

Immediate Changes in Phosphate Excretion Following Parathyroidectomy in the Rat.* (20901)

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In previous studies(1-4) the effect of parathyroid extract on phosphate metabolism in bone has been emphasized. These works argue convincingly for a direct action of the parathyroid hormone on bone. In addition to this, Stewart and Bowen(5) have suggested that the phosphuria produced by parathyroid extract administration might possibly be an artefact resulting from the methods of extraction. In the light of these studies, it seemed advisable to re-examine the effect of the parathyroids on phosphate excretion by the kidney in order to determine, if possible, whether in addition to the effect on bone there could be a direct effect on the kidney.

One valid objection that has been raised to the use of experiments dealing solely with the administration of parathyroid extract is that available commercial extracts of these glands are inhomogenous preparations containing a large amount of inert protein(5,6). In view of this objection it would appear that the best method of showing that the results achieved by this use of the extract could be attributed to the hormone of the parathyroid glands would be to undertake similar studies in parathyroidectomized animals. By removing the source of the hormone from the body, opposite effects from those produced by the addition of the extract should be seen if they are true effects produced by the parathyroid hormone; and administration of parathyroid extract to such hypoparathyroid animals should reverse these changes and return the animals toward their normal physiological state. Tweedy *et al.*(7) reported such effects utilizing radiophosphorus as an indicator of phosphorus excretion. Their work, however, is based on long range studies and does not in itself show that these effects could not have resulted indirectly due to changes in bone metabolism. The following studies were therefore undertaken to study the changes,

and the reversibility thereof, occurring in the first few hours after parathyroid removal. The results obtained in these studies indicate very strongly that in the rat at least one of the sites of direct action of the hormone of the parathyroids is in the kidney.

Materials and methods. Approximately 125 male Sprague-Dawley rats, weighing between 180 and 225 g were used in these experiments. The following procedure was used in each of the individual experiments: On the morning of the experimental day, the animals were parathyroidectomized under ether anesthesia by the method of Richter and Birmingham(8). One hour after completion of the operation, approximately 7 μ C of radio-phosphorus were administered intraperitoneally; each animal was given a water dose equivalent to 5% of its body weight and placed in metabolism cages for the collection of urine. Control animals similarly treated were run at the same time. The 3 test materials, parathyroid extract,[†] calcium chloride, and disodium phosphate were injected as indicated in the particular experiments. Urine was collected either hourly or at 3-hour intervals. When collected hourly, an additional 5 ml of water was administered at the third hour to enhance the flow of urine. Experiments were terminated at 3, 7, 11, and 13 hours after parathyroidectomy, at which time blood was removed for serum calcium and inorganic phosphate analyses. In one experiment, sham operations were performed on the control animals used, and in a second they were anesthetized without surgery. Since the serum calcium and phosphate levels and the phosphorus excretion values for the control animals of these 2 experiments were identical with those in which controls were not subjected to anesthesia or surgery, it was concluded that neither of these was a factor in the results obtained. Injection of radio-activity was delayed until one hour after

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[†] Lilly's Parathyroid Extract.

TABLE I. Effect of Parathyroidectomy upon Radio-Phosphorus Excretion by the Kidney.*

Hr after parathyroidectomy→	Excretion of radioactivity following parathyroidectomy; P-32 inj. 1 hr after surgery									Total phosphorus exc./hr, mg
	Total							Total 4-7 hr	Total 6 hr	
	2	3	2 hr	4	5	6	7			
A) Normals (8)	553	165	718	53	107	76	46	282	1000	.34 ± .05
B) PTX (8)	4	3	7	3	4	2	2	11	18	.02 ± .007
C) PTX (16)	—	—	—	—	—	—	—	—	22	—
D) 50 i.u. PTH admin. at operation (4)	—	—	—	—	—	—	—	—	284	—
E) 75 i.u. PTH—½ at operation, ½ 2 hr after (4)	—	—	—	—	—	—	—	—	725	—
F) 75 i.u. PTH—admin. 3 hr after surgery (4)	—	—	21	—	—	—	—	842	863	—

* For comparative purposes, radioactivity excreted by control animals was set at 1000 for the 6 hr collection period, and values for experimentals adjusted to these standards. Since the values for controls run with Groups C-F are therefore all 1000, they are omitted from the table. Numbers in parentheses are No. of animals.

removal of the parathyroids to allow full recovery from the anesthesia. Radio-activity determinations were made by standard procedures with a Geiger-Müller counter. Serum calcium determinations were made using the Clark-Collip modification of the Kramer-Tisdall Method. Urinary and serum inorganic phosphate determinations were made by the method of Fiske and SubbaRow.

