Chapter 9

Effect of dexamethasone treatment on kinetic attributes of rat liver mitochondrial ATPase during postnatal development.

Introduction

Consistent with the data in the previous Chapter, the dexamethasone treatment resulted in a generalized decrease in the ATPase activity and 2 week and 4 week group were most sensitive The data also showed changes in the lipid phospholipid occurs Since ATPase is a membrane bound enzyme and its activity is modulated, it was of interest to study the kinetic properties modulated

Materials and Methods

Assay of ATPase activity

ATPase activitiy were 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 (1) 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 (2)

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 (3) The values of Km and Vmax obtained by the three methods were in close agreement and were averaged Hill plot analysis was carried out where indicated (3)

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 (4)

All the kinetics data were computer analyzed employing Sigma plot version 5 0 (5)

Protein estimation was by the method of Lowry et al. with bovine serum albumin used as the standard (6).

Results are given as mean ± SEM

Statistical evaluation of the data was by Students` t-test

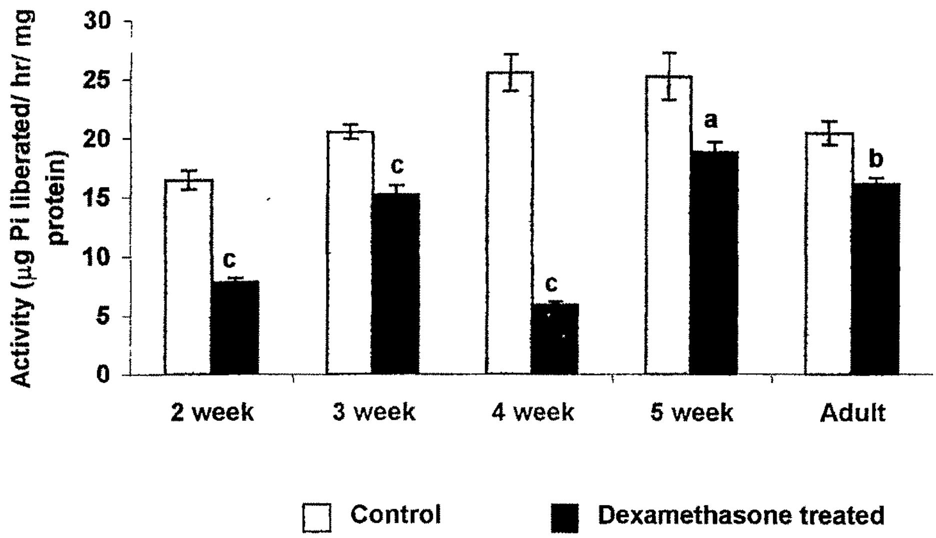
Results

The data in the Fig 1 showed that dexamethasone treatment resulted in general decrease in ATPase activity Hence in the next set of experiments we examined the substrate and temperature dependence of the enzyme activity Fig 2-6 shows the substrate saturation kinetics and their corresponding Eadie-Hofstee plot for control and dexamethasone treatment graph as shown in Fig 2-6 As can be seen except for the 3 week dexamethasone treatment group, normal substrate saturation pattern was seen in the rest of the groups The 3 week dexamethasone group showed significant saturation curve The corresponding Eadie-Hofstee plots are also shown in Fig. 2-6 For the 3 week dexamethasone animals the Hill plot analysis was done As can be noted the Eadie-Hofstee plots resolved the ATPase activity in 3 components In the control group in all

Fig 1. Effect of dexamethasone treatment on liver mitochondrial ATPase activities during development

[a] $p < 0.01$, [b] $p < 0.002$, [c] $p < 0.001$ as compared with corresponding control group

Fig. 1



Activity (µg Pi liberated/ hr/ mg protein)
30
25
20
15
10
5
0
c
c
c
a
b
2 week
3 week
4 week
5 week
Adult
Control
Dexamethasone treated

Fig. 2 to 6. Typical substrate saturation curves and the respective Eadie-Hofstee plots for rat mitochondrial ATPase in controls and dexamethasone treated animals Experimental detail as given in the text For determination of substrate kinetics of ATPase, ATP was used substrate over a concentration range of 0 01 to 5mM The abscissa represents the rea velocity v, while the ordinate represents [S] For the Eadie-Hofstee plots the abscissa repre the reaction velocity v, while the ordinate represents v/[S] Reaction velocity v == μmol liberated hr^{-1} mg $protein^{-1}$ V/[S]= reaction velocity divided by the corresponding subs concentration Hill plot analysis were made for sigmoidal curves [log (v/V-v) versus log [S] mM

