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Studies on Tissue Cholinesterase in Domestic Animals

I. Method in Determination of Tissue Cholinesterase Activities in Domestic Animals

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Introduction

Concerning the bodily distribution of pseudo-cholinesterase (pseudo-ChE) in the domestic animals, reportings have, hitherto, been confined only to those dealing with some parts of viscera and tissues in some species: dog[7,16] (pancreas, liver, and cerebrum), horse[7] (pancreas), cat[7] (liver, salivary glands, stomach, small intestine, pancreas and heart), cattle[2] (liver, kidney, spleen, and cornea), with seeming non-presentation of any report about the active distributions of pseudo-ChE in viscera and tissues examined in comparison, among the domestic animals.

The authors carried out the measurement of tissue cholinesterase (T-ChE) activity by their own method[12] in each domestic animal for the purpose of investigating the comparative relationships between serum cholinesterase (S-ChE) and T-ChE in the domestic animals. But it was proved that the authors' method could not be used directly for the measurement of T-ChE not only due to the fact that there is a conspicuous difference in the activity among each animal but to the fact that there is a remarkable difference in it in the tissues of the same animals. Then, in accordance with the above investigations of the measurement method[12] for S-ChE activity, examinations were carried out about the substrate specificity of T-ChE in each animal on viscus and tissue. According to the result obtained, determination was made on the measurement method of T-ChE activity, with the investigation executed on the S-ChE activity in comparison with the T-ChE activity obtained by the method.

Materials and Methods

1. Experimental animals and required tissue

Experiments were made in healthy dogs (three), a horse, cats (three), pigs (five) and cattle (five) and upon liver, kidney, heart, spleen, pancreas and lung, in each animal.

2. Separation of tissue cholinesterase

According to Saito's method[13], separation was carried out to the following effect: (in accordance with Saito's method[13]) fresh viscera and tissues of each animal slaughtered by bloodletting were stored in a freezer, after one or two days, they were dissolved and homogenized.

(1) Preparation of homogenate

A fragment of tissue was cut off strictly by surgical knife in size of about 2 mm, wrapped in gauze and rinsed till blood in it was removed fully in physiological saline. The gauze was wrung

and thereafter moisture of the materials was excluded with filter paper as much as possible, and they were weighed. To the material was added 2 ml/g of physiological saline and they were mixed. After that, the liquid was homogenized with homogenizer. At that occasion, the vessel containing materials should be cooled beforehand. Moreover, it must be homogenized fully until fragments of tissue became invisible to the naked eye.

3. Procedure and preparation of reagent

(1) Buffer

According to Dietzs' method[4], phosphate buffer keeping pH=7.6, $\mu=0.1$ was used.

(2) Substrate and its concentration

Bytyrylthiocholine iodide (BTC), Propionylthiocholine iodide (PTC), Acetylthiocholine iodide (ATC)

The above mentioned three substrates were divided into seven classes with such concentrations as 2.0×10^{-4}, 3.0×10^{-4}, 5.0×10^{-4}, 1.0×10^{-3}, 2.5×10^{-3}, 5.0×10^{-3}, 1.0×10^{-2} M/l.

(3) Solutions for giving color development and for stopping reaction

According to Iuchi's method[9], 1.0×10^{-3} M/l 5, 5′-dithiobis (DTNB) as the solution for giving color development and 0.6% sodium lauryl sulfate as the solution for stopping reaction, were used, respectively.

4. Instruments for the measurement

ULTRA-TURRAX, TP18–10 as a homogenizer, KUBOTA, Model KR–200B as a centrifuge, Water-bath incubator made at Yamato Science as a incubator, and 101-spectrophotometer made at Hitachi for the measurement of absorbance, were used, respectively.

5. Procedure for the preparation of enzyme activity curve

The dilution multiple for homogenate was decided with the intention of holding transmittance, spectroscopically, within the range of 15–70%. In a case when the transmittance was not kept within the range, owing to the difference of enzyme activity in each substrate the percentage limits in transmittance were extended from 10% to 80%. In each homogenate the amount of 0.04 ml was used, and the dilution multiple for those are listed in Table 1.

Table 1. Dilution of tissue extracts for assay

animals / tissues	Dog	Horse	Cat	Pig	Cattle
Liver	41	26	26	9	2
Kidney	8	5	3	3	un-diluted
Heart	3	un-diluted	un-diluted	un-diluted	un-diluted
Spleen	3	2	5	2	un-diluted
Pancreas	402	5	2	2	un-diluted
Lung	3	8	5	un-diluted	un-diluted

6. Experimental procedure

Some explanation as to the procedure for the preparation of enzyme activity curve is as follows. (Cf. Table 2)

Table 2. Procedure for assay

	Blank	Test
Diluted extract	—	0.04 ml
H_2O	0.04 ml	—
Substrate	2 ml	2 ml
Incubation	15 min at 30°C	
DTNB+Inhibitor	2 ml	2 ml
	Read the absorbance at 412 nm	

(1) Preparation of diluted homogenate.

(2) As to the three substrates (BTC, PTC and ATC) they were separated into those with 7 kinds of concentrations, and 2 ml of which was prepared in the test tube, respectively.

(3) 0.04 ml of diluted homogenate and distilled water for blank, were added to the above test tubes, and mixed.

(4) The mixed homogenate was incubated for 30 minutes at 30°C.

(5) Immediately after the incubation, 2 ml of 0.6% sodium lauryl sulfate which was mixed with the equal amount of 1.0×10^{-3} M/l DTNB to make the solution to give color-development as well as to stop enzyme-reaction, was added to the respective test tube and blank, and the mixed solution was made to be colored in yellow.

(6) Transmittance was calibrated by a spectrophotometer at 412 nm against blank, and the absorbance was fixed by the absorbance table.

(7) Preparation of standard curve (calculation of K factor)

1.0×10^{-4} M/l of DTNB containing 1.5, 1.0 and 0.5 ml in each of the test tube A, B and C was mixed with the same amount of 1.0×10^{-3} M/l of G.S.H, respectively. The absorbance; α, β and γ of A, B and C respectively was read against phosphate buffer, each tube containing 0.3 μM, 0.2 μM and 0.1 μM of SH groups, respectively.

$$\text{K factor } (\mu\text{M/absorbance unit}) = \frac{\frac{0.3}{\alpha} \quad \frac{0.2}{\beta} \quad \frac{0.1}{\gamma}}{3}$$

(8) Expression of activity value

According to the method in serum, activity was to be expressed by using 1 g in the viscus in place of 1 g in serum. Then, activity value was calculated as follows.

$$\text{Activity } (\mu\text{M/g/min/30°C}) = \frac{\text{absorbance change}}{\text{incubation time (30)}} \times \text{K factor} \times \frac{\text{extract dilution} \times 2}{\text{ml of diluted extract}}$$

Results

1. Substrate specificity to the three substrates

(1) Substrate specificity and enzyme activity curve

(a) Figs. 1, 2 and 3 show enzyme activity curve in dogs. In the tissues excepting spleen the resolution ratio was high in BTC, PTC and ATC, in this order, while in spleen ATC, PTC and BTC, in this order. The reaction curve in other tissues, excepting lung, reached plateau in the concentration of 5 mM, drawing straight in lung; showing typical bell shaped curve in ATC and PTC in spleen.

