THE INDUCTION OF AN ATP-ENERGIZED MITOCHONDRIAL VOLUME CHANGE BY THE COMBINATION OF THE -S-S- COMPOUND, ELLMAN'S REAGENT WITH EITHER A RESPIRATORY INHIBITOR OR AN UNCOUPLING AGENT*,**

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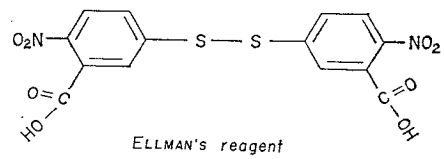
When the -S-S- compound ELLMAN's reagent was combined either with the respiratory inhibitor antimycin or the uncoupling agent 2,4-dinitrophenol an ATP-energized mitochondrial volume change was induced. Thus ELLMAN's reagent behaved as an electrophilic thiol reagent analogous to showdomycin and N-ethylmaleimide. These results encouraged the search for possible reactions between the pivotal mitochondrial thiol group and biologically important substances which contain the -S-S- grouping even though older reports in the literature of the induction of mitochondrial swelling by biologically important -S-S- compounds were recently discounted.

When either a respiratory inhibitor[1] or an uncoupling agent[2] was combined with a thiol reagent and added to rat liver mitochondria, an ATP-energized mitochondrial volume change was induced. We have attributed this reaction to the conjugation of a strategically located pivotal mitochondrial thiol group with the thiol reagent[3]. It was reasoned that the thiol group was exposed by either the respiratory inhibitor or the uncoupling agent. The pivotal mitochondrial thiol group has been located between two cycles[3]. One of the cycles meshes with the respiratory chain and the other cycle meshes with a cycle involving ATP, ADP, and Pi.



ELLMAN'S reagent

Agents which exposed the mitochondrial

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** Abbreviations used :

ELLMAN : ELLMAN'S reagent *i.e.*, 5,5'-dithio-bis-(2-nitrobenzoic acid) also known as 3,3'-dithio-bis-(6-nitrobenzoic acid). C. A. registry number 69-78-3.
ATP : Adenosine-5'-triphosphate.
ADP : Adenosine-5'-diphosphate.
Pi : Inorganic phosphate.
DNP : 2,4-Dinitrophenol.
pFCCP : Carbonyl cyanide *p*-trifluormethoxyphenylhydrazone.
NEMI : N-Ethylmaleimide.
N-OH-AAF : N-Hydroxy-N-acetyl-2-aminofluorene.
DI-OH-DBAQ : 4',8'-Dihydroxy-1,2,5,6-dibenz-9,10-anthraquinone.
N-OH-AABIP : N-Hydroxy-N-acetyl-4-aminobiphenyl.

thiol group included antimycin[1], DNP[2], rotenone[2], lapachol[4], dicoumarol[5], pFCCP[5], N-OH-AAF[6], DI-OH-DBAQ[7], and N-OH-AABIP[8]. Many of these agents are of biological interest as they include an antibiotic[1], an antitumor agent[4] and metabolites of carcinogens[6,7,8]. Only a limited number of thiol reagents have been examined in our system thus far. These have included showdomycin and NEMI. Showdomycin, which is an antibiotic and antitumor agent is of biological interest[9].

The disulfide [-S-S-] moiety possibly could be an additional functional group which could behave like a thiol reagent in our ATP-energized mitochondrial volume change system. This would be significant as there are many compounds with biological activity which possess the -S-S- function. In order to examine this possibility, we have studied in this investigation the action of ELLMAN's reagent, as a model compound which contains the -S-S- grouping, in our ATP-energized mitochondrial volume change system.

There have been several reports—using experimental procedures quite different than ours—which investigated the interaction of ELLMAN's reagent with mitochondria. These reports concluded that ELLMAN's reagent interacted with the thiol group between the DNP and oligomycin-sensitive[10,11,12] sites. Thus the strategically located pivotal mitochondrial thiol group postulated in our scheme could be a target for ELLMAN's reagent. It is worthy to mention that the previously reported induction of mitochondrial swelling by biologically important -S-S- compounds such as insulin, oxidized glutathione, oxytocin and 8-lysine vasopressin have recently been discounted and were ascribed to metallic ion contaminants by CASH *et al.*[13]

