findings to the Pappenheimer proposals relating diphtherial toxin to cytochrome b is discussed.

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Received May 6, 1952. P.S.E.B.M., 1952, v80.

Effect of Certain Plant Growth Substances on Oxidative Phosphorylation In Rat Liver Mitochondria.* (19681)

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Recent work has resulted in technics whereby mitochondria from a number of mammalian tissues can be isolated by differential centrifugation and their metabolism studied using conventional procedures(1). It appears that the mitochondria are the chief intracellular sites of oxidative phosphorylation(2). A number of compounds, among them dinitrophenol(3,4) and the barbiturates (5), have been shown to uncouple phosphorylation from oxidative processes with a concomitant increase in respiration. Bonner(6) reported that 2,4-dinitrophenol in concentrations which inhibited section growth was a powerful promoter of *Avena* respiration. The effects of plant growth substances on certain plants is reflected in increased total respiration, with or without accelerated growth and development(7). It was suggested(8) that perhaps 2,4-dichlorophenoxyacetic acid (2,4-D) and other plant growth substances might be uncoupling agents. The above considerations led us to investigate the effects of various plant growth substances on a mammalian tissue system which would test this possibility.

Experimental. General procedures and methods were similar to those previously described(5,9). Rat liver mitochondria were isolated according to the methods of Schneider and Hogeboom(10). In the experiments designed to demonstrate a stimulatory effect on respiration the phosphate-deficient system of Judah(3) was used. The mitochondria used in these experiments were washed 3 times with 0.25 M sucrose in order to remove most of the inorganic phosphate. Other experimental details are given in the figure legends.

Results. In the experiments designed to show the effect of 2,4-D on oxidative phosphorylation (Fig. 1) it was found that this compound was a potent uncoupling agent. At concentrations of 2,4-D as high as 1×10^{-3} M there was little effect on respiration in the test system and yet the P:O ratio fell to 20%

* Supported in part by a grant from the Rockefeller Foundation.

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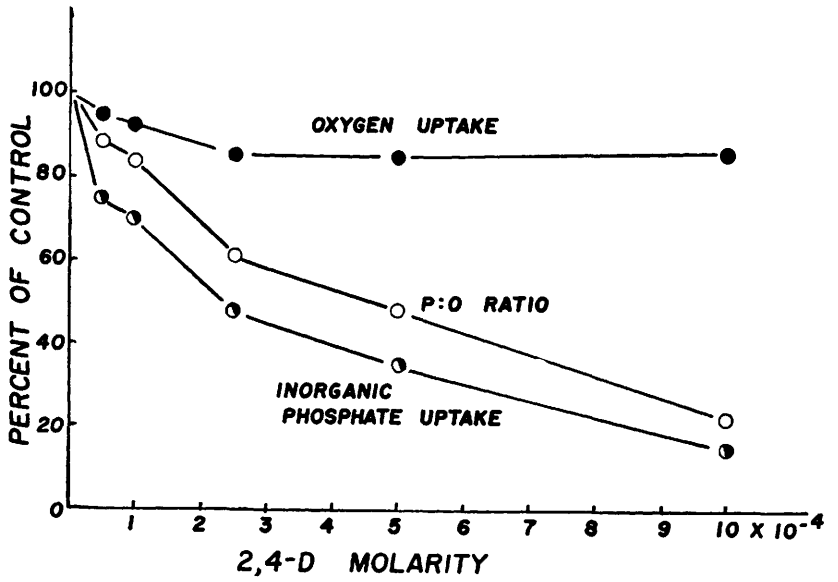

FIG. 1. Effect of 2,4-D on oxidative phosphorylation in rat liver mitochondria. Vessel contents: 1.5 x 10^{-2} M Na-glycylglycine, pH 7.4, 2.0 x 10^{-2} M K-phosphate, pH 7.4, 2.8 x 10^{-2} M glucose, 5.0 x 10^{-2} M KCl, 8.0 x 10^{-3} M $MgCl_2$, 2.5 x 10^{-3} M K-adenosinetriphosphate, 8.3 x 10^{-6} M cytochrome c, 1.3 x 10^{-2} M Na-pyruvate, 4.3 x 10^{-3} M Na-malate, 0.1 ml yeast hexokinase(9), 2,4-D concentration as indicated, 0.4 ml rat liver mitochondria suspension(9), 1.2 x 10^{-2} M NaF, total volume 2.0 ml, 0.2 ml 2 N KOH in center well with fluted filter paper, 0.2 ml 50% trichloroacetic acid in sidearm. Solutions added in the order listed. Gas phase-air, temperature 20°C. Duration of the experiment 30-40 minutes. Each point is the average of 3 separate experiments, percent activity for any individual vessel being calculated from its respective control. P:O ratios in the controls ranged from 2.34 to 2.44.

