CHAPTER : 6

Effect of alloxan-diabetes on the kinetics attributes of serum butyrylcholinesterase in male and female rats.

Introduction

Cholinesterases are responsible for the rapid elimination of acetylcholine, within one millisecond after its release at cholinergic synapses, thus allowing precise temporal control of muscle contraction (1). Vertebrates in particular possess two cholinesterases corresponding to two distinct genes : acetylcholinesterase (AChE, E.C. 3.1.1.7.) and butyrylcholinesterase (BChE, E.C. 3.1.1.8.). The two enzymes are distinguished primarily on the basis of their substrate specificity : AChE hydrolyzes acetylcholine faster than other choline esters and is much less active on butyrylcholine. In contrast, BChE hydrolyzes butyrylcholine well but also hydrolyzes acetylcholine. The two enzymes may also be distinguished by their affinity for, or reactivity with, various selective inhibitors, such as BW 284C51 for AChE and ethopropazine, iso-OMPA and bambuterol for BChE (2). The distribution of BChE transcripts was analyzed by northern blots in human tissues : their level is very high in liver, high in the lungs, low in brain and heart, barely detectable in muscle, kidney and pancreas and apparently absent in placenta (3). BChE is synthesized in the liver and secreted into the plasma (4).

The major component of human plasma BChE corresponds to tetramers, composed of two disulfide-linked dimers (5). Non-denaturing electrophoresis reveals the presence of monomers (called C1 in this system) and dimers (C3) in addition to the

tetramers (C4) (6).

The function of BChE remains a puzzle. BChE is involved in the degradation of succinylcholine, used as a myorelaxant in surgical operations. It also hydrolyzes drugs such as heroin (7, 8). Although it has been suggested that it is involved in the detoxification of plant esters and phytotoxins ingested in the diet (9) or in the regulation of plasma choline levels (10), other evidences suggest that BChE is involved in lipid and lipoprotein metabolism (11-13). Serum BChE activity was positively correlated with serum triacylglycerol concentration in hypertensive patients (14), obese patients (15) and patients with hypercholesterolemia (16) and hyperlipoproteinemia (17, 18).

The induction of diabetes with streptozotocin in rats caused a significant rise in serum BChE activity in diabetic rat, mouse and human (19). BChE activity increased significantly in liver and adipose tissue in alloxan-diabetic rats (20). Sex difference was observed in both basal and increase in activity in plasma, liver, pancreas and adipose tissue (20).

The object of the present study was to investigate the effect of alloxan diabetes on serum BChE activity in rats belonging to both the sexes. The substrate kinetics of serum BChE was also checked. Studies were extended to check temperature dependence of the enzyme activity.

Materials and Methods

Animals were made diabetic as described in Chapter 4 of the thesis (21). Insulin treatment (22) was given to diabetic rats as described in chapter 4 of the thesis.

Isolation of serum

The blood was allowed to clot at room temperature and sera were collected after centrifugation a 475 X g for 8 min in a clinical centrifuge. The straw colored supernatant was carefully decanted. Measurements of BChE activity were carried out as described earlier (23, 24) in Chapter 3 of the thesis. substrate (25) and temperature kinetics (26) determinations were as described in Chapter 3 of the thesis.

Results

Data in Table 1 show the BChE activity in serum of rats belonging to both the sexes. In control male rats the BChE activity in the serum was 215 n mole/min/ml, while it was 4.4 fold higher in the control female rats. In diabetic male rats the activity increased significantly by 2.3 fold and after insulin treatment the activity decreased by 21 %. However the change was not statistically significant. By contrast, in the females the activity was more or less the same in the three groups, i.e. control, diabetic and insulin-treated diabetic.

Table 1. Effect of alloxan-diabetes on serum BChE activity in the male and female rats.

Animals	Control	Diabetic	Insulin treated diabetic
Male	214.7±10.0(9)	497.1±45.3(10)[a]	394.3±30.8(7)[a]
Female	941.2±95.2(9)	957.9±83.3(10)	887.5±90.3(7)

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

BChE activity = n mole / min / ml serum.

a, $p<0.001$ compared with corresponding control.

