Parathyroid Hormone and Mitochondrial Metabolism

SPECIFICITY, SENSITIVITY, AND PHYSIOLOGICAL CORRELATES*

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SUMMARY

The effects of parathyroid hormone upon substrate decarboxylation and ion uptake and release in isolated mitochondria in terms of specificity, sensitivity, and the correlation between action in vivo and in vitro were studied. Parathyroid hormone added to isolated liver or kidney mitochondria in vitro stimulated the decarboxylation of a variety of Krebs' cycle intermediates. This decarboxylation required specific ion environments and in particular was not seen if chloride was the only anion present. In contrast, polylysine, histone, dinitrophenol, Zn++, and a basic non-hormonal peptide from the parathyroid glands all stimulated decarboxylation in a chloride medium. The effects of these basic polypeptides were inhibited completely by polyglutamic acid which did not greatly alter the effect of parathyroid hormone. The actions of these basic polypeptides were partially blocked by bovine serum albumin or by serum from parathyroidectomized animals, both of which enhanced the effect of parathyroid hormone.

Specificity was also examined by comparing the effect of four different chemical modifications of the parathyroid hormone molecule upon succinate decarboxylation in vitro with their effects in a standard rat assay in vivo. There was a comparable loss of potency in the two assays with each derivative examined.

Under optimum conditions in vitro, $2 \times 10^{-8}$ M hormone produced a 2-fold increase in rate of succinate decarboxylation. This is well above the concentration found in normal plasma, but approaches that found in the plasma of patients with chronic secondary hyperparathyroidism.

Conditions were defined under which the addition of parathyroid hormone to rat kidney mitochondria in vitro led to either enhanced or decreased uptake of calcium. The factor determining which of these effects was produced appeared to be the ionic composition of the medium.

These data led to the following conclusions: (a) the effect of parathyroid hormone upon mitochondrial metabolism is highly specific; (b) concentrations of hormone which approach those seen in vivo are effective; and (c) there is a significant correlation between the effects that the hormone produces in vivo and those that it induces in vitro.

The energy-linked transport of cations across the mitochondrial membrane is a well established phenomenon (1–4). However, the physiological function of this mitochondrial activity is not well understood. One possible lead has been the discovery that several hormones, including vitamin D (5), cortisol (6), estrogens (6), and parathyroid hormone (3, 7–14), influence this mitochondrial activity. Most attention has been focused on the effects of the parathyroid hormone because these have some similarities to those that the hormone produces upon the renal tubule (15), and they are highly specific (15) and restricted to mitochondria of certain organs (3). However, these effects are usually seen only with relatively large and unphysiological concentrations of hormone (8–13), and many of these same effects are produced by purified histones (16) or other basic polypeptides (17) and Zn++ (18). In addition, there does not appear to be a simple and clear correspondence between the effects of this hormone upon the shift in ions across the membranes of the renal tubule and those of the isolated mitochondria (15). This is particularly clear in the case of the vitamin D-deficient animals in which it has been shown that the renal tubule responds normally to parathyroid hormone (19), but that mitochondria from these kidneys do not release calcium in a normal manner following addition of parathyroid hormone in vitro (7). Likewise, it has not been possible to alter the function of isolated mitochondria by the prior administration of large doses of parathyroid hormone to animals several hours before their organs are removed and mitochondria prepared therefrom (20). Thus three major problems, those of sensitivity, specificity, and physiological correlation, require further exploration before a decision can be made concerning the rel-
evance of the effects of this hormone upon isolated mitochondria to its mode of action in vivo.

The present studies were designed to explore further these three aspects of parathyroid hormone action and mitochondrial function.

**EXPERIMENTAL METHODS**

Rat liver mitochondria were prepared as previously described (13) with the use of 0.37 m sucrose and 0.05 mM EDTA, pH 7.4, for preparing the homogenate. Rat kidney mitochondria were prepared with or without 0.05 mM EDTA as previously described (7). Decarboxylation was measured by the method of Tain, Scow, and Chernick (21) as employed by Aurbach, Houston, and Potts (14). The standard medium contained: sucrose, 160 mm; potassium phosphate (pH 7.0), 32 mm; DPN, 1 mm; MgCl₂, 5 mM; oligomycin, 5 μg per ml; 2% bovine serum albumin; and substrate, 8 mM. The radioactive substrates employed were uniformly labeled 14C-malate, 1,4-14C-fumarate, 1,4-14C-succinate, 1,4-14C-asparate, 5,6-14C-isocitrate, 5-14C-oxalosuccinate, 5,6-14C-isocitrate, 1,5-14C-citrate, 1,5-14C-oxalate, and uniformly labeled 14C-oxalacetate. These were added at a content of 0.2 μC per ml. They were obtained from Nichen, Inc., Nuclear-Chicago, New England Nuclear, Calbiochem, and Volk.

