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ION TRANSPORT AND PHOSPHOLIPID LABELLING IN RAT-LIVER MITOCHONDRIA

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SUMMARY

Parathyroid hormone and valinomycin are both highly specific agents which alter the rate of translocation of specific cations across the inner mitochondrial membrane. They were employed to test the proposal that phospholipids, particularly diphosphoinositide, are involved in mitochondrial ion translocations. Both agents had profound effects upon the rate and extent of phospholipid labelling from [^{32}P]orthophosphate. Some of these effects, however, were also produced by dinitrophenol. It was concluded that the rate and extent of phospholipid labelling was a reflection of the rate of turnover of the intramitochondrial adenine nucleotides, and that parathyroid hormone and valinomycin influenced phospholipid labelling by influencing the rates of substrate and oxidative phosphorylation.

INTRODUCTION

A considerable amount of literature has recently been amassed concerning the accumulation of divalent cations[1–14], monovalent cations[15–20] and a variety of anions[21–24] by isolated mitochondria. Cation accumulation can be supported by the same pool of high-energy intermediates as that used for ATP synthesis, and it would appear that transport across the mitochondrial membrane involves carrier-mediated systems similar to those seen in cell membranes. However, the nature of these mechanisms remains unresolved. In the case of the transport of sodium across the plasma membrane, HOKIN AND HOKIN[25,26] have suggested that phospholipids are intimately involved in this process. More recently, GARBUS, DELUCA AND LOOMANS[27] have detected a lipid fraction from liver and kidney mitochondria which is rapidly labelled by radioactive inorganic phosphate in the presence of substrate oxidation. This has been identified by GALLIARD AND HAWTHORNE[28–30] as diphosphoinositide. Inhibitors of oxidative phosphorylation were found to block the labelling of diphosphoinositide, but oligomycin, an inhibitor of terminal phosphorylation, did not. These facts led GARBUS, DELUCA AND LOOMANS[27] to propose that phosphoinositides might be intermediates in oxidative phosphorylation. On the other hand, MICHELL, GALLIARD AND HAWTHORNE[29] showed that the conditions for phosphorylation of phosphatidyl inositol

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and for accumulation of phosphate *plus* Ca^{2+} or Mg^{2+} were very similar. This led HAWTHORNE[31] to propose that di- or triphosphoinositide acted as an ion carrier in this membrane.

Because of these observations and theories, a study of the effects of parathyroid hormone and valinomycin upon the phospholipid turnover in isolated mitochondria was undertaken in an effort to define, if possible, the relationship between cation transport and phospholipid turnover in isolated liver mitochondria. Parathyroid hormone and valinomycin were used as specific agents to increase cation transport (see refs. 8, 17–23, 32–36).

MATERIALS AND METHODS

Rat-liver mitochondria were prepared as previously described[18]. The standard incubation medium consisted of mitochondria from 0.65 g of liver tissue, 40 μmoles of Tris–HCl buffer (pH 7.4), 30 μmoles of substrate (sodium glutamate, sodium succinate or sodium β-hydroxybutyrate), 20 μmoles of $MgCl_2$, 2.5 μmoles of sodium phosphate (*plus* ^{32}P), 250 μmoles of sucrose and 10 μg oligomycin in a total vol. of 2.40 ml. The reaction medium without mitochondria and orthophosphate was pre-incubated in a 25-ml erlenmeyer flask at 30° for 5 min. Aliquots of mitochondrial suspension were added to the incubation flasks 2 min before the reaction was initiated by the addition of [^{32}P]orthophosphate. Parathyroid hormone was prepared by the method of RASMUSSEN, SZE AND YOUNG[37] and dissolved in 0.1 mM acetic acid. Valinomycin was dissolved in ethanol. The hormone, valinomycin, or an appropriate solvent was added to the mitochondrial suspension. The incubation was terminated rapidly by the addition of 9 ml of $CHCl_3$–methanol (1:2, v/v). The mixture was homogenized 20 times in a Potter–Elvehjem homogenizer, following which 3 ml of chloroform were added and then 3 ml of 2 M KCl in 0.5 M potassium phosphate buffer (pH 7.4). The mixture was homogenized 20 times after each of these additions. The mixture of extracted mitochondria was filtered through a plug of glass wool into a separatory funnel equipped with a Teflon stopcock. The two phases were allowed to separate overnight. The lower organic phase containing the phospholipid was removed and allowed to run through a filter paper. Aliquots of this lipid extract were used for analysis. The blank was treated in the same way except that the mixture of $CHCl_3$–methanol (1:2, v/v) was added prior to the addition of orthophosphate.

