Parathyroid Hormone and Renal Cell Metabolism*

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ABSTRACT: The early effects of parathyroid hormone, calcium, and EGTA infusion upon renal 3′5′-cyclic adenosine monophosphate and glycolytic and Krebs cycle intermediates was studied in normal animals as well as those made vitamin D deficient or treated with an excess of corticosterioids. Hormone infusion caused an inhibition and EGTA a stimulation of isocitric dehydrogenase in normal animals. Calcium infusion led to an inhibition of pyruvate kinase in normal animals as well as in those made vitamin D deficient or those treated with excess corticoids. Hormone infusion also led to an inhibition of pyruvate kinase in the latter two types of animals.

The hormone produced the same rise in 3′5′-cyclic adenosine monophosphate in all three types of animals, but neither calcium nor EGTA had any effect upon the concentrations of 3′5′-adenosine monophosphate. It was concluded that a rise in intracellular Ca^{2+} may be one of the earliest consequences of parathyroid hormone action.

In an effort to gain further insight into the mechanism of parathyroid hormone action, the early effects of this hormone upon the concentrations of glycolytic and Krebs cycle intermediates in renal tissue were examined. Studies were carried out in control animals, and in animals treated with excess glucocorticoids or made vitamin D deficient by dietary restriction. The latter two groups were included because it is known that both glucocorticoids and vitamin D influence calcium metabolism and the renal response to parathyroid hormone (Rasmussen, 1968).

The changes in metabolic intermediates following infusion of calcium chloride, magnesium chloride, acetazolamide, EGTA, and $NaHCO_3$ were also examined, and compared with those seen after hormone infusion.

Finally, because of the recent studies implicating cyclic 3′5′-AMP as an intermediate in parathyroid hormone action (Wells and Lloyd, 1967; Chase and Aurbach, 1967, 1968; Rasmussen *et al.*, 1968), the changes in concentration of this cyclic nucleotide were measured under all experimental conditions in an effort to correlate, if possible, changes in its concentration with the other metabolic changes observed.

Experimental Section

Male Wistar or Holtzman rats weighing 120–140 g were maintained on a standard laboratory chow diet for at least 1 week before being subjected to parathyroidectomy. The animals were parathyroidectomized by hot wire cautery under ether anesthesia and were studied either 2.5 hr or several days after operation. Some animals were parathyroidectomized, allowed to recover from surgery for 2 days, then given cortisone acetate, 2.5 mg/day for 3 days before being used for study.

Each individual experiment employed 8–12 rats divided into a control and treatment group. Each particular experimental procedure was repeated at least three times. Thus, each experimental value represents the mean of values from 12 to 18 animals.

Vitamin D deficient animals of approximately the same weight were raised from male weanling rats obtained from low D-breeding strain of the Holtzman Co. (Madison, Wis.). These animals were raised in isolation on a D-deficient diet containing a normal Ca/P ratio as previously described (Rasmussen *et al.*, 1963). They were used for study 2.5 hr after either parathyroidectomy or a sham operation. This short time interval was selected because these parathyroidectomized animals developed severe tetany approximately 4 hr after operation.

For the metabolic studies, all animals were anesthetized with dialurethane (CIBA Pharmaceutical Co.) given intraperitoneally in a dose of 0.6 ml/kg. The femoral vein was exposed and perfused under direct vision with either isotonic saline (control), parathyroid hormone (hormone treated), or an agent such as $CaCl_2$, $MgCl_2$, EGTA (ethylene bisoxyethylenenitrilotetraacetate), Diamox (acetazolamide), or HCl. Initially, perfusions were carried out for varying lengths of time but after some experimentation a standard time of 5 min was selected. In all cases, one-third of the total dose of the agent to be given was infused rapidly over a few seconds and then a sustaining infusion was maintained over the remaining time interval. Parathyroid hormone, prepared as previously described (Hawker *et al.*, 1966), was given in a total dose of 10 μg. $CaCl_2$ and $MgCl_2$ were given as a 60 mM solution using a total of 60 μmoles. This was sufficient to elevate plasma calcium (6.5 ± 0.5 to 11.5 ± 1.0 mg per 100 ml of plasma) in the parathyroidectomized animals, but a large dose of $CaCl_2$ (100 μmoles) was necessary to bring about a

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similar elevation of plasma calcium in the D-deficient parathyroidectomized animals. EGTA was given as a 20 mM solution neutralized with NaOH; HCl was given as a 0.125 M solution; $HaHCO_3$ was given as a 0.25 M solution; and Diamox as a solution of 3 mg/ml. In all instances the amount of fluid administered was the same in the experimental and control animals, and never exceeded 1.5 ml in total volume.