In certain experiments, anions other than phosphate were employed, and in other cases Mg²⁺ or K⁺ or both were eliminated from the medium. The specific conditions of particular experiments are noted on the appropriate figures.

The effect of a variety of substances was examined on the rate of decarboxylation. These included: (a) dinitrophenol, 5 × 10⁻⁴ M; (b) parathyroid hormone and Peak 2, a basic non-hormonal peptide from the parathyroid glands, both prepared as previously described (22); (c) valinomycin, a gift from Dr. Henry Lardy; (d) histone, a, an arginine-rich histone prepared by the method of Hnilica and Busch (23), and a gift from Dr. Arnold Schwartz; (e) polylysine, with an average molecular weight of 2,500 and obtained from Sigma; (f) Zn⁺, as described by Brierly, Bhattacharya, and Walker (18); and (g) four chemically modified derivatives of parathyroid hormone. The first was oxidized hormone prepared by the method of Rasmussen and Craig (24) with HzO₂. The second was carboxymethyl-S-methionyl parathyroid hormone prepared by the method of Tashjian, Ontjes, and Munson (25). The third was nitrated to various compounds was also examined. It had an average molecular weight of 77,000, and was obtained from Pilot Chemicals, Inc. (Watertown, Massachusetts).

Serum from vitamin D-fed parathyroidectomized animals was obtained 4 days after complete removal of the parathyroid glands. Vitamin D-deficient rats were grown as previously described (7). These animals were parathyroidectomized or subjected to a sham operation 3 to 4 hours before being decapitated for use in preparation of mitochondria. From the time of operation until decapitation, they were perfused with a standard electrolyte solution (19).

The rate of uptake and release of mitochondrial ions was measured by our previous methods (10, 19). The incubation medium for these experiments was slightly different from that used for the decarboxylation studies and contained 290 mm sucrose, 16 mm sodium succinate, 6.8 mm KCl, 0.05 mM CaCl₂ with 0.1 μg of ⁴⁵Ca, 6 mm MgCl₂, 17 mm Tris-HCl, 3 mm potassium phosphate, and oligomycin, 2.5 μg per ml. In addition to measuring ⁴⁵Ca, ³²P, and ⁴K were added in some experiments. These two isotopes were added at 0.01 μCi per ml. In experiments with all three isotopes, three batches of medium were made up, each containing a single isotope, and three simultaneous experiments conducted. At the end of the incubation, 1 ml was filtered on Celite, washed with 3 ml of cold 0.25 m sucrose, dried, and either extracted with perchloric acid or counted directly. Direct counting was employed for the isotopes of potassium (³⁴K) and phosphate (³²P).

The assay of parathyroid hormone in vivo was carried out by a modification of the method of Munson (31).

**RESULTS**

As shown in Fig. 1, the rate of succinate decarboxylation was a function of parathyroid hormone concentration between 0.2 μg per ml and 125 μg per ml. In a magnesium-containing medium, the hormonal response was a function of the nature of the anion. When chloride replaced phosphate, parathyroid hormone, even at a concentration of 100 μg per ml, had no effect upon succinate decarboxylation (Fig. 2). However, arsenate and sulfate, although less effective, could replace phosphate. Their order of effectiveness was similar to their effectiveness in supporting hormone-dependent respiration (9), which, in turn, was related to their rates of accumulation (32). The other requirements for hormonal effect were similar to those reported by Aurbach et al. (14). Also, as noted by them (14), hormone-stimulated decarboxylation in a magnesium-free environment as long as phosphate was present. This is similar to the hormonal stimulation of succinate oxidation under similar circumstances (12, 13). However, it was found in the present studies that by increasing the K⁺ concentration to 20 mM this effect of parathyroid hormone could be abolished.

To examine further the specificity of the relationship between ion transport and decarboxylation, a study was made of valinomycin-induced transport and succinate decarboxylation. The rate of decarboxylation was found to be a function of valinomycin concentration between 1 and 1000 μg per ml. The decarboxylation was seen with either phosphate, acetate, or chloride, but in the latter case the time of onset was considerably delayed. The effect was also cation-specific, being seen with K⁺, Cs⁺, Rb⁺, but not with Na⁺ or Li⁺, in keeping with the known specificity of transport seen with this agent (33).

