THE INDUCTION OF ATP ENERGIZED MITOCHONDRIAL VOLUME CHANGES BY THE COMBINATION OF THE TWO ANTITUMOR AGENTS SHOWDOMYCIN AND LAPACHOL*

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The plant substance, lapachol, which is a hydroxyquinone, an antitumor agent, and an uncoupling agent, mimics the behaviour of the classical uncoupling agent 2,4-dinitrophenol in the following manner. Firstly, an ATP energized mitochondrial volume change is induced by the combination of lapachol with showdomycin (a nonmercurial thiol reagent which is an antibiotic and also an antitumor agent). Secondly, an appropriate concentration of lapachol is capable of inhibiting the ATP energized mitochondrial volume change induced by gramicidin in the presence of the permeant ions potassium and L-malate. The further addition of showdomycin reinstates an effect of gramicidin. Thus lapachol exposes a strategically located mitochondrial thiol group which occupies a pivotal position between a cycle which meshes with the respiratory chain and a cycle which meshes with ATP. This role for lapachol is in agreement with our previous hypothesis that the cycle meshing with the respiratory chain involves quinones, hydroxyquinones and hydroquinones and that lapachol acting as a foreign hydroxyquinone interferes with this cycle and exposes the mitochondrial thiol group normally meshing with the cycle. The hydroxyquinone lawsone also mimics 2,4-dinitrophenol. Lawsone is decidedly less effective than lapachol. Lawsone unlike lapachol does not possess an isopentenyl side chain. A simple test has been devised for selecting combinations of antitumor agents which act against a single intracellular target, such as mitochondria.

Antibiotics of known structure have been used to identify in the mitochondria, functional groups which are involved in oxidative phosphorylation and the related phenomena of ion transport and energized mitochondrial volume changes.[1,2,3,4,5,6] The induction of ATP (adenosine-5′-triphosphate) energized mitochondrial volume changes by the combination of either a respiratory inhibitor such as antimycin or an uncoupling agent such as DNP (2,4-dinitrophenol) with a nonmercurial thiol reagent such as showdomycin (an antibiotic and an antitumor agent) lead to the rationalization that a mitochondrial thiol group is exposed either by the respiratory inhibitor or the uncoupling agent.[4,5] Studies with various combinations of gramicidin, DNP, and showdomycin havc shown that the exposed thiol group occupies a pivotal position betwen two catalytic cycles[6]. One cycle meshes with the respiratory chain and one cycle meshes with ATP.

The exposure of a mitochondrial thiol group which interacts with the cycle which meshes with the respiratory chain agrees with our previous hypothesis regarding oxidative phosphorylation[7]. This hypothesis postulated a cyclic sequence involving

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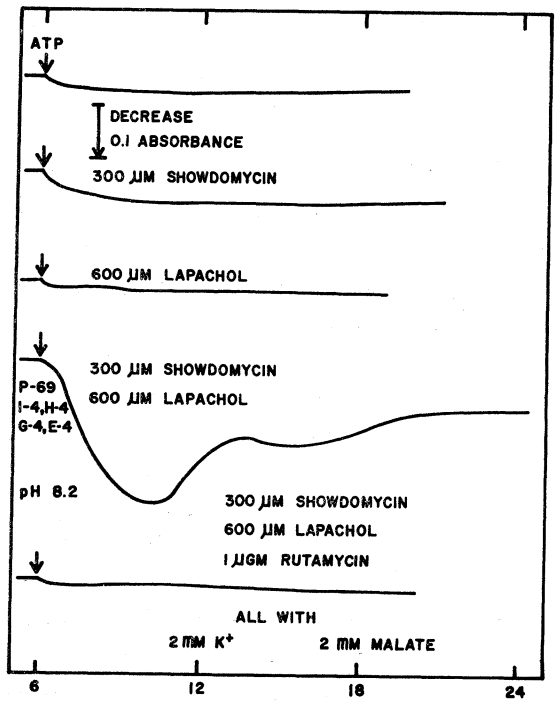
quinones, hydroxyquinones, quinones conjugated with thiol groups and quinones conjugated with phosphate groups. Both our hypothesis and our rationalization regarding the pivotal position of the mitochondrial thiol group point to the possibility that a foreign hydroxyquinone might compete with the endogenous hydroxyquinone and thus interfere with the cycle which meshes with the respiratory chain. If such an interaction occurred the foreign hydroxyquinone might mimic the respiratory inhibitor antimycin or the uncoupling agent DNP and also expose the pivotal mitochondrial thiol group. The exposed mitochondrial thiol group could then be detected, as before,[4,5] by inducing an ATP energized mitochondrial volume change on the further addition of the nonmercurial thiol reagent showdomycin.

