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Research Article

CALCIUM AND PHOSPHORUS HOMEOSTASIS IN THE PARATHYROIDECTOMIZED DOG; EVALUATION BY MEANS OF ETHYLENEDIAMINE TETRAACETATE AND CALCIUM TOLERANCE TESTS *

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In an experiment designed to determine whether or not homografts of parathyroid tissue would survive and function in millipore diffusion chambers, Wilson, Zollinger, Mahan and Brooks (1) subjected female mongrel dogs to parathyroidectomy either in two stages or as a single procedure. Twelve animals with transplants survived the acute postoperative period with its attendant severe hypocalcemia, but all required intensive parenteral calcium support for periods ranging from 19 to 78 days. This supportive therapy could eventually be stopped, and 11 of these dogs were perfectly well in all respects for two to five months prior to removal of their transplants. The range of the serum calcium level was 9 to 11 mg per 100 ml in six of them, and 7 to 8 mg per 100 ml in the other five. However, after all of the millipore chambers were removed, there was little or no alteration in their clinical status or their serum calcium levels. The dogs were followed for two and one-half to four months after the chambers were excised, and the only shift in the level of serum calcium that occurred was in one dog, which, with a serum calcium initially in the 7 to 8 mg per 100 ml range, was later able to maintain a consistent value of 9.0 mg per 100 ml.

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It might be argued that these animals were being kept alive by accessory parathyroid tissue. However, the usual site of any such tissue (the thyroid gland) was always removed. In addition, whenever one parathyroid gland was deliberately left in place, the serum calcium level remained normal for as long as 140 days; yet, in every dog in which parathyroidectomy was felt to be complete, a precipitous fall in the level of serum calcium occurred within 48 hours, and animals that were not given calcium injections at this stage died within eight days.

A further possibility is that small amounts of parathyroid tissue, insufficient at the time of operation to sustain life, were left and subsequently underwent hypertrophy while the animal was being kept alive by calcium injections. In view of the high proportion (55 per cent) of dogs in which this explanation would need to be invoked we regard it as somewhat unlikely. We believe, therefore, that the surviving dogs became adapted to existence without any parathyroid tissue whatever. While all had normal appearance and did not exhibit latent tetany, six of the eleven dogs were able to maintain serum calcium values in the normal range, and the other five stabilized their serum calcium at a lower level.

These findings pose two major problems. First, how is parathyroid function assessed in the dog, and how do these adapted animals differ from the normal in their regulation of calcium and phosphorus metabolism? Secondly, what are the mechanisms whereby aparathyroid dogs can exert control over the metabolism of calcium and phosphorus? It has been the purpose of this study to concentrate primarily on the first of these questions.

MATERIALS AND METHODS

Ten female mongrel dogs, with weights ranging from 10 to 20 kg, had previously undergone total thyro-para-

thyroidectomy and had reached the adapted state just described (1). These are referred to below as the experimental or parathyroidectomized dogs. Eight normal female mongrel dogs, with weights between 11.8 and 19 kg, served as controls. Perineotomy was performed on all animals, both experimental and control, to facilitate urethral catheterization. All the dogs received a normal kennel diet, which consisted of Ken-L-Bisket (Quaker Oats), kibbled medium, 2 to 4 cups per day, with water *ad libitum*. Their estimated daily calcium intake was of the order of 2.5 g.

Measurement of maximum tubular reabsorption of phosphorus (T_mP). Fifty ml of water per kg of body weight was administered by stomach tube. One hour later, an intravenous infusion was set up and a priming injection, consisting of 4 ml of 4 per cent creatinine and 3.5 ml of 0.5 M sodium phosphate per kg of body weight, was given. This was followed by a sustaining injection of 4 ml of 4 per cent creatinine and 4 ml of 0.5 M sodium phosphate per kg, made up to 500 ml with 0.9 per cent sodium chloride solution and given at the rate of 6 ml per minute. The sodium phosphate consisted of $Na_2HPO_4 \cdot 12\ H_2O$ and $NaH_2PO_4 \cdot H_2O$ in the proportion of 10.9 : 1 Twenty minutes was allowed for the establishment of a steady state, and then three or four 15 to 20 minute urine collections were made via an indwelling Foley catheter. Blood samples were drawn at the midpoint of each period.

