The role of the parathyroid hormone in the regulation of calcium and phosphate metabolism has been recognized for many years (1). At the cellular level, the hormone is thought to regulate calcium and phosphate transport reactions. It is now known that (a) increase calcium reabsorption in the renal tubule (2, 3), (b) increase the transport of calcium across intestinal membranes (4, 5), (c) increase bone mineral mobilization (6), and (d) stimulate the renal excretion of phosphate (7). Moreover, it has been suggested by several sources that the primary biochemical action of the hormone may be on some aspect of phosphate metabolism (7, 8).

Studies with isolated mitochondria have provided a new approach to the study of parathyroid hormone action. DeLuca, Engstrom, and Rasmussen (9) have demonstrated that parathyroid hormone stimulates a vitamin D-dependent release of calcium from rat kidney mitochondria. In addition, it has been found that the hormone stimulates inorganic phosphate uptake (10) and a phosphate-dependent, nonphosphorylative respiration (11). These effects, which were observed in oligomycin-inhibited mitochondria fortified with adenosine triphosphate, were highly specific for the parathyroid hormone and did not depend upon the presence of vitamin D. These findings suggested that the primary effect of this hormone may be to control the translocation of phosphate across cellular and subcellular membranes.

It is the purpose of this paper to describe in detail the characteristics of the parathyroid hormone-dependent uptake of inorganic phosphate by isolated mitochondria.

**EXPERIMENTAL PROCEDURE**

Thirty-day-old male rats weighing 130 g were obtained from the Rolfsmeier Company, Madison, Wisconsin. They were maintained on a stock diet and used within 1 week. The rats were killed by stunning and decapitation. Liver mitochondria were prepared as previously described (12).

Mitochondria equivalent to 0.7 mg of N were incubated in a medium containing 13.3 mM sodium phosphate buffer, pH 7.2, 0.19 M sucrose, 6.7 mM MgCl₂, and 10 mM L-glutamate in a total volume of 3.0 ml. The incubations were carried out at 30°C in a Dubnoff metabolic shaker with air as the gas phase. After a 5-minute equilibration period, 200 µg of purified bovine parathyroid hormone (13) contained in 0.2 ml of 0.0001 N acetic acid was added to the flasks. Control flasks received the appropriate amount of acetic acid.

At the indicated times (20 minutes unless otherwise stated), a 1.0-ml aliquot of the reaction mixture was removed and rapidly filtered by suction through a pad of Celite as described earlier (10). The Celite-retained mitochondria were washed twice with 1.0-ml portions of ice-cold isotonic sucrose, and the pad was then dried at 110°C for 1 hour. Inorganic phosphate was determined by the method of Fiske and SubbaRow (14) after extraction of the Celite pad with 5 N H₂SO₄. Parathyroid hormone-stimulated phosphate uptake was calculated by subtracting phosphate uptake in control mitochondria from the phosphate taken up in the presence of parathyroid hormone. Magnesium measurements were made with the Perkin-Elmer atomic absorption spectrometer on 0.1-N HCl extracts of the Celite-retained mitochondria.

**RESULTS**

**Requirements**—As revealed in Fig. 1, addition of hormone to the reaction mixture resulted in a striking accumulation of P_i within 20 minutes. As much as 1 µmole of P_i was accumulated per mg of mitochondrial protein. The fact that in the absence of glutamate or Mg²⁺ there was no significant P_i uptake suggests that both these factors are necessary for the hormone effect. It was also apparent that KCl was unable to substitute for sucrose in the medium and that there was little accumulation of P_i in the absence of added hormone (Fig. 7). Addition of ATP to the complete medium appeared to reduce rather than enhance the hormone effect. When mitochondria were incubated in the presence of 10⁻⁴ M oligomycin, hormone failed to produce a stimulation of P_i uptake. After the addition of 0.67 mM ATP to the oligomycin-inhibited mitochondria, however, the hormonal effect was again fully restored. It is important to emphasize, however, that in an oligomycin-blocked system in which ATP is supplied, an oxidizable substrate is still required for the parathyroid hormone-dependent phosphate transport.