The data which follows indeed shows that the hydroxyquinone lapachol when combined with showdomycin induces an ATP energized mitochondrial volume change. The work is of additional interest because the plant substance lapachol[8] is an antitumor agent[9] and these studies have yielded a combination of two antitumor agents which act, in a concerted fashion, on mitochondria. HOWLAND[10] has already characterized lapachol as an uncoupling agent.

Methods

The procedures and methods have been previously described,[1,2,3,5] however, the pH of the trischloride buffer is indicated on the diagrams. Incubations were at 27°C in standard rectangular quartz cuvettes with a 1-cm light path. The final basic reaction mixture had a volume of 3 ml and contained 1.5 mg of mitochondrial protein (prepared from rat liver[1]) 333 μM tris ATP which was added in 0.05 ml by means of the adding-mixing device[2] as indicated by an arrow on the diagrams; 75 mM sucrose; and 75 mM trischloride buffer. A decrease in absorbancy at 620 mμ was considered to be a measure of mitochondrial swelling. The contribution of lapachol to the absorbance was eliminated by reading the spectrophotometer at 620 mμ rather than at the customary value of 520 mμ. A model 2000 automatic spectrophotometer manufactured by Gilford Instrument Laboratories, Incorporated, Oberlin, Ohio, was used. All cations were added in the form of chloride salts and all anions were added in the form of tris salts neutralized to pH 7.4[1]. The figures and legends provide further experimental details.

Fig. 1. Lapachol combined with showdomycin. Effect of rutamycin. Basic medium (see methods).



Results

It is seen in Fig. 1 that the combination of 300 μM showdomycin with

Fig. 2. Various concentrations of lapachol and showdomycin. Basic medium (see methods).

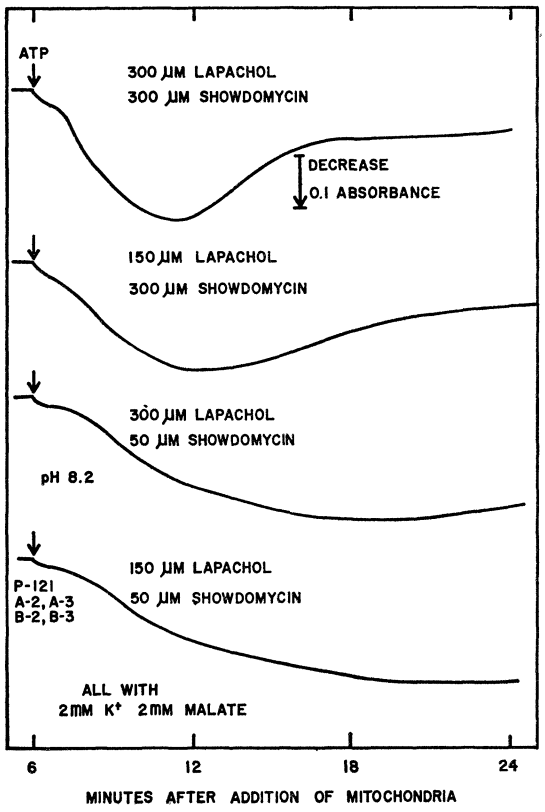


Fig. 3. Role of patassium ion and L malate. Basic medium (see methods).

