THE CATALYTIC EFFECT OF 2,4-DINITROPHENOL ON ADENOSINETRIPHOSPHATE HYDROLYSIS BY CELL PARTICLES AND SOLUBLE ENZYMES*

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One of the first clues to the mechanism by which DNP[1] stimulates metabolism was the discovery by Ronzoni and Ehrenfest (2) that, in frog muscles, DNP increases the rate of disappearance of phosphocreatine and makes the metabolic mechanism for the resynthesis of phosphocreatine less effective than normal. These results together with other metabolic data (summarized by Lardy and Elvehjem (3)) led to the hypothesis that DNP and similar agents "act by allowing oxidations to occur without phosphorylation or actually cause dephosphorylation of high energy phosphate" ((3) p. 16; *cf.* (4)). Apparent support for the latter possibility was obtained in the finding (3) that DNP accelerated the rate of ATP hydrolysis by minced rat muscle in the absence of added substrates and under anaerobic conditions.

Loomis and Lipmann (5) and Cross *et al.* (6) found DNP to prevent the uptake of phosphate associated with oxidations of Krebs' cycle intermediates. However, it was reported by Teply (7) that phosphate is required for the oxidation, but that DNP causes the release of the phosphate and thus permits a small quantity to serve catalytically. It appeared that a reinvestigation of the effect of DNP on ATP breakdown might yield some information as to the mechanism by which this agent prevents oxidative phosphorylation. Independently, similar work was undertaken by Hunter (8) and by Potter and Recknagel (9).

In the present work a study has been made of the influence of ionic environment, aging, and certain inhibitors on the hydrolysis of ATP by rat liver fractions, especially mitochondria, in the presence and absence of DNP. Soluble preparations from acetone-desiccated mitochondria hydrolyze ATP if fortified with Mg^{++}. This hydrolysis is greatly enhanced by DNP.

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[1] DNP will be used to designate 2,4-dinitrophenol; ATP, adenosinetriphosphate.

EXPERIMENTAL

Tissue Preparations—Livers from adult male rats of the Sprague-Dawley strain were the source of all preparations. They were removed immediately after decapitating the rats and were placed in chilled 0.25 M sucrose in an ice bath. The cellular components were fractionated by the procedure of Schneider (10) with the following modifications. *Nuclei* were washed four times with 0.25 M sucrose at a centrifugal force of 600 × *g* for the first two sedimentations and 400 × *g* for the last three sedimentations to facilitate separation of the mitochondria still present. *Mitochondria* were separated as described before (11). Preaging of mitochondria (12, 13) was accomplished by holding the twice washed particles in sucrose at 37° for 20 minutes. The suspension was then cooled in ice until used.

Acetone-desiccated mitochondria were obtained as described elsewhere (14), and the powder was stored in a desiccator at −5° until used. Enzyme extracts were prepared by homogenizing 0.05 gm. (the approximate yield from 1 gm. of fresh liver) of the acetone powder per ml. of water or 0.25 M sucrose and allowing the suspension to stand 20 minutes at 0°. A clear supernatant solution was obtained by centrifuging at 18,000 × *g* for 30 minutes at 0°.

Other Reagents—Other reagents were obtained from the following sources: ATP as the disodium salt, Pabst Laboratories; adenosinediphosphate (ADP), inosinetriphosphate, *p*-chloromercuribenzoate, and *o*-iodosobenzoate, Sigma Chemical Company; DNP, Eastman, recrystallized.

Assay Procedure—The substrate and buffer were supplied by 0.06 M ATP brought to pH 7.4. The reaction mixture contained ATP, 6 μM, 0.3 ml. of enzyme source in 0.25 M sucrose, and, as indicated in the legends, either 0.15 M KCl, 0.25 M sucrose, or water was added to give a final volume of 1 ml. Other additions were made at the expense of the diluent. All reaction components except ATP were added to chilled centrifuge tubes. When the enzyme source had been added, the tubes were immediately immersed in the 25° bath and, after the contents had come to the temperature of the bath, the reaction was started by addition of ATP.[2] Zero time tubes were deproteinized by addition of 1 ml. of chilled 10 per cent trichloroacetic acid before addition of ATP. All tubes were chilled after deproteinization and analyses for inorganic phosphate were made by the procedure of Lowry and Lopez (15). The nitrogen content of the enzyme sources was determined by a micro, direct nesslerization procedure. An attempt was made to use a quantity of enzyme source containing very nearly 0.2 mg. of nitrogen per tube in each assay. Unless otherwise designated, all results are calculated and reported on the basis of this quantity of nitrogen.