Fig. 2

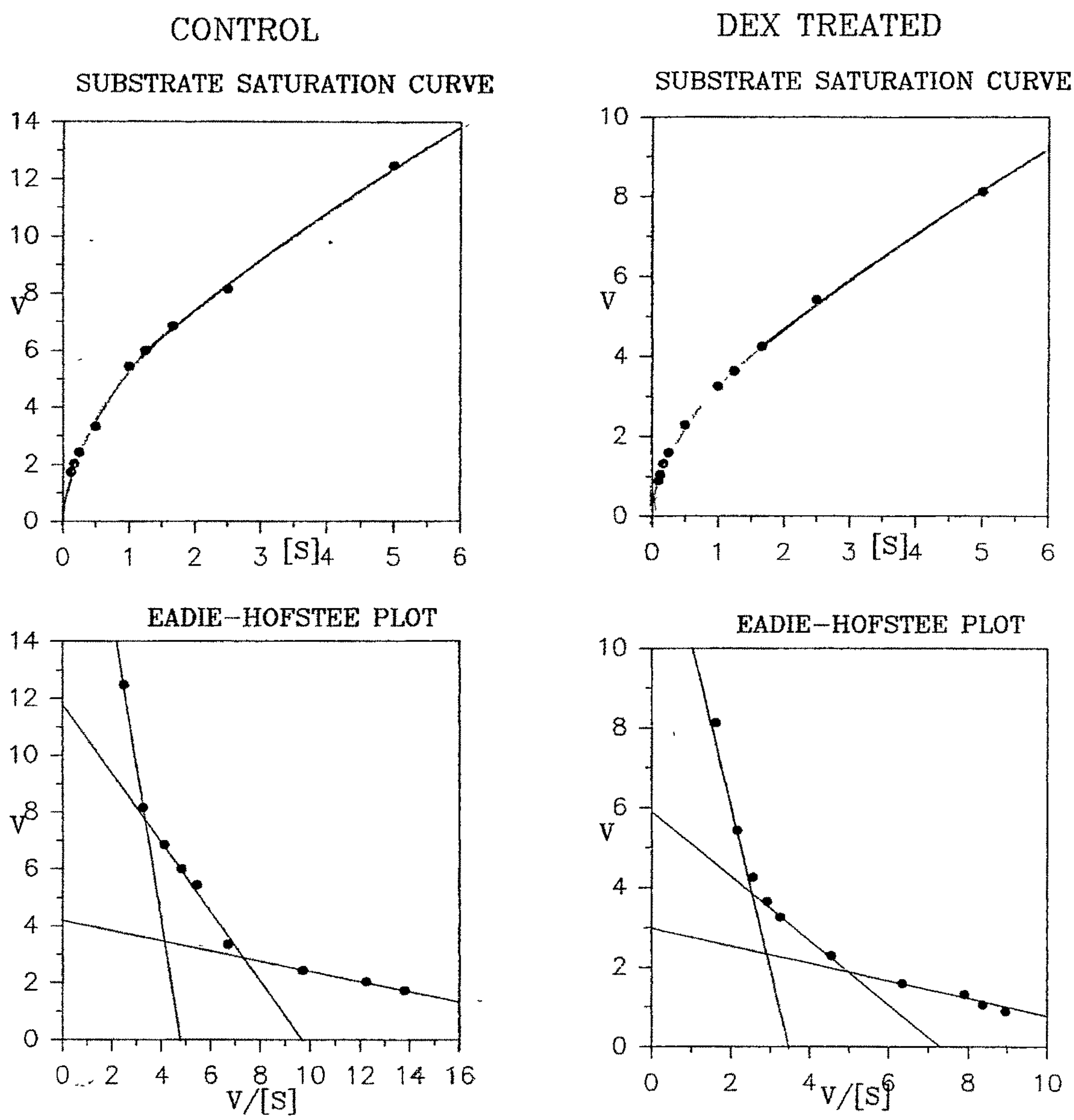

CONTROL
DEX TREATED
SUBSTRATE SATURATION CURVE
SUBSTRATE SATURATION CURVE
V
[S]
EADIE-HOFSTEE PLOT
EADIE-HOFSTEE PLOT
V
V/[S]

Fig. 3

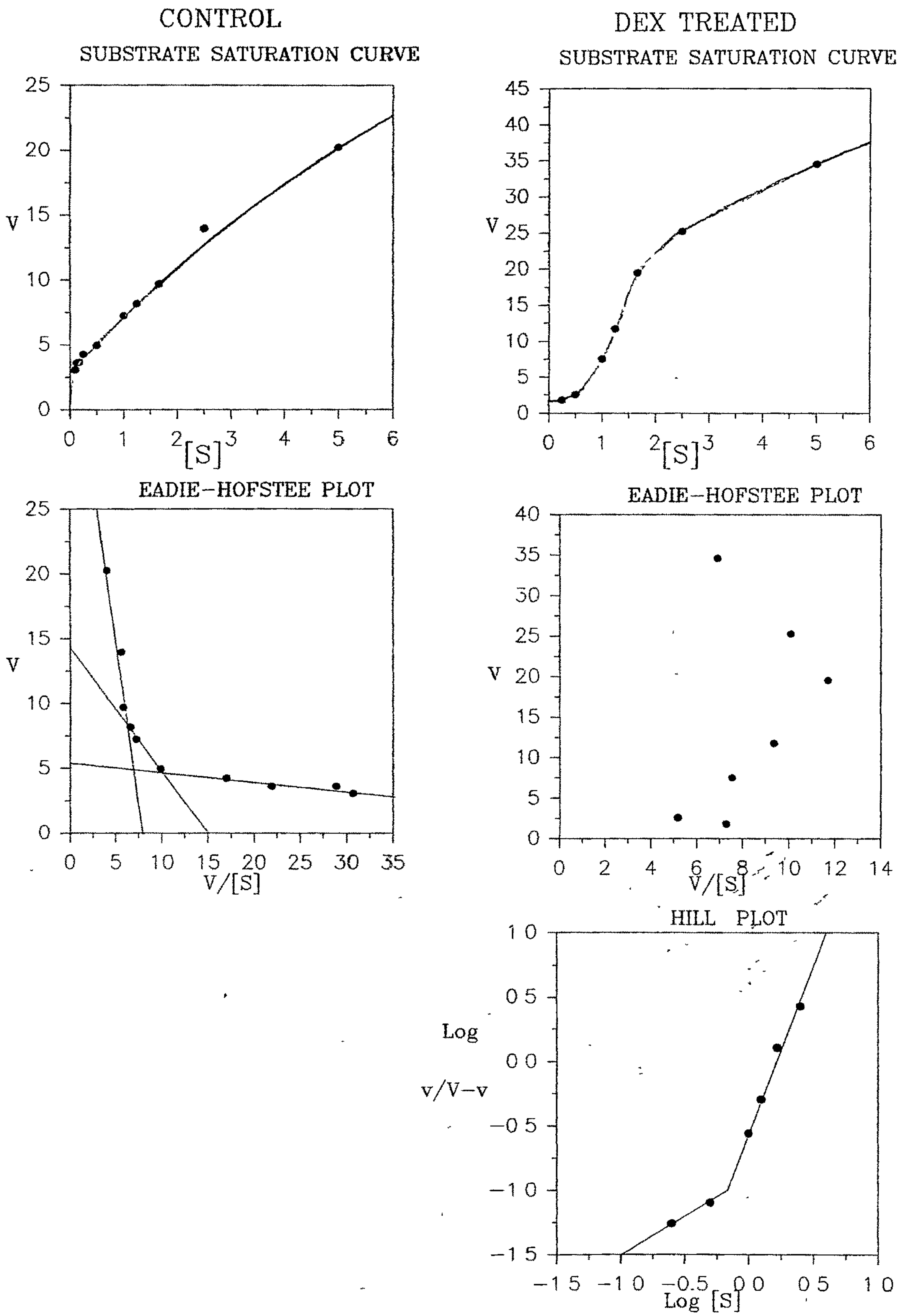

CONTROL
SUBSTRATE SATURATION CURVE
V
[S]
EADIE–HOFSTEE PLOT
V
V/[S]
DEX TREATED
SUBSTRATE SATURATION CURVE
V
[S]
EADIE–HOFSTEE PLOT
V
V/[S]
HILL PLOT
Log v/V–v
Log [S]