Aliquots of the chloroform layer were plated directly on planchets, dried and counted under a thin end-window counter for total ^{32}P present in phospholipids. Suitable aliquots of lipid in chloroform were chromatogramed on a silicic acid-impregnated paper[38] and developed with diisobutyl ketone–acetic acid–water (40:30:7, by vol.)[39] or with water-satd. phenol–ammonium[40]. The radioactive spots on the chromatograms were counted with a strip scanner.

RESULTS

The effects of parathyroid hormone and valinomycin upon the incorporation of [^{32}P]orthophosphate into phospholipid are shown in Figs. 1 and 2. When succinate served as substrate, either parathyroid hormone or valinomycin inhibited the incorporations of [^{32}P]orthophosphate into phospholipids. When glutamate or β-hydroxy-

butyrate served as substrate, parathyroid hormone had no effect upon the formation of labelled phospholipid whereas valinomycin inhibited their formation. However, in all cases it was shown, by independent measurements, that cation transport into mitochondria was increased by parathyroid hormone as well as by valinomycin.

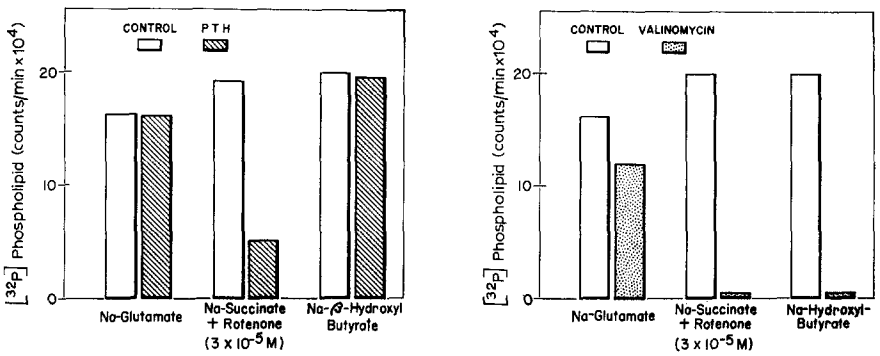


Fig. 1. The effect of parathyroid hormone (PTH) upon the incorporation of [^{32}P]orthophosphate into phospholipid with different substrates. The reaction mixture contained 30 μmoles substrate (sodium glutamate, sodium β-hydroxybutyrate or sodium succinate *plus* 30 μM rotenone), 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 250 μmoles sucrose, mitochondria equivalent to 0.65 g of liver tissue, and 200 μg parathyroid hormone or an equivalent amount of solvent. 2 min after the addition of mitochondrial suspension, 2.5 μmoles sodium phosphate buffer (pH 7.4) (*plus* ^{32}P) were added. The total vol. was 2.4 ml. The reactions were terminated after 40 min of incubation at 30°.

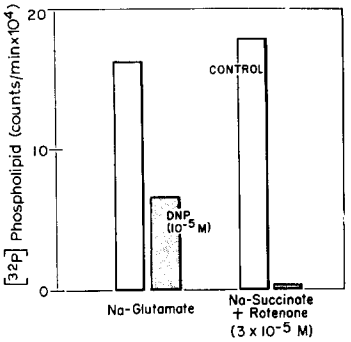
Fig. 2. The effect of valinomycin upon the incorporation of [^{32}P]orthophosphate into phospholipid. The reaction mixture contained 30 μmoles substrates (sodium glutamate, sodium β-hydroxybutyrate or sodium succinate *plus* 30 μM rotenone) 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 125 μmoles sucrose, 100 μmoles KCl, mitochondria equivalent to 0.65 g of liver tissue and 1 μg valinomycin or equivalent amount of solvent. 2 min after the addition of mitochondrial suspension, 2.5 μmoles sodium phosphate buffer (pH 7.4) (*plus* ^{32}P) were added. The final vol. was 2.40 ml. The reactions were terminated after 40-min incubation at 30°.

