Onderstepoort J. vet. Res. (1967), 34 (2), 647–672
Printed in the Repub. of S. Afr. by The Government Printer, Pretoria

A STUDY OF THE EFFECTS OF THYRO-PARATHYROIDECTOMY IN YOUNG AND MATURE SHEEP*

PETER CHARLES BELONJE, Veterinary Research Institute, Onderstepoort

CONTENTS

INTRODUCTION

The results of thyro-parathyroidectomy in man and laboratory animals are well documented and are the subjects of review articles such as those of Geschwind (1961) and Talmage (1962). Briefly these results include hypocalcaemia, hyperphosphataemia, a decrease in both urinary calcium and phosphorus and a decreased intestinal absorption of calcium.

Classically parathormone has been considered to be formed in the parathyroid gland and released into the blood during hypocalcaemic conditions. The hormone then stimulates the osteoclastic breakdown of hydroxyapatite to release calcium and phosphorus from the bones to correct the hypocalcaemia. In addition, parathormone increases both the gut- and renal tubular absorption of calcium. Concurrently it has a phosphaturic effect to rid the body of excess phosphate (Guyton, 1966). However, recently a second hormone, thyrocalcitonin, has been isolated from the thyroid gland. In a comprehensive review of the literature by Munson (1966), it appears that this hormone is stimulated by hypercalcaemic conditions and it lowers plasma calcium by rapidly inhibiting bone resorption, whether this resorption was stimulated by parathormone or not. However, synergistically with parathormone, thyrocalcitonin has a hypophosphataemic effect which has been demonstrated (Milhoud & Moukhtar, 1966; Robinson, Martin & MacIntyre, 1966) to be as a result of a phosphaturic effect.

* Submitted in partial fulfilment of the requirements for the degree of M. Med. Vet. (Physiol.), in the Faculty of Veterinary Science, University of Pretoria, Pretoria, December 1966

Received for publication on 12 April 1967.—Editor

Except for the goat, the effects of thyro-parathyroidectomy in the ruminant species in general are scanty but I quote Payne & Chamings (1964): " In summary, the literature on thyro-parathyroidectomy in ruminants suggests that: (1) hypocalcaemia occurs regularly, (2) tetany is rare except in young calves, (3) the effect of the operation on the plasma inorganic phosphate is variable, (4) there is no evidence concerning the effect of the operation on plasma magnesium or the way in which the loss of parathyroid glands might affect the future health of the animal ".

Using thirty-two goats these workers demonstrated that thyro-parathyroidectomy resulted in hypocalcaemia, hypophosphataemia (contrary to man and laboratory animals) and transient hypomagnesaemia. Long term effects included poor growth and decreased bone density in young goats and abnormalities of gestation and lactation in female goats. Tetany occurred only rarely but there was a high incidence of a " drowsiness syndrome ".

The work on goats was continued by Payne & Sansom (1966) who, by employing stable calcium balance techniques and the isotopes ^{45}Ca, ^{47}Ca, and ^{85}Sr, found that whereas thyro-parathyroidectomy resulted in a decreased gastrointestinal absorption of calcium there was no change in either the endogenous intestinal secretion or the urinary calcium excretion. These animals, whether receiving thyroxin sodium or not, were in a negative calcium balance, whereas normal or thyroidectomized animals were in a positive balance. Bone calcium resorption was greater in thyro-parathyroidectomized animals whether receiving thyroxin or not, whereas thyroxin supplements restored the reduced bone accretion rates in both thyroidectomized- and parathyroidectomized goats.

The only work that could be traced on sheep was an article by Lotz, Talmage & Comar (1954) in which they concluded that the administration of parathyroid extract removed phosphorus but not calcium from the long bones, increased serum phosphorus, but not serum calcium levels, and caused a slight rise in urinary phosphorus excretion.

The present experiment was therefore designed to study the effects of thyro-parathyroidectomy on the plasma, urine and total balance of calcium, phosphorus and magnesium of the sheep. However, as there seems to be a difference in reaction between the young and the mature animal (Dukes, 1955), particular emphasis will be placed on the effect of age on the parameters measured. As thyroxin sodium was supplemented in the operated animals the results obtained reflect the effects of the removal of both parathormone and thyrocalcitonin.

Materials and Methods

Animals

Male Merino sheep were used throughout.

During the balance trial periods the animals were kept in metabolism cages whilst at other times they were housed in individual pens with wire mesh floors.

The feed consisted of teff hay and meal concentrates of the composition and analysis as shown in Tables 1 and 2 respectively.

TABLE 1.—*Composition of the meals used*

Ingredient	Control meal	High calcium meal	High phosphate meal
	%	%	%
Samp	88	86	86
Sunflower oil cakemeal	5	5	5
Blood meal	3	3	3
Bone meal	3	—	—
Salt	1	1	1
$CaCO_3$	—	5	—
NaH_2PO_4	—	—	5

TABLE 2.—*Analysis of foodstuffs*

Foodstuff	Ca	P	Mg
	%	%	%
Control meal	0·68	0·44	0·08
High calcium meal A*	1·60	0·18	0·06
High calcium meal B*	1·90	0·30	0·09
Low calcium meal	0·18	0·60	0·60
Teff	0·28	0·17	0·06

* Two different consignments

Although each sheep will be dealt with individually, in general the experiments comprised:—

(*a*) A preoperative balance trial of 5 days to obtain a base-line with which to compare subsequent trials.

(*b*) Thyro-parathyroidectomy within 2 days of the termination of the above trial.

(*c*) Four 5-day balance trials commencing immediately after the operation.

(*d*) Following on (*c*), a sudden change of diet to a low Ca high P meal to study the effects of such a change.

Surgical procedure

Thyro-parathyroidectomy was performed as outlined by Payne & Chamings (1964). In the four animals used in this experiment, the superior parathyroids were always found in the most anterior dorsal portion of the thymus, which lay immediately ventral to the carotid, lateral to the trachea, medial to the jugular and posterior to the mandibular salivary gland. The inferior parathyroids were found embedded in the thyroid gland.

Intravenous pentobarbitone sodium was used throughout and gave good results when the induction was very slow. To prevent excess salivation, 1/16 to 1/8 gr atropine sulphate was injected subcutaneously about 15 minutes prior to the operation. Identification of the tissues was most difficult if blood stained the area, but this was overcome by douching the site with normal saline at 37°C.

Immediately after the operation and for a further two days, daily administrations of one million units of penicillin, 1 gm streptomycin and 4 ml 5 per cent promethazine maleate (" Phenergan ", M & B) intramuscularly prevented sepsis and tissue reactions. In all cases the wounds healed by first intention.

Five milligrams thyroxin sodium was injected at weekly intervals throughout the rest of the trial (Payne & Chamings, 1964).

Collection and preparation of specimens

1. *Feed:* During the balance trials, daily intake of teff and meal was recorded. In order to obtain a representative sample, aliquots of the teff and meal being fed were periodically taken. These were then well mixed, ground fine and between 3 to 5 gm was accurately weighed and ashed overnight at 450°C. After cooling, the ash was washed into Erlenmeyer flasks with 50 ml 1N HCl, boiled vigorously for 15 minutes and cooled. This, with water washings, was filtered into a 200 ml volumetric flask and made up to the mark. This solution was then suitably diluted for each particular determination.

2. *Faeces:* The daily faecal output, collected in canvas bags carried by the animals, was dried at 100°C for 24 hours and stored. Once the trial was over, each sample was mixed well, weighed and a 1/10th aliquot taken. The five 1/10th aliquots for each 5-day period were then pooled, ground fine and well mixed. About 3 to 5 gm of this powder was then accurately weighed, ashed overnight at 450°C and treated further as described under " Feed ".

3. *Urine:* This was collected in clean plastic or glass vessels fitted with muslin covered funnels and containing 100 ml 1N HCl as preservative. The daily volume was recorded and a 1/100th aliquot was taken and frozen. At the end of the trial, the daily aliquots of each 5-day period were pooled. The pH was set at 7·5 with NaOH and they were allowed to stand overnight during which time any precipitate, which might have formed due to collection in an acid medium, had dissolved. This stock urine was then suitably diluted for each determination.

4. *Blood:* This was collected after jugular venupuncture into heparinized McCartney bottles, and then centrifuged for plasma. Plasma inorganic phosphate (Pl.I.P.), total plasma proteins (T.Pl.P.) and alkaline phosphates (A.P.) were determined immediately, while the remaining plasma was kept frozen for calcium (Pl.Ca), magnesium (Pl.Mg), sodium (Pl.Na) and potassium (Pl.K) determinations.

Analytical methods

Calcium: By precipitation with naphthalhydroxamic acid (Trinder, 1960) as described by Wootton (1964).

Phosphorus: By a variant of the phosphomolybdic acid reaction (Delsal & Manhouri, 1958) as described by Wootton (1964).

Magnesium: The titan yellow method of Neill & Neely (1956). In the determination of food and faeces magnesium, the tungstic acid precipitation step was omitted and 0·5 ml of the test solution was made up to 6 ml with water and then the gum ghatti, titan yellow and sodium hydroxide solutions were added as usual.

