Chapter 5
Effect of Antimalarials Treatment on Rat Liver Mitochondrial ATPase Kinetics Properties

Introduction

In earlier chapters it was shown that the three antimalarials CQ, PQ and Q adversely affected the oxidative phosphorylation in rat liver mitochondria as well as the mitochondrial FoF1 ATPase activity (Chapter 4). Inhibition of FoF1 ATPase in *S. Pneumoniae* by Q has also been reported (1). The process of energy transduction i.e. oxidative phosphorylation is dependent on the membrane phospholipids as is the function of the FoF1 ATPase (2). Requirement of acidic phospholipids for FoF1 ATPase is well established (3). Antimalarials are reported to alter the lipid/phospholipid profiles. It has also been reported that following treatment with CQ, the ratio of acidic phospholipids to basic phospholipids had increased in rat liver (4). In a recent report Ross et al. have shown that CQ treatment resulted in the induction of cardiolipin synthesis (5). Thabrew et al. have reported that treatment with CQ and Q resulted in alterations in the lipid/ phospholipid composition of the rat liver microsomes (6). Deepalakshmi et al. showed that oral feeding of CQ resulted in increase in the total phospholipids and decrease in cholesterol in rat liver mitochondria (7). Hence it was of interest to find out that what will be the effect on the kinetic properties of FoF1 ATPase in liver mitochondria. The results of these investigations are summarized in this chapter.

Materials and Methods

Details of chemicals used are as described in Chapter 4.

The treatment with antimalarials and isolation of mitochondria were essentially the same as described in Chapter 3 and Chapter 4 respectively.

Assay of ATPase activity

ATPase activitiy was measured in the assay medium (total volume 0.4 ml) containing 50 mM Tris-HCl buffer pH 7.4, 75 mM KCl and 0.4 mM EDTA. The assays were performed in the presence of 6 mM $MgCl_2$ and 100 μM DNP. After pre-incubating the mitochondrial protein (Ca. 100μg) in the assay medium at 37 °C, the reaction was initiated by the addition of ATP at a final concentration of 5 mM (8). The reaction was carried out for 10 min and then terminated by the addition of 0.1 ml of 5% (w/v) sodium dodecyl sulfate (SDS) solution and the amount of liberated inorganic phosphorus was estimated by the method of Fiske and Subba Row (9).

For substrate kinetics studies concentration of ATP was varied in the range from 0.1 mM to 5 mM

For temperature kinetics studies, experiments were carried out with fixed ATP concentration of 5 mM and the temperature was varied from 5°-53°C with an increment of 4°C at each step.

The data for substrate kinetics were analyzed by the Lineweaver-Burk, Eadie-Hofstee and Eisenthal and Cornish-Bowden methods for the determination of Km and Vmax (10). The values of Km and Vmax obtained by the three methods were in close agreement and were averaged

The data on temperature kinetics were analyzed for determination of energies of activation in the high and low temperature ranges (E_1 and E_2 respectively) and phase transition temperature (Tt) according to the method described previously (11).

All the kinetics data were computer analyzed employing Sigma plot version 5.0 (12,13).

Protein estimation was by the method of Lowry et al. with bovine serum albumin used as the standard (14).

Results are given as mean ± SEM.

Statistical evaluation of the data was by Students' t-test.

Results

As was evident from Table 5 (Chapter 4) the antimalarials had differential effect on rat liver mitochondrial ATPase. Treatment with CQ for 7 days resulted in 28 % increase in the activity whereas 14 day treatment had a reverse effect i.e. 33 % decrease. PQ treatment caused a progressive decrease of 24 to 32 % while Q treatment resulted in elevation (29 and 13 % increase) in the activity.

Since the effects of the three antimalarials were different, in the next series of experiments we checked the dependence of the enzyme activity on substrate i.e. ATP concentration The substrate saturation curves for the control and antimalarials treated

groups are shown in Fig. 1. It is evident that the typical substrate saturation patterns were obtained for the control and all the antimalarials treated groups.