Particularly important, parathyroid hormone increased the rate and extent of [^{32}P]-orthophosphate accumulation into mitochondria as has been reported previously[22,32]. Thus in spite of increased rates of respiration, cation translocation, and orthophosphate accumulation, there was either no change or a decrease in rate of ^{32}P incorporation into phospholipids. The results are in keeping with the known uncoupling effects of parathyroid hormone[32] and valinomycin[41] in the presence of glucose–hexokinase system, and suggested that the formation of ^{32}P-labelled phospholipid is controlled by the level of phosphorylated high-energy compounds in mitochondria.

To test this possibility further, the effect of 2,4-dinitrophenol upon phospholipid labelling was examined. The addition of dinitrophenol (10 μM) inhibited the incorporation of [^{32}P]orthophosphate into phospholipid when either glutamate or succinate was the substrate. The extent of inhibition differed considerably with different substrates: when glutamate was the substrate, 2,4-dinitrophenol (10 μM) inhibited the labelling of phospholipid about 60 %, whereas with succinate as the substrate, the inhibition was about 98 % (Fig. 3).

Oligomycin, which inhibits oxidative phosphorylation[42], has no effect upon valinomycin or parathyroid hormone-mediated cation transport when energy is supplied by substrate oxidation (see refs. 17, 20, 22, 32 and 36). However, oligomycin

completely inhibited the incorporation of [^{32}P]orthophosphate into phospholipid, when succinate or β-hydroxybutyrate was the substrate; but inhibition was only partial if glutamate served as substrate (Fig. 4). The effects of parathyroid hormone and valinomycin upon the phospholipid labelling were also altered in the presence of oligomycin. When succinate was the substrate, the addition of either parathyroid



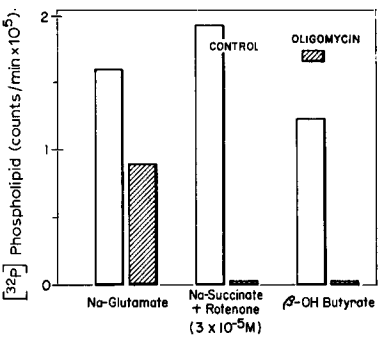


Fig. 3. The effect of 2,4-dinitrophenol (DNP) (10 μM) upon the incorporation of [^{32}P]orthophosphate into phospholipid. The reaction mixture contained 30 μmoles substrate (sodium glutamate or sodium succinate *plus* 30 μM rotenone), 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 250 μmoles sucrose, mitochondria equivalent to 0.65 g of liver tissue. 0.1 ml of 0.24 mM 2,4-dinitrophenol or an equivalent amount of solvent was pipetted into the reaction flask. 2 min after the addition of mitochondrial suspension, 2.5 μmoles sodium phosphate buffer (pH 7.4) (*plus* ^{32}P) were added. The final vol. was 2.40 ml. The reactions were terminated after 40-min incubation.

Fig. 4. The effect of oligomycin upon the incorporation of [^{32}P]orthophosphate into phospholipid with different substrates. The reaction mixture contained 30 μmoles sodium succinate (*plus* 30 μM rotenone), 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 250 μmoles sucrose, mitochondria equivalent to 0.65 g of liver tissue, and 10 μg oligomycin dissolved in 0.1 ml of ethanol or equivalent amount of ethanol. 2 min after the addition of mitochondria, 2.5 μmoles sodium phosphate buffer (pH 7.4) (*plus* ^{32}P) were added. The final vol. was 2.40 ml. The reactions were terminated after 40-min incubation.

TABLE I

THE EFFECT OF DIFFERENT SALTS UPON THE VALINOMYCIN AND PARATHYROID HORMONE-STIMULATED INCORPORATION OF [^{32}P]ORTHOPHOSPHATE INTO PHOSPHOLIPID IN THE PRESENCE OF OLIGOMYCIN

The basic reaction medium contained 30 μmoles sodium glutamate, 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 10 μg oligomycin, 125 μmoles sucrose and mitochondria equivalent to 0.65 g of liver tissue. An additional 200 μmoles of sucrose was present in the control flasks, and this was replaced by 100 μmoles of the respective salt (potassium acetate, KCl, sodium acetate, and NaCl). Either valinomycin, 1 μg, or parathyroid hormone, 200 μg, were added to appropriate flasks. The reaction was terminated after 40 min, and the results are expressed as radioactivity incorporated into phospholipid.