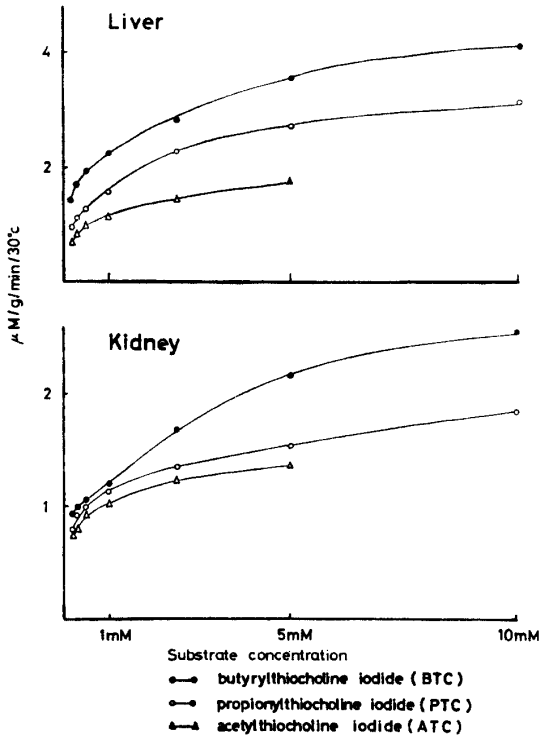

Fig. 1. Hydrolysis of ATC, PTC and BTC by dog tissue ChE.

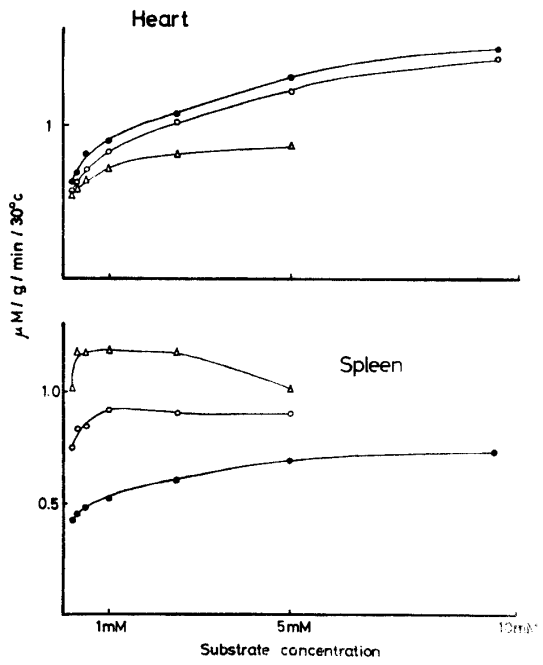

Fig. 2. Hydrolysis of ATC, PTC and BTC by dog tissue ChE.

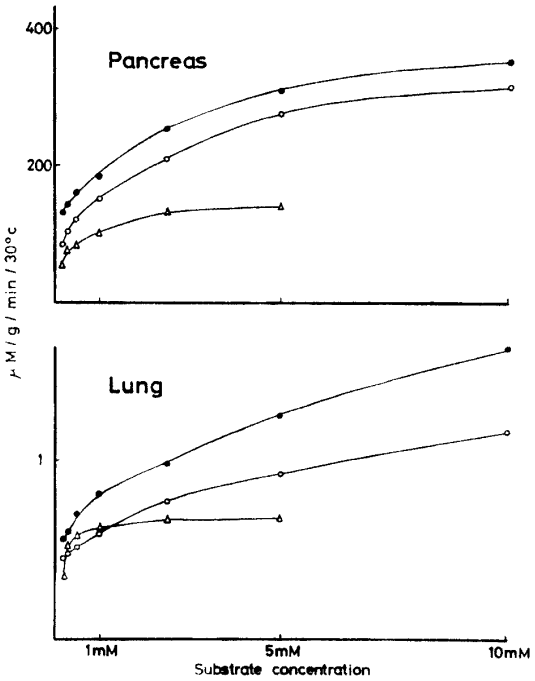

Fig. 3. Hydrolysis of ATC, BTC and PTC by dog tissue ChE.

Fig. 4 shows the comparison made, in the resolution ratios on the three substrates, by each T-ChE, and S-ChE in 5 mM of substrate, liver activity in BTC being defined as 100. BTC is most specific in the tissue, excepting spleen and serum, being most specific in kidney. As to the respective tissue activity, that of pancreas in almost 35 times as high as that in liver.

(b) Figs. 5, 6 and 7 show enzyme activity curve in horses. Likewise in case of dogs, the

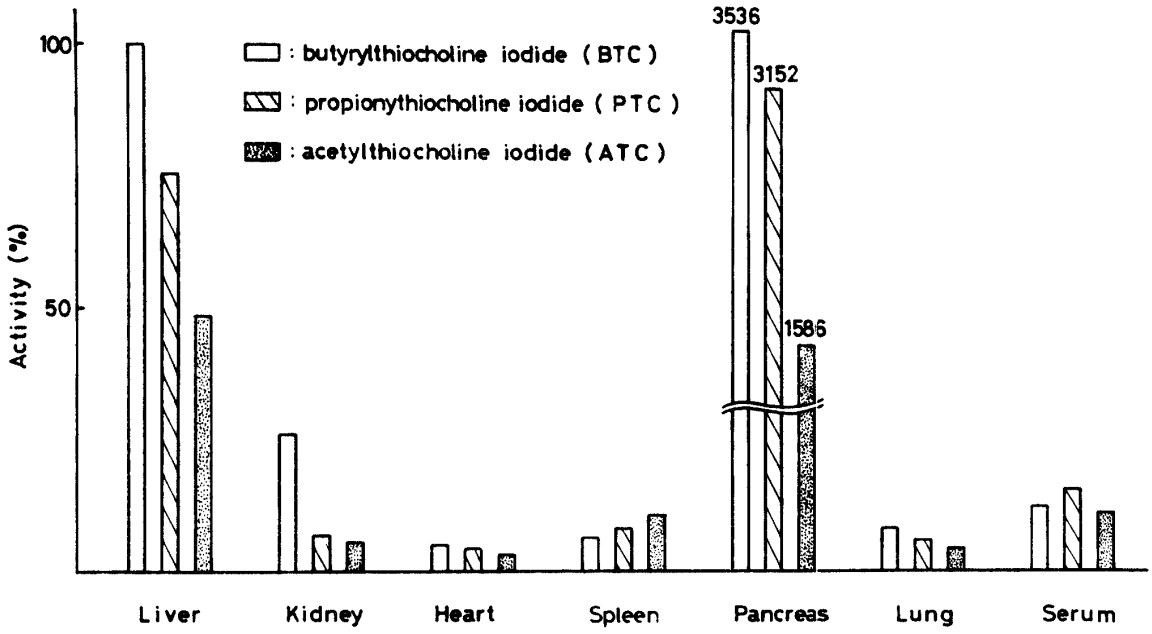

Fig. 4. Substrate specificity patterns of Dog tissue ChE.
Activity as % of liver activity using BTC (5×10^{-3} M)

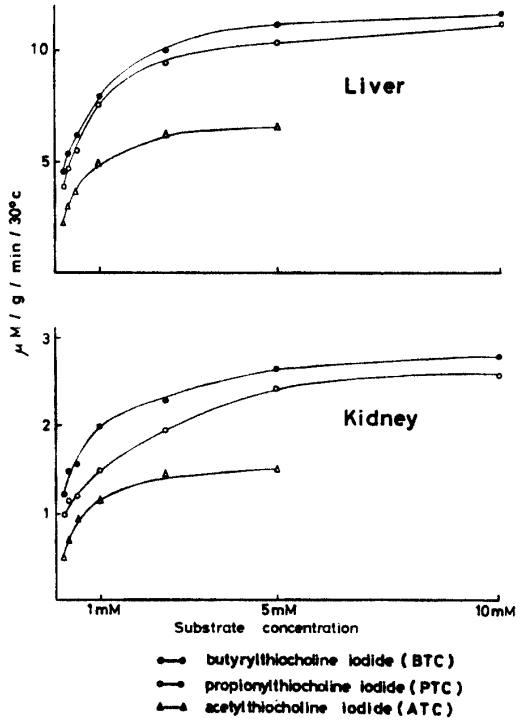

Fig. 5. Hydrolysis of ATC, BTC and PTC by horse tissue ChE.