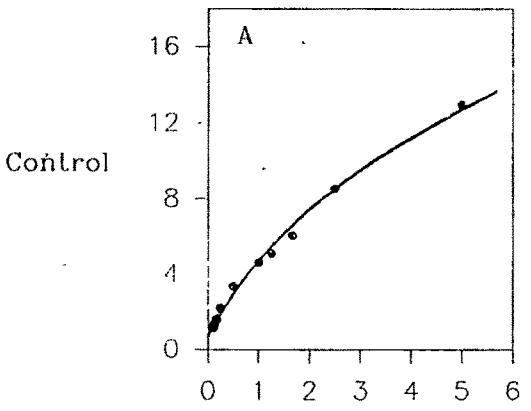
The data were then analyzed in terms of Lineweaver-Burk, Eadie-Hofstee and Eisenthal and Cornish-Bowden methods (10). For the sake of brevity only the corresponding Eadie-Hofstee plots are shown in Fig. 2. From the Eadie-Hofstee plots it is clear that in the control group the mitochondrial ATPase activity resolved in three components. This is consistent with previously reported observations by us and other investigators (15,16). A similar three component pattern was seen even for CQ-7 group. However, CQ treatment for 14 days resulted in abolishment of one component. Only two components were evident. A pattern similar to that of the CQ-14 group was also seen following PQ treatment. In the animals treated with Q the typical three component pattern was evident.

The values of Km and Vmax for the components of ATPase are given in Table 1. These values for the control group are consistent with our previously reported observations (16) CQ treatment for 7 days resulted in the lowering of Km of the first component whereas the Km of the third component had increased. The Vmax values for all the components almost doubled. This is consistent with the data in Table 5 (Chapter 4). CQ treatment for 14 days, on the other hand, resulted in increased value of Km of component I as well as component II; the Vmax of both the components increased. Apparently, the third component was absent

PQ treatment for 7 days brought about a small decrease in Km and increase in Vmax of the first component. Similar changes were noted even for component II. For the PQ-14 group the pattern was almost the same

Fig.1. Typical substrate saturation curves for rat liver mitochondrial ATPase in controls and antimalarials treated animals. Experimental details are as given in the text. For determination of substrate kinetics of ATPase, ATP was used as a substrate over a concentration range of 0.01 to 5mM. The abscissa represents the reaction velocity v, while the ordinate represents [S]. Reaction velocity v = μmol of Pi liberated hr^{-1} mg $protein^{-1}$.
[S]= mM

