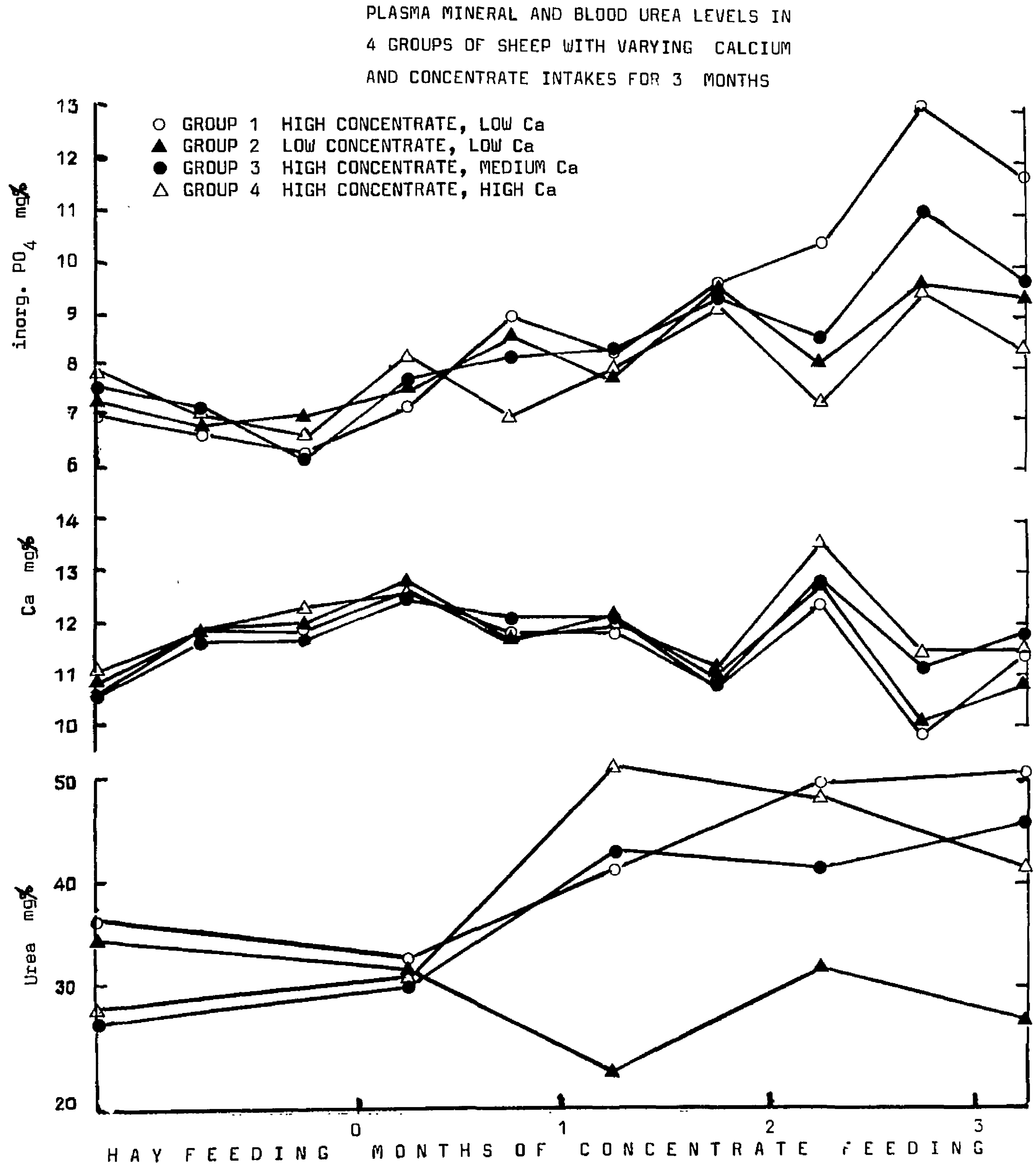


FIG. 10. PLASMA MINERAL AND BLOOD UREA LEVELS IN FOUR GROUPS OF SHEEP WITH VARYING CALCIUM AND CONCENTRATE INTAKES FOR 3 MONTHS (EXPT. 4).

The group intakes of water were estimated over a 5-day period of concentrate feeding to check the normality of water intake in metabolism cages. Groups 1 and 2 had an intake of 900 ml, Groups 3 and 4 990 ml per lamb daily, corresponding well with the value of 873 ml in metabolism cages.

When the urine volume is expressed as a percentage of the water intake (Table 32), the only marked variation was a considerable drop in Group 2.

7. Urinary Phosphorus and Calcium : Urinary phosphorus concentrate increased fourfold on concentrate feeding, compared with control samples from the same animals (Table 32). This increase occurred in all groups except Group 3. Since the urinary volume varied considerably on concentrate feeding, these figures are also inserted in the table and demonstrate that an absolute increase in urinary phosphorus excretion took place in Groups 1, 2 and 4; a decrease in Group 3 was not statistically significant.

Urinary calcium was lower in all groups except Group 4, when concentrate feeding is compared to hay feeding.

Table 32 : Water Intake and Urinary Volume, and Urinary Ca and P Outputs during Hay and Concentrate Feeding

Each group consists of 5 individuals, and each was sampled for 3 days, i.e. 15 samples per group.

		Water Intake ml	Urine Volume ml	Urine Volume / Water Volume %	Urinary P mg%	Urinary P mg/day	Urinary Ca mg %	Urinary Ca mg/day
All Groups	Hay	854	380	45	1.76	6.68	8.3	31.54
	Concentrate	873	300	34	7.6	22.80	6.4	17.20
Group 1 : 1000 g low Ca	Hay	976	358	37	0.99	35.44	11.9	42.60
	Concentrate	1156	453	39	14.2	54.32	6.7	30.35
Group 2 : 250 g low Ca	Hay	757	490	65	2.17	10.63	9.8	48.02
	Concentrate	793	145	18	11.6	16.82	7.0	10.15
Group 3 : 1000 g med.Ca	Hay	857	317	37	2.6	6.59	7.3	23.14
	Concentrate	828	245	30	1.7	4.18	4.9	12.00
Group 4 : 1000 g high Ca	Hay	825	356	43	1.8	6.40	4.0	14.24
	Concentrate	715	357	50	7.23	25.81	6.7	23.91

8. Urinary pH : Values for urinary pH, recorded on 24-hour samples, are presented in Table 33. Consistent results were obtained on any one day, but variations between individuals on different days were marked.

Table 33 : Mean Urinary pH of Wethers during Hay and Concentrate Feeding

Groups	n	Hay	Concentrate
1	15	6.8	7.2
2	15	7.05	6.4
3	15	6.6	7.9
4	15	6.3	7.3

n = number of samples

There was some tendency for the urine to become slightly more alkaline on heavy concentrate feeding.

Anaerobic urine samples were collected by direct bladder puncture at the time of slaughter (4 specimens per group). In all cases the mean value of the group was slightly alkaline (pH 7.05 - 7.6). There were 4 slightly acidic samples, 3 of them being in Group 1. The range of pH values was pH 6.3 - 8.1.

9. Gross Pathology

(a) Urolithiasis : In one animal (25849/2) calculi were found in the left ureter as well as left kidney. The calculi occupied the renal pelvis and the proximal 15 cm of ureter, which was dilated over the same distance, compared with the right ureter. The left and right kidneys weighed respectively 90 and 48 g.

Another sheep (25849/23) with several calculi (2 mm x 2mm) causing urethral obstruction, a urethral rupture posterior to this point, chronic cystitis and cystic calculi, and with numerous calculi in each kidney, showed hypertrophy and slight hydronephrosis of the left kidney, discrete localised haemorrhages in the mucosa of the proximal half of the left ureter and some haemorrhages and inflammatory reaction in the peri-urethral tissues. The bladder and penis are shown in Plate 5. Weight and dimensions of the kidneys were :

	Weight	Dimensions
Left kidney	73 gm	8 x 5 x 3 cm
Right kidney	47 gm	6 x 4.5 x 2.7 cm

A third sheep (25849/32) showed numerous scattered white areas on the cortical surface of the right kidney. The capsule at the anterior pole had adhered to the surrounding fat. On cross-section, the fibrosed areas were seen to extend through the cortex into medulla. This kidney contained no calculi. The left kidney contained calculi but otherwise appeared macroscopically normal.

(b) Kidney Weights in relation to Renal Lithiasis (Fig. 12): In 40 sheep, the weights of the left and right kidneys did not vary by more than 5 g. In 18 animals, the left kidney was heavier, in 15 the right kidney, and in 9 cases weights were identical to within 1 gm. In both sheep with a great discrepancy in renal weight, calculi were found in one or both kidneys. In one case of hydronephrosis (25849/2), the left kidney weighed approximately twice as much as the right kidney, the larger kidney containing calculi. It could not be demonstrated that any discrepancy in weight over 1 g difference was statistically significantly correlated with the presence of calculi.

10. Histopathology: The appendage in the lambs where the organ was occluded by calculi showed superficial necrosis and an extensive area of subepithelial inflammation with

massive invasion by polymorphs and a few plasma cells. Sections of the urethra above the site of rupture showed no abnormality.

Mild lesions of chronic pyelonephritis were found in 9 animals with renal calculi. Active lesions, with no evidence of surrounding fibrosis were found in 2 sheep. Pyelonephritis was also evident unilaterally in 3 sheep without renal calculi.

11. Nature of Vermiform Appendage: When examined initially, every animal had all but 1 - 3 mm of the appendage adherent to the prepuce. At the conclusion (140 days later), in one case of unilateral renal lithiasis the appendage was free along all its length. In another case with no calculi the appendage was free along the distal 10 mm. In a third case (25849/38), the adhesed appendage was dilated to about 3 mm diameter, but contained no calculi. The results expressed in terms of the length of free appendage in millimetres, related to the presence or absence of calculi are given in Table 34.

Table 34 : Length of Free Appendage in Experimental Sheep related to Incidence of Urolithiasis

	Length of Free Appendage (mm)				Entirely Free
	1	2	3	10	
Calculi present	21	4	2	1	1
Calculi absent	8	3	0	1	0

There was no relationship between urolithiasis and the degree of adhesion. In one case the appendage was accidentally removed at slaughter.

The diameter of the appendage was 1.5 - 2 mm internally, and no difference was found between sections from the organ when free and when still adhesed to the prepuce. When the urethral orifice was occluded and when the organ was forcibly dilated by irrigation of water by a syringe inserted in the more proximal urethra, the effective internal diameter increased to about 3 mm in the body of the appendage, but remained about 2 mm in the most distal section.

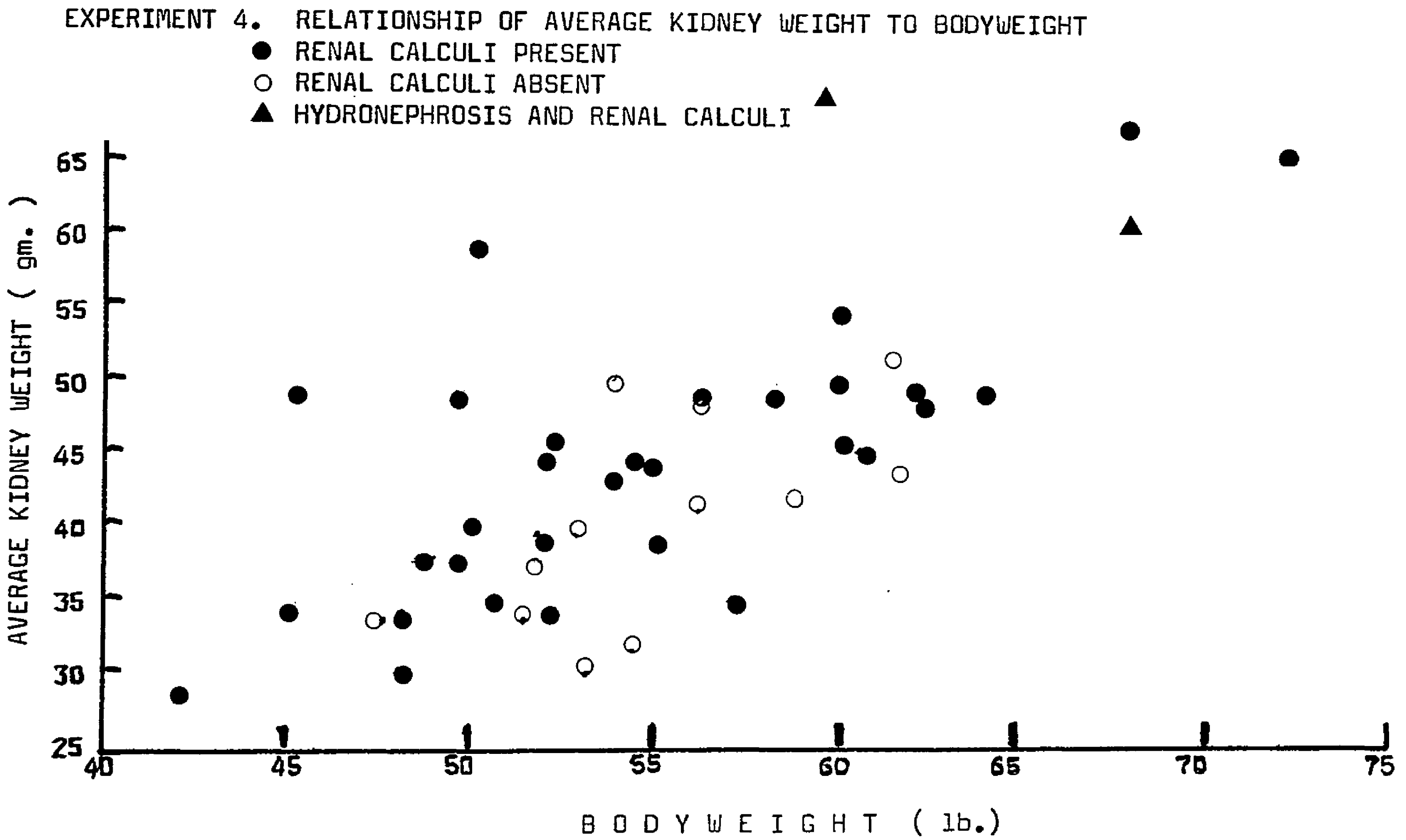


FIG. 12. RELATIONSHIP OF AVERAGE KIDNEY WEIGHT TO BODYWEIGHT, WITH REFERENCE TO THE PRESENCE OF RENAL CALCULI. KIDNEY WEIGHT (gm.) IS AVERAGE OF LEFT AND RIGHT KIDNEYS; BODYWEIGHT IS AVERAGE OF LAST 2 WEIGHINGS (EXPT 4)

Discussion

The dietary regime achieved a wide variation in daily calcium intake (1.52-25.60 g), and a similarly variable Ca : P ratio (0.6 : 1 - 11.3 : 1), these figures including the hay. The mineral intake of Groups 1 and 2 (unsupplemented by ground limestone) was below the stated nutrient requirements of calcium and phosphorus - 2.9 and 2.6 g (Agricultural Research Council, 1965). Conversely, the highest Ca intake was 8 times the recommended requirement while the phosphorus level remained at the lower limit.

Though no cases of fatal obstructive urolithiasis developed, 2 animals had obstruction of the urethra but were not seen with any clinical signs and were detected only at *post mortem* examination. This evidence of passage of calculi from kidneys in 2 animals, together with the high incidence of calculi in all groups (30/42 = 71%) suggests that the feeding in all groups was potentially liable to lead to the development of obstruction.

The data on the size and number of calculi in different groups fails to reveal any significant difference, but confirms that a low Ca, low P diet is not innocuous in relation to urolithiasis.

Based on the measurements of the diameter of the appendage there existed in all groups animals with calculi of a size sufficient to occlude the organ. From this experiment no conclusion can be reached as to <u>why</u> further obstructive cases did not develop. Some possible explanations may be put forward. Calculi may have broken up during movement from the kidney to the bladder and urethra. Calculi may sometimes have redissolved in the bladder urine. Their movement down the urinary tract may not have been accompanied by the type of microtrauma likely effectively to reduce the internal diameter of the urinary pathway. Movement of the animals may never have been so violent and prolonged, so as to provoke a migration of several calculus particles simultaneously down one ureter, or simultaneously from the bladder neck down through the urethra.

Weight gain was negligible in the low concentrate group, but no clinical evidence of rickets was seen, despite the inadequate Ca intake. It is possible that rickets would have developed if these animals had grown. In those animals given more concentrates the mean weight gain in 3 months was 4.5 kg and was depressed in the high calcium group (3 kg) compared with the others (5 kg). The protein level was adequate for sheep (12%), and the reduced palatability of the mixture may be partly responsible for this performance. Having regard for the poor weight gain, it is unexpected that no difference was seen between calculus and non-calculus sheep.

The plasma mineral figures show the anticipated pattern, with calcium values increased in response to the higher intake, and with a corresponding depression of phosphorus. The marked differences in calcium did not develop until 7 weeks on the variable Ca intake.

The effect of the concentrate diet on water intake was not striking but while intake varied little, the urinary volume, expressed as a percentage of the intake, was lowest in these sheep given little concentrates.

The urinary pH figures are subject to criticism in view of the possibility of pH changes during the 24-hour period before estimation. The samples collected anaerobically at slaughter showed the pH values in which the calculi formed in this experiment – magnesium ammonium phosphate – would remain out of solution, i.e. a predominantly alkaline reaction.

The histopathological and gross pathological findings are interesting in that pyelonephritic lesions were of variable chronicity and the majority were not active, none had affected widespread areas of renal tissues, and there was therefore no effect on renal function as judged by measurement of plasma urea. The distribution of pyelonephritic lesions suggested no direct relationship with renal calculi. The significance of pyelonephritis is discussed further in the general discussion.

The occurrence of ureteral calculi in sheep is rare and tends to be overlooked in favour of the obstructive syndrome involving the appendage, and the collection of calculi in the renal pelvis and bladder. Diagnosis in the live sheep of ureteral calculi is difficult if not impossible, and it is not possible to prove that in case 25849/2 calculi were present in the left ureter in life, and did not move down the proximal ureter from the renal pelvis at the time of slaughter. Supporting evidence that the ureteral calculi were present in life is (a) that a degree of hydroureter was also seen and (b) that hydronephrosis was clearly evident. It is, therefore, hypothesised that the calculi caused a partial ureteral urinary obstruction with hydroureter and hydronephrosis as consequences. The degree of hydronephrosis tends to indicate that the condition had persisted some weeks.

The abnormal right kidney of case 25849/32 is an example of chronic interstitial nephritis which is a frequent incidental finding in otherwise normal sheep and, as in this case, not necessarily associated with the presence of renal calculi.

The range of kidney weights was wide (27 g - 90 g) with the majority (77/84) falling in the 30 - 55 g range. The 2 kidneys were usually very nearly the same weight, but, while discrepancy was associated with hydronephrosis or renal lithiasis, many kidney pairs of similar weight also contained calculi.

Since it has been suggested that the prepubertal adhesion of the vermiform appendage to the prepuce proper may be significant in causing obstruction by a reduction of the urethral diameter (Belonje, 1965a), the length of free (i.e.

unadhesed) appendage was recorded for each animal at slaughter. No consistent pattern was found between the degree of appendage adhesion and the particular calculus status. Since few animals had any significant length of the organ separated from the preputial epithelium, comparison is difficult between those animals in which the appendage was wholly free, and those in which part was still attached. The suggestions of Johnson (1948) and Belonje (1965a) that urolithiasis might be more liable to develop in wethers, where separation of the appendage occurs later than in rams, cannot be supported by this experiment. Belonje (1965a) further agreed with the suggestion of Wiggens and Terrice (1953) that live-weight gain plays an important part in the separation of the appendage and that separation was almost invariably complete by the time animals reached 27 kg live-weight, but these observations referred to ram lambs only. Belonje (1965a) found complete fusion of the appendage in all wethers, irrespective of age and weight which had been castrated at 3-6 weeks old. Again, these South African findings on Merino and German Merino sheep cannot be directly compared with Scottish Blackface wethers, and further work may be indicated to follow the precise pattern of breakdown of the adhesions in local breeds.

(vi) Experiment 5

The aim of this experiment was to determine the effect on the incidence of urolithiasis in tup lambs of (a) hay feeding and (b) hay plus varying levels of a similar concentrate mixture to that in Experiment 4.

Material and Methods

Forty-two normal Blackface tup lambs, aged 9 months, were obtained from 6 hill farms in the Newton Stewart area of Kirkcudbrightshire. Weights ranged from 22 to 40 kg.

Clostridial vaccine was injected initially and repeated after 1 month. Vitamin A and D were injected at monthly intervals. All sheep received Methyridine 90% solution (Promintic, I.C.I.) at commencement, and Thiabenzole (Thiabendazole, Merck, Sharp and Dohme) 3 months later.

The sheep were selected at random and placed in 4 groups. All the animals received meadow hay ad lib. for 135 days. After this time, 3 sheep were selected at random from each group of 11, 11, 10 and 10 animals and slaughtered. The urinary tracts were examined for calculi.

During the 135 days, 31 sheep were affected with acute bronchopneumonia. Five sheep died in the period day 67 to day 69. All sick animals received antibiotic treatment. Examination of sheep post mortem revealed a typical pasteurella pneumonia. Five animals of a similar type were purchased in Glasgow Market to replace the dead sheep.

After the period of hay feeding (135 days), a concentrate mixture and hay was fed for a further 96 days, as follows :

Group 1 : ad lib. low Ca concentrate + hay to appetite
Group 2 : approx. 250 g low Ca concentrate + hay to appetite
Group 3 : ad lib. medium Ca concentrate + hay to appetite
Group 4 : ad lib. high Ca concentrate + hay to appetite

All animals were killed after 96 days on this diet.

During the course of the diet, metabolic cage studies were made on 2 sheep from each group, selected at random. The animals were allowed to become acclimatised for 10 days before the studies, which continued for 4 days, were carried out.

Results

A. Hay Feeding

1. Feed and Mineral Intake : Hay consumption in all

groups was about 1600 g per head daily. Since the analysis was Ca : 0.15%, P : 0.13%, with a 20% dry matter content, intake was 1.92 g Ca and 1.67 g P. Mean weight at the start was 33.6 kg and at the end was 33.2 kg.

2. Development of Obstructive Urolithiasis and Incidence of Calculi : In the period of hay feeding alone (135 days) no deaths resulted from urolithiasis. However, 7 of the 12 sheep had renal calculi at *post mortem* examination (58%). The distribution and number of calculi are shown below in Table 35.

Table 35 : Distribution, Size and Numbers of Calculi after Hay Feeding

Number of animals		12
Number with calculi		7
Distribution :		
Bilateral		3
Unilateral		4
Calculi :		
Number* :	+++	1
	++	5
	+	1
Size* :	+++	3
	++	1
	+	3

* Number and size categories +++, ++ and + are given on page 60.

The amount of calculus material varied considerably. One sheep had a solitary 1-2 mm long calculus at the proximal end of the ureter; while another had over 100 small calculi in various calices of both kidneys.

3. Plasma Mineral Changes (Fig. 13) : Initial values on hay feeding (mean ± S.D.) were : Ca 11.41 mg% ± 0.77; Mg 1.96 mg% ± 0.52; P 7.62 mg% ± 0.91.

After hay feeding, and immediately before concentrate feeding started, values were : Ca 10.64 mg% ± 0.50; Mg 1.74 mg% ± 0.49; P 7.62 mg% ± 0.88.