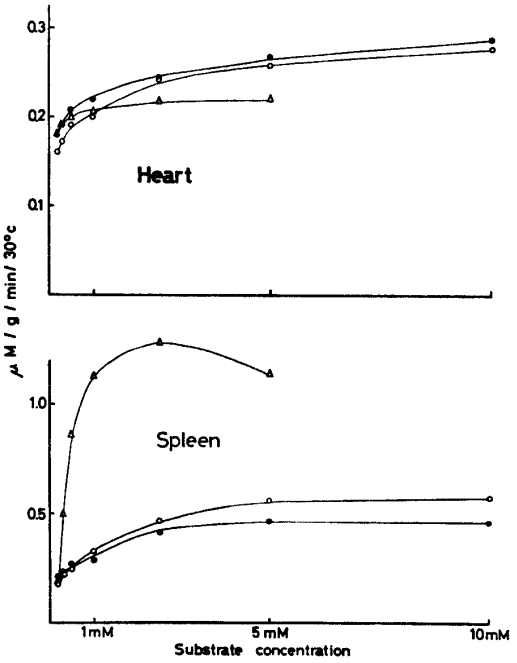

Fig. 6. Hydrolysis of ATC, BTC and PTC by horse tissue ChE.

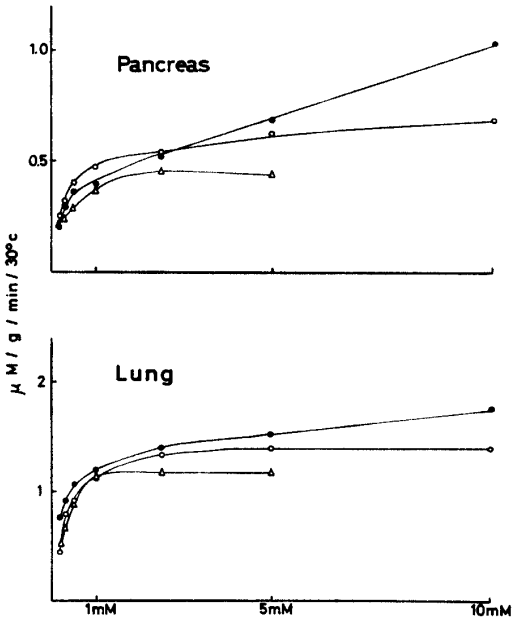

Fig. 7. Hydrolysis of ATC, BTC and PTC by horse tissue ChE.

resolution ratio was high in BTC and ATC in this order in the tissue excepting spleen; while in spleen it was high in ATC, PTC and BTC in this order. And that, the resolution ratio of ATC in spleen is remarkably higher than in the other two substrates. Only in ATC it showed typical bell shaped curve. The resolution ratios of both BTC and PTC were noted to be nearly approximate. The reaction velocity in BTC began to get faster once it was beyond 3 mM in pancreas. The difference between the reaction velocities in BTC and PTC tended to become separated. Fig. 8 shows

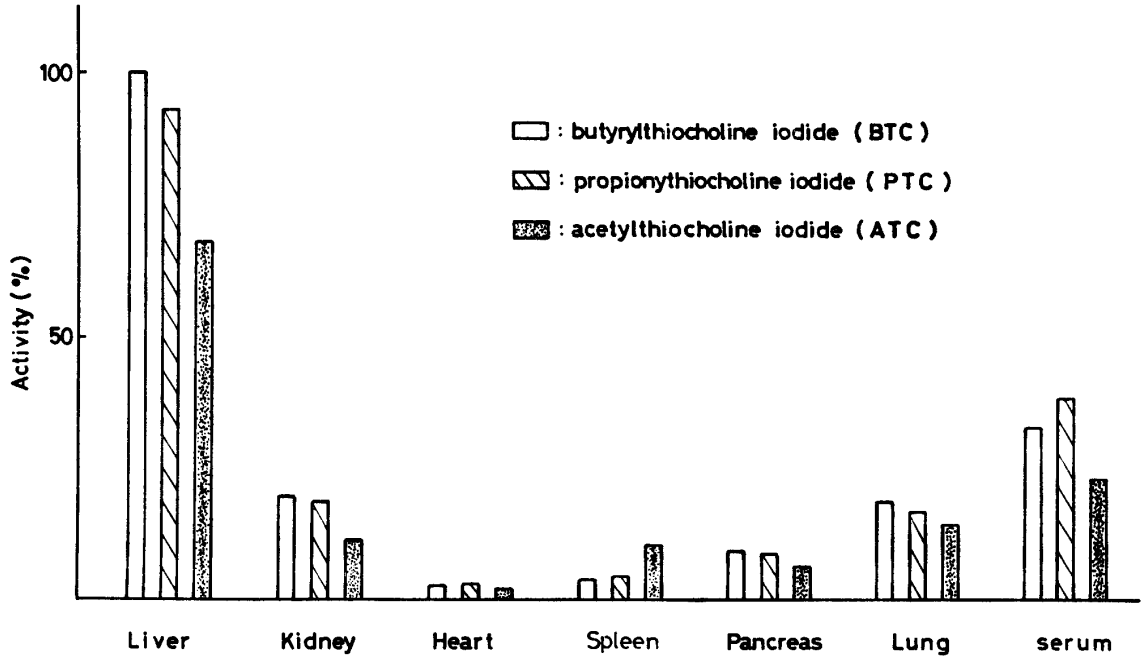

Fig. 8. Substrate specificity patterns of horse tissue ChE.

the comparison of activities in 5 mM of each substrate. Activity was high in liver and serum in this order. In serum, PTC was fixed to be the most specific substrate.

(c) Figs. 9, 10 and 11 show enzyme activity curve in cats. The resolution ratio was high in BTC, PTC and ATC in this order in liver, heart, pancreas and lung; and kidney, in BTC, ATC and PTC in this order; and in spleen, in ATC, PTC and BTC in this order. Likewise in case of dogs

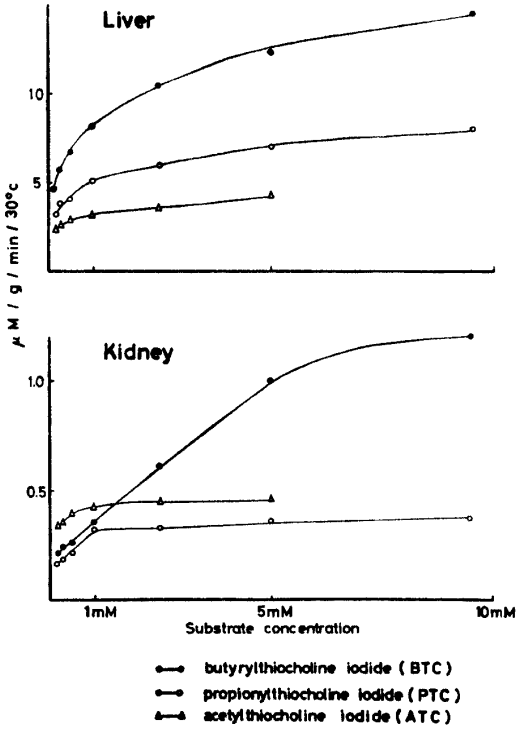

Fig. 9. Hydrolysis of ATC, BTC and PTC by cat tissue ChE.