Methods

The general procedures, methods, and purification of the water have been previously described, for the mitochondrial volume change experiments.[7] The pH of the trischloride buffer is indicated on the figures. Incubation was at 27°C in standard rectangular glass cuvettes with a 1-cm light path. The basic reaction mixture for the volume change experiments had a final volume of 3 ml and contained 0.75 mg mitochondrial protein (prepared from rat liver); 75 mM sucrose; and 75 mM trischloride buffer. A decrease in absorbancy at 520 nm was taken as a measure of mitochondrial swelling. A Model 2,000 automatic spectrophotometer manufactured by Gilford Instrument Laboratories Incorporated, Oberlin, Ohio was used. All cations were added in the form of chloride salt and anions including ELLMAN's reagent were added in the form of tris salts neutralized to pH 7.4. Solutions of oligomycin were prepared in dimethyl sulfoxide (distilled *in vacuo*) and added to the incubation mixture in a volume of 0.03 ml by means of the adding-mixing device. Antimycin was dissolved in 95 % ethanol (0.02 ml). All controls contained the appropriate amount of dimethyl sulfoxide and 95 % ethanol. The figures and legends provide additional experimental details.

Results

It is seen (Fig. 1) that while neither antimycin nor DNP nor ELLMAN's reagent by themselves induced an ATP-energized mitochondrial volume change an appropriate combination of antimycin plus ELLMAN's reagent or DNP plus ELLMAN's reagent induced marked responses. The effects were enhanced when the pH was raised and the effects were inhibited by oligomycin. Attention is directed at the observation

that at the higher pH and at the higher concentration of ELLMAN's reagent there was an incipient lack of inhibition by oligomycin.

In these experiments the range of effective concentration of ELLMAN's reagent

Fig. 1. The combination of ELLMAN's reagent with antimycin or DNP.
Basic medium see methods.