Typical substrate saturation curves and the corresponding typical Eadie-Hofstee plots for serum BChE for both male and female rats are shown in Figure 1 and 2 respectively. From Figure 2 it can be inferred that two components of BChE are present in the serum of the male rats from the three groups.

Data in Table 1 show Km and Vmax values derived from substrate kinetic analysis for serum BChE from the male rats. It is clear that component I has the Km of about 0.5 mM and Vmax of 146 n mole/min/ml serum. The second component has high Km (2.16 mM) and high Vmax (264 n moles/min/ml serum). In diabetic condition Km of component II increased by 65% and the Vmax of both the components increased by 63 % and 144% respectively. All the changes were statistically significant. After insulin treatment Km of component II decreased (35 % decrease) and became comparable with the controls. The Vmax of component I was comparable with the diabetic male rats and thus was 65 % higher than the control male rats. However, the Vmax of component II decreased by 27 % as compared to the diabetics, but was sill about 80 % higher than in the controls.

Typical substrate saturation curves and Eadie-Hofstee plots for the serum BChE from female rats are also shown in the Figure 1 and 2. As can be seen from the typical Eadie-Hofstee plots (Figure 2) two components of BChE were present even in the serum of the female rats. Data in Table 3 summarize the

Figure 1.

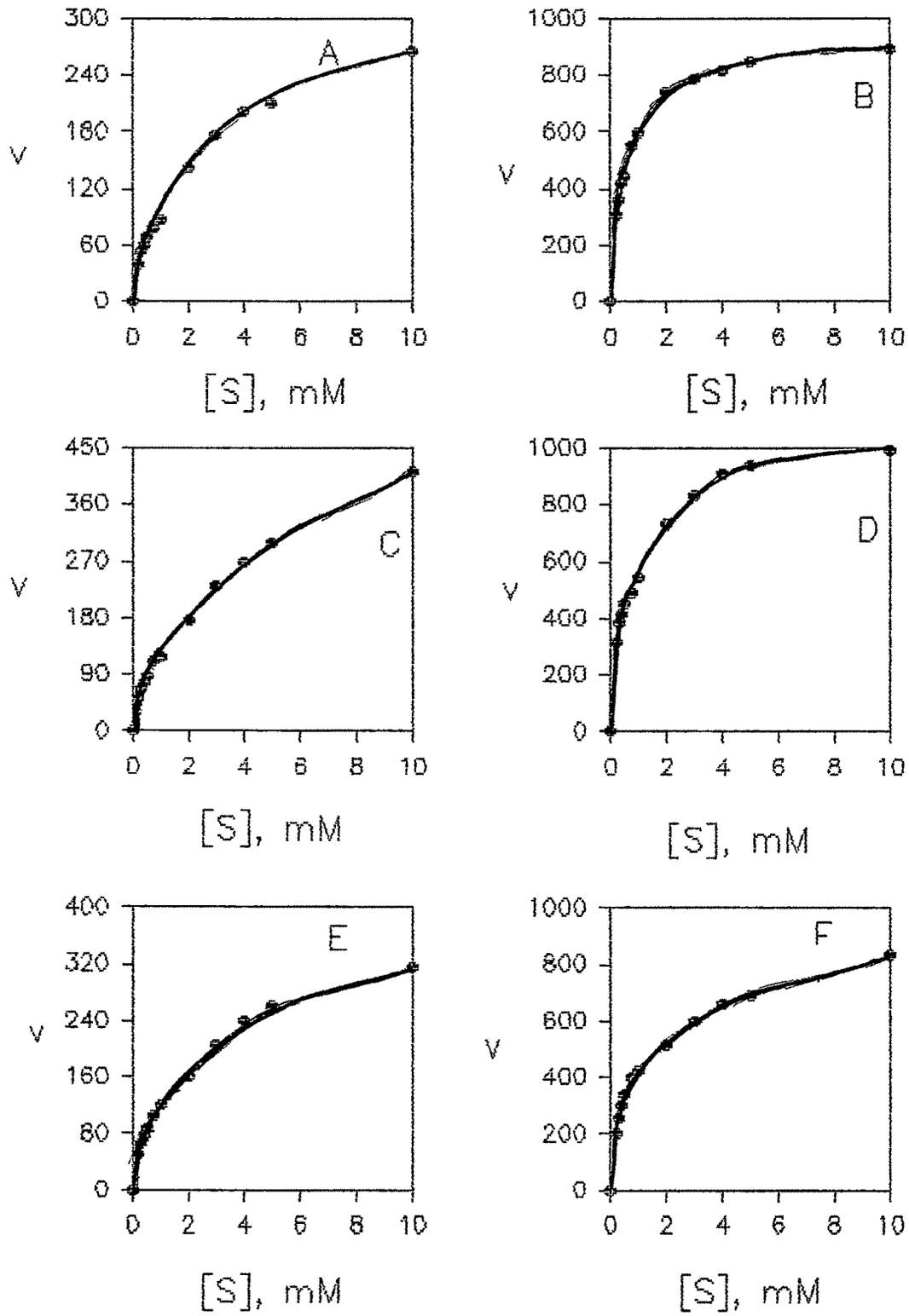
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Figure 2.