The differences between levels before and after hay feeding were not significant.

Water consumption and urinary volume were respectively 1788 ml and 512 ml daily. Urinary volume was thus 28% of the water intake. If the water content of hay is included, total fluid intake was 2100 ml.

4. Kidney Size : Renal size was closely related to body-weight. Renal weights ranged from 34 to 84 g. At slaughter of 12 sheep after hay feeding, 4 cases without calculi had identical weights (recorded to 500 mg) of left and right kidney,

and one case differed by 1 g. In the 7 calculus cases, the differences in weight were 0.5 g (3 cases), 1, 2, 4 and 28 g. The details of the last case were :

Right kidney : 84 g (hydronephrosis, ureteral calculus)

Left kidney : 56 g (hydronephrosis, no calculi).

No other gross abnormalities were found in the kidneys of the 12 sheep after hay feeding.

5. Renal Pathology : With one exception, the kidneys of the sheep without renal calculi showed no abnormality (7, 17, 39, 44). Sheep 30, though macroscopically normal (left and right weights and dimensions respectively 38.5 g., 38.0 g, 6.5 x 4.5 x 3.5 cm - both), showed on microscopic examination of the left kidney a mild widespread cellular infiltration in the cortical interstitial tissue and in the right kidney an acute cortical interstitial nephritis with abscessation and necrosis, also hyperplasia of cortical arteries with proliferation of the endothelium and perivascular cuffing. No intratubular abnormalities were seen.

The calculus-containing sheep were divided into 4 showing microscopic kidney changes, and 3 which were normal, except for the presence of calculi. The abnormalities were as follows: one sheep (25) showed gross and microscopic evidence of hydronephrosis. The thickness of pelvis, medulla and cortex were 2.5 cm, 0.5 cm and 1.5 cm (compared with normal values of about 1.5 cm, 1.0 cm and 1.5 cm), evidence of pelvic dilation and loss of medullary substance. The hydronephrotic lesion was bilateral, but calculi were confined to the right kidney. No lower urinary obstruction was found to account for the hydronephrosis. One sheep (10) showed mild glomerulonephritis, another (3) slight cortical tubular degeneration which could have been attributed to mild post mortem decomposition, and the last (14) showed a subcapsular (cortical) interstitial nephritis, with 2 areas of polymorph invasion, in the right kidney. The left kidney appeared normal.

B. Concentrate Feeding

1. Feed and Mineral Intake : The daily average quantities of concentrates consumed in the total period was less than in the last 4 weeks of the experiment, as below :

	Day 0 - 96	Day 68 - 96
Group 1 : *ad lib*. low concentrate	1000 g	1360 g
Group 2 : 250 g low concentrate	260 g	260 g
Group 3 : *ad lib*. med. concentrate	950 g	1700 g
Group 4 : *ad lib*. high concentrate	1050 g	1950 g

Hay consumption was very low in Groups 1, 3 and 4, about 400 g daily. In Group 2, consumption was 1400 g daily.

Daily Ca and P intakes for individuals in different groups were as follows :

	Group 1	Group 2	Group 3	Group 4
Ca	3.5	5.7	10.7	41.4
P	4.2	3.7	3.6	3.7
Ca : P ratio	0.8 : 1	1.5 : 1	2.9 : 1	11.2 : 1

During the last 28 days, the daily Ca and P intakes were increased as below :

	Group 1	Group 2	Group 3	Group 4
Ca	4.3	5.7	18.0	75.5
P	5.4	3.7	5.9	6.3
Ca : P ratio	0.8 : 1	1.5 : 1	3.0 : 1	12.0 : 1

2. Development of Obstructive Urolithiasis and Incidence of Calculi : Obstructive urolithiasis developed in 3 cases in 2 of which the condition was fatal. Clinical details are given later (p.179).

There were renal calculi in 20 of the 25 sheep at the end of concentrate feeding so that the incidence of calculi was 81%. The distribution and character of calculi are detailed in Table 36, including also the 2 fatal cases.

Table 36 : Blood Urea (mg%) and Distribution, Size and Number of Calculi after Concentrate Feeding

		Group 1	Group 2	Group 3	Group 4	Total
Blood urea (mg%)		31.8 ± 10.7	32.6 ± 8.4	41.9 ± 6.4	32.0 ± 7.8	
No. of animals		7	5	8	7	27
No. with calculi		6	5	5	6	22
Distribution :						
Bilateral		4	3+	3*	5*	15
Unilateral		2	2	2	1	7
Calculi :						
Number** :	+++	1	2	2	3	8
	++	2	3+	1*	2*	8
	+	3	0	2	1	6
Size** :	+++	0	1+	3*	2	6
	++	2	1	0	2*	5
	+	4	3	2	2	11

* including fatal obstructive case

\+ including non-fatal obstructive case

** number and size categories +++, ++ and + are given on p. 60.

3. Relationship of Weight to Urolithiasis and Weight Gain

(a) There was no relationship between lamb weight and the presence of urolithiasis. At the conclusion, 5 lambs without uroliths had gained (mean and S.D.) 12 $\pm$ 2 kg (26 $\pm$ 5 lb), 20 lambs with uroliths weighed 12 $\pm$ 4 kg (26 $\pm$ 8 lb).

(b) Mean weight gain and S.D. of groups after concentrate feeding were as follows :

Group 1	14.5 $\pm$ 2.1 kg
Group 2	8.6 $\pm$ 1.5 kg
Group 3	12.7 $\pm$ 1.4 kg
Group 4	12.7 $\pm$ 1.8 kg

The increase in weight of Groups 1, 3 and 4 was significantly greater than that of Group 2 ($p < 0.05$).

4. Plasma Mineral Changes : After starting concentrate feeding there was rise of the mean phosphorus of all groups. Excluding one animal subsequently killed for unthriftiness (see later), values ranged from 6.1 - 9.6 mg% (mean 7.99 mg%) after the first week, and 7.7 - 11.5 (mean 9.4 mg%) after 2 weeks, and remained slightly elevated throughout. There was no differences between groups or between urolithiasis and non-urolithiasis sheep.

Calcium values showed no significant changes. Figures ranged from 10 to 12 mg%. In the 2 high Ca intake groups, there were occasional high values about 13 mg% but at the conclusion of the experiment Groups 1, 2, 3 and 4 had respective means of 11.6, 11.4, 12.2 and 11.9 mg%. Again there were no significant differences between urolithiasis cases and others.

Plasma magnesium showed considerable irregularity and no consistent pattern was detected.

5. <u>Blood Urea</u> : The mean urea ($\pm$ S.D.) at the commencement of the experiment was 18.3 $\pm$ 7.4 mg%. At the conclusion the figures showed no significant differences between groups (Table 36).

6. <u>Water Intake and Urinary Volume</u> : The values given in Table 37 indicate that the water intake was very similar on hay and on concentrate feeding, and likewise only minor differences were noted in the urinary output.

Table 37 : Water Consumption and Urinary Volume during Hay and Concentrate Feeding (ml/day) : Mean Values

Group	Control (Hay)	Groups 1	2	3	4
Water	1788	1964	2086	1836	1549
Urine	512	582	587	526	482
$\frac{\text{Urine Volume}}{\text{Water Volume}}$ %	28	30	28	28	41

If the urinary volume is expressed as a percentage of the water intake, very little difference is observed between groups, or between 2 types of feeding.

However, if the figures are expressed on a basis of body-weight, animals on concentrate in Groups 1, 2 and 3 drank significantly less than when they were on hay feeding ($p < 0.05$). The difference for Group 4 is not significant. Considering the urine volume on the same ml/kg basis, the difference between urine volume on hay and concentrate feeding is significant for all groups ($p < 0.05$).

7. Haematology : At slaughter, blood samples were also collected in E.D.T.A. tubes for estimation of haemoglobulin, packed cell volume (PCV), total and differential white cell count.

Of the 25 samples, 3 clotted, 17 were from urolithiasis-containing animals and 5 from negative animals. There was no significant difference in the Hb concentration, PCV, total or differential white cell counts between the 2 groups (Table 38).

Table 38 : Hb Concentration, PCV, Total White Cell and Differential Counts of 17 Urolithiasis Animals and 5 Negative Animals

	Urolithiasis +ve (17 animals)	Urolithiasis -ve (5 animals)
Hb (mean and range) g/100 ml	11.5 (11 - 13)	13.0 (11 - 15)
PCV %/100 ml	34 (29 - 38)	39 (34 - 46)
Total WBC/cu.mm	9.5 (6 - 17)	9 (6 - 13)
% neutrophils	28 (1 - 73)	22 (16 - 33)

8. Examination of Urine at Post Mortem : Sterile samples of bladder urine obtained at slaughter from 8 animals revealed the following organisms on culture :

Staphylococci, coagulase negative (all samples)

B. coli (2 samples)

Non-haemolytic streptococii (1 sample).

None of these isolates were considered likely to be of significance.

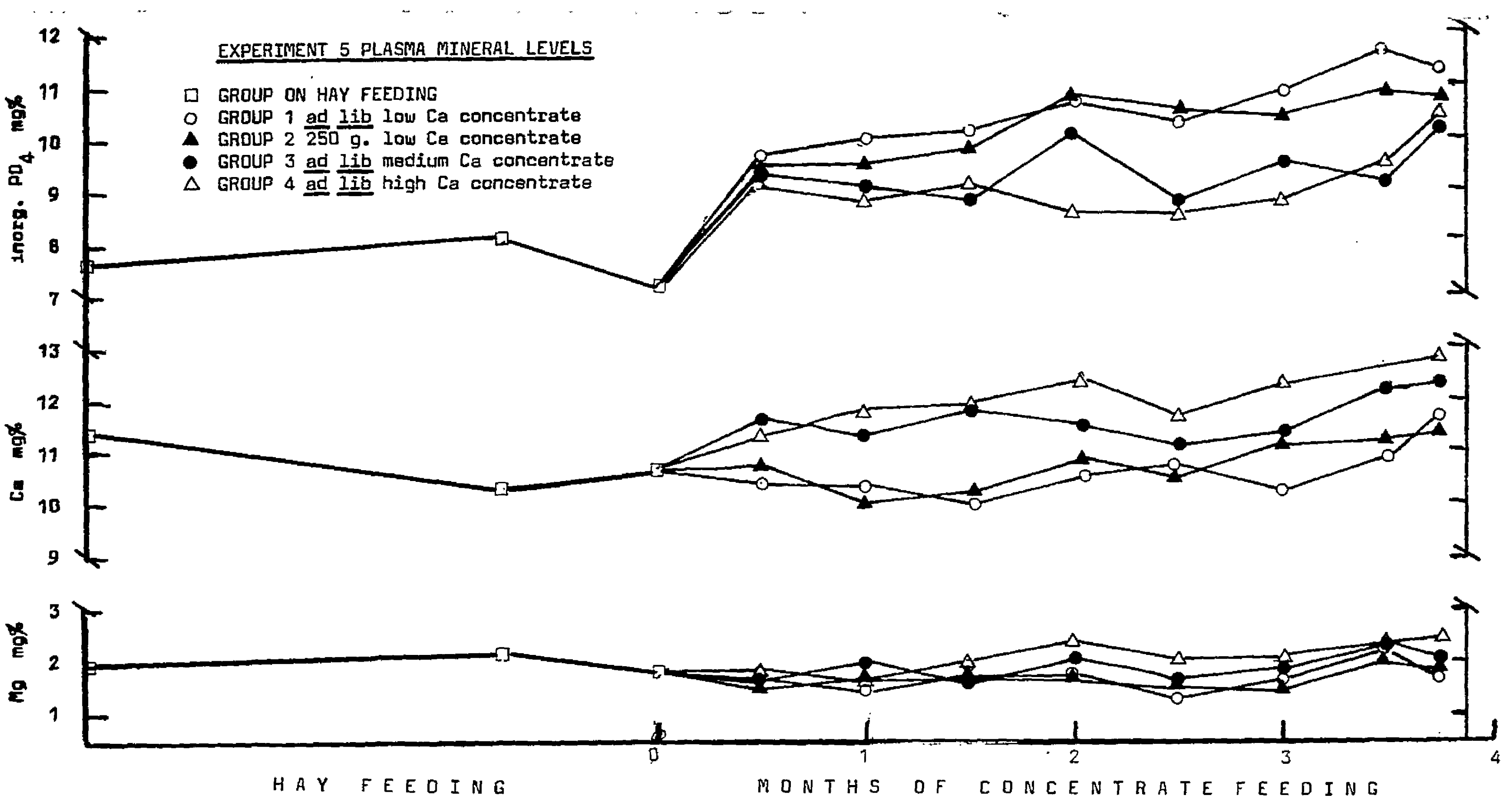


FIG. 13. PLASMA MINERAL LEVELS IN SHEEP ON HAY FEEDING AND THEN TRANSFERRED TO CONCENTRATE FEEDING WITH DIFFERING CALCIUM INTAKES (EXPT 5)

9. Kidney Size : After concentrate feeding, all 6 non-calculus cases varied by 2.5 g or less, while 6 of 19 calculus cases varied by more than this amount. The mean (and S.D.) of the 2 groups was 1.2 $\pm$ 0.8 g and 2.5 $\pm$ 1.8 g. The difference is insignificant.

10. Clinical Features, Pathology and Biochemistry of 2 Obstructive Sheep and 1 Sheep dying of Other Causes :

Case 26695/36 : This animal died suddenly after 39 days of concentrate feeding (Group 3). Death was considered due to uraemia following rupture of the bladder.

Case 26695/23 : This animal was seen to be dull on the 89th day of concentrate feeding (Group 4). The head was held low, the respiratory rate was 60/minute and there was total anorexia. The penis was difficult to extrude and was swollen in the distal 18 cm to a diameter of 5 cm (normal 2 cm). Urine dripped from the tip of the glans, and not from the tip of the vermiform appendage, in the base of which a calculus could be palpated. The animal was still able to stand at 3.30 p.m. the same day and died at 4 p.m. Further details on the pathology of these cases are now given.

Case 36 : Both kidneys showed extensive degenerative changes. The weight and dimensions were :

	Weight (g)	Dimensions (cm)
Right kidney	66	7.5 x 4 x 3
Left kidney	62	7.0 x 4 x 3

Each contained large numbers of calculi up to 3 x 2 mm, occupying several calices, all white in colour. Biochemical and microscopic examination showed that they consisted of magnesium ammonium phosphate (struvite) with little amorphous material.

The bladder wall rupture had led to extravasation of urine dorsally along the left periureteral region to involve the left perirenal area. The left ureter was occluded by an acute inflammatory reaction near the ureteral orifice. The right ureter was dilated and patent.

The bladder showed some discolouration from post mortem change.

The penis appeared relatively normal except for one calculus lodged in the extreme tip of the appendage and one or two smaller, non-obstructing calculi in the penis below the sigmoid flexure. The kidneys and bladder were unsuitable for histopathological examination.

Case 23 : Kidney measurements were :

	Weight (cm)	Dimensions (cm)
Right kidney	175	11 x 7 x 5
Left kidney	180	10 x 7 x 5

The gross swelling of the kidneys was associated with bilateral perirenal oedema and probable urine around the left kidney, from which the capsule was only separated with great difficulty. The outer cortical surface showed many pale yellow specks 1-3 mm in diameter. On section, the left kidney showed similar lesions throughout the cortex. There was a subcapsular fibrinous exudate. Gross hydronephrosis of the left kidney had resulted in the cortex forming a band varying from 7-17 mm in width. The epithelium of the pelvis was bright scarlet. About 6 flaky calculi measuring 2 x 3 mm were recovered (Plate 6).

The right kidney showed early hydronephrosis. The medullary width was 3 times that on the left kidney. There was similar, but fewer nodules. A large infarct was present. A few calculi were visible, the largest being 4 x 4 mm (Plate 7).

The bladder contained 300 ml yellow urine, with a few blood clots and cellular debris, and some particles of sand-like material (i.e. calculi under 1 mm long).

The dorsal surface of the penis was very inflamed in the distal 20 cm and there was an oedematous reaction along the whole pre-sigmoid portion.

Histologically the foci in the renal cortex were abscesses, characterised by the presence of very large numbers of polymorphs in the centre of foci of necrosis. Sections of the mucosa of bladder and urethra showed simple congestion. Table 39 summarises the pathological data of these 2 cases.

Table 39 : Pathological Details of 26695/36 and 26695/23

	36	23
Abdominal urine (ml)	About 1500 (retroperitoneal)	None
Abdominal calculi	None	None
Site of urinary tract rupture	Bladder left lateral wall 2 x 0.5 cm	? left renal ureteral junction
Kidneys	Enlarged, flabby; bilateral calculi	Very enlarged, perirenal Oedema, bilateral calculi
Ureters	Left ureter occluded	Mild dilatation and congestion; ureteral calculi (right side)
Bladder	Localised haemorrhage at rupture site; extensive petechiae	Dilated (300 ml); congestion, frank haemorrhage at neck, calculi
Urethra	Much extravasated blood in lumen below sigmoid flexure; severe congestion	Blood and cellular debris below ischium; glans penis very inflamed, with calculus in appendage

Case 23 : Biochemistry

Biochemical examination of plasma from this sheep on the day of illness, sampled $5\frac{1}{2}$ hours before death, showed the following abnormalities : urea 219.6 mg%, Mg 4.4 mg%, P 4.4 mg%. Other estimations (Ca 11.7 mg%, protein 7.5 mg% and Na 148 meq/L) were considered normal.

Urine taken by bladder puncture immediately after death gave the following values : Ca 2.1 mg%, Mg 8.0 mg%, P 2.6 mg%, urea 1.2 mg%, protein 121.5 mg%.

The urine sample contained large numbers of organisms and a moderate number of red cells.

Case 26695/46 : Death from Other Causes

A further animal (in the concentrate group) was slaughtered on account of unthriftiness. No precise diagnosis was made. The only obvious biochemical abnormality just before slaughter was a very elevated plasma inorganic phosphate of 15.3 mg%, unassociated with a raised blood urea (normal at 16 mg%). The phosphate values of this lamb had frequently been very low and in the 6 previous weeks had been as follows : 4.3, 4.6, 4.0, 9.9, 4.4 mg%. Five of these values were at least 2 mg% below all other animals of the same group.

At *post mortem* examination no cause for the loss of condition was apparent. The viscera appeared normal. Both kidneys contained a few small calculi. The kidneys were dull brown and the pelvis contained some gelatinous material. They were normal on histopathological examination.

11. Gross Pathology: Abnormalities were found in 5 animals on gross examination of the urinary tract (calculi +ve bilateral). The kidneys of no. 6 on section were a slightly mottled grey colour. The capsule separated easily from the cortex. No changes were found on histology.

Sheep no. 16 (calculi +ve bilateral) showed changes in the left ureter which was slightly dilated at the renal-ureteric junction, and the bladder and urethra. The bladder contained a few small calculus particles. The appendage was almost totally obstructed at its tip by a small calculus about 2 x 1 mm. When pressure by a water-filled syringe was applied behind the appendage, water dripped very slowly from the tip (one drop per second), i.e. past the obstruction. The more posterior portion of the appendage ballooned grossly. The bladder contained 300 ml of urine.

Sheep no. 19 (-ve calculi) showed discrete 2 mm diameter yellow nodules distributed evenly throughout the cortex of both kidneys. In addition, there were fine yellow streaks running through the cortex into the medulla. On histology these lesions proved to be pyelonephritic, there being multiple small abscesses throughout the cortex and destruction of collecting nodules with severe necrosis and a neutrophilic reaction.

The bladder of sheep no. 32 contained a few small cystic calculi but there was no reaction in the epithelium; case 33 was similar. Both had renal calculi.

Concentrate Feeding

Sheep nos. 23 and 36 are excluded since their renal pathology is described elsewhere. Four of the 6 sheep which died from pasteurella pneumonia had kidneys which showed extensive degeneration and were unsuitable for histology (20, 29, 31, 34). One of the others (41) contained no calculi but showed an acute pyelitis in the left kidney with a severe localised inflammatory lesion in the pelvic epithelium, neutrophil infiltration and necrosis. The right kidney had an inflammatory exudate in several dilated tubules of the ascending loop of Henle and in the collecting ducts, the principal areas being near the cortico-medullary junction. The kidneys of no. 40 were normal.

Three of 5 animals without calculi had normal kidneys macro- and microscopically (13, 28, 37). One animal (15), though there was no evidence grossly, histologically showed mild hydronephrosis of one kidney with dilatation of numerous collecting tubules. The other animal (19) showed an eosinophilic exudate in several of the larger collecting tubules, with no reaction in the renal substance.

Histological Findings

No histological changes were found in 10 of the 20 sheep. Case 30 showed an acute cortical interstitial nephritis, with abscessation and areas of necrosis. There was also hyperplasia of branches of the renal artery, arterial endothelial proliferation, and perivascular cuffing. A second section of the same kidney showed milder changes confined to the cortex, with some cellular infiltration.

Smaller and more scattered inflammatory foci were found in the cortex of 8 further cases (10, 11, 14, 33, 38, 41, 43, 45). Tubular necrosis was seen in 2 cases (18, 33). Tubular dilatation was detected in 2 cases, unassociated with any other abnormality. Hydronephrosis had not been apparent grossly.

Case 32 presented a calcium or phosphate deposit on the renal papillae of one kidney. This animal also had renal calculi in the same kidney. One case (41) had an acute inflammatory focus on the papillary border with extensive tissue destruction, and another section showed inflammatory exudate in several dilated collecting tubules. This was classified as pyelitis.

Pyelitis and pyelonephritis were seen together in one case (43).

Focal areas of cortical degeneration, unassociated with any inflammatory response were found in case 11, which also showed hyperaemia.

Four animals had prominent deposits of hyaline material in the cortical tubules (6, 9, 12, 19) but most animals had smaller quantities of similar staining material, without evidence of an inflammatory focus.

Discussion

For the first time in this series of experiments an attempt was made to determine whether calculi could be recovered post mortem from sheep which had only been fed hay. It is impossible to state absolutely that these calculi were not present at the commencement of the experiment. Since the calculi are liable to be relatively radio-translucent in sheep, due to the low calcium content, they do not show up well on radiography of the kidney. Any detail is masked by the overlying rumen and its contents, and even starvation fails to give conditions suitable for useful radiographs. Surgical exploration of the renal pelvis in sheep is difficult under any conditions, due to the depth at which the kidneys are located distant from a midline laparotomy wound and due to the intervening rumen. Surgery would be liable to lead to some fibrosis and possible impairment of renal function.

The belief that the kidneys of the sheep in this experiment were free of calculi initially rests on the following evidence. Firstly, the sheep were obtained from various hill farms where they had spent all but the last days at grass. Secondly, none of the supplying farmers had noted any urinary abnormality in any sheep in the groups from which the experimental animals were drawn. Thirdly, the texture of the calculi at slaughter was soft and crumbly, suggesting that they had formed relatively rapidly, i.e. recently. Fourthly, the evidence, previously presented, that few sheep kept outdoors and appearing to be healthy, have been found to have calculi at slaughter, suggests that these sheep were normal sheep in this particular sense.

In a nagative sense, therefore, the appearance of calculi in sheep fed hay only for 135 days suggests that concentrates are not an essential factor in urolithiasis. Though no calculi were found in some cases, some calculi may have formed and have been passed out with the normal urinary flow.