Medium	^{32}P *incorporated into phospholipid (counts/min* × 10^{-4})			
	Control	*Valinomycin*	*Control*	*Parathyroid hormone*
Sucrose	2.31	2.56	2.74	3.63
Potassium acetate	2.92	0.27	3.80	1.27
KCl	2.41	3.86	2.82	3.91
Sodium acetate	2.32	2.32	3.32	3.30
NaCl	2.03	2.02	2.81	2.88

hormone or valinomycin in the presence of oligomycin had no effect upon the incorporation of [^{32}P]orthophosphate into phospholipids. When glutamate was the substrate, the addition of parathyroid hormone, valinomycin or 2,4-dinitrophenol increased the incorporation of [^{32}P]orthophosphate into phospholipid (Fig. 5).

The effects of both valinomycin and parathyroid hormone upon ion translocation are highly specific: Valinomycin influences only K^+ and parathyroid hormone K^+ and Mg^{2+} movements[7,20,36]. To demonstrate that the effects of these two agents upon phospholipid turnover were related to their effects upon ion movements, the effect of altering the ionic environment was examined employing glutamate as substrate in the presence of oligomycin. The results obtained with valinomycin are shown in Table I. When K^+ was absent or replaced by Na^+ no significant effect was noted. When KCl was employed (phosphate was also present), there was a significant increase in labelling. However, when potassium acetate was employed, valinomycin led to a marked inhibition. In the latter instance, this inhibition was attributed to the marked mitochondrial swelling seen under this circumstance. A study of the time course of labelling in the presence of potassium acetate showed that valinomycin led to an early stimulation of phospholipid labelling followed by an abrupt cessation of labelling simultaneous with marked mitochondrial swelling.

In the case of parathyroid hormone, the results with potassium were quite similar (Table I), as would be predicted from its effects upon K^+ uptake[20]. However, in the absence of K^+, parathyroid hormone also stimulated phospholipid labelling (80 mM sucrose; Table I), but this was abolished by the addition of either sodium

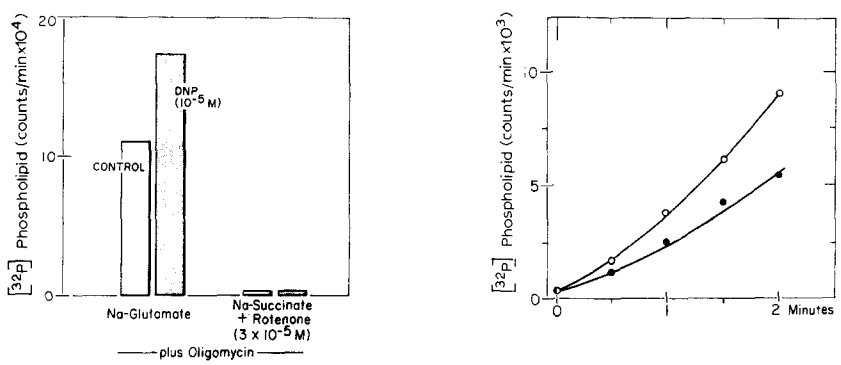


Fig. 5. The effect of 2,4-dinitrophenol (DNP) (10 μM) upon the incorporation of [^{32}P]orthophosphate into phospholipid in the presence of oligomycin. The reaction mixture contained 30 μmoles substrate (sodium glutamate, or sodium succinate *plus* 10 μM rotenone), 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 250 μmoles sucrose, mitochondria equivalent to 0.65 g of liver tissue, 10 μg oligomycin and 0.1 ml of 0.24 mM 2,4-dinitrophenol or equivalent amount of solvent. The final vol. was 2.40 ml. The reactions were terminated after 40-min incubation. Similar results were obtained if either valinomycin or parathyroid hormone was added in place of DNP.