Thus, in the case of both parathyroid hormone and valinomycin, the rate of succinate decarboxylation was an indirect but apparently reliable index of the effect of these agents upon energy-dependent ion translocations across the mitochondrial membrane. This is also borne out by the fact that these effects were not confined to succinate alone. The decarboxylation of a variety of other mitochondrial substrates was enhanced by...
parathyroid hormone under appropriate conditions. The only Krebs cycle intermediate whose rate of decarboxylation was not affected was oxaloacetate, in keeping with the recent evidence that oxaloacetate does not cross the mitochondrial membranes.

Uncoupling agents should also enhance succinate decarboxylation. This proved to be the case, as shown in Fig. 3. The addition of $5 \times 10^{-4} \text{ M}$ dinitrophenol led to a marked stimulation of succinate decarboxylation which, however, was not ion-dependent, being demonstrable with a wide variety of anions and cations, and thus lacking the ion specificity seen with parathyroid hormone.

**Specificity**

The effect of a variety of agents reported to have effects similar to parathyroid hormone was tested. Many similarities were noted between their effects and those of parathyroid hormone. However, two very striking differences were noted. As shown in Fig. 4, the addition of either parathyroid hormone, polylysine, Peak II peptide, histone, or Zn$^{++}$ at appropriate concentrations in a phosphate medium (containing magnesium) led to a significant increase in the rate of decarboxylation in all cases. However, when the experiments were repeated in a

![Fig. 1](http://www.jbc.org/)

**Fig. 1.** The rate of succinate decarboxylation as a function of parathyroid hormone concentration (■■■■) and polylysine (●●●●) concentration. Incubations were carried out with 1.1 mg of mitochondrial protein per ml in a medium containing 8 mM sodium succinate, 0.2 μC of $^{14}$C-1,4-succinate, 160 mM sucrose, 1 mM DPN, 32 mM potassium phosphate (pH 7.6), 5 mM MgCl$_2$, 5 μg per ml of oligomycin, and 2% bovine serum albumin. Various concentrations of parathyroid hormone or polylysine were dissolved in 0.1 ml of a mixture of which 5% was serum from parathyroidectomized rats and 95% was pH 7.6 phosphate buffer sufficient to give a final phosphate concentration of 32 μM. Liver mitochondria were added to the incubation flask and this was followed by the addition of hormone or polylysine in a volume of 0.1 ml, giving a final serum concentration of 0.5%. The total time of incubation was 30 min.

![Fig. 2](http://www.jbc.org/)

**Fig. 2.** The rate of succinate decarboxylation as a function of time in the presence (□□□□) and absence (●●●●) of a variety of agents. Parathyroid hormone, $2 \times 10^{-4} \text{ M}$; polylysine, $6 \times 10^{-4} \text{ M}$; basic peptide Peak II, $4 \times 10^{-4} \text{ M}$; histone $\text{H}2a$, $1.5 \times 10^{-4} \text{ M}$; Zn$^{++}$, $5 \times 10^{-4} \text{ M}$. These concentrations were chosen because they induced approximately the same degree of stimulation as parathyroid hormone in the phosphate medium. The chloride medium contained 20 mM Tris-Cl, pH 7.4, and 200 mM sucrose. It was otherwise identical with the phosphate medium. The phosphate medium was that described in Fig. 1.

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** The effect of $5 \times 10^{-4} \text{ M}$ dinitrophenol (DNP) upon succinate decarboxylation in media containing predominantly phosphate (□□□□), acetate (○○○○), and chloride (●●●●). Experiments were carried out as described in Fig. 2.

![Fig. 4](http://www.jbc.org/)

**Fig. 4.** The rates of succinate decarboxylation in phosphate (upper) and chloride media (lower) in the presence (□□□□) and absence (●●●●) of a variety of agents. Parathyroid hormone, $2 \times 10^{-4} \text{ M}$; polylysine, $6 \times 10^{-4} \text{ M}$; basic peptide Peak II, $4 \times 10^{-4} \text{ M}$; histone $\text{H}2a$, $1.5 \times 10^{-4} \text{ M}$; Zn$^{++}$, $5 \times 10^{-4} \text{ M}$. These concentrations were chosen because they induced approximately the same degree of stimulation as parathyroid hormone in the phosphate medium. The chloride medium contained 20 mM Tris-Cl, pH 7.4, and 200 mM sucrose. It was otherwise identical with the phosphate medium. The phosphate medium was that described in Fig. 1.
chloride medium, all of the agents except parathyroid hormone still enhanced the rate of decarboxylation. This was interpreted as evidence for a more general change in membrane structure induced by the agents, other than the hormone, because it is known that the membrane is normally impermeable to chloride (34). In keeping with this interpretation was the fact that at these or slightly higher concentrations all of these substances caused mitochondrial clumping or agglutination, an effect not produced even by large amounts of the hormone. Also as shown in Fig. 1, higher concentrations of these other agents led to significant inhibition of the maximal rates of decarboxylation.