Experimental data. Changes in phosphate excretion. The experimental procedure described above consistently produced an immediate and marked drop in phosphate excretion determined both by excretion of P-32 and total urine inorganic phosphate. Out of 55 animals parathyroidectomized not one rat failed to show a drop in phosphate excretion of less than a factor of 10, and all but 8 showed a drop in P-32 by a factor of 30 or more at all the times checked between 2 and 13 hours after parathyroidectomy. In order to determine the time between parathyroidectomy and the time urinary excretion was effected, hourly collections were made. Since the radio-activity was injected one hour after the operation, the 6 hourly collections represent the second through the seventh hour after the removal of the parathyroids. The results are shown in Table I, A and B. It will be noted that by the first hour of collection, the effect on the kidney was maximal, indicating that the effect on phosphate excretion was produced immediately upon the loss of circulating parathyroid hormone, which

appears to occur within the first hour after parathyroidectomy. In further experiments, parathyroid extract was administered subcutaneously to parathyroidectomized animals to determine the ability of the extract to prevent changes seen in parathyroidectomy and to reverse these once they had occurred. When administration of the extract was started immediately after the removal of the parathyroids, phosphorus excretion was maintained at or below normal level, depending upon the dose of the extract and the frequency of its administration (Table I, D and E). When the extract was administered 2 hours after parathyroidectomy, the rapid fall in phosphate excretion which had occurred by this time was dramatically reversed by the production of a marked phosphuria (Table I, F).

Reliability of P-32 values as an indication of PO_4 ion excretion. In the routine experiments, the radioisotope was injected one hour after parathyroidectomy. To establish conclusively that the P-32 excretion values were a reliable indicator of inorganic phosphate changes, 2 checks were made. First, a control experiment was run identical to those described above except that the radiophosphorus was injected 18 hours before parathyroidectomy. By 18 hours the specific activities of P-32 in the various compartments of the body are relatively stable in comparison to the rapidly changing specific activities encountered immediately after injection. In this ex-

TABLE II. Comparison of Serum Values to Phosphorus Excretion of Parathyroidectomized and Control Rats at Various Time Intervals Following Parathyroidectomy.

Time after parathyroidectomy, hr	No. of animals	Urinary P-32	Serum Ca, mg %	Serum P, mg %
Controls	40	1000*	10.7 $\pm$.17	9.0 $\pm$.28
3	20	15	9.2 $\pm$.19	9.6 $\pm$.33
7	24	22	7.0 $\pm$.24	10.6 $\pm$.33
11	9	—	7.3 $\pm$.59	12.4 $\pm$.24
13	4	7	7.6 $\pm$.11	15.1 $\pm$.61

* For comparative purposes, radioactivity excreted by control animals was set at 1000 for each of the experimental periods, and values for experimental animals adjusted to these standards.

periment, the P-32 excretion following parathyroidectomy was altered to the same extent as that seen when the radioisotope was administered one hour after parathyroidectomy. With the total radioactivity of the controls for the same 6-hour period set at 1000, the excretion value of the experimentals was 38.3. For the second check, inorganic phosphates in the urine were determined in several groups of animals. These values are given in Table I on the basis of mg of phosphorus excreted per hour. The ratio between the experimental and the controls is in the same range as for the P-32 values.

Comparison of phosphate excretion rates to calcium and phosphate serum levels. In order to determine the relationship between the changes in phosphate excretion and those in serum levels of calcium and phosphate produced by parathyroidectomy, determinations were made of both serum and urine values at intervals of 3 to 11 hours after parathyroid removal. The results are shown in Table II. The urinary excretion of phosphate which, as pointed out above, was curtailed within the first hour after parathyroidectomy, remained low throughout the experimental period. Serum phosphate and calcium changes were more gradual. The serum calcium level appeared to drop faster than the phosphate level rose. The rise in serum phosphate, while indicated at 3 hours, was not significant until the 7-hour period. It is interesting to note that the amount of phosphate (approximately 2 mg phosphorus) which the parathyroidectomized animal failed to excrete during the

7-hour experimental period is over 10 times that which would be required to raise the serum phosphorus level to the 10.6 mg % noted if all the withheld phosphate had remained in the serum.