Phosphorus: creatinine clearance ratio (C_P/C_{Cr}) after intravenous calcium infusion. A Foley urethral catheter was passed, and the bladder was emptied and washed out with two 50 ml volumes of distilled water; the catheter was then clamped and the urine and bladder washings discarded. One hour later, the bladder was again emptied and washed out; the combined urine and washings constituted the pre-infusion specimen. An intravenous infusion containing 15 mg of calcium per kg of body weight (as calcium gluconate[1]), made up to a total volume of 120 ml with 0.9 per cent sodium chloride solution, was then started. The rate of flow was adjusted so that the infusion was completed in 1 hour. Urine samples were collected, with washings, at the end of the infusion, 1 hour later, and thereafter at intervals of 2 hours for 6 more hours. Blood samples were collected at the midpoint of the pre-infusion period, at the end of the infusion, and at intervals of 2 hours thereafter for 6 more hours. In most of these experiments a further blood sample was collected 24 hours after the end of the infusion.

Infusion of ethylenediamine hydrogen trisodium acetate (EDTA trisodium salt, Trisodium Versenate). The procedure was similar to that for calcium infusion, except that urine samples were collected for 1 hour before the infusion, and at intervals of 2 hours after the start of the infusion for a total of 8 hours. During the one hour infusion period, 50 mg per kg of EDTA trisodium salt, or the equivalent weight of the disodium salt,[2] dissolved in 120 ml of 0.9 per cent sodium chloride solution, was injected. Blood samples were drawn at the midpoint of the pre-infusion period, at the end of the infusion, and 1, 2, 4, 6 and 24 hours afterwards.

Chemical methods. Serum calcium was determined by complexiметric titration, with ammonium purpurate as the indicator, as devised by Munson and associates (2). Phosphorus in the serum and urine was measured by the method of Gomori (3). Creatinine was determined in two ways. In the T_mP experiments, with exogenous creatinine loading and high serum and urine creatinine levels, the method of Folin and Wu (4) was used, modified as follows: serum proteins were removed by "acid tungstate" precipitation, with 1 : 4 dilution, as recommended by Owen, Iggo, Scandrett and Stewart (5) and the color developed by taking 0.2 ml filtrate, 3.8 ml water, and 2 ml of alkaline picrate. Urines were diluted appropriately and treated with alkaline picrate in the same manner. In the calcium and EDTA infusion experiments, where the serum creatinine level was not artificially elevated, the adsorption-elution procedure with Lloyd's reagent, described by Owen and co-workers (5), was followed exactly, except that in most of the experiments serum proteins were removed by trichloroacetic acid at a 1 : 4 dilution, with a final concentration of 10 per cent trichloroacetic acid.

RESULTS

Serum calcium. The mean serum calcium in the normal dogs, calculated from two determinations in each of six animals, was 10.8 mg per 100

TABLE I

Tubular maximal reabsorption of phosphorus (T_mP)

Dog	Range of serum inorganic phosphorus	Periods	T_mP*
no.	*mg/100 ml*	*no.*	
Parathyroidectomized			
2	24.0–24.3	3	85
4	21.8–22.3	3	148
5	21.8–23.6	4	167
6	26.2–26.6	3	154
12	24.0–25.2	4	92
13	18.8–21.4	4	79
			Mean 121
Normal			
102	18.8–20.3	3	111
104	15.7–17.1	4	97
106	17.4–18.2	3	169
			Mean 125

* Expressed as micromoles of phosphorus per minute, corrected to a glomerular filtration rate of 100 ml per minute.