A number of other diphosphopyridine nucleotide-linked substrates besides glutamate were able to support this hormonal effect (Table 1), but none was as effective as glutamate. Succinate was an even poorer substrate, and there was no net accumulation of P_i when citrate was used as the substrate. However, in the case of citrate, parathyroid hormone-dependent phosphate accumulation could be restored by merely increasing magnesium ion concentration; this finding suggests that the citrate may have interfered with the availability of magnesium ion.

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Influence of Pi Concentration—To study phosphate uptake when mitochondria were incubated in the presence of varying amounts of Pi, it was necessary to add a suitable buffer to the reaction mixture. Initial studies with phosphate buffer indicated an optimal pH of 7.2 for the reaction. Both imidazole and tris(hydroxymethyl)aminomethane buffers were found to be suitable replacements, but imidazole was preferred because of its greater buffering capacity in this pH range. When either imidazole or Tris were added to a phosphate-containing medium, they slightly inhibited the hormonal effect. Phosphate accumulation as a function of external phosphate concentration is illustrated in Fig. 2. When 6.7 mM Pi was present in the reaction mixture, the accumulation of Pi in the mitochondria reached a maximum within 20 minutes. However, it is possible that the point of maximal accumulation would vary with incubation time. Lower levels of Pi in the medium reduced proportionately the amount of Pi appearing in the mitochondria.

Influence of Parathyroid Hormone Concentration—The rates of Pi accumulation as a function of hormone concentration are shown in Fig. 3. They demonstrate a direct, but not linear, relationship between uptake of Pi and parathyroid hormone concentration. With increasing amounts of hormone, greater accumulation of Pi occurred within a shorter time period, the maximal response to 200 µg being obtained within 20 minutes. With the existing conditions it was not possible to show any significant Pi accumulation when less than 8 x 10^{-4} M hormone was present.

Influence of Mg²⁺—As shown in Fig. 4, with increasing amounts of Mg²⁺ there was an increased accumulation of Pi in the presence of hormone. A maximal response was obtained with 10 mM MgCl₂. At higher levels of MgCl₂ a definite inhibition of Pi accumulation occurred.

The fact that the uptake of Pi was a result of hormonal stimulation suggested that a simultaneous movement of a cation in the same direction might occur. It was possible to demonstrate that magnesium is accumulated with the phosphate. When mitochondria were incubated in a medium containing hormone and 0.7 mM MgCl₂ the uptake of magnesium was a function of phosphate accumulation (Table II). The mean ratio of magnesium to phosphate accumulated was found to be 1.5:1. In the absence of added Pi, no magnesium was accumulated in response to parathyroid hormone.

Effect of Metabolic Inhibitors—The data presented in Table III summarize the effects of a number of metabolic inhibitors upon the hormone-stimulated phosphate transport. Inhibitors of electron transport (CN⁻, antimycin A, azide, and amnophosphosides) and several uncouplers of oxidative phosphorylation (2,4-dinitrophenol, gramicidin, and Dicumarol) effectively prevented the parathyroid hormone-dependent Pi uptake. Warfarin surprisingly did not inhibit to any significant degree, and general glycolytic inhibitors were also ineffective. Rotenone was found to inhibit glutamate-supported Pi transport, but failed to inhibit...
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**FIG. 4** (left). P$_1$ accumulation in mitochondria as a function of magnesium concentration. Incubations were carried out as described in the text.

**FIG. 5** (right). Inhibition of the parathyroid hormone-stimulated P$_1$ uptake by either cyanide or oligomycin. Experimental conditions were as described in the text. Cyanide (10$^{-4}$ M) or oligomycin (10$^{-4}$ M) was added at the point indicated.

**TABLE II**

<table>
<thead>
<tr>
<th>Ion</th>
<th>Accumulation</th>
<th>Ratio of Mg$^{2+}$ to P$_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.7 mM MgCl$_2$</td>
<td>1.34</td>
<td></td>
</tr>
<tr>
<td>P$_1$</td>
<td>0.90</td>
<td>1.49:1.00</td>
</tr>
</tbody>
</table>

**TABLE III**

The effect of metabolic inhibitors on the parathyroid hormone-dependent phosphate uptake

Incubations were carried out as described in the text. Inhibitors were preincubated with the mitochondria for 5 minutes before the addition of the hormone.