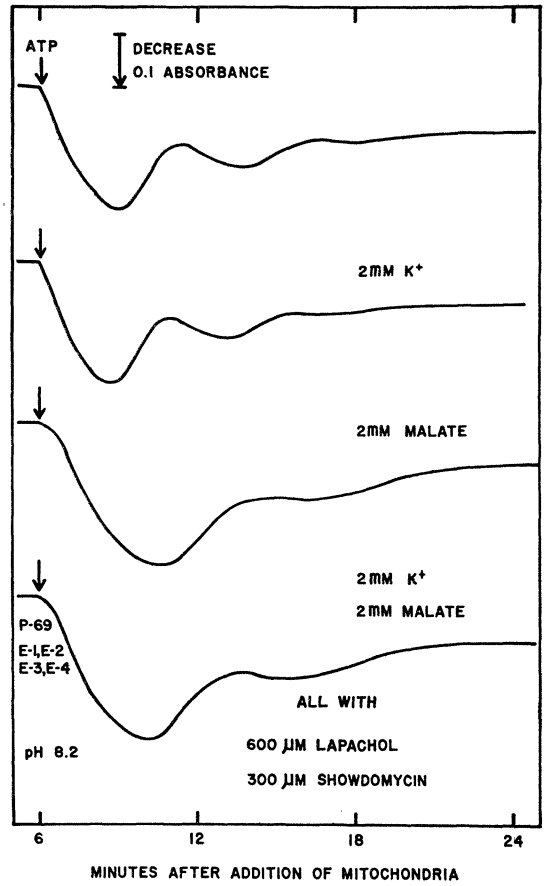
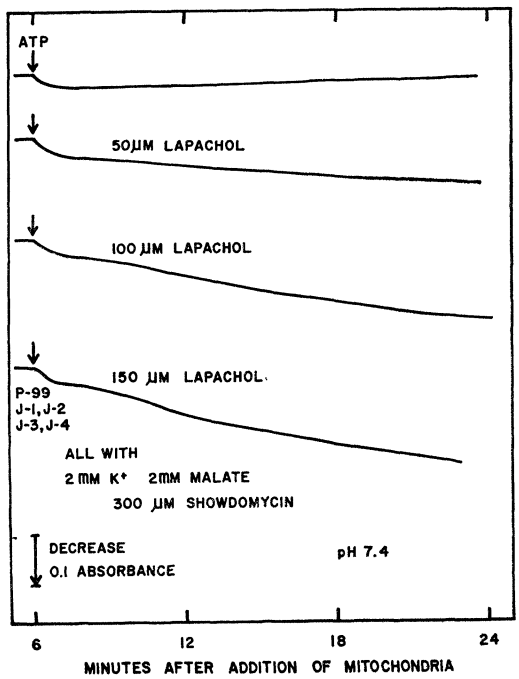
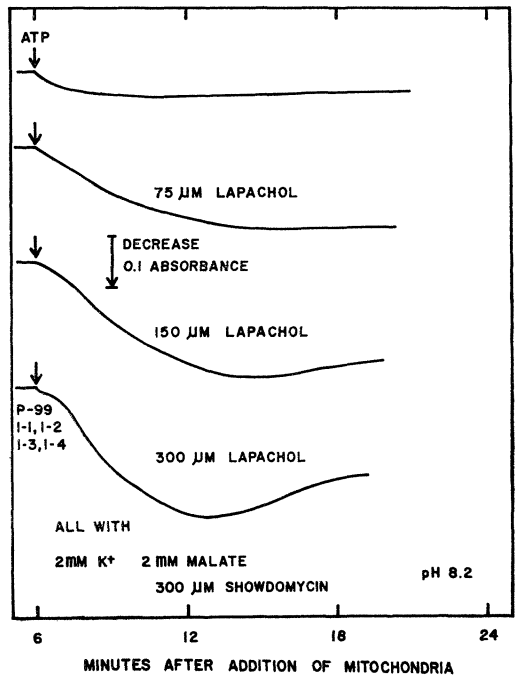


Fig. 4. Effect of pH on lapachol system. Basic medium (see methods).