Fig. 4

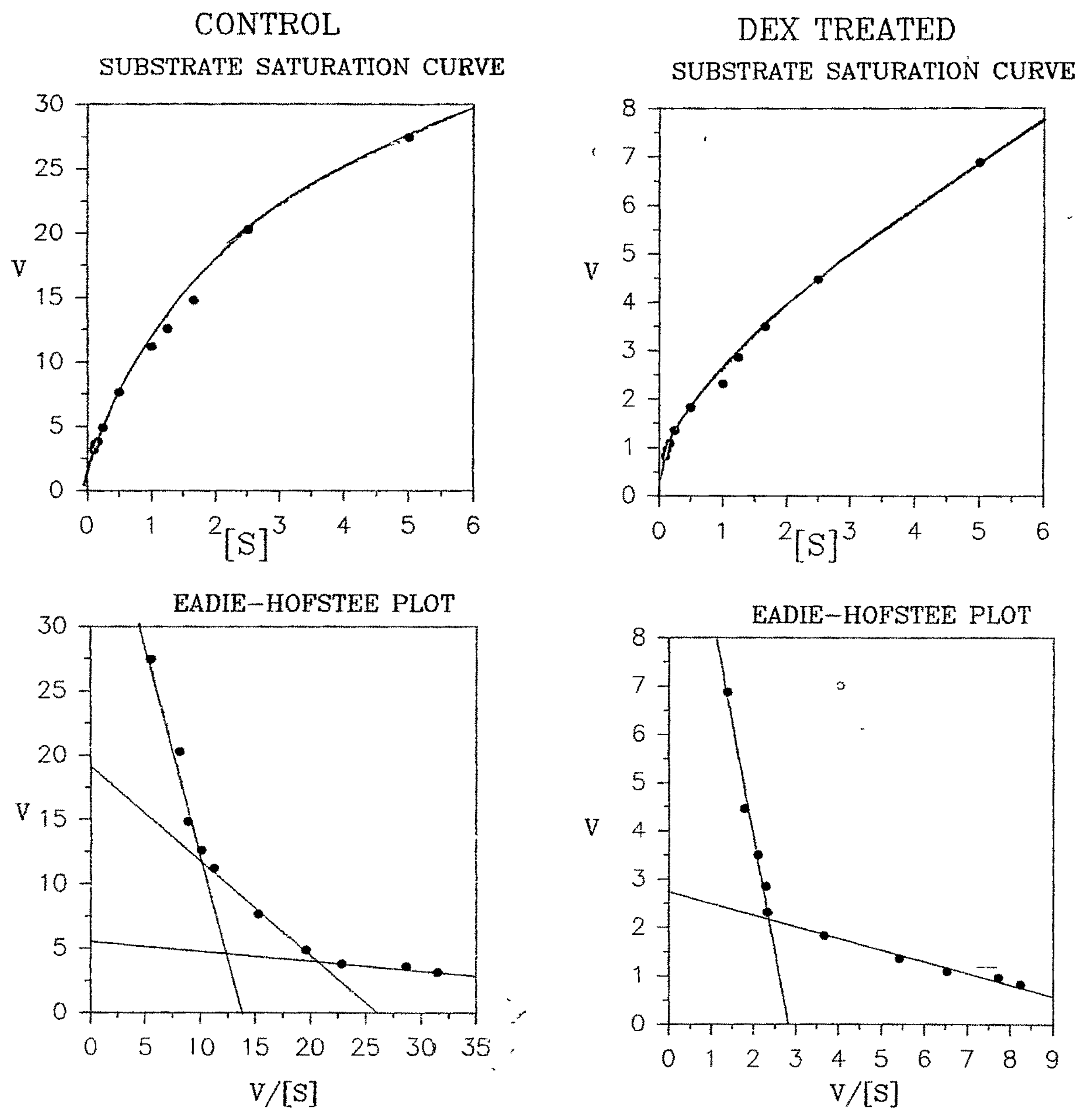
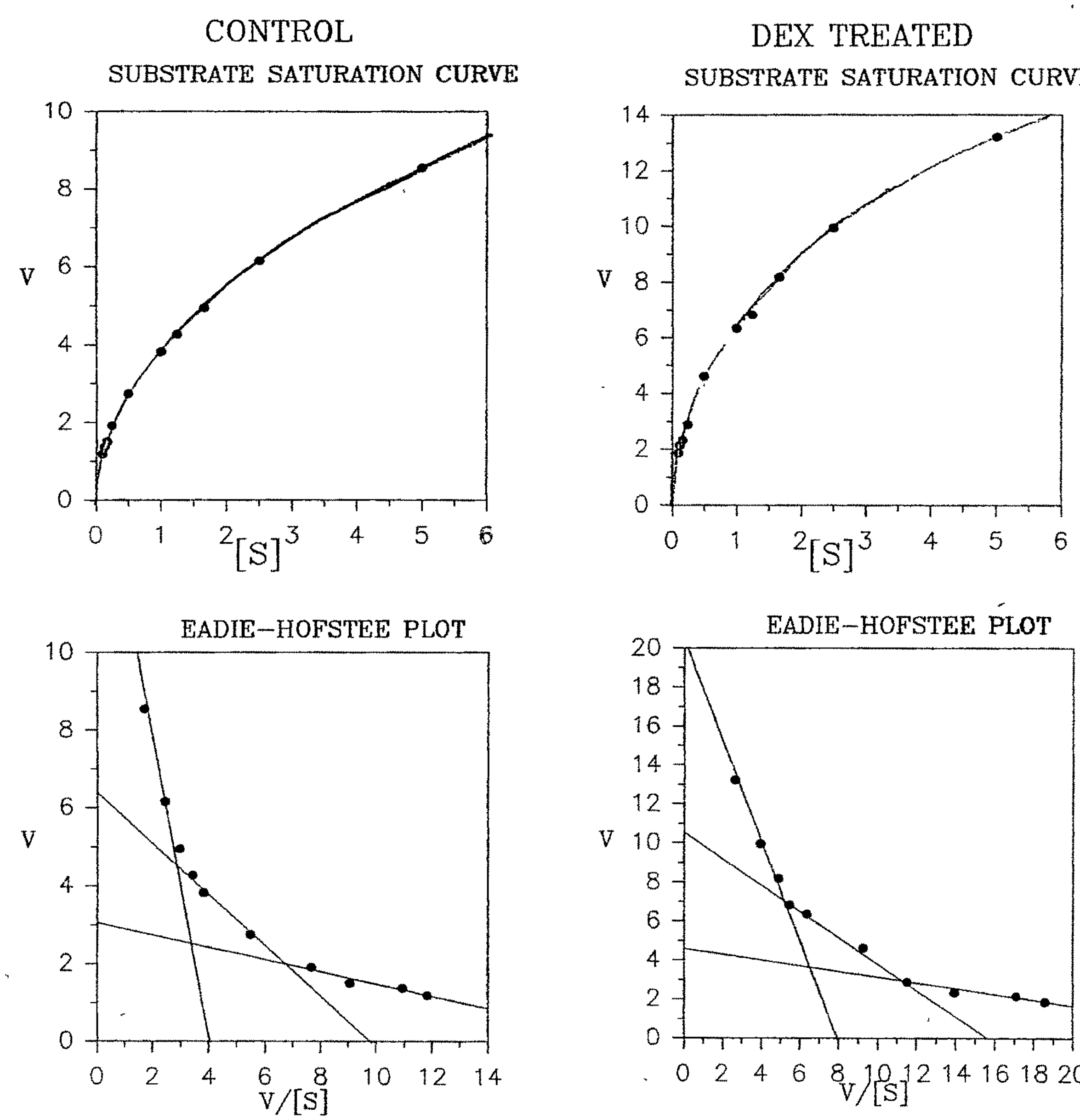