The other observation of possible significance on hay feeding is the renal pathology. As in previous experiments, renal disease was often associated with a difference of weight between the kidneys. One case of hydronephrosis was observed but since calculi were only present in one kidney, the nature of the chronic obstructive lesion is uncertain. Since no mechanical factor, for example stricture or "kinking" of the ureter or an obstruction of the ureteric orifice, was found, it is possible that ureteric calculi may have been involved earlier in the experiment.

Cellular infiltration of the interstitial areas of the cortex may only be detected on histology, and was not associated with the presence of calculi. Renal abnormalities were commoner in those sheep with calculi, but changes were relatively mild. The lesion of glomerulonephritis in one sheep may conceivably have led to later widespread areas of cortical fibrosis and uraemia. The aetiology of glomerulonephritis is obscure in man and animals though hypersensitivity had been suggested to play a role in pathogenesis and experimental models in laboratory animals have involved group A Streptococcal infections (Rammelkamp, 1967).

The results of the second part of the experiment (concentrate feeding) should be compared with those found in the previous experiment, since high calcium intakes, and high Ca : P ratios were a feature of 2 groups. While 2 fatal cases developed from obstructed urolithiasis, the distribution of animals with calculi was apparently quite random throughout the 4 groups, regardless of the Ca intake and the level of concentrate feeding, just as in the previous experiment. The incidence of calculi was slightly higher overall, 81% compared with 76%.

As in previous experiments, no relationship was found between weight gain and the occurrence of urolithiasis, which appears therefore to have no detrimental effect until clinical obstruction, or chronic cystitis (which was not seen in any animal) develops.

The rise in plasma phosphorus is attributed to the considerable increase in the phosphorus intake. The persistently high values are surprising in view of the very high calcium intake sustained for some months, which nevertheless had only a minor, and non-significant effect on the plasma calcium levels.

The water intake on hay feeding was almost double that of the sheep in the previous experiment, and the significance of this is not known. It cannot be attributable only to the slightly larger size of these animals. On concentrate feeding the intake varied little, as in the earlier experiment, and the urinary volumes, expressed as percentages of the water intake showed little variation from one group to another, whereas in experiment 4 the values had shown great changes.

The typical cases of obstructive urolithiasis, which occurred in this experiment, were sudden in onset and it was not possible to follow the biochemical changes from the very onset of obstruction. Before obstruction occurred, there were no abnormalities detected in plasma minerals recorded in the regular sampling procedures.

The presence of microabscesses, and therefore a pyelonephritis, provoked the examination of blood for haematology at *post mortem*, in an attempt to correlate abnormalities in the white cell picture with the presence of calculi, and with lesions of pyelonephritis in these sheep. Though several cases of focal infiltration by polymorphs were found on histology, these were in animals both with and without calculi. The absence of a correlation with the white cell count suggests that these foci were insignificant, as far as the systemic reaction was concerned.

D. RENAL PATHOLOGY

Twenty-five male sheep were examined pathologically in a 5-year period, out of a total of 35 sheep received in the Surgery Department of Glasgow University Veterinary Hospital, and affected with urolithiasis.

The material from 5 animals was unsuitable for histological examination as the animals had died some hours previously and the carcass was partly decomposed. While the general changes, together with the clinical history of these cases, have been published elsewhere (Weaver, 1969), details of the renal findings are given and discussed in relation to the findings in experimental cases of this work. Details of the 20 cases suitable for examination are as follows.

Case 17309

The left kidney was surrounded by an area of gelatinous material, urine and haemorrhage. The kidney showed a severe pyelonephritis with necrotic areas up to 5 mm in diameter, distributed throughout the cortex and medulla. One necrotic area had ruptured to give a communicating tract from renal pelvis to the exterior of the cortex, on top of which was a urine-filled pseudo-cyst, formed due to adhesions produced by a surrounding peritonitis.

The right kidney was similarly affected with pyelonephritis. No renal calculi were found.

(Other findings : severe cystitis; severe urethritis. Ischial urethrotomy 10 days previously).

Case 19545

Both kidneys were grossly enlarged and were affected with severe chronic pyelonephritis. Left kidney weighed 650 g (15 x 11 x 8 cm), right kidney 550 g (12 x 11 x 8 cm). The renal capsule was thickened and firmly adhesed to the cortex. Both cortical surfaces were rough and pitted by areas of fibrosis. Both kidneys showed severe hydronephrosis and attendant pus and tissue debris filled the pelvis. The cut surface of the kidney was dark, mottled by diffuse grey areas with radial streaking. The cortico-medullary junction was ill-defined. Histology revealed severe lesions of chronic pyelonephritis and fresh areas of acute inflammation, necrosis and abscessation alteration in both medulla and cortex.

Case 19597

The kidneys showed early hydronephrosis, the left being surrounded by urine and gelatinous material. Size and weight were not recorded. No intrarenal calculi were found.

Case 21591

The kidneys were very wet and flabby, and no calculi were detected. Macroscopically, the kidneys were normal but histological examination showed nephrosis of the proximal convoluted tubules, together with slight periglobular fibrosis.

(Other findings : moderate haemorrhagic cystitis and urethritis).

Case 21632

Both kidneys showed localised areas of necrosis in the cortex, characterised by foci of neutrophils, congestion and haemorrhage.

(Other findings : acute haemorrhagic cystitis; acute urethritis; acute urethral obstruction).

Case 20353

The pelvices of both kidneys contained numerous small calculi, up to 2 mm long, interspersed between the calyces.

The kidneys appeared congested. Histology revealed areas of haemorrhage in the cortex and pelvis, slight interstitial nephritis and some areas of Von Kossa positive-staining material in the collecting tubules of the medulla.

(Other findings : severe haemorrhagic cystitis, severe urolithiasis with calculi in distal 5 cm).

Case 23273

The left kidney weighed 138 g (10.5 x 8 x 3 cm), the right kidney 132 g (8.5 x 5.7 x 3.5 cm). The left kidney was surrounded by a mass of subperitoneal gelatinous urine. Both kidneys showed early hydronephritis, and were congested. No calculi were found in the kidneys.

Histologically, both kidneys showed severe foci of acute cortical and medullary inflammatory reaction, and there were several areas of infarction. There was marked tubular dilatation and inflammatory debris was noted in several collecting tubules.

(Other findings : acute ureteritis; severe cystitis and bladder rupture, with calculi in urethra).

Case 24746

The left kidney showed slight hydronephrosis and the pelvis contained many (over 40) calculi, the largest measuring 5 x 4 mm. The colour was yellow; the consistency hard.

Both kidneys showed nephrosclerosis with a longstanding inflammatory reaction and organised fibrous tissue in the cortex. Polymorphs and small mononuclear cells were associated with collecting tubules in the pelvis. These findings were indicative of chronic pyelonephritis. Some collecting tubules contained calculi, which were closely associated with and appeared continuous with the flattened, thin and sometimes absent epithelium of these tubules. Nephrosis and hyaline cast formation was present. Pigmentation was seen in the cytoplasm of tubular cells.

(Other findings : organising chronic cystitis and bladder rupture (Plate 8), urethritis and granulation tissue reaction).

Case 24852

The right kidney was ruptured one year previously and was removed. No calculi were detected in pelvis or calyces. The blocks for histology were accidentally destroyed.

The left kidney (128 g, 9 x 6.5 x 3.8 cm) showed a small 2 cm long, irregular scar on the anterior pole. Histologically, there was severe interstitial cortical fibrosis. Many tubules in the area had been destroyed and intact tubules contained protein casts. There was some destruction of glomeruli, with periglomerular and glomerular fibrosis. A slight mononuclear cell infiltration was associated with this localised lesion (Plate 9). The medulla showed slight fibrosis close to the cortico-medullary junction and associated tubules also contained protein casts.

The lesion was classified as chronic nephritis as it was impossible to judge whether the lesions were focal, interstitial or pyelonephritic in character.

(Other findings : bladder normal; urethra moderately congested).

Case 25337

The left kidney (removed 5 days before death) weighed 309 g (11.5 x 9.7 cm) and contained a few small calculi between several calyces. Haemorrhage was present around one calyx. The kidney showed early hydronephrosis, confirmed histologically, and areas of mononuclear lymphocyte and polymorphonuclear infiltration of the cortex (Plate 10), indicative of a mild degree of pyelonephritis.

The right kidney (109 g, 10 x 6 x 3 cm) contained a large recent infarct in the middle of the cortex (Plates 11, 12). Calculus material was present similar in character to the left kidney. Bacterial culture from the right kidney was negative.

(Other findings : localised haemorrhagic cystitis; congestion and necrosis of distal urethra).

Case 27345

The left kidney weighed 100 g and the right 90 g measuring about 10 x 6 cm.

Both kidneys were pale, soft, swollen and mottled white, with frank pus in the pelvis. There was bilateral hydronephrosis.

Histologically, both kidneys showed diffuse necrosis of both cortical and medullary tissue, and a granulocyte and mononuclear cell infiltration, especially in the medulla. There was some post mortem decomposition. Some renal collecting tubules were dilated and contained casts, which were mainly eosinophilic in nature, but which sometimes contained refractile material of definite crystalline structure, resembling calcium crystals. Von Kossa did not stain this material uniformly black, but some stain was incorporated with this material. Conclusion : severe pyelonephritis.

(Other findings : purulent, almost obliterative urethritis).

Case 27659

The left kidney (180 g) was enlarged to about twice normal size. The right kidney weighed 130 g while both kidneys showed severe hydronephrosis. Pus was present in their pelvices. The cortical surfaces were paler than normal and there were large, irregular, well-demarcated paler areas of fibrosis. These findings were indicative of the chronic phase of severe pyelonephritis.

Case 27831

The left kidney weighed 110 g (9 x 6 x 4 cm), the right kidney 108 g (8 x 6 x 4 cm). Macroscopically both kidneys were normal. No calculi were found in the kidneys. Histologically the kidneys were normal.

(Other findings : acute haemorrhagic cystitis and acute urethritis).

Case 28024

The left kidney weighed 82 g (8.0 x 4.5 x 3.3 cm), the right kidney 72 g (7.5 x 4.5 x 3 cm). The kidneys appeared macroscopically normal, except for the presence between the calyces of several grey calculi up to 3 mm long, and slight congestion of the left kidney.

(Other findings : chronic cystitis; urethral congestion and necrosis proximally).

Case 30465

The left kidney weighed 85 g, the right 81 g, and each measured 9 x 6 x 3 cm. The kidneys showed a moderate degree of hydronephrosis and congestion. Small yellow calculi were found in the pelvis of the right kidney.

Histological examination was not possible.

(Other findings : bladder was normal; distal urethra was very inflamed and necrotic).

Case 30635

The left kidney weighed 64 g (7 x 5 x 3 cm), the right kidney 74 g (8 x 5 x 3 cm). Both kidneys contained a few small yellowish calculi and showed slight hydronephrosis.

Macroscopically they appeared otherwise normal.

Histologically, the kidneys showed with PAS stain bright red masses of amorphous material in tubules, especially proximal convoluted tubules. The material was located in the cytoplasm or in the tubular lumen, never in both simultaneously. Some Von Kossa positive staining material was present, but the PAS material did not stain with Von Kossa.

(Other findings : chronic cystitis and necrotising purulent urethritis).

Case 30644

Pathological examination took place 3 days after death, the dead animal having been kept in a chill. Post mortem degeneration was advanced. The left and right kidneys weighed respectively 76 g and 74 g. The right kidney presented an irregular surface with pale areas of fibrosis. Calculi were present in both kidneys. Histology was not performed.

Case 30710

The left kidney weighed 150 g (9 x 7 x 4.5 cm), the right kidney 101 g (9.5 x 6.5 x 3.5 cm). The right kidney was congested and presented slight hydronephrosis. Measurements were as follows :

	Right Kidney	Left Kidney
Width of cortex	1.0	1.5
Width of medulla	1.5	1.5
Width of pelvis	2.5	2.0

Histologically, there was necrosis of the renal papillae in the right kidney, with a ring of haemorrhage demarcating the necrotic area (Plate 13). There was patchy interstitial fibrosis with little cellular reaction in the cortex. A very severe diffuse fibrosis was present in the cortico-medullary region, with hyaline casts in the tubules (Plate 14). This was classified as focal chronic interstitial nephritis. The left kidney was normal histologically.

Case 30859

The kidneys showed a fairly widespread purulent nephritis. Extensive areas of the cortices were pale and in some of these areas, abscess formation was apparent. Additionally, radial streaks of fibrosis were apparent. A mild degree of hydronephrosis was apparent. No calculi were found.

Histologically, the kidneys showed the characteristic features of severe pyelonephritis. The reaction appeared to be longstanding, with areas of chronic fibrosis and other areas where the reaction was extremely acute. The acute changes were extensive with an intense polymorph infiltration of the pelvic mucosa and submucosa by inflammatory cells and areas of fibrosis (Plates 15 - 19).

Case 31041

The left kidney weighed 85 g (8 x 5.5 x 3 cm) and the right kidney 98 g (8 x 6 x 3.5 cm). Left and right kidneys contained respectively about 30 and 5 yellow calculi, maximum size being 2 x 2 mm. No other renal abnormality was detected macroscopically. Histologically, there were no significant lesions in the kidneys.

(Other findings : bladder had 3 discrete areas of necrosis and superficial haemorrhages; lower urethra was moderately inflamed).

The principal renal lesions are listed with the degree of uraemia, abscessation in the urinary tract, and presence or absence of an ischial urethrotomy wound (Table 40).

Table 40 : The Renal Lesions in 20 Cases of Urolithiasis in relation to the Degree of Uraemia and Abscessation associated with the Urinary Tract and Presence of Urethrotomy Wound

Case No.	Renal Lesion	Uraemia	Abscessation	Ischial Urethrotomy	Renal Calculi
17309	pyelonephritis+++	+++	++	+	-
19545	pyelonephritis+++	+++	+++	+	-
21591	cortical nephrosis	+++	-	-	-
19597	hydronephrosis	+++	-	-	-
21632	cortical necrosis	+++	-	-	-
20353	interst. nephritis	++	-	-	+
23272	pyelonephritis++	+++	-	+	-
24746	chron.pyelonephritis+++	+++	-	-	+
24852	chronic nephritis	-	-	-	-
25337	pyelonephritis+	+++	-	-	+
27345	pyelonephritis+++	+++	+++	+	-
27659	pyelonephritis+++	+	-	+	-
27831	normal	+++	-	-	-
28024	pyelonephritis+	+++	+++	-	+
30465	no diagnosis	+++	-	-	+
30635	hydronephrosis	+++	+++	-	+
30644	atypical pyelonephritis+	+++	-	-	+
30710	focal interst. nephritis	+++	-	-	-
30859	pyelonephritis+++	+++	+++	-	-
31041	normal	+++	-	-	+

N.B. Pyelonephritis lesion is graded + (slight), ++ (moderate), +++ (severe). Uraemia is graded - (none), + (blood urea 80 mg%), ++ (200 mg%), +++ (> 200 mg%)

Discussion

The renal findings in 20 cases of naturally occurring urolithiasis indicate that pyelonephritis, generally severe, affected 10 animals. A further case may have been included but was classified as chronic nephritis as a distinction between focal interstitial nephritis and pyelonephritis was not possible. Other cases proved to have cortical nephrosis and cortical necrosis, and 2 animals only showed hydronephrosis. While the less common lesions may in part be attributable to various disease processes in the kidneys, not least the severe uraemia which was associated with all but 2 cases, further consideration is given to the pyelonephritis lesion.

Classifying the results differently, all 5 animals with a posterior urethrotomy wound had pyelonephritis, while, excluding case 24746 whose classification is doubtful, 4 of the remaining 14 cases had pyelonephritis, and in 2 of these 4 cases the degree was slight (+).

Obstructed kidneys are very prone to infection and therefore to pyelonephritis (Smith and Jones, 1961) and in cattle can result from either an ascending or descending type of infection, while hydronephrosis is a common corollary and is the only lesion mentioned in association with urolithiasis by Beveridge (1942). Jubb and Kennedy (1963) believe that following impaction of urethral calculi, stasis and bacterial growth, inflammation may ascend to bladder, even to kidneys.

No other detailed histological reports have been found, but it seems likely that infection may rather readily spread up from a urethrotomy incision.

From Table 40 it may also be seen that frank abscessation was present in the urinary tract of 3 of 5 animals with a posterior urethrotomy wound. The abscesses were adjacent and ventral to the urethrotomy incision. In 3 further cases abscessation was present, but in a different locus, around the sigmoid flexure and more distal urethra. Abscessation was associated with pyelonephritis in 5 of 6 animals, again suspicious evidence of an ascending urinary infection. Haematogenous spread cannot be ruled out, however, since 4 of the 5 animals with abscessation had shown pyrexia and an increased total white cell count ($>$ 18000/cm mm), suggestive of pyaemic spread.

The known difficulty in urination experienced for several weeks by 3 animals with a posterior urethrotomy wound is further evidence of persistent urinary stasis and the likelihood of chronic cystic distension and chronic ureteral dilation, so that conditions may well have been ideal for the development of an ascending infection.

Bacteriological attempts at isolation of a specific organism from various kidneys showing pyelonephritis were unsuccessful and mixed cultures including B. coli, staphylococci and Proteus species were often obtained.

Turning now from pyelonephritis to the glomerulus, in this series the glomerulus and periglomerular area remained relatively normal. Two cases showed glomerular and periglomerular fibrosis associated with other cortical lesions, but these minor changes contrast sharply with the recent observations of Adamesteanu et al. (1969), who have claimed that glomerulonephritis was the predominant histological feature of their series of 12 sheep. This glomerulonephritis was characterised by glomerular endothelial hyperplasia and accumulation of proteinaceous material in Bowman's capsule, sometimes associated with endothelial desquamation. Their calculi were, as in this work, primarily of the magnesium ammonium phosphate variety. Egoshin et al. (1967) noted primarily an interstitial nephritis in uncomplicated cases. Interstitial tissue could in some cases replace individual glomeruli, and in others, Bowman's capsule became distended with urine as a result of pressure. Textbooks either give no details of microscopic lesions in urolithiasis (Smith and Jones, 1961), or claim that pyelonephritis or interstitial nephritis are the main lesions (Jubb and Kennedy, 1963; Blood and Henderson, 1960). Glomerulonephritis is stated to be rare in animals (Blood and Henderson, 1969; Langham and Hallman, 1941) and the description of a ram with glomerulonephritis by Langham and Hallman suggests that interstitial nephritis was the primary lesion.

As is shown in Table 40, neither glomerulonephritis nor chronic interstitial nephritis was a common lesion in the Scottish sheep. While 2 animals had normal kidneys - though, as in all cases, calculi were found elsewhere in the urinary system - the most common lesion was a pyelonephritis, and it has been hypothesised that in some cases urogenous spread took place from a lower site. It is also possible that some animals had a pyelonephritic lesion before they developed urinary calculi, and that the pyogenic process flared up as a result of further renal damage, for example, the development of a hydronephrosis. It is evident that there is no evidence for a common pathological lesion in this genis, and that even renal calculi do not form a constant feature of sheep with urolithiasis, when seen at post mortem examination.

E. COMPOSITION OF CALCULI

Results

In both experimental and natural cases of urolithiasis, the composition was principally of phosphate, namely magnesium ammonium phosphate (struvite) or magnesium hydrophosphate - trihydrate (or Newberyite) as has been previously reported briefly (Weaver, 1966). The detailed results are given in Table 41. One specimen consisted principally of calcium carbonate, and 2 contained some calcium oxalate.

The results of x-ray crystallography showed that many specimens were non-crystalline. The x-ray photographs showed no sharp lines, but only the diffuse band which is always given by Durofix. It seems the material must be made of some amorphous substance which could not be identified (Lonsdale, 1965).

Identification of 25849/2 gave a weak Newberyite pattern (Newberyite = magnesium hydrophosphate - trihydrate, $M_gHPO_43H_2O$) and P.927 was also Newberyite.

These results are discussed later.

Table 41 : Results of Analysis of Calculi for Various Radicals

Case No.	Ca^{++}	Mg^{++}	NH_4^{+}	PO_4'''	C_2O_4''	CO_3''
17309	+	+	+	+	+	+
19699	+	+	+	+	+	–
19778) 20012)	NS	NS	NS	–	NS	NS
20353	+	+	+	+	NS	–
21591	–	–	+	+	NS	NS
21787	+	–	+	+	–	–
21893	+	+	+	+	–	–
21896	–	+	+	+	–	–
21897	+	+	+	+	–	–
22070	+	+	+	+	–	–
22137	–	+	+	+	NS	–
23272	NS	NS	NS	+	NS	NS
23416	–	+	+	+	–	–
23988/23	NS	–	+	–	NS	–
23924/26	–	+	+	+	NS	–
23951	–	+	+	+	–	NS
28024	+	–	+	–	NS	+
30523	NS	–	+	+	–	–
31040	+	+	+	+	–	NS
33575	+	+	+	+	–	–

Abbreviations : + positive : – negative : NS not sampled.

X-ray crystallograph series (results in text): 23989/26, 23988/11, 25233, 25338, 25849/2, 25849/25, 26311.

P A R T I I I

GENERAL DISCUSSION AND CONCLUSIONS

Major difficulties inherent in this investigation included firstly, the problem of whether ovine urolithiasis under British conditions correspond to that under American conditions, and secondly, the sheer number of possible aetiological factors, the majority of which could not be examined in this investigation.

INCIDENCE

The 1969 report of the Animal Breeding Research organisation gives the latest figures for the British sheep population, which includes about 350,000 rams. It is stated that about 0.01% are liable to die in any one year (while 0.1% are slaughtered). It seems that these percentages represent estimates only, and it is unlikely that only 35 rams die in one year, since figures from insurance companies give much higher numbers, and a higher incidence is also confirmed by local slaughterhouses. If the death rate is raised to 0.04%, there is a hypothetical average of 140 rams dying in Britain in any one year. An annual loss of 12 such animals from urolithiasis would represent a rate of about 7%. The survey carried out among veterinary practitioners indicates that the disease is commonly encountered in Scotland and, judged on the recovery rate in one series (Weaver, 1969), it could be maintained that deaths from urolithiasis in rams may form a small but significant proportion of total ram deaths. The figure for rams denotes breeding animals, and therefore animals over one year old. The majority of affected animals, however, are younger than one year, and the official figures for this population are not available. An explanation is required for the fact that the disease is not even listed in a recent publication of the Ministry of Agriculture (1964) giving an analysis of sheep deaths, 1959-1961. This survey refers to sheep examined at Veterinary Investigation Centres in England and Wales, and thus excluded Scotland, with its relatively higher incidence of the disease in a large sheep population. Since symptoms of this disease are characteristic, it may, in many cases, have been thought unnecessary to have the diagnosis confirmed by a veterinary investigation. Reports from 2 Veterinary Investigation Centres (Cambridge - J. B. Spence, personal communication, 1965; Newcastle - C. R. Findley, personal communication, 1968), have confirmed suspicions that some outbreaks of urolithiasis are investigated in England.

Environmental factors have rarely been studied in ruminant urolithiasis, and climatic factors, for example, temperature and humidity, were impossible to control in the present study. Though not presented among the results, it appeared that the sporadic cases of obstruction seen in these experimental sheep occurred at times when there had been a sharp fall in temperature. This has been a common observation elsewhere but, as confirmed by Udall and Chow (1969), it has been impossible to find a report of an epidemiologic study of urolithiasis in animals to substantiate the observation that the majority of clinical outbreaks occur during the periods of worst weather.