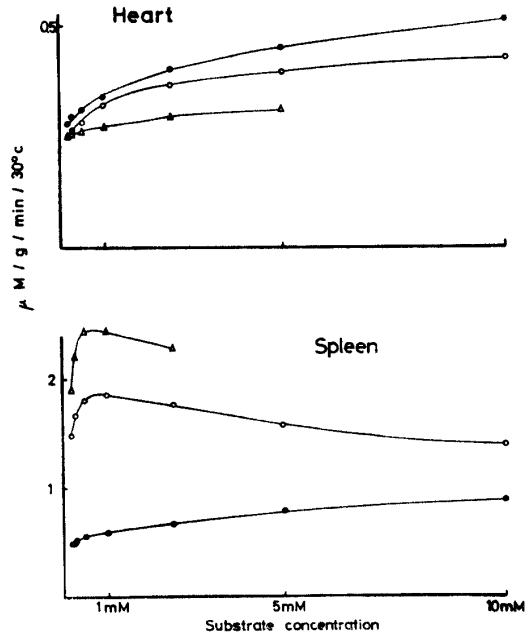

Fig. 10. Hydrolysis of ATC, BTC and PTC by cat tissue ChE.

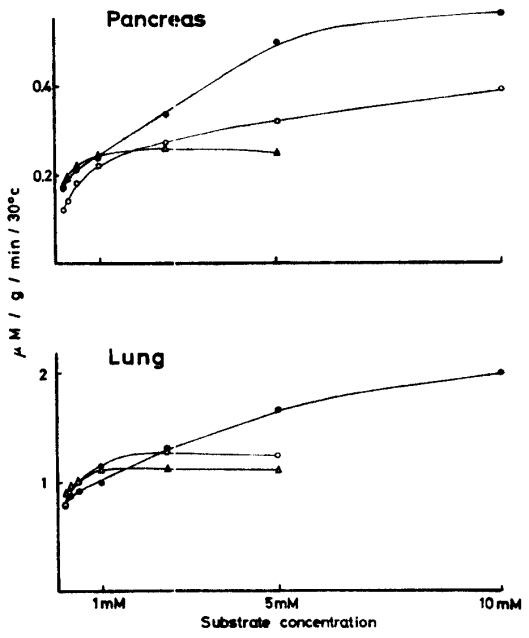

Fig. 11. Hydrolysis of ATC, BTC and PTC by cat tissue ChE.

it showed bell shaped curve in ATC and PTC. Fig. 12 shows the comparison of activities in 5 mM of each substrate. In liver and kidney, BTC activity was higher than that in others, while in spleen, ATC activity was much higher than that in others; and in serum PTC was the most specific substrate.

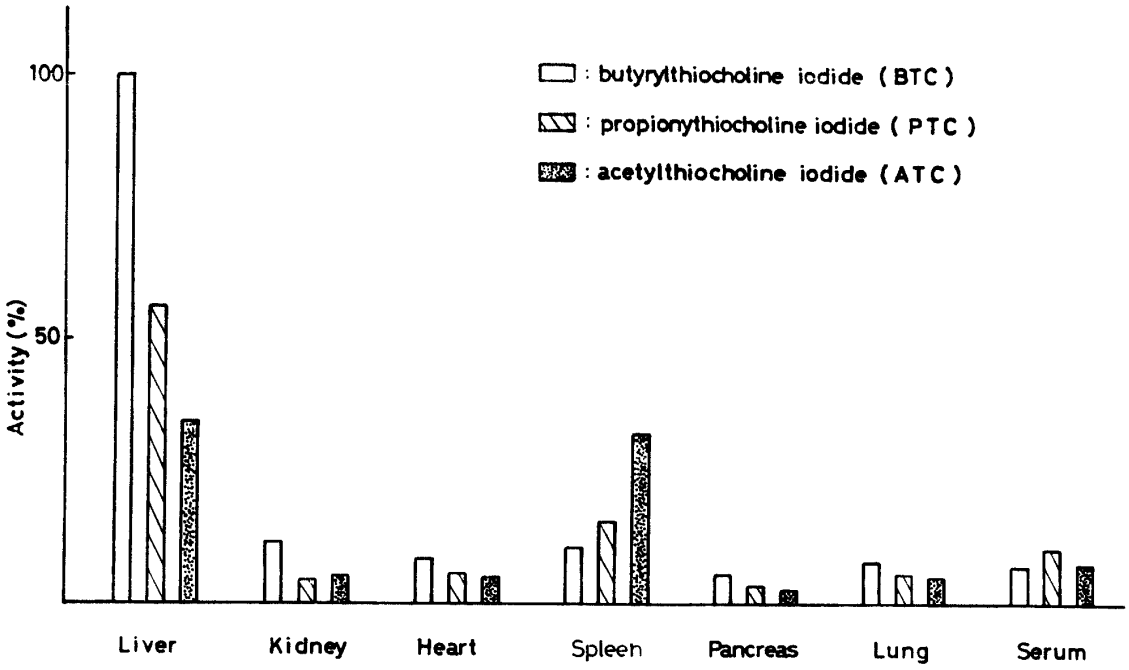

Fig. 12. Substrate specificity patterns of cat tissue ChE.

(d) Figs. 13, 14 and 15 show enzyme activity curve in pigs. In liver, kidney and lung the resolution ratio was high in BTC, ATC and PTC in this order, and in pancreas, heart and spleen in

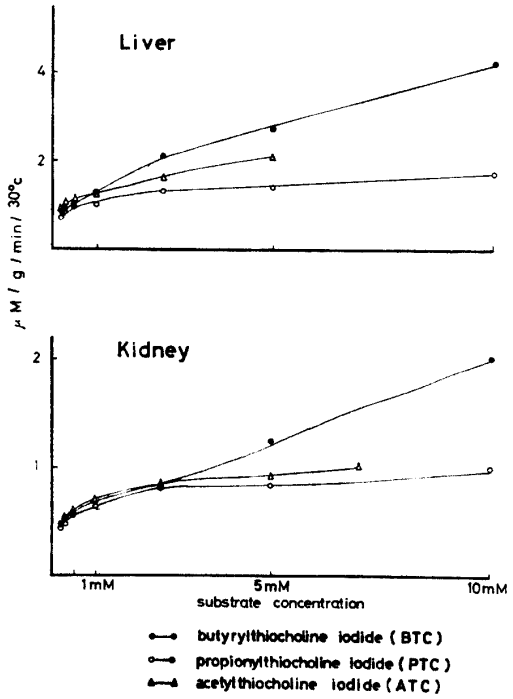

Fig. 13. Hydrolysis of ATC, BTC and PTC by pig tissue ChE.

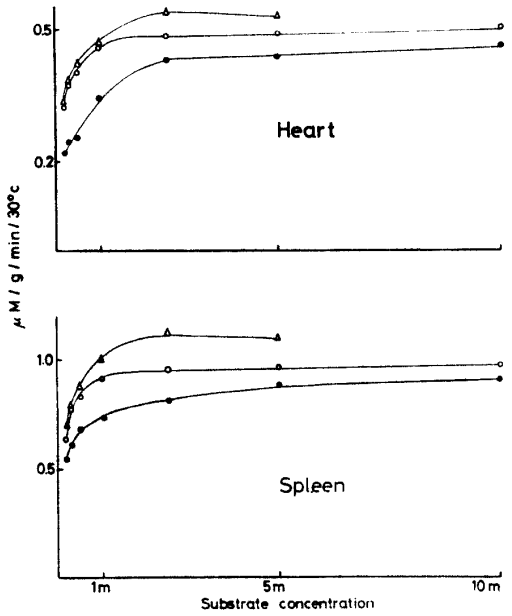

Fig. 14. Hydrolysis of ATC, BTC and PTC by pig tissue ChE.