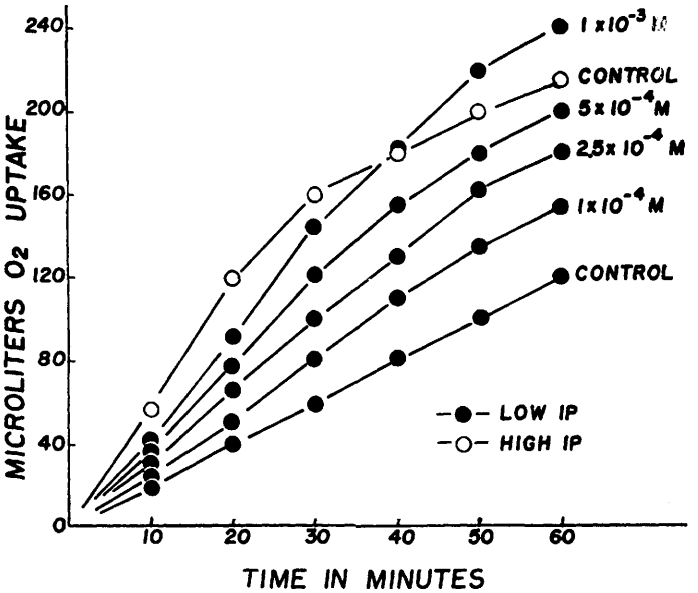

FIG. 2. Effect of 2,4-D on the respiration of rat liver mitochondria in a phosphate deficient system. Vessel contents the same as in Fig. 1, except that the low phosphate vessels contained 5 x 10^{-4} M K-phosphate, pH 7.4. The difference in ionic strength between low and high phosphate vessels was made up by the addition of KCl to the low phosphate vessels. Temperature 30°C.

TABLE I. Effect of some Plant Growth Substances on Respiration of Rat Liver Mitochondria in a Phosphate-Deficient System.*

Compound	Molar concentration	Oxygen uptake†
High phosphate control		200
Low phosphate control		110
		114
2,4-dichlorophenoxyacetic acid	1×10^{-3}	226
p-chlorophenoxyacetic acid	5×10^{-4}	116
	1×10^{-3}	142
Indole-3-acetic acid	5×10^{-4}	97
	1×10^{-3}	102
Indole-3-butyric acid	5×10^{-4}	103
	1×10^{-3}	100
α-naphthaleneacetic acid	5×10^{-4}	105
	1×10^{-3}	112

* Conditions as in Fig. 1 and 2.
† Microliters per 40 min.

of the control level. Uncoupling effects can be seen at concentrations of 2,4-D as low as 5×10^{-5} M.

Since we were able to show that 2,4-D could dissociate oxidative and phosphorylative mechanisms, this compound and other plant growth substances were studied in the phosphate deficient system. The results with 2,4-D are shown in Fig. 2. Increasing the concentrations of the plant growth substance resulted in increasing respiratory rates. Analysis at the end of the run showed that the vessels exhibiting the greatest stimulation also contained the highest amounts of inorganic phosphate. In a typical experiment (Table I), indole-3-acetate, indole-3-butyrate and α-naphthaleneacetate were ineffective in producing respiratory stimulation in concentrations as high as 1×10^{-3} M. While 2,4-D in concentrations of 1×10^{-3} M doubled the rate of the low phosphate system (equalling or exceeding the high phosphate control rate), p-chlorophenoxyacetate produced about a 30% increase in rate above the low phosphate control level (Table I). Thus it was possible by limiting the phosphate concentration to demonstrate a stimulatory effect with 2,4-D and to a lesser extent with p-chlorophenoxyacetate.

