CHAPTER : 8
Effect of alloxan-diabetes on the temperature kinetics of cardiac butyrylcholinesterase in male and female rats.

Introduction

As described in the previous Chapter 7 studies were extended our study to find out the effect of diabetic state and insulin treatment on temperature kinetics of BChE in soluble and membrane-bound fraction of heart.

Materials and Methods

Animals were made diabetic as described in Chapter 4 of the thesis (1). Insulin treatment (2) was given to the diabetic rats as described in Chapter 4 of the thesis. Isolation of soluble and membrane-bound forms of cholinesterases was achieved essentially by following the procedure of Bisso et al (3) as described earlier (5) in the Chapter 7 of the thesis. Butyrylcholinesterase (BChE) activity was assayed as described earlier (4, 5) in Chapter 3 of the thesis. Temperature kinetics of BChE was performed as described in Chapter 3 of the thesis (6, 7).

Results

Typical temperature curves for BChE from soluble fraction of male heart are shown in the Figure 1, and the typical Arrhenius plots are presented in Figure 2. Data in Table 1 give results on energies of activation and phase transition temperature for BChE from soluble fraction of male rat heart. It is evident that in the controls for higher temperature

Figure 1.

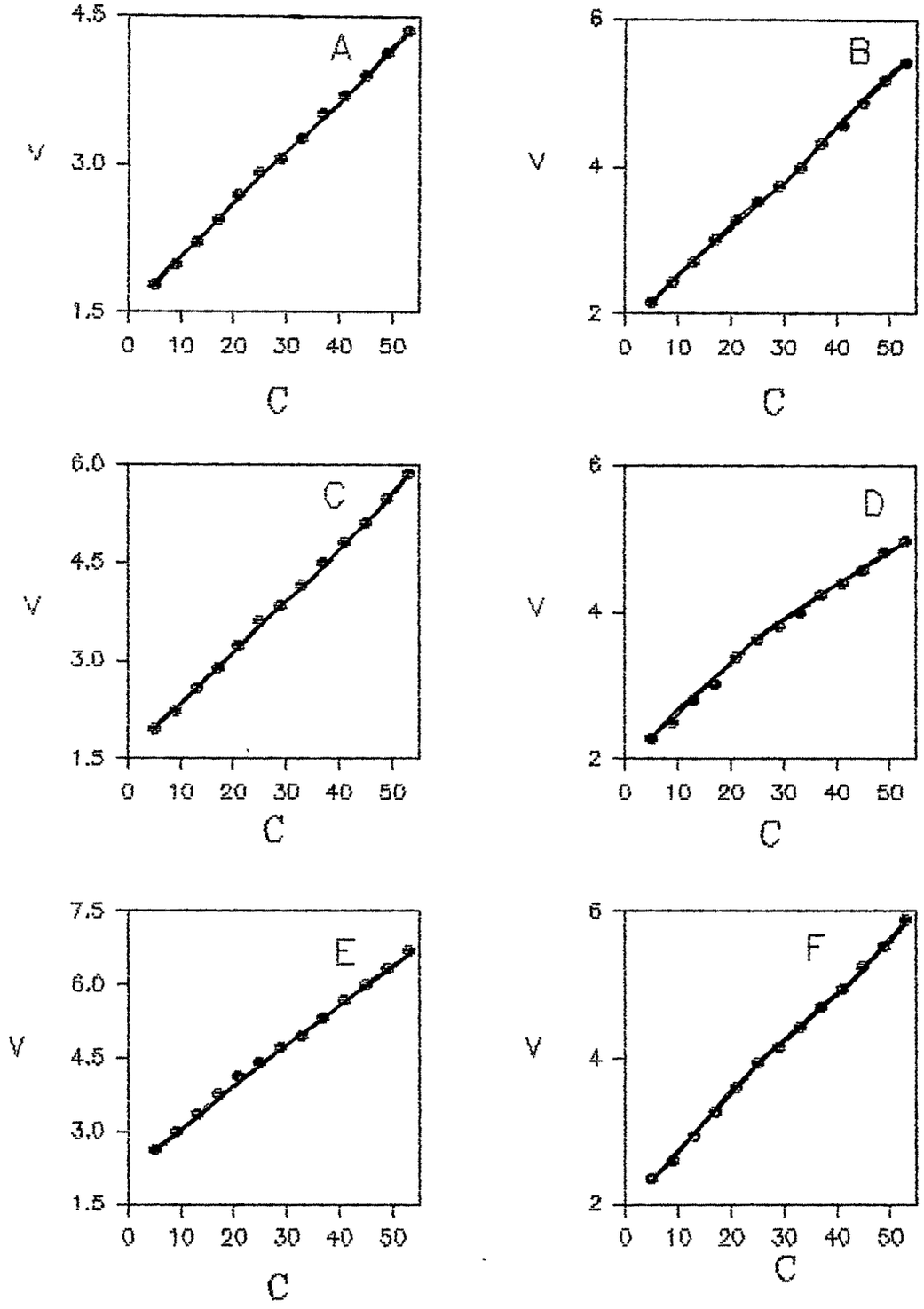

Figure 2.