Effect of calcium and phosphate injection on phosphate excretion. Two experiments were run to determine whether or not the changes in phosphate excretion produced by parathyroidectomy could be influenced by the presence of a surplus of calcium or phosphate ions in the blood. In the first, 2 mg of calcium, as calcium chloride, were injected hourly beginning the first hour after parathyroid removal. In the second experiment, 2 mg of phosphorus, as di-sodium phosphate, were injected at the conclusion of the operation and at one, 3, and 4 hours after parathyroidectomy. In both experiments the animals were killed at the seventh hour and blood removed for analysis. The results of these experiments are summarized in Table III. The flooding of the animal with calcium had no effect on the changes seen in phosphate excretion following parathyroidectomy. It did, however, not only prevent the rise in serum phosphate normally seen, but suppressed this value slightly below that seen in the control rats used in these experiments. The control animal was able to compensate for the most part for the excess phosphate administered. This was done by a high phosphate excretion rate with the result that serum phosphate was only mildly and, presumably, temporarily elevated and the serum calcium level was not affected. The parathyroidectomized animal was unable effectively to take care of the injected phosphate. The serum phosphate rise produced by parathyroidectomy was considerably exaggerated, yet the phosphate excretion was still below that seen in the normal rat (Table III).

Discussion. While the Albright and Reifenshtein theory of parathyroid function(9) can no longer be accepted *in toto*, as has been demonstrated previously(1,10), it can not be denied that kidney excretion of phosphorus is markedly influenced by parathyroid function. These phosphate changes have recently been studied by such workers as Tweedy *et al.*(7), Handler and Cohen(11,12), without

TABLE III. Comparison of Serum Values to Phosphorus Excretion at 7 Hours after Parathyroidectomy under Various Experimental Conditions.

Condition of rats	No. of animals	Urinary P-32*	Serum Ca, mg %	Serum P, mg %	Urinary phosphorus	
					No. of rats	mg phosphorus
Normal	40	1000	10.7 ± .17	9.0 ± .28	16	2.38 ± .36
PTX	24	22	7.0 ± .24	10.6 ± .33	12	.14 ± .04
PTX + 12 mg Ca	4	16	10.4 ± .28	7.9 ± .56	—	—
Normal + Na ₂ HPO ₄	4	3300	10.3 ± .23	10.7 ± .31	4	7.1 ± 1.7
PTX + Na ₂ HPO ₄	4	683	7.6 ± .6	14.2 ± .31	4	1.2 ± .6

* For comparative purposes, radioactivity excreted by control animals was set at 1000 for each of the experimental periods, and values for experimental animals adjusted to these standards.

conclusively showing either a direct action of the hormone on the kidney or a definite site of action within the kidney.

Since evidence is accumulating to show a direct action in bone attributable to the hormone of the parathyroids, it is very important to establish whether the changes seen in kidney function are *direct* or *indirect* effects. The results of this study give convincing data that, at least in the rat, the kidney is directly involved. Following parathyroidectomy, reduction in kidney excretion of phosphate occurs within the first hour and can be increased within an equally short time by the injection of parathyroid extract. These marked (up to 50-fold) changes in phosphate excretion can occur with negligible changes in serum calcium and phosphate values. What is most convincing is that the decrease in phosphate excretion occurs even though either excess calcium or phosphate ions are injected at or immediately following parathyroid removal. In view of the above data, therefore, direct involvement of the kidney in parathyroid function must be considered.

Summary. Parathyroid function has been studied in rats by determination of the changes produced immediately after parathyroid removal. Phosphate excretion by the kidney is markedly reduced within one hour after parathyroidectomy. This impairment

of phosphate excretion is apparent in rats flooded with either calcium or phosphate ions after removal of the parathyroids. It is concluded, therefore, that in the rat the parathyroids at least in part control phosphate and calcium metabolism by a direct action on kidney excretion of phosphate.

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