Animals housed exclusively had a significantly higher incidence of cases of urinary obstruction than animals part-housed or pastured, but calculations on figures from clinical cases seen in the Glasgow University Veterinary Hospital have shown no difference between the incidence of obstructed animals housed exclusively or part-housed (Weaver, 1969).

In this series of experiments, all experimental animals were housed, but the size of the pens permitted adequate exercise. In this respect the conditions were different from the majority of American experiments in which the sheep were maintained outdoors, and may, though this is nowhere stated, have been subject to a greater variation in climate (temperatures, humidity, wind). The amount of exercise undertaken by sheep has been suggested (Beveridge, 1942) as a factor important in the occurrence of obstructive urolithiasis, as well as in their formation. Beveridge doubted whether this factor operated in sheep confined to pens, but Nairn's (1939) observations, the only one commenting on Scottish conditions, were completely contrary, since he found that the obstructive syndrome was most important in young rams with limited exercise. The figures given in Part I underline Nairn's observations, so that a rational recommendation in animal husbandry would be the importance of ensuring that all sheep liable to urolithiasis were given exercise by being turned outside for some hours each day.

Little attempt was made to discover on a larger scale the proportion of animals in the population containing urinary calculi, as opposed to developing the obstructive syndrome. Personal reports from other research establishments where housed male or castrated sheep are kept, and where routine *post mortem* examination of the kidneys is carried out, indicate that calculi only occur in a large proportion of animals at risk in occasional experiments (Preston, 1962).

Unfortunately ovine kidneys are not routinely sectioned in the slaughterhouse by the meat inspector, unless there is an obvious external abnormality apparent. It appears unlikely that renal calculi are anything but a very rare finding in normal slaughterhouse material, which consists, it must be recalled, very largely of castrated male lambs or wethers, and ewes, reared on extensive systems.

URINE

The values obtained for urinary mineral excretion in normal housed male sheep fall within the range obtained by most other workers. The figures in experimental production of the disease, involving the feeding of excessive quantities of calcium and/or phosphorus, failed to show the significant increases demonstrated by other workers in urinary phosphorus and magnesium (Lindley et al., 1953; Cornelius et al., 1959; Packett and Hauschild, 1964; Robbins et al., 1965; Packett et al., 1968). There was no indication that estimation of urinary minerals could be used as an indicator for the presence of calculi in an individual, as suggested by Lamprecht et al.(1969).

NON-CRYSTALLINE COMPONENT

Calculi tend to be regarded as macroscopic accumulations of mineral concretions. The amorphous, non-crystalline and non-mineral component has been examined in some detail (Boyce and King, 1963; Cornelius, 1963) and the results have formed the basis of a hypothesis for the initiation of calculus formation. This component has not been investigated at all in this work, largely because the complexity of the necessary biochemical method lies outside the field of investigation, but the spectographic studies indicated the presence of an amorphous component, which was not apparent in the less sophisticated biochemical studies. Even in the calculi identified as struvite, a proportion of amorphous material may have been present, and in all instances this may possibly have corresponded to the material examined by Boyce and King (1963). Support for the admixture of amorphous material is given by the histopathological features of pyelitis and the debris located in the renal pelvis, as well as the PAS-positive material identified in the kidneys of Experiment 2.

CALCIFICATION

Ever since Handall's plaques were noted in the mid-thirties, areas of calcification have been observed in some apparently otherwise normal human kidneys and this incidence may reach 20% (Pyrah and Raper, 1955). It is also claimed that minute deposits of calcium may be found in all human kidneys, whether stone-formers or not (Anderson, 1946). These areas of calcification may appear anywhere in the kidney, including the epithelium of the renal pelvis. Artificial reproduction of oxamide papillary lesions supported the theory that the origin of calculi is related to this structure (Vermeulen et al., 1967). No sign of pelvic plaques was seen in this series. Recent work has demonstrated that a somewhat similar process may be found in some sheep: examination of kidneys of 11 sheep on experimental feeding showed that 8 had intramedullary renal calcification, either intratubular or interstitial in position (Davis et al., 1969). This material was considered to be different from normal calculi and it was suggested that it could be a normal finding in domestic sheep. None of these animals had calculi, and none showed pyelonephritis.

In this work, kidneys were stained by haematoxylin and eosin, but also by Von Kossa to demonstrate areas of calcification and intratubular mineral deposits. This method was found to be effective in both interstitial and intratubular areas and black deposits were easily identified. These areas were found in both calculus and non-calculus sheep, confirming the findings of Davis et al. (1969) on calcified material. In the vast majority of personal observations the Von Kossa-positive material was located in distal tubules or collecting ducts. Invariably, several deposits were present on any one section. But the presence of these deposits raises the question: "when is a concretion a calculus?" If a more thorough examination of the calyces, perhaps using a dissecting microscope, had been carried out, would calculi have been detected in sheep classified as having none? The forced flow of water used to wash out calculi appeared effective and dissection into the minor as opposed to the 10 to 16 major calyces (Ellenberger and Baum, 1943), would have made the kidneys difficult to utilise for histology.

CALCULI

Though the point is discussed again later, it is worth contrasting the small number of fatal and non-fatal obstructive cases (respectively 7 and 3) with the total of 92 sheep with renal calculi, out of a total of 172 experimental sheep. The non-fatal obstructive cases may have been greater, but this group is dependent on personal observation of signs of obstruction associated with a demonstrable calculus, or with demonstration of a calculus at slaughter of a healthy animal, in an obstructing position. All 3 cases were found to have a ruptured urethra behind an obstruction in the vermiform appendage. The difference in incidence of fatalities, compared with American work, may be dependent partly on calculus size, partly on animal size and growth rate, and partly on anatomical factors.

The location of the calculi in relation to the renal pelvis requires consideration. The kidneys in the sheep have a relatively short hilus so that their movement is limited (May, 1964). The gas and food-filled rumen normally keeps the kidneys closely applied to the ventral aspect of the anterior lumbar vertebrae. Since the convex surface of the kidneys is ventral, solid material will tend to stay within the major calyces and in the ventrolateral part of the renal pelvis. Only as a result of sudden movement, such as in running or fighting, will there be a tendency for solid material to move to the entrance of the ureter. Restriction of exercise may enhance the possibility of continued growth of microcalculi by reducing the chance of their early passage down to the bladder, and this point is further developed later.

The types of calculi found in Britain are on biochemical and x-ray crystallography very similar to those reported from the United States by Elam et al. (1956) on a high phosphorus intake, where the calculi were principally composed of magnesium and phosphorus. The authors do not give a value for ammonium content, so that their identifications as struvite is impossible. In later work in California, Cornelius et al. (1959) reported that, while wethers excreted myriads of triple phosphate crystals spectographic analysis revealed magnesium and potassium phosphate, and x-ray diffraction studies indicated that the material was quite amorphous. On the other hand, illustrations of the amount of calculus material in the bladder and kidneys demonstrate clearly that in the American experimental work, the quantity was considerably greater than in Scotland. The factor of quantity may explain why obstruction in the American cases was often at the bladder-neck, while in Scotland, both in experimental and natural cases, the vermiform appendage was usually obstructed first. There is difficulty in making useful observations on the quantity of calculus material. It may be recorded simply on a weight basis, but when groups are considered, the effect of one individual with a particularly large amount of material may make the comparative results ridiculous. The number and size of calculi, not previously utilised in any study, appears more useful. Until things are proved otherwise, it is reasonable to assume that the more calculi are present, the greater the risk of obstruction occurring in that individual. Again, since in the male or castrated sheep the unusually small diameter of the vermiform appendage has to be tolerated, the size of calculi, as measured in millimetres, appears a likely indication of the chance of a particular calculus causing an obstruction. Thus, the American sheep (Cornelius et al., 1959) produced rapidly a large quantity of calculi, which passed rapidly down to the bladder and formed a plug-like blockage in the bladder-neck, a syndrome quite unlike either the clinical cases or the experimental sheep affected with obstruction in this study.

OTHER FACTORS

The literature on factors involved in the aetiology of urolithiasis was briefly considered earlier, and it is necessary to substantiate the omission of various possible factors from specific study in this work.

Vitamin A, claimed to be a causative factor in urolithiasis in man (McCarrison, 1931), rats (Steiner et al., 1939) and goats (Schmidt, 1939), was ignored since all animals were given vitamin A, either incorporated in the feed (which was checked for potency at intervals) or later, more conveniently, by injection of amounts substantially greater than those recommended by the National Research Council (1957).

Vitamin D was similarly given in quantities similar to those recommended by the National Research Council. Though hypovitaminosis D has never been alleged to be a contributory factor to urolithiasis, hypervitaminosis D is known to lead in man and animals to widespread calcification in blood vessels and also in the kidney (Jubb and Kennedy, 1963; Heptinstall, 1966), but pathological changes in animals have only followed massive doses which were not given prophylactically in this study.

Urinary stasis was unlikely to develop when fluid intake was maintained, and while the sheep remained clinically healthy. Urinary infection was examined in one experiment, but samples of urine taken <u>post mortem</u> under sterile conditions failed to demonstrate any significant urinary tract pathogens in experimental sheep.

It appears unlikely that urinary infection plays an important role in the first phase of calculus formation, and the urine of sheep which were affected with cystic phosphatic calculi, and which were destroyed without any attempt at surgery, were rarely found to have infected urine, in contrast to the findings in the dog (Jubb and Kennedy, 1963).

FEEDING

The results obtained in connection with the incidence of urolithiasis under different feeding conditions are in considerable conflict with most experimental work, as already discussed under the individual experiments. Just as all clinical cases here reported were in sheep receiving some form of concentrate feeding, and a high concentrate intake is considered essential for the experimental production of urolithiasis, the final experiment revealed that calculi could be recovered from housed sheep receiving only hay. The only report of hay as a predisposing factor is the claim by Gardiner et al. (1968) that oestrogens present in some forms of Australian clover hay may lead to urinary obstruction in wethers following development of secondary sex organs. In the present study, no clover hay was ever fed. In previous experiments, likewise, the incidence was often as high in sheep on low levels of concentrate as high levels.

Very recently, in a survey (unpublished) of old male sheep purchased from different parts of Scotland, 3 of 50 animals contained macroscopic calculi, and many had small renal lesions of healed or chronic pyelonephritis and chronic nephritis, which may have indicated a previous episode of acute renal disease, possibly associated with calculi. All these animals had been on hay diet for many weeks prior to slaughter. Clearly, the use of such older animals for experimental urolithiasis studies might have led to misleading results, but in the final experiment the sheep were young, and there was no sign of lesions of pyelonephritis in the sheep, fed on hay, which showed renal calculi on slaughter.

The occurrence of calculi in sheep on a diet of hay only has not been previously recorded. The obvious significance is that any male sheep fed indoors under such a regime is liable to develop calculi. As shown in other work the quantity of concentrate feed does not appear to play an important role in promoting the situation of calculus formation. These observations add to the already extensive contradictory findings, and, as in other work, on ruminant urolithiasis, as Field (1969) has written "the differences are impossible to explain because of the few parameters measured." In this series of experiments, little attention was paid to the potassium intake, though this has been demonstrated to be intimately involved in urinary pH, which in turn may be an important factor in urolithiasis. Potassium has likewise remained unsuspected as a contributary factor in urolithiasis until Lamprecht et al. (1969) investigated retrospectively, the significance of the potassium intake in several years' experiments in which the main interest had been in the relationship of various minerals to urolithiasis. They reported a curvilinear relationship of K intake to percentage urolithiasis, with the maximum incidence at a point between minimal and maximal K intake, this point depending on the intake of other elements. It was claimed that this curvilinear effect might be the reason for some inconsistencies in the effect of the element on urolith formation (Crookshank, 1966; Bushman et al., 1968). The role of potassium has been considered in a hypothesis of Chen-Chow et al. (1968) who think potassium may compete for binding sites or poly-electrolytes which provide nuclei for inorganic ions. When the cation K has been compared with Na in respect of their relative effectiveness in reducing urolithiasis, K has afforded better protection (Crookshank, 1966).

Since the daily requirements of any sheep in these experiments (National Research Council, 1957; Agricultural Research Council, 1965) were unlikely to exceed 3 g calcium (0.20% in total feed), and 2.7 g phosphorus (0.18%), it is worthwhile considering the absorption and excretion of these elements when given in larger quantities, as here. If the mineral requirements are assumed for ram lambs, as opposed to fattening lambs (since the rate of growth was slow in all experiments), the requirements of Ca and P are very similar (N.R.C., 1957).

In a review, Smith (1969) stated that both cattle and sheep appear to be able to tolerate quite a wide range of Ca and P ratios in their diet, without any serious interference in the utilisation of either (quoting Bohstedt, 1942; Young et al., 1966). It is known that some precipitation of calcium phosphate occurs in the small intestine of calves, even on normal mineral intakes (Smith and McAllen, 1966) but only when the pH exceeds 6.5 and not, therefore, in the middle and upper small intestine where most absorption of Ca and probably also phosphate occurs. Such findings may also apply to growing sheep. The nett absorptive efficiency with varying P intakes has been demonstrated in sheep by Young et al. (1966), but its mechanism is not clear. The quoted phosphorus requirements may be high, in the light of the recent studies of Benzie et al. (1960).

No evidence exists for a true excretion of either calcium or phosphorus into the gut, and since published work and these observations indicate that little is excreted in the urine, the major control of their quantities in the body tissues is presumably through absorption (Hill, 1961), and depends on the animal's requirements and the form in which the elements are fed.

Phosphorus generally appears to be more readily available than calcium (Lofgreen and Kleiber, 1953; Shroder and Hansard, 1958; Thomson et al., 1959), and phosphorus absorption appears relatively unaffected by other dietary constituents, and influenced primarily by the type of phosphorus fed (Hill, 1961). Thus, Lucker and Lofgreen (1961), using a radio-isotope technique, found that six-month-old wether lambs absorbed both calcium and phosphorus to an extent directly dependent on the amount fed.

The experimental animals used in Experiment 4 were this type of subject, and, by analogy, it would appear that large, excessive quantities of calcium and magnesium should have been absorbed. Without the use of more sophisticated techniques involving radio-isotopes, it is not possible to state definitely that this absorption took place.

Most reported periods of experimental feeding were in the range of 3-5 months, as in this study. While one report quoted the onset of acute obstruction as early as 14 days after the commencement of feeding K_2HPO_4 (Cornelius et al., 1959) and a second report quoted a first obstruction on day 32, these are exceptions. Most records have failed to detail when cases occurred. Further, examinations of the incidence in the experimental groups with most calculi has often shown that, while this has been as high as 80% (Packett et al., 1968), 90% (Elam et al., 1956), 79% (Crookshank et al., 1966), the incidence has often been much lower in other work, e.g. 56% or 39% (Packett and Hauschild, 1964). When acute urinary obstruction is considered, few reports give a high incidence (an exception of 50% by day 16 is given by Cornelius et al., 1959) and a figure of 36 cases out of 237 animals, in several experiments, representing 15% mortality, may be fairly typical (Emerick and Embry, 1964a). Urolithiasis rarely affects all individuals in a susceptible group, but this fact is common to most diseases, animal or human. Obstruction, likewise, affects only a small proportion of a given group at risk, whether as field cases, or experimental subjects.

The poor apparent repeatability of previous work has been remarked on by Idaho studies (Annual Report of Regional Project W - 41, 1964, by Dr. R. Mason, unpublished). This group used a similar feed mixture in successive years and found that 10/10 animals in one year, and 2/10 animals in the second year, contained calculi. The weather, genetic make-up or labile factors in the feed pellets were suggested as an explanation. Similarly in this work Experiment 2 showed that an American mixture which had been amply demonstrated to be calculogenic in the U.S.A. proved disappointing in Scotland even when the proportion of the K_2HPO_4 supplement was increased. Reynolds and Lindahl (1968) have reported a total failure of rations, shown elsewhere to be calculogenic, to produce calculi. Climate, type of sheep, and undetected differences in trace elements might all hypothetically play a role in explaining this discrepancy. For example, an increase in the water intake has been found to increase phosphorus excretion in urine (Suttle and Field, 1966). Since it is known that certain strains of Scottish Blackface - the breed used throughout this work - have a genetically associated water consumption different from the other strains of Blackface, and recognisable by the potassium content of the red cells (Evans, 1954, 1957), it is easy to see how such strain variations may result in a different predisposition to urolithiasis. Further investigation of the total water intake in experimental sheep on calculogenic outlets may prove useful.

A low Na intake might result in a lowered water intake and its possible consequences, but in all mixtures fed in these experiments the Na level exceeded the minimum requirement given by the A.R.C. (1965) and levels below those recommendations have been shown to produce no apparent effect on the well-being of sheep (Jones et al., 1967).

SOURCES OF ERROR

Possible sources of error arise from any method of experimentation. These errors fall under several main headings in urolithiasis work:

(a) absence of verification of calculus-free status initially;

(b) infrequent and possibly inaccurate sampling of blood and urine;

(c) differing methods of recording the degree of urolithiasis, as already discussed.

Some criticism of previous work is given at this point. Results of blood examinations for various constituents have sometimes been based on infrequent sampling, e.g., 3 times in 181 days by Lindley et al. (1953), on pre-slaughter values only (Belonje, 1965), and sometimes only from random animals (Crookshank et al., 1967). In no instances has blood been examined at weekly or fortnightly intervals. Urine collection methods were sometimes crude, with no attempt to use preservatives, to reduce the activity of urea-splitting organisms and allegedly statistically significant results were drawn from cases which showed pH differences of much less than 1 pH unit.

The examination of methods of preservation of urine has here demonstrated that pH changes of 1 pH unit may occur in samples exposed to the atmosphere for 24 hours and the validity of results based on unsophisticated methods of collection appears questionable. Other methods of urine collection may be unsophisticated - involving training the animals to urinate reflexly when their hindquarters are gently touched -- but leave the problem of diurnal variation unsolved (Stacy, 1969), or involve a delicate surgical preparation such as an exteriorised ureter (Stacy and Brook, 1964). In other experiments (Elam et al., 1956; Lindley et al., 1953; Robbins et al., 1965), lack of acclimatisation to, and very limited periods in metabolic cages place a limited value on results of urine examinations from such animals (Robbins et al., 1965; Packett et al., 1968). In cases of mild hydronephrosis it is often found that calculi are no longer within the renal calices, but in the renal pelvis, ureter and bladder. In some experiments only one kidney was examined macroscopically and the results based on this finding and the presence of microcalculi in the second kidney (Crookshank et al., 1967). Other workers examined only a proportion of their experimental animals for calculi (Kunkel et al., 1961).

COMPARATIVE RESULTS

Turning to a comparison of the results of the different experiments in terms of the incidence of fatal obstuction and of renal calculi, this information is summarised in Table 42 which summarises the results of experiments P to 5. It will be seen that the percentage of fatal obstruction is given as 0-20.

In every case this percentage represents a single individual of a small group, e.g. one in five. Likewise the single instance of 100% for the recovery of renal calculi represents that 5 of 5 possible animals were affected.

There is no consistent pattern in the results as presented in Table 42. In fact, the calcium and phosphorus intake which resulted in the 100% incidence in Experiment 5 is identical with one group of Experimant 3 where the incidence was 57%. A very similar intake in Group 4 of Experiment 3 resulted in the lowest incidence of any experiment except the preliminary study. In Experiment 5, massive intakes of calcium failed to result in significantly increased numbers of animals with calculi.

Table 42 : Summarised Data of Experimental Feeding : Intakes of Calcium and Phosphorus related to Fatal Urinary Obstruction and Incidence of Renal Calculi expressed as Percentage

Experiment and Group		Daily Mineral Intake Ca g	Ca %DM	P g	P %DM	Fatal Obstruction Total	Fatal Obstruction %	Renal Calculi Total	Renal Calculi %
P		0.70	0.08	3.81	0.47	0	0	0	0
1		13.6	1.70	8.2	1.02	2	20	6	60
2	A	7.5	1.10	2.8	0.72	0	0	1	*
	B	9.0	0.92	5.9	0.34	0	0	1	*
	C	17.7	1.27	16.8	1.20	0	0	6	30
3	1	4.0	0.4	2.7	0.27	1	14	3	43
	2	4.2	0.4	2.9	0.27	1	14	4	57
	3	5.7	0.5	3.7	0.33	0	0	4	57
	4	6.2	0.5	4.0	0.40	0	0	2	28
	5	7.8	0.6	4.9	0.39	0	0	3	43
	6	8.0	0.6	5.1	0.40	1	14	3	43
4	1	1.5	0.18	2.4	0.27	0	0+	7	63
	2	1.6	0.14	1.9	0.16	0	0	8	72
	3	6.9	0.79	2.4	0.27	0	0+	7	63
	4	25.6	3.40	2.2	0.28	0	0	8	72
5	H	1.9	0.15	1.7	0.13	0	0	7	58
	1	3.5	0.25	4.2	0.30	0	0	6	86
	2	5.7	0.34	3.7	0.22	0	0+	5	100
	3	10.7	0.80	3.6	0.27	1	12	5	62
	4	41.4	2.80	3.7	0.25	1	14	6	86

* insufficient animals

\+ one case of non-fatal obstruction

The potassium intake may have been different in sheep which had similar calcium and phosphorus intakes. The result of a higher K intake would be an increased urinary K excretion (Scott, 1969) and hence an increased urine alkalinity of direct relevance to the likelihood of calculus formation. Similar differences in other elements could alternatively account for the variation in results: Hoar, Emerick and Embry (1969) have demonstrated this difference in the case of sodium bicarbonate.

Packett and Hauschild (1964) suggested, in attempting to explain certain mineral relationships, that "the actual formation of gross calculi was determined by the degree of change of certain metabolic variables, i.e. high phosphorus levels in the serum and urine accompanied by high magnesium levels." In this study no evidence was found that high urinary and plasma phosphorus were associated with gross calculus formation, neither was a high plasma magnesium a useful indication of the likelihood of calculi. Indeed, utilising a large volume of data from Texas, in discriminant analysis, Lamprecht et al. (1969) were only able to predict with approximately 75% certainty based on urinary excretion, and urinary and plasma mineral and electrolyte levels the likelihood of a given animal having calculi.

It might be hypothesised from these differences that the development of calculi was entirely different under Scottish conditions, yet if so, the analysis of calculi would probably be different from that of the American studies. This was not so, though formation may have been slower in Scotland. Similar small quantities have been reported from France by Jean-Blain et al. (1968), where the clinical condition appears to correspond more closely than in U.S.A. to that recognised in Scotland.

The number of variable factors which cannot be controlled under these experimental conditions is such that it would be unwise to pursue a comparison with the U.S.A. too far. The evidence presented amply demonstrates that urinary calculi may develop under numerous conditions of concentrate or hay feeding in housed sheep, either entire or castrated.

