Mem. Fac. Agr. Kagoshima Univ., **17**, 219–234, 1981

Studies on Tissue Cholinesterase in Domestic Animals

II. Detection of Tissue Cholinesterase Isoenzymes in Domestic Animals

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Received for Publication September 10, 1980

Introduction

Since former times, we generally have recognized the theory that in serum cholinesterase (S-ChE) there hardly is substrate specificity only with partial exception, this is to say that both the S-ChE and tissue cholinesterase (T-ChE) show the same pattern in identical species, not having such an item as visceral specificity[15].

However, in isoenzyme level, basing on the result got by the examination of experimental animals it is being clarified that similar patterns are not always observed between the S-ChE and the respective T-ChE[4].

Authors[11,12] proved that S-ChE was specific to propionylthiocholine (PTC) in dog, horse and cat, while it was specific to acetylthiocholine (ATC) in pig and cattle, and using these substrates examined S-ChE on isoenzyme level. But as to the T-ChE isoenzyme even concerning that in human body, reporting has been made only in a part of viscera (Liver etc.)[10,19].

Svensmark[19] examined liver ChE isoenzyme of human and suggested the existence of a precursor of S-ChE, and judging from the nature against the in the inhibitor Lidell *et al.*[10] indicated that both T-ChE and S-ChE were under the control of the same gene. It was assumed that the both were derived from the same origin and varied on the way transforming itself from the former to the latter. Presumably the variation was occasioned to be different in each domestic animals.

Prior to the clarification of the relevancy in the close relationship between S-ChE and T-ChE was deemed to be necessary.

There has been hardly any report published on this point in veterinary science.

Then authors carried out this experiment for the purpose of investigating the relevancy of the relationship T-ChE and S-ChE in animals.

Materials and Methods

1. Experimental animals and required tissue

Experiments were performed in healthy dogs, horses, cats, pigs and cattle and on liver, kidney, heart, spleen, pancreas and lung in each animal.

2. Separation of tissue cholinesterase

Separation was carried out in accordance with the previous report[14].

3. Detection of T-ChE isoenzyme

The separation of tissue protein was executed by disc electrophoresis on polyacrylamide gel. After the finish of electrophoresis, a gel was divided into 4 equal parts, and one of those was used for the detection of protein fraction (examination of the mobility of albumin), the others were used for the detection of T-ChE isoenzyme fraction in the three substrate (which will be mentioned later). The density of ChE isoenzyme fraction and protein fraction of the stained gel were measured by densitometer.

(1) Disc electrophoresis

(a) Reagents; The preparation of the reagents to prepare gel and buffer for electrophoresis was performed in accordance with Juul's methods[5].

(b) Method to prepare; The method used by us was a sort of method modified partially from the technique described by Nakamura[16], basing on the method originated by Ornstein and Davis in preparing gel. That is, author's method and Nakamura's are same in preparing separation gel and spacer gel, but in our case in preparing the sample gel it was modified as in the following: after being divided into three types (10 μl, 50 μl and 100 μl) as additional amounts, viscera (tissue) extracts were poured directly into column according to the degree of each activity.

This differs from the original method in the point of having no sucrose added.

(c) Current; The used instrument is as follows.

Electrophoresis chamber: MS KIKI CO., LTD.

Stabilized power supply: TOYO SOLID STATE POWER SUPPLY MODEL 1510 TOYOKAGAKU SANGYO CO., LTD.

The current was adjusted to 3 m Amp per column. The time for electrophoresis was set about from 1.5 to 2.5 hours[5].

(2) Staining procedure of T-ChE isoenzyme in gel

In staining T-ChE isoenzyme we roughly followed the modified method of Juul[5], but it was modified in a few points as follows.

We used three substrates [butyrylthiocholine (BTC), PTC and ATC], the concentrations of which were unified in 5.0×10^{-3} M/l. Incubation was performed on the standard of 3 hours till detecting band (Cu-thiocholine) got conspicuously beclouded.

According to the original method, below mentioned procedure was performed.