[2] The tube contents were exposed to air but they were not shaken.

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Results

Experiments with Various Cellular Components—Rat liver homogenized in 0.25 M sucrose and the various fractions therefrom did not hydrolyze ATP at an appreciable rate (Table I). The addition of DNP (3×10^{-4} M) caused a rapid liberation of inorganic phosphate by the whole homogenate and the nuclear and mitochondrial fractions. DNP had no appreciable effect on the hydrolysis of ATP by the microsomal or supernatant fractions. The hydrolysis of ATP by the whole homogenate and by the N_w fraction[3] was strongly enhanced by 0.0075 M Mg^{++}; the activity of the P_w and S_2 fractions was affected much less by Mg^{++}, while that of M_w was only slightly enhanced. These latter results are in agreement with those recently published by Novikoff *et al.* (17).

TABLE I

Effect of DNP on Phosphate Liberation from ATP by Liver Cell Fractions

Fraction	Phosphate liberated per 0.2 mg. N			
	No Mg^{++} added		With 0.0075 M Mg^{++}	
	Control	+ 3×10^{-4} M DNP	Control	+ 3×10^{-4} M DNP
	μM	μM	μM	μM
Original homogenate	0.02	3.16	1.03	2.60
N_w	0.06	0.89	0.71	1.00
M_w	0.04	1.34	0.12	1.29
P_w	0.11	0.22	0.46	0.47
S_2	0.00	0.04	0.27	0.26

Incubated 10 minutes at 25°.

Sedimentation of some mitochondria with the nuclei, even after several washings, may account for some of the DNP effect in N_w. However, nuclei prepared by the citrate extraction procedure of Dounce (18) also showed a DNP effect which was enhanced 2-fold by replacing isotonic sucrose with isotonic KCl to give a final molarity of 0.09 M KCl. The "ATPase" activity of this nuclear preparation was high and was further activated by the addition of 0.0015 M Ca^{++} or Mg^{++}.

Studies with Mitochondria—Subsequent experiments in the present series were performed with M_w. With these particles as the source of the enzyme, phosphate liberation was directly proportional to DNP concentration until a maximal rate was achieved with 6×10^{-5} M DNP; higher concentrations neither increased nor diminished the response. For most further studies a concentration of 3×10^{-4} M DNP was arbitrarily chosen to insure the maximal possible effect. At this concentration of DNP, with

[3] The conventional abbreviations (16) are employed.

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isotonic KCl as the diluent, the amount of phosphate liberated increased linearly with time over a 15 minute period if the amount of M_w was such that the ATP concentration did not fall below 2 μM per ml. The rate was directly proportional to the amount of M_w (Fig. 1) until about two-thirds of the terminal phosphate of ATP had been liberated. At higher concentrations of M_w the rate of phosphate liberation was probably limited by the rapidly diminishing ATP concentration and the rate at which the mitochondrial myokinase (13, 19) forms ATP from ADP. The ATP-hydrolyzing activity of M_w was stimulated by Ca^{++}, but only very slightly by

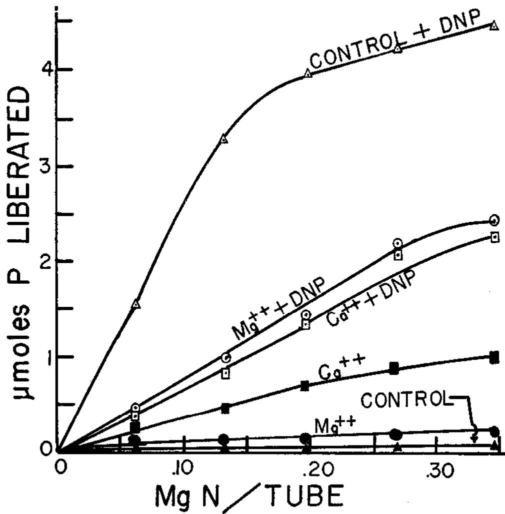

Fig. 1. Relationship of enzyme concentration to ATP hydrolysis. All tubes contained 6 μM of ATP in 0.25 M sucrose. Dilution to 1 ml. made with 0.15 M KCl or divalent salts. Ca^{++} and Mg^{++}, when added, were 0.0075 M. Incubated 10 minutes at 25°.