Exactly 30 sec after the cessation of the infusion, the abdominal cavity was rapidly opened by a midline incision, and the left kidney was grasped by its vascular root with a pair of curved forceps, avulsed, and immediately frozen by compression with aluminum tongs precooled in liquid nitrogen. This procedure was accomplished in less than 5 sec. After the kidney had been removed, a sample of blood was obtained by cardiac puncture for measurement of plasma calcium and phosphate.

The frozen renal tissue was powdered in a mortar and pestle cooled in Dry Ice. When reduced pyridine nucleotides were to be assayed, exposure of the tissue to CO_2 was avoided by cooling the mortar in liquid N_2.

To assay Krebs cycle and glycolytic intermediates and adenine nucleotides, approximately 400 mg of tissue powder was weighed in a cooled (in Dry Ice) 10-ml Potter-Elvejhem homogenizer and then homogenzied in 2 ml of 8% perchloric acid in 40% ethanol (v/v). After homogenization, the suspension was centrifuged and the pellet was reextracted with 1.5 ml of 6% perchloric acid. The combined extracts were neutralized to pH 5.5 ± 0.5 with 3 M K_2CO_3 containing 0.5 M triethanolamine. The $KClO_4$ precipitate was removed by low-speed centrifugation.

Assays of the metabolic intermediates were carried out on suitable aliquots of this neutralized extract. The assays employed appropriate enzyme systems which coupled all measurements to changes in oxidation or reduction of pyridine nucleotides. These changes were measured fluorometrically in a metabolite fluorometer (designed and constructed in the instrument shop of the Johnson Research Foundation, University of Pennsylvania). The precise details of all of the assays of the intermediates of the citric acid cycle have been thoroughly described by Williamson and Herczeg (1968). Glycolytic intermediates were measured by the methods of Matria and Estabrook (1964).

A different tissue extract was used to measure reduced pyridine nucleotides. Approximately 100 mg of cold powdered tissue was heated at 55° for 2 min in 1 ml of 0.2 N KOH in 50% (v/v) ethanol. After cooling in ice, the pH of the mixture was adjusted to pH 8.5 ± 0.5 with 1 M triethanolamine HCl (pH 5.5) and then centrifuged. Suitable aliquots of this mixture were assayed for DPNH and TPNH by the methods of Williamson and Herczeg (1968).

For the measurement of 3′,5′-AMP in renal tissue, approximately 100 mg of tissue was extracted with 2.5 ml of 5% trichloroacetic acid to which had been added a standard amount of [^{3}H]3′5′-AMP. The insoluble residue after centrifugation was reextracted with 1.0 ml of 5% trichloroacetic acid. The trichloroacetic acid was removed by repeated (four times) extraction of the aqueous extract with four volumes of

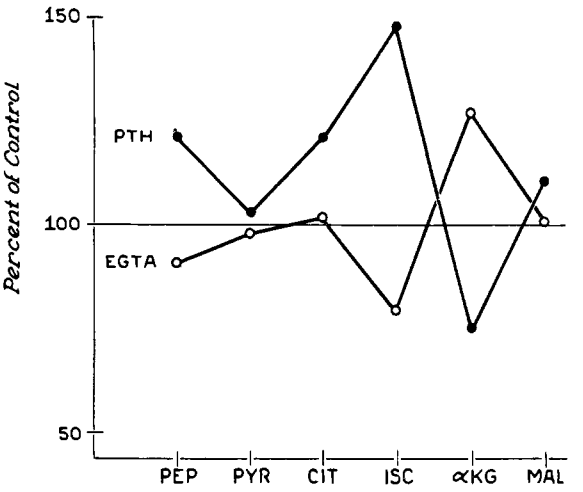


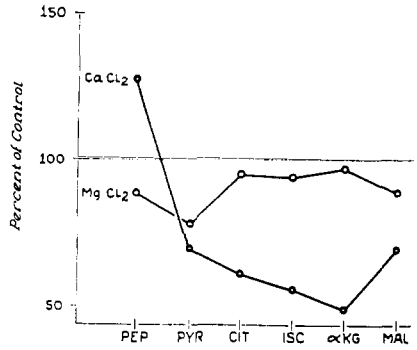
FIGURE 1: The pattern of change in metabolic intermediates in renal tissue seen 6 min after the intravenous infusion of parathyroid hormone (●—●) or EGTA (○—○) into parathyroidectomized rats. The results are plotted as per cent of control values obtained from parathyroidectomized animals given a saline infusion. Each point represents the mean value of six animals.