600 μM lapachol induces an ATP energized mitochondrial volume change at pH 8.2. The phenomena is inhibited by the antibiotic rutamycin. The effect is enhanced (Fig. 2) either by increasing the concentration of lapachol when the concentration of showdomycin (300 μM) is kept constant or by increasing the concentration of showdomycin when the concentration of lapachol (300 μM) is kept constant, at pH 8.2. The ATP energized mitochondrial volume change, at pH 8.2 (Fig. 3), is induced by the combination of 600 μM lapachol with 300 μM showdomycin without the addition of potassium or malate ions. The addition of 2 mM potassium ions has a minimal effect. However, the addition of 2 mM malate ions increases both the amplitude and the period of oscillation, in a characteristic empirical manner.[5,6]

Fig. 5. Inhibition of the gramicidin system by lapachol. Basic medium (see methods).

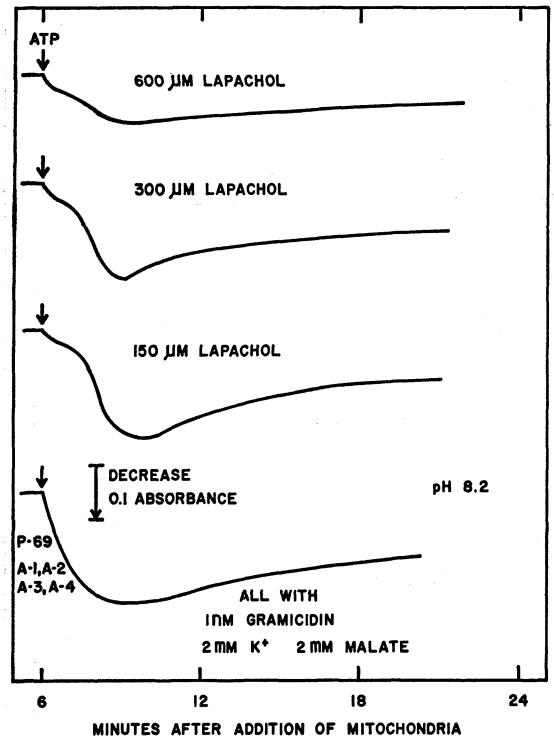


Fig. 6. Showdomycin plus the inhibited system. Basic medium (see methods).