Alkaline phosphatase: Estimation of phenol released from enzymatic hydrolysis of phenyl phosphate (Wootton 1964). In the determinations mentioned above a Unicam S.P. 500 spectrophotometer was used.

Sodium and potassium: Were determined using an E.E.L. flame photometer (Model A) employing the methods described by Wootton (1964).

Total plasma protein: By the biuret method of Weichselbaum (1946), read on an E.E.L. portable photoelectric colorimeter (Model B).

General: All the reagents used were of analytical quality, and de-ionized water was used throughout. All the glassware was chromic acid washed and triple rinsed in de-ionized water before use.

Results

Sheep No. 1

An eight months old ram weighing 25 Kg.

This sheep had been fed on teff *ad lib.* and about 500 gm per day of the control meal for 5 months prior to the operation on 17.2.1966. The superior parathyroids together weighed 34·2 mg, i.e. 1·37 mg parathyroid tissue/Kg body weight.

Twenty-four hours after the operation the Pl.Ca had dropped from 15·88 to 7·15 mg per cent, the Pl.I.P. had risen from 11·40 to 19·66 mg per cent and the Pl.Mg had fallen slightly from 1·87 to 1·77 mg per cent. Four hours later violent clinical tetany developed; the symptoms included frothing at the mouth, tremor in the jaw, a wild frenzied facial expression, hyperaesthesia, spasm of the limbs and arching of the back which was so severe as to cause the animal to sit like a dog with spastic hindlimbs projecting in front of and between straddled forelimbs. An infusion of 120 ml " M.F.C." intravenously and 120 ml subcutaneously relieved the symptoms, but as a precaution a further 120 ml was administered subcutaneously 14 hours later. (" M.F.C.", M & B = calcium borogluconate 20·9 per cent w/w, magnesium hypophosphite 4·2 per cent w/w, dextrose 16·7 per cent w/w). During the first five post-operative days the animal ate very little but after dosing a rumen fluid mixture (rumen fluid 650 ml, sugar 60 gm, propionic acid 5 ml, water up to 1 litre) *per os* on the fifth day, its intake gradually increased and by ten days after the operation had reached normal levels. This inappetence precluded the intended balance trial.

The sheep was maintained in good health on the original diet for a further 5 months, i.e. to 19.9.1966, when the high phosphate meal was substituted. (See Graph 7a for plasma values). During the first twenty-four hours the animal ate 200 gm of teff and 492 gm of the high phosphate meal, whereas during the following twenty-four hours the teff intake had risen to 400 gm and the meal intake had dropped to 120 gm. This selection suggested a natural tendency to decrease phosphate intake and it was decided to raise this artificially by dosing one litre of a phosphate buffer at hour 56 (18·16 gm $NaH_2PO_4.2H_2O$ + 26·52 gm $Na_2HPO_4.12\ H_2O$ up to 1 litre with water, pH 7·0, containing 5·90 gm P). At hour 72 the animal was lethargic and there was a slight tremor of the forelimbs. Gradually weakness set in and at hour 79 the animal was unable to stand or move but could pick up its head. At hour 96 this " downer " syndrome had advanced to complete flaccid paralysis of the whole body and neck; the eyelids responded slowly to the corneal reflex, the ears did not twitch and respiration (± 35/minute) and heartbeat (± 40/minute) were barely discernible. Analyses showed that while both Pl.Na and Pl.I.P. were elevated, Pl.Ca, Pl.K and Pl. Mg were lowered. It was therefore decided to first administer potassium *per os* and follow this a few hours later with calcium borogluconate intravenously. The effect of dosing 25 gm KCl in one litre of water was remarkable as, within five minutes of dosing, the head was raised, the corneal reflex

was normal, the animal looked alert, flicked flies away from its ears but was unable to rise. During the next hour the respiratory rate had increased to a panting 100/minute, the heartrate had increased to 120/minute and the animal even nibbled at some teff. Blood specimens, however, actually revealed a decrease in P1.K from 2·9 to 2·7 m. eq/litre.

At hour 101, 150 ml " Calcium borogluconate " (M & B 22·5 per cent w/w) was administered intravenously over a period of forty minutes. Within five minutes of completion of the infusion the animal staggered up and attempted to walk. There was an intense spasm in extension of all four limbs, but a flexion of the phalanges with the result that it had to walk on its fetlocks. A few seconds later it collapsed and, while the heart had stopped beating, spasmodic breathing continued for about two minutes. Blood taken ten minutes after death showed a rise in P1.K, P1.I.P. and a tremendous rise in P1.Ca to 79 mg per cent.

A subsequent post-mortem examination failed to reveal any remaining parathyroid tissue nor any macroscopic lesions.

Sheep No. 2

A nine months old ram weighing 39 Kg

After the experience of postoperative tetany with Sheep 1 it was decided to use a young animal which had previously been kept on a high calcium diet. In this case, Sheep 2 had been maintained on a diet of teff *ad lib.* and about 500 gm per day of the high calcium meal A for six months prior to the experiment. This proved to be successful as, although there was a sharp postoperative drop in P1.Ca, tetany did not develop after the operation on 7.3.1966, and it was possible to complete the planned schedule of one 5-day preoperative and four 5-day postoperative balance trials on the diet of teff *ad lib.* and a daily maximum of 700 gm of high calcium meal A. The results are presented in Table 3 and Graphs 1, 2 and 3 and will be dealt with in the discussion.

TABLE 3.—*Sheep* 2. *The calcium, phosphorus and magnesium intake and output during periods* 1 *to* 5

Element	Period	Intake (gm)			Output (gm)		
		Teff	Meal	Total	Faeces	Urine	Total
Calcium	1	3·86	56·00	59·86	45·27	2·12	47·39
	2	4·68	56·00	60·68	41·06	1·66	42·72
	3	4·69	56·00	60·69	65·05	2·67	67·72
	4	6·26	55·76	62·02	59·77	4·44	64·21
	5	6·26	56·00	62·26	50·80	4·95	55·75
Phosphorus	1	2·35	6·30	8·65	8·98	0·05	9·03
	2	2·84	6·30	9·14	9·84	0·02	9·86
	3	2·85	6·30	9·15	14·37	0·02	14·39
	4	3·78	6·27	10·05	15·80	0·04	15·84
	5	3·78	6·30	10·08	14·43	0·03	14·46
Magnesium	1	2·21	2·10	4·31	1·40	1·21	2·61
	2	2·67	2·10	4·77	0·51	1·44	1·95
	3	2·68	2·10	4·78	0·76	1·41	2·17
	4	3·58	2·09	5·67	1·03	2·15	3·18
	5	3·58	2·10	5·68	1·28	1·97	3·25

The superior parathyroids together weighed 31·5 mg, i.e. 0·81 mg parathyroid tissue per Kg body weight.

After the abovementioned balance trials, the sheep was returned to its pen for 5 weeks and maintained on the same diet.

On 4.5.1966 Sheep 2 and a control of the same age which had also been fed teff and high calcium meal A for eight months previously were placed into separate metabolism cages and a 5-day balance trial conducted. On the sixth day (9.5.1966) the high phosphate diet was substituted with the intention of proceding to a further balance trial. Within 24 hours of the change of diet Sheep 2 developed tetany, the symptoms being as described for Sheep 1. The intended balance trial therefore had to be abandoned but it was possible to compare the blood and urine figures between the two sheep during the completed 5-day period and the first 24 hours after the change in diet. These comparisions are presented in Graph 5 and will be dealt with in the discussion.

Rapid relief of clinical symptoms followed an intravenous infusion of 350 ml " Calcium borogluconate " (22·5 per cent w/w) but as a precaution this was followed by a further 350 ml subcutaneously.

Twenty-four hours later the Pl.Ca had risen from 5·80 to 20·13 mg per cent, the Pl.I.P. from 8·90 to 10·90 mg per cent while the Pl.Mg had dropped from 1·08 mg per cent to below a detectable figure. Although the animal looked fairly normal, it refused to eat and there were no signs of ruminal contractions, so neostigmine was injected intramuscularly.

Forty-eight hours after the attack of tetany, the Pl.I.P. and Pl.Mg appeared to be returning towards normal but the Pl.Ca had dropped to 7·76 mg per cent. There were signs of tremor in the hindquarters and the rumen was still static. Further treatment consisted of administering 100 gm $CaCl_2$ in 1 litre of water together with 1 litre of rumen fluid mixture as previously described, *per os.* In addition an intramuscular injection of neostigmine was given.

The following day the animal still refused to eat and treatment with rumen fluid mixture and neostigmine was repeated.

The next day the animal was very weak and additional treatments of cortisone, antibiotics and antihistamines over the following three days were of no avail.

On 20.5.1966 a laparotomy was performed when it was found that the rumen wall was atrophied and that this organ was filled with teff which was fresh smelling and even had a green colour, indicating a complete absence of ruminal flora activity. The animal died during the operation and, but for the ruminal atrophy, no further macroscopic lesions were noticed during a subsequent post-mortem examination. No remaining parathyroid tissue could be found.