H_2O-saturated ether. The resulting solution was then passed through a 2 × 0.35 cm column of Dowex 50-X8 in the H^+ form, and the column was washed with 5 ml of H_2O. The eluent was either lyophilized or evaporated to dryness in a Buchler rotoevaporator, and the residue was taken up in a small volume (50 μl) of H_2O and separated by thin-layer chromatography using cellulose (MN-Cellulosepulver 300 HR, Macherey, Nagel & Co.) as the supporting medium, and isopropyl alcohol–H_2O–NH_4OH (7:1.5:1.5) as developing solvent. The cyclic nucleotide was eluted from the cellulose with 2 ml of H_2O and assayed by first converting 3′5′-AMP into 5′-AMP using the brain phosphodiesterase (prepared by the method of Cheung, 1966). The resulting AMP was converted into ADP with myokinase, and then into glucose-6-PO_4 with hexokinase, excess phosphenolpyruvate, and glucose in a cycling enzyme system. The final product, glucose 6-phosphate, was assayed by the method of Matria and Estabrook (1964). This method represents a modification of the techniques of Goldberg *et al.* (1967).

Results

As shown in Figure 1 and Table I, the intravenous infusion of parathyroid hormone into a parathyroidectomized rat led to a prompt and significant increase in isocitrate, a less dramatic increase in citrate, and a significant fall in α-ketoglutarate. The changes shown were seen 6 and 12 min after initiation of infusion, but similar changes were seen as early as 2 min after PTH infusion as shown in Table II where the values of isocitrate, α-ketoglutarate, their ratios, and the values of 3′5′-AMP are recorded as a function of time after PTH infusion.

One of the possible explanations for this change in metabolite profile was an increase in intracellular



FIGHRE 2: The pattern of change in metabolic intermediates in renal tissue seen 6 min after the intravenous infusion of $CaCl_2$ (●—●) or $MgCl_2$ (○—○) into parathyroidectomized rats.

calcium. To examine this possibility, infusions of EGTA were given to lower, and infusion of calcium given to raise, extracellular calcium. As shown in Figure 1, the infusion of EGTA led to a pattern opposite to that seen with PTH, namely, a fall in isocitrate and a rise in α-ketoglutarate. However, calcium infusion led to quite a different pattern of change as shown in Figure 2 and Table III. In this instance the predominant changes were an increase in phosphenolpyruvate and 3-phosphoglycerate, and a fall in pyruvate and all the Krebs cycle intermediates.

It was also considered possible that an increase in intracellular Mg^{2+} might produce the changes seen after PTH. However, as shown in Figure 2, magnesium infusion led to less striking changes than those seen after calcium infusion, with little change in PEP and pyruvate, and no significant change in the level of Krebs cycle intermediates.

To rule out the possibility that the changes seen after PTH or Ca^{2+} were due to changes in intracellular pH, the effect of metabolic alkalosis, acidosis, and respiratory acidosis upon metabolite profiles was also examined. The results are shown in Figure 3. In each instance the pattern was quite different from that seen after either PTH or calcium infusion.

It was also considered possible that these changes might be due to changes in either adenine or pyridine

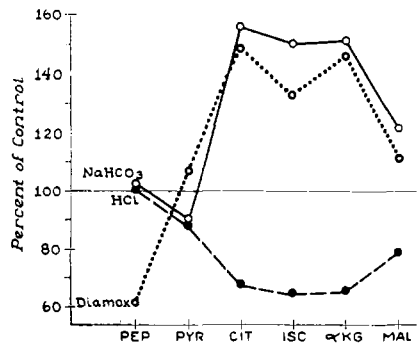


FIGURE 3: The pattern of change in renal cell metabolites seen 5 min after the intravenous infusion of HCl (●--●), $NaHCO_3$ (○—○), or Diamox (○···○) into parathyroidectomized rats.