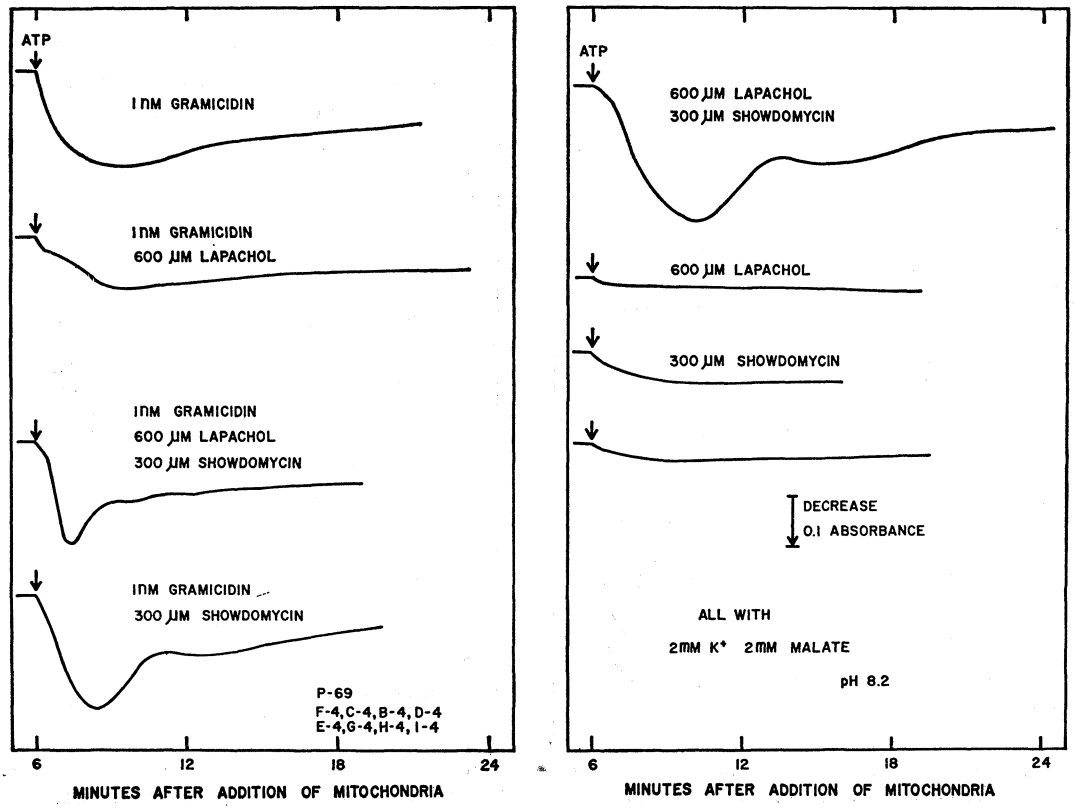
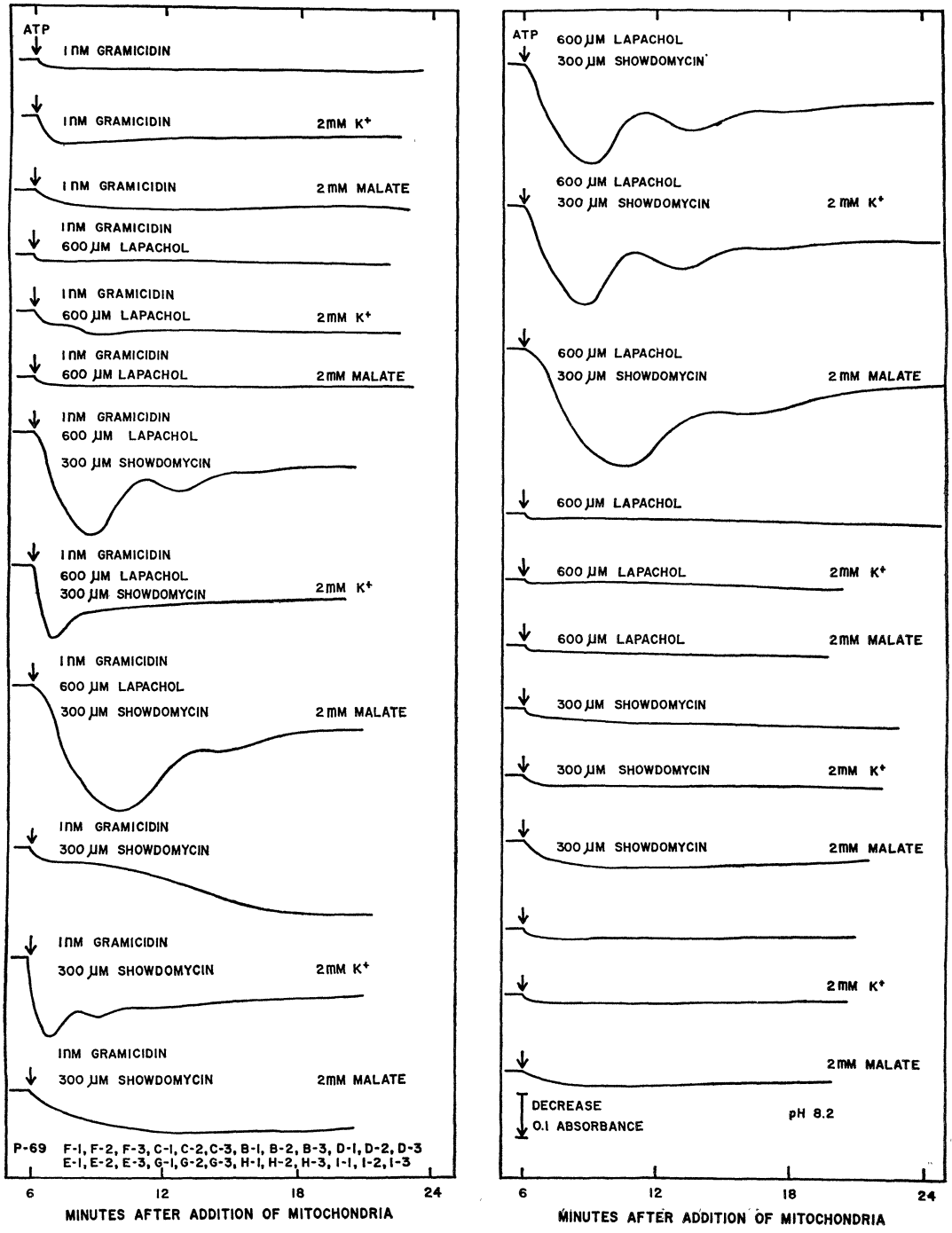