Mg^{++} (Fig. 1). When 0.052 M KCl was present, Mg^{++} and Ca^{++}, at 0.0075 M, depressed the effect of DNP on ATP hydrolysis.

During the course of this investigation Kielley and Kielley (12) reported the effect of preaging M_w in the absence of substrate on their "ATPase" activity. When this technique was applied, the effect of DNP was found to be considerably different from that in fresh M_w. As is shown in Figs. 2 and 3, DNP had relatively little effect on ATP breakdown in M_w which had been preaged for 20 minutes at 37°. In fresh M_w, additions of Mg^{++} or Ca^{++} were found to depress phosphate liberation in the presence of DNP (Fig. 2). After preaging the M_w, Mg^{++} accelerated phosphate liberation and, at optimal concentrations of 0.001 to 0.005 M, enhanced the effect of DNP. With preaged M_w, Ca^{++} did not alter appreciably the effect of DNP.

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Replacing 0.25 M sucrose in the medium by 0.15 M KCl enhanced the effect of DNP on phosphate liberation (Fig. 3). This effect was much less pronounced in preaged mitochondria. These effects of KCl on mitochondria are not specific, since NaCl gave similar results. Potter and Reck-

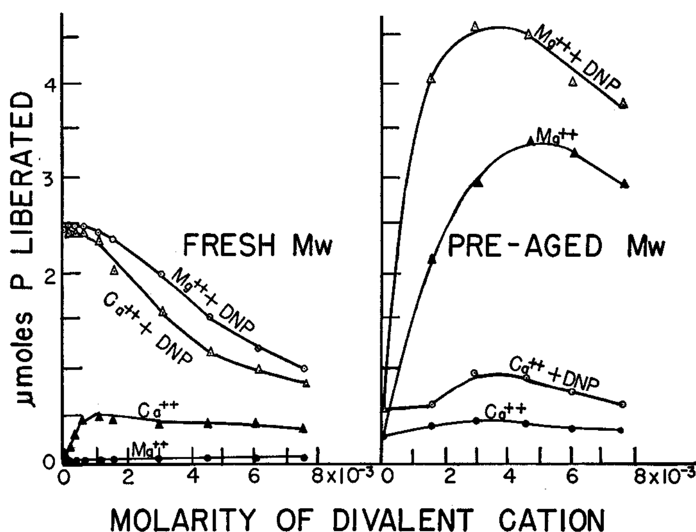

FIG. 2. Effect of increasing the molarity of divalent cations on phosphate liberation from ATP by fresh and preaged M_w. All tubes contained 6 μM of ATP and 0.3 ml. of M_w (0.2 mg. of N) in 0.25 M sucrose. Additional dilution to 1 ml. made with 0.15 M KCl or divalent cation. Incubated 10 minutes at 20°.

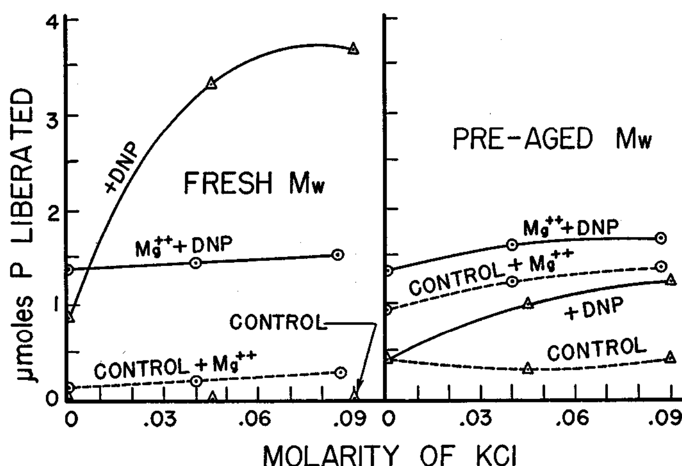

FIG. 3. Influence of increasing the molarity of KCl on hydrolysis of ATP by M_w. All tubes contained 6 μM of ATP and 0.3 ml. of M_w (0.224 mg. of N) in 0.25 M sucrose. Mg^{++} when added was at a level of 7.5×10^{-3} M. Additional volume to 1 ml. made up with 0.15 M KCl and 0.25 M sucrose. Incubated 10 minutes at 25°.