TABLE I: Metabolite Concentrations in Renal Tissue 6 min after Intravenous Infusion of PTH into Parathyroidectomized Rats.

	mμmoles/g of Fresh Tissue (Mean ± Std Error)	
Compd	PTX Control	PTX + PTH after 6 min
G-6-P	92.6 ± 2.8	106.5 ± 3.4[a]
F-6-P	22.3 ± 0.9	32.3 ± 2.3[a]
3-PG	49.4 ± 2.1	54.9 ± 4.3
PEP	21.9 ± 0.6	22.6 ± 1.7
Pyr	31.4 ± 1.3	31.3 ± 2.2
Lact	1500.0 ± 54.0	1400.0 ± 70.0
CoA	34.6 ± 1.7	34.7 ± 1.3
AcCoA	14.6 ± 0.9	13.3 ± 1.6
CIT	270.0 ± 8.2	322.0 ± 28.0[a]
ISC	24.0 ± 0.6	40.0 ± 3.6[a]
α-KG	263.0 ± 6.6	174.5 ± 10.5[a]
Mal	137.0 ± 4.0	150.0 ± 7.8
DPN	430.0 ± 10.0	414.0 ± 10.6
TPN	24.7 ± 0.9	21.7 ± 3.6
DPNH	38.9 ± 3.3	36.3 ± 2.0
TPNH	62.3 ± 4.8	60.4 ± 1.9
ATP	1755.0 ± 43.0	1710.0 ± 45.0
ADP	295.0 ± 8.0	313.0 ± 14.0
AMP	50.5 ± 2.0	50.0 ± 2.1
3′,5′-AMP	0.82 ± 0.06	1.73 ± 0.07[a]
Pyr/Lact	0.021	0.022

[a] Significantly different from control ($P < 0.01$). Abbreviations: PTH, parathyroid hormone; PTX, parathyroidectomized; 3-PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; CoA, coenzyme A; AcCoA, acetyl coenzyme A; Cit, citrate; Isc, isocitrate; α-KG, α-ketoglutarate; Mal, malate; RPN, reduced pyridine nucleotides; PN, pyridine nucleotides.

nucleotide levels in the whole cell or in one or more cellular compartments. However, as shown in Table I, there was no significant change in the concentration of either ATP, ADP, AMP, DPN, TPN, DPNH, or TPNH following parathyroid hormone infusion. Furthermore, the pyruvate/lactate ratio did not change significantly (Table I) indicating that there was no significant change in cytoplasmic redox potential. Because of the fact that neither total nor cytoplasmic redox potential changed, it was concluded that the mitochondrial redox potential had also not changed significantly.

At this point it was concluded that the effect of PTH upon the metabolite profile might be accounted for by a change in intracellular calcium except for the fact that calcium infusion led to a different change from that seen after PTH infusion. This could have meant either that the changes seen after the two agents were due to changes in different cell populations or were affecting different compartments within the same cells.

TABLE II: The Time Course of Changes in Isocitrate, α-KG, and 3′,5′-AMP Concentrations of Renal Tissue after Intravenous Infusion of PTH into Parathyroidectomized Rats.

	mμmoles/g of Fresh Tissue			
	ISC	α-KG	ISC/α-KG	3′,5′-AMP
0 time (control)	24.0 ± 0.6	263 ± 6.6	0.091	0.82 ± 0.06
2-min infusion	43.4 ± 3.0	177 ± 10.1	0.250	3.16 ± 0.41
6-min infusion	40.0 ± 3.6	174 ± 11.0	0.230	1.73 ± 0.07
12-min infusion	32.4 ± 1.8	186 ± 8.5	0.170	1.62 ± 0.19

TABLE III: Metabolite Concentrations in Renal Tissue 5 min after Intravenous Infusion of $CaCl_2$ into Parathyroidectomized Control and Vitamin D Deficient Rats.

	mμmoles/g of Fresh Tissue (Mean ± Std Error)			
Compd	PTX	PTX + $CaCl_2$	Vitamin D (−)[b] PTX	Vitamin D (−)[b] PTX + $CaCl_2$
G-6-P	92.6 ± 2.8	84.0 ± 8.4	73.5 ± 1.6	73.4 ± 4.0
F-6-P	22.3 ± 0.9		20.8 ± 1.0	19.9 ± 1.1
3-PG	49.4 ± 2.1	73.8 ± 6.3[a]	36.0 ± 2.5	55.0 ± 4.7[a]
PEP	21.9 ± 0.6	29.1 ± 2.5[a]	28.7 ± 1.5	35.7 ± 0.9[a]
Pyr	31.4 ± 1.3	21.9 ± 3.2[a]	47.7 ± 2.2	34.0 ± 1.7[a]
Cit	270.0 ± 8.2	167.0 ± 9.3[a]	334.0 ± 18.0	264.0 ± 14.0[a]
ISC	24.0 ± 0.6	13.4 ± 1.2[a]	23.0 ± 1.2	18.7 ± 1.8[a]
α-KG	263.0 ± 6.6	126.5 ± 7.0[a]	286.0 ± 2.0	191.0 ± 34.0[a]
MAL	137.0 ± 4.0	90.7 ± 11.0[a]	154.0 ± 15.0	128.0 ± 11.0[a]
3′,5′-AMP	0.82 ± 0.06	0.75 ± 0.08	0.71 ± 0.08	0.96 ± 0.09