A second difference (Fig. 5) was that seen in the presence and absence of magnesium in a chloride medium. In this case,

![Fig. 5](image_url)

**Fig. 5.** The rate of succinate decarboxylation in a chloride medium as a function of the log dose of parathyroid hormone, histone, and polylysine in the presence (●-●) and absence (●-●) of 1 mM MgCl₂. Incubations were carried out at 30° for 30 min in the standard medium with or without MgCl₂.

...histone, polylysine, and parathyroid hormone all enhanced decarboxylation of succinate in the absence of magnesium, but in its presence only histone and polylysine produced such an effect. In fact, increasing concentrations of the hormone led to a slight inhibition of succinate decarboxylation. Other basic polypeptides gave effects similar to those seen with polylysine.

Specificity was also examined by employing four chemically modified preparations of parathyroid hormone. The first was carboxymethyl-S-methionyl parathyroid hormone prepared as described by Tashjian (25). The substitution of the carboxymethyl groups is thought to be confined to the sulfur atoms of the 2 methionine residues. Also, parathyroid hormone was oxidized at pH 4.8 with H₂O₂ as previously described, leading to specific oxidation of the methionine residues to the sulfoxides (24). Both of these derivatives were totally inactive in the mitochondrial system (Fig. 6) (only the data from the study with the alkyl derivative are plotted), even when added at a concentration of 200 μg per ml, nearly 10 times the concentration of unmodified hormone needed to produce a maximal response. There was a similar loss of activity in vivo (Fig. 6). In this case 300 μg given to a thyroparathyroidectomized rat led to no change in plasma calcium, whereas 15 to 20 μg of unmodified hormone normally induced a maximal response.

The third analogue was parathyroid hormone which had been reacted with tetranitromethane under conditions thought to be specific for modifying the tyrosine residue. The modified hormone (N-PTH (nitrated parathyroid hormone), Fig. 6) showed a similar loss of potency in vitro as compared to its loss in vivo. It is not known whether these results mean that all of the hormone reacted with the reagent and that the modified product has 30% of the intrinsic activity of the hormone, or that only 70% of the hormone reacted under the experimental conditions, and that all the hormone which did react is totally inactive. This matter is being investigated.

The fourth analogue was prepared by reacting the hormone with 2-hydroxy-5-nitrobenzyl bromide under conditions thought to be specific for reacting with the tryptophan residue (27, 35). Again, there was a comparable loss of activity in the two assay systems (Fig. 7).
The problem of specificity was examined in another way. Aurbach et al. (17) have proposed that the effects of parathyroid hormone upon mitochondrial metabolism are due to the basic character of the hormonal polypeptide, and are therefore non-specific. If so, it was reasoned that the action of all of these basic polypeptides including the hormone should be blocked by the addition of an acidic agent capable of binding them. To examine this possibility the effect of polyglutamic acid upon the responses of the mitochondria to the addition of parathyroid hormone and these other basic substances were examined. The results are shown in Table 1. The addition of $1 \times 10^{\text{-}6}$ M polyglutamate completely blocked the effect of $4 \times 10^{\text{-}5}$ M polylysine or $2 \times 10^{\text{-}4}$ M histone f2a, but had only a minor effect upon the action of either valinomycin or parathyroid hormone. The polyglutamate was present in a concentration 5 times that of the hormone, yet the hormone produced a 9-fold stimulation of decarboxylation compared to a 12-fold increase in the control flasks.

Sensitivity

Recent reports (36, 37) indicate that parathyroid hormone has a considerable propensity to bind to glass. Because of this fact, a study was made of the effects of adding bovine serum albumin or serum from parathyroidectomized rats to the incubation flasks. The addition of either bovine serum albumin or serum from parathyroidectomized animals led to a significant increase in the sensitivity of the response to parathyroid hormone, but to a striking decrease in the sensitivity of the response to polylysine or histone.

With 0.5% serum the hormonal potency was 150% of control, that of polylysine was 80%, and that of histone was 75%. With 5% bovine serum albumin the hormonal potency was 130% of control, that of polylysine was 2.5%, and that of histone was 5%.