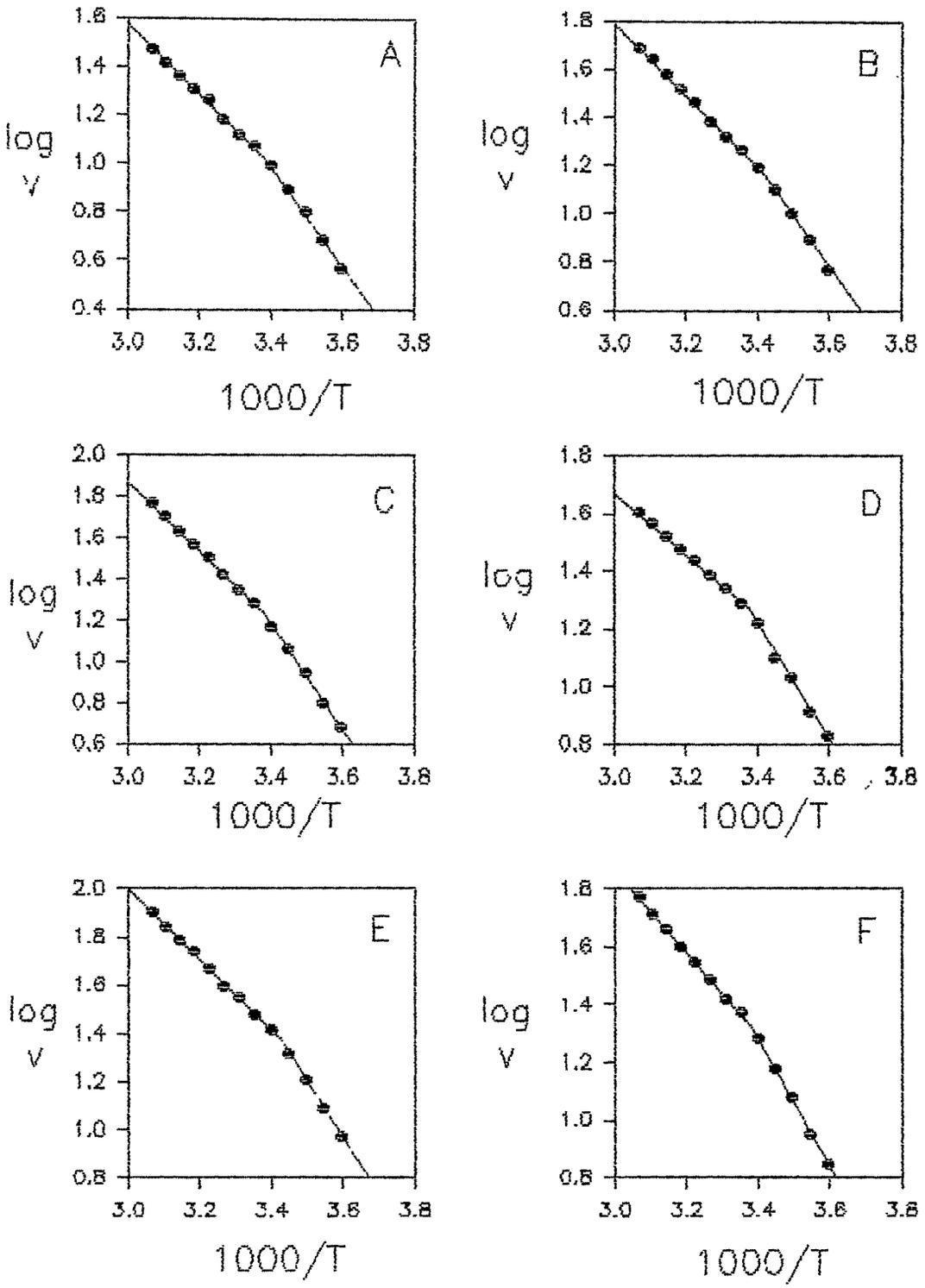

Table 1. Temperature kinetic of soluble form of cardiac BChE from male rats.

Animals	Energy of activation (KJ / mole)		Phase transition temperature (Tt), ^{o}C
	E1	E2	
Control(10)	27.0±0.78	41.1±1.18	23.8±0.46
Diabetic(8)	31.0±0.80 [a]	47.7±1.17 [b]	23.8±0.98
Insulin treated diabetic(10)	25.7±1.04 [#]	45.7±2.80	19.5±0.90 [b,@]

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

a, $p<0.005$ and b, $p<0.001$ compared with corresponding control.

@, $p<0.005$ and #, $p<0.001$ compared with corresponding diabetic.

range the energies of activation (E1) was 27.0 KJ/mole, while for lower temperature range the value of E2 was 41.1 KJ/mole and the phase transition temperature (Tt) was 23.8^{o}C. In diabetic condition there was a small (15 and 16 %) increase in the values of E1 and E2, while the phase transition temperature (Tt) was comparable with the control rats. After insulin treatment E1 and E2 values were normalized to the control level but the phase transition temperature decreased significantly by about 4^{o}C.

Typical temperature curves for BChE from soluble fraction of female rat heart are also shown in Figure 1 and Figure 2 shows the typical Arrhenius plots. Data in Table 2 summarize temperature kinetics results. In the control females for higher temperature range energy of activation E1 was 28.0 KJ/mole, while for lower temperature range energy of activation E2 was 42.2 KJ/mole and phase transition temperature Tt