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nagel (9) found increasing tonicity (sucrose) to decrease the response of M_w to DNP. It now appears that both the amount and the nature of the solutes influence the phosphate-liberating activity.

Substrate Specificity—Phosphate liberation from ADP by mitochondria is enhanced by DNP. However, the rate with ADP as substrate is only one-eighth to one-fifth that achieved with ATP in either fresh or preaged mitochondria. Inosinetriphosphate was hydrolyzed at an extremely slow rate and this hydrolysis was not enhanced by DNP.

Influence of Metabolites—In the discussion it is postulated (see Hunter (8)) that phosphate liberation under the influence of DNP may involve enzymes which function normally in oxidative phosphorylation. Since these enzymes may interact with various metabolites, the influence of the latter on phosphate liberation was studied (Table II). Oxalacetate enhanced phosphate liberation from ATP by mitochondria, which is in agreement with earlier experiments with soluble extracts of muscle (20). However, it exerted a striking inhibition of the DNP effect in fresh mitochondria. L-Malate and succinate also depressed phosphate liberation in the presence of DNP with both fresh and preaged mitochondria. These effects are not the result of providing oxidizable substrates with subsequent uptake of inorganic phosphate. Glutamate, which is rapidly oxidized, having a P:O ratio of 3 (11), does not depress the DNP effect in either fresh or preaged mitochondria. Furthermore, addition of 10^{-4} M KCN, which is sufficient to inhibit cytochrome oxidase completely, did not alter the effects of these compounds. Fatty acids such as butyrate, crotonate, and caprylate depressed the liberation of phosphate in the presence of DNP. Caprylate itself greatly enhanced phosphate liberation, probably because of its surface activity (21, 22). Fluoride partially inhibited the effects of caprylate and of DNP in fresh mitochondria and completely eliminated these effects in preaged mitochondria.

Influence of —SH Agents—In view of the probable rôle of —SH groups in phosphate transfer, the influence of various agents known to react with thiol groups was determined. As is shown in Fig. 4, *p*-chloromercuribenzoate at 5×10^{-5} M almost completely inhibited phosphate liberation by mitochondria in the presence of DNP. When Mg^{++} was added, inhibition was less striking. The influence of this inhibitor on mitochondria is strikingly dependent on the salt concentration of the suspension medium. In the absence of both KCl and $MgSO_4$, 10^{-5} M *p*-chloromercuribenzoate did not depress phosphate liberation at all, while 10^{-4} M caused 20 per cent inhibition in fresh mitochondria and stimulated phosphate liberation in preaged mitochondria.

o-Iodosobenzoate, which inactivates thiol groups by oxidation, did not inhibit phosphate liberation from either fresh or preaged mitochondria,

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either in the presence or absence of DNP with or without 1.5×10^{-3} M Mg^{++}. In the absence of DNP, but with 1.5×10^{-3} M Mg^{++}, iodosoben-

TABLE II

Influence of Various Substrates on Phosphate Liberation by Mitochondria

Experiment	Additions	Phosphate liberated			
		Fresh M_w		Preaged M_w	
		Control	+ DNP 3×10^{-4} M	Control	+ DNP 3×10^{-4} M
		μM	μM	μM	μM
A	None	0.14	4.72	0.79	1.22
	Oxalacetate 0.01 M	0.68	1.63	0.45	0.92
	Butyrate 0.005 M	0.07	4.01	0.69	0.74
	Crotonate 0.005 M	0.15	2.41	0.47	0.91
	Cinnamate 0.005 M	0.47	3.09	1.42	0.85
	Caprylate 0.003 M	2.48	3.37	1.11	0.98
	Fluoride 0.0075 M	0.39	2.86	1.19	0.92
	Caprylate + fluoride	0.95	3.05	1.21	0.99
B	None	0.04	4.97	0.01	1.65
	Glutamate 0.03 M	0.00	4.75	0.00	1.83
	L-Malate 0.03 M	0.13	3.07	0.00	0.85
	Succinate 0.03 M	0.13	3.30	0.09	0.79
	Oxalacetate 0.03 M	0.32	3.40	0.35	1.21
C	None	0.00	4.68	0.59	1.67
	KCN 1×10^{-4} M	0.19	4.58	0.67	1.63
	L-Malate 0.03 M	0.00	3.53	0.21	0.79
	" + KCN	0.30	3.32	0.52	0.82
	L-Glutamate 0.03 M	0.00	4.56	0.53	1.60
	" + KCN	0.18	3.99	0.43	1.58
D	None	0.01	2.73	0.62	1.33
	KCN 1×10^{-4} M	0.20	2.03	0.43	0.84
	Oxalacetate 0.03 M	0.20	1.58	0.40	0.93
	" + KCN	0.28	1.58	0.38	0.90