Physiological Correlates

Calcium-binding and Release—One of the most difficult problems is that of relating these effects in vitro of the hormone to its effects in vivo. The first effect which parathyroid hormone was found to exert upon isolated mitochondria was that of increasing the rate of calcium release when added to kidney mitochondria from vitamin D-fed but not from those obtained from vitamin D-deficient animals (7). This observation led to a subsequent re-investigation of the relationship between the activities of parathyroid hormone and vitamin D in vivo (10, 38). These investigations established that parathyroid hormone acts upon bone and intestine only when vitamin D is present, but that it continues to exert its characteristic effects upon renal tubular function even in a vitamin D-deficient animal. These results led to a dilemma. On the one hand, an effect in vitro of parathyroid hormone, i.e. calcium release from mitochondria, was demonstrable in mitochondria from vitamin D-fed but not in those from D-deficient rats (7). Nevertheless, in vivo, the hormone was found to produce its characteristic effect upon the renal excretion of calcium, phosphate, potassium, and magnesium in vitamin D-deficient animals (31). This would imply that the effect in vitro of the hormone upon calcium release was in no way related to that that the hormone produces in vivo. However, there was one important difference between the experiments in vivo and in vitro. In order to demonstrate the effect of hormone in vitamin D-deficient rats in vivo, it was first necessary to remove the animals’ own parathyroid glands.

Table 1

<table>
<thead>
<tr>
<th>Agent</th>
<th>Without polyglutamate</th>
<th>With polyglutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2,807</td>
<td>2,506</td>
</tr>
<tr>
<td>Parathyroid hormone</td>
<td>33,383</td>
<td>22,354</td>
</tr>
<tr>
<td>Polylysine</td>
<td>46,612</td>
<td>23,628</td>
</tr>
<tr>
<td>Valinomycin</td>
<td>89,990</td>
<td>61,505</td>
</tr>
<tr>
<td>Histone f2a</td>
<td>46,400</td>
<td>2,110</td>
</tr>
</tbody>
</table>

Effect of polyglutamic acid upon response of kidney mitochondria to parathyroid hormone, polylysine, valinomycin, and histone

The incubation medium contained 160 mM sucrose, 1 mM DPN, 32 mM potassium phosphate (pH 7.6), 5 mM MgCl2, 5 μg per ml of oligomycin, 8 mM succinate with 0.2 μC of 14C, 14 succinate, and 0.01 mM Tris-polyglutamate, pH 7.6. The concentrations of parathyroid hormone, valinomycin, and polylysine were 2 $\times$ 10$^{-6}$ M, 4 $\times$ 10$^{-6}$ M, and 4 $\times$ 10$^{-5}$ M, respectively. Incubation was at 30° for 30 min.
Fig. 10. In this experiment the total intramitochondrial magnesium and the uptake of \(^{32}\)P, \(^{42}\)K, and \(^{46}\)Ca were measured simultaneously with kidney mitochondria prepared from vitamin D-deficient parathyroidectomized animals. The addition of hormone \(\text{in vitro}\) led to a marked increase in magnesium accumulation, a slight reduction in phosphate uptake, and no change in \(K^+\) uptake during the same time interval that it led to calcium release.

A second difficulty in correlating the effect of the hormone upon the transport of ions across the renal tubule with those that it induces in isolated mitochondria relates to the fact that in mitochondria under the usual conditions of study (Fig. 10) calcium and magnesium appear to move in opposite directions when hormone is present, whereas in the renal tubule the reabsorption of both is enhanced by the hormone (19). However, by the proper selection of conditions \(\text{in vitro}\), it is possible to induce concurrent uptake of both \(Ca^{++}\) and \(Mg^{++}\) into mitochondria.

![Graph](image1.png)

**Fig. 8.** The binding of \(^{45}\)Ca by isolated liver mitochondria from vitamin D-fed (D(+)) and vitamin D-deficient rats (D(-)) as a function of time in the presence (● ● ●) and absence (O O O) of \(5 \times 10^{-7}\) M parathyroid hormone. The incubation medium contained 250 mM sucrose, 6 mM MgCl\(_2\), 16 mM succinate, 6.5 mM KCl, 3 mm potassium phosphate (pH 7.4), 17 mM Tris-HCl (pH 7.4), 2.5 \(\mu\)g per ml of oligomycin, 0.05 mM CaCl\(_2\) with 0.1 \(\mu\)M of \(^{45}\)Ca, and 1.18 mg per ml of mitochondrial protein. The results are plotted as counts per min per mg of mitochondrial protein.