PATHOLOGY

The pathological features in calculi obstruction are dramatic and largely the result of the mechanical effects, the acute reaction of the urinary epithelium to the presence of fine calculi, and the reaction of different tissues to a uraemia. What is worthy of further discussion is the development of chronic pyelonephritis in some sheep. The veterinary literature gives no indication of the incidence of pyelonephritis in sheep (Marsh, 1965; Jubb and Kennedy, 1963). Focal lesions, of uncertain significance but without evidence of bacteria, have been observed in a few cases of urolithiasis in sheep by Davis _et al._ (1969). The disease, acute or chronic, is common in man (Heptinstall, 1966) and in this series appeared in both experimental and natural cases. The pyelonephritis associated with urinary obstruction was invariably acute, while subacute lesions were found in animals showing no symptoms and with no calculi, but also in sheep with calculi. Chronic pyelonephritis was an incidental finding in numerous sheep unassociated necessarily with calculi. It is tempting to hypothesise that pyelonephritis may be an important predisposing factor for urolithiasis, leading to the production of desquamated material, tubular blockage, urinary stasis, and the creation of further favourable environmental circumstances. The distribution of pyelonephritic lesions in subacute and chronic cases is not convincing evidence for an ascending infection from the lower urinary tract, since the lesions tended to be located in the cortex, and pyelitis was not a feature.

If, therefore, the lesion was a result of blood-borne infection, one must postulate a pyaemia resulting from a local septic focus elsewhere in the body. Older rams frequently have abscessation about the head, perhaps as a result of fighting or other injury, but such injuries are uncommon, or at any rate rarely reported, in younger sheep.

The severe pyelonephritis, of recent origin, or a flare-up of an old lesion, was doubtless the result of an ascending infection. The distension of ovine kidneys in acute obstruction lower down the tract is probably the result of back-pressure up the ureters, in which circumstances there is likely to be vesico-ureteric reflux, and infected urine in the bladder could be redistributed to the kidneys, a factor which could be significant in cases of infection of posterior urethrotomy wounds.

A recent paper (Adamesteanu et al., 1969) claimed that glomerulonephritis and hydronephrosis were the outstanding pathological features of ovine kidneys in urolithiasis caused by overfeeding with concentrates, and described masses of albumen occupying Bowman's capsule and compressing the glomerulus, leading to glomerular hyperplasia in some instances, and also associated with tubular degeneration. In this series such changes were never seen either in clinical cases or in experimental animals, and the reason for this anomaly is not clear.

The studies on renal weights indicated that urolithiasis did not in itself lead to any increase or decrease in size, and that differences were attributable to other renal changes such as hydronephrosis, pyelonephritis or other abnormality. The poor radiographic opacity of the material from Scottish sheep has been mentioned, and similar material appears to be present in Texas sheep with urolithiasis. Together with the known difficulties of achieving radiographic detail through the ovine abdomen, these points indicate the uselessness of radiography as an aid to diagnosis of ovine urolithiasis, in sharp contrast to other species and to man.

The following conclusions are drawn from this work :

1. Ovine urolithiasis is a sporadic but significant cause of disease, often terminating fatally, on Scottish farms breeding male sheep.

2. The disease is almost entirely confined to sheep which are housed for at least part of the day. It may have a higher incidence in farms breeding ram lambs.

3. The clinical disease is characterised by an obstructive syndrome. Though obstruction is usually in the vermiform appendage of the penis, renal changes are severe, and hydronephrosis, pyelonephritis and pyelitis are prominent.

4. The calculus material varies in colour, size and form, but biochemical and x-ray crystallographic examination show that it is struvite with organic debris.

5. Since this crystalline component will only precipitate in an environment above pH 7, urinary pH is likely to be an important predisposing cause of calculus formation.

6. Urine pH was found to vary little when samples were preserved in screw-topped glass bottles, occluding any air, and preferably with an oil seal. Conversely, samples left exposed to the atmosphere, even if uncontaminated by bacteria, were subject to rapid changes of pH, so that conventional methods of urine collection for 24-hour periods were unsuitable for consideration of pH.

7. Urine pH of grazing ewes was found to vary seasonally, but, apart from a few months in the summer, it was usually acidic. Assuming that grazing wethers and rams excreted similar urine, struvite calculus formation would be impossible.

8. Urine pH of rams and wethers in metabolism cages, where samples were protected from rapid pH change by the use of thymol, was generally alkaline but results were variable.

9. Urine mineral excretion by sheep was found to be similar to values reported in the literature. Individuals, both on control and calculogenic diets were liable to excrete increased amounts of phosphorus in urine.

10. In a series of experiments involving 172 entire or castrated sheep a series of mixtures, aimed at giving a variable calcium and phosphorus intake, failed to give clearcut evidence that either a high calcium, or a high phosphorus intake was liable to lead to a high incidence of obstructive urolithiasis. Seven sheep died from acute obstruction due to calculi, and a further three had a non-fatal obstruction. Ninety-two of the 172 experimental

sheep had renal calculi at slaughter. The highest incidence (5 of 5 animals contained renal calculi) was obtained when sheep were fed 5.7 g Ca and 3.7 g P, but none of the 5 sheep showed the obstructive syndrome. A high Ca intake did not, on the other hand, have a protective effect, as claimed by other workers. It appears that sheep which are housed and which are fed only hay may develop calculi. The difficulty of ensuring initially that the sheep do not already contain calculi is the limiting factor.

11. The presence of PAH-positive material was confirmed in the collecting tubules of the kidney. Von Kossa-positive material was located in distal tubules or collecting ducts. Both types of material were found not necessarily associated with the presence of macroscopic renal calculi, but this fact in no way invalidates the hypothesis that such material may be a precursor to calculi.

12. Despite the experimental design, it proved impossible adequately to eliminate a large number of variable

factors, and differences with the results of American workers, now also been reported by other workers in the U.S.A., may be the result of variations in such factors as the intake of potassium or sodium, or different environmental conditions (e.g. climate), or uncontrollable genetic factors such as the water consumption in particular sheep strains.

SUMMARY

Ovine urolithiasis is alleged to be associated with intensive feeding of concentrates, a type of husbandry which has similarities to methods of rearing male sheep in Scotland, and which may become increasingly common in intensive feeding systems in ruminants in Britain.

The thesis is divided into three parts. Part I reviews the distribution and predisposing factors of ovine urolithiasis, composition of ovine urine, both normal and in urolithiasis, experimental production by dietary means and finally the renal pathology and the composition of calculi.

In Part II various surveys revealed that obstructive urolithiasis was uncommon in Britain except in Scotland, where the incidence varied from 1-10% principally among housed male sheep aged 6 months to 2 years and where, since cases were often fatal, the economic loss was significant.

In determination of the liability of ovine urinary pH to change under different conditions of storage, samples kept in screw-topped containers to exclude air underwent no significant pH change in 24 hours.

In catheterised samples taken at monthly intervals for seven years, from healthy Cheviot and Half-bred ewes, a seasonal variation was shown in urinary pH, which, except in a few summer months, was acidic, a finding contrary to most textbook statements. Urinary pH was significantly correlated ($p < 0.01$) with urinary potassium concentration. Little calcium, magnesium and phosphorus was excreted in urine, and no seasonal pattern was apparent. Proteinuria and acetonuria occurred occasionally.

Studies on four housed Blackface rams (half-brothers) showed that their urinary pH was generally alkaline. Urinary mineral concentrations, especially magnesium, were higher than in grazing ewes. The urinary phosphorus concentration of one tup was significantly ($p < 0.01$) less than in the others.

Feeding experiments were carried out on 172 Blackface sheep to determine the incidence of clinical (obstructive) and subclinical urolithiasis (presence of calculi). Seven animals developed a fatal obstruction with calculi, a further three had non-fatal obstruction, and 92 sheep had renal calculi.

No calculi developed in six male lambs fed a mixture grossly inadequate in calcium (0.7 g daily) and adequate in phosphorus (3.81 g), for 120 days. Two clinical cases of obstruction by calculi developed and four others had renal calculi when ten castrated lambs were fed a high calcium (2.90%) and phosphorus (1.70%) concentrate mixture with roots and wheat straw. Kidneys of obstructed sheep demonstrated hydronephrosis and pyelonephritis. Other sheep showed early foci of pyelonephritis.

Experiment 2 employed a feed composition known in the United States to be calculogenic but, despite a subsequent increase of the potassium acid phosphate concentration in the mixture from 2.14 to 4.28%, no animal developed obstruction. Eight of 30 sheep had calculi at slaughter.

On a relatively normal and narrow range of Ca and P intakes, differing quantities of concentrates and roots had no effect on the development of urolithiasis. Nineteen of 42 sheep (45%) contained calculi, including three fatalities. Liveweight increase was unrelated to the presence of renal calculi.

The calcium intake was then varied in 42 castrated lambs (1.51 g to 25.60 g daily), while the phosphorus intake was low (1.9 - 2.4 g). While 71% of the animals had calculi, the incidence was unrelated to calcium intake.

The previous experiment was repeated with ram lambs, and initially twelve were killed after 135 days of hay feeding alone, seven of which contained renal calculi. After 96 days of concentrate feeding, renal calculi were found in 22/27 sheep, including two animals with fatal urinary obstruction.

No significant differences in plasma mineral values were demonstrable between calculus-containing and other sheep. The pathological features of obstruction were similar to those in the natural disease. Microcalculi were demonstrated in renal collecting tubules.

Pyelonephritis was common in obstructive urolithiasis and was considered sometimes to result from ascending infection from a urethrotomy wound, and sometimes from blood-borne infection. Glomerulonephritis and chronic interstitial nephritis were rare. Intracytoplasmic PAS-positive globules occurred in the proximal convoluted tubules of sheep with renal calculi.

On biochemical analysis, ovine urinary calculi consisted of triple phosphate together with some calcium phosphate and in two cases with calcium oxalate and carbonate. X-ray crystallography showed that some calculi were amorphous in structure while others were struvite (magnesium ammonium phosphate) or newberyite (magnesium hydrophosphate-trihydrate).

The results are discussed in Part III. As struvite is an important crystalline component in ovine calculi in Scotland, urinary pH is likely to be a significant factor in its production, and precipitation is unlikely at the urinary pH encountered in grazing sheep. From the results of these experiments it appears unlikely that extreme Ca and P intakes, high K_2HPO_4, or a high root intake are significant in producing an increased incidence of calculi in indoor sheep. Since animals developed calculi on hay feeding alone, it appears that factors such as climate, genetic profile, or elements such as K and Na may explain certain discrepancies in these results in Scottish sheep.

REFERENCES

Adamesteanu, I., Adamesteanu, C., Ghergariu, S., Angi, E., Danielesco, N., Moldovan, A. and Morar, R. (1969) Revue Méd. vét. 128, 353.

Agricultural Research Council (1965) Nutrient Requirements of farm livestock. No.2. Ruminants. London.Agricultural Research Council.

Albright, F. (1936). Trans. Assn. Am. Physicians 51, 199.

—— and Reifenstein, E.C. (1948) "Parathyroid glands and metabolic bone disease". Baltimore. Williams and Wilk

Anaud, R.S. and Parker, H.R. (1966) Am. J. vet. Res. 27, 899.

Anderson, L. (1946) Surgery Gynaec. Obstet. 82, 275.

—— and McDonald, J.R. (1946) Surg. Gynec. and Obstet. 82,2

Animal Breeding Research Organisation (1969) Annual report. Jan. 19 London.Agricultural Research Council.

Armstrong, R.H. and Cameron, A.F. (1959) J.Brit. Soc. Anim. Prod. I, 37.

—— —— (1961)Ibid. 3, 295

Bailey, C.B., Bezeau, L.M. and Lawson, J.E. (1963) Canad. J. Anim. Sci. 43, 150.

Baker, L.H., Reid, J.J., and Owen, M. (1928) J. Amer. vet. med. Ass. 79. 97. quoted by Habisreitinger (1961).

Barr, D.P., Bulger, H.A. and Dixon, H.H. (1929) J.Amer. med. Ass. 92, 951.

Beesom, W.M., Pence, J.W. and Holm, G.C. (1943) Am.J.vet.Res. 4,120.

Belonje, P.C. (1965a) J.S. Afr. Vet. med. Ass. 36, (3), 381.

___ (1965b) Ibid. 36, (3), 377.

Bell, T.D., Smith, W.H. and Erhart, A.B. (1954). J. Anim. Sci. 13, 425.

Belscher, H.W. (1957) "Sheep management and diseases". 5th Edition. Sydney. Angus and Robertson.

Bennetts, H.W. (1950). J. Dept. Agric. West Aust. 27, 129.

Bentinck-Smith, J (1963). "Clinical Biochemistry of Domestic Animals", edited by C.E. Cornelius and J.J. Kaneko. New York Academic Press.

Best, C.H. and Taylor, N.B. (1955). "Physiological basis of medical practice". Baltimore. Williams and Wilkins.

Beveridge, W.I.B. (1942). Aust. vet. J. 18, 127.

Bharadwaj, M.D. and Calhoun, M.L. (1959) Am. J. vet.Res. 20, 841.

Biggs, A.W., Norfleet, C.M., and Garvey, F.K. (1955) Amer. Urol. Assoc. 19, 76.

Black, W.H., Ellis, N.R., Jones, J.M. and Keating, F.E. (1947) U.S. Dept. Agr. Tech. Bull. 945.

Blaxter, K.L., Graham, N. McL. and Wainman, F.W. (1959). J. Agric. Sci. 52, 41.

Blood, D.C. and Henderson, J.A. (1960) Veterinary Medicine, Bailliere, Tindall and Cox.

Bohstedt, G. (1942) J. Dairy Sci. 25, 440.

Bollwahn, W. (1961) Tierarztl. Umsch. 16, 8.

Boyce, W.H. (1966) Proc. 3rd Int. Congr. Nephrol. Washington. 2, 354.

——— Garvey, F.K. and Norfleet, C.M. (1955). Amer. J. Med. 19, 87.

——— Pool, C.S., Meschan, I. and King, J.S.Jnr. (1958). Acta Radiologica 50, 543.

Bromner, F. (1964) In "Mineral Metabolism" edited by C.L. Comar and F. Bromner. New York. Academic Press.

Bushman, D.H. (1968) "Ovine phosphatic urolithiasis". Thesis. Abstr. in Diss. Abstr. 28, no. 2672B.

——— Emerick, R.J. and Embry, L.B. (1965a) J. Anim. Sci. 24, 671.

——— ——— ——— (1965b) Ibid. 24, 876.(abstract)

——— ——— ——— (1968) Ibid 27, 490.

Butt, A.J. (1956) "Etiologic factors in renal lithiasis" Springfield, Illinois. Thomas.

——— and Hauser, E.A. (1952) Science 115, 308.

——— ——— and Seifter, J. (1952) J. Amer. med. Ass. 150, 1096.

Butt, A.J., Seifter, J. and Hauser, E.A. (1952) New Orleans Med. and Surgical J. 104, 754.

Campbell, L.D. and Roberts, W.K. (1965) Can. J. Anim. Sci. 45, 147.

Chen-Chow, F.H., Hamar, D.W. and Udall, R.H. (1968) Invest. Urol. 5, 557.

Christoph, H. (1965) "Arbeitsmethoden des Laboratoriums in der Veterinärmedizin". Leipzig. S. Hirzel.

Coburn, S.P. and Packett, L.W.Jnr. (1962) J. Nutr. 76, 385.

Coles, E.H. (1967) "Veterinary Clinical Pathology" Philadelphia, Saunders.

Cornelius, C.E. (1963) Annals. N.Y. Acad. Sci. 104, 638.

——— and Bishop, J.A. (1961), J. Urol. 85, 842.

——— Moulton, J.E. and McGowan, B. (1959). Am. J. vet. Res. 20, 863.

——— Bishop, J.A., Berger, L.R. and Pangborn, J. (1961) Am. J. vet. Res. 22, 1000.

——— and Kaneko, H. (1963) "Clinical Biochemistry of Domestic Animals". New York. Academic Press.

Cornfield, A.H. and Pollard A.G. (1950). J. Sci. Fd. Agr. 1, 260.

Cottereau, P., Froget, J., Magat, A., Jaillardon, C. and Monard, G. (1966) Rev. Med. Vet. Toulouse, 117, 13.

Cottet, J. (1961) "Encyclopaedia of Urology". Edited C.E. Aiken et al. Berlin, Springer.

——— and Vittu, C. (1955) La Presse medicale 63, 878.

——— Vittu, C. and Catarelli, G. (1962) Rein et Foie, Vittel 4, 11 29.

Counsellor, V.S. and Priestley, J.T. (1935) J. Amer. med. Ass. 104, 1309.

Cotran, R.S. (1964) Proc. 14th Annual Conference on the Kidney. Edited by J. Metcalf. Boston. Little, Brown & Co.

Crookshank, H.R. (1962) J. Anim. Sci. 21, 993.

——— (1966) Ibid. 25, 1005

——— (1963) Personal communication

——— Packett, L.V.Jnr. and Kunkel, H.O. (1965) J. Anim. Sci. 24, 638.

——— Keating, F.E., Burnett, E., Jones, J.H. and Davis, R.E. (1960) Ibid. 19, 595.

——— and Robbins, J.D. (1962) Nature 196, 1343.

——— ——— and Kunkel, H.O. (1967). J. Anim. Sci. 26, 1179.

Cunningham, I.J., and Cunningham, M. (1938). N.Z.J.Sci.Tech. 19, 529.

Davis, W.D., Scott, R., Crookshank, H.R. and Spjut, H.J. (1969). J. Urol. 100, 383.

Dewhirst, J.K., Harrison, F.A. and Keynes, R.D. (1968). J. Physiol. 195, 609.

Dick, W. (1869). "Occasional papers on various subjects". Edinburgh. W. Blackmore and Sons.

Dickens, C. (1842). Veterinarian 15, 357.

Dixon, W.E. and Hoyle, J.L. (1928). Brit. med. J. 5, 832.

Dukes, H.H. (1955) "The physiology of domestic animals". 7th Edition. New York. Cornell Univ. Press.

Dutt, B., Majamdar, B.N. and Kehar, N.D. (1959). Brit. Vet. J. 115, 63.

Easterfield, T.H., Rigg, T., Askew, T.U. and Bruce, J.A. (1930). Vet. J. 86, 251.

——— ——— ——— ——— Ibid. and (1931) J. Comp. Path. & Ther. 44, 297.

Ebstein, W. (1884) "Die Natur und Behandlung der Harnsteine". Wiesbaden, Steinkopff.

——— and Nicolaier, A. (1891) "Experimentelle Erzeugung von Harnsteinen". Wiesbaden. J.F. Bergmann.

Edwards, N.A. and Hodgkinson, A. (1965). Clin. Sci. 29, 327.

Egoshin, I.S., Ryskulov, K.R. and Vidomskii (1967). Veterinariya (Moscow). 44, (8), 90.

Elam, C.J. (1957) Thesis. Washington State Univ. Abstract in Diss. Abst. 18, 12. 1958.

Elam, C.J. (1961) Personal communication.

____ Ham. W.E. and Dyer, J.A. (1959) J. Anim. Sci. 18, 383.

____ ____ and Schneider, B.H. (1957). Proc. Soc. Exp. Biol. Med. 95, 769.

____ Schneider, B.H. and Ham, W.E. (1956), J. Anim. Sci. 15, 800.

Ellenberger, L. and Baum, H. (1943) Handbuch der Vergleichende Anat. der Haustiere. 18th Edit. Berlin. Springer.

Elliott, J.S., Quaide, W.L., Sharp, R.F. and Lewis, L. (1958). J. Urol. 80, 269.

____ Sharp, R.F. and Lewis, L. (1959). Ibid. 81, 366.

____ Todd, H.E. and Lewis, L. (1961). Ibid. 85, 428.

Emerick, R.J. and Embry, L.B. (1962) J. Anim. Sci. 21, 995.

____ ____ (1964) Ibid. 23, 1079.

England, G.J. (1954) Brit. J. Anim. Behav. 2, 56.

Evans, J.V. (1954) Nature 174, 931.

____ (1957) Ibid 180, 756.

Evereth, D.F., Bolin, F.M., Goldby, A.E. and Ford. K.D. (1948). N. Dakota Agr. Exp. Sta. Bull. 10, 49.

____ and Miller, T.W. (1939) Vet. Med. 34, 106.

Evvard, J.M., Culbertson, C.C. and Wallace, I.W. (1923) Iowa Agr. Expt. Sta. Bull, 215, 369.

Field, A.C. (1969). Proc. Nutr. Soc. 28, 198.

Fish, A.R. et al (1948). Brit. Med. J. I, 7. Quoted by Tayson, E.D. (1961).

Fiske, G.H. and Subbarow, Y. (1925). J. Biol. Chem. 66, 375.

Fleish, H. (1965). Urol. int. 19, 372.

——— and Bisaz, S. (1964) "Symposium über die posttraumatisch Entzündung und ihre Behandlung". Davos. 1964. 68. Basel Karger.

Fleming, G. (1902) Operative Veterinary Surgery. Vol. 1. 2nd Editi London. Bailliere, Tindall and Cox.

Flocks, R.H. (1939). J. Amer. med. Ass. 113, 1466.

——— (1944) Am. J. of Nursing 44, 207.

Frank, F.W. (1962) Personal communication

——— Meinershagen, W.A., Baron, R.R., Scrivner, L.H. and Keith, T.B. (1961) Am. J. vet. Res. 22, 899.

Friedmann H.S., and Rubin, M.A. (1955) Clin. Chem. I, 125.

Fujimaki, Y. (1927) Japan. Med. World 6, 29. Quoted Pontius, Carr and Doyle (1930).

Gardiner, M.R. (1965) J. Agr. West. Australia 6, (12), 731.

——— Nairn, M.E. and Meyer, E.P. (1966). Aust. vet. J. 42, 315.

Garner, R.J. (1961) "Veterinary Toxicology". 2nd Edn. London. Bailliere, Tindall and Cox.

Glenn, H.W., Hamilton, J. and Tucker, J.O. (1956). Wyoming Agr. Exp. Sta. Circ. no. 73.

Godard, J. (1948) "Lithiase urinaire du mouton". These. doct. vet. Paris (Alfort).

Gough, D.W.P. (1942) Agriculture 50, 230.

Gould, C.M. (1965) Information from Veterinary Clinical Observation Unit, Southampton.

Grünberg, W. (1964a). Zbl. allg. Path. 105, 256.

____ (1964b) Path. vet. 1, 258.

Guersel, A.E. (1936) J. Urol. 42, 447.

Gustafsson, B.E. and Norman, A. (1962) J. Exp. Med. 116, 273.

Gutteridge, J. (1840) Veterinarian 8, (new series). 576.

Habisreitinger, K. (1961) "Harnuntersuchungen bei gesunden Schafen". Inaug. Diss. Giessen.

Haag, J.R. and Palmer, L.S. (1928) J. Biol. Chem. 76, 367.

Hager, R.H. and McJath, T.R. (1928) J. Amer. med. Ass. 85, 1352.

Halstead, S.B. (1961) Amer. J. Trop. Med. Hyg. 10, 918.

Harris, L. and Alexander, M.A. (1953). Nebraska Exp. Sta. Qu. 2, 1.

Haugh, I., Lonsdale, K., Mason, P. and White, E.G. (1966) J. Small Anim. Pract. 7, 565.

Hawkins, W.W. Jnr. (1965) J. Amer. vet.med. Ass. 147, 1321.

Healy, D.J., Bullard, J.F. and Spears, H.D. (1928) J. Amer. vet.med. Ass. 73, 87.

Heaton, F.W. and Anderson, C.K. (1965) Clin. Sci. 28, 99.

Hellström, J. (1955) Rev. Surg. 22, 381.

Helmy, N. and Fouad, K. (1964). Vet. Med. J. (Giza), 10, 245.

Heptinstall, R.H. (1966) "Diseases of the Kidney", Boston. Little, Brown.