<table>
<thead>
<tr>
<th>Addition to medium</th>
<th>Inhibitor concentration</th>
<th>Inhibition of hormone-stimulated P$_1$ uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligomycin</td>
<td>10$^{-4}$ M</td>
<td>100</td>
</tr>
<tr>
<td>Phloridzin</td>
<td>10$^{-3}$ M</td>
<td>30</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>3 x 10$^{-3}$ M</td>
<td>25</td>
</tr>
<tr>
<td>Fluoride</td>
<td>10$^{-3}$ M</td>
<td>10</td>
</tr>
<tr>
<td>p-Chloromercuribenzoate</td>
<td>10$^{-3}$ M</td>
<td>0</td>
</tr>
<tr>
<td>Cyanide</td>
<td>10$^{-3}$ M</td>
<td>100</td>
</tr>
<tr>
<td>Dinitrophenol</td>
<td>2 x 10$^{-4}$ M</td>
<td>100</td>
</tr>
<tr>
<td>Antimycin</td>
<td>10$^{-7}$ M</td>
<td>100</td>
</tr>
<tr>
<td>Warfarin</td>
<td>7 x 10$^{-4}$ M</td>
<td>25</td>
</tr>
<tr>
<td>Gramicidin</td>
<td>7 x 10$^{-4}$ M</td>
<td>100</td>
</tr>
<tr>
<td>Aside</td>
<td>10$^{-3}$ M</td>
<td>90</td>
</tr>
<tr>
<td>Rotenone</td>
<td>10$^{-5}$ M</td>
<td>100</td>
</tr>
<tr>
<td>Rotenone (succinate as substrate)</td>
<td>10$^{-5}$ M</td>
<td>0</td>
</tr>
<tr>
<td>Anaerobiosis</td>
<td>7 x 10$^{-4}$ M</td>
<td>100</td>
</tr>
<tr>
<td>Dicumarol</td>
<td></td>
<td>100</td>
</tr>
</tbody>
</table>

that supported by succinate. This might be expected from the fact that rotenone blocks DPNH oxidation but not succinate oxidation. These data also suggest that the succinate-supported binding of phosphate is not mediated through the energy-linked reduction of DPN.

Inhibition of the parathyroid hormone-dependent P$_1$ uptake by 1 mM CN was more dramatically illustrated when cyanide was added during the course of the hormone-stimulated P$_1$ uptake (Fig. 5). CN caused an immediate cessation of P$_1$ accumulation by the mitochondria. Of great interest is the fact that the accumulated phosphate remained constant in the mitochondria after CN inhibition, which suggests that the phosphate is not rapidly lost by some nonspecific means, such as a disrupted membrane system. Identical data were obtained when cyanide was added to oligomycin-blocked mitochondria in the presence of ATP.

In a further demonstration of the energy requirement of the phosphate transfer system, oligomycin was added during the parathyroid hormone-stimulated P$_1$ uptake. In the absence of added ATP, the result was also an immediate inhibition of P$_1$ uptake. Again the accumulated phosphate was maintained throughout the remainder of the incubation period.

**Temperature Dependence**—Fig. 6 shows that the parathyroid

**FIG. 6.** Temperature dependence of the parathyroid hormone-dependent P$_1$ accumulation. Experimental conditions were as described in the text. Incubations were carried out in the presence or absence of 10$^{-4}$ M oligomycin. ATP, 0.67 mM, was added when oligomycin was present.
hormone-dependent Pi uptake is temperature-dependent, whether it is carried out in the presence or absence of oligomycin. No hormonal effect was observed at 0°C.