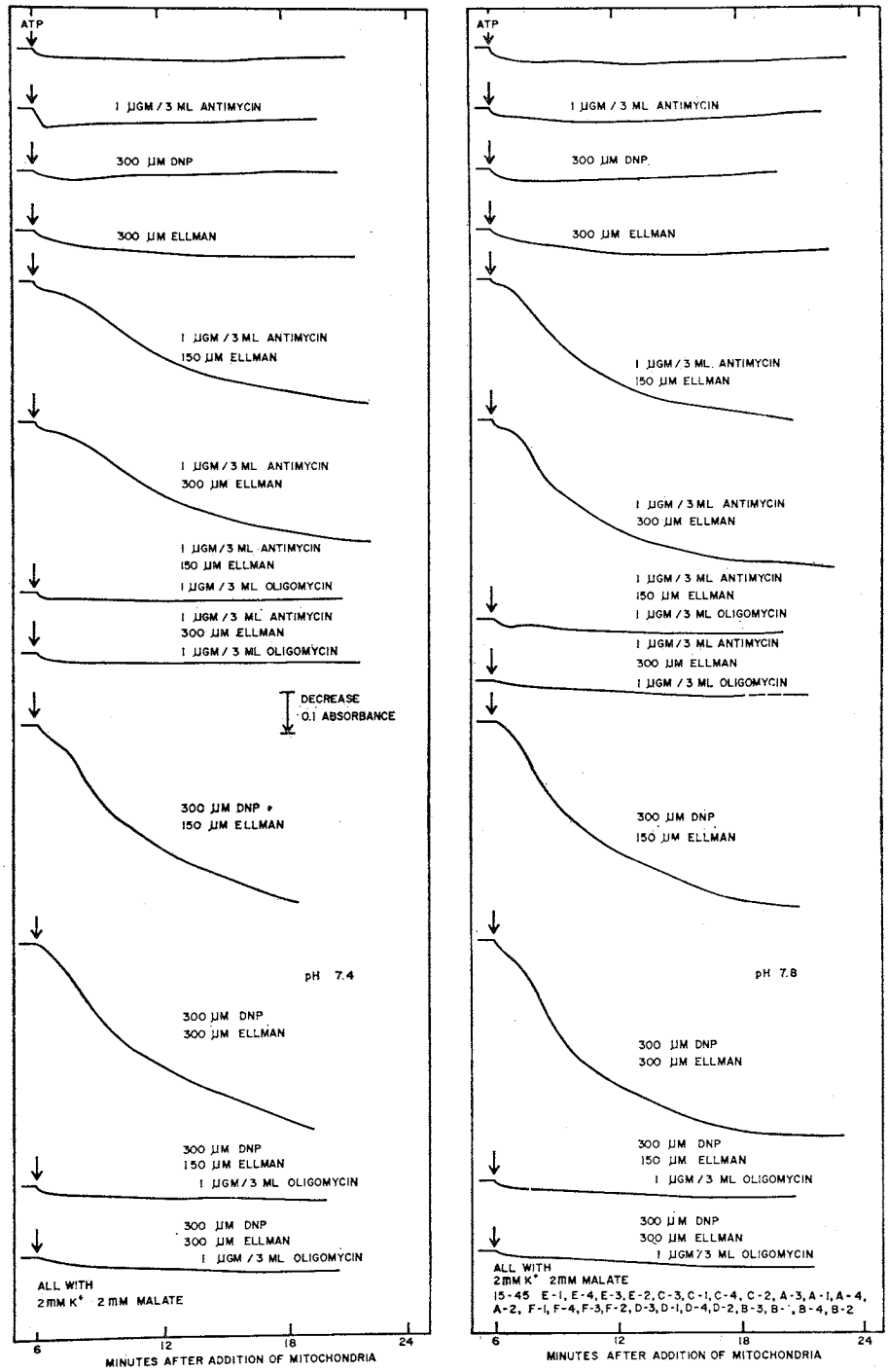


Fig. 2. Various concentrations of ELLMAN's reagent combined with antimycin.
Basic medium see methods

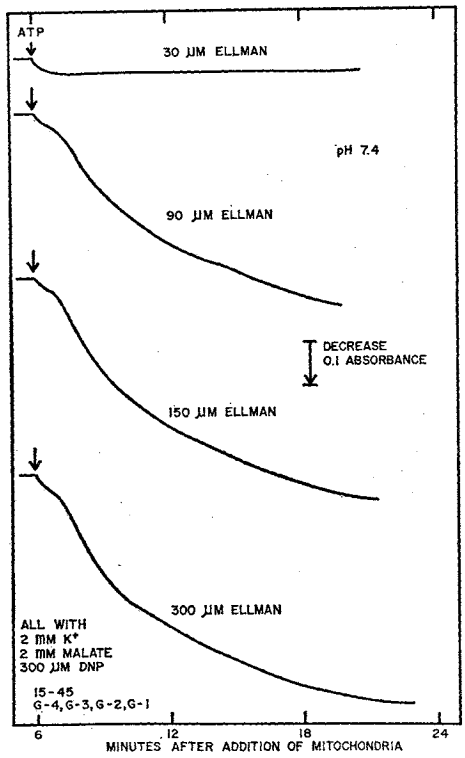
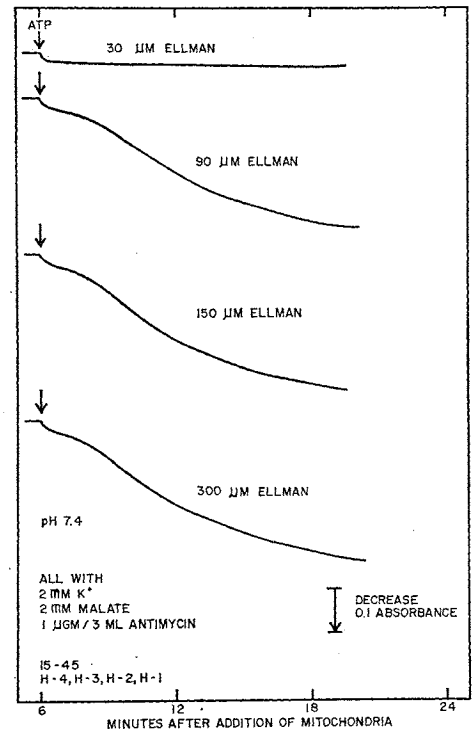


Fig. 3. Various concentrations of ELLMAN's reagent combined with DNP.
Basic medium see methods.