Herrick, E.H. and Vatne, R.D. (1961) Trans. Kansas Acad. Sci. 64, 2

Heuberger, W. (1962) Urol. internat. 14, 319.

Higgins, C.C. (1935) New England J. Med. 213, 1007.

——— (1951) J. Amer. vet. med. Ass. 118, 81.

Hill, R. (1961) World Rev. Nutr. Diet. 1961, 133.

Hjelle, A. (1969) Acta. vet. Scand. 10, 1.

Hjerpe, C.A. (1968) Am. J. vet. Res. 29, 143.

Hoar, D.W., Emerick, R.J. and Embry, L.B. (1968) J. Anim.Sci. 27, 1165.

Hobday, F. (1922) Vet. Rec. 34, 519.

Hodgkinson, A. and Pyrah, L.N. (1958) Brit. J. Surg. 46, 10.

Höfer, M. and Kleinzeller, A. (1964) Proc. II. Int. Congr. Nephrolo Amsterdam, Excerpta Med. Foundation.

Howard, J.E. (1962) Canad. med. Ass. J. 86, 1001.

——— Thomas, W.C., Mukai, T., Johnstone, R.A. Jnr. and Pascoe, B.J. (1962). Trans. Ass. Amer. Phys. 75, 301.

Jaworski, Z.F., Brown, E.I., Fedoruk, S. and Seitz, H. (1963) New England J. Med. 269, 1103.

Jean-Blain, M., Jean-Blain, A., and Jean-Blain, C. (1968). Rev. Méd. Vét. 31, 623.

Johnson, D.W., Palmer, Z.S. and Nelson, J.W. (1940) Vet. Med. 34, 3

Johnstone, I.L. (1948) Aust. vet. J. 24, 86.

Jones, J.H., Keating, F.E., Ellis, N.R. and Crookshank, H.R. (1962). Texas Agr. Exp. Sta. Report No. 1570, Cattle Series 10

Jones, D.I.H., Miles, D.G. and Sinclair, K.B. (1967). Br. J. Nutr. 21, 391.

Jordan, R.M. (1959). J. Anim. Sci. 12, 670.

Jubb, K.V. and Kennedy, P.C. (1963) "Pathology of Domestic Animals". Vol 2. New York. Academic Press.

Kachmann, K. (1937) "Hat die Methode der einfachen quantitativen Harnzuckerbestimmung .... Veterinärmedizin?" Inaug. Diss Leipzig.

Kale, S.M., Bhane, V.J. and Jamkhedkarp, P. (1966). Indian Vet. J. 43, 200.

Ketz, H.A. (1960) Zbl. Vet.Med. 7, 411.

Keyser, L.D. (1935) J. Am. med. Ass. 104, 1299.

King, E.J. and Haslewood, G.A.D. (1936) Lancet, 2, 1153.

King, J.S.Jnr. (1967) J. Urol. 97, 583.

King, J.S.Jnr. and Boyce, W.H. (1957) Proc.Soc.exp. Biol. (N.Y.) 95, 183.

____ Jackson, R. and Ashe, B. (1964). Invest. Urol. I, 555.

Kinne, R., MacFarlane, W.V. and Butz-Olsen, O.G. (1961). Nature, 192, 1084.

Kleeman, C.R., Bohaninan, J., Bernstein, D., Ling, S. and Maxwell, M.H. (1964). Proc. Soc. exp. Biol. (N.Y.) 115, 29.

Kolb, E. (1962) "Lehrbuch der Physiologie der Haustiere". Jena. Fischer.

____ (1967) "Lehrbuch der Physiologie der Haustiere" Jena. Fischer. 2nd Edition.

Kraft, H. (1964). "Labormethoden der Veterinarmedizinbei Haussäuget 2nd Edition. Published by H. Kraft, München and Leik-Druck Kelheim.

Kunkel, H.O., Whitaker, E.S., Packett, L.V.Jnr. and Crookshank, H.R (1961). J. Anim. Sci. 20, 940.

Lagergren, C. (1956) Acta. Radio Suppl. No. 153.

Lamprecht, W.O., Darroch, J.G. and Crookshank, H.R. (1969) J. Anim. Sci. 28, (3) 386.

Langham, R.F. and Hallman, E.T. (1941) J. Amer. vet. med. Assoc. 99, 471.

Lathem, J.E. and King, J.S. Jnr. (1963) J. Urol. 89, 541.

____ ____ and Stanton, J. (1963) Ibid 89, 871.

Latimer, J.K. (1948) Amer. J. Med. 5, 256.

Laughlin, V.C. (1944) Ohio State Med. J. 40, 1147.

Leblanc, P. and Sevigne, H. (1899) J. Med. veter. 50, 703. quoted by Habisreitinger (1961).

Lichtenstein, R. (1926) Wien. med. Wschr. 39, 1156.

Lichtwitz, L. (1944) The formation of Concretions" in "Colloid Chemistry", edited by J. Alexander, New York, Reinhold.

Lillie, R.D. (1954) "Histopathologic technique and practical histochemistry". New York. Blakiston.

Lindley, C.E., Tayson, E.D., Ham, W.E. and Schneider, B.H. (1953) J. Anim. Sci. 12, 704.

Linton, R.G. (1927) "Animal Nutrition and Veterinans Dietetics", Edinburgh, Green.

Lonsdale, K. (1965). Personal communication.

Lueker, C.E. and Lofgren, G.P. (1961) J. Nutr. 74, 233.

MacFarlane, W.V., Morris, R.J.H. and Howard, B. (1956). Nature 178, 304.

McCarrison, R. (1931). Brit. Vet. J. 87, 411.

McGregor, J., Nordin, B.E.C. and Robertson, W.G. (1965). Nature, 207, 861.

McIntosh, G.H. (1966). J. Agric. South Austral. 69, 316.

McQueen, E.G. (1962) J. Clin. Path. 15, 367.

Marek, J. and Mocsy, J. (1958) "Lehrbuch der Klinischen Diagnostik der inneren Krankheiten der Haustiere". Jena. Fischer. 5th Edition.

Marsh, H. (1961) J. Amer. vet. med. Ass. 139, 1019.

____ (1965) "Sheep Diseases" 3rd Edition. Baltimore, Williams and Wilkins.

____ and Safford, J.W. (1957) J. Amer. vet. med. Ass. 130, 342

Martin, J.K. (1969) Personal communication

May, N.D.S. (1964) "The Anatomy of the Sheep." 2nd Edition St. Lucia Univ. of Queensland.

Mia, A.S. and Cornelius, C.E. (1964) Invest. Urol, I, 439.

Michael, L.B. (1910) Iowa Exp. Sta. Bull. 112.

Milks, H.J. (1935) Cornell Vet. 25, 153.

Morrison, F.B. (1959) "Feeds and Feeding" 22nd Edition. Clinton Iowa. Morrison Publ. Co.

Miller, G.H. Chapman, W.H., Seibutis, L. and Vermeulen, C.W.(1956) J. Urol 76, 42.

Ministry of Agriculture (1964) Animal Disease Surveys. Report No.3. Analysed results of post-mortem examination of Sheep. 1959-61. H.M.S.O. London.

Moussu, G. (1923) "Les Malades du Mouton". Paris. Vigot Frères.

Nairn, W. (1939) Brit. vet. J. 95, 360.

National Research Council (1957) Nutrient requirements of domestic animals. No.5. Sheep. Nat. Acad. Sci. Nat. Research Council Publ. No. 504.

Newsom, I.E. (1938) J. Amer. vet. med. Ass. 92, 495.

——— Tobiska, J.W. and Osland, H.B. (1943). Colorado Agr Exp. Sta. Bull. 31.

Nordstoga, K. (1962) Nord. Vet.Med. 4, 280.

Nutbourne, D.M. and De Wardener, H.E. (1960) Clin. Sci. 20, 63.

Oehme, F.W. and Tillmann, H. (1965). J. Amer.vet.med. Ass. 147, 1331.

Oppenheimer, G.D. and Pollock, H. (1937). J. Amer. med. Ass. 108,

Oppermann, I. (1950). "Lehrbuch der Krankheiten des Schafes". 2. Aufl. Hannover, Schaeper.

Osborne, T.B. and Mendel L.B. (1917) J. Amer.med.Ass. 69, 32.

Packett, L.V. and Coburn, S.P. (1965) Amer.J.vet.Res. 25, 1508.

——— and Hauschild, J.P. (1964)J. Nutr. 84, 185.

——— Lineberger, R.O. and Jackson, H.D. (1968). J. Anim. Sci. 27, 1716.

Passmore, R. (1953) Lancet I, 638.

Peacock, M., Hodgkinson, A. and Nordin, B.E.C. (1967), Brit. med. J. 3, 469.

Pearse, A.G. (1954) "Histochemistry". London. Churchill.

Peirce, A.W. (1945) Aust. J. exp. Biol.Sci. 23, 295.

Petrichev, M. (1964) Nauch. trud. Vissh. vet.med. Inst.Sofia, 12, 111.

—— Lazarn, V. and Sotirov, N. (1958) Izv. Inst.Pat. Zhivotni Sofia, 7, 149.

Piper, C.S. (1950). "Soil and Plant Analysis". Adelaide, Univ. of Adelaide.

Pontius, B.E., Carr, R.H. and Doyle, L. (1930). J. Agric. Res. 42, 7.

Pope, G.S. (1963). In Annual Report, N.I.R.D., Shinfield, Reading, 63.

Potter, B.J. (1961). Aust. J. Agr. Res. 12, 440.

—— (1963) Ibid. 14, 518.

Preston, T.R. (1962) Personal communication

—— Greenhalgh, I. and McLeod, N.A. (1959). Anim. Prod. I, 135.

—— —— —— (1960) Ibid. 2, 11.

Prien, E.L. (1949) J. Urol, 61, 821.

—— (1955) Ibid. 73, 627.

Puntriano, G.O. (1954) J. Amer. vet. med. Ass. 124, 55.

Pyrah, L.N. (1958) Proc. Roy. Soc. Med. 51, 183.

—— and Raper, F.P. (1955) Brit. J. Urol. 27, 333.

Randall, A. (1937) Ann. Surg. 105, 1009.

Rammelkamp, C.H. (1967). Aetiology of glomerulonephritis in "Renal Disease", 2nd Edition, Ed. D.A.K. Black, Oxford, Black

—— (1940) J. Urol. 44, 580.

Read, R. (1840) Veterinarian 8, (new series) 160.

Reinhold, J.G. (1953) "Standard methods in clinical chemistry". Edited M. Reiner, Vol.1. New York. Academic Press.

Reynolds, P.J. and Lindahl, I.L. (1968) J. Anim. Sci. 27, 1516. (Abstract)

Robbins, J.D., Kunkel, H.O. and Crookshank, H.R. (1965) Ibid. 24, 76.

Rook, J.A.F., and Storry, J.E. (1962) Nutr. Abstr. Rev. 32, 1055.

Rosenheim, M.L. (1963) Br.med. J. I, 1433.

Schaffrath, W. (1941) "Versuche zur Diagnose und Behandlung mechanischer Harnabflusshindernisse in der Harnröhre bei Schafböcken". Dissertation. Berlin.

Scheunert, A. and Trautmann, A. (1965) "Lehrbuch der Veterinärphysiol Berlin, Parey.

Schilling, E. (1962) Zbl. Vet.Med. 9, 282.

Schmidt, H. (1941) Am. J.vet. Res. 2. 373.

Schmidt-Nielsen, K. (1964) "Desert Animals". London. Oxford Universi Press.

Schneider, B.H., Taysom, E.D. and Ham, W.E. (1952). Washington. State College, Agr. Exp. Sta. Circ. 205.

Smith, H.A. and Jones, T.C. (1961) "Veterinary Pathology", 2nd Editi London, Bailliere, Tindall and Cox.

Smith, R.H. (1969) Proc. Nutr. Soc. 28, 151.

____ and McAllan, A.B. (1966) Br. J. Nutr. 20, 703.

Shirley, R.L., Easley, J.F., Ammerman, C.B., Kirk, W.G., Palmer, A.Z. and Cunha, T.J. (1964) J. Anim. Sci. 23, 298. (Abstr).

Sisson, S. (1938) "Anatomy of Domestic Animals". Philadelphia. W.B. Saunders.

Spooner, W.C. (1844) "Diseases of the Sheep". 2nd Edition. London. Simpkin, Marshall & Co.

Stacy, B.D. (1969) Q.Jl. exp. Physiol. 54, 1.

____ and Brook, A.H. (1964) Aust. J. Agr. Res. 15, 289.

Stewart, J. (1929) Vet.Rec. 9, 443.

Steyn, D.G. and Reinach, N. (1939). Onderst. J. Vet. Sci. 12, 167.

Steiner, M. Zuger, B. and Kramer, B. (1939) Arch. Path. 27, 104.

Strauss, M.B. and Welt L.G. (1963) "Disease of the kidney". London. Churchill.

Sutherland, A.K. (1958). Aust. Vet. J. 34, 44.

Suttle, N.F.& Field, A.C. (1967) Br. J. Nutr. 21, 819.

Swingle, K.F. and Marsh, H. (1953). Am. J. vet. Res. 14, 16.

____ ____ (1956) Ibid. 17, 415.

Tas, D. (1963). Personal communication.

Tayson, E.D. (1961) "Urinary calculi in sheep". Thesis. Michigan Sta University.

____ Lindley, C.E., Schneider, B.H. and Ham, W.E. (1951) Washington Agr. Exp. Sta. Circ. 162.

Telle, P.P., Preston, R.L., Kintner, L.D. and Pfander, W.H. (1964) J. Anim. Sci. 23, 59.

Thomas, W.C.Jnr., Bird, E.D. and Tomita, A. (1963) J. Urol. 90, 521.

—— and Howard, J.E. (1959) Trans. Assoc. Amer. Physiol. 72, 181.

Tindal, J. (1842) Veterinarian 15, 150.

Tribe, D.E. (1949) Engl. J. Exp. Agric. 17, 105.

Udall, R.H. (1959a). Am. J. vet. Res. 20, 423.

—— (1959b) Ibid. 20, 426.

—— and Chen-Chow, F.H. (1963). Ann. N.Y. Acad. Sci. 104, (

—— —— (1969). Adv. Vet. Sci. 13, 29.

—— Deem, A.W. and Maag, D.D. (1958). Am. J. vet. Res. 19, 825.

—— and Jensen, R. (1958) J. Am. vet. med. Ass. 133, 514.

—— Seger, C.L. and Chen-Chow, F. (1965), Cornell Vet. 55, 198.

Underwood, E.J. (1941). Aust. Vet. J. 17, 202.

Van Leersum, E.C. (1928). J. Biol. Chem. 76, 137.

Varley, H. (1964). "Practical Clinical Biochemistry". London. Heinemann.

Vermeulen, C.W., Goetz, R., Ragin, H.D., and Grove, W.J. (1950). J. Urol. 66, 6.

—— C.W. Lyon, E.S., Ellis, J.E. and Borden, T.A. (1967) Ibid 97, 573.

Vermeulen, C.W., Lyon, E.S., and Miller, G.H. (1958). Ibid. 94, 576.

Vermeulen, C.W., Lyon, E.S. and Fried, F.A. (1969) Ibid. 104, 176.

Walser, M. (1961) J. Phys. Chem. 65, 159.

Watchorn, E. (1932) J. Hyg. 32, 156.

——— and McCance, R.A. (1937). Biochem. J. 31, 1379.

Weaver, A.D. (1963) Vet. Rec. 75, 300.
——— (1966) Ibid 78, 820.
——— (1969) Ibid. 84, 320.

Whedon, G.D. and Shore, E. (1957) J. Clin. Invest. 36, 966.

Wiggins, E.L. and Terrill, C.E. (1953) J. Anim. Sci. 12, 524.

Wilkinson, W.S., O'Mary, C.C., Wilson, G.D., Bray, R.W., Pope, A.L. and Casida, L.E. (1955) Ibid 14, 866.

Williams, W. (1884). "Principles and Practice of Veterinary Medicine" 5th Edition. Edinburgh. Menzies.

Witzigmann, - (1940). "Harnuntersuchungen bei Haussäugetieren". Hannover, Schaper.

Youatt, W. (1893) Quoted by Pontius, Carr and Doyle (1930).

Young, V.R., Lofgreen, G.P. and Luick, J.R. (1966). Br. J. Nutr. 20, 795.

——— Richards, W.P.C., Lofgreen, G.P. and Luick, J.R. (1966) Br. J. Nutr. 20. 783.

Zweifach, B. (1959) In "The Arterial Wall", edited by A. Lancing, Baltimore. Williams and Wilkins.

APPENDIX

1. ANALYTICAL METHODS

ANALYTICAL METHODS FOR FOOD

Analysis was carried out on the fresh food which was ground through a Christy and Norris mill. If necessary, the food samples (i.e. hay, straw, etc.) were first dried (95-100°C) before grinding, and moisture content calculated from these figures. Estimations were made on dry, ashed material.

Calcium was estimated using an oxalate precipitation technique and potassium permanganate titration (Piper, 1950).

Magnesium was estimated colorimetrically using Titan yellow (Cornfield and Pollard, 1950).

Phosphorus was estimated using the Lorenz sulphate-molybdic acid reagent (Piper, 1950).

These methods are fully described below.

ESTIMATION OF CALCIUM IN FOOD AND FAECES (Piper, 1950)

The aliquot of the silica-free solution of food or faeces ash was taken from about 5 g original material, and was placed in a 250 ml beaker to which was added 5-10 ml concentrated HCl and 1 drop of methyl red indicator solution.

1. The solution is neutralised in the cold by the addition of dilute (1 + 4) ammonia solution, stirring well to avoid local precipitation of Ca and Mg phosphates through excessive local alkalinity.

2. Dilute (1 + 4) HCl solution is carefully added until the indicator just turns back to red.

3. After 5-10 minutes, 10 ml dilute (1 + 19) HCl solution is added, followed by 10 ml oxalic acid solution.

4. The mixture is boiled and then, using a dip pipette, 10 ml hot ammonium oxalate solution is added drop by drop, with constant stirring.

5. Boiling is continued for 1-2 minutes to assist formation of the coarse-grained precipitate which may not always take place at this stage.

6. The solution is allowed to cool, and then neutralised to pH 5 to complete precipitation of calcium oxalate. Sodium acetate is added drop by drop, stirring well, until the indicator is orange-pink: if too yellow, a few drops of acetic acid are added to produce the correct colour.

7. The solution pH is adjusted to pH 5, allowed to stand overnight and filtered through an 11 cm Whatman No. 44 filter paper. The precipitate is washed until chlorine-free. If too much magnesium is present in the original sample, the precipitate is washed in dilute HCl solution, cooled, neutralised and reprecipitated as in the first precipitation.

8. The precipitated calcium oxalate is washed from the filter paper, and an excess of dilute (1 + 4) sulphuric acid and warm water is added until all the oxalic acid is in solution. The solution is warmed to 70°C and titrated with 0.1 N potassium permanganate.

Finally, the filter paper is added and the solution is stirred until the colour is discharged and titration is continued to the final end-point.

1 ml 0.1 N $KMnO_4$ = 0.0020 g Ca.

ESTIMATION OF MAGNESIUM IN FOOD (Cornfield and Pollard, 1950)

The aliquot of the ash solution representing 20-40 mg food or faeces is placed in a large test tube.

1. 1 ml of "Magnesium Compensating Solution" is added to the aliquot. (This solution is prepared as follows : 13.9 g of pure anhydrous CaCl and 0.88 g of potash alum are dissolved in water, and 0.96 g of potassium dihydrogen phosphate added and the volume made up to 1 litre. The solution contains 5 mg Ca, 500 µg P_2O_5 (as PO_4) and 50 µg Al per ml).

2. 1 ml of freshly prepared 2.5% Mannitol solution is added to prevent interference from Mn.

3. The volume is made up to exactly 20 ml by distilled water.

4. 2 ml of a fresh 0.05% Titan Yellow solution is added and then 2 ml 3 N NaOH, after which the mixture is shaken well.

5. The mixture is left undisturbed for exactly 10 minutes, and again shaken and transferred to an 8 ml EEL colorimeter tube.

6. The reading is made immediately using a green filter No. 625, a blank of distilled water being 0.

CALCULATION

If x mg of Mg are present in the aliquot, representing y mg food or faeces, then % mg $= \frac{x}{y} \times 100$.

ESTIMATION OF PHOSPHORUS IN FOOD (Piper, 1950)

Lorenz reagent I (sulphate-molybdic acid) : dissolve 100 g ammonium sulphate in 1 litre nitric acid of S.G. 1.36 at 15°C in a 2-litre flask. Then 300 g ammonium molybdate is dissolved in hot water, transferred to a litre measuring flask, cooled to about 20°C, diluted to the mark, and mixed well. The molybdate solution is added in a thin stream, shaking well, to the contents of the 2-litre flask. It is allowed to stand for 48 hours at room temperature, filtered and stored in a stoppered reagent bottle in a refrigerator.

1. A suitable aliquot of 25 ml food or faeces ash solution (= 0.05 g original material) is transferred to a tall shaped beaker and evaporated to dryness on a hotplate.

2. 5 ml water + 5 ml conc. nitric acid are added and the mixture again evaporated to dryness.

3. The residue is dissolved in 15-15.5 ml conc. nitric acid and 34 ml water and 1 ml conc. sulphuric acid is added.

4. The mixture is heated just to boiling, stirring well to dissolve most of the calcium sulphate. The beaker is removed from the flame, stirred for 10 seconds to cool the overheated sides of the beaker, and 50 ml of the Lorenz reagent is rapidly added, the contents are stirred, the beaker is covered and left to stand at least 2 hours.

5. The beaker contents are filtered through a Gooch crucible fitted with a small Whatman No. 42 filter paper to cover the holes only. (The crucible and filter paper are dried for 15 minutes in an oven before use).

6. The precipitate is washed 4 times with 2% ammonium nitrate made just acid to litmus with a drop of nitric acid if necessary.

7. The precipitate is washed 3 times with acetone and air is drawn through the crucible for $\frac{1}{2}$ - 1 minute.

8. The crucible is finally placed in a dessicator, without dehydrating agents, evacuated to about 200 mm and left for 30 minutes before weighing.

CALCULATION

The amount of precipitate is multiplied by 0.0144 to give the amount of P.

ESTIMATION OF CALCIUM IN BIOCHEMICAL FLUIDS BY THE ETHYLENEDIAMINE TETRA-ACETIC ACID (E.D.T.A.) PROCEDURE

Principle

Ammonium purpurate produces a red colour with calcium ions. The mixture is titrated with E.D.T.A., which forms a stable unionised complex with the calcium ions. When all the free calcium ions have been bound in this way, the indicator returns to its original blue colour. The end point is detected on the galvanometer of the EEL titrator. Magnesium does not interfere at the degree of alkalinity produced by 6 ml $\frac{N}{10}$ NaOH. Phosphate interferes only above 12 mg%. Removal of the phosphate from urine by means of an ion exchange resin may be necessary.

Method

1. Into a 10 ml cuvette was pipetted 0.5 ml serum, 3.0 ml ammonium purpurate solution, 6.0 ml $\frac{N}{10}$ NaOH (freshly prepared). A small metal stirrer platform of the EEL titrator. Switch on stirring mechanism. The tip of an automatic 2 ml reservoir burette - E-ml G.S.2852 - containing 0.036% E.D.T.A. solution was placed just below the surface of the solution in the cuvette. Care was taken to avoid obstructing the light path through the cuvette.