Fig. 4 clearly shows that raising the pH from 7.4 to 8.2 markedly enhances the effect of the combination of lapachol with showdomycin.

The ATP energized mitochondrial volume change induced by gramicidin in the presence of 2 mM potassium ion and 2 mM malate is inhibited by progressively

Fig. 7. Role of ions in the reinstated system. Basic medium (see methods).



increasing concentrations of lapachol (Fig. 5). It is seen in Fig. 6 that the inhibitory effect imposed by 600 μM lapachol is removed by the addition of 300 μM showdomycin. In all responsive systems (Fig. 7) containing gramicidin, that is, gramicidin alone, gramicidin plus lapachol plus showdomycin, and gramicidin plus showdomycin, the addition of potassium ion without malate has a decided effect in enhancing the phenomenon in agreement with our previous observation[6]. It is also seen that the addition of malate to the responsive systems containing showdomycin, that is, lapachol plus showdomycin, and lapachol plus showdomycin plus gramicidin, causes a characteristic[5,6] increase in the amplitude and period of oscillation.

The combination of 300 μM showdomycin with 2.4 mM lawsone induces an ATP energized mitochondrial volume change at pH 8.2 (Fig. 8). At least 1.2 mM lawsone in combination with 300 μM showdomycin at pH 8.2 is necessary for an appreciable ATP energized mitochondrial volume change. The phenomena dependent upon the combination of lawsone with showdomycin is markedly increased (Fig. 9) when the pH is raised from 7.4 to 8.2.

Fig. 8. Lawsone combined with showdomycin. Basic medium (see methods).

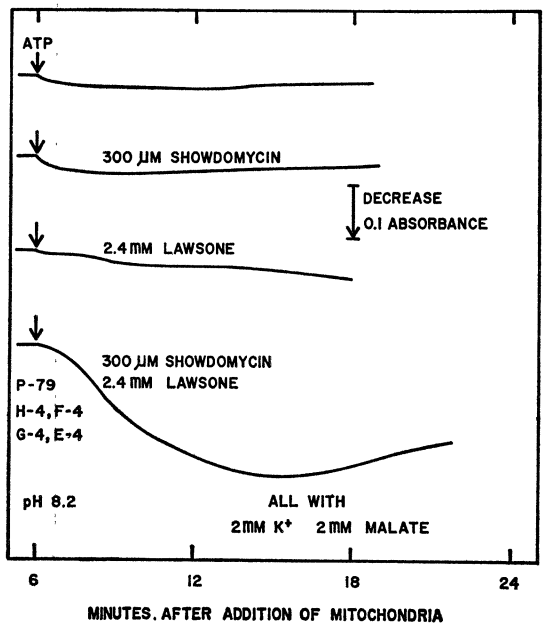
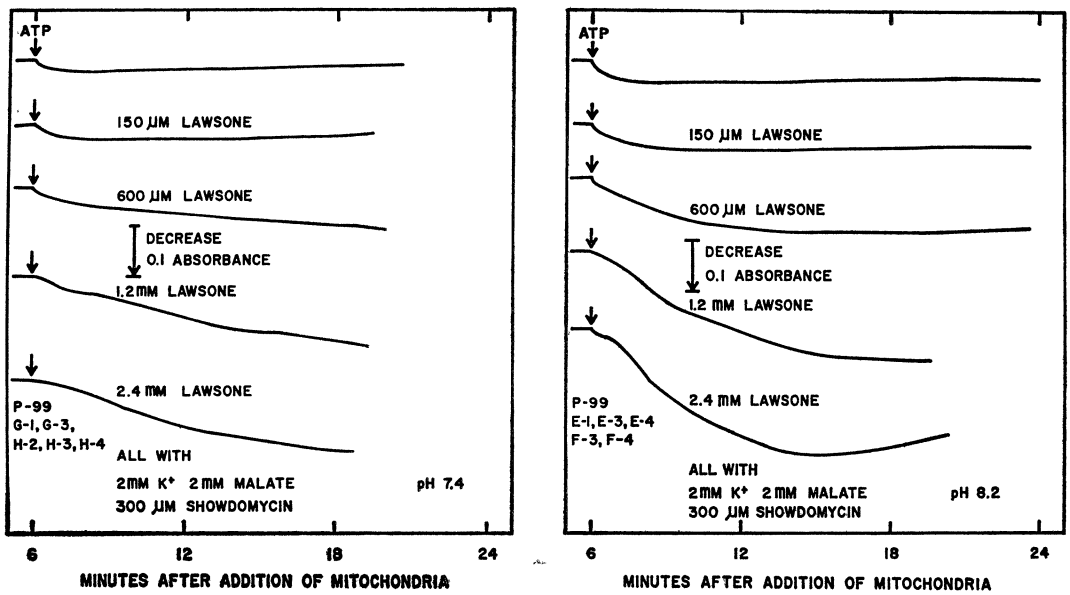