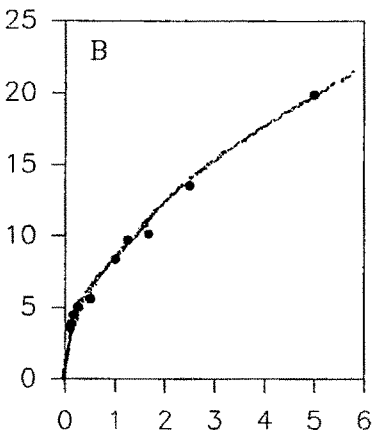
Fig. 1



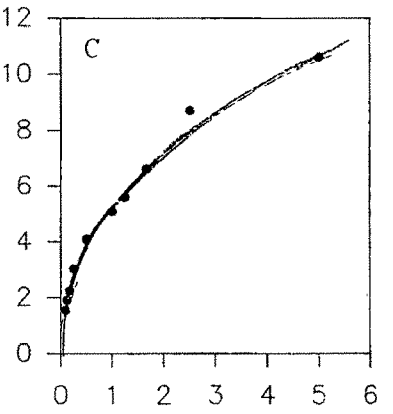
Control

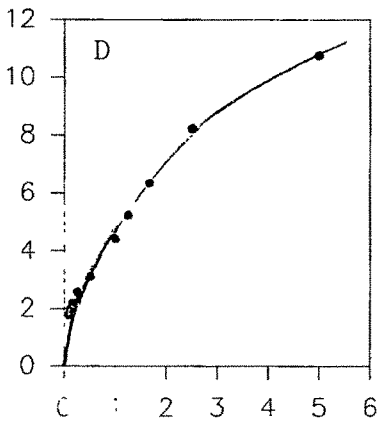
7 day

14 day

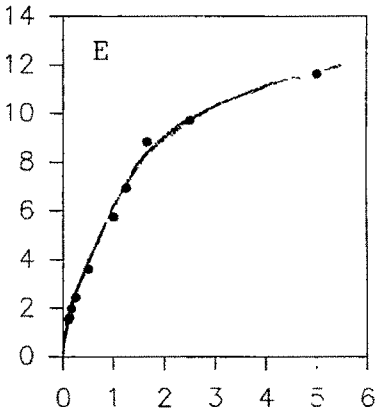


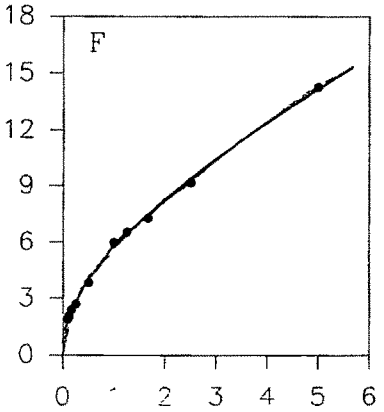
CQ





PQ





Q

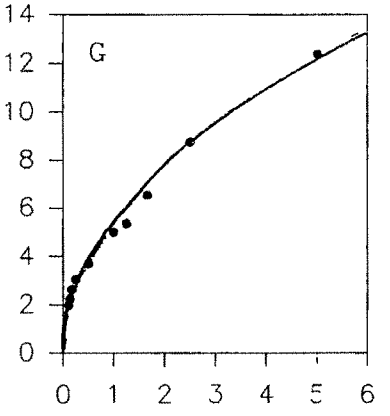


Fig.2. The respective Eadie-Hofstee plots for rat liver mitochondrial ATPase in controls and antimalarials treated animals. Experimental details are as given in the text and in Fig. 2. The abscissa represents the reaction velocity v, while the ordinate represents v/[S]. Reaction velocity v == μmol of Pi liberated hr^{-1} mg $protein^{-1}$. V/[S]= reaction velocity divided by the corresponding substrate concentration.