[1] Kindly supplied by Parke, Davis & Company, Detroit, Mich.

[2] A generous supply of the latter substance was provided by Riker Laboratories, Inc., Los Angeles, Calif.

TABLE II

Clearance data following infusion of calcium (15 mg/kg) during one hour

Dog	Periods 1 1 Hr before infusion	2 1 Hr during infusion	3 0–1 Hr after infusion	4 1–3 Hrs after infusion	5 3–5 Hrs after infusion	6 5–7 Hrs after infusion
no.			Creatinine clearance values			
Parathyroidectomized						
1	45.5	40.8	40.2	45.0	42.6	54.0
4	33.8	31.8	25.0	*	27.3	35.3
5	70.2	74.5	65.2	67.0	63.3	72.5
12	42.3	51.0	49.5	45.4	44.1	36.0
15	33.3	39.6	39.4	38.3	34.2	40.8
21	50.7	40.0	43.3	39.0	35.4	47.1
23	*	*	23.4	22.0	24.3	30.2
Normal						
102	22.1	36.8	36.0	32.6	32.2	27.0
104	33.1	35.6	38.4	33.3	31.3	33.5
105	44.0	42.7	47.3	47.4	49.5	59.0
106	36.7	43.5	41.4	*	*	*
107	46.1	47.3	51.2	55.3	45.6	56.8
109	52.5	54.4	43.6	50.1	43.0	38.4
			Phosphorus: Creatinine clearance ratios†			
Parathyroidectomized						
1	0.293		0.280	0.171	0.081	0.072
4	0.282	0.172	0.103	0.137	0.148	0.146
5	0.160	0.125	0.111	0.100	0.132	0.096
12	0.166	0.087	0.192	0.154	0.146	0.129
15	0.210	0.090	0.051	0.0435	0.057	0.050
21	0.113	0.072	0.058	0.076	0.0795	0.0644
23	0.183	0.027	0.096	0.138	0.109	0.090
Normal						
102	0.164	0.060	0.019	0.0053	0.0037	0.0022
104	0.228	0.116	0.0428	0.0474	0.250	0.195
105	0.241	0.096	0.055	0.142	0.08	0.046
106	0.0502	0.0053	0.00224	0.00155	0.00296	0.0509
107	0.0317	0.0029	0.0344	0.212	0.125	0.515
109	0.0416	0.044	0.079	0.133	0.182	0.115

* Incomplete collection of urine.

† Calculated as $\frac{P_u \times Cr_p}{Cr_u \times P_p}$. This ratio may be converted to percentage of tubular reabsorption of phosphorus by the following relation: $\%\ T.R.P. = 100 - (C_P/C_{Cr} \times 100)$.

ml (SD ± 0.55). Of the parathyroidectomized dogs, six usually had serum calcium levels ranging between 9.0 and 11.0 mg per 100 ml, although slightly lower values were sometimes obtained. The levels in the remaining four (nos. 4, 5, 21 and 23) were always lower and ranged between 5.8 and 8.3 mg per 100 ml.

Serum phosphorus. The mean serum inorganic phosphorus level in normal dogs (based upon 11 determinations in 6 animals) was 4.6 mg per 100 ml (SD ± 0.8). In the parathyroidectomized dogs (10 animals, 19 determinations) the mean level was 5.6 mg per 100 ml (SD ± 1.3). The difference between these means was statistically significant ($p < 0.05$). However, in the six parathyroidectomized dogs with more normal serum calcium values, the mean phosphorus level was 4.7 mg per 100 ml, whereas in the four with lower calcium levels, the mean phosphorus value was 6.8 mg per 100 ml ($p < 0.001$).

Tubular maximal reabsorption of phosphorus (T_mP). The results are shown in Table I. It will be seen that in all the experiments the serum inorganic phosphorus levels were over 15 mg per 100 ml and in the majority were near 20 mg per 100 ml. These levels were found satisfactory by Hogben and Bollman (6) for the measurement of phosphorus Tm in the dog. There was essentially

no difference in the findings for the normal and the parathyroidectomized dogs.