All tubes contained 6 μM of ATP (disodium salt, pH 7.4) and 0.3 ml. of M_w in 0.25 M sucrose. 0.15 M KCl made up the additional volume to 1 ml. No Mg^{++} was added in any of the experiments. The quantities of M_w used were 0.18, 0.3, 0.35, and 0.23 mg. of N respectively. Incubated for 10 minutes at 25° (Experiments B, C, and D) or 30° (Experiment A).

zoate enhanced phosphate liberation slightly in fresh mitochondria and markedly in preaged mitochondria.

Iodoacetate (3×10^{-4} M) or diphenyliodonium chloride (3×10^{-4} M) depressed the DNP effect on phosphate liberation from 5 to 30 per cent in various experiments.

DNP and Phosphate Liberation from ATP by "Soluble" Enzymes—To

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facilitate the study of the mechanism by which DNP enhances phosphate liberation, an attempt was made to obtain particle-free systems which

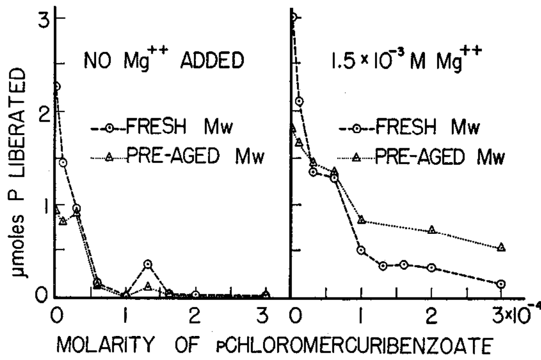

FIG. 4. *p*-Chloromercuribenzoate inhibition of the liberation of phosphate from ATP by M_w in the presence of DNP. 3×10^{-4} M DNP, 6 μM of ATP, and 0.3 ml. of M_w (0.2 mg. of N) in 0.25 M sucrose present in all tubes. Additional volume to 1 ml. made up with 0.15 M KCl (highest concentration, 0.09 M). Incubated 10 minutes at 25°.

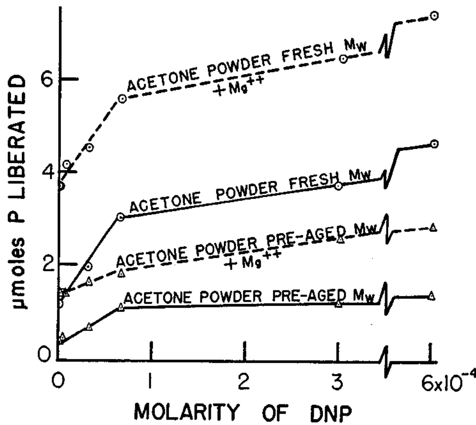

FIG. 5. Effects of increasing the molarity of DNP on ATP hydrolysis by extracts of acetone powder of M_w. All tubes contained 6 μM of ATP, 0.3 ml. (0.906 mg. of N) of extract of acetone powder of M_w, and 0.15 M KCl to a volume of 1 ml. Incubated 10 minutes at 20°.

would respond to DNP. Aqueous extracts[4] of acetone-desiccated mitochondria possess an Mg^{++}-requiring enzyme which hydrolyzes ATP. DNP greatly increased the rate of ATP hydrolysis by this preparation (Fig. 5). The effect of DNP increased linearly with increasing concentrations of

[4] The supernatant fraction after centrifuging at 18,000 × *g* for 30 minutes was used in all experiments.

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DNP up to 6×10^{-5} M; the rate of phosphate liberation continued to increase more slowly with increasing concentrations of DNP greater than 6×10^{-5} M.