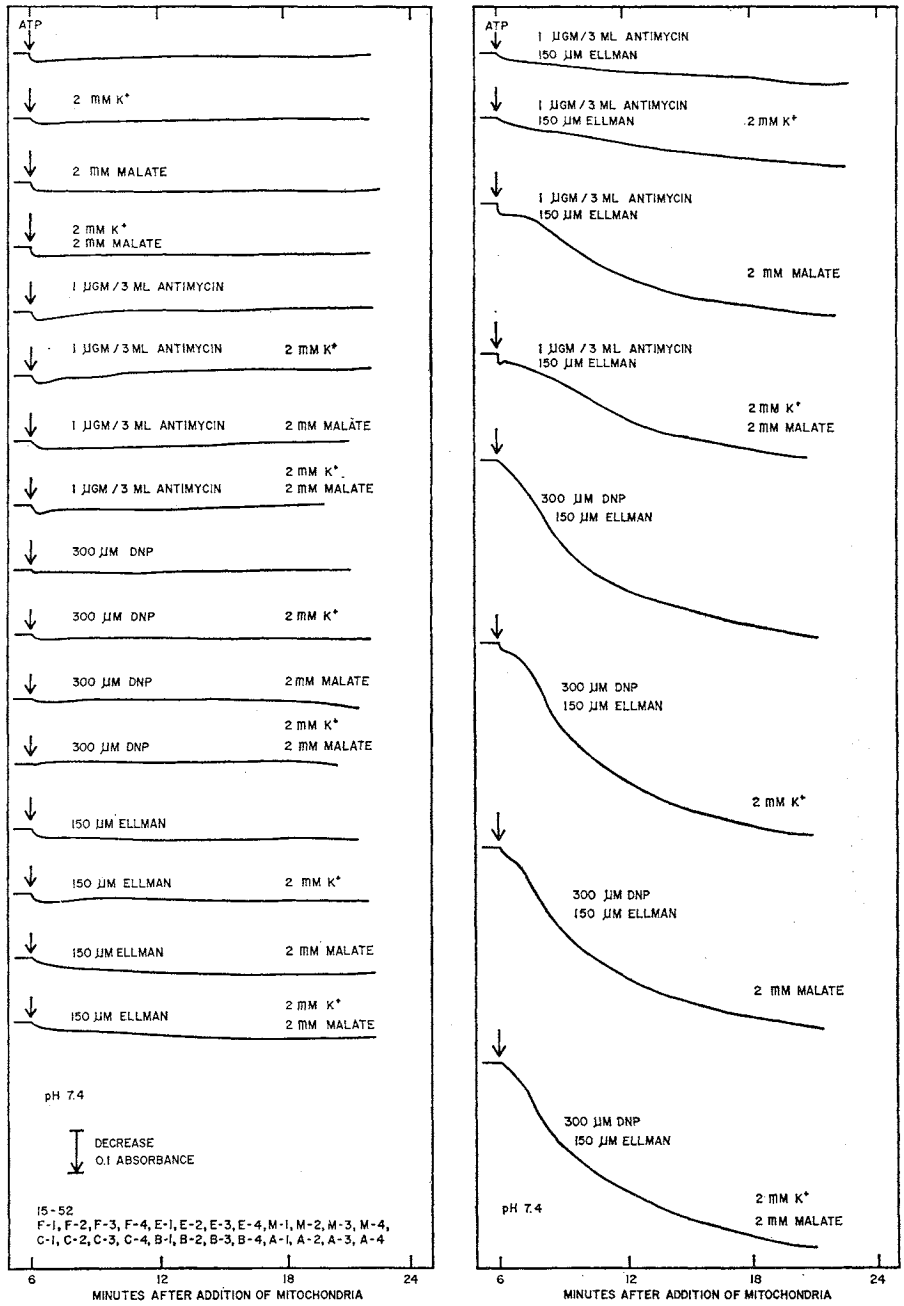
was between 30 μM to 300 μM with either antimycin or DNP (Figs. 2, 3). The addition of potassium ion had negligible effects on either the combination of antimycin plus ELLMAN's reagent or the combination of DNP plus ELLMAN's reagent (Figs. 4, 5). On the other hand, the addition of malate markedly increased the mitochondrial volume change induced by ELLMAN's reagent plus antimycin and the addition of malate extended the time course of the phenomena and dampened the oscillation (Figs. 4, 5) induced by the combination of DNP plus ELLMAN's reagent.

Discussion

ELLMAN's reagent when combined with a respiratory inhibitor such as antimycin or an uncoupling agent such as DNP induced an ATP-energized mitochondrial volume change phenomena. The phenomena required adequate levels of ELLMAN's reagent and were enhanced when the pH was raised. Potassium ion did not affect the phenomena while malate ion markedly altered the induced effect. These results are analogous to those observed previously with showdomycin or NEMI[1,2,5]. Thus ELLMAN's reagent behaved like a thiol reagent in our ATP-energized mitochondrial volume change system and we attribute this to the conjugation of ELLMAN's reagent with a nucleophilic mitochondrial thiol group exposed by either a respiratory inhibitor or an uncoupling agent.

These results and explanations are completely compatible with the data and conclusions of HAUGAARD *et al.*[10], and by MIYAHARA[11]. In addition, as we have ascertained a significant reaction of ELLMAN's reagent with the pivotal mitochondrial thiol group located in our

Fig. 4. The role of ions with the combination of ELLMAN's reagent and antimycin or DNP.
Basic medium see methods.