CONTROL
SUBSTRATE SATURATION CURVE
V
[S]
EADIE–HOFSTEE PLOT
V
V/[S]
DEX TREATED
SUBSTRATE SATURATION CURVE
V
[S]
EADIE–HOFSTEE PLOT
V
V/[S]

Fig. 5


CONTROL
SUBSTRATE SATURATION CURVE
V
[S]
DEX TREATED
SUBSTRATE SATURATION CURVE
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EADIE–HOFSTEE PLOT
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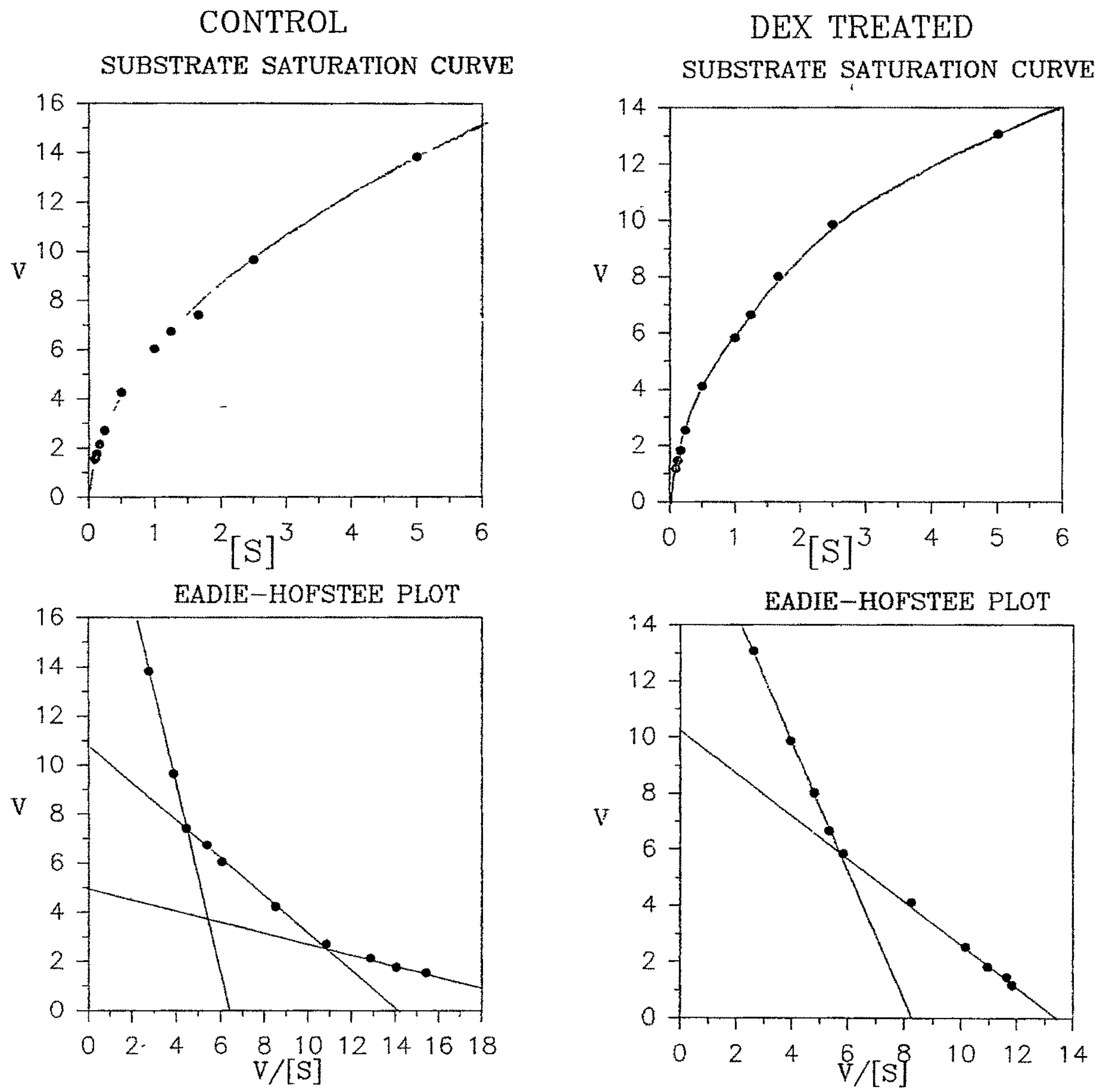
V/[S]

Fig. 6



CONTROL
SUBSTRATE SATURATION CURVE
V
[S]
EADIE-HOFSTEE PLOT
V
V/[S]
DEX TREATED
SUBSTRATE SATURATION CURVE
V
[S]
EADIE-HOFSTEE PLOT
V
V/[S]

the ages the three component had comparable value of Km and Vmax (Table 1). In 2 week group dexamethasone treatment resulted in general decrease of Vmax and lowering of Km of component II , in 4 week group component II was absent and the Vmax of the remaining two component decreased drastically, Km of I[st] component increases In the 5 week group, dexamethasone treatment resulted in a generalized decrease in Vmax The Km of component III decreased by half The decrease in Km of component III in 5 week and adults may be a compensatory mechanism The 3 week dexamethasone group showed allosteric pattern which is shown in corresponding Hill plot The Hill plot analysis revealed that at low concentration range (up to 0 63 mM) the enzyme bound 1 ATP molecule and at high range 2 ATP molecules were bound (Table 2).

Dexamethasone treatment also affected the temperature dependence of the enzyme Which is depicted in Fig 7-11 The corresponding Arrhenius plots are also included The effect of dexamethasone treatment in the different age group were differential As can be seen the value of energy of activation (Table 3) were more or less comparable belonging to different age groups and the phase transition temperatures were also more or less comparable Dexamethasone treatment was effective in lowering the energy of activation especially in the 5 week and adult animals Dexamethasone treatment resulted in a substantial increase in the phase transition temperature in the 5 week group, in the adults the effects was of opposite nature

Discussion

From the data presented, it can be noted that in all the control groups the ATPase activity resolved in 3 component and the Km and Vmax values were more or less comparable (Fig 2-6, Table 1) Dexamethasone treatment had variable effects. Thus dexamethasone