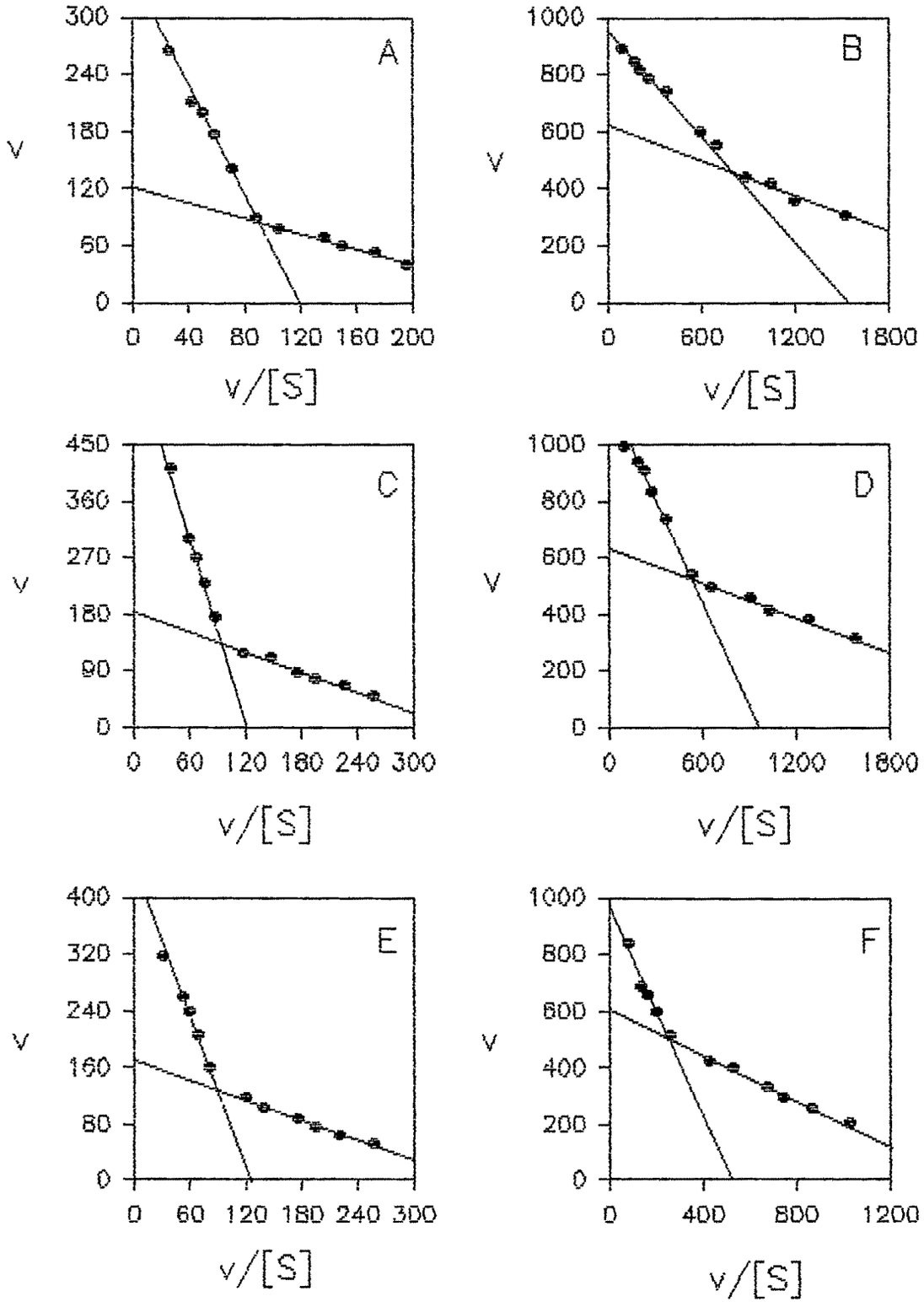
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Table 2. Effect of alloxan diabetes on the substrate kinetics of serum BChE in the male rats.