Phosphorus: creatinine clearance ratio (C_P/C_{Cr}) after calcium infusion. These results are shown in Table II. It will be seen that in all the dogs studied, except 109, the ratio fell during the experiment. However, the results do not provide a clear-cut distinction between the normal and the parathyroidectomized dogs. In Dogs 102, 104 and 106, the ratio decreased more than in any of the others, falling to 1.3, 3.2 and 6.4 per cent, respectively, of the level before the calcium infusion. This pronounced decrease is in harmony with the results obtained in normal man, and it probably indicates the presence of functioning parathyroid tissue. Its absence, under the conditions of our experiments, does not indicate the contrary.

Serum calcium levels after calcium infusion. The results are shown in Table III and Figure 1. The usual statistical techniques have been applied to these data, and the results are shown in the values for p at the foot of the table. These indicate highly significant differences between the normal and the parathyroidectomized dogs for all periods of the experiment. Parathyroidectomized dogs with low initial serum calcium levels demonstrated the same response as the other parathyroidectomized dogs when the results were expressed as percentages of the initial value.

Serum phosphorus levels after calcium infusion. The results are shown in Table IV. It is clear that a conspicuous rise in serum phosphorus occurred in both groups, reaching a peak one hour after the end of the infusion, and then declining. No difference in this respect could be discerned between the two groups.

Serum calcium levels (non-EDTA-bound) after infusion of EDTA sodium salt. The results are shown in Table V and Figure 1. The

TABLE III

Serum calcium levels following infusion of calcium (15 mg/kg) during one hour

Dog	Initial value	End of infusion		2 Hrs after infusion		4 Hrs after infusion		6 Hrs after infusion		24 Hrs after infusion	
			% Init. value		% Init. value		% Init. value		% Init. value		% Init. value
no.	*mg/100 ml*	*mg/100 ml*		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*	
Parathyroidectomized											
1	9.6	12.4	129	10.9	114	10.3	108	9.4	98	9.4	98
2	9.4	15.3	163	13.6	145	12.6	134	12.0	128	10.9	116
4	7.1	11.5	162	11.1	156	10.5	148	9.1	128		
5	8.3	11.9	144	11.2	135	11.1	134	10.5	127	9.6	116
6	8.8	13.2	150	11.9	135	11.2	127	10.8	123	9.7	110
12	10.3	16.7	162	15.1	147	13.1	127			10.8	105
13	10.8	14.5	134	13.3	123	12.7	118	11.9	110		
15	8.5	13.3	157	12.3	145	11.5	135	11.4	134	10.3	121
21	6.7	10.9	163	10.3	154	9.9	148	10.0	149	8.2	122
23	7.2	14.1	196	11.8	164	9.3	129	8.4	117	7.8	108
Mean			156.0 ±18.8		141.8 ±15.4		130.6 ±12.2		123.8 ±14.4		112.0 ± 8.1
Normal											
102	11.4	15.1	133			11.5	101	10.0	88	10.6	93
104	10.7	12.1	113	10.9	102	10.4	97	10.7	100	10.8	101
105	10.5	14.8	141			12.4	118			10.8	103
106	10.1	12.7	126	11.0	109	10.1	100	10.2	101	9.6	95
107	10.9	12.4	114	11.6	105	11.0	101	10.7	98	10.9	100
109	11.0	14.3	130	11.8	107	10.6	96	10.9	99	10.9	99
Mean			126.2 ±11.5		105.8 ± 2.9		102.2 ± 6.6		97.2 ± 5.3		98.5 ± 5.4
p Values for differences between the means											
Upper limit			0.01		0.005		0.001		0.005		0.01
Lower limit			0.005		0.001				0.001		0.005