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

Group	Treatment	Component I Km 1	Component I V max 1	Component II Km 2	Component II V max 2	Component III Km 3	Component III V max 3
2 week	Control	0 24±0 05	4 51±0 32	1 16±0 12	11 27±0 80	6 03±0 31	33 89±2 50
	Dex	0 24±0 03	3 01±0 19 [b]	0 63±0 05 [b]	5 79±0 12 [c]	4 13±0 41 [b]	14 37±0 61 [c]
3 week	Control	0 10±0 02	5 90±0 52	0 76±0 08	13 02±1 20	6 08±0 25	40 14±2 86
	Dex	-- --	-- --				
4 week	Control	0 18±0 04	6 92±0 36	0 98±0 15	19 44±2 13	3 37±0 16	42 09±2 81
	Dex	0 37±0 08 [a]	2 75±0 25 [c]	--	--	5 19±0 72 [a]	12 81±1 62 [c]
5 week	Control	0 17±0 04	8 67±0 69	0 58±0 08	15 23±0 81	4 94±0 81	47 95±5 10
	Dex	0 11±0 02	3 87±0 26	0 58±0 10	8 56±1 00 [a]	2 84±0 44 [a]	22 70±2 39 [c]
Adult	Control	0 17±0 03	5 75±0 86	0 71±0 07	12 54±0 96	4 32±0 81	29 18±3 36
	Dex	--	--	0 70±0 13	11 44±1 39	2 48±0.40 [b]	24 27±2 55

The experimental details are as given in the text

The results are given as mean ± SEM of the 8 independent observations For the substrate ATP was used in the concentration range of 0 01 to 5 mM

Km, mM,

Vmax , 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] $p < 0\ 002$ and [c] $p < 0\ 001$ compared with the corresponding control

Table 2. Effect of dexamethasone treatment on Hill plot analysis of rat liver mitochondrial ATPase.

Group	Treatment	n_1	$S_{0\ 5\ (1)}$	Transition mM	n_2	$S_{0\ 5\ (2)}$
3 week	Dex (8)	0 496±0 12	3 400±0 240	0 62±0 042	1 757±0 21	2 876±0 64

The experimental details are as given in the text

The results are given as mean ± SEM of the 8 independent observations For the substrate ATP was used in the concentration range of 0 01 to 5 mM

$S_{0\ 5(1)}$ and $S_{0\ 5\ (2)}$, mM, n_1 and n_2 are Hill co-efficients

Fig. 7 to 11. Typical temperature curves and the respective Arrhenius plots for rat li mitochondrial ATPase in controls and dexamethasone treated animals Experimental details as given in the text The ATPase activity was determined with 5 mM ATP The absc represents the reaction velocity v, while the ordinate represents the temperature in °C Reac velocity v = μmol of Pi liberated hr^{-1} mg $protein^{-1}$ For the Arrhenius plots abscissa represents log 'of reaction velocity v, while the ordinate represents reciprocal of absolute tempera T*1000 Reaction velocity v = μmol of Pi liberated hr^{-1} mg $protein^{-1}$ Absolute temperature °Kelvin

Fig.7.

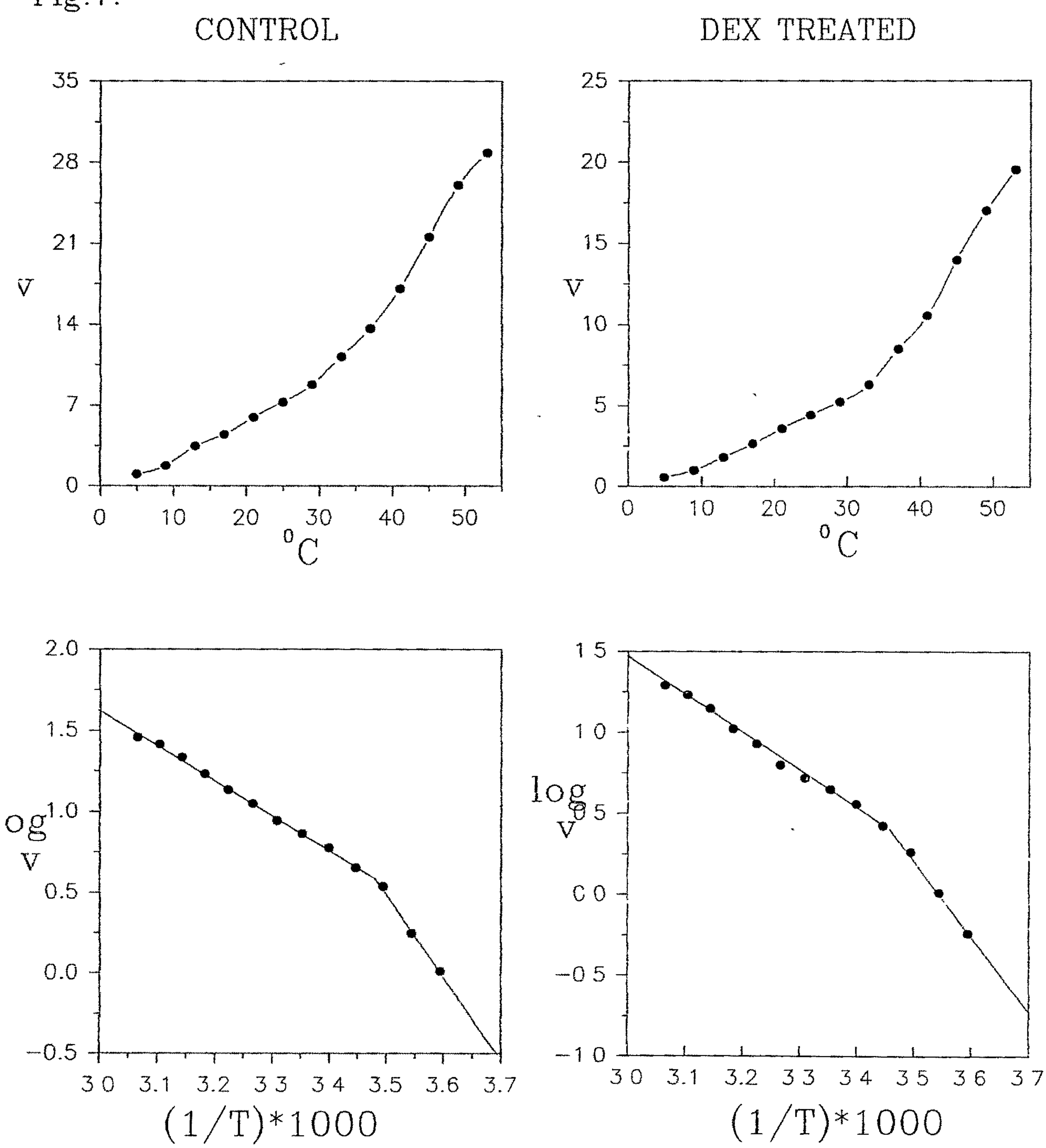


Fig.8.