Fig. 2

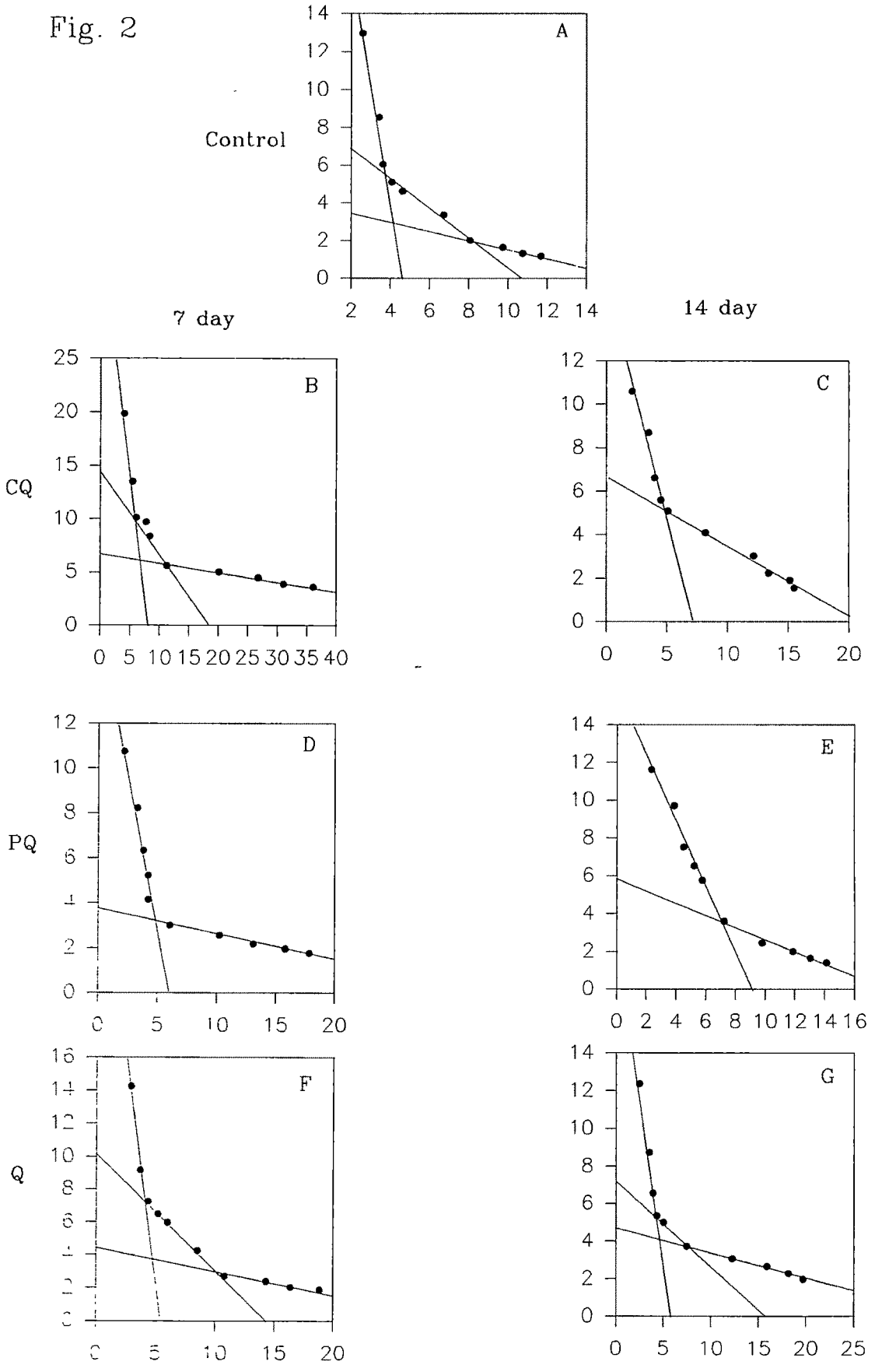


Table 1. Effect of antimalarials treatment on substrate kinetics properties of rat liver mitochondrial ATPase.

	Component I		Component II		Component III	
	Km	V *max*	Km	V *max*	Km	V *max*
Control (17)	0.212±0.008	3.362±0.299	0.928±0.037	8.115±0.528	2.717±0.116	14.780± 0.692
CQ-7 (8)	0.149±0.012[d]	6.464±0.271[d]	0 964±0.084	14.127±1.073[d]	3.865±0.225[d]	27.085±2.350[d]
CQ-14 (8)	0.388±0.028[d]	6.478±0.365[d]	1.774±0.150	13.510±1.235	--	--
PQ-7 (8)	0.179±0.013[a]	4.570±0.150[c]	2 353±0.236	14.241±0.803	--	--
PQ-14 (8)	0.233±0.041	6.663±0.088[d]	1.286±0.112	17.170±1.320	--	--
Q-7 (8)	0.235±0.030	4 637±0 471[a]	0.977±0.099	10.652±0.986[a]	3.415±0.178[d]	19.764±1.488[b]
Q-14 (8)	0.198±0.013	6.076±0.329[d]	0.819±0 096	11.456±1 383[a]	3.666±0.103[d]	24.177±0 725[d]

The experimental details are as given in the text.

The results are given as mean ± SEM of the number of observations indicated in the parentheses. The substrate used for kinetics determination of the rat liver mitochondrial ATPase was ATP over the concentration range of 0.01 to 5 mM.
Km is expressed as mM
Vmax is expressed as µmole of Pi liberated /hr/ mg protein
The Km and Vmax values were calculated by three different methods of analysis as described in the text using Sigma Plot version 5.0 and averaged for calculating the mean ± SEM values.
[a] $p< 0.05$, [b] $p<0.01$, [c] $p<0.002$ and [d] $p<0.001$ compared with the corresponding control.

Q treatment resulted in increased values of Km of component III and increase in Vmax in all the three components (Table 1), which is consistent with the results shown in Table 5 (Chapter 4)

In the light of compositional changes in the lipids and phospholipids profiles as reported (5-7) and altered substrate saturation kinetics (Table 1and Fig. 1,2) it was of interest to find out the effect of antimalarials treatment on temperature dependent changes in the ATPase activity. The typical plots depicting the temperature dependence of ATPase activity are shown in Fig. 3. It can be noted that the pattern was comparable for control, CQ-7 and Q treated animals where a progressive increase in the activity with increasing temperature was noted. For CQ-14 group and PQ treated groups the pattern deviated from the control in that over the temperature range of 21°C to 37°C the rate of increase was lowered considerably thus producing a plateau region.