Sheep No. 3

A four years old wether weighing 56·8 Kg

This animal had been maintained on a diet of teff *ad lib.* and a daily maximum of 700 gm high calcium ration B for three weeks prior to and during the experiment to be described. A 5-day preoperative balance trial was conducted. Mild inappetence followed the operation performed on 19.7.1966 but was transient and all four postoperative balance trials could be successfully carried out. The results are presented in Table 4 and Graphs 1, 2 and 3.

TABLE 4.—*Sheep* 3. *The calcium, phosphorus and magnesium intake and output during periods* 1 *to* 5

Element	Period	Intake (gm)			Output (gm)		
		Teff	Meal	Total	Faeces	Urine	Total
Calcium	1	5·54	64·30	69·84	64·31	2·30	66·61
	2	1·93	19·95	21·88	11·75	0·44	12·19
	3	5·32	29·62	34·94	32·52	1·34	33·86
	4	5·45	42·20	47·65	38·68	2·95	41·63
	5	7·17	51·70	58·87	46·83	2·64	49·47
Phosphorus	1	3·37	10·15	13·52	10·72	0·14	10·86
	2	1·17	3·15	4·32	4·30	0·10	4·40
	3	3·23	4·68	7·91	8·19	0·07	8·26
	4	3·31	6·66	9·97	9·45	0·12	9·57
	5	4·35	8·16	12·51	11·49	0·16	11·65
Magnesium	1	3·17	3·05	6·22	3·66	1·28	4·94
	2	1·10	0·95	2·05	1·01	0·71	1·72
	3	3·04	1·40	4·44	2·58	1·21	3·79
	4	3·11	2·00	5·11	2·30	1·16	3·46
	5	4·10	2·45	6·55	2·79	1·61	4·40

The superior parathyroids together weighed 64 mg, i.e. 1·13 mg parathyroid tissue per Kg body weight.

Immediately after the last of these balance trials, the high phosphate meal was substituted. No clinical effects followed this change, and the animal was allowed to equilibrate to the new diet for a fortnight. During this time, the P1.Ca varied between 10·43 and 10·34 mg per cent, P1.I.P. between 5·52 and 6·62 mg per cent and P1.Mg between 1·84 and 1·63 mg per cent with no significant trends. After this equilibration period three 5-day balance trials (Periods 6, 7 and 8) were performed to establish reaction of the animal to the diet. The results are presented in Table 5 and Graph 6 and will be dealt with under the discussion.

TABLE 5.—*Sheep* 3. *The calcium, phosphorus and magnesium intake and output, balance and apparent absorption during periods* 6 *to* 8

Element	Period	Intake (gm)			Output (gm)			Bal. (gm)	App. Abs. %
		Teff	Meal	Total	Faeces	Urine	Total		
Calcium	6	7·28	6·27	13·55	11·37	0·87	12·24	+1·31	16·09
	7	7·87	6·21	14·08	11·73	0·89	12·62	+1·46	16·69
	8	3·77	6·30	10·07	8·31	0·83	9·14	+0·93	17·48
Phosphorus	6	4·42	20·90	25·32	20·34	0·27	20·61	+4·71	19·67
	7	4·78	20·70	25·48	21·42	0·26	21·68	+3·80	15·93
	8	2·29	21·00	23·29	17·83	0·26	18·09	+5·20	23·44
Magnesium	6	4·16	2·09	6·25	4·59	1·30	5·89	+0·36	26·56
	7	4·50	2·07	6·57	3·76	1·46	5·22	+1·35	42·77
	8	2·15	2·10	4·25	2·50	0·98	3·48	+0·77	41·18

$$\text{Apparent Absorption per cent} = \frac{\text{Intake} - \text{Faeces Output} \times 100}{\text{Intake}}$$

As the high phosphate diet had not produced any clinical effect on the animal it was decided on 26.9.1966 to dose two litres of the phosphate buffer (i.e. 11·8 gm P) mentioned previously. Hour 0 was taken as 8.30 a.m. of that morning and the phosphate buffer was administered at hour 6 with no immediate clinical result (see Graph 7b). During the first twenty-four hours 500 gm teff and 680 gm high phosphate meal was eaten after which no further food was taken.

At hour 24 the animal was " down " but the lips and cheeks were foamy, suggesting that the animal had had tetany some hours previously. The respiration was shallow and panting ($\pm$ 150/minute) and the heartrate elevated to 180/minute.

At hour 28 a slow intravenous infusion of 0·588 gm $CaCl_2.2H_2O$, 0·677 gm $MgCl_2.6H_2O$, 0·076 gm KCl in 200 ml of water was administered in an attempt to correct the deficiencies found by blood analysis. The heartrate decreased from 180 to 120/minute, but no other response was detectable.

At hour 30 the animal was still " down " so a further infusion of 1·176 gm $CaCl_2.2H_2O$, 2·25 gm Calcium borogluconate, 1·354 gm $MgCl_2.6H_2O$ and 0·832 gm KCl in 200 ml water was administered intravenously and 20 ml " Calcium borogluconate " (22·5 per cent w/w) subcutaneously. There was no clinical response, but the heartrate remained at a constant 120/minute throughout the infusion.

At hour 31 the animal was still " down ", although blood analyses showed a marked improvement in P1.Ca. P1.I.P. had also returned to more normal levels, but P1.K and P1.Mg were still low and almost unaffected by the infusions.

The animal died during the night and a post-mortem revealed neither macroscopic pathology nor any remaining parathyroid tissue.

Sheep No. 4

A six year old wether weighing 36 Kg.

This animal had been maintained on a diet of only teff hay *ad lib.* for three weeks prior to the trial. No postoperative tetany occurred and five balance trials, during which the animal received only teff *ad lib.* were conducted. The results are presented in Table 6 and Graphs 1, 2 and 3.

TABLE 6.—*Sheep* 4. *The calcium, phosphorus and magnesium intake and output during periods* 1 *to* 5

Element	Period	Intake (gm)	Output (gm)		
		Teff	Faeces	Urine	Total
Calcium	1	11·68	13·83	1·09	14·92
	2	11·70	11·68	1·68	13·36
	3	11·98	10·15	1·59	11·74
	4	13·97	8·57	2·74	11·31
	5	13·78	9·86	2·90	12·76
Phosphorus	1	7·09	8·27	0·17	8·44
	2	7·11	8·29	0·14	8·43
	3	7·28	6·49	0·21	6·70
	4	8·48	5·62	0·24	5·86
	5	8·36	6·14	0·25	6·39
Magnesium	1	6·67	4·12	1·01	5·13
	2	6·69	5·82	1·24	7·06
	3	6·85	3·76	1·21	4·97
	4	7·98	2·66	1·40	4·06
	5	7·87	3·44	1·33	4·77

The superior parathyroids removed at the operation (1.8.1966) weighed 43·8 mg, i.e. 1·22 mg parathyroid tissue/Kg body weight.

Immediately after the last of the above balance trial periods the high phosphate meal was offered in addition to the teff ration. Although the animal ate the meal avidly, no clinical tetany resulted and three further balance trials (6, 7 and 8) were conducted. These results are presented in Table 7 and Graph 6 and will be dealt with under the discussion.

TABLE 7.—*Sheep* 4. *The calcium, phosphorus and magnesium intake, output, balance and apparent absorption during periods* 6 *and* 8

Element	Period	Intake (gm)			Output (gm)			Bal. (gm)	App. Abs. %
		Teff	Meal	Total	Faeces	Urine	Total		
Calcium	6	10·36	4·16	14·52	12·02	2·28	14·30	+0·22	17·22
	7	9·58	5·96	15·54	13·24	2·10	15·34	+0·20	14·80
	8	3·76	6·18	9·94	6·22	1·47	7·69	+2·25	37·42
Phosphorus	6	6·29	13·88	20·17	10·83	0·25	11·08	+9·09	46·31
	7	5·81	19·88	25·69	16·90	0·28	17·18	+8·51	34·21
	8	2·28	20·62	22·90	12·86	0·27	13·13	+9·77	43·84
Magnesium	6	5·92	1·39	7·31	3·56	0·80	4·36	+2·95	51·30
	7	5·47	1·99	7·46	6·64	1·24	7·88	−0·42	10·99
	8	2·15	2·06	4·21	2·17	0·81	2·98	+1·23	48·46

$$\text{Apparent Absorption per cent} = \frac{\text{Intake} - \text{Faeces Output} \times 100}{\text{Intake}}$$

As in the case of Sheep 3 the high phosphate diet had no clinical effect so two litres of the phosphate buffer (11·8 gm P) was dosed on 26.9.1966. Hour 0 was taken as 8.30 a.m. of that morning and the phosphate buffer was dosed at hour 6 (see Graph 7c). During the first twenty-four hours 500 gm teff and 525 gm of the high phosphate meal were eaten. Subsequently only a small amount of teff was taken as will be indicated.

At hour 24 the animal was loath to move but trembled and staggered uncertainly when prompted to do so.