was 21.6^{o}C. Values of E1, E2 and Tt compared well with those for the male rats. In diabetic condition E1 value decreased significantly by 18 % and after insulin treatment E1 value normalized and was comparable to the control female rats. E1 value was comparable in three female groups. Tt increased in the diabetic group by 3.2^{o}C and insulin treatment was ineffective in restoring this near to control value.

Typical temperature curves and Arrhenius plots of BChE from membrane-bound fraction of male rat heart are shown in

Table 2. Temperature kinetic of soluble form of cardiac BChE from female rats.

Animals	Energy of activation (KJ / mole)		Phase transition temperature (Tt), ^{o}C
	E1	E2	
Control(10)	28.0±1.36	42.2±2.35	21.6±0.74
Diabetic(8)	22.9±1.20[a]	39.2±1.00	24.8±0.60[b]
Insulin treated diabetic(9)	25.1±1.23	41.5±1.46	25.3±0.78

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

a, $p<0.02$ and b, $p<0.005$ compared with corresponding control.

Figures 3 and 4 respectively. Data in Table 3 summarize temperature kinetics analysis. In the control male, for the higher temperature range energy of activation E1 was 29.9 KJ/mole, while for lower temperature range energy of activation E2 was 41.4 KJ/mole and phase transition temperature Tt was 25.1^{o}C. In diabetic condition no significant change was observed in E1, E2 and Tt values. After insulin treatment there was a small (9%) but reproducible increase in the E1 value; other parameters were not affected.