The data were then analyzed in terms of Arrhenius plots to find out the energies of activation and phase transition temperature(s). The corresponding Arrhenius plots are shown in Fig 4. As can be noted typical biphasic plots were obtained for control, CQ-7 and Q-7 groups; the plots were monophasic for Q-14 group. In CQ-14 and PQ treated animals the plots were chair shaped with two breaks. Other reserchers have also reported chair shaped Arrhenius plots for mitochondrial ATPase and cytochrome oxidase (17,18).

The data on energies of activation and phase transition temperature are given in Table 2. As can be noted CQ treatment for 7 days resulted in increased energy of activation without any change in phase transition temperature. Following CQ treatment for 14 days

Fig. 3. Typical temperature curves for rat liver mitochondrial ATPase in controls and antimalarials treated animals. Experimental details are as given in the text. The ATPase activity was determined with 5 mM ATP. The abscissa represents the reaction velocity v, while the ordinate represents the temperature in °C. Reaction velocity v = μmol of Pi liberated hr^{-1} mg $protein^{-1}$.

Fig. 3

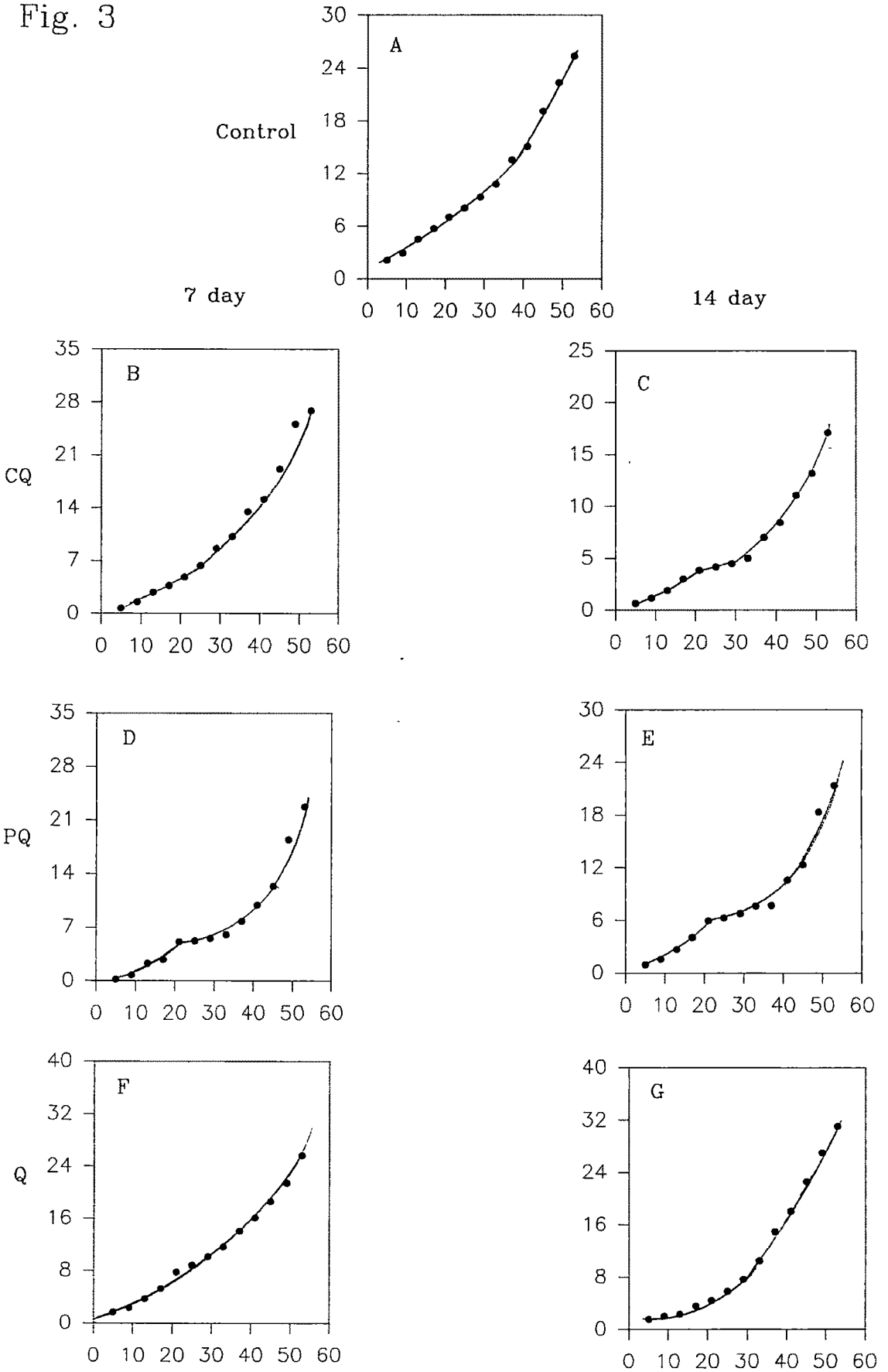


Fig. 4. The respective Arrhennius plots for rat liver mitochondrial ATPase in controls and antimalarials treated animals. Experimental details are as given in the text and Fig. 5. The abscissa represents the log of reaction velocity v, while the ordinate represents reciprocal of absolute temperature T*1000. Reaction velocity v = μmol of Pi liberated hr^{-1} mg $protein^{-1}$. Absolute temperature T= °Kelvin.