![Graph](image2.png)

**Fig. 9.** The binding of calcium by kidney mitochondria from vitamin D-fed (+D) animals (left) either sham-operated or parathyroidectomized, from vitamin D-deficient (-D) sham-operated animals (center), and from vitamin D-deficient parathyroidectomized animals (right) in the presence (O O O) or absence (□ □ □) of \(5 \times 10^{-7}\) M parathyroid hormone. Incubations were carried out as described in Fig. 8, except that mitochondrial protein concentration was 0.65 mg per ml.

![Graph](image3.png)

**Fig. 10.** The simultaneous measurement of magnesium, phosphate, potassium, and calcium binding in rat kidney mitochondria incubated in the medium described in Fig. 8 and containing either 0.05 \(\mu\)C of \(^{32}\)P, 0.05 \(\mu\)C of \(^{42}\)K, or 0.1 \(\mu\)C of \(^{46}\)CaCl\(_2\) with (● ● ●) or without (O O O) \(2 \times 10^{-7}\) M parathyroid hormone. Separate flasks containing the separate isotopes were incubated simultaneously. The analysis of magnesium was carried out on extracts prepared from the mitochondria used for \(^{42}\)K accumulation after decay of this isotope.

![Graph](image4.png)

**Fig. 11.** The effect of parathyroid hormone upon magnesium, potassium, and calcium binding by rat kidney mitochondria in the presence of substrate and ATP. Magnesium content, \(^{42}\)K uptake, and \(^{46}\)Ca uptake were measured as a function of time in the presence (● ● ●) and absence (□ □ □) of \(2 \times 10^{-7}\) M parathyroid hormone in a medium containing CaCl\(_2\) (0.25 mM) with 0.1 \(\mu\)C of \(^{46}\)Ca, 6 mM MgCl\(_2\), 17 mM Tris-HCl (pH 7.2), 3 mM ATP, 0.27 mM cytochrome c, 250 mM sucrose, 3 mM potassium phosphate (pH 7.2), 15 mM sodium succinate, and 6.5 mM KCl. Mitochondrial protein, 0.80 mg per ml.

chondria under the influence of the hormone. Thus the addition of ATP (0.5 to 3.0 mM) to a medium containing succinate without oligomycin led to enhanced calcium binding in the presence of hormone (Fig. 11). The effect was observed with calcium concentrations between 0.06 and 0.3 mM. This effect of hormone upon \(Ca^{++}\) accumulation was related specifically to the presence of both ATP and \(Mg^{++}\), and it was not induced by the addition of valinomycin or the other basic polypeptides.

**Decarboxylation**—Because succinate decarboxylation proved to be a sensitive indicator of hormone action, it was of interest to determine the effect of parathyroidectomy upon the rate of succinate decarboxylation in subsequently isolated mitochondria. For this purpose, vitamin D-deficient rats were subjected to sham operation or parathyroidectomy and killed 4 hours later. The kidneys were removed and mitochondria were
prepared. Prior parathyroidectomy led to a significant reduction in the rate of succinate decarboxylation (compare sham with parathyroidectomized group). This difference corresponded to a hormone concentration of 0.6 to 1.2 \( \mu \text{g} \) per ml. The addition of parathyroid hormone in vivo produced a much greater effect upon the mitochondria from the parathyroidectomized as compared to its effect upon ones obtained from sham operated animals.

However, similar changes in succinate decarboxylation were not seen when mitochondria were prepared from vitamin D-fed, sham, and parathyroidectomized rats. Studies were also carried out measuring succinate decarboxylation with mitochondria obtained from parathyroidectomized rats and parathyroidectomized animals treated with 10 to 100 \( \mu \text{g} \) of purified hormone. No consistent difference was found. Because of this it was decided to examine the possibility that the hormone in the kidney was being washed out or inactivated during the course of the preparation of the mitochondria. For this purpose homogenates of kidney were prepared in the usual fashion. Then from one aliquot of the homogenate, mitochondria were prepared in the normal fashion, and to another parathyroid hormone was added and mitochondria were then prepared. Mitochondria prepared from this hormone-fortified homogenate had the same rate of succinate decarboxylation as those from the control homogenate. In other words, the addition to the initial homogenate of doses of hormone (6 to 30 \( \mu \text{g} \) per ml) sufficient to stimulate decarboxylation markedley in isolated mitochondria (Fig. 1) led to no detectable effect upon succinate decarboxylation in the subsequently isolated mitochondria. This meant either that the hormone was inactivated or that it was washed out during the preparation of the mitochondria. To test these alternatives, larger concentrations of hormone were added to the homogenate and mitochondria were then prepared and tested. When 100 \( \mu \text{g} \) per ml of hormone were added to the initial homogenate, and mitochondria were spun out and washed without further washing, the rate of decarboxylation was approximately twice that seen in control mitochondria (Fig. 12). This response is normally produced by a concentration of hormone in the range 0.5 to 1.0 \( \mu \text{g} \) per ml. Thus approximately 0.01% of the hormone had been effective. This is approximately that which would have been present if dilution were the only factor. The expected hormone concentration on the basis of dilution was 0.8 to 1.4 \( \mu \text{g} \) per ml.