2. A 606 yellow filter was placed in the titrator, and by turning the sensitivity control clockwise, the galvanometer was set to 100 on the lower (linear) scale.

3. The mixture was titrated with the E.D.T.A. until the galvanometer spot which slowly moves to the left during the titration, remained stationary. The titration was started by adding 0.1 ml volumes of E.D.T.A. and reduced to volumes of 0.01 ml as the end point approached.

4. A blank, and a 10 mg% Ca standard solution were titrated as above using 0.5 ml 10 mg% Ca standard respectively. Since the method is linear up to at least 40 mg% only one standard solution was necessary.

5. The result in mg% was calculated from the formula below, where 0.5 ml of both unknown and standard was titrated.

$$\frac{\text{Titration of unknown} - \text{titration of blank}}{\text{Titration of standard} - \text{titration of blank}} \times 10$$

DETERMINATION OF PLASMA PHOSPHORUS BY THE METHOD OF FISKE AND SUBBAROW (1925)

1. There was pipetted 6 ml distilled water, 0.3 ml plasma and 1.2 ml 2% trichloracetic acid into a 15 ml centrifuge tube. After mixing well, it was allowed to stand for a few minutes and centrifuged at 3000 rpm for 5 minutes.

2. Then 5 ml of the clear supernatent was pipetted into a clean, dry test tube and had added 0.8 ml of 5% ammonium molybdate in 15% sulphuric acid, followed by 0.2 ml amino-naphtholsulphuric acid. It was allowed to stand exactly 5 minutes and compared in either the EEL colorimeter with filter 205 or the spectrophotometer model 600 (Unicam) at wavelength 680 mμ.

3. Preparation of the standard. Take 5 ml of standard phosphate solution (0.02 mg P in 5 ml) instead of the 5 ml of clear supernatent and proceed as above.

4. Preparation of the blank. Take 5 ml of distilled water in place of the clear supernatent and proceed as above.

5. The result in mg per 100 ml was calculated as below:

$$0.02 \times \frac{\text{test}}{\text{standard}} \times \frac{7.5}{5} \times \frac{1}{0.3} \times 100$$

$$= \frac{\text{test}}{\text{standard}} \times 100$$

DETERMINATION OF BLOOD UREA BY UREASE NESSLERISATION METHOD (Varley, 1964)

Principle

The sample of blood is incubated with the enzyme urease which converts the urea to ammonia. The proteins are precipitated and the colour produced with Nessler's reagent compared colorimetrically with the colour produced under the same conditions with a standard urea solution.

Method

1. Test solution : add 0.2 ml plasma to a 15 ml tapered centrifuge tube containing 3 ml isotonic sodium sulphate solution.

2. Similar quantities of a standard urea solution and of distilled water are prepared in the same way.

3. Tubes are placed in water bath which is heated to 37°C.

4. Add to each tube 0.2 ml of urease suspension prepared by grinding one urease tablet in 5 ml of 30% methanol. Stopper tubes with rubber bungs, mix and incubate for 20 minutes at 37°C.

5. To each tube add 0.3 ml of zinc sulphate solution and 0.3 ml of 0.5 N sodium hydroxide to precipitate proteins, mixing by inversion after each addition.

6. Centrifuge or filter through a small (7 or 5.5 cm) Whatman No. 41 filter paper. Treat 2 ml of supernatent with 5 ml of ammonia-free distilled water and 1 ml Nessler's reagent and read against water immediately in the spectrophotometer model 600 (Unicam) at wavelength 450 mμ.

Urine urea is measured by the same method.

PLASMA AND URINARY SODIUM AND POTASSIUM

Plasma and urinary sodium and potassium were estimated by a Sterne photometer (EEL) technique (Varley, 1964).

PLASMA PROTEIN

Plasma protein was estimated by Biuret method, involving a modification of the method of Reinhold (1953) for total protein only.

URINE PROTEIN

Urine protein was estimated using sulfosalicylic acid precipitation method of King and Haslewood (1936).

HISTOLOGICAL TECHNIQUES

Sections of tissues were fixed in 10% formalin, trimmed after an interval varying from 1 week to 3 months, and were then put through a double embedding series, involving the following stages :

(i) 80% spirit : 4-8 hours;
(ii) 8% Phenol Meth. : 18-24 hours;
(iii) 3 changes of absolute alcohol: 2 hours each;
(iv) equal parts of absolute alcohol/amyl acetate: 1-2 hours;
(v) 3 changes amyl acetate;
(vi) 3 changes 1% Celloidin in methyl benzoate: 24 hours each;
(vii) Benzene 10-12 minutes;
(viii) 2 changes paraffin wax : 3 hours each;
(ix) embed in paraffin wax.

Staining methods employed were haematoxylin and eosin, periodic acid - Schiff (Carleton, 1957) and Von Kossa (Pearse, 1954).

2. PHOTOGRAPHIC ILLUSTRATIONS

PLATE 1. OPENED BLADDER OF RAM SHOWING HAEMORRHAGIC CYSTITIS. PROBE POINT IS SITUATED OVER RUPTURE SITE, ALMOST OCCLUDED BY BLOOD

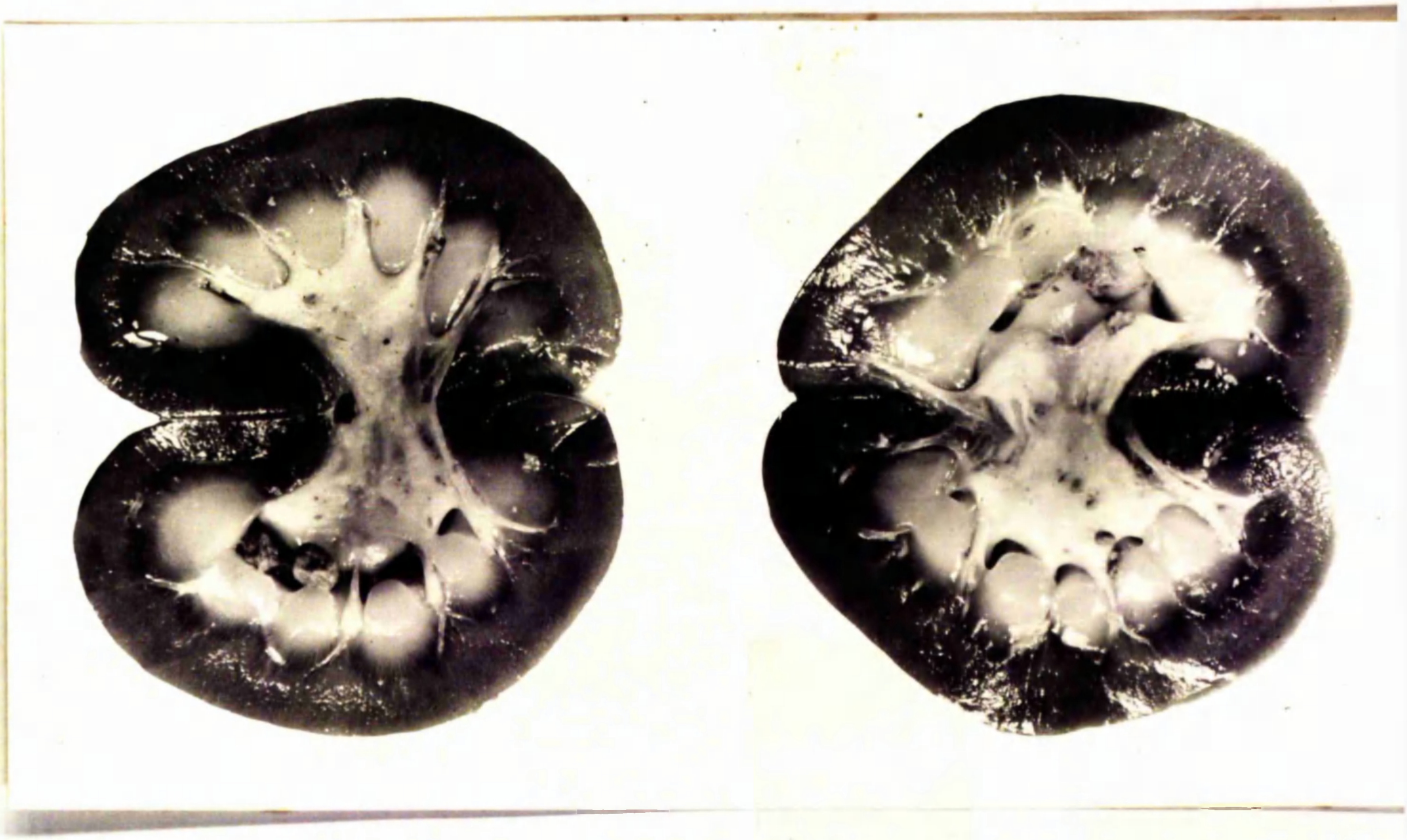


PLATE 2. LONGITUDINAL SECTION THROUGH TWO KIDNEYS SHOWING TWO DISCRETE CALCULI IN ONE CASE, AND SMALLER AMOUNTS OF CALCULUS DEBRIS IN SECOND CASE. KIDNEYS ARE MACROSCOPICALLY NORMAL (23988/19)

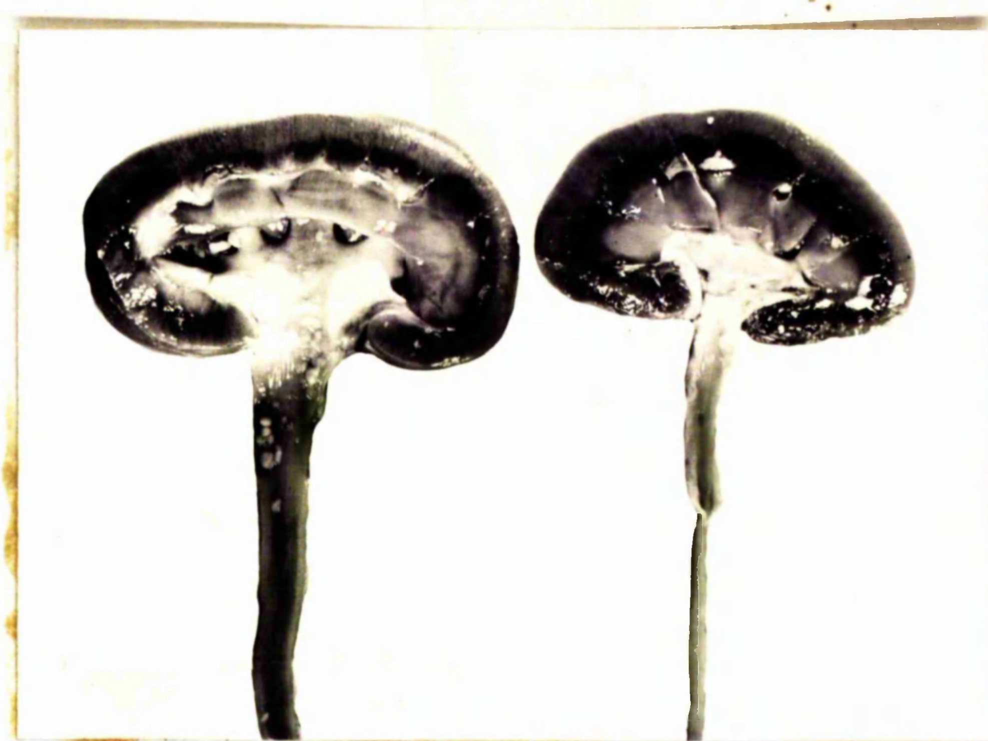


PLATE 3. LEFT: RENAL AND URETERAL CALCULI. DILATATION WITH OEDEMA AND INFLAMMATORY REACTION IN URETER. RIGHT: RENAL CALCULI WITH CONGESTION AT CORTICO-MEDULLARY JUNCTION; NORMAL URETER

PLATE 4A. KIDNEY, URETER AND BLADDER, SHOWING SEVERE HYDRONEPHROSIS, PELVIC AND URETERAL CALCULI, AND MARKED THICKENING OF THE URETER. RENAL MEDULLA IS ALMOST NON-EXISTENT (25849/2)

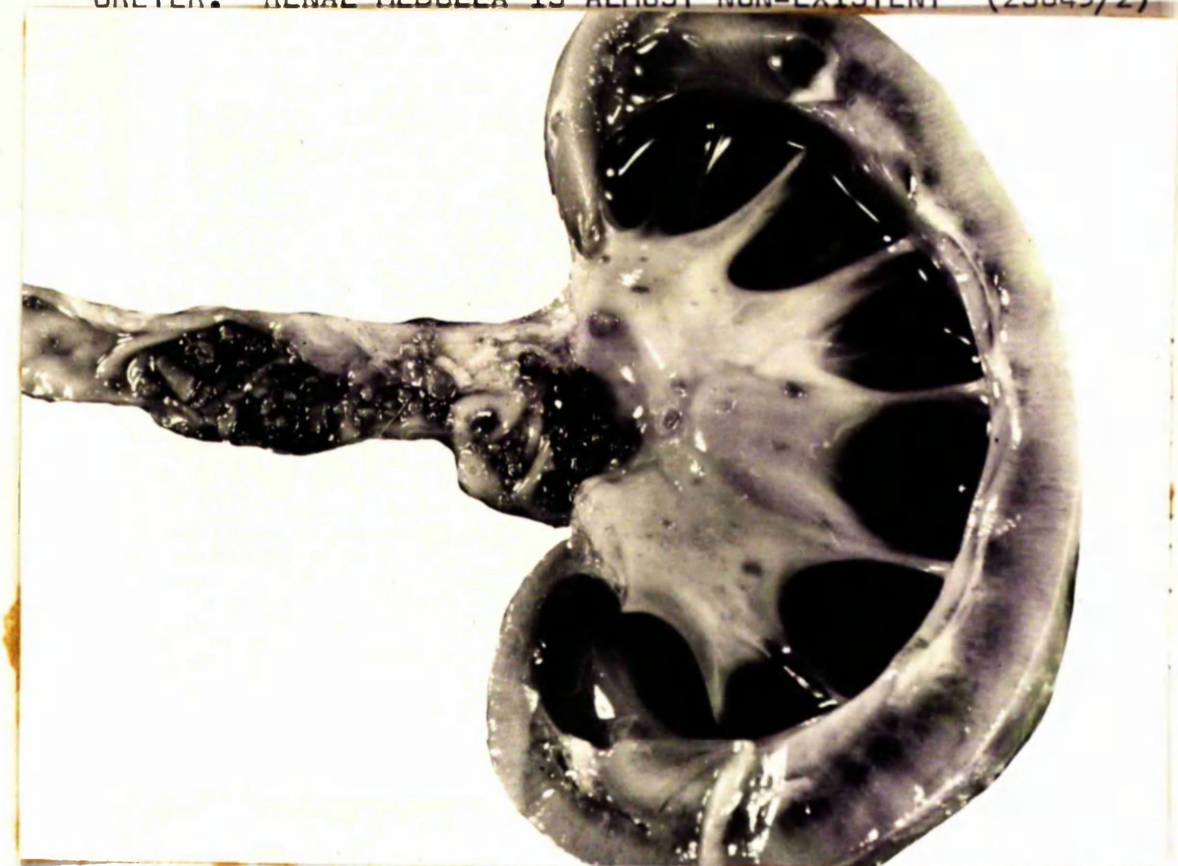


PLATE 4B. ENLARGED VIEW OF PLATE 4A, SHOWING MASS OF URETERAL CALCULI. KIDNEY APPEARS PALE AND FIBROTIC.

PLATE 5. OPENED BLADDER AND DISTAL PENIS, INCLUDING GLANS AND VERMIFORM APPENDAGE OF RAM WHICH DIED OF OBSTRUCTIVE UROLITHIASIS. A. COLLECTION OF SEMI-TRANSLUCENT CALCULI B. THICKENED AND ROUGHENED BLADDER EPITHELIUM C. SEVERAL CALCULI IMPACTED IN APPENDAGE

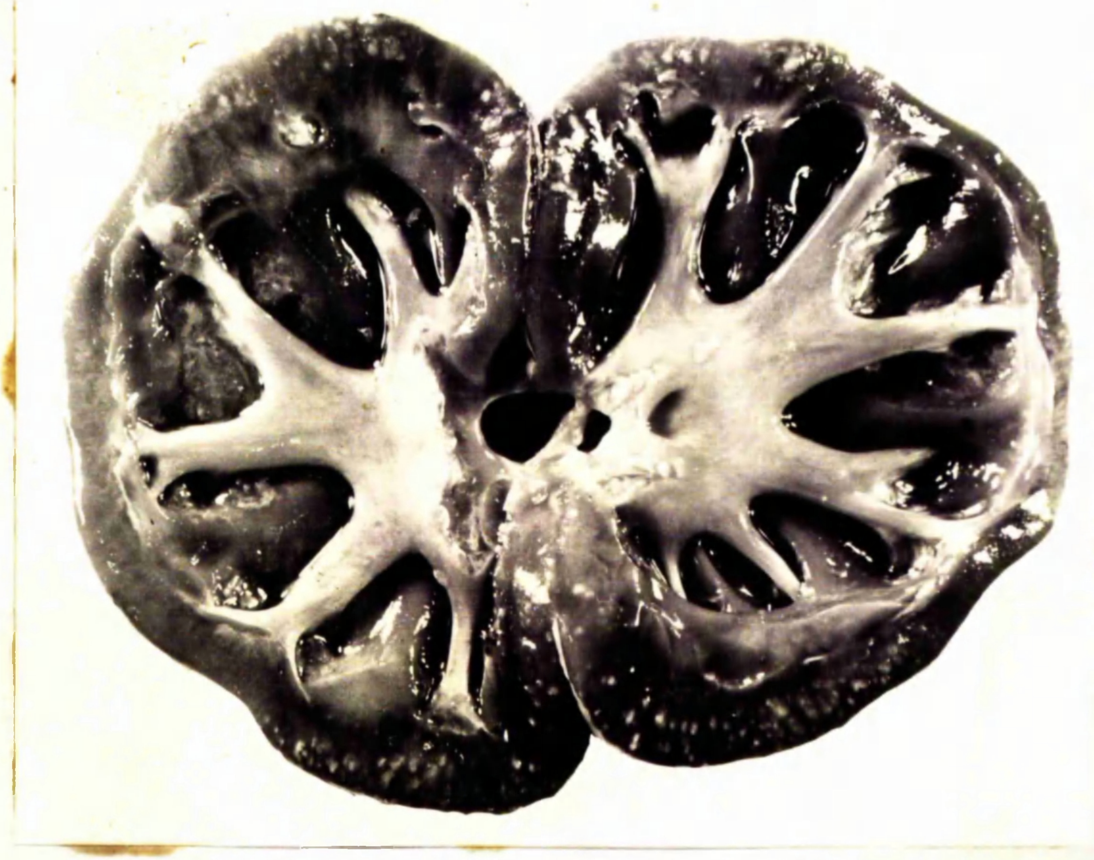


PLATE 6. LEFT KIDNEY OF LAMB WITH SEVERE HYDRONEPHROSIS, SMALL RENAL CALCULI, AND MULTIPLE SUPPURATIVE CORTICAL FOCI. SEVERE PYELONEPHRITIS. (26695/23L)

PLATE 7. RIGHT KIDNEY OF SAME LAMB, SHOWING EARLY HYDRONEPHROSIS. FAINT PALE AREAS ARE VISIBLE IN CORTEX. PYELONEPHRITIS. (26695/23R)