By hour 28 the animal was " down " with the head turned to the right. Breathing movements were easily discernible (28/minute) and the heartrate easily audible (80/minute). It was decided to treat with " Calcium borogluconate " (22·5 per

cent w/w) intravenously. As the first few drops entered the vein, the heartrate dropped to 40/minute and heart sounds were loud and thudding. It was considered unsafe to proceed with the infusion and a total of only 10 ml was given. This had a beneficial effect as, although the animal was unable to stand, it became alert and could move its head.

Two hours later (hour 30) the animal had relapsed with the head once again resting on the right flank. " Calcium borogluconate " (90 ml) was infused subcutaneously.

Blood taken at hour 31 showed that although the Pl.Ca was normal, Pl.Mg had dropped precipitously to below detectable levels.

At hour 54 the sheep was still " down " with the head turned to the right. It was able to kick strongly, was keen to eat but could not right itself. Blood figures showed that Pl.Ca had once again dropped, while Pl.Mg was rising slowly. Further treatment consisted of subcutaneous infusions of 2 gm KCl in 50 ml water, 6 gm $MgSO_4.6H_2O$ in 50 ml water and 50 ml " Calcium borogluconate ". A further 50 ml " Calcium borogluconate " was administered into the peritoneal cavity.

By hour 56 the animal was interested in its surroundings, ate well, but still could not right itself or move its head away from its right flank. A mild tremor was present in the limbs and neck.

By hour 72 the animal looked well, ate well and could move its head at will. The muscles of the legs offered good resistance and although it could kick, it could not stand. Despite this seemingly excellent recovery the animal was found dead at hour 96.

A post-mortem examination was conducted and no unusual patholgy was noted nor could any remaining parathyroid tissue be found.

Other parameters measured

(*a*) *Ratio of parathyroid weight: body weight:* This ratio has already been mentioned under the results of each individual sheep. In order to assess the results a further two animals, with the following statistics, were used. Sheep A, a thirteen months old ram who had been kept on the low calcium ration for ten months prior to slaughter, and Sheep B, of the same age and sex, which had been kept on a high calcium ration for ten months prior to slaughter. A summary of the results obtained is to be found in Table 8.

TABLE 8.—*The age, body weight, superior parathyroids weight and ratio weight of superior parathyroids: body weight in Sheep* 1, 2, 3, 4, *A and B*

Animal	Age	Wt. of Sup. Para. (mg)	Body wt. (Kg)	Ratio mg/Kg	Remarks
Sheep A	13 months	85·0	35·0	2·43 : 1	Low Ca ration for 10 months
Sheep 1	8 months	34·2	25·0	1·37 : 1	Control ration for 5 months
Sheep 4	6 years..	43·8	36·0	1·22 : 1	Teff for 3 weeks
Sheep 3	4 years..	64·0	56·8	1·13 : 1	High Ca ration for 3 weeks
Sheep 2	9 months	31·5	39·0	0·81 : 1	High Ca ration for 6 months
Sheep B	13 months	26·0	49·0	0·53 : 1	High Ca ration for 10 months

(*b*) *Plasma alkaline phosphatase:* According to Allcroft & Folley (1941) (cited by Cornelius & Kaneko, 1963) the normal alkaline phosphatase in apparently normal ewes varies between 3·0 and 166·1 units per 100 ml plasma. In this experiment the alkaline phosphatases remained well within this limit but all the animals showed a slow rise throughout. This was particularly noticeable in the young sheep (see Table 9).

TABLE 9.—*The alkaline phosphatase values at the beginning and end of the experiments.* (*Expressed in King Armstrong Units*/100 *ml plasma*)

Sheep	Beginning	End
1	16·5	40·0
2	15·0	32·6
3	8·0	14·7
4	6·0	10·0

Further work on the alkaline phosphatase in sheep is necessary before any interpretation of the above figures can be made.

(*c*) *Total plasma proteins:* It is well known that certain fractions of both Pl.Ca and Pl.Mg are attached to, and can be affected by, changes in the plasma proteins (Stevenson & Wilson, 1963). Total plasma proteins were therefore determined as a control and, in all the experiments conducted, were found to have but a small variation with no particular trends.