Discussion. There is little doubt that the stimulation of the respiration of liver mitochondria observed under the influence of 2,4-D is a result of its uncoupling properties maintaining in the phosphate deficient system an effective level of inorganic phosphate. Whether or not this is the mechanism by which 2,4-D produces stimulation of plant respiration is a question which cannot be answered at this time. A suggestion has been made to the effect that inorganic phosphate might form an ester phosphate link with phenoxyacetic acids which on cyclization of the compound would result in the generation of a high energy bond(11). Plant tissues apparently contain systems for energy transfer analogous to the adenylate systems of animal tissues(6,12) and it is possible that the phenoxyacetic acids, at least, interact with these systems. In a discussion of this sort it must be remembered that there are at least 2 ways in which respiration of intact tissues can be stimulated: 1. An increased demand on the energy supplying sources by the requirements of increased growth or function, or 2. An uncoupling action disrupting the control over respiration which is ordinarily mediated by the level of inorganic phosphate and acceptors therefor. Conceivably plant growth substances might act upon either or both of these postulated mechanisms depending upon the concentration of the substance present at any given time either *in vitro* or *in vivo*. Whatever the relation of the uncoupling effect to plant metabolism, 2,4-D may perhaps prove to be a useful tool for the investigation of the coupling mechanisms of tissues in general.

Summary. 2,4-dichlorophenoxyacetic acid was shown to be an uncoupling agent for oxidative phosphorylation and to stimulate the respiration of rat liver mitochondria in a phosphate-deficient medium. p-chlorophenoxyacetic acid was much less potent in producing the above effects. Indole-3-acetic acid, indole butyric acid, and α-naphthaleneacetic acid were inactive. The results are discussed in relation to the metabolism of plant and animal tissues.

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Received June 23, 1952. P.S.E.B.M., 1952, v80.

Placental Transfer of Radioactive Digitoxin in Rats and Guinea Pigs.* (19682)

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The placental transfer of digitalis has received little or no attention in the past. Since the availability of radioactive digitoxin, studies on the penetration of digitoxin across the placental membrane in laboratory animals have become possible.

Method. Rats and guinea pigs were selected as test animals since their placenta is in some respects morphologically similar to that of man(1). Uniformly labeled C^{14}-digitoxin prepared by biosynthesis(2) and having a specific activity of 620,000 counts per minute per milligram (0.43 μc per mg) was administered intravenously to each animal. The dose used for each Sprague-Dawley rat was 0.175 mg (approximately 0.5 μg/g) and for each guinea pig 0.25 mg (approximately 0.25 μg/g).

The animals were sacrificed one hour after intravenous administration of the drug and the fetuses and maternal hearts were removed. In addition the guinea pig fetal hearts were isolated. The tissues were thoroughly washed in isotonic saline to remove blood, then dried between filter paper, weighed, and homogenized in Potter and Elvehjem homogenizers with 50% ethanol. Guinea pig fetuses (minus the hearts) were prehomogenized in a Waring blender and an aliquot transferred to Potter and Elvehjem homogenizers. The fetal hearts of the rats were not isolated because of their small size.

The extraction procedure employed for the isolation of unchanged digitoxin and fractionation of its metabolic products consisted of diluting the homogenates with 50% ethanol, adding a few milligrams of non-radioactive digitoxin as carrier, and making a liquid-liquid extraction with chloroform(2). The chloroform fraction was evaporated to dryness, taken up in 50% methanol, extracted with carbon tetrachloride to remove fats and lipids, and re-extracted with chloroform. The latter chloroform fraction was evaporated to dryness, again taken up in chloroform (C.P.), and then allowed to pass through an alumina column. Successive elutions were made with the following solvents: 1% ethanol in chloroform, 10% ethanol in chloroform, and 40% aqueous ethanol. Unchanged digitoxin was found to occur in the 10% ethanol in chloroform eluate. Identification of the drug was accomplished using paper partition chromatography(2), polarographic analysis(3), and color reaction tests(2). All radioactive samples were analyzed using an internal gas-flow Geiger counter(4). The maximum counting error was 10% with the error for most counting samples being less than 5%. The various samples showing radioactivity other than unchanged digitoxin were considered to contain metabolic products of the parent drug. The

* This work was done under a contract between the Atomic Energy Commission and the University of Chicago. It was aided in part by a grant from the Life Insurance Medical Research Grant.

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