Typical temperature curves of BChE from membrane-bound fraction of female rat heart are also shown in Figure 3. Corresponding typical Arrhenius plots are shown in Figure 4. Data in Table 4 give the results on temperature kinetics analysis. In control female rats the pattern was almost comparable to the males except that the E2 was somewhat lower (13%). In diabetic condition the E1 and Tt value were comparable to the control but the E2 value increased by 14 %. In insulin treated diabetic females, the value of E1 was comparable to the diabetic group.

Discussion

The data in the earlier chapter (Chapter 7) suggest that the cardiac membranes were differentially affected in diabetic state and after insulin treatment in the male and the female

Figure 3.

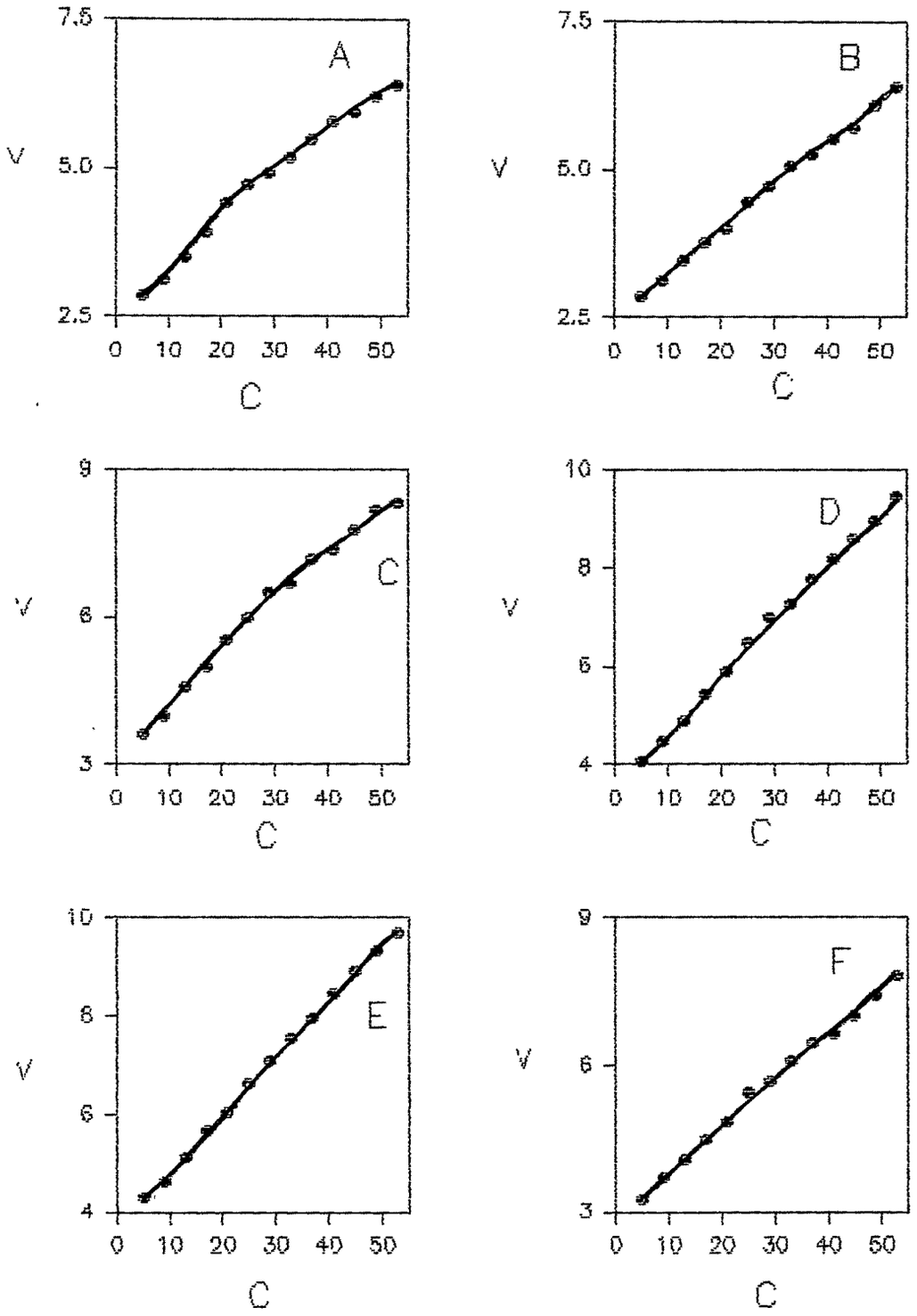

Figure 4.