**Influence of Ca Ions**—As revealed in Fig. 7, the addition of 3 x 10^{-4} m Ca to the medium containing ATP and no oligomycin resulted in an increased Pi transport in either the presence or absence of hormone. However, when Pi accumulation was studied in the presence of Ca ions, ATP, and 1 mM CN, the calcium-induced Pi transport was still observed, but the hormone-dependent Pi transport was completely eliminated. In studies on Ca uptake by mitochondria, DeLuca and Engstrom (12) have reported that 1 mM CN does not prevent Ca uptake from occurring provided an external source of ATP is supplied. These data then suggest that the mechanism of the hormone-dependent magnesium phosphate transport is somewhat different from the nonhormone-dependent transport of calcium phosphate into mitochondria.

**Effect of Vitamin D**—The stimulation of Pi uptake by parathyroid hormone was clearly evident in mitochondria from vitamin D-deficient rats (Table IV). This effect of the hormone is therefore in contrast to the previous observation of a vitamin D-dependent parathyroid hormone-stimulated release of calcium (9).

**Correlation of Respiration with Pi Uptake**—Because parathyroid hormone is known to stimulate both respiration and Pi uptake by mitochondria, an effort was made to establish the relationship between Pi uptake and respiration. During the initial 5-minute period following the addition of the hormone,

![Graph showing Parathyroid hormone (PTH) stimulation of Pi uptake](image)

**Fig. 7.** Parathyroid hormone (PTH) stimulation of Pi uptake in the presence of calcium ions. Incubations were carried out as described in the text, except that the medium contained 0.67 mM ATP and no oligomycin. Ca^{++} (3 x 10^{-4} m) and cyanide (10^{-4} m) were added where indicated.

Table IV

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hormone-dependent Pi accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No vitamin D</td>
<td>2.00</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>2.31</td>
</tr>
</tbody>
</table>

Rats were fed a diet that was adequate in calcium and phosphate but deficient in vitamin D as previously described (12). Where indicated, each rat received 75 i.u. of crystalline calciferol in oil every 3 days. Incubations were carried out as described in the text, and the above results are representative of experiments involving three separate groups of rats. The values given are the averages of triplicate determinations.

**Discussion**

The present and previous (10) findings demonstrate a highly specific effect of parathyroid hormone upon a phosphate transport system in isolated mitochondria. The energy for phosphate translocation comes apparently from the oxidative phosphorylation process because inhibitors of the respiratory chain, anaerobiosis, or uncouplers of oxidative phosphorylation completely block the phosphate uptake. In agreement with this, an oxidizable substrate and magnesium ions are absolute requirements for the translocation. Oligomycin, an inhibitor of a terminal reaction of the phosphorylative chain, prevents the hormone-supported phosphate uptake in the absence of external ATP, but when external ATP is supplied the phosphate uptake is restored (Fig. 1). However, even under these conditions respiration is required for the transfer reaction. The exact biochemical meaning of this dual requirement for ATP and respiration in oligomycin-blocked mitochondria is unclear at the present time. The fact that the hormone-induced uptake of phosphate can occur in oligomycin-blocked mitochondria suggests that an intermediate of oxidative phosphorylation before the oligomycin block may provide the energy for the translocation or that it may serve directly as the phosphate carrier in this system. These possibilities are currently under study. It should be noted that the hormone-dependent translocation of phosphate differs markedly from the phosphate translocation system described by Brierley, Bachmann, and Green (15) in heart mitochondria with regard to its behavior toward ATP and oligomycin. It should also be noted that a small amount of phosphate is accumulated in the absence of hormone (Fig. 7). This accumulation can be accelerated greatly by the addition of large quantities of magnesium ion, as has been observed by Brierley et al. with heart mitochondria (15). The relationship of this and the hormone-dependent transport must await further experimental examination.

The relationship between oxygen consumption and phosphate transport in mitochondria deserves comment. Under our most
favorable conditions, it was found that 2 g atoms of oxygen are consumed for each mole of inorganic phosphate taken up by the mitochondria. This transfer reaction would appear to represent a great expense of energy. However, the experimental approach to this measurement involved assumptions that may not be completely valid. It was assumed that all of the phosphate transported into the mitochondria was held there and eventually measured. Unless the intramitochondrial concentration of magnesium phosphate was such as to permit the immediate formation of the magnesium salt, it is possible that much of the phosphate transported into the mitochondria was not deposited but was returned to the medium, either by its route of entry or by some other means. This would result in the high oxygen to phosphate ratios observed. It is also possible that the hormone has, in addition to the phosphate transfer reaction, an uncoupling effect on oxidative phosphorylation independent of the phosphate translocation reaction.