Fig. 6. The effect of valinomycin upon the incorporation of [^{32}P]orthophosphate into phospholipid. The reaction mixture contained 30 μmoles sodium glutamate, 40 μmoles Tris–HCl buffer (pH 7.4), 250 μmoles sucrose, mitochondria equivalent to 0.65 g of liver tissue with (●) or without (○) 1 μg valinomycin. 2 min after the addition of mitochondrial suspension, 5 μmoles $MgCl_2$, 10 μmoles KCl and 0.15 $\mu\mu$moles carrier-free [^{32}P]orthophosphate were added to the reaction mixture. The total vol. per flask was 2.40 ml. The reaction was terminated with chloroform–methanol mixture at desired time. Similar results were seen when succinate replaced glutamate, and when parathyroid hormone replaced valinomycin.

acetate or NaCl (Table I). This stimulation is undoubtedly related to the presence of Mg^{2+} (see refs. 20, 32 and 36), and it is known from previous unpublished work that the presence of 40 mM NaCl or sodium acetate blocks the effect of parathyroid hormone upon magnesium-dependent respiration and magnesium phosphate accumulation.

All of the above effects were noted in rather prolonged incubations. It seemed possible that short-term experiments with carrier-free [^{32}P]orthophosphate might reveal a small rapidly labelled pool of phospholipid which was influenced by these agents and more directly related to ion translocations. Accordingly, short-term experiments were done. As shown in Fig. 6, significant labelling was found with either glutamate or succinate as substrate. The addition of either valinomycin or parathyroid hormone decreased this rate with either substrate. When oligomycin was added, there was a significant decrease in the early labelling of phospholipid. This was increased by parathyroid hormone (Fig. 7) when glutamate but not when succinate served as substrate. Similar results were obtained with valinomycin. The effects of various cations upon the early labelling seen when glutamate was substrate were similar to those noted with longer periods of incubation (Table I). Either Mg^{2+} or K^+ was required for parathyroid hormone action and K^+ for valinomycin action.

The labelled phospholipids in both the short-term and more prolonged incubations were characterized. In the latter, 2 radioactive spots were noted when chromatography of the lipid extracts was carried out. The results of the chromatography in 2 separate systems led to the conclusion that of the two materials one corresponded to phosphatidic acid, and the other to a spot identified as diphosphoinositide by HOKIN AND HOKIN[40]. In the short-term incubations only diphosphoinositide was labelled. The extent of its labelling corresponded to the extent of total labelling.

All of these results suggested that either parathyroid hormone or valinomycin influenced phospholipid labelling indirectly by an effect upon intramitochondrial ATP pools. If this conclusion is correct, then the addition of α-glycerophosphate should abolish the effect of these agents, and these agents should have no effect upon the

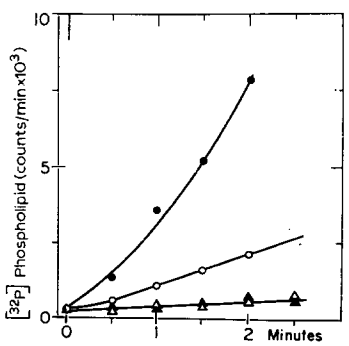


Fig. 7. The effect of parathyroid hormone upon the incorporation of [^{32}P]orthophosphate into phospholipid in the presence of oligomycin. The reaction mixture contained 30 μmoles substrate (30 μmoles sodium glutamate (●, ○) or 30 μmoles sodium succinate and 30 μM rotenone (▲, △)) 40 μmoles Tris–HCl buffer (pH 7.4), 325 μmoles sucrose, 10 μg oligomycin and mitochondria equivalent to 0.65 g of liver tissue. Parathyroid hormone, 200 μg, was added (●, ▲) or equivalent amount of solvent (○, △) per flask were added to the mitochondrial suspension. 2 min after the addition of mitochondrial suspension, 5 μmoles $MgCl_2$ and 0.15 $\mu\mu$mole carrier-free [^{32}P]orthophosphate were added to the reaction mixture. The total vol. per flask was 2.40 ml. The reaction was terminated with the chloroform–methanol mixture at the desired time.