	Component I		Component II	
	Km	Vmax	Km	Vmax
Control (9)	0.49±0.055	145.6±13.5	2.16±0.19	263.6±13.1
Diabetic (10)	0.51±0.077	236.8±34.6[a]	3.56±0.48[a]	643.7±57.3[c]
Insulin treated diabetic (7)	0.31±0.070	240.4±22.9[b]	2.32±0.36	471.3±38.6[c,#]

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

Units : Km = mM; Vmax = n mole / min / ml serum.

a, $p<0.05$; b, $p<0.005$ and c, $p<0.001$ compared with corresponding control value.

#, $p<0.05$ compared with corresponding diabetic value.

Table 3. Effect of alloxan diabetes on the substrate kinetics of serum BChE in the female rats.

Animals	Component I		Component II	
	Km	Vmax	Km	Vmax
Control(8)	0.34±0.05	708.4± 80.8	1.13±0.12	1049.1±106.7
Diabetic(10)	0.32±0.05	601.1±105.3	1.73±0.24[a]	1019.5±141.9
Insulin treated diabetic (6)	0.27±0.03	697.9± 56.1	1.32±0.14	1061.7± 64.6

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

Units : Km = mM; Vmax = n mole / min / ml serum.

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

substrate kinetic analysis. In control group component I was having low Km 0.34 mM and low Vmax (708 n moles/min/ml serum), while component II has high Km (1.13 mM) and high Vmax (1049 n mole/min/ml serum). Diabetic state or insulin treatments had only marginal effects.

Typical temperature curves for serum BChE are shown in Figure 3 and corresponding typical Arrhenius plots are shown in Figure 4. The data on temperature kinetic are given in the Table 4.

In the serum from control males, in the higher temperature range the energy of activation E1 was low (23.8 KJ/mole), while for the lower temperature range the energy of activation E2 was high (50.9 KJ/mole) with the phase transition occuring at 21.6^{o}C. In the diabetic male rats E1 value increased by 22 % which was statistically significant. After insulin treatment E1 became comparable to the controls but the E2 value decreased by 29 % compared to the diabetic males and was also somewhat lower (19%) as compared to the control males. The phase transition temperature in three groups was comparable.

Typical temperature curve for serum BChE from female rats are shown in Figure 3 and the typical Arrhenius plots are shown in Figure 4. Data in Table 5 summarize the temperature kinetics properties. Thus in the control female rats also for

Figure 3.