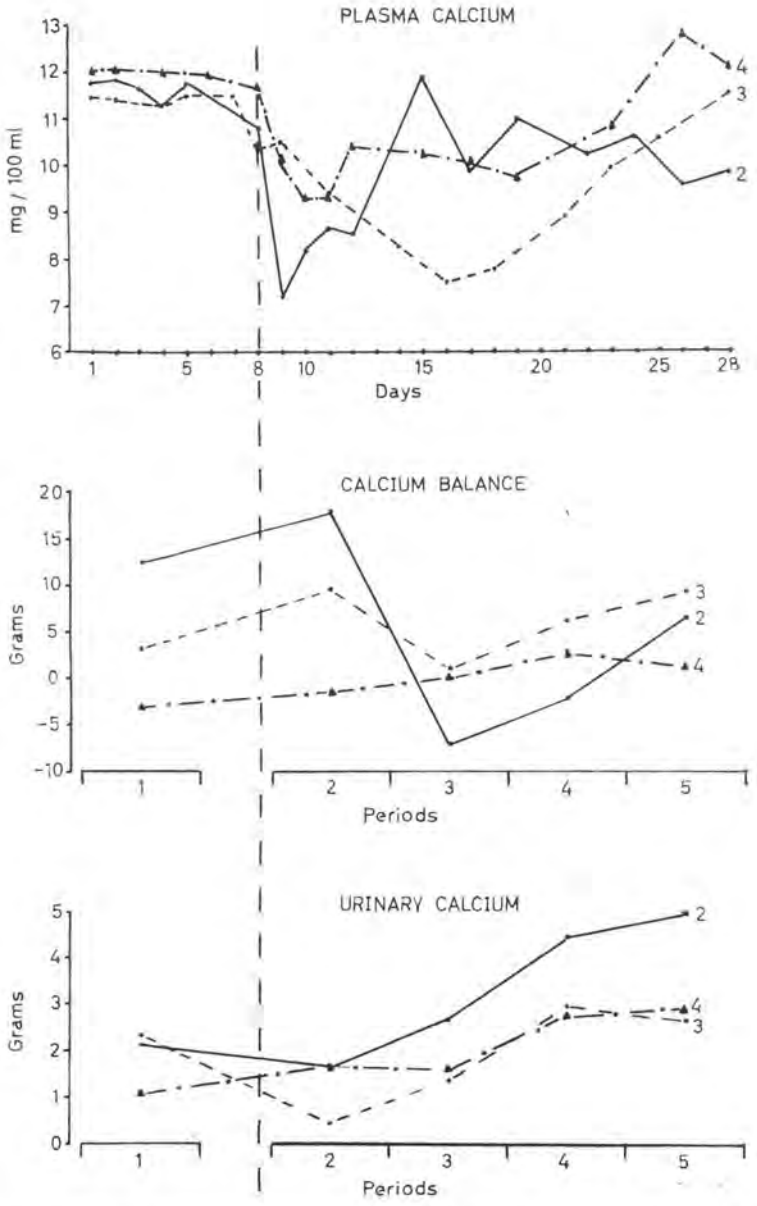
These variations were:—

Sheep 1..... 7·20 to 7·72 gm per cent

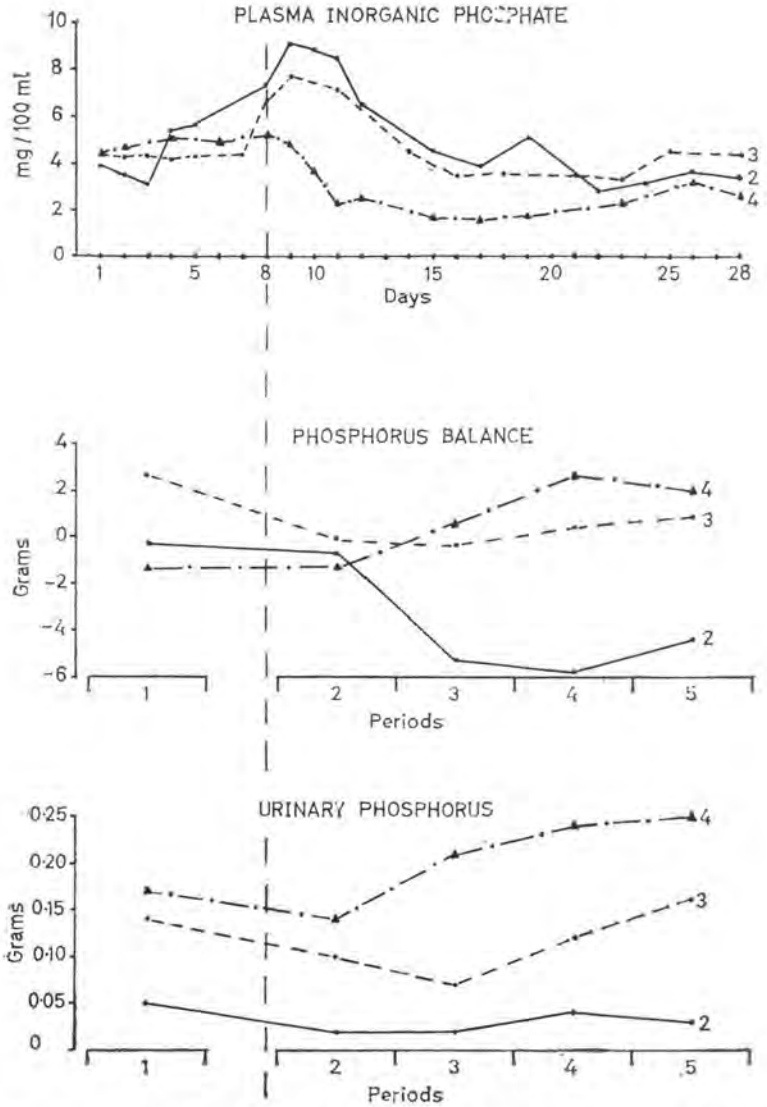
Sheep 2..... 6·16 to 6·82 gm per cent

Sheep 3..... 6·16 to 6·82 gm per cent

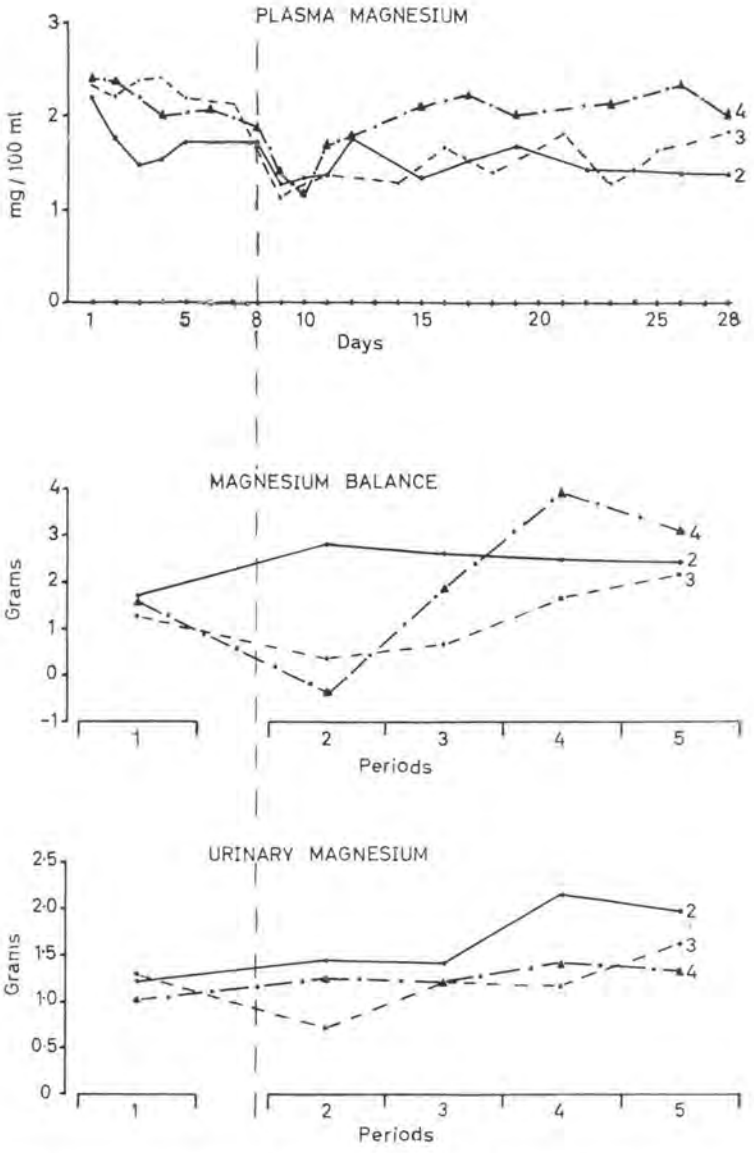
Sheep 4..... 6·16 to 7·20 gm per cent



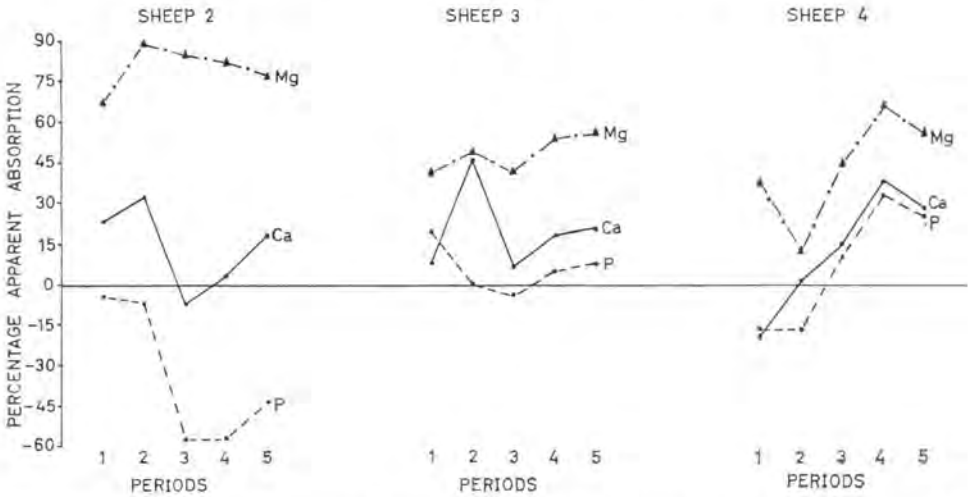
GRAPH 1.—The calcium results from Sheep 2, 3 and 4 during periods 1 to 5 (vertical dashed line denotes operation date)



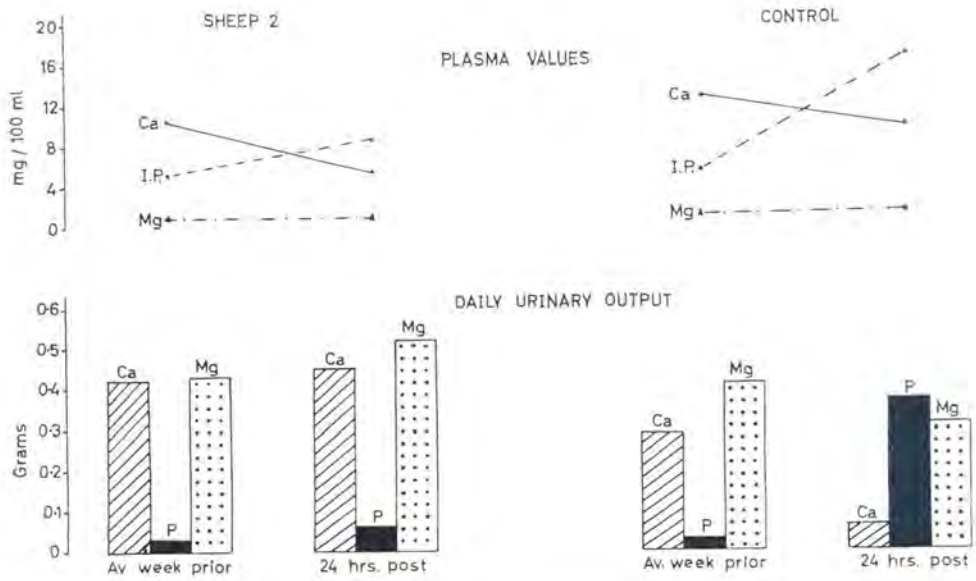
GRAPH 2.—The phosphorus results from Sheep 2, 3 and 4 during periods 1 to 5 (vertical dashed line denotes operation date



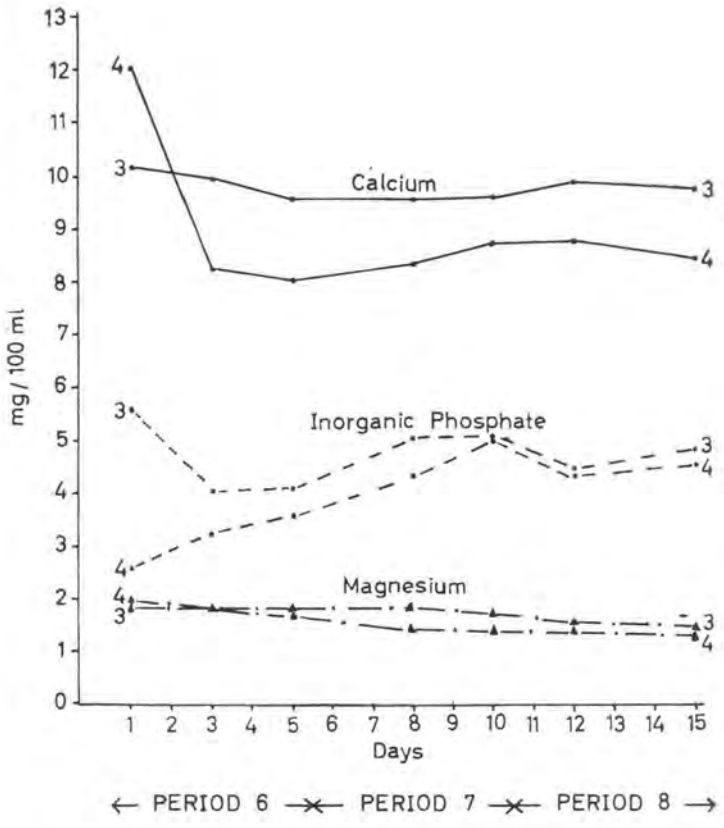
GRAPH 3.—The magnesium results from Sheep 2, 3 and 4 and during periods 1 to 5 (vertical dashed line denotes operation date)