Fig.4

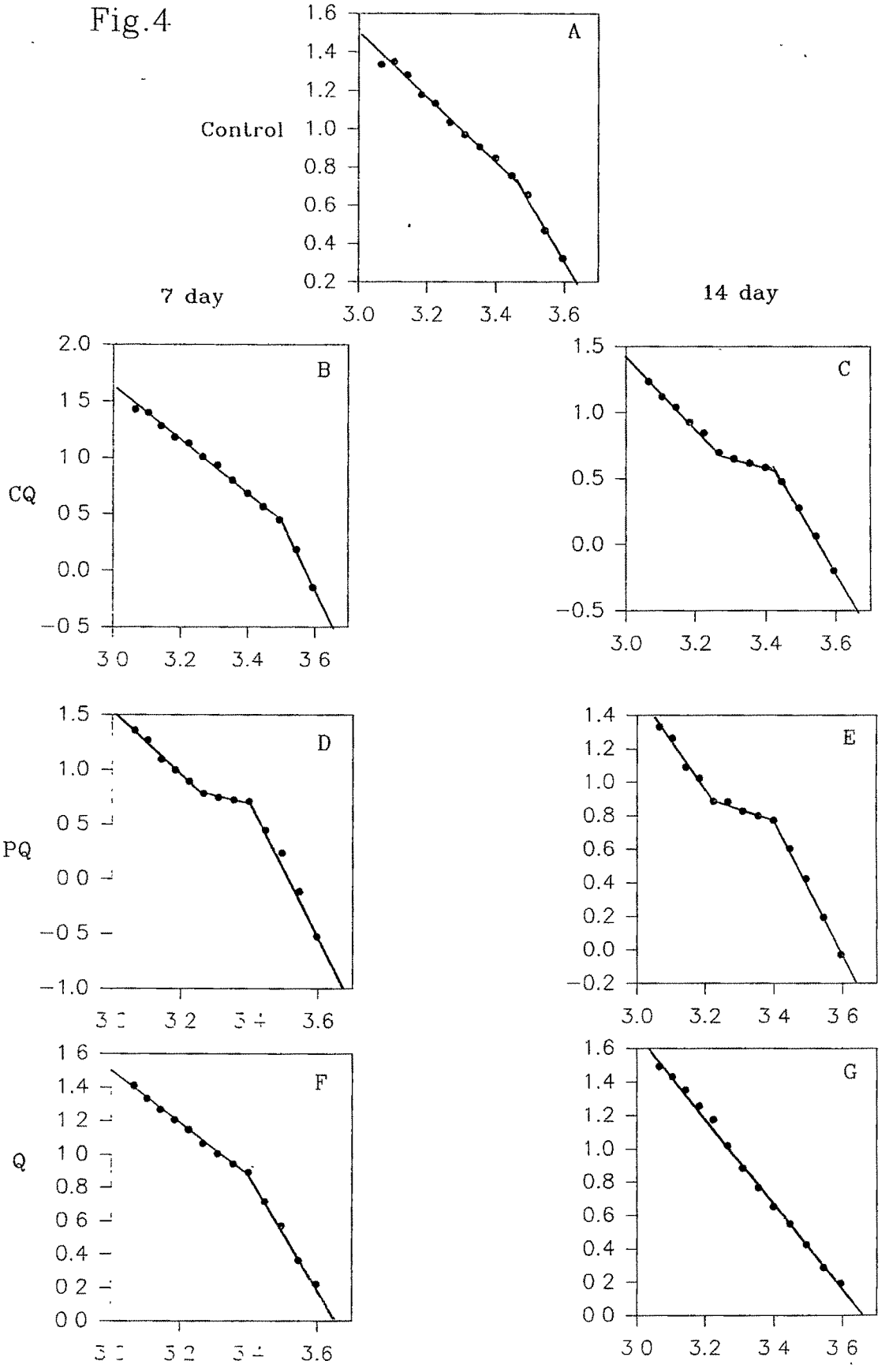


Table 2. Effect of antimalarials treatment on Arrhenius kinetics properties of rat liver mitochondrial ATPase.

	(Energy of activation, KJ/mole)			Phase transition temperature Tt (°C),	
	E_1	E_2	E_3	Tt_1	Tt_2
Control (16)	34.60±0.75	--	57.96±2.24	13.84±0.63	--
CQ-7 (8)	43.80±0.69[d]	--	97.33±6.15[d]	13.59±0.16	--
CQ-14 (8)	50.02±2.68[d]	21.90±1.97	82.51±6.39[d]	18.28±1.01[c]	34.00±1.13
PQ-7 (8)	52.06±1.53[d]	14.65±1.23	65.31±1.77[b]	22.80±0.42[d]	33.27±0.70
PQ-14 (8)	53.81±3.30[d]	16.39±1.11	86.55±3.15[d]	20.00±1.14[d]	39.50±1.12
Q-7 (8)	34.51±1.73	--	67.23±1.82[a]	20.12±0.74[d]	--
Q-14 (8)	50.09± 0.60[d]	--	--	--	--

The results are given as mean ± SEM of the number of observations indicated in the parentheses.

[a] $p< 0.02$, [b] $p<0.01$, [c] $p<0.002$ and [d] $p<0.001$ compared with the corresponding control.

a transient energy of activation E_2 of about 28 Kcal became evident; at the same time the value of E_1 and E_3 increased. Phase transition temperature was elevated by 4.5° and additional break in the plot appeared at 34°C. Almost similar pattern was obtained in PQ-7 and PQ-14 groups. Q treatment for 7 days resulted in increase in the value of E_3 with a 6.3°C increase in the phase transition temperature. Interestingly, in the Q-14 group the phase transition was abolished and a comparatively higher value of energy of activation was observed (Table 6).