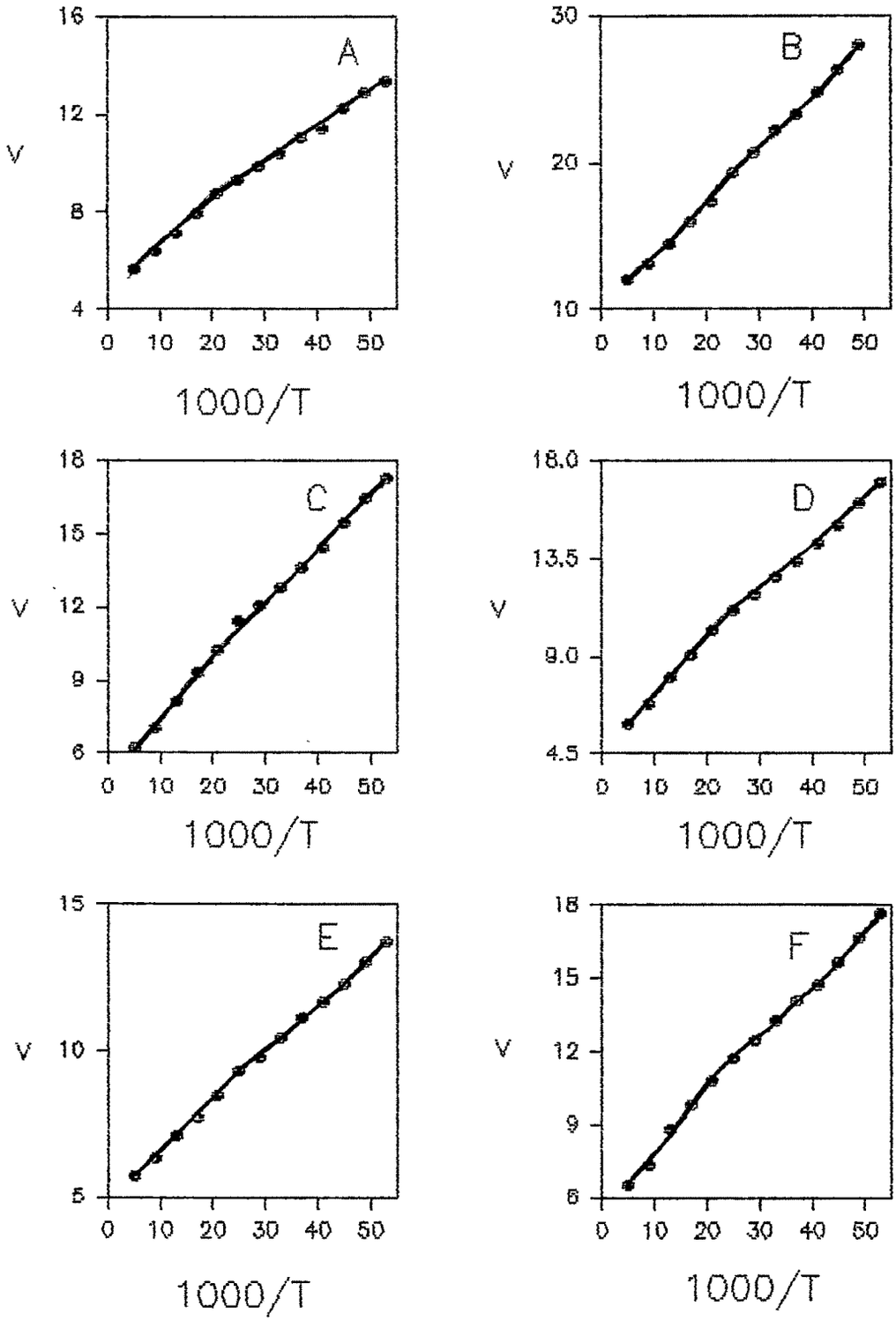
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Figure 4.