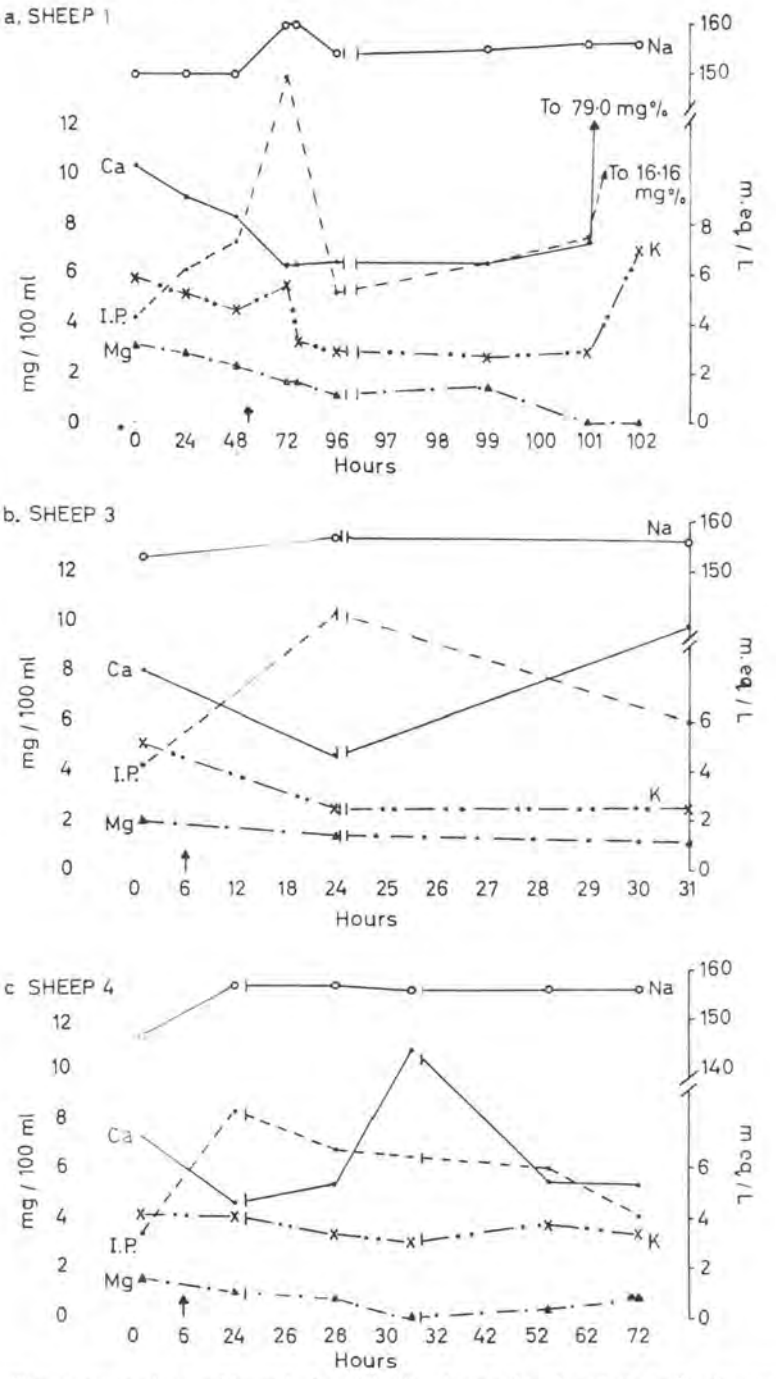
GRAPH 4.—The percentage apparent absorption of calcium phosphorus and magnesium of Sheep 2, 3 and 4 during periods 1 to 5. [Percentage apparent absorption $= \frac{\text{intake–faeces output}}{\text{intake}} \times 100$]



GRAPH 5.—The comparison between Sheep 2 and control before and after the change in diet



GRAPH 6.—The plasma calcium, inorganic phosphate and magnesium values of Sheep 3 and 4 during periods 6, 7 and 8



GRAPH 7.—The plasma values in Sheep 1, 3 and 4 after dosing the phosphate buffer (arrow denotes hour of dosing)

Discussion and Conclusions

As the main theme of these experiments was to determine whether the results of thyro-parathyroidectomy differed in young and mature sheep, the results will mainly be discussed in the light of such dissimilarities.

Calcium

1. *Pre- and postoperative balance trials (periods 1–5)*

(*a*) *Plasma calcium figures (Pl.Ca)*

(i) *Immediate reaction:* Twenty-four hours after the operation the Pl.Ca of the young Sheep 1 had dropped from 15·88 to 7·15 mg per cent and tetany occurred four hours later. This animal had been ingesting about 5 gm calcium per day which is far higher than the 2·8 gm per day recommended for a 60 lb sheep (Maynard & Loosli, 1962).

In the case of the other young Sheep 2 there was also a sudden postoperative drop in Pl.Ca from 10·79 to 7·15 mg per cent during the first twenty-four hours, but tetany was evidently averted by a calcium intake of about 10 mg per day.

No marked immediate drop in Pl.Ca was noted in either of the two mature animals (No. 3, 10·32 to 10·48 mg per cent and No. 4, 11·64 to 10·09 mg per cent) although they were on widely varying calcium intakes (No. 3, $\pm$ 10 gm and No. 4, $\pm$ 2·5 gm Ca per day).

This difference in the reaction to parathyroidectomy indicates that the high demand for calcium in the young animal renders it much more dependent on the action of parathormone than the mature animal.

(ii) *Delayed reaction:* As will be noticed in Graph 1, the Pl.Ca of Sheep 2 returned to normal limits about eight days after the operation, i.e. during Period 3 when both the balance and percentage apparent absorption figures were at their lowest. In the case of Sheep 3, however, the Pl.Ca fell with the total balance, and both were at their lowest during Period 3. Subsequent improvement in Pl.Ca coincided with an increase in the calcium balance. The Pl.Ca of Sheep 4 decreased only slightly within two days after the operation but was soon restored to normal, coinciding with an ever increasing positivity of the calcium balance.

This difference in the delayed reaction suggests that the young animal was able to call on its soluble bone reserves to supplement the Pl.Ca in the face of a decreased balance, while the mature animals had to rely mainly upon intestinal absorption. This correlates well with the findings in cattle that the percentage of soluble bone decreases with advancing age (Hansard, Comar & Davis, 1954; Luick, Boda & Kleiber, 1957).

(*b*) *Urinary calcium*

Although the daily loss of calcium in the urine appears insignificant as compared to that of the faeces, it does represent a considerable proportion of the net gain in the intestinal tract.

In all cases the urinary loss of calcium increased after the operation, markedly so in Sheep 2 and 4. This would indicate an anti-calciuric effect probably of parathormone. As will be discussed later this was not accompanied by a significant change in urinary phosphorus excretion.

The immediate decrease in calcium excretion, shown particularly in Sheep 3 and to some extent in Sheep 2, might be attributable to the release of parathormone from the glands during operation.

There was no direct correlation between Pl.Ca concentration and urinary calcium excretion.

(*c*) *General remarks*

Although the balance trends during the five periods are similar in Sheep 2 and 3 (Graph 1) it is important to note that, although on similar dietary intakes, the young animal had a far higher preoperative percentage apparent absorption than the mature sheep. More important is the fact that at the end of the five periods, the balance and percentage apparent absorption of the young sheep had not yet returned to the preoperative level, whereas both Sheep 3 and 4 were far above the levels recorded in Period 1. If one assumes that endogenous loss of calcium is unchanged after thyro-parathyroidectomy, as has been demonstrated in the goat (Payne & Sansom, 1966), then it can be postulated that removal of these glands removed a facilitatory absorption mechanism in the young animal, but an inhibitory mechanism in the mature animal. This could perhaps explain the decreasing true absorption of calcium that occurs with increasing age in bovines (Hansard, Comar & Plumlee, 1954; Smith, 1962).

No definite reasons can be found for the increased balance during Period 2 in Sheep 2 and 3, but it is conceivably the result of handling the glands during the operation.