TABLE IV

Serum phosphorus levels following infusion of calcium (15 mg/kg) during one hour

Dog	Initial value	End of infusion	% Init. value	2 Hrs after infusion	% Init. value	4 Hrs after infusion	% Init. value	6 Hrs after infusion	% Init. value	24 Hrs after infusion	% Init. value
no.	*mg/ 100 ml*	*mg/ 100 ml*		*mg/ 100 ml*		*mg/ 100 ml*		*mg/ 100 ml*		*mg/ 100 ml*	
Parathyroidectomized											
1	4.9	4.8	98	4.3	88	4.2	86	3.3	67		
2	5.1	5.1	100	6.5	128	6.8	134	6.4	126	6.3	124
4	7.6	7.2	95	10.0	132	9.1	120	9.0	118		
5	6.0	7.3	122	8.4	140	7.5	125	8.2	137	7.4	123
6	6.3	5.6	106	6.9	130	6.1	115	5.9	111	5.3	100
12	4.5	6.0	133	6.2	138	5.8	129			6.2	138
13	3.6	4.3	119	5.9	164	6.2	172	6.4	178		
15	4.8	5.1	106	6.5	135	6.9	144	6.7	139	5.7	119
21	6.1	6.5	107	7.7	126	8.2	134	6.5	107	6.2	102
23	7.5	8.8	117	9.4	125	9.9	132	8.5	113	7.3	98
Mean			110.3 ±12.5		130.6 ±19.2		129.1 ±22.3		121.8 ±30.3		114.9 ±15.2
Normal											
102	4.8	4.6	96	5.5	115	5.2	108	5.5	115	4.8	100
104	6.2	6.4	103	7.0	113	6.8	110	6.1	99	6.2	100
105	5.3	5.0	94	6.8	128	5.8	109	6.3	119	4.7	89
106	4.9	5.4	110	4.8	98	4.8	98	4.6	94	5.2	106
107	3.9	6.3	162	6.9	177	6.7	172	6.2	159		
109	4.1	5.3	129	6.2	151	5.6	137	4.5	110		
Mean			115.7 ±25.7		130.3 ±28.2		122.3 ±25.2		116.0 ±21.9		98.8 ± 7.2

values of p for the differences between the two groups have been determined as in Table III, and it can be seen that these differences are highly significant, except for the levels at the end of the infusion. The slow restoration of the original calcium level in the parathyroidectomized dogs is obvious from Figure 1. As with the calcium infusions, the responses of the parathyroidectomized dogs with a low initial serum calcium level could not be separated from those of the other parathyroidectomized dogs, when the results are expressed as percentages of the initial values. The serum calcium level was not followed in every dog to the point of restoration of the initial value. In one animal, however, the process was studied in detail, and the results (Table VI) give a good indication of the sluggishness of this response.

Serum inorganic phosphorus levels after infusion of EDTA sodium salt. No consistent changes were seen in either the normal or the parathyroidectomized dogs.

Effect of thyroid replacement. It might be argued that the altered response to calcium and to EDTA infusions in the operated animals was due