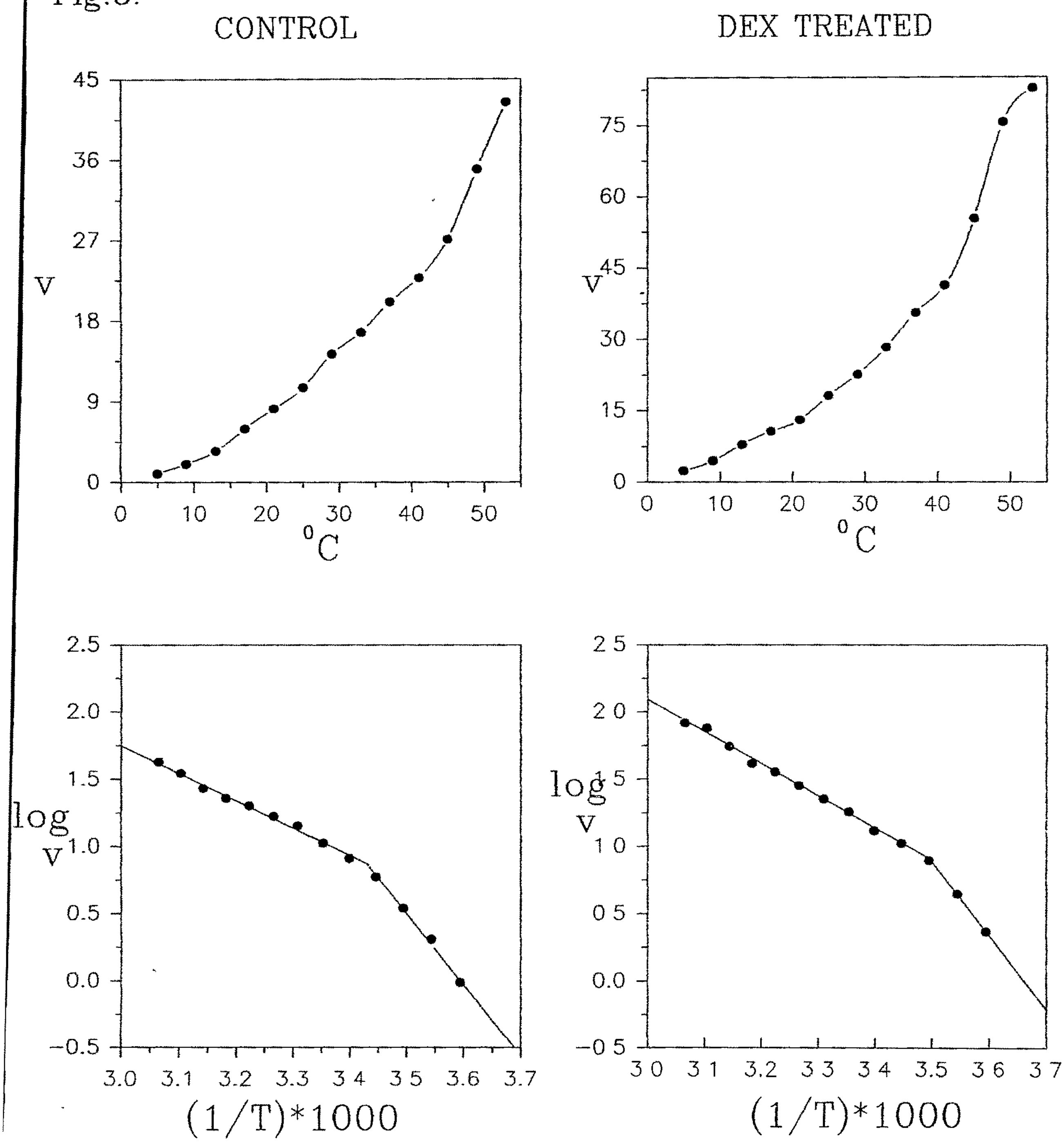


Fig.9

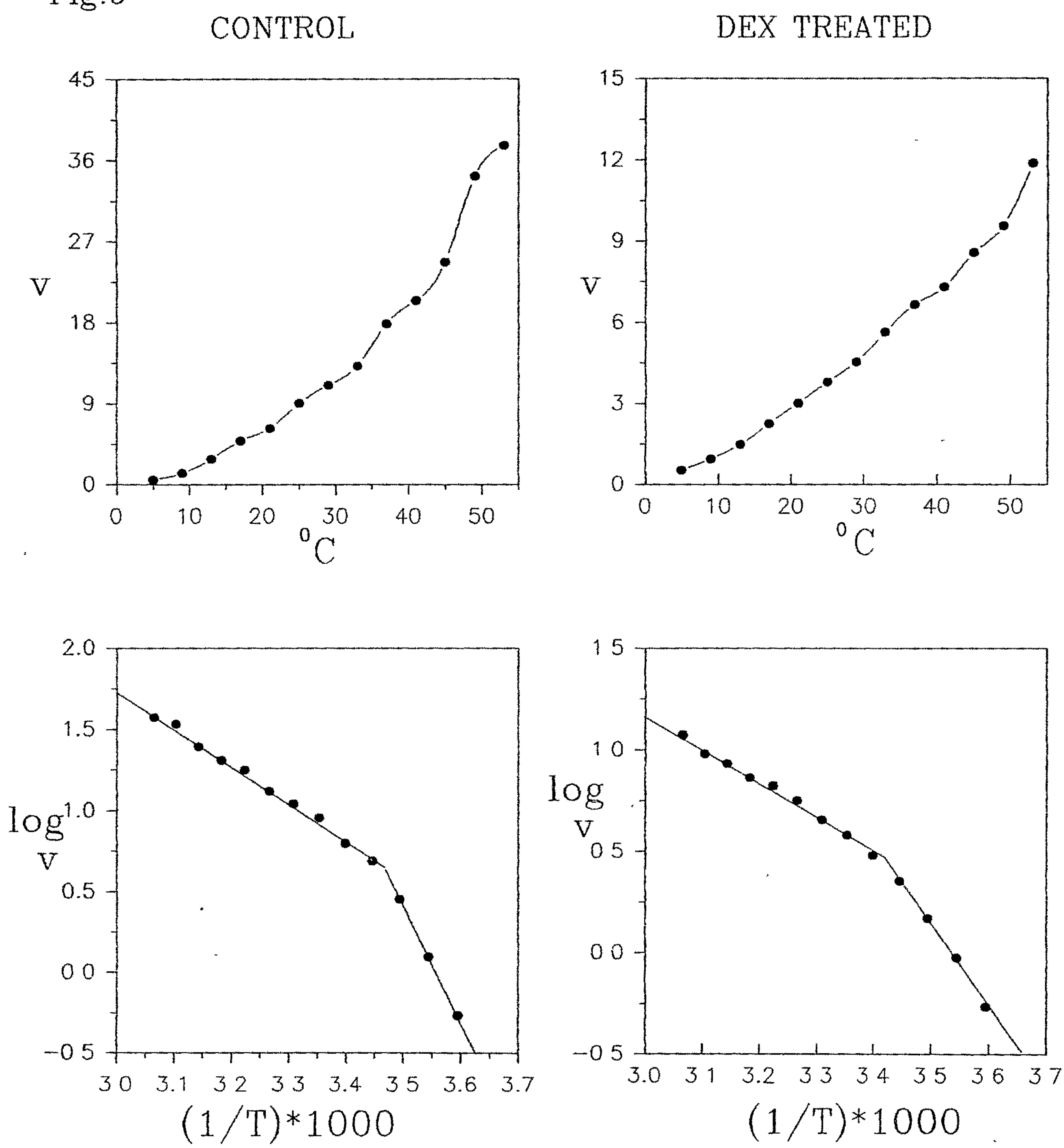

CONTROL
DEX TREATED
45
36
27
18
9
0
V
0
10
20
30
40
50
°C
15
12
9
6
3
0
V
0
10
20
30
40
50
°C
2.0
1.5
1.0
0.5
0 0
−0 5
log
V
3 0
3.1
3 2
3 3
3.4
3 5
3.6
3.7
(1/T)*1000
1 5