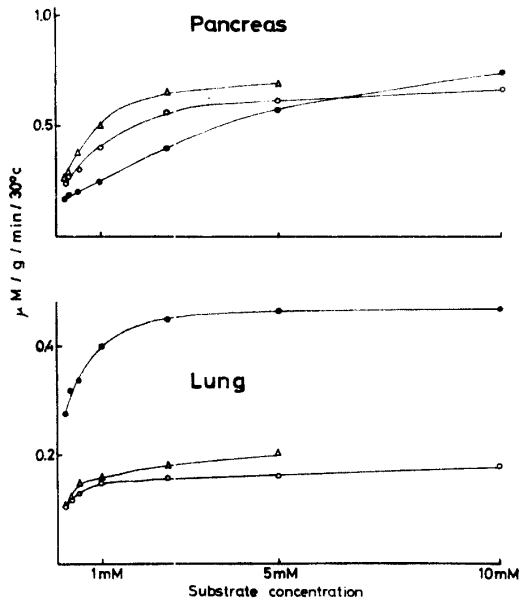

Fig. 15. Hydrolysis of ATC, BTC and PTC by pig tissue ChE.

ATC, PTC and BTC in this order. In the concentration beyond 7 mM pancreas activity of BTC was higher than that of PTC. In heart, spleen and lung, it was about at 3 mM that the reaction curve in BTC reached plateau, and in other viscera, it rose straight before approaching the concentration counting less than 10 mM. In spleen, it showed no bell shaped curve in ATC and PTC resembling those in dogs, horses and cats mentioned above.

Fig. 16 shows the comparison of activities in 5 mM of each substrate. Lung activity in BTC was remarkably higher than that in other viscera. Likewise in the cases of spleen, lung and heart, serum activity was high in ATC, PTC and BTC in this order.

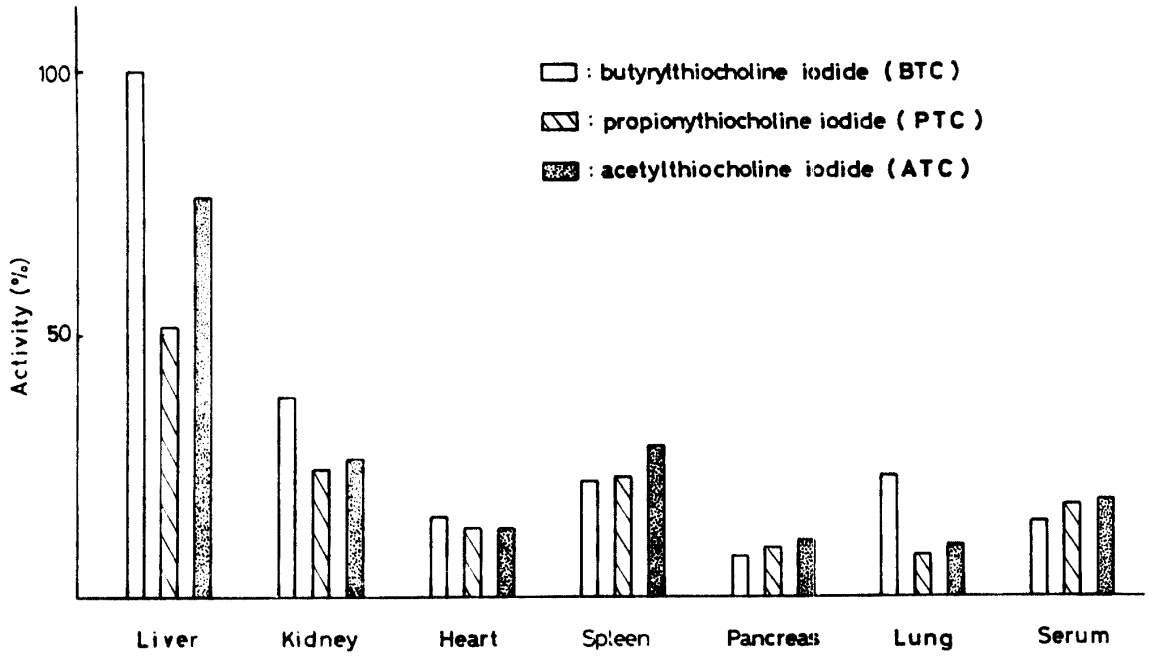

Fig. 16. Substrate specificity patterns of pig tissue ChE.

(e) Figs. 17, 18 and 19 show enzyme activity curve in cattle. In kidney, the resolution ratio was high in BTC, PTC and ATC in this order, and in liver and pancreas in BTC, ATC and PTC in this order. The resolution ratio in spleen was different from the cases of above four domestic

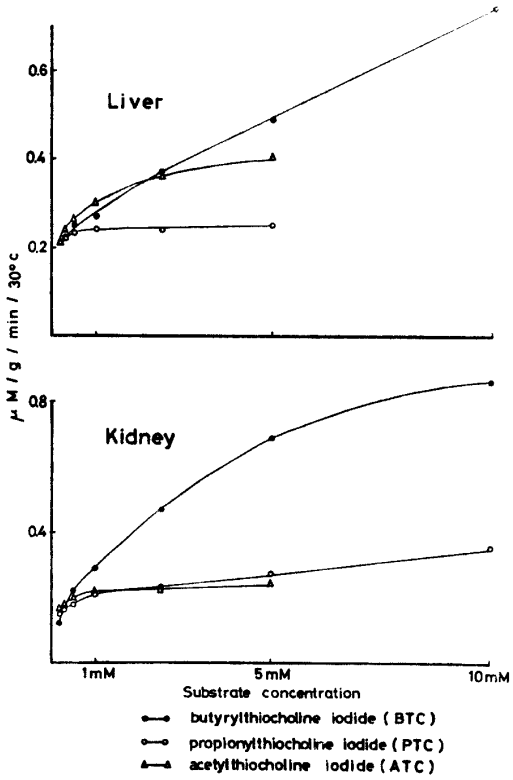

Fig. 17. Hydrolysis of ATC, BTC and PTC by cattle tissue ChE.

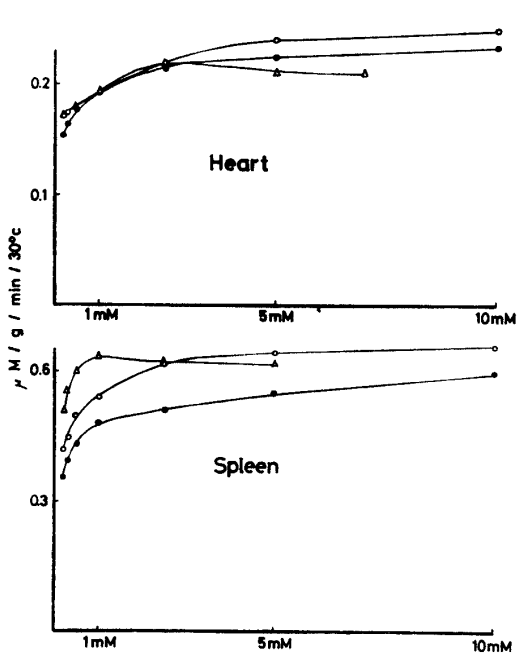

Fig. 18. Hydrolysis of ATC, BTC and PTC by cattle tissue ChE.