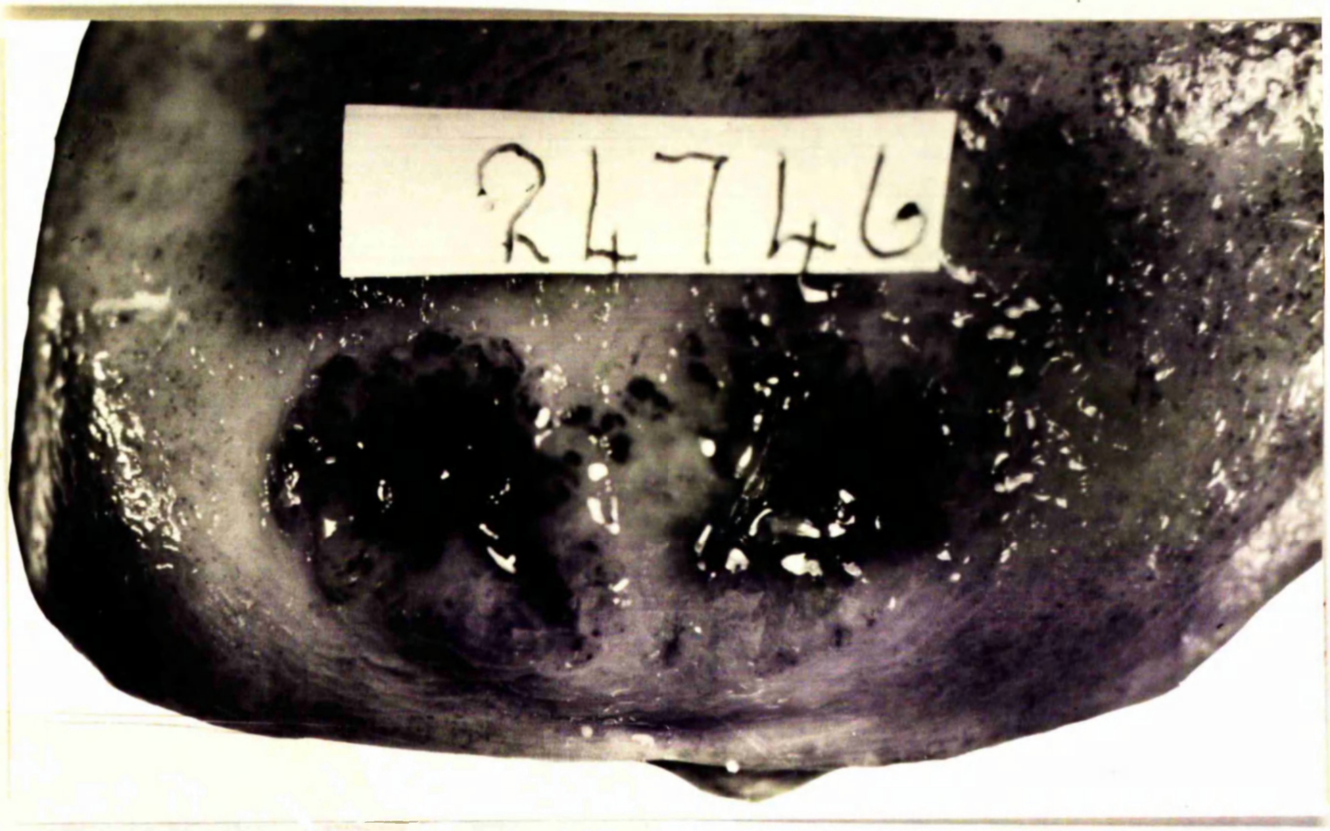


PLATE 8. BLADDER EPITHELIUM SHOWS TWO AREAS OF BLADDER WALL RUPTURE, WITH SURROUNDING HAEMORRHAGE

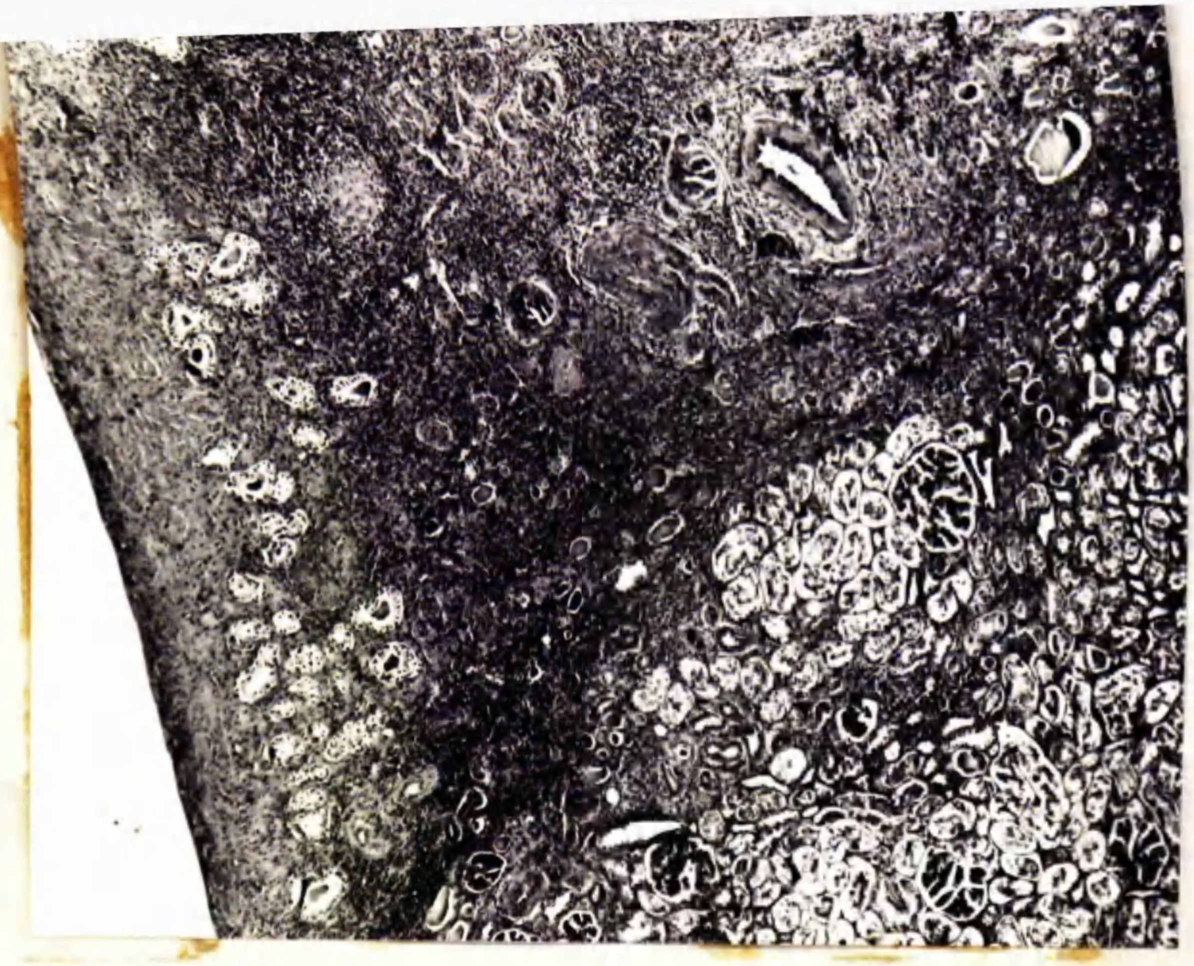


PLATE 9. SECTION OF LEFT KIDNEY SHOWING SEVERE CORTICAL FIBROSIS, AND EXTENSIVE TUBULAR DILATATION, ALSO TUBULAR CASTS AND A MONONUCLEAR CELLULAR INFILTRATION. H & E x45 (24852)

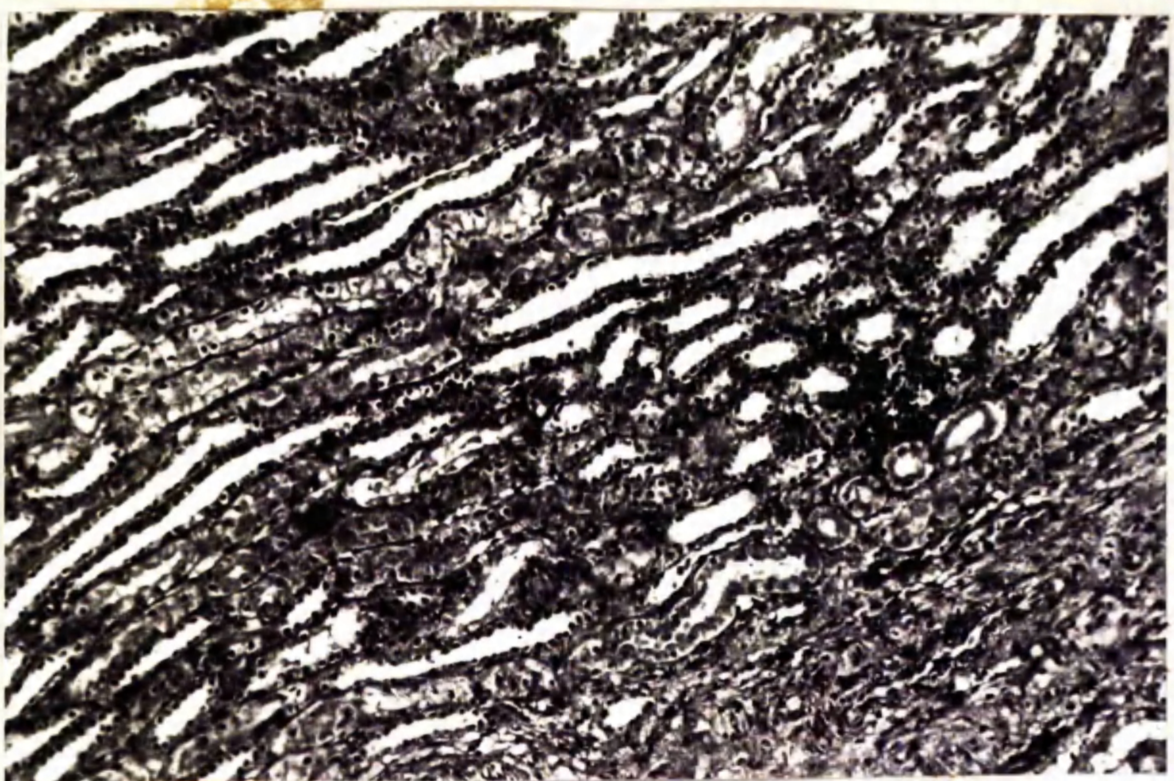


PLATE 10. SECTION OF KIDNEY SHOWING TUBULAR DILATATION INDICATIVE OF HYDRONEPHROSIS, AND MILD LYMPHOCYTE INFILTRATION. H & E. x150 (25337L)

PLATE 11. RENAL INFARCT VISIBLE ON CORTICAL SURFACE (25337)

PLATE 12. SECTION THROUGH KIDNEY ABOVE, SHOWING PALE CORTICAL AREA AT A, AND DARKER MEDULLARY AREA, B. FURTHER RECENT AND OLDER INFARCTS ARE VISIBLE AT C AND D.

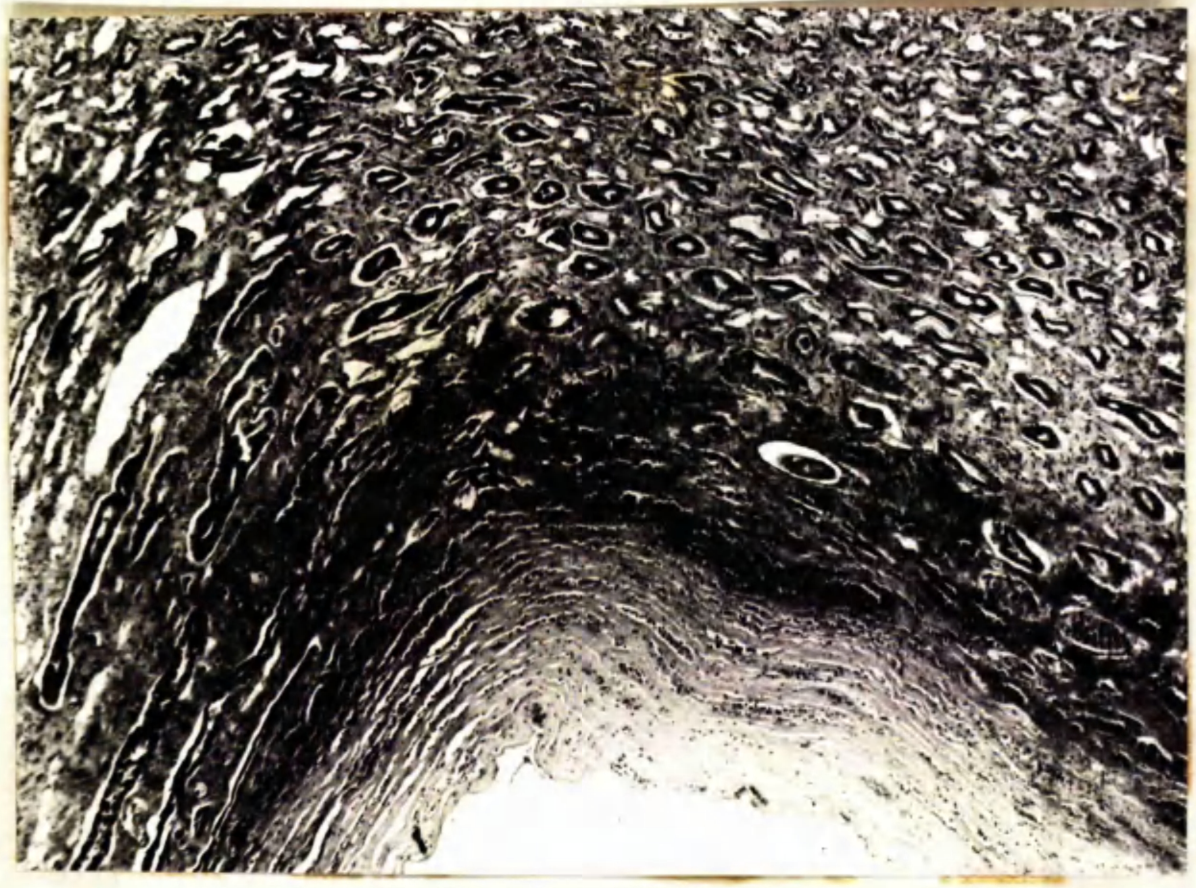


PLATE 13. SECTION OF PELVIC EPITHELIUM SHOWING NECROSIS, WITH LINE OF HAEMORRHAGE (A) DEMARCATING NECROTIC AREA. A FEW TUBULES CONTAIN CASTS. H & E x 45 (30710).

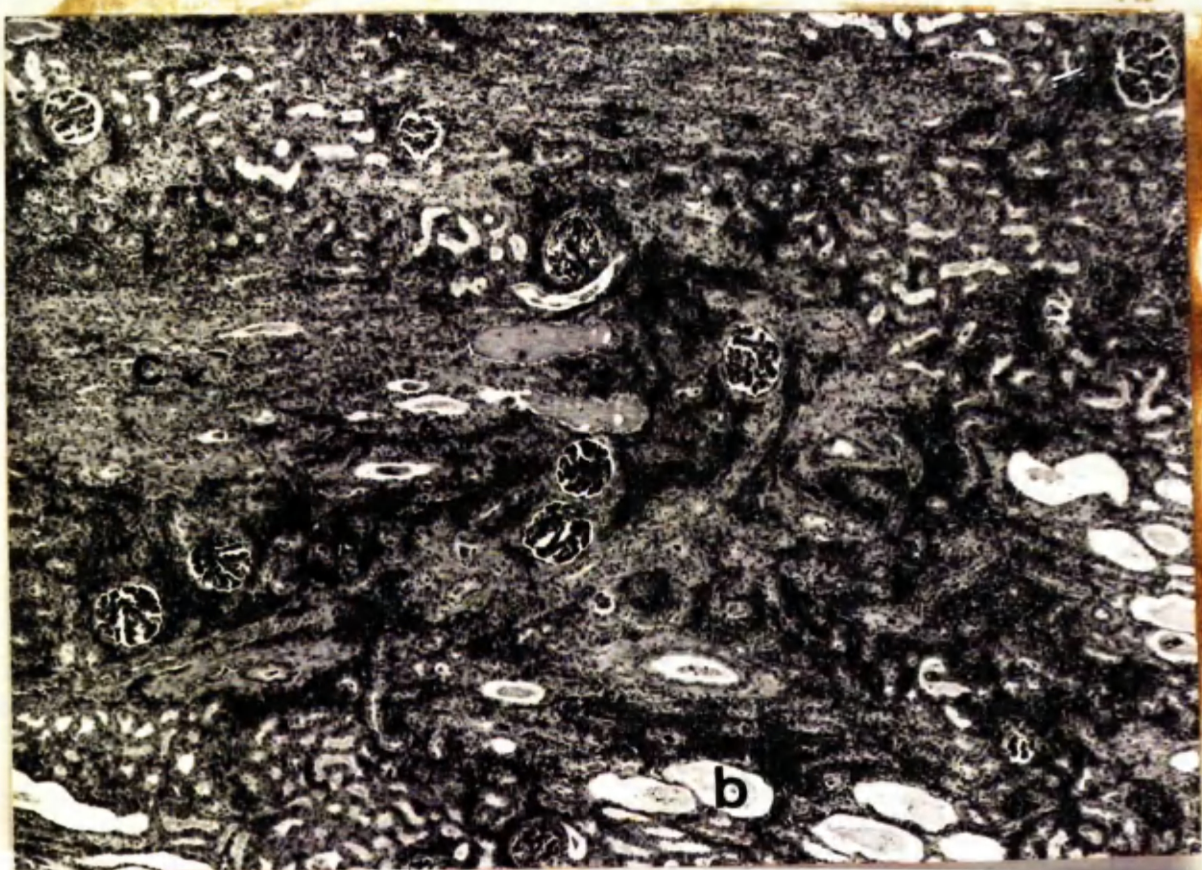


PLATE 14A. SECTION OF RENAL CORTEX SHOWING SEVERE PYELONEPHRITIS WITH AREAS OF INTENSE CELLULAR REACTION (A), TUBULAR DILATATION WITH CASTS (B), AREAS OF FIBROSIS (C) AND SOME RELATIVELY UNALTERED TUBULES (D). H & E x 45 (30859).

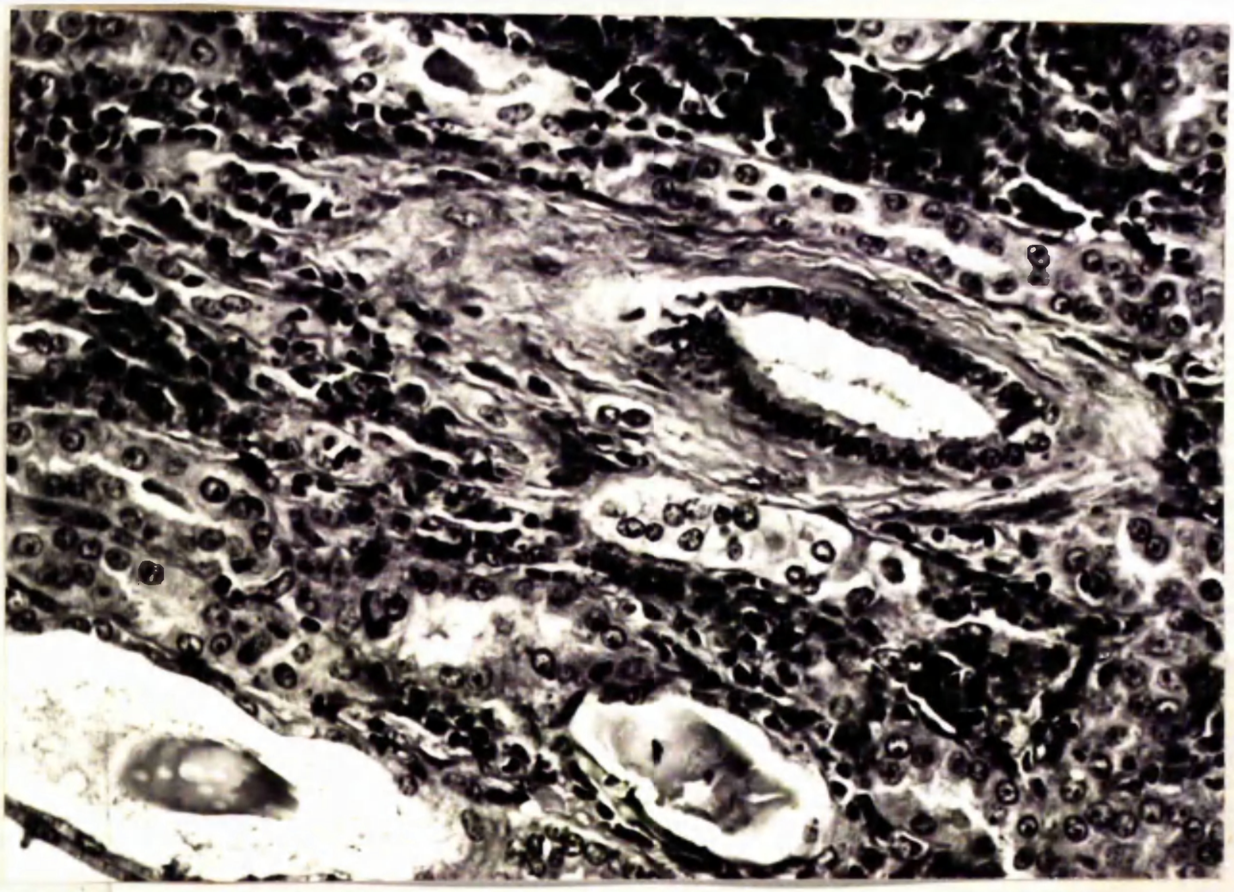


PLATE 14B. SECTION OF RENAL CORTEX WITH SEVERE PERITUBULAR FIBROSIS AND PROMINENT TUBULAR CASTS. H & E x 300 (30859).

PLATE 15. SECTION OF RENAL PELVIS WITH MODERATE PYELONEPHRITIS, INVOLVING POLYMORPH INVASION, DESTRUCTION OF GLOMERULI AND TUBULES. NOTE DEBRIS IN DILATED TUBULES. H & E x 150 (30859).

PLATE 16. SECTION OF RENAL CORTEX SHOWING ACUTE INTERSTITIAL REACTION. SOME TUBULES APPEAR RELATIVELY NORMAL. OTHERS SHOW EVIDENCE OF SURROUNDING CHRONIC INTERSTITIAL NEPHRITIS. H & E x 150 (30859).

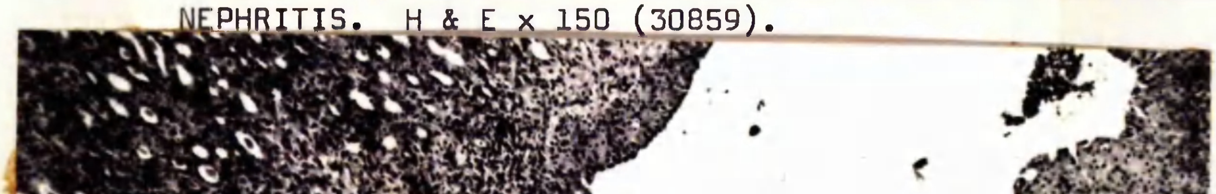


PLATE 17. PELVIC EPITHELIUM, SHOWING ACUTE PYELITIS, AREAS OF FIBROSIS (A), AND INFLAMMATORY CELLULAR DEBRIS IN LUMEN. H & E x 50.

PLATE 18. HIGH POWER VIEW OF PART OF PLATE 17, SHOWING EPITHELIAL DESQUAMATION AND CELLULAR DEBRIS. H & E x 150.

PLATE 19. PORTION OF CALYX WITH PYELITIS. FOCA. AREAS OF POLYMORPHONUCLEAR INVASION, AND DESQUAMATION OF EPITHELIUM. H & E x 50.

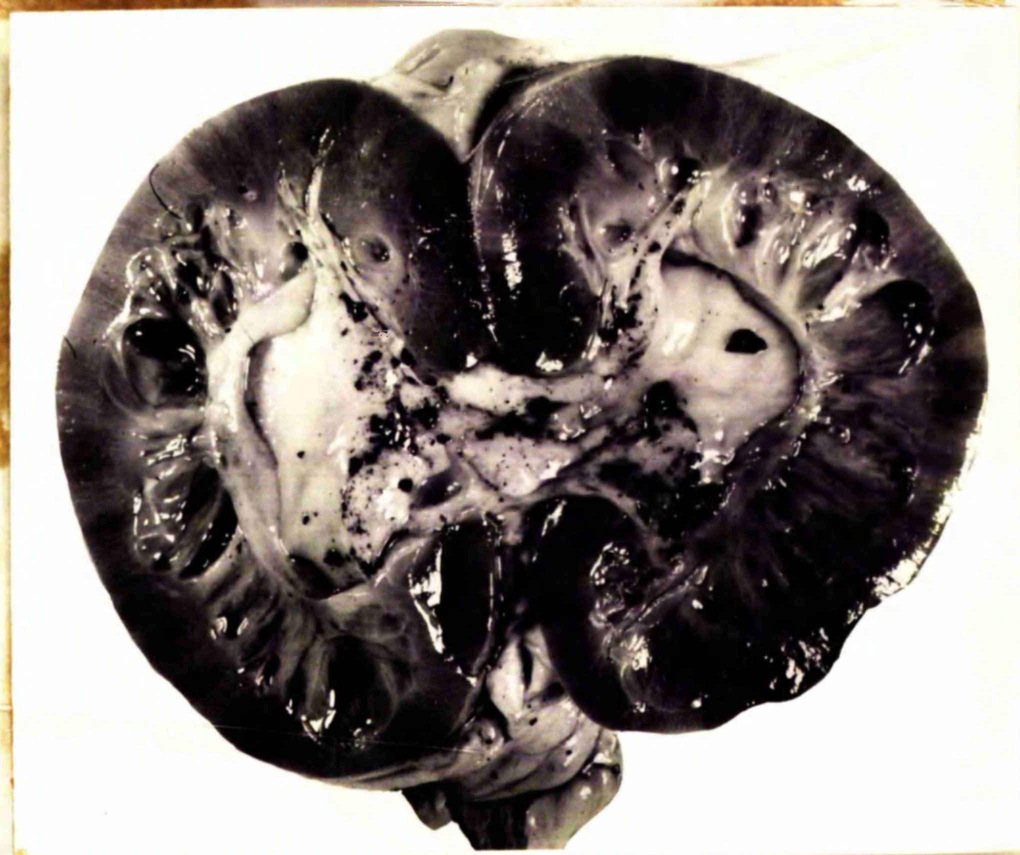


PLATE 20. DARK RED CALCULUS MATERIAL IN KIDNEY, WHICH SHOWS MILD HYDRONEPHROSIS, MULTIPLE CYST FORMATION AND PALE RADIAL STREAKS FROM CORTEX INTO MEDULLA. CALCULI WERE COMPOSED OF TRIPLE PHOSPHATE CRYSTALS, BLOOD AND CELLULAR DEBRIS. CHRONIC NEPHRITIS.