1 0
0 5
0 0
−0 5
log
V
3.0
3 1
3.2
3 3
3 4
3 5
3 6
3 7
(1/T)*1000

Fig.10.

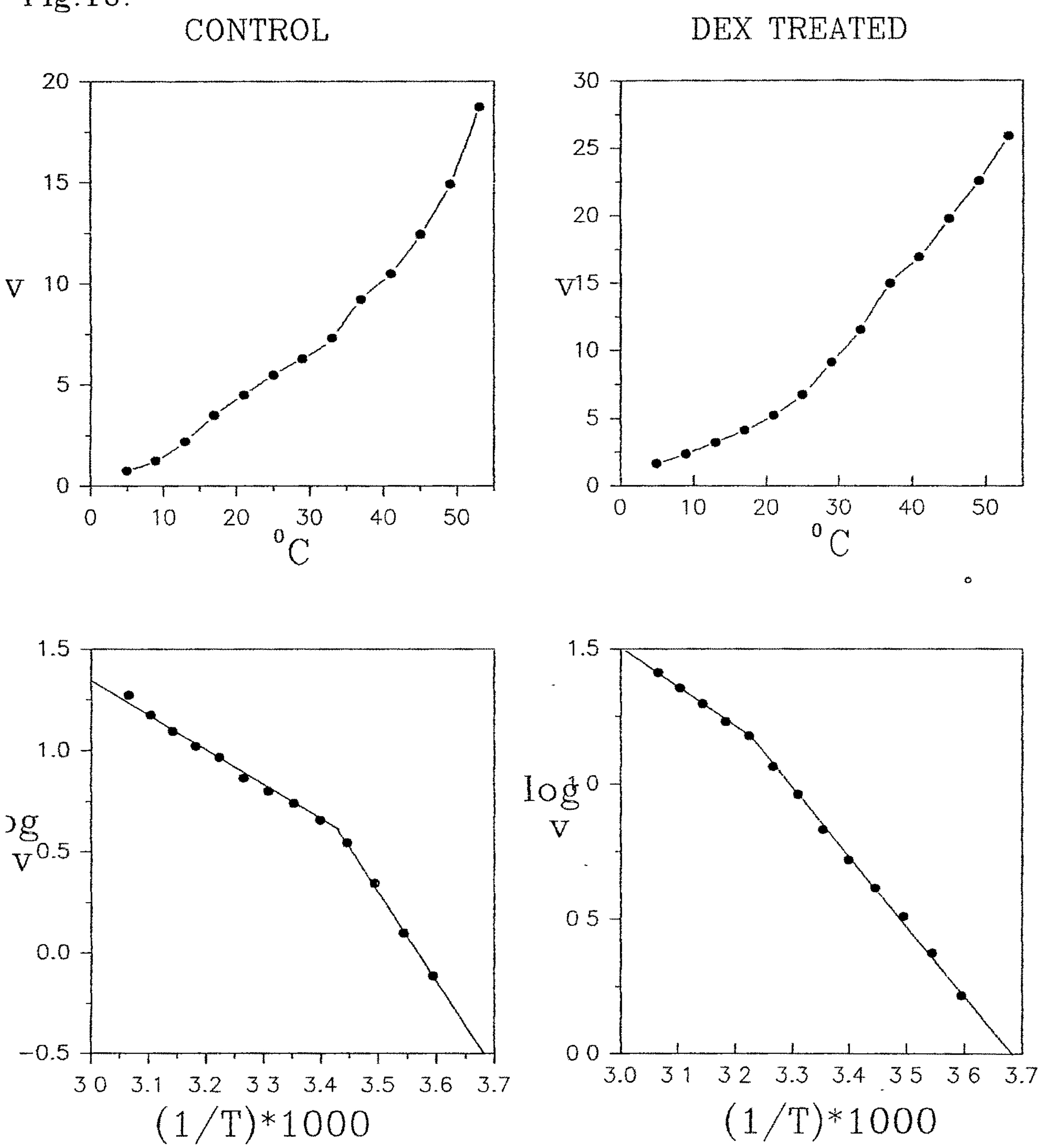

CONTROL
DEX TREATED
V
0
5
10
15
20
25
30
°C
log
V
1.5
1.0
0.5
0.0
-0.5
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
(1/T)*1000

Fig.11.