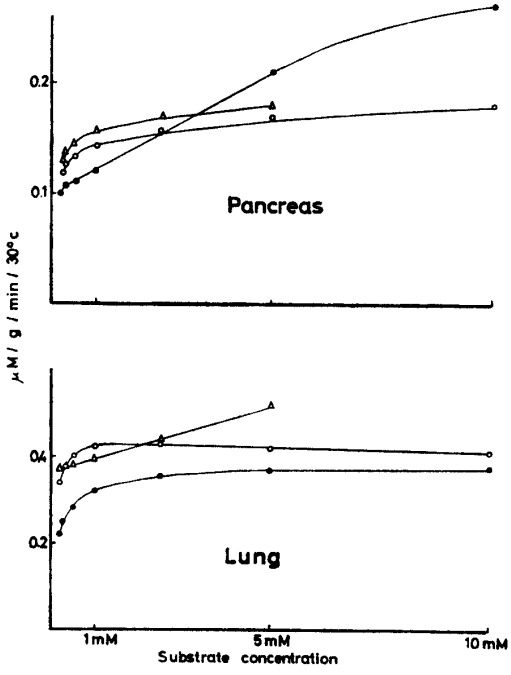

Fig. 19. Hydrolysis of ATC, BTC and PTC by cattle tissue ChE.

animals. That is, in spleen, it was high in PTC, ATC and BTC in this order; and in heart, in PTC, BTC and ATC in this order; and in lung, in ATC, PTC and BTC in this order. In heart, spleen and lung, it was about at 5 mM that the reaction curve in BTC reached plateau. In other viscera, it rose almost straight. When it was beyond 5 mM in liver, kidney and pancreas the reaction curve of ATC and PTC reached plateau, but in BTC the reaction velocity tended to become faster according to the increase of substrate concentration. The reaction curve of ATC in viscera, excepting that in lung, almost reached plateau within the range of 1–5 mM. In lung, the reaction curve of ATC was nearly straight, being specific in comparison with those in the other domestic animals. Fig. 20 shows the comparison of activities at 5 mM of each substrate. Serum activity was lower than that in case of each viscus. Liver and kidney activities in BTC were higher than those of other viscera. When the difference in activities noted between liver and other viscera in cattle was compared with those of other domestic animals, the former was remarkably smaller than the latter. This was assumed to be due to the fact that liver activity of cattle is lowest among these domestic animals as may be noted when it is compared with the liver activities of other domestic animals.

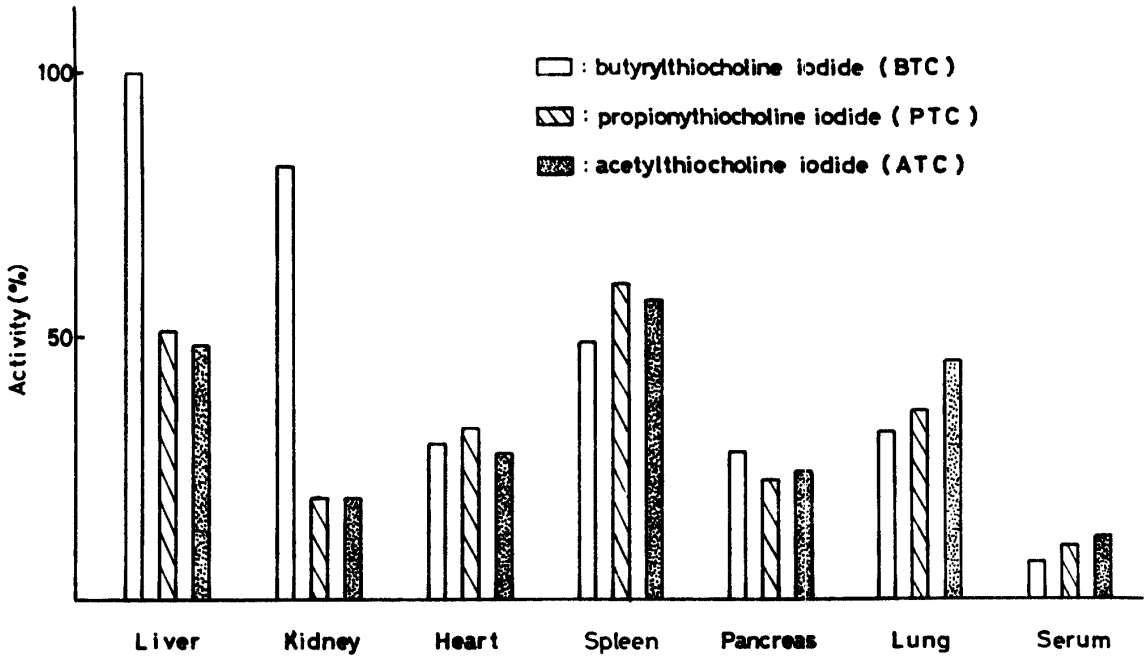

Fig. 20. Substrate specificity patterns of cattle tissue ChE.

(2) Comparative activity in each viscus of domestic animals

Figs. 21, 22, 23 and 24 showed the comparison of ChE activity at 5 mM of BTC in each domestic animal with the separation of serum from viscera. As may be noted on Fig. 21 in comparison of S-ChE activity was high in horses, dogs, cats, pigs and cattle in this order. In kidneys of horses, it was higher than in those of dogs, and there was no difference among other domestic animals. In heart, activity was high in dogs, cats, horses, pigs and cattle in this order. In spleen, it was comparatively low in horses and in dogs, cats, pigs, cattle and horses in this order. In pancreas, it was overwhelmingly higher in dogs than that in other domestic animals, being more than a hundred times as high as that in the cats fixed in the second order. There was no difference among other domestic animals. In lung, likewise in case of serum activity was high in horses, dogs, cats, pigs and cattle in this order. Therefore, unlike in serum activity in viscera, excepting the one in lung, is highest in dogs among other domestic animals, surpassing that of horse viscera.

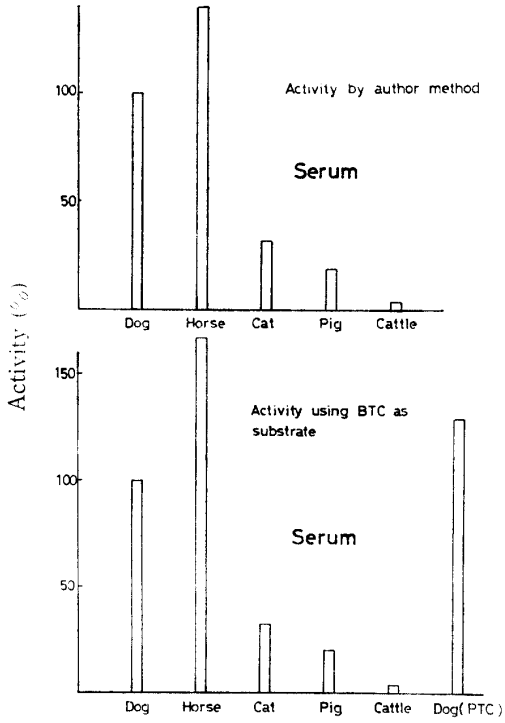

Fig. 21. Relative S-ChE activity of different animals against it of dog.