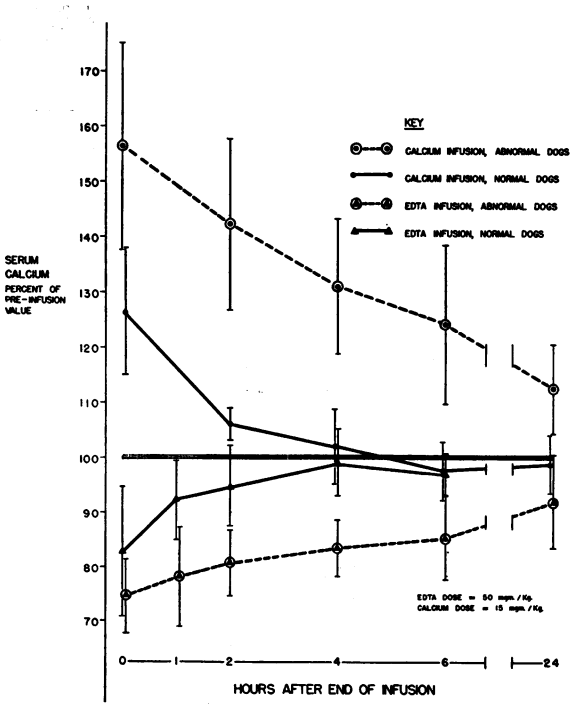

FIG. 1. MEAN SERUM CALCIUM LEVELS, EXPRESSED AS PERCENTAGES OF PRE-INFUSION VALUES, FOLLOWING CALCIUM AND EDTA INFUSIONS IN NORMAL AND IN ADAPTED PARATHYROIDECTOMIZED DOGS. The vertical lines represent one standard deviation above and below the means.

TABLE V

Serum calcium levels (non-EDTA-bound) following infusion of EDTA trisodium salt (50 mg/kg) during one hour

Dog	Initial value	End of infusion	% Init. value	1 Hr after infusion	% Init. value	2 Hrs after infusion	% Init. value	4 Hrs after infusion	% Init. value	6 Hrs after infusion	% Init. value	24 Hrs after infusion	% Init. value
no.	*mg/100 ml*	*mg/100 ml*		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*		*mg/100 ml*	
Parathyroidectomized													
1	10.9	8.7	80	9.0	83	8.9	82	8.6	79	8.4	77	8.9	82
2	10.1	8.3	82	7.8	77	7.3	79	7.9	78	7.7	76	7.7	76
4	6.9	4.8	70	4.0	58	5.0	72	5.8	84			6.5	94
5	5.8	4.3	74	4.7	81	4.8	83	4.9	84	5.1	88	5.5	95
6	8.2	5.6	68	6.5	79	6.6	81	7.0	85	7.2	88	8.0	98
12	9.6	7.0	73	7.7	88	7.8	81	7.8	81	8.4	88	8.7	91
13	9.9	8.3	84	9.1	92	9.1	92	9.2	93	9.7	98	9.9	100
15	9.0	6.9	77	7.3	81	7.5	83	7.4	82	7.7	86		
21	8.2	6.3	77	6.7	82	6.8	83	7.3	89	7.4	90	8.4	102
23	6.3	4.1	65	4.7	75	5.3	84	5.2	83	5.2	83	6.0	95
Mean			75.0 ± 6.8		78.8 ±9.1		81.3 ±6.0		83.8 ±5.1		86.0 ±7.5		92.6 ±8.5
Normal													
102	10.2	9.2	90	10.1	99	10.5	103	11.0	108	9.6	94		
104	11.3	9.8	87	10.6	94	11.1	98	10.7	95			10.7	95
105	10.5	7.8	74	8.4	80	9.1	86	9.7	92	10.1	96	10.2	97
106	10.7	10.1	94	10.6	99	10.5	98	10.3	96	10.0	94		
107	11.6	10.4	90	10.2	88	10.6	91	11.7	101	11.6	100		
109	10.0	6.3	63	9.4	94	9.6	96	10.3	103	10.3	103		
Mean			83.0 ±11.9		92.4 ±7.3		95.3 ±7.3		99.2 ±6.1		97.4 ±3.9		
p Values for differences between the means													
Upper limit			0.2		0.01		0.005		0.001		0.01		
Lower limit			0.1		0.005		0.001				0.005		

to lack of thyroid rather than parathyroid hormone, since, in every case, the thyroid gland was removed as completely as possible. In some of the parathyroidectomized dogs, the studies were carried out while the animals were receiving thyroid extract orally (30 mg three times a week, from the time of thyroidectomy). The remaining dogs were studied at periods up to four months after thyroid replacement had been discontinued. No difference could be found between the responses of dogs with and without thyroid replacement.