In conclusion it can be said that treatment with the three antimalarials effected the mitochondrial membrane functions in the host tissue. As is evident, the kinetic properties in terms of Km and Vmax were indeed greatly influenced by antimalarial treatment. Likewise, the Arrhenius plots also revealed significant alterations following antimalarial treatment. Of particular interest was the chair shaped Arrhenius plots in CQ-14 and PQ treated animals (both 7 day and 14 day treatment groups). Significance of these changes remains obscure at this stage but could perhaps relate to changes in the mitochondrial lipid/ phospholipid compositions. This possibility is further checked in the next chapter.

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Summary

The effect of antimalarials (chloroquine, primaquine and quinine) treatment on mitochondria FoF1 ATPase kinetics properties were studied. Treatment with antimalarials resulted in altered substrate and temperature kinetics properties of mitochondrial ATPase. Treatment with antimalarials increased the Vmax of almost all the three components. Prolonged treatment with chloroquine and treatment with primaquine resulted in loss of one component; quinine treatment was least affective. Interestingly the group which showed loss of one component showed a chair shaped Arrhenius plots with three energies of activation and two phase transition temperatures. The corresponding energies of activation i.e. E_1 and E_3 were higher when compared to controls. Similarly Tt_1 was also higher than that in the controls. The results thus suggest that long-term treatment with antimalarials alters the function of membrane bound enzyme such as FoF1 ATPase.