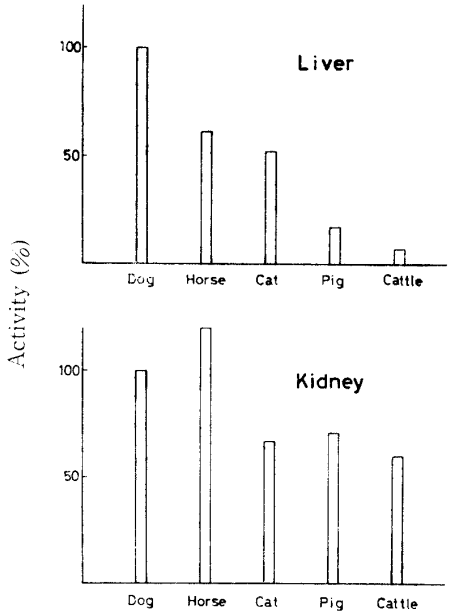

Fig. 22. Relative T-ChE activity of different animals using BTC. (T-ChE activity of dog being defined as 100)

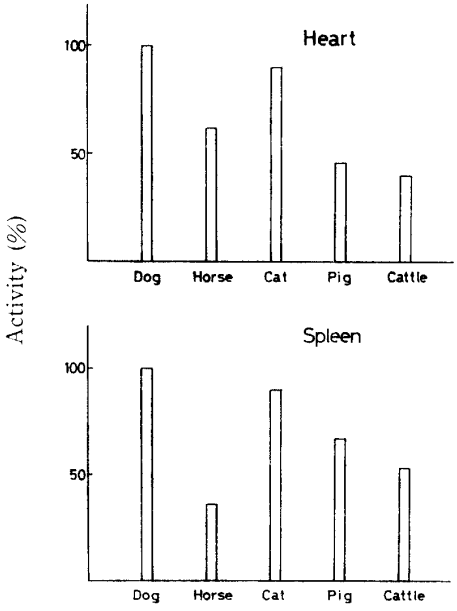

Fig. 23. Relative T-ChE activity of different animals using BTC. (T-ChE activity of dog being defined as 100)

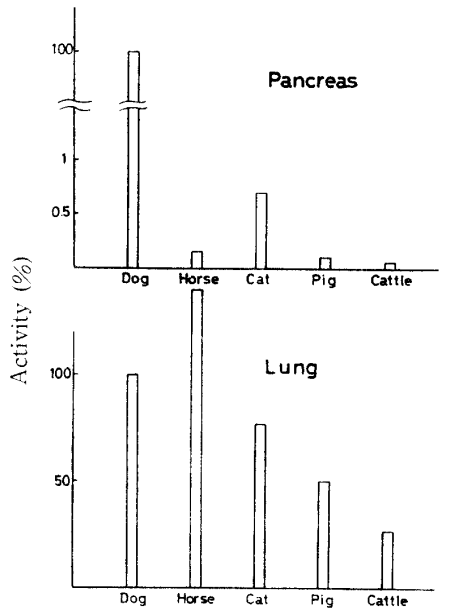

Fig. 24. Relative T-ChE activity of different animals using BTC. (T-ChE activity of dog being defined as 100)

2. Michaelis constant (Km)

The Km value in the respective T-ChE was determined by the method of Lineweaver Burk using 4 kinds of substrate concentrations (2.0×10^{-4}, 3.0×10^{-4}, 5.0×10^{-4}, 1.0×10^{-3} M/l) in the enzyme activity curve. Fig. 25 showed as an example the Lineweaver Burk plot of BTC to the

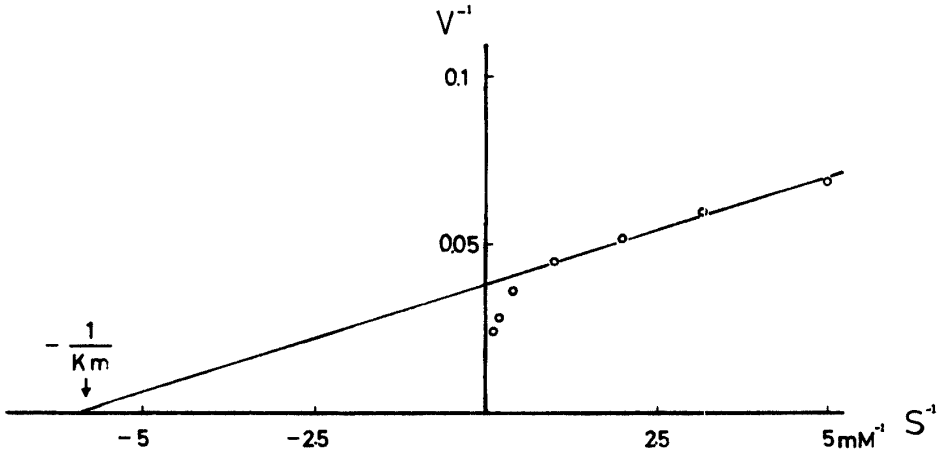

Fig. 25. Lineweaver-Burk plot of BTC for dog liver cholinesterase.

liver ChE in dogs. Km values in BTC, PTC and ATC were showed in Table 3, Table 4 and Table 5, respectively. Within the range of 6.5×10^{-5}–5.0×10^{-5} M/l Km value in BTC was noted to be closely resembled with that in serum reported by authors[12], Das and Liddell[3] and Ecobichon and Comeau[5]. In PTC, Km value exists within the range of 2.4×10^{-5}–9.0×10^{-4} M/l and in ATC it exists within 2.0×10^{-5} M/l. Moreover, PTC and ATC showed the tendency to extend further the range of Km value. Km value of T-ChE in dogs with BTC was nearly similar to that of serum.

Table 3. Km values of tissue ChE of domestic animals in BTC $\times10^{-5}$

tissues \ animals	Dog	Horse	Cat	Pig	Cattle
Liver	14	20	22	7.7	8.3
Kidney	13	15	17	13	50
Heart	13	8.5	87	17	22
Spleen	7.4	13	6.5	17	17
Pancreas	13	30	9.5	15	11
Lung	13	17	10	13	8.0
Serum	13	29	—	31	19

Table 4. Km values of tissue ChE of domestic animals in PTC $\times10^{-5}$

tissues \ animals	Dog	Horse	Cat	Pig	Cattle
Liver	14	25	14	1.3	2.4
Kidney	11	15	20	14	28
Heart	10	6.9	8.7	13	2.5
Spleen	5.9	20	90	11	7.6
Pancreas	33	33	27	22	5.1
Lung	8.3	22	11	11	6.9
Serum	13	33	8.8	21	9.9

Table 5. Km values for tissue ChE of domestic animals in ATC $\times10^{-5}$

tissues \ animals	Dog	Horse	Cat	Pig	Cattle
Liver	20	25	8.7	11	11
Kidney	11	40	7.7	13	9.5
Heart	6.9	3.8	2.9	11	4.9
Spleen	2.0	100	13	11	6.3
Pancreas	20	18	8.1	33	6.7
Lung	91	50	6.1	14	2.0
Serum	18	55	16	27	5.0

3. Measuring method for T-ChE and activity value in the respective tissue in domestic animals

From the above experimental results, using BTC as substrate was assumed to be most convenient and finally best for the execution of comparisons of activities among tissues and of those among domestic animals. Accordingly, the dilution multiples for the homogenate were so decided as to hold transmittance within the range of 30–60%. And then the T-ChE activities of the domestic animals were measured after having unified the substrate concentration within the normal state of 5.0×10^{-3} M/l. Reagents and measurement procedure are nearly similar to those in the preparation of enzyme activity curve. The points differing from the latter are as follows; First, unifying the substrate concentration in a normal state of 5.0×10^{-3} M/l of BTC; second, using 0.05 ml of the diluted homogenate, third, adding 3 ml of substrate and the same amount of solution to give color development as well as to stop reaction, respectively. Table 6 shows dilution multiples of the homogenate. Table 7 shows measurement procedure. Table 8 shows the mean value of T-ChE activities in the respective domestic animal, measured by the above method. Activity in liver was higher than that in any other tissue of each domestic animal and it was secondarily high in kidney. Each activity in the other tissues was nearly similar. But pancreas activities of dogs were exceptionally high and was quite specific.