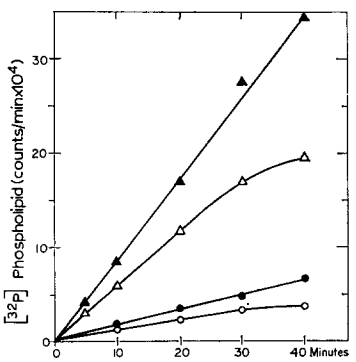
incorporation of ^{32}P-labelled α-glycerophosphate into phospholipids. This proved to be the case. The addition of 1 mM unlabelled α-glycerophosphate inhibited the incorporation of [^{32}P]orthophosphate into phospholipid. When α-[^{32}P]glycerophosphate was employed neither valinomycin nor parathyroid hormone had any significant effect upon its incorporation (Table II). Of particular interest was the fact that the presence

TABLE II

THE INCORPORATION OF ^{32}P LABELLED α-GLYCEROPHOSPHATE INTO PHOSPHOLIPID

The reaction mixture contained 30 μmoles sodium glutamate, 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 125 μmoles sucrose, 100 μmoles KCl, 10 μg oligomycin, 8.4 μmoles ^{32}P-labelled α-glycerophosphate, and mitochondrial equivalent to 0.65 g of liver tissue; and either no sodium phosphate or 1 mM sodium phosphate buffer (pH 7.4). Parathyroid hormone, 200 μg, valinomycin, 1 μg, or equivalent amount of solvent were added to the mitochondrial suspension. The reaction was carried out for 20 min at 30°.

Condition	*^{32}P incorporation into phospholipid (counts/min × 10^{-3})*		
	Control	*Valinomycin*	*Parathyroid hormone*
No orthophosphate	12	11.5	—
1 mM orthophosphate	1.2	1.2	—
No orthophosphate	16.0	—	16.6
1 mM orthophosphate	1.5	—	1.6



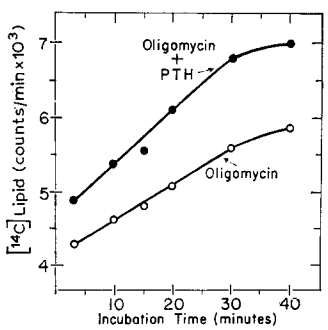


Fig. 8. The effect of glycerol upon the incorporation of [^{32}P]orthophosphate into phospholipid in the presence and absence of parathyroid hormone. The reaction mixture contained 30 μmoles sodium glutamate, 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 10 μg oligomycin, 250 μmoles sucrose, mitochondria equivalent to 0.65 g of liver tissue and 0.24 μmole glycerol (△, ▲) or equivalent amount of water (○, ●), and 200 μg parathyroid hormone (▲, ●) or equivalent amount of solvent (△, ○) per flask. 2 min after the addition of mitochondria, 2.5 μmoles sodium phosphate buffer (pH 7.4) labelled with ^{32}P were added. Similar changes were observed when valinomycin replaced parathyroid hormone.

Fig. 9. The effect of parathyroid hormone (PTH) upon the incorporation of [1,3-$^{14}C_2$]glycerol into mitochondrial phospholipid. The reaction mixture contained 30 μmoles sodium glutamate, 20 μmoles $MgCl_2$, 40 μmoles Tris–HCl buffer (pH 7.4), 10 μg oligomycin, 250 μmoles sucrose and mitochondria equivalent to 0.65 g of liver. Parathyroid hormone, 200 μg (●), or equivalent amount of solvent (○) per flask was added to the mitochondrial suspension. [^{14}C]Glycerol (0.24 μmole) and 2.5 μmoles of sodium phosphate (pH 7.4) were added 2 min after incubation was started. The final vol. was 2.40 ml.

of inorganic phosphate greatly enhanced the rate of α-glycerophosphate incorporation into phospholipid, but even under these circumstances parathyroid hormone had no further effect.

The effect of glycerol upon this system was also examined. The addition of 0.1 mM glycerol stimulated the uptake of orthophosphate into phospholipid (Fig. 8) in the glutamate–oligomycin system. Both valinomycin and parathyroid increased phospholipid labelling in the presence of glycerol. Because of this striking increase in phosphate incorporation induced by glycerol, the uptake of [^{14}C]glycerol was examined. Neither valinomycin nor parathyroid hormone increased the rate of total [^{14}C]glycerol accumulation by mitochondria. However, both agents stimulated [^{14}C]-glycerol conversion into phospholipid (Fig. 9), in a medium containing glutamate as substrate and oligomycin to inhibit oxidative phosphorylation. Of particular interest was the fact that the effect of valinomycin was apparent even when K^+ was absent from the medium, and the addition of K^+ led to no further stimulation of glycerol incorporation.