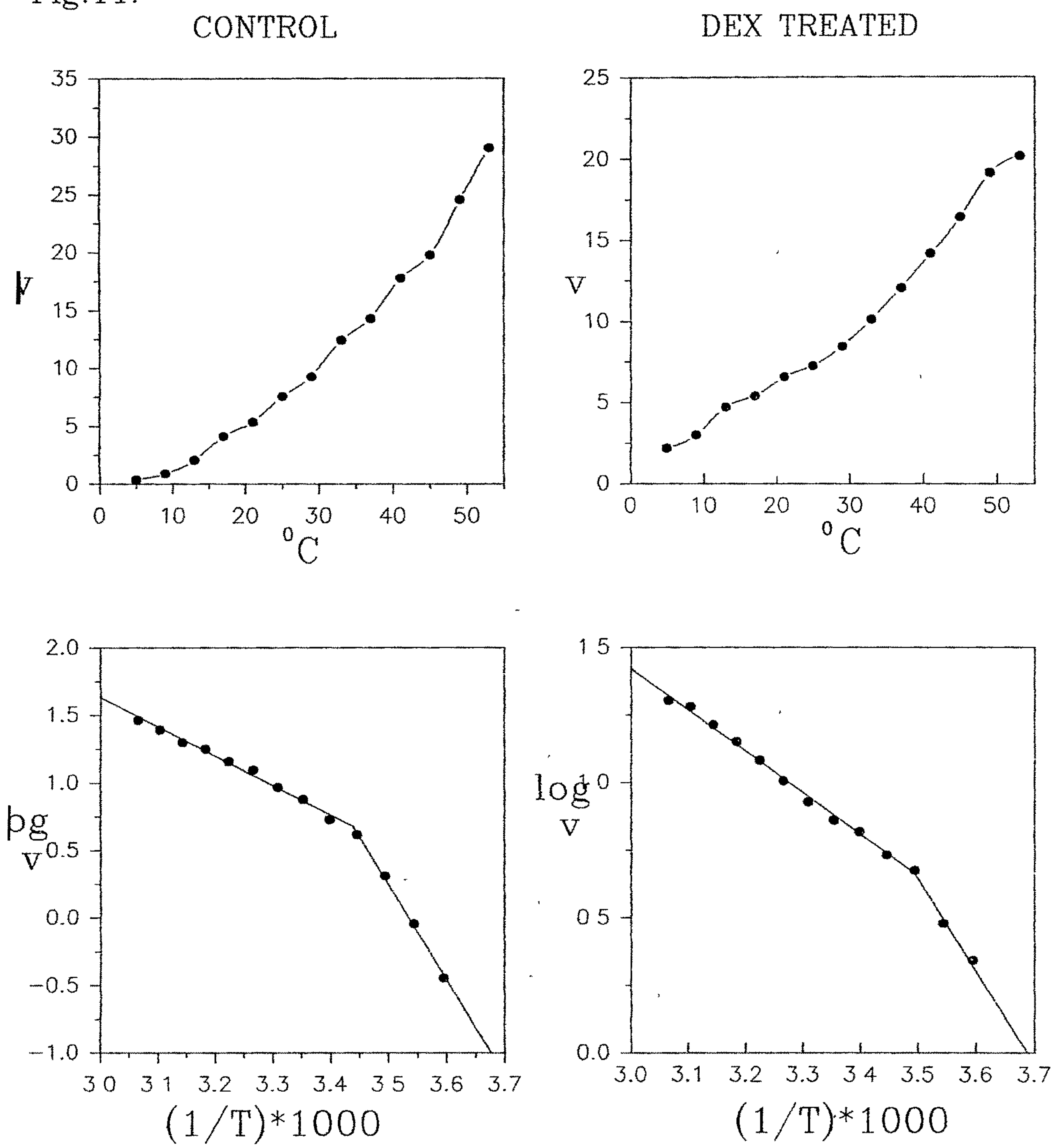

CONTROL
DEX TREATED
V
°C
log V
(1/T)*1000
35
30
25
20
15
10
5
0
0
10
20
30
40
50
2.0
1.5
1.0
0.5
0.0
−0.5
−1.0
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7

Table 3 Effect of dexamethasone treatment on Arrhenius kinetics properties of rat liver mitochondrial ATPase

Group	Treatment	(Energy of activation, KJ/mole) E_1	E_2	Phase transition temperature Tt (°C), Tt_1
2 week	Control	41 42±1 34	97 56±6 96	14 99±0 79
	Dex	43 48±4 82	94 34±5 88	17 21±0 70
3 week	Control	32 31±1 38	102 38±2 40	17 79±0 55
	Dex	37 82±1 71 [a]	84 43±5 35 [a]	17 47±0 61
4 week	Control	51 63±1 81	160 36±12 87	15 74±0 73
	Dex	38 78±1 70	93 91±8 52	16 82±1 28 [a]
5 week	Control	39 58±1 46	108 85±10 70	11 54±1 05
	Dex	23 22±1 01 [d]	60 91±2 001 [d]	32 01±2 82 [d]
Adult	Control	35 85±2 19	132 29±11 45	15 29±1 10
	Dex	25 71±1 47 [c]	93 23±12 09 [a]	11 27±0 53 [a]

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

[a] p< 0 05, [b] p<0 02, [c] p<0 01 and [d] p<0 001 compared with the corresponding control

treatment in 2 week group resulted in a general lowering of the Km and Vmax of all the components except for the Km of component I In the 3 week group the characteristic changed to allosetric pattern In the 4 week group component II was abolished, while for the remaining components Km increased and Vmax decreased In 5 week group Vmax of component III decreased in the adults component I was abolished

The observed changes however did not correlate with changes in the membrane lipids Dexamethasone treatment had a generalized effect of lowering of energies of activation these changes correlated positively with TPL/PS and DPG/BPL (basic phospholipid) The changes in E_2 correlated positively with APL (acidic phospholipid) and APL/BPL ratio Interestingly, the phase transition temperature correlated negatively with APL and positively with BPL Since major correlation were seen with total acidic phospholipid and total basic phospholipid but not with individual acidic or basic phospholipids hence it may have a modulatory role (Table 4)

It is possible that the changes in phase transition might correlate with changes in fatty acid composition which dexamethasone has been reported to bring about (7), however this possibility have not been examined in present studies

In conclusion dexamethasone treatment resulted in age specific changes in the kinetic properties of liver mitochondrial Fo F1 ATPase The generalized decrease in the ATPase activity correlate well with decrease ADP phosphorylation reported in Chapter 5

Table 4 Regression analysis of ATPase parameters with lipid parameter

Parameter	Regression coefficient (r)
Energy of activation E_1	
TPL/PS	0 5773
DPG/BPL	0 7071
Energy of activation E_2	
APL	0 7032
APL/BPL	0 6777
Phase transition temperature Tt	
APL	-0 5728
BPL	0 7300

APL, acidic phospholipid, BPL, basicc phospholipid, DPG, Diphosphotidylglycerol, PS, Phosphatidylserine, TPL, total phospholipid

Summary

Dexamethasone treatment resulted in generalized decrease in ATPase activity in all the age groups The normal substrate saturation pattern was noted for all the age groups irrespective of treatment except in the three week dexamethasone group where sigmoidal pattern was observed Eadie-Hofstee plot for all the group except three week animals, resolved ATPase activity in three components and age-specific changes were observed in Km and Vmax after dexamethasone treatment Hill plot analysis for 3 week dexamethasone suggested that at lower concentration one ATP molecule binds and at concentration higher than 0 6mM 2 ATP molecules bind with enzyme Arrhenius kinetics revealed that the dexamethasone treatment lowers the energy of activation in 5 week and adult animals Also the dexamethasone treatment resulted in a substantial increase in the phase transition temperature in 5 week group whereas in adults opposite effect was observed

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In conclusion dexamethasone treatment resulted in age specific changes in the kinetic properties of liver mitochondrial Fo F1 ATPase The generalized decrease in the ATPase activity correlate well with decrease ADP phosphorylation reported in Chapter 5