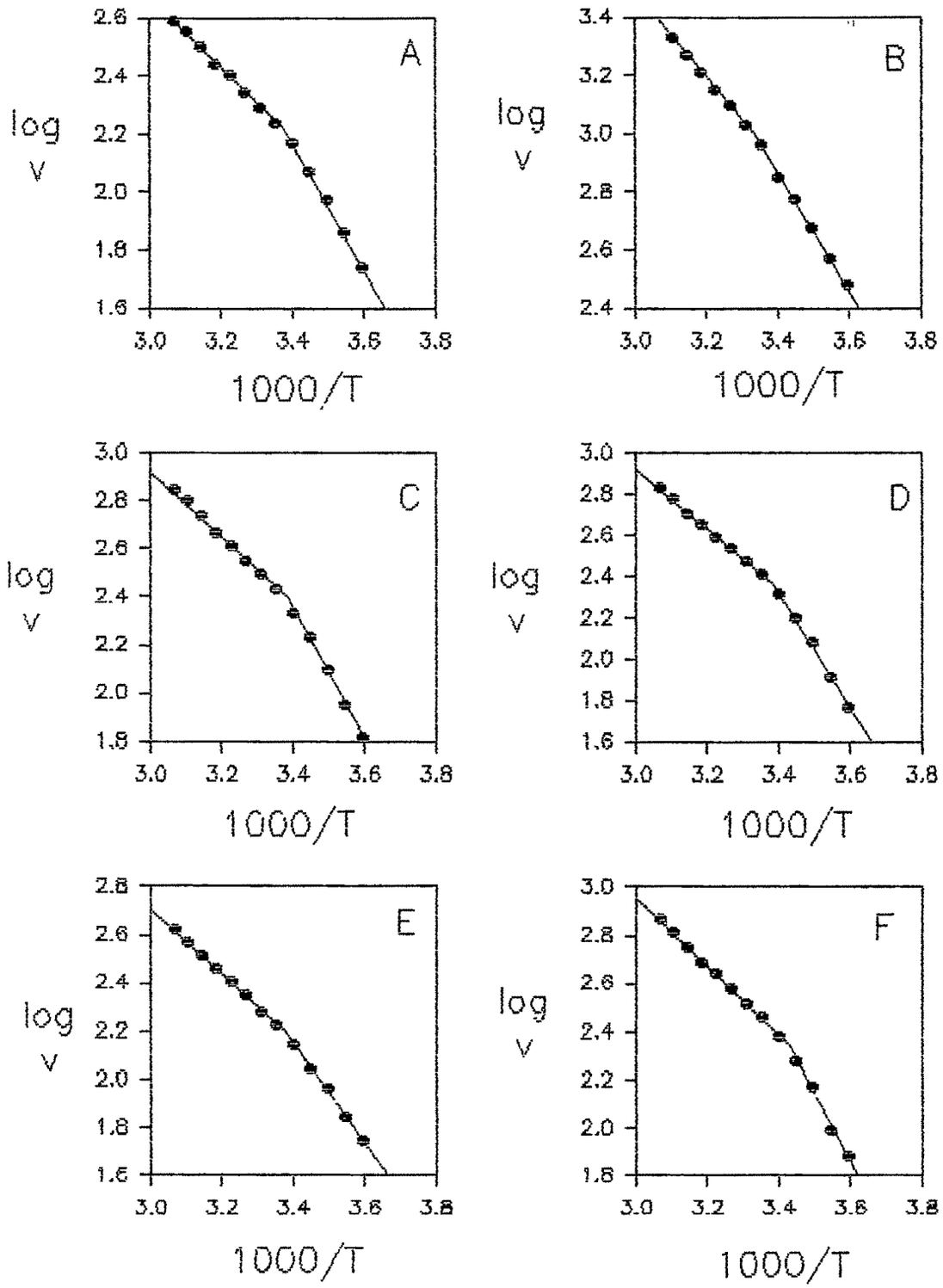

Table 4. Effect of alloxan diabetes on the temperature kinetic of serum BChE in the male rats.

Animals	Energy of activation (KJ / mole)		Phase transition temperature (Tt), ^{o}C
	E1	E2	
Control(10)	23.8±1.02	50.9±4.04	21.6±1.22
Diabetic(10)	29.0±0.90[a]	58.2±5.41	22.7±0.57
Insulin treated diabetic(10)	25.5±1.15[@]	41.4±2.70[#]	24.2±1.16

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

a, $p<0.002$ compared to corresponding control value.

@, $p<0.01$ and #, $p<0.001$ compared to corresponding diabetic value.

Table 5. Effect of alloxan diabetes on the temperature kinetic of serum BChE in the female rats.