**DISCUSSION**

The present results are further confirmation of the fact that the effects of parathyroid hormone upon the mitochondrial membrane are highly specific (13). When analyzed by appropriate means, it is possible to distinguish between its effects and those of a variety of basic polypeptides (Figs. 1, 4, and 5) and other substances known to affect mitochondrial behavior. Of particular interest is the fact that most of these other agents alter the membrane structure in such a way that it becomes permeable to chloride ion (Fig. 4) and that their effects in a chloride medium are not influenced to any significant extent by the addition of Mg\(^{++}\) which has a profound effect upon parathyroid hormone action (Fig. 5). It is also noteworthy that the dose-response curves seen with the basic polypeptides are qualitatively different than that seen with parathyroid hormone (Fig. 1 and Reference 17).

The specificity of the hormonal effect is also clearly demonstrated by the results shown in Table I. Aurbach et al. (17) claimed that the effect of parathyroid hormone upon mitochondrial metabolism was due to a nonspecific effect of this basic polypeptide similar to the effects produced by other basic polypeptides and proteins. However, the results in Table I clearly show that polyglutamic acid inhibits the action of the basic polypeptides, e.g. polylysine, but does not have a similar effect upon parathyroid hormone action.

Perhaps the most striking confirmation of the specificity of parathyroid hormone action is afforded by the data with the chemically modified derivatives of the hormone (Figs. 6 and 7). Four such derivatives were prepared and in each instance there was a significant loss of hormonal potency when measured in a standard assay in vivo. With each of the four derivatives there was a strictly comparable loss of potency in the mitochondrial assay in vivo and the standard assay in vivo. This rather close analogy could hardly be coincidental, nor could it be readily explained by assuming that the mitochondrial responses were being produced by a nonhormonal contaminant in the hormonal preparation. Further derivatives of this kind should surely be tested, and those already tested analyzed chemically. This work is in progress.

Having accepted the fact that the effects of this hormone upon isolated mitochondria are specific, one is then faced with the more difficult question of deciding whether these effects are of physiological significance, and, if so, how they are related to the mechanism of action of this hormone. One immediate concern is that of sensitivity. Under the best of circumstances the present hormone was effective at 200 \( \mu \text{g} \) per ml (2 \( \times 10^{-4} \) M) when assayed by measuring succinate decarboxylation. Aurbach et al. (14) have reported responses at 10\(^{-7} \) M. A similar sensitivity is achieved when calcium release is measured under appropriate circumstances (7). This concentration of hormone is above that thought to exist in normal plasma (0.5 to 6 \( \mu \text{g} \) per ml), but approximates that seen in severe hyperparathyroidism, approximately 60 \( \mu \text{g} \) per ml (39). In ad-
dation, the data of Orimo and Fujita (40) suggest that the kidney may concentrate this agent. Based on their data, the concentration of parathyroid hormone in the kidney may be as high as 350 to 500 μg per ml. However, the significance of this apparent accumulation of hormone in a target tissue must be better established because the kidney is known to concentrate a variety of other peptide hormones, and another report indicates that kidney homogenates inactivate parathyroid hormone (41).

A second aspect of the sensitivity problem is that of determining the true concentration of hormone in our incubation flasks. The hormone has been found to bind avidly to glass under the conditions of our assay (36), and this binding is not completely prevented by amounts of serum and bovine serum albumin used in the present system in vitro. These several facts point up the difficulty in finally determining what the effective concentration of hormone is at the mitochondrial membrane in vivo, as well as the true concentration of the hormone in the present experiments in vitro. All that can be said at present is that hormone concentrations similar to those seen in the plasma of individuals with severe hyperparathyroidism must be added in order to observe an effect upon the behavior of mitochondria. During the subsequent preparation of mitochondria and that these concentrations are well above the usual physiological concentration.