2. *The effects of decreasing dietary calcium and/or increasing dietary phosphate (Periods 6 to 8)*

The effect of decreasing dietary calcium and increasing dietary phosphate was dramatic in Sheep 2, resulting in tetany within twenty-four hours of the change (see Graph 5). During this time the Pl.Ca had dropped from 10·58 to 5·80 mg per cent, while the daily urinary calcium excretion was almost unchanged (0·42 to 0·45 gm per 24 hours). Comparable results in a normal young control show a smaller drop in Pl.Ca from 12·92 to 10·00 mg per cent associated with a rapidly acting conservation of urinary calcium (0·29 to 0·06 gm per 24 hours).

This shows that in the absence of parathyroids the animal was able to maintain a normal Pl.Ca level on a high intake, but had no regulatory mechanisms to reduce loss or probably to facilitate absorption when on a lower intake.

As mentioned previously, Sheep 3 was allowed to equilibrate on the low calcium, high phosphate diet for a fortnight. During this time the Pl.Ca had slowly decreased from 11·62 to 10·34 mg per cent. After this equilibration the three metabolism trials (6 to 8) were conducted during which the Pl.Ca gradually fell to 9·53 mg per cent and then rose to 9·87 mg per cent (see Graph 6). During these three periods it was found that although the calcium intake had decreased from 10 to 12 gm to $\pm$ 2·5 gm per day, the animal still managed to remain in a slightly positive balance (Table 5). The almost constant and low urinary calcium output seems to denote a basal or obligatory excretion.

In Sheep 4, which was not allowed to equilibrate, there was a sharp decline in Pl.Ca from 12·06 to 8·23 mg per cent within two days of the change in diet (Graph 6). This is surprising as the change in diet did not affect calcium intake,

nor was there an increase in urinary calcium. The effect of the increased dietary phosphate was only gradually reflected in an increase in Pl.I.P. which could therefore not have been instrumental in depressing Pl.Ca. The only explanation possible is therefore to be found in the gut where the phosphate would appear to have interfered with calcium absorption. This is substantiated by the finding that between Periods 5 and 6 the percentage apparent absorption of phosphate rose from 26·55 per cent to 46·31 per cent while the apparent absorption of calcium decreased from 28·44 per cent to 17·22 per cent (Table 7).

During the rest of Periods 6 to 8, Pl.Ca remained between 8·02 and 8·73 mg per cent, while there was an ever decreasing urinary calcium output towards the "basal level" set by Sheep 3.

The conclusion drawn from this experiment is that, once again, there is evidence to prove that the requirements for calcium are far greater in the young animal and necessitate more active controlling mechanisms than in the mature sheep.

Phosphorus

1. *Pre- and postoperative balance trials* (*Periods* 1 *to* 5)

(*a*) *The plasma inorganic phosphate figures* (*Pl.I.P.*) *Graph* 2

It is most interesting to note that in both the animals (Sheep 2 and 3) fed a high calcium ration (Ca: P ratio roughly 6: 1) 24 hours of starvation prior to operation resulted in a drop in Pl.Ca and a sharp rise in Pl.I.P. Sheep 4 was on a far lower calcium intake (Ca: P ratio 1·6: 1) and no comparable changes were recorded.

Subsequent to the preoperative rise in Pl.I.P. in Sheep 2 and 3, there was a peak on the first postoperative day and then a gradual fall to normal limits during the rest of the trial. The figures for Sheep 4 show a steady decline to a low point during Period 3, followed by a gradual rise. This decline paradoxically took place while the phosphorus balance was steadily improving.

A possible explanation of this is that in the case of Sheep 2 and 3 the discontinuation of the large intake of calcium tended to depress the Pl.Ca, which stimulated either bone dissolution or release of the phosphate from body tissues. The position in Sheep 4 is the reverse in that the calcium balance improved slightly faster than the phosphorus balance and may have promoted the deposition of the phosphate into the bone or its influx into the body tissues.

(*b*) *Urinary phosphorus* (*Graph* 2)

Although the three sheep were on widely varying calcium and phosphorus intakes, the average daily output of urinary phosphorus during Period 1 only varied from 0·01 to 0·05 gm which is exceptionally low when compared to the daily output in the human of approximately 1·5 gm (Wootton, 1964). This demonstrates a remarkable phosphate conservation in the sheep. While acknowledging the findings in laboratory animals that both parathyroid hormone and thyrocalcitonin have phosphaturic effects, and the findings in this experiment that there is in fact a decline, with subsequent rise, in urinary phosphorus after thyro-parathyroidectomy, the actual amounts are so small that it is felt that in sheep on a relatively low phosphorus intake, the main control of phosphorus balance is mediated through the intestine and not through the kidney.

(*c*) *General remarks*

In general the balance figures for calcium and phosphorus show a tendency to follow one another, the main exception being in Sheep 3, Period 2. However, as mentioned previously, in the urine a fair amount of calcium is excreted compared to only a small amount of phosphorus. A much closer comparison is found in the percentage apparent absorption of these two elements, where, in fact, the parallelism is remarkable (Graph 4). This indicates that the absorption of calcium and phosphorus is mediated by the same mechanisms.

Reverting to Period 2 of Sheep 2 and 3 one finds that the decreased Pl.Ca (Graph 1) is associated with an increased positive balance, whereas the increased Pl.I.P. (Graph 2) is associated with a decreased balance which appears paradoxical. It may be postulated that during this period the Pl.I.P. was mediated through the Pl.Ca level in a reciprocal manner. As mentioned before in Part (*a*) of this section, this is substantiated by the findings in Sheep 4.

2. *The effects of decreasing dietary calcium and/or increasing dietary phosphate* (*Periods* 6 *to* 8)

The comparison between young and mature animals has already been drawn under calcium metabolism, as this was the most dramatic and important consequence.

When comparing Sheep 2 with its control (Graph 5) it is noteworthy that whereas the control's Pl.I.P. rose from 5·73 to 17·20 mg per cent, that of Sheep 2 only rose from 5·32 to 8·90 mg per cent. As has been demonstrated previously, a drop in Pl.Ca is usually associated with a rise in Pl.I.P. and this should theoretically have caused a greater rise in Pl.I.P. in Sheep 2. The reason that it did not may be that whereas the control had an intact facilitatory absorption mechanism, Sheep 2 had not. Furthermore, while the urinary phosphorus in Sheep 2 only doubled itself to a response of less than a twofold rise in Pl.I.P., the urinary phosphorus of the control was ten times increased to a threefold rise in PlI.P. This suggests that in the intact young animal phosphaturic principles were present to rid the body of excess phosphate.

In the mature animals this change in diet led to an increased phosphorus balance, demonstrating the ability of the body to utilize this element fairly well after thyro-parathyroidectomy (Tables 5 and 7). In Sheep 4 there was a gradual increase in Pl.I.P. from 2·60 to 4·34 mg per cent while in Sheep 3 the Pl.I.P. fluctuated between 4·02 and 5·57 mg per cent (Graph 6). The urinary phosphorus during these three periods was, however, only slightly higher than that recorded during Periods 1 to 5 (Tables 5 and 7).

These results once again demonstrate the rapidity of the course of events in the young animal when compared to the mature sheep.

Magnesium

Before discussing the effects of the experiment on this element, it must be mentioned that I am well aware of the limitations of the analytical method employed, and realize that during faecal analysis the large amount of calcium present may well have interfered. The results are, however, reasonable and the discussion will proceed with the above facts in mind.

1. *Pre- and postoperative balance trials* (*Periods* 1 *to* 5)

(*a*) *The plasma magnesium figures* (*Pl.Mg*) (*Graph* 3)

In all three sheep there was a definite fall in Pl.Mg after the operation. This was followed by a gradual but variable rise. This corresponds to the findings in goats (Payne & Chamings, 1964). Although there was a slight fall in Pl.Mg during the starvation period in Sheep 2 and 4, this was fairly marked in Sheep 3, an effect which is evidently pronounced in lactating ewes (Herd, 1966).

(*b*) *Urinary magnesium* (*Graph* 3)

Although there was a slight increase in urinary magnesium in all three sheep, this was not very pronounced, but there was a greater increase in urinary magnesium during the five periods in the young animal than in the two mature animals. Inexplicably the lowered Pl.Mg noticed during Period 2 was only reflected in a lowered urinary magnesium in Sheep 3.

(*c*) *General remarks*

In Sheep 2 the magnesium balance (Graph 3) shows a slight increase during Period 2 and a subsequent decline. This is not correlated to either the calcium or phosphorus balances (Graphs 1 and 2). In the mature sheep the opposite effect is seen, with a decline and subsequent rise, corresponding fairly well with the phosphorus balance of Sheep 3 (Graph 2) and both the phosphorus (Graph 2) and calcium balances (Graph 1) of Sheep 4. When considering the percentage apparent absorptions, very much the same picture is seen in Sheep 2, but in Sheep 3 and, except for Period 1, Sheep 4 this parameter is very closely associated with the percentage apparent absorption of calcium (Graph 4).

2. *The effects of decreasing dietary calcium and/or increasing dietary phosphate* (*Periods* 6 *to* 8)

Although there were fairly marked changes in plasma and urine calcium and phosphorus in Sheep 2 and its control, no significant changes occurred in the plasma and urine magnesium (Graph 5).