	Energy of activation (KJ / mole)		Phase transition temperature (Tt), ^{o}C
	E1	E2	
Control(10)	24.9±1.59	41.2±2.60	23.2±1.28
Diabetic(10)	26.6±1.40	57.4±5.56[a]	21.5±0.91
Insulin treated diabetic(8)	27.9±1.56	58.0±7.51	22.0±0.62

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

a, $p<0.02$ compared with corresponding control.

higher temperature range energy of activation E1 was low (24.9 KJ/mole), while for the lower temperature range energy of activation (E2) was high (41.2 KJ/mole) and phase transition temperature (Tt) was 23.2^{o}C. In the control group the E2 was somewhat low (19%) in the females than in the male group (eg. Table 4). In diabetic female rats only E2 value increased by 39 % which was statistically significant. After insulin treatment also E2 value remained elevated as compared to the control female rats, but this difference was not statistically significant. The phase transition temperature was not influenced under any of the experimental conditions.

Discussion

Compared to the males, serum BChE activity was found 4.4 fold higher in the control females, which agrees well with the earlier report (20). These authors reported increase in plasma BChE activity in both male and female diabetic rats. However we were able to observe increase in BChE activity only in the male but not in the female rats. The observed differences may possibly due to different strain of rats. Increase in plasma BChE activity in diabetic males agrees with earlier report (20). Insulin treatment proved to be ineffective in rectifying secondary defects of diabetes i.e. no improvement was observed in plasma BChE activity.

To check whether kinetic properties of the serum BChE is altering or not, experiments were carried out to examine

substrate and temperature kinetics of this enzyme. In the male rats, substrate kinetic data showed presence of two components : one with high affinity and second having low affinity. In diabetic male rats Vmax of both the components increased which is consistent with the observed increase in enzyme activity (Table 1). Km of component II increased in diabetic male rats as compared to the control males, after insulin treatment the Km of component II became comparable to that in the controls. In female rats in diabetic condition only Km of component II increased, while no effect was observed after insulin treatment. The results thus suggest that the female rats may be more resistant than the males as far as the diabetes induced changes in serum BChE are concerned.

It may be pointed out here that the Km values of component I were comparable in the males and females. However, the Km of component II differed considerably between the males and the females.

As refereed to above in the "Introduction" section, BChE is synthesized in the liver (4) and three molecular isoforms C1, C3 and C4 are present in the human serum (6).

It may hence be suggested that the observed differences in the kinetic properties of serum BChE in the male and female rats (Table 2-5) could be attributed to the possibility that

in the males and females different molecular isoforms of the enzymes may be released in to the serum from the liver. The temperature kinetic data discussed below, may also be considered in the above context.

Temperature kinetic data showed decrease in E1 under diabetic condition in male rats. After insulin treatment E1 was still lower than the control male rats and E2 value decreased significantly. On the other hand, only decrease in E2 was observed in diabetic female rats, which once again proves that the female rats respond differently than the males.

In conclusion in diabetic male rats but not in the females the serum BChE activity increased. The substrate and temperature kinetic properties of serum BChE in the male rats were affected to a greater extent in the males than in the females. The results thus emphasize the basic physiologic differences between the two sexes.

Summary

Serum BChE activity was found to be higher (4.4 fold) in the control female than in the control male rats.

Serum BChE activity increased only in the male diabetic rats but not in the female diabetic rats. No changes were observed in the serum BChE activity after insulin treatment.

In both male and female rats two components of serum BChE were present.

In the diabetic male rats Vmax of both the components increased, with increase in the Km of component II; after insulin treatment this value was comparable with the control male rats.

In the female rats, in diabetic condition only Km of component II increased, while no effect was observed after insulin treatment.

Temperature kinetics data showed decrease in E1 under diabetic condition in the male rats, while in the female rats E2 decreased.

In the male rats after insulin treatment E1 value was lower than in the control male rats and E2 value decreased significantly.

Figure legends

Figure 1 Typical substrate saturation curves for rat serum 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 / ml serum.

Figure 2 Typical Eadie-Hofstee plots for rat serum 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 Figure 1.

Figure 3 Typical temperature curves for rat serum 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 / ml serum.

Figure 4 Typical Arrhenius plots for rat serum 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 Figure 3.

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