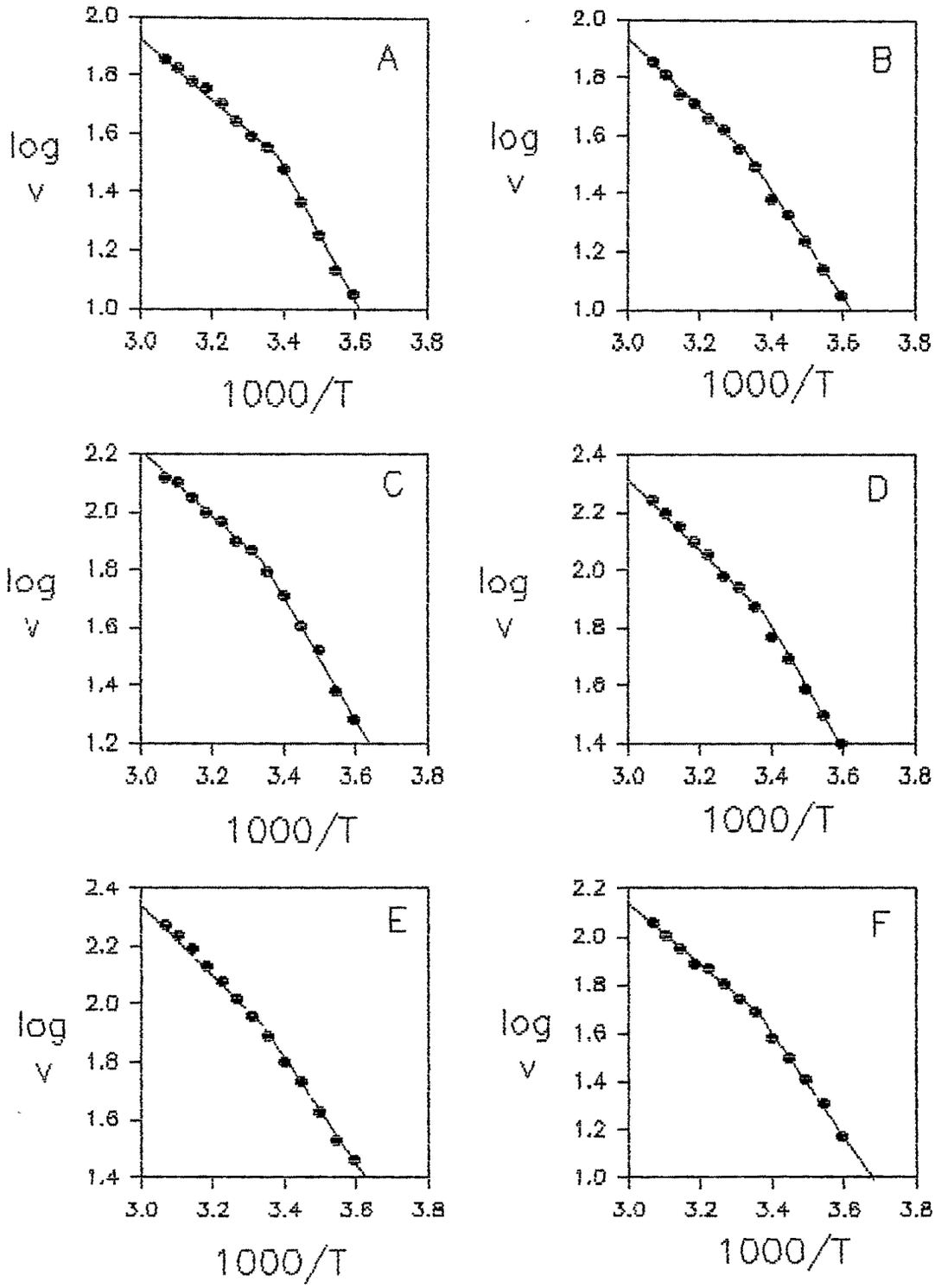

Table 3. Temperature kinetic of membrane-bound form of cardiac BChE from male rats.