Preaged mitochondria yielded acetone powder extracts with much less of the phosphate-liberating enzyme (Fig. 5). The response of these extracts to DNP was of less magnitude than that obtained with extracts of fresh mitochondria but was proportional to the phosphate-liberating ability in the absence of DNP.

The hydrolysis of ATP by the extract of acetone powder of fresh mitochondria could be stimulated considerably by the addition of Mg^{++} (Figs. 5 and 6). This stimulation by Mg^{++} occurred both in the presence and absence of dinitrophenol. As Fig. 6 shows, calcium at a very low level

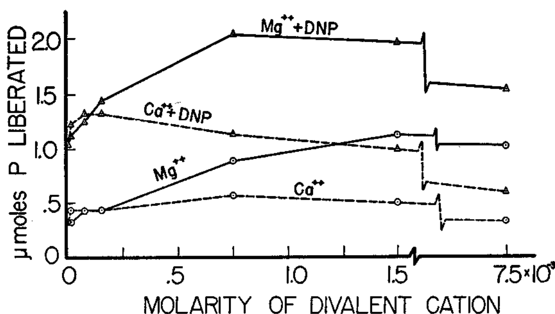

FIG. 6. Effect of increasing the molarity of divalent cations on ATP hydrolysis by extracts of acetone powder of M_w. All tubes contained 6 μM of ATP, 3×10^{-4} M DNP, 0.3 ml. of extract (0.686 mg. of N) from acetone powder of M_w, and 0.15 M KCl as a diluent to a final volume of 1 ml. Incubated 10 minutes at 20°.

(0.15×10^{-3} M) gave a slight increase in the phosphate liberated in the absence of dinitrophenol, but calcium did not maintain the stimulation by dinitrophenol as well as did magnesium.

Dialysis of the extract against 0.001 M cysteine, pH 7.3, at 0° overnight resulted in a partial loss of ability to respond to dinitrophenol in the absence of potassium and magnesium. The addition of magnesium, and also potassium, partially restored this ability. The addition of a boiled extract of acetone powder or of 8 units of coenzyme A (The Upjohn Company) did not enhance the response. If the undialyzed extract were prepared in 0.001 M cysteine, pH 7.3, its activity was the same as that of a sucrose extract; therefore the cysteine itself was not responsible for the loss of activity.

When the enzyme extract was dialyzed against 0.02 M tris(hydroxymethyl)aminomethane, pH 7.4, at 0° for 2 hours in a system made anaerobic with nitrogen, there was also a loss of activity as measured by

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phosphate liberated in the presence of dinitrophenol. Magnesium with potassium restored the phosphate-liberating activity; the combination was slightly more effective than was Mg^{++} alone. The addition of tris(hy-

TABLE III

ATP Hydrolysis by "Soluble" Enzyme Systems

Experiment	Enzyme preparation*	Enzyme protein	Phosphate liberated				
			Without added Mg^{++}		With 0.0015 M Mg^{++}		
			Control	3×10^{-4} M DNP	Control	3×10^{-4} M DNP	$\frac{+DNP}{-DNP}$
		mg.	*μM*	*μM*	*μM*	*μM*	
A (0.037 M KCl)	Undialyzed extract	4.74	0.17	0.49	0.76	1.37	1.8
	Dialyzed extract	4.35	0.17	0.34	0.78	1.50	1.9
	Pellet from 144,000 × g, 1 hr.	2.40	0.09	0.35	1.47	2.77	1.9
	Supernatant from pellet	4.14	0.10	0.21	0.34	0.50	1.4
B	Undialyzed extract in						
	0.092 M KCl	5.34	0.36	1.28	1.33	2.56	1.9
	0.092 " NaCl		0.21	0.64	1.15	2.12	1.8
	0.15 M sucrose		0.90	1.17	1.86	2.08	1.1
	Dialyzed extract in						
	0.092 M KCl	4.71	0.03	0.16	0.73	1.29	1.8
	0.092 " NaCl		0.06	0.06	0.63	0.98	1.6
	0.15 M sucrose		0.09	0.20	0.80	1.00	1.2
C	Dialyzed extract in						
	0.077 M KCl	4.74	0.17	0.46	0.86	1.77	2.1
	0.077 " NaCl		0.17	0.33	0.75	1.67	2.2
	0.15 M sucrose		0.25	0.47	1.04	1.64	1.6
D	Pellet from 144,000 × g, 1 hr., in						
	0.077 M KCl	0.60	0.04	0.12	0.59	1.33	2.2
	0.077 " NaCl		0.03	0.09	0.48	1.05	2.2
	0.15 M sucrose		0.10	0.24	0.84	1.24	1.5