(3) Protein staining procedure in gel

Protein staining in gel was performed by immersing half of gel in anilin blue 1% for 2 hours, and there after the gel was destained in acetic acid 7%.

(4) Densitometry

The density of each band in isoenzyme-stained-gel and protein-stained-gel was recorded by densitometer (OZUMOR 82, ASUKA MFG CO., LTD.), making use of the slit (width 0.2 mm, length 4 mm) through the filter No. 61 (610 nm). Gel measured, was kept in acetic acid 7% and in measurement the transparent part of the solution was looked upon as a blank.

Results

To make some comparisons among the three substrates as well as to measure the mobility each substrate in the viscera of the respective domestic animal the patterns of isoenzyme-staining in the

three substrates and protein staining using gel were divided into the 4 equal parts and were illustrated.

Corresponding to the appellation of S-ChE isoenzyme in the IV report[12] and on obediance to Lamotta and Woronicks[9] advice each isoenzyme fraction was designated C_1, C_2,..., in the order of decreasing mobility.

In case of the indistinct band, for example in the case when both C_1 and C_2 fractions appeared in a form of one band, that was named C_{1-2}. In the following we describe the results obtained in viscera of each domestic animal.

1. Figs. 1–4 show isoenzyme patterns of ChE in the three substrates (BTC, PTC and ATC) in dog; (illustrating serum in Fig. 1, liver and kidney in Fig. 2, heart and spleen in Fig. 3, pancreas and lung in Fig. 4). Fig. 5 shows pancreas ChE isoenzyme patterns of fresh extract and of the one in which dissolution and freezing were repeated mutually for the period of 6 months.

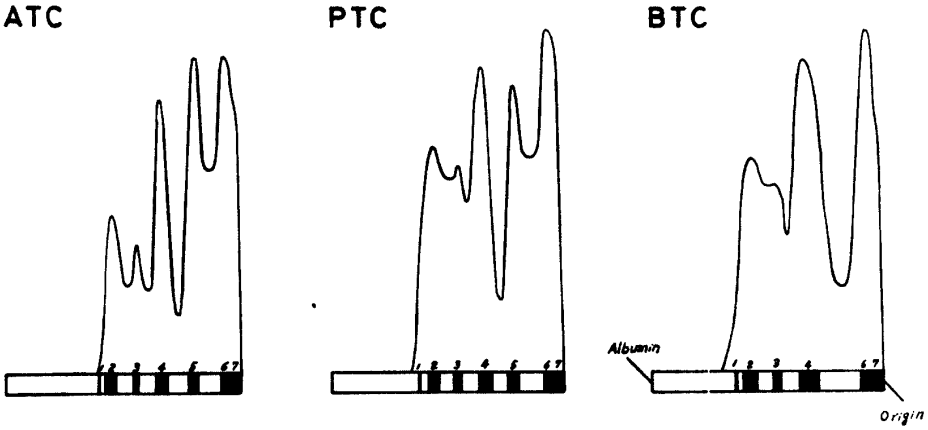

Fig. 1. Isoenzyme patterns of dog serum cholinesterase (ChE) using ATC, PTC and BTC as substrate.

(1) In the IV[12] and the VII[13] reports, in case of serum seven bands were detected in each substrate, but in this measurement, concerning C_5 band in BTC almost naught was detected, moreover, the separation between C_1 and C_2 bands in each substrate was quite indistinct. This may be due to the fact that the amount of used in this measurement is larger than the amount of 0.008 ml adopted in IV[12] and VII[13] reports. C_5-band-activity was higher in ATC, PTC and BTC in this order. C_6- and C_7-band activities were similar through the respective substrates. C_1- and C_2-band-activities in ATC were apparently low.

(2) In liver, the three bands (C_{1-4}, C_5 and C_{6-7}) were detected in each substrate. Likewise in case of serum C_5 band activity was higher in ATC, PTC and BTC in this order, but was low in each substrate. In dog activity in the other viscera was comparatively high, only activity in liver was low. C_{1-4} band-activity was higher in BTC, PTC and ATC in this order, which was the reverse order in case of C_5 band-activity