Table 6. Dilution of tissue extracts for assay

animals / tissues	Dog	Horse	Cat	Pig	Cattle
Liver	51	26	26	7	undiluted
Kidney	5	5	3	3	undiluted
Heart	3	undiluted	undiluted	undiluted	undiluted
Spleen	3	undiluted	3	2	undiluted
Pancreas	808	3	undiluted	2	undiluted
Lung	5	5	3	undiluted	undiluted

Table 7. Procedure for assay

	Blank	Test
Diluted extract	—	0.05 ml
H_2O	0.05 ml	—
Substrate (BTC, 5×10^{-3} M)	3 ml	3 ml
Incubation	15 min at 30°C	
DTNB+Inhibitor	3 ml	3 ml
	Read the absorbance at 412 nm	

Table 8. Mean activities of tissue ChE in domestic animals

Unit: μM/g/min/30°C

Substrate: Butyrylthiocholine iodide (5×10^{-3} M)

animals / tissues	Dog (3)	Horse (1)	Cat (2)	Pig (5)	Cattle (5)
Liver	16.42	10.02	8.65	2.79	1.09
Kidney	1.50	1.79	1.0	1.06	0.9
Heart	0.81	0.50	0.73	0.37	0.32
Spleen	1.02	0.37	0.92	0.68	0.54
Pancreas	580.6	0.91	0.45	0.69	0.31
Lung	1.30	1.85	1.01	0.65	0.35

( ): No. tested

Discussion

Substrate specificity of T-ChE in the respective domestic animal, differing from that of S-ChE, was noted to be optimal in almost all the tissues excepting spleens in dogs, horses and cats. In pigs, BTC was optimal in liver, kidney, pancreas and lung, while ATC was optimal in heart and spleen. But in pancreas the optimality was quite similar through the three substrates. That is, in pigs, substrate specificity showed remarkable differences among the respective tissues. In cattle, BTC was clearly optimal in liver, kidney and pancreas. In both BTC and PTC in spleen and heart a close resemblance was noted in the optimality. ATC was optimal in lung of cattle, and it was proved that as in the case of pigs the substrate optimality showed difference among the tissues. By this it was proved that the substrate specificity of T-ChE was not only differed from that of serum but also was more or less different in each tissue in accordance with the variety in the kind of animal. The optimality in spleen was great in ATC, PTC and BTC in this order in dogs, horses and cats; especially it was remarkable in ATC. In cattle and pigs the optimality of ATC was somewhat greater than that of PTC. By this it was indicated that the optimality was strongly under the influence of true-ChE in blood, especially of erythrocyte. However, it was reported that Km values of S-ChE in cattle and pigs with ATC are 1.8×10^{-4} (by authors) and 1.86×10^{-4} [17], respectively. Km value of erythrocyte-ChE in dogs with ATC was 9.5×10^{-5} [17]. On the other hand, the Km value of visceral ChE in dogs obtained by this experiment was 2.0×10^{-4} in liver and pancreas. This value is close to that of serum. In spleen, it is 2.0×10^{-5} and about of 1/5 Km value of erythrocyte-ChE noted by Ward and Hess[17]. The Km value of S-ChE in dogs with BTC is 1.7×10^{-5} [12] and those of tissues excepting spleen are within the range of 1.3×10^{-4}–1.4×10^{-4}, which is close to that of serum, while the value in spleen is of such a low value of 7.4×10^{-5}. This shows that spleen ChE is not solely under the influence of blood. T-ChE activity is uniformly high in the liver of each domestic animal, being from two to eight times as high as S-ChE activity. This result agrees with the reports of many scholars[14),16)]. ChE activity in other tissues is equal to, or less than, that of serum; and in cattle, there was no great difference in the activity in each tissue. Moreover, the activity was noted to be higher than that in serum. In dogs pancreas ChE activity was remarkably high, being about 280 times as S-ChE activity; while in other domestic animals it was rather low, agreeing with the report of Hebb and Hill[7]. Augustinsson[1] reported that S-ChE of pigs was originally derived from pancreas and pancreas ChE belonged to the specific pattern, but in our experiments in pigs no remarkable difference between pancreas ChE and other tissue ChE was ascertained. Marx and Carter[11] reported that through the histological examination of kidney ChE they ascertained the existence of pseudo-ChE in all sorts of animals, though in dogs and rats its existence was indistinct. However, in this experiment, kidney ChE existed in each domestic animal, its activity being nerve generally low but was high in horses, dogs, pigs, cats and cattle in this order. It turned out that dogs are one of the animals having high ChE activity. And this differs from Marx and Carter's[11] histological research result in these animals. In heart the activity was low in each domestic animal. But Vlk and Tuček[15] indicated that in atrium the activity was higher than that in ventricle activity, in dogs and cats. Hegab and Ferrans[8] reported that the activity was high in *fasciculus atrioventricularis* of rats. In view of those facts, it was assumed that in heart the ChE activity was obviously differed in the respective inward parts of the heart, concerning such difference no investigation was made by the authors. About the heart ChE activity of each domestic animal, the activity in dogs and cats belonging to beasts of prey was much

higher than that of others. In lung, Gerebtzoff[6] and Mann[10] ascertained, by the histological method, the existence of butyryl ChE in trachea muscle, muscus gland and in the secretion from the gland. Adding to this, the measurement by authors proved that in dogs and horses, lung ChE activity was 1/4 of S-ChE activity, and in cattle, it was a little higher than S-ChE activity, and in other domestic animals it was almost to S-ChE activity. The above results supported[14] the theory that there was biochemical difference between S-ChE and T-ChE and there was not any identity between the two. But with these it is impossible for us to solve the question whether T-ChE was originated in blood, or whether S-ChE was originated in tissues and that at what location the body ChE containing S-ChE was synthesized and by what route it was transported.

In the future, it will be quite necessary for us to make extensive investigations concerning T-ChEs.

Summary

The authors carried out some measurements of ChE activity and substrate specificity in the respective tissue, with the comparative investigations made concerning the relationships between S-ChE and T-ChE. The obtained results are summarized as follows.

1. The optimal substrate fit for S-ChE was PTC in dogs, horses and cats, while it was ATC in pigs and cattle. The optimal substrate fit for T-ChE was in dogs, horses and cats excepting ATC in spleen of each animal, BTC in pigs excepting ATC in spleen and heart, BTC in cattle excepting PTC in spleen and heart and ATC in lung. BTC seemed to be the optimal substrate through the 5 domestic animals.

2. Km values of T-ChE in each domestic animal with three substrate were in the range of 6.5×10^{-5}–5.0×10^{-4} M/l in BTC, 2.4×10^{-5}–9.0×10^{-4} M/l in PTC, 2.0×10^{-5}–1.0×10^{-3} M/l in ATC, respectively.

3. As may be noted in the result of the measurement made on T-ChE activity in each domestic animal by the method using 5.0×10^{-3} M/l of BTC, T-ChE activity in liver was ascertained to be high in each domestic animal. Secondarily in kidney, it was comparatively high and in other viscera it was low. Exceptionally, T-ChE activity in pancreas in dogs was 35 times as high as that in liver.

4. The T-ChE activity in lung and kidney, and the S-ChE activity were high in horses, dogs, cats, pigs, and cattle in this order. The T-ChE activities in liver and other tissues were highest in dogs.

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