* All extracts of mitochondrial acetone powders were centrifuged at 18,000 × g for 30 minutes to remove insoluble material. Incubated 10 minutes at 20°.

droxymethyl)aminomethane buffer to the assay system with undialyzed extract did not alter its activity.

Much of the enzyme which responds to dinitrophenol could be sedimented from the dialyzed extract by centrifugation at 144,000 × g for 1 hour. The supernatant solution contained relatively little "ATPase," and the stimulation by DNP was not as great as in the pellet or unfractionated extract (Experiment A, Table III).

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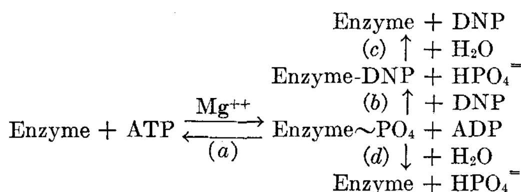
The phosphate-liberating activity of the soluble enzyme was usually slightly greater in the presence of KCl than of NaCl. Phosphate liberation was approximately doubled by the addition of DNP. When isotonic sucrose was used as the diluent, the ATPase activity was greater than in the presence of salt, but DNP was less effective in enhancing the activity. Desoxycholic acid, which increases ATP hydrolysis with fresh mitochondria (22), had no effect on phosphate liberation by the extract. Investigations of the soluble fraction are continuing.

DISCUSSION

Conversion of the mitochondrial ATP-hydrolyzing enzymes from a *latent* (9) to an *active* state may involve liberation of bound (inactive) enzymes or it may result from partial denaturation of phosphate-transferring enzymes, with the result that they may be enabled to react with water as an acceptor (*cf.* (23) p. 484). Incubation of mitochondria at 37° in the absence of substrate (12, 13), washing mitochondria with water (8), or incubation in hypo- or hypertonic mediums (9) activate the ATP-hydrolyzing system. Each of these treatments results also in making DNP less effective in inducing hydrolysis of ATP. Although the detrimental effect of DNP on maintenance of mitochondrial structure would probably have an effect similar to the above treatments, DNP is assumed to have a specific effect not dependent upon liberation of a bound enzyme for the following reasons: (*a*) the effect of DNP is immediate and continues linearly with time; (*b*) DNP enhances ATP hydrolysis by a soluble enzyme system.

During the course of this work we have been guided by the hypothesis that DNP displaces inorganic phosphate[5] from phosphorylated enzymes or coenzymes, as shown in the accompanying scheme. In keeping with the experimental findings, reactions (*a*), (*b*), and (*c*) are assumed to proceed



rapidly in intact mitochondria, while reaction (*d*) does not. Preincubation without substrate or suspension of the mitochondria in water so alters the enzymes that reaction (*d*) is readily measurable. In such mitochondria and in the soluble enzyme preparation liberation of phosphate occurs by

[5] The possibility that DNP accepts $\sim PO_4$ to form dinitrophenyl phosphate and that this compound spontaneously liberates phosphate is eliminated by the finding (Dr. Paul D. Boyer, personal communication) that dinitrophenyl phosphate is a relatively stable compound.

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both reactions (*d*) and (*b*) + (*c*). In the soluble preparation, KCl and NaCl appear to increase the proportion of the reaction proceeding by (*b*) + (*c*) (Table III).