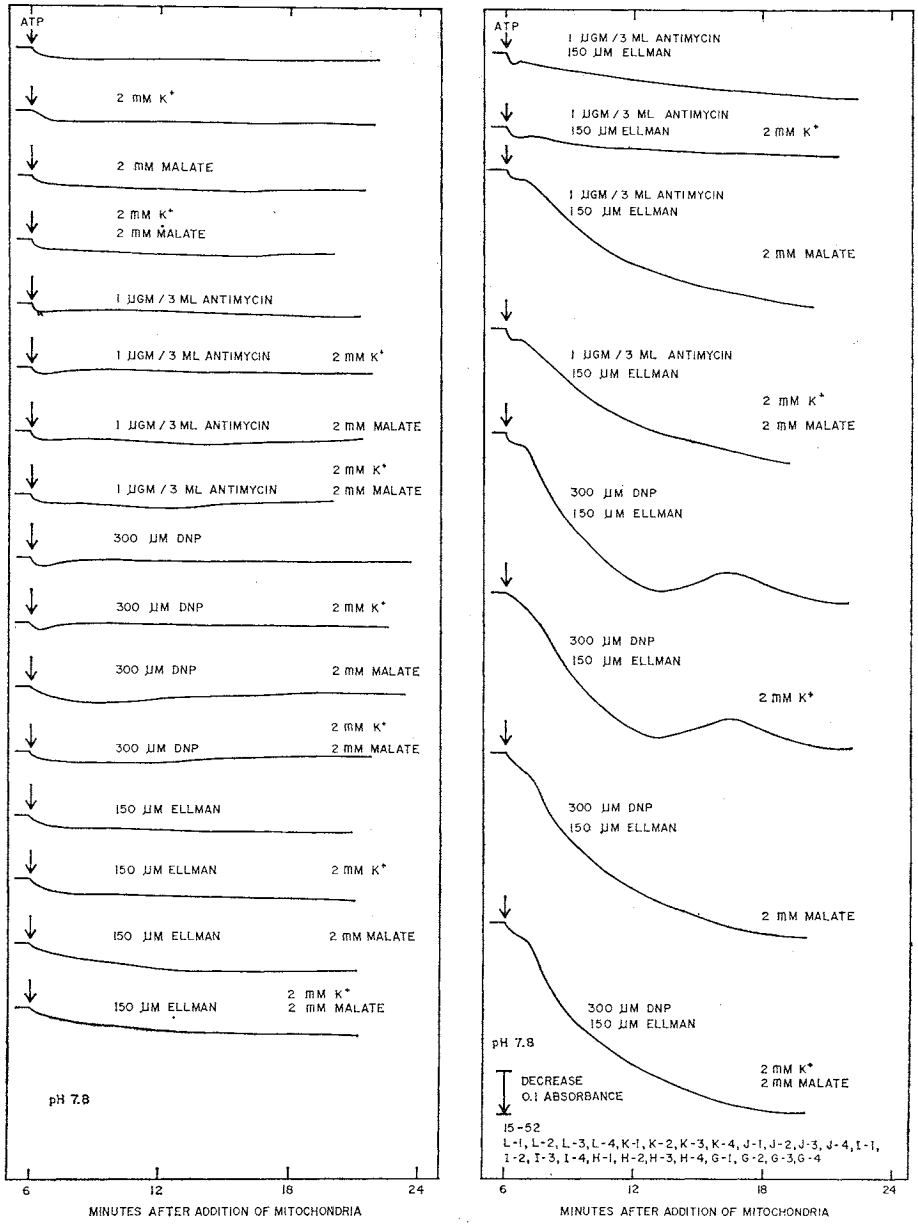
scheme between two cycles[3], the observation of MIYAHARA[11] that the ATP-Pi exchange reaction was not readily inhibited by ELLMAN's reagent becomes understandable. Our conclusions also agree with those of SABADIE-PIALOUX and GAUTHERON[12] who believe an -SH group is involved in the coupling mechanism of oxidative phosphorylation; however, it is difficult to relate their measurements of -SH levels with the scheme we have proposed. This difference could easily be explained by the different conditions of SABADIE-PIALOUX

and GAUTHERON and their possible inability to distinguish between the various thiol groups involved in oxidative phosphorylation.

Our generalization of a strategically located pivotal mitochondrial thiol group coupling the processes of two cycles, one cycle which meshes with the respiratory chain and one which meshes with ATP, ADP, and Pi has been satisfactory in explaining the behavior of yet another reagent which reacts with mitochondria. These results do encourage the search for possible reactions between the pivotal mitochondrial thiol group and biologically important substances which contain the -S-S- grouping.

Fig. 5. The role of ions with the combination of ELLMAN's reagent and antimycin or DNP.

Basic medium see methods.



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