DISCUSSION

The present study was concerned with the interrelationship between phospholipid turnover and ion transport in isolated rat-liver mitochondria. The results indicate that phospholipids probably do not serve as ion carriers in the translocation of Mg^{2+} or K^+ across mitochondrial membranes. All of the present data show that intramitochondrial ATP or GTP is the immediate precursor for phospholipid labelling, and that the rate and extent of this labelling is a measure of the rate of turnover of the intramitochondrial nucleotide pool. Conditions which uncoupled oxidative phosphorylation (Figs. 1–3) led to a decrease or abolishment of ATP synthesis and phospholipid labelling. Similarly, inhibition of ATP synthesis by oligomycin (Figs. 4 and 5) led to an inhibition of phospholipid labelling. In both instances, *i.e.* with or without oligomycin, uncoupling (Figs. 1–5) led to complete inhibition of labelling with succinate as substrate, but only a partial loss when glutamate was substrate. Furthermore, with glutamate as substrate, it was possible to enhance phospholipid labelling by uncoupling with either parathyroid hormone (Mg^{2+} and K^+), valinomycin (K^+), or dinitrophenol when oxidative phosphorylation was blocked by oligomycin (Table I), *i.e.* in the presence of oligomycin, the rate of substrate phosphorylation and of phospholipid labelling are enhanced by any mechanism which uncouples electron transport.

Even in short-term experiments there was no evidence for an increase in the specific labelling of a mitochondrial phospholipid which can be attributed specifically to translocation of ions.

The data obtained with α-glycerophosphate (Table II) and glycerol (Figs. 8 and 9) can also be accounted for in terms of control being exerted at the level of substrate phosphorylation. In the system where glutamate was substrate, oligomycin was present, and no glycerol was added, the rate of substrate phosphorylation was limiting because of coupled electron transport. When electron transport was uncoupled, the rate of substrate phosphorylation increased and then the availability of glycerol became limiting. When glycerol was added, further synthesis was possible and substrate phosphorylation increased further. In the case of [^{14}C]glycerol, increased

incorporation into phospholipid, induced by parathyroid hormone or valinomycin, was a consequence of increased high-energy phosphate from substrate phosphorylation due to the uncoupling effect of ion translocation (Fig. 9).

There were some differences between the effects of parathyroid hormone and valinomycin (Fig. 2 and Table I). However, most of these can be accounted for by the known differences in their effects upon ion permeabilities[18,20,36]. The differences noted in Figs. 1 and 2 are less easy to account for, particularly the fact that when β-hydroxybutyrate was substrate parathyroid hormone had little effect upon phospholipid labelling whereas valinomycin had a marked effect. This may indicate a difference in sensitivity of this particular dehydrogenase to the effects of these two agents, and is a point which deserves further study for it clearly implies that the metabolic consequences of a particular change in membrane permeability may have specific effects unrelated to the general uncoupling action which is the usual consequence of increased ion translocation.

One additional fact which cannot be readily explained is why valinomycin stimulated the incorporation of [^{14}C]glycerol into phospholipids when K^+ was absent, but did not stimulate ^{32}P incorporation into phospholipids under the same circumstances. This observation deserves further study.