Fig. 9. Effect of pH on lawsone system. Basic medium (see methods).



Discussion

The plant substance lapachol[8], an uncoupling agent[10] and an antitumor[9] agent, clearly mimics the behaviour of DNP. Like DNP[5] lapachol exposes a mitochondrial thiol group which can conjugate with the nonmercurial thiol reagent showdomycin and induces an ATP energized mitochondrial volume change (Fig. 1). The effect is enhanced either by increasing the concentration of lapachol or by increasing the concentration of showdomycin (Fig. 2). The addition of potassium ion, as before[5], has a minimal effect while again the addition of malate increases the amplitude and period of the oscillation (Fig. 3). The phenomenon again is markedly enhanced by raising the pH (Fig. 4). The mitochondrial thiol group exposed by lapachol is also capable of inhibiting the gramicidin system (Fig. 5). Again[6] when this exposed mitochondrial thiol group has the opportunity to react with showdomycin the gramicidin system is reinstated (Fig. 6). In the reinstated gramicidin system, that is, a system composed of gramicidin plus lapachol plus showdomycin, potassium ion has an enhancing effect, thus, as before[6], the gramicidin system has remained intact. Accordingly, based upon our previous rationalization[6] lapachol exposes a mitochondrial thiol group which occupies a pivotal position between the cycle which meshes with the respiratory chain and the cycle which meshes with ATP. This role for the hydroxyquinone lapachol is in harmony with our previous hypothesis for oxidative phosphorylation[7]. This hypothesis implies that a mitochondrial thiol group could be exposed when the cycle meshing with the respiratory chain is disturbed by a foreign hydroxyquinone competing with the endogenous hydroxyquinone.

The hydroxyquinone lawsone which does not possess an isopentenyl side chain also exposes a mitochondrial thiol group, in agreement with our hypothesis for oxidative phosphorylation. However, there is no question that lawsone is much less active than lapachol. While the significance of the side chain is obscure we would like to suggest that a lipid side chain could act as a swinging arm when the hydroxyquinone moiety is embedded in a lipid matrix and thus fit the dynamic geometry of the catalytic cycle.

This data is of interest not only because it is related to our hypothesis for oxidative phosphorylation and presents a biochemical activity for lapachol, but also a simple experimental technique has been evolved for collecting combinations of antitumor agents which act in a concerted fashion against a single intracellular target such as mitochondria. This simple assay furthermore may also be used to monitor synthetic variations of hydroxyquinones and aid in the synthesis of uncoupling agents which are potent and useful antitumor agents.

The antitumor activity and lack of toxicity of lapachol has merited consideration of lapachol in clinical trials[9]. Our data indicates that combinations of nonmercurial thiol reagents such as showdomycin with antitumor agents such as lapachol should be considered in experimental cancer chemotherapy.

Acknowledgement

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