In the mature sheep however, there was a definite depression in the magnesium balance after this change. In both cases the calcium balance had declined, but the phosphorus balance had increased markedly, and it is felt that this had adversely affected the magnesium balance (Tables 5 and 7). This effect is also noticed in the plasma picture of Sheep 4 where there was a dramatic drop in the Pl.Ca followed by a fairly constant plateau, whereas Pl.I.P. and Pl.Mg gradually and reciprocally increased and decreased respectively.

Sheep 3 had been allowed to equilibrate and the Pl.Ca, Pl.I.P. and Pl.Mg figures were all fairly constant (Graph 6).

The Effect of Dosing the Sodium Phosphate Buffer

From the results obtained (Graph 7) the following course of events is postulated. The rising Pl.I.P. caused a decrease in both Pl.Ca and Pl.Mg and resulted in tetany. During tetany the Pl.K was normal, whether it was normal (Sheep 4) or low

(Sheep 1) prior to the spasms. After tetany there was a fall in P1.K which is interpreted as both an intracellular loss of potassium as a result of the low P1.Ca (Morrill, Kaback & Robbins, 1964) together with an extracellular loss of potassium possibly via the kidneys into the urine. The net result was a loss of cell membrane potential and the " downer paralysis " observed clinically.

Contrary to the marked clinical improvement after treatment during tetany (Sheep 1, 28 hours after thyro-parathyroidectomy, and Sheep 2, 24 hours after the change of diet) the treatments during this " downer syndrome " were singularly ineffectual. This suggests that the events outlined above resulted in some enzymatic disturbances with subsequent inability to adequately utilize the elements infused.

Thus in Sheep 1 the KCl *per os* had a slight clinical effect, and although P1.Ca rose, P1.K declined and P1.Mg dropped to below detectable levels. At this point an intravenous infusion of " Calcium borogluconate " less than and at a slower rate than that given to Sheep 2 during tetany, elevated the P1.Ca to 79 mg per cent and caused death.

In Sheep 3 the intravenous calcium, magnesium and potassium mixture, while not affecting the heart, caused only a rise in P1.Ca with no beneficial clinical result.

In the case of Sheep 4, 10 ml " Calcium borogluconate " intravenously severely affected the heart and, together with a subcutaneous injection, caused a marked rise in P1.Ca; this reverted to pre-infusion levels. Subsequent subcutaneous calcium, magnesium and potassium resulted in very little change in the plasma picture and, although the animal was clinically better, it too died.

From the above it is suggested that, in order to reinstate the intracellular potassium concentration, an injection of insulin should be followed by an intravenous infusion of potassium and glucose (Harper, 1965), after which calcium and magnesium may be administered intravenously and possibly subcutaneously.

Other Parameters Measured

(*a*) The ratio of the weight of the superior parathyroids to body weight (see Table 8).

This ratio, which has been described in bovines (Stott & Smith, 1964), shows that a high calcium diet suppresses parathyroid weight and, by inference, parathyroid function. This is consistent with the accepted findings that hypercalcaemic conditions inhibit parathyroid secretion.

Insufficient material precluded statistical analysis.

(*b*) Plasma alkaline phosphatase (Table 9).

As mentioned in the results, the normal variation given for sheep is so wide that no conclusions can be drawn.

(*c*) Total plasma proteins.

In all the experiments performed the variations were small and probably had no effect on either P1.Ca or P1.Mg.

Summary

The effects of thyro-parathyroidectomy with subsequent thyroxin replacement have been studied in two young and two mature sheep.

Observations made included the effects of the operation on the absorption, excretion and plasma levels of calcium, phosphorus and magnesium, when the animals were kept on diets of different calcium and phosphorus levels.

It was found that in the young sheep postoperative hypocalcaemic tetany occurred unless the dietary intake of calcium was extremely high. In the mature sheep there was a temporary drop in plasma calcium levels after the operation, but tetany did not occur irrespective of the calcium intake.

It is maintained that in the young sheep the hormones of the thyroparathyroid complex governing calcium and phosphorus and magnesium metabolism, play a much more essential role than they do in mature sheep. Actions of these hormones which have been demonstrated are: (1) increase of calcium absorption in the young animal and inhibition of absorption in the mature ones; (2) increase of urinary phosphate excretion and retention of calcium.

Acknowledgements

I wish to express my sincere thanks to:

The Department of Agricultural Technical Services for seconding me to Onderstepoort for two years for purposes of postgraduate study; the Chief, Veterinary Research Institute, Onderstepoort, for research facilities; Prof. R. Clark for his guidance and interest during the course of the experimental studies and in the preparation of this thesis; Dr. J. M. M. Brown for his help with, and criticism of, the laboratory aspects of the work; the Misses Adriana Wagner and Anna Brink, Mrs. Lulu van Zyl and Messrs. R. Gray, R. J. J. Briel and P. J. de Wet for their technical assistance; my parents for their encouragement during the course of my academic career and to my wife, Judy, without whose inspiration, patience and moral support this work could not have been completed.

References

CORNELIUS, C. E. & KANEKO, J. J., 1963. Clinical biochemistry of domestic animals. New York & London: Academic Press.

DUKES, H. H., 1955. The physiology of domestic animals. 7th ed., London: Bailliere, Tindall and Cox.

GESCHWIND, I. I., 1961. *In* Mineral metabolism, 1, pt. B. Eds. C. L. Comar & F. Bronner, New York: Academic Press.

GUYTON, A. C., 1966. Textbook of medical physiology. 3rd ed., Philadelphia & London: W. B. Saunders Co.

HANSARD, S. L., COMAR, C. L. & DAVIS, G. K., 1954. Effects of age upon the physiological behaviour of calcium in cattle. *Am. J. Physiol.*, 177, 383–389.

HANSARD, S. L., COMAR, C. L. & PLUMLEE, M. P., 1954. The effects of age upon the calcium utilization and maintenence requirements in the bovine. *J. Anim. Sci.*, 13, 25–36.

HARPER, H. A., 1965. Review of physiological chemistry. 10th ed., Los Altos, California: Lange Medical Publications.

HERD, R. P., 1966. Fasting in relation to hypocalcaemia and hypomagnesaemia in lactating cows and ewes. *Aust. vet. J.*, 42, 269–272.

LOTZ, W. E., TALMAGE, R. V. & COMAR, C. L., 1954. Effect of parathyroid extract administration in sheep. *Proc. Soc. exp. Biol. Med.*, 85, 292–295.

LUICK, J. R., BODA, J. M. & KLEIBER, M., 1957. Some bio-kinetic aspects of calcium metabolism in dairy cows. *Am. J. Physiol.*, 189, 483–488.

MAYNARD, L. A. & LOOSLI, J. K., 1962. Animal nutrition. 5th ed., New York, Toronto, London: McGraw-Hill Book Company Inc.

MILHOUD, G. & MOUKHTAR, M. S., 1966. Antagonistic and synergistic actions of thyrocalcitonin and parathyroid hormone on the levels of calcium and phosphate in the rat. *Nature, Lond.*, 211, 1186–1187.

MORRILL, G. E., KABACK, H. R. & ROBBINS, E., 1964. Effect of calcium on intracellular sodium and potassium concentration in plant and animal cells. *Nature, Lond.*, 204, 641–642.

MONSON, P. L., 1966. Thyrocalcitonin. *Ann. intern. Med.*, 64, 1353–1357.

NEILL, D. W. & NEELY, R. A., 1956. The estimation of magnesium in serum using Titan Yellow. *J. clin. Path.*, 9, 162–163.

PAYNE, J. M. & CHAMINGS, J., 1964. The effect of thyro-parathyroidectomy in the goat with particular respect to clinical effects and changes in the concentrations of plasma calcium, inorganic phosphate and magnesium. *J. Endocr.*, 29, 19–28.

PAYNE, J. M. & SANSOM, B. F., 1966. Calcium metabolism in normal and thyro-parathyroidectomized goats. *J. Physiol.*, 184, 433–443.

ROBINSON, C. J., MARTIN, T. J. & MacINTYRE, I., 1966. Phosphaturic effect of thyrocalcitonin. *Lancet*, 2, 83–84.

SMITH, R. H., 1962. Net exchange of certain inorganic ions and water in the gastro-intestinal tract of the milk fed calf. *Biochem. J.*, 83, 151–163.

STEVENSON, D. E. & WILSON, A. A., 1963. Metabolic disorders of domestic animals. 1st ed. Oxford: Blackwell Scientific Publications.

STOTT, G. H. & SMITH, V. R., 1964. Histology, cytology and size of the parathyroid in the bovine related to age and function. *J. Dairy Sci.*, 47, 426–432.

TALMAGE, R. V., 1962. Parathyroid function: A calcium replacement mechanism. *Am. Zool.* 2, 353–360.

WEICHSELBAUM, T. E., 1946. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am. J. clin. Path.*, 16, 40–49.

WOOTTON, I. D. P., 1964. Micro-analysis in medical biochemistry. 4th ed., London: J. & A. Churchill Ltd.