[a] Significantly different from control ($P < 0.01$). [b] Vitamin D (−) indicates vitamin D deficient rats.

The former was definitely possible since Caulfield and Schrag (1964) had shown that renal calcification following PTH occurs in a different portion of the nephron from that seen after excess calcium infusion. The latter was also possible in view of the well-established evidence that parathyroid hormone alters the permeability of the mitochondrial membrane *in vitro* (Rasmussen and Ogata, 1966; Rasmussen *et al.*, 1967).

It is of course not possible to decide between these two alternatives, but the results, obtained when PTH was infused into parathyroidectomized rats which had been maintained on a D-deficient diet or treated with excess cortisone, favor the second alternative. In both of these instances (Figure 4), the most striking change was a rise in PEP and a fall in pyruvate with a less striking fall in isocitrate, and several other Krebs cycle intermediates. The results obtained in these two instances were quite comparable and were qualitatively similar to those seen after calcium infusion in D-deficient animals (Figure 4). It was not possible to study the effect of EGTA infusion in D-deficient animals because the administration of this agent led to their death.

The question of the tissue specificity of this effect of PTH was also examined by analyzing the changes in liver metabolites following PTH. Neither PTH nor EGTA infusion produced the same changes in liver as in kidney; in fact the only significant changes were a fall in malate after PTH and a fall in α-ketoglutarate after EGTA infusion. In addition, there was no significant change in liver 3′5′-AMP after parathyroid hormone infusion in contrast to a significant rise in the kidney.

Because of the recent observations concerning the role of 3′5′-AMP in parathyroid hormone action (Wells and Lloyd, 1967; Chase and Aurbach, 1967), it seemed possible that the different patterns of response seen in D-deficient, cortisone-treated, and control animals might be accounted for by differences in 3′5′-AMP production in response to parathyroid hormone. However, as shown in Figure 5, this was not the case. There was nearly an identical increase in 3′5′-AMP following a standard dose of hormone in the three different types of animals. Also, neither Ca^{2+}, phosphate, acid, alkali, Diamox, nor EGTA infusion led to any significant change in 3′5′-AMP concentrations in the kidneys of parathyroidectomized rats.

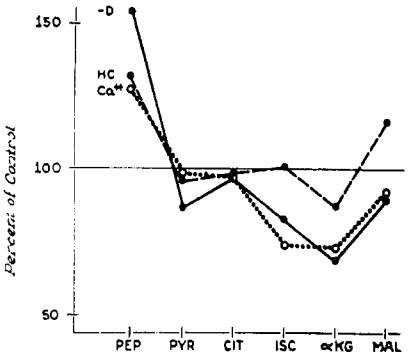


FIGURE 4: The pattern of change in renal cell metabolites seen 4 min after the intravenous infusion of parathyroid hormone into parathyroidectomized animals maintained on a D-deficient diet (●—●) or treated with cortisone (●- - -●). O. . .O indicates the results obtained when calcium was infused into parathyroidectomized animals maintained on a D-deficient diet. Note the striking similarity between the response to PTH and calcium infusion in the D-deficient animals.

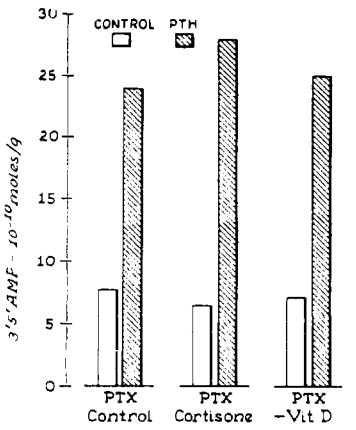


FIGURE 5: The concentration of cyclic 3′5′-AMP per gram-weight of renal tissue, before and 4 min after PTH infusion in parathyroidectomized animals maintained, respectively (left to right), on a normal diet, on a normal diet plus cortisone, and on a vitamin D deficient diet.