Animals	Energy of activation (KJ / mole)		Phase transition temperature (Tt), ^{o}C
	E1	E2	
Control(8)	21.9±0.48	41.4±1.31	25.1±0.97
Diabetic(10)	22.1±0.57	44.0±1.45	22.9±0.57
Insulin treated diabetic(11)	24.0±0.24 a,#	40.3±1.65	24.4±1.15

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

a, $p<0.002$ compared with corresponding control.

#, $p<0.01$ compared with corresponding diabetic.

Table 4. Temperature kinetic of membrane-bound form of cardiac BChE from female rats.

Animals	Energy of activation (KJ / mole)		Phase transition temperature (Tt), ^{o}C
	E1	E2	
Control(7)	22.9±0.90	36.6±2.09	23.3±1.45
Diabetic(10)	22.0±0.97	42.4±1.56[a]	22.8±0.76
Insulin treated diabetic(9)	24.8±0.43[#]	40.1±0.71	24.3±0.70

Results are expressed as mean±S.E.M. of number of independent observations indicated in the parentheses.

a, $p<0.05$ compared with corresponding control.

#, $p<0.02$ compared with corresponding diabetic.

rats. The present studies are extension of these observations. Temperature kinetics data when analyzed in terms of the Arrhenius plots reflect on the membrane function (7).

From the data presented it is clear that the differential effects of diabetic state and insulin treatment could be noted in the soluble as well as the membrane-bound form of BChE in the males and females.

Thus in the males for the soluble BChE, the trend was increased value of E1 and E2 which was partly restored by insulin treatment; simultaneously the Tt decreased significantly in the insulin-treated group. For the females the effect was that the value of E1 only decreased in diabetic state.

Compared to this, the picture for the membrane-bound BChE was very different. In the males, there was a small but reproducible increase in E1 especially after insulin-treatment. As against this, in the females the values of both E1 and E2 increased and the trend continued even after insulin-treatment.

The results thus substantiate the notion that the cardiac membrane and membrane function are differentially affected in the males and females in diabetes (eg. see Chapter 7 of the thesis).

The observed high incidences of coronary heart disease (CHD) and congestive heart failure (CHF) in diabetic females, may possibly relates to these differential effects.

Summary

Temperature kinetics of cardiac BChE revealed that in the male rats for the soluble for of BChE, E1 and E2 values increased in diabetics and were partly restored by insulin treatment; simultaneously the Tt decreased significantly in the insulin-treated group.

In the female rats for the soluble BChE only value of E1 decreased in diabetic state.

In the male rats for the membrane-bound BChE a small but reproducible increase was observed in the value of E1 after insulin treatment, while in the females, for membrane-bound BChE E1 and E2 value increased in diabetic condition and the trend continued even after insulin-treatment.

Figure legends

Figure 1 Typical temperature curves for rat heart soluble fraction of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text.
Enzyme activity v in n mole/min/mg protein.

Figure 2 Typical Arrhenius plots for rat heart soluble fraction of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text and in legend to Figure 1.

Figure 3 Typical temperature curves for rat heart membrane bound form of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text.
Enzyme activity v in n mole/min/mg protein.

Figure 4 Typical Arrhenius plots for rat heart membrane bound form of BChE. (A) Control male (B) Control female (C) Diabetic male (D) Diabetic female (E) Insulin treated diabetic male (F) Insulin treated diabetic female rats. Other experimental details are as described in the text and legend to Figure 3.

References

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