On the positive side, however, are the data recorded in Fig. 9 showing that the chronic hyperparathyroidism of vitamin D deficiency leads to a situation in which kidney mitochondria are no longer responsive to parathyroid hormone in vitro, as measured by calcium release, but do respond if the animal has been parathyroidectomized several hours prior to the preparation of the kidney mitochondria. This situation is completely analogous to the situation in vivo in which the infusion of hormone into a vitamin D-deficient rat leads to no change in renal function unless the animal has been subjected to prior parathyroidectomy (19). This similarity is rather compelling evidence that this hormone affects mitochondrial behavior in vivo, and also supports the notion that the change in mitochondrial activity is involved in the normal change in cellular function induced by the hormone. However, it is difficult to reconcile the data shown in Fig. 9 with those shown in Fig. 12. In the latter experiment, it was clearly shown that hormone added to homogenates was washed out during the subsequent preparation of mitochondria and that relatively massive concentrations of hormone, 100 μg per ml, must be added in order to observe an effect upon the behavior of the subsequently isolated mitochondria. It seems most unlikely that these concentrations of hormone are present in vivo, even in vitamin D-deficient rats with secondary hyperparathyroidism. Hence one must consider the possibility that hormone remains bound to the mitochondria of hyperparathyroid vitamin D-deficient animals during the preparation of these organelles. In any case, it is now clear why many of our experiments failed in their attempt to show that parathyroid hormone alters mitochondrial function in vivo when the hormone is given to an experimental animal in vivo. It is equally clear why Cohn, Levy, and Eller (20) obtained negative results when examining the same question. These results might have been predicted. The action of parathyroid hormone upon the kidney is rapid and short lived. Hence, it would be most unlikely that the hormone would alter the properties of the mitochondrial membrane in a stable and prolonged way which would allow examination several hours after these organelles had been removed from the animal and washed free of parathyroid hormone.

In this light, the results obtained with the mitochondria from the vitamin D-deficient parathyroidectomized and sham-operated animals (Fig. 9) are even more impressive, and point to the possibility that long continued hyperparathyroidism leads eventually to a more fixed change in mitochondrial membrane function. Similarly, the recent data of Kimberg and Goldstein (42) show that chronic hypoparathyroidism leads to a significant increase in the calcium binding of subsequently isolated kidney mitochondria when compared to those obtained from control animals. Both their data and those in the present study indicate that under appropriate conditions either vitamin D-deficiency or parathyroid excess or deficiency leads to a change in the steady state level of calcium bound to kidney mitochondria. This differs from previous observations that the vitamin or hormone increases only the rate or release of bound calcium (5, 7). However, this difference is due to the fact that under the conditions of the previous studies the composition of the incubation medium and the mitochondrial concentrations were such that nearly 100% of the calcium was bound; hence it was impossible to show a change in steady state level. In the present experiments, as well as those of Kimberg and Goldstein (42), the complete binding of calcium did not occur; hence changes in the steady state binding were observable.

Another apparent difference between responses in vivo and in vitro has been resolved by the present study. When parathyroid hormone acts upon the kidney, the excretion of both calcium and magnesium diminishes; i.e., the rate of their reabsorption by the renal tubule increases (19). Thus the 2 cations are apparently moving in the same direction across the renal tubular cell membranes. Previous mitochondrial studies led to the conclusion that under the influence of parathyroid hormone these 2 cations moved in opposite directions across the mitochondrial membrane (7, 13, 15). Thus it seemed difficult to reconcile the facts in vivo with the model system in vitro. Yet the present results (Fig. 11) clearly show that under a different set of environmental circumstances the effect of parathyroid hormone is to promote both Ca++ and Mg++ accumulation by isolated kidney mitochondria. The present data taken in conjunction with those reported previously (1, 3, 5, 7–15) and those obtained recently by Kimberg and Goldstein (6, 42) all indicate a striking similarity between the effects of cortisone, vitamin D, and parathyroid hormone upon calcium metabolism in vivo with those these same agents produce upon the uptake and release of calcium by the mitochondrial membrane in vitro. Taken in toto they imply that the changes in membrane function which these hormones produce in vitro are related in some fashion to their action in vivo. However, the difficulty of defining this relationship remains a formidable task because it is so clearly evident that the mitochondrial environment has a profound effect upon the expression of inherent mitochondrial activities, and all mitochondrial experiments in vitro are carried in environments which differ considerably from that in which these organelles normally function in vivo.

Also, the possibility remains that this mitochondrial system in vitro is merely a model which reflects in part the type of response that the parathyroid hormone elicits when interacting with the plasma membranes of the target cells, and that this hormone never interacts directly with the mitochondrial membrane in
This, of course, is a point of crucial importance, and, given present day experimental techniques, one which is exceedingly difficult to resolve. Thus for the present, at least, one must continue to examine this possibility by indirect means in the hope that sufficient information can be gathered to allow for a reasonable conclusion.

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