Discussion

There are many complex and unknown factors which regulate carbohydrate metabolism in renal tissue as well as the well-established effects of changes in acid–base balance (Lotspeich, 1967; Kamm *et al.*, 1967; Simpson, 1967). This means that any interpretation of the present data is open to the possibility of error. There are several additional types of data which one would like to have in the present study. One is the effect that changes in extracellular [Ca^{2+}] would have on the metabolic response to PTH. The other is the changes in over-all flux through the citric acid cycle produced by these various metabolic changes. Both of these data are difficult to obtain *in vivo* but studies are now underway utilizing slices of renal cortex *in vitro* in an attempt to answer these questions. Accepting that 3′5′-AMP is an intracellular mediator, one is still left with the problem of deducing the mechanism by which it brings about its changes in renal tubular function. Of particular importance are the present observations that under conditions where the response of the tubule to hormone is altered, vitamin D deficiency and excess glucocorticoids, there is no change in the magnitude of the rise in cyclic AMP following parathyroid hormone infusion (Figure 5). However, there is a change in the metabolic response to the hormone as indicated by the differences in metabolite profiles (compare Figures 1, 2, and 4). It is important to emphasize that these changes in metabolite profiles are observed as soon as the changes in cyclic AMP.

The striking similarity in the changes in metabolite profiles seen following calcium and PTH infusion in D-deficient and cortisone-treated animals leads naturally to the possibility that PTH acts by increasing intracellular calcium, as does calcium infusion, with a consequent inhibition of a caclium-sensitive enzyme (Bygraves, 1967), pyruvate kinase (Figures 2 and 4). The question then arises as to how one is to interpret the metabolic effects of PTH in a control animal in which little change in pyruvate kinase is seen, but a striking inhibition of isocitric dehydrogenase is in Figure 1.

This latter enzyme from kidney[1] is inhibited by Ca^{2+}. Furthermore, if the situation in renal tissue is similar to that seen in liver, then most of the citrate, isocitrate, and α-ketoglutarate is in the mitochondrial and not in the cytoplasmic compartment of the cell (J. R. Williamson, personal communication). Thus the possibility must be entertained that in this instance, the inhibition of enzyme activity is due to a rise in Ca^{2+} concentration particularly because no change in ATP/ADP or RPN/PN was observed which could account for this change. If this is a correct interpretation, it means that the increase in intracellular calcium is primarily mitochondrial when PTH is infused into a parathyroidectomized control animal, but primarily cytoplasmic when the hormone is infused into a D-deficient or cortisone-treated animal.

This conclusion is of considerable interest in view of the observations with isolated mitochondria showing that excess cortisone or vitamin D deficiency lead to an impaired ability of the mitochondria to accumulate calcium (Kimberg and Goldstein, 1966; DeLuca *et al.*, 1962; Rasmussen *et al.*, 1967), and the observations that under appropriate circumstances parathyroid hormone can enhance the accumulation of calcium by isolated mitochondria (Rasmussen *et al.*, 1967), and that this effect is dependent upon the presence of vitamin D (DeLuca *et al.*, 1962).

It is obvious that other mechanisms might account for the changes in metabolite profiles observed. One possibility which was seriously considered, was that these changes could be due to a fall in intracellular pH. However, neither alkalosis, acidosis, nor acetazolamide infusion led to metabolite changes similar to those produced by PTH which argues against the notion of Hellman *et al.* (1965) that the initial effect of the hormone on renal function is a change in bicarbonate excretion.

[1] Unpublished data.

A final conclusion concerning the mechanism by which the hormone exerts these changes will depend eventually upon the ability to measure directly the concentration of Ca^{2+} within the renal cell. Unfortunately, no direct methods are presently available. However, it has been shown that parathyroid hormone increases total renal calcium and tubular reabsorption of calcium (Rasmussen, 1968; Widrow and Levinsky, 1962). Also studies measuring plasma calcium (Copp, 1965), the uptake of calcium by isolated cells grown in tissue culture (Borle, 1968a,b), the metabolic responses of bone to parathyroid hormone and calcium (Talmage, 1967a,b), or the morphological changes seen in bone cells after parathyroid hormone infusion (Cameron *et al.*, 1967) are all consistent with the hypothesis that an early effect of parathyroid hormone upon responsive cells is a rise in intracellular calcium ion concentration. The possible relationship between cyclic 3′5′-AMP and intracellular $[Ca^{2+}]$ has been discussed elsewhere (Rasmussen and Tenenhouse, 1968).

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