The present experiments indicate that, in the presence of hormone, magnesium is transported, or at least deposited, with the phosphate. The ratio of magnesium to phosphate (1:5:1) is not surprising since this deposition would be expected if a solution of magnesium and phosphate were saturated at the pH values used in these studies. Although it is clear that both phosphate and magnesium are necessary to demonstrate a hormonal effect, it is not possible to decide whether the hormone stimulates primarily the transfer of magnesium, of phosphate, or of magnesium phosphate.

Previously it was shown that the parathyroid hormone stimulates the release of calcium (as calcium phosphate) from the mitochondria, but only when vitamin D is present (9). In the present study it is shown that the hormone stimulates the uptake of phosphate (as magnesium phosphate) by isolated mitochondria by a process independent of vitamin D. Although it would be premature to attempt to describe the physiological significance of these findings, it is instructive to note that they have made possible certain predictions with regard to the physiological interrelation of these two agents. It was suggested, on the basis of the mitochondrial studies, that the parathyroid hormone would not be able to exert its characteristic effects on calcium transport reactions in vitamin D deficiency, whereas it would continue to exert an effect upon phosphate metabolism. Positive support for this supposition has now been obtained (16).

In view of the role of parathyroid hormone in calcium and phosphate metabolism in the intact organism, the influence of calcium ions on the hormone-dependent Pi transport was studied. Although an increased transport of Pi occurred in the presence of calcium ions, the uptake of phosphate coupled to calcium translocation did not appear to be hormone dependent. Furthermore, the difference in CN sensitivity suggests that the phosphate translocation in the two systems occurs by different mechanisms.

As reported previously (10), the stimulation of phosphate translocation is highly specific for the parathyroid hormone. This suggested the possibility that this system could be used as an assay for parathyroid activity. This system is now being used in our laboratories as an index of hormonal activity in crude fractions from bovine parathyroid glands. However, because of its lack of sensitivity, it has not yet proven useful for detecting hormonal activity in biological fluids.

The specificity of the hormone and vitamin D action in the mitochondrial systems, and the parallel between the findings with mitochondria and the physiological actions of the two agents, indicate that in all likelihood the mitochondrial systems are physiologically significant. On the other hand, the rather unphysiological concentrations of the hormone used in these studies and the fact that liver is not recognized as a site of parathyroid hormone action raise questions that must be answered before a complete understanding of the significance of these systems can be obtained. At the present time, they must be looked upon as model systems that seem to offer possibilities of determining the biochemical mechanisms of action of the two agents. They further indicate that a primary effect of the hormone is on translocation of phosphate across membranes.

**SUMMARY**

Pure parathyroid hormone added in vitro markedly stimulates the accumulation of inorganic phosphate by isolated liver mitochondria (1 μmole of Pi per mg of mitochondrial protein) by a process that is independent of vitamin D. This highly specific effect of the hormone requires the presence of an oxidizable substrate and magnesium ions. The magnesium ions are translocated with the phosphate, and a magnesium to phosphate ratio of 1.5:1.0 is obtained in the mitochondria. This process is blocked by inhibitors of the electron transport chain, by anaerobiosis, by low temperature, and by uncouplers of oxidative phosphorylation. Oligomycin, an inhibitor of a terminal reaction of the phosphorylative chain, also inhibits the hormone-dependent Pi translocation. This inhibition can be overcome by the addition of adenosine triphosphate, but even under these conditions, the energy of oxidative phosphorylation is required for the parathyroid hormone-dependent phosphate accumulation.

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Parathyroid Hormone-dependent Uptake of Inorganic Phosphate by Mitochondria
John D. Sallis, H. F. DeLuca and Howard Rasmussen


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