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REFERENCES

1 G. P. BRIERLEY, E. BACHMANN AND D. E. GREEN, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 1928.
2 E. CARAFOLI, C. S. ROSSI AND A. L. LEHNINGER, *Biochem. Biophys. Res. Commun.*, 19 (1965) 609.
3 B. CHANCE, *Energy-linked Function of Mitochondria*, Academic, New York, 1963, p. 253.
4 B. CHANCE, *J. Biol. Chem.*, 240 (1965) 2729.
5 B. CHANCE AND T. YOSHIOKA, *Federation Proc.*, 24 (1965) 425.
6 J. B. CHAPPELL, M. COHN AND G. D. GREVILLE, in B. CHANCE, *Energy-linked Function of Mitochondria*, Academic, New York, 1963, p. 219.
7 H. F. DELUCA AND G. W. ENGSTROM, *Proc. Natl. Acad. Sci. U.S.*, 47 (1961) 1744.
8 H. F. DELUCA, G. W. ENGSTROM AND H. RASMUSSEN, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 1604.
9 G. W. ENGSTROM AND H. F. DELUCA, *J. Biol. Chem.*, 237 (1962) 974.
10 G. W. ENGSTROM AND H. F. DELUCA, *Biochemistry*, 3 (1964) 379.
11 A. L. LEHNINGER, *Physiol. Rev.*, 42 (1962) 467.
12 H. RASMUSSEN, B. CHANCE AND E. OGATA, *Proc. Natl. Acad. Sci. U.S.*, 53 (1965) 1069.
13 C. S. ROSSI AND A. L. LEHNINGER, *J. Biol. Chem.*, 239 (1964) 3971.
14 F. D. VASINGTON AND J. V. MURPHY, *J. Biol. Chem.*, 237 (1962) 2670.
15 J. B. CHAPPELL AND A. R. CROFTS, *Biochem. J.*, 95 (1965) 393.
16 J. L. GAMBLE, JR., *J. Biol. Chem.*, 228 (1957) 955.
17 C. MOORE AND B. C. PRESSMAN, *Biochem. Biophys. Res. Commun.*, 15 (1964) 548.
18 C. MOORE AND B. C. PRESSMAN, *Biochem. Biophys. Res. Commun.*, 15 (1964) 558.
19 B. C. PRESSMAN, *Proc. Natl. Acad. Sci. U.S.*, 53 (1965) 1076.
20 H. RASMUSSEN, J. FISCHER AND C. ARNAUD, *Proc. Natl. Acad. Sci. U.S.*, 52 (1964) 1198.
21 H. RASMUSSEN, J. D. SALLIS, M. FANG, H. F. DELUCA AND R. YOUNG, *Endocrinology*, 74 (1964) 388.
22 J. D. SALLIS, H. F. DELUCA AND H. RASMUSSEN, *J. Biol. Chem.*, 238 (1963) 4098.
23 J. D. SALLIS AND H. F. DELUCA, *J. Biol. Chem.*, 239 (1964) 4303.
24 S. R. MAX AND J. L. PURVIS, *Biochem. Biophys. Res. Commun.*, 21 (1965) 587.
25 L. E. HOKIN AND M. R. HOKIN, *Federation Proc.*, 22 (1963) 8.

26 L. E. HOKIN AND M. R. HOKIN, *Ann. Rev. Biochem.*, 32 (1963) 553.
27 J. GARBUS, H. F. DELUCA, M. E. LOOMANS AND F. M. STRONG, *J. Biol. Chem.*, 238 (1963) 59.
28 T. GALLIARD AND J. N. HAWTHORNE, *Biochim. Biophys. Acta*, 70 (1963) 479.
29 R. H. MICHELL, T. GALLIARD AND J. N. HAWTHORNE, *Biochem. J.*, 92 (1964) 45 p.
30 T. GALLIARD, R. H. MICHELL AND J. N. HAWTHORNE, *Biochim. Biophys. Acta*, 106 (1965) 551.
31 J. N. HAWTHORNE, in R. M. C. DAWSON AND D. N. RHODES, *Metabolism and Physiological Significance of Lipids*, Wiley, London–New York–Sydney, 1964, p. 197.
32 M. FANG AND H. RASMUSSEN, *Endocrinology*, 75 (1964) 434.
33 H. RASMUSSEN AND H. F. DELUCA, *Ergeb. Physiol. Biol. Chem. Exptl. Pharmakol.*, 53 (1963) 109.
34 H. RASMUSSEN, *Science*, 128 (1964) 431.
35 H. RASMUSSEN, in P. KARLSON, *Mechanism of Hormone Action*, Academic, New York, 1965, p. 131.
36 H. RASMUSSEN AND E. OGATA, *Biochemistry*, 5 (1966) 733.
37 H. RASMUSSEN, Y. L. SZE AND R. YOUNG, *J. Biol. Chem.*, 239 (1964) 2852.
38 G. V. MARINETTI, *J. Lipid Res.*, 3 (1962) 1.
39 G. V. MARINETTI, J. ERBLAND, M. ALBRECHT AND E. STOTZ, *Biochim. Biophys. Acta*, 26 (1957) 130.
40 L. E. HOKIN AND M. R. HOKIN, *Biochim. Biophys. Acta*, 84 (1964) 563.
41 W. C. MCMURRAY AND R. W. BEGG, *Arch. Biochem. Biophys.*, 84 (1959) 546.
42 H. A. LARDY, D. JOHNSON AND W. C. MCMURRAY, *Arch. Biochem. Biophys.*, 78 (1958) 587.