TABLE VI

EDTA infusion in Dog. no. 2

Time		Non-EDTA-bound serum calcium
	Days	*mg/100 ml*
Before infusion		10.1
At end of infusion		8.3
After infusion:	1	7.7
	2	8.6
	3	8.7
	4	8.9
	5	9.2
	6	9.6
	7	9.8
	8	10.2

In order to explore this point more thoroughly, two dogs, which had previously been tested without thyroid replacement were given triiodothyronine orally and then tested. One dog received 25 μg daily for 5 days, then 50 μg daily for 16 days; the other was given 50 μg daily for 16 days. The responses of these animals to calcium and EDTA infusions were unchanged by triiodothyronine in this dosage. To exclude the possibility that the drug was not being absorbed properly, the same two dogs were treated with intramuscular injections of 120 μg of triiodothyronine daily for 7 days, and the infusions were then repeated. The responses were again essentially unchanged.

DISCUSSION

It is clear that, from the standpoint of separating normal from parathyroidectomized dogs, some criteria were more satisfactory than others. The level of serum calcium, for example, was quite unreliable; six out of the ten parathyroidectomized dogs consistently had normal values after an initial period of critical and life-endangering hypocalcemia. It is of interest that the same six dogs also had normal levels of serum inorganic phosphorus.

The tubular maximal reabsorption of phosphorus was also an unsatisfactory criterion. However, this finding was not entirely unsuspected, since attempts to detect hypoparathyroidism in humans by means of this test, in the experience of most workers (7), have been unsuccessful. Hogben and Bollman (6) demonstrated a raised T_mP in parathyroidectomized dogs, but these measurements were made some 14 days after operation, and it is therefore probable that their animals had not reached the adapted state.

The phosphorus: creatinine clearance ratio after calcium infusion has been used for some years to evaluate parathyroid function in man, and is a valuable clinical test (8, 9). The response seen in normal subjects is a rise in serum inorganic phosphorus and a fall in C_P/C_{Cr}, while in hypoparathyroid subjects, although the rise in serum phosphate occurs, the C_P/C_{Cr} remains unchanged or may even rise.

Our observations in dogs are not consistent with the findings in man. It is possible that a species difference is responsible, but it should also be noted that the conditions of our test were somewhat different from those originally proposed by Howard, Hopkins and Connor (8). While the dose of calcium (15 mg per kg) was the same, we have given the injection over a period of one hour rather than four hours, and we have confined our measurements to the six hours following the infusion. Some preliminary studies of C_P/C_{Cr}, 24 hours after the infusion, showed that no additional information was obtained. It is possible that the longer stimulus of the four hour infusion would have produced a sharp reduction in C_P/C_{Cr} in all of our normal dogs, instead of in only half of them. The rise in serum phosphorus in both the normal and the parathyroidectomized dogs after calcium infusion is comparable with the changes seen in normal humans and in patients with hypoparathyroidism by Nordin and Fraser (9) and supports the view that the parathyroid glands are not responsible for this phenomenon.

The changes in serum calcium levels produced by infusions of calcium and of EDTA were more satisfactory as a means of discriminating between normal and parathyroidectomized dogs. Many investigators have given infusions of calcium to normal humans and to patients with hypoparathyroidism (8–10); but attention seems to have been focused mainly on the changes in urinary phosphate excretion, and little account has been taken of the serum calcium levels. This consideration, and the fact that our calcium infusions were given in one quarter of the time customary for studies in man, make it difficult to relate our findings to human parathyroid physiology. In the dog, however, it is clear that while the parathyroid glands may not be necessary for life or even for the maintenance of a normal serum calcium level, in their absence the animal is unable to deal so effectively with an acute intravenous load of calcium.