The phosphorylated enzyme or coenzyme would correspond to the Y~ phosphate of Hunter (8) and to the high energy phosphate compound, other than ATP, whose breakdown is the rate-limiting factor in mitochondrial respiration (11). It is postulated that usually three such phosphorylated compounds are formed at specific sites during the transport of electrons from the substrate to cytochrome oxidase (*cf.* (24)). Whether DNP

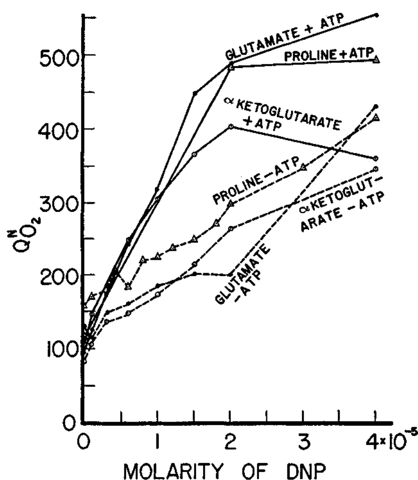

FIG. 7. Effect of DNP on mitochondrial respiration in the presence and absence of ATP. The flasks contained 0.01 M substrate, 0.0075 M Mg^{++}, and 1×10^{-5} M cytochrome *c*. ATP, when added, was 0.0018 M. Final volume 2.0 ml. Duration of experiment 60 minutes; temperature 30°.

causes phosphate liberation from each of three different Y~phosphates or from a single Y~PO_4 which is common to each of the three phosphorylations cannot yet be determined.

We have previously presented arguments (25) and data (11) supporting the concept that rates of oxidation in mitochondria are limited by the rates at which phosphate is transferred from the enzyme~PO_4 (Y~PO_4) to acceptors. Agents which cause (directly or indirectly) liberation of inorganic phosphate from the Y~phosphates also accelerate mitochondrial respiration. As is shown in Fig. 7, the presence of added ATP enhances the ability of low concentrations of DNP to stimulate mitochondrial respiration. When higher levels of DNP were employed (4×10^{-5} M or greater), oxygen consumption, without added ATP, was as rapid as when ATP was added. One possible interpretation of these data is that the

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different Y~phosphates in the electron transport chain differ in their susceptibility to attack by DNP. At low concentrations of DNP, and in the absence of added ATP, respiration would be limited by the rate of dephosphorylation of the least sensitive Y~PO_4. In the presence of sufficient ATP to permit transphosphorylation between different Y~phosphates, stimulation of respiration would be expected to be greater and directly proportional to DNP concentration as observed. Other interpretations which have been thought of do not fit the available data (see Hunter (8) and above) as well as this tentative hypothesis does.

A discussion of the nature of the soluble magnesium-stimulated "ATPase" which can be obtained from mitochondria must await further work, which is now in progress.

SUMMARY

2,4-Dinitrophenol greatly enhanced the liberation of inorganic phosphate from ATP by the nuclear and mitochondrial fraction of rat liver. The microsomal and supernatant fractions did not exhibit this effect.

With mitochondria (M_w) the rate of phosphate liberation was proportional to the DNP concentration up to 6×10^{-5} M. In the presence of excess DNP the rate was proportional to the quantity of M_w and to time.

With both fresh and preaged M_w the response to DNP was much greater in mediums containing salt (either NaCl or KCl) than in isotonic sucrose. Magnesium salts in appreciable concentrations depressed the response of fresh M_w to DNP, but enhanced the response in preaged M_w. Calcium salts, which activate ATP hydrolysis by fresh M_w in the absence of DNP, also depressed the effect of DNP on phosphate liberation. Magnesium salts enhanced phosphate liberation by preaged M_w both in the presence and absence of DNP. Calcium was virtually without effect in preaged M_w.

Oxalacetate enhanced phosphate liberation from ATP by fresh M_w. This dicarboxylic acid as well as succinate and L-malate depressed the effect of DNP on phosphate liberation. Fatty acids also depressed the effect of DNP. Caprylate enhanced phosphate liberation, probably because of its surface activity.

The thiol inhibitor, *p*-chloromercuribenzoate, strongly depressed the effect of DNP; iodoacetate and *o*-iodosobenzoate did not.

Water extracts of acetone-desiccated M_w contain an Mg^{++}-activated enzyme which hydrolyzes ATP. The activity was enhanced about 100 per cent by DNP. Extracts from preaged M_w contain less of this enzyme than do extracts from fresh M_w.

DNP is more effective in enhancing respiration of M_w in the presence of added ATP than in its absence. A possible explanation for this effect is discussed.

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THE CATALYTIC EFFECT OF 2,4-DINITROPHENOL ON ADENOSINETRIPHOSPHATE HYDROLYSIS BY CELL PARTICLES AND SOLUBLE ENZYMES

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