In interpreting the changes in serum calcium following EDTA infusion, it must be recognized that the method used to determine serum calcium under these circumstances, measures not the total calcium but only that fraction of it that is not already bound by EDTA. Had the EDTA in the serum been destroyed by preliminary ashing before the estimates were made, the figures obtained for serum calcium would have been substantially higher. However, EDTA infused *in vivo* probably binds the ionized fraction of serum calcium more effectively than any other, and the fall in non-EDTA-bound calcium in these dogs was almost certainly accompanied by a fall in the ionized fraction. There is some evidence (11, 12) that variations in this fraction may be responsible for regulating the output of parathyroid hormone. Our results show that the normal animal rapidly restores the non-EDTA-bound fraction (and probably the ionized fraction) to its original level, while the parathyroidectomized animal does so only slowly and imperfectly.

Somewhat similar findings were recorded by

Patt and Luckhardt (11) and Stewart and Bowen (12) who infused sodium oxalate solutions into normal and parathyroidectomized dogs, and showed that in the latter group restoration of the serum calcium level was slow and incomplete. The thyroid and parathyroid glands of these dogs had been removed shortly before the infusions; in the study of Patt and Luckhardt, just prior to the infusions, and in that of Stewart and Bowen, one to five days previously. From our results it is evident that this disability in calcium regulation persists indefinitely. On the other hand, Copp (13) performed similar experiments on acutely parathyroidectomized dogs, using larger doses of EDTA (equivalent to 86 mg per kg of the trisodium salt). He was unable to discern any obvious alteration in the response to EDTA infused 20 hours after parathyroidectomy.

If the results from the infusions of calcium and EDTA are considered together, it becomes clear that the homeostatic mechanisms responsible for maintaining the level of serum calcium constant are vulnerable to acute challenge in the parathyroidectomized dog as compared with the normal. This is the case whether the serum calcium is raised or lowered from its initial level. At the same time, the parathyroidectomized dogs do possess a mechanism capable of restoring their level of serum calcium toward the original value.

Our study does not permit any conclusion as to the mechanism of this homeostasis in the intact dog. Only two basic processes appear possible in such a rapid adjustment. There is either a renal mechanism, whereby the rate of urinary calcium excretion is increased or decreased according to the needs of the situation, or an alteration of the equilibrium between the circulating calcium and the calcium in the skeleton. Previous work (14–16) indicates that parathyroid hormone may take effect through both mechanisms. In the parathyroidectomized dogs, as has been noted above, there is good reason to believe that no parthyroid tissue remained; yet the animals were still able to regulate their serum calcium levels, although with less than normal efficiency. This implies one of two possibilities. On the one hand, these dogs may have been obtaining parathyroid hormone, or some substance with the same biological action, from other tissues, as has been postulated in some human patients with neoplasms (17, 18). On the other hand, the removal of all parathyroid tissue may have allowed another, more sluggish mechanism to come to light—for example, an alteration in the net calcium flux across the intestinal wall.

Whether a similar adapted state develops in humans deprived of all parathyroid tissue is uncertain. Mammalian parathyroid physiology, and the status of parathyroid grafted tissue in particular, should be reviewed in the light of these results.

SUMMARY

1. Six normal dogs and ten adapted parathyroidectomized dogs have been studied with a view to defining the differences in calcium and phosphorus homeostasis in the two groups. The adapted animals required support during the acute hypocalcemic period after removal of their parathyroid glands for from 19 to 78 days. For the five months that these dogs were studied in the course of this experiment, no special support was required.

2. Parathyroidectomized dogs could not be distinguished with certainty from normal dogs either by clinical observation or by studies of serum calcium or phosphorus levels, phosphorus: creatinine clearance ratios after a calcium infusion, or tubular maximal reabsorption of phosphorus.

3. Parathyroidectomized dogs receiving intravenous infusions of either calcium gluconate (15 mg per kg in one hour) or EDTA (50 mg per kg in one hour) demonstrated markedly impaired ability to restore their serum calcium levels to the initial value, as compared with normal dogs. This response was unaltered by thyroid replacement therapy.

4. These findings imply that the parathyroid glands play an important part in dealing with acute disturbances of the serum calcium level. However, a secondary mechanism, capable of more sluggish regulation, seems to be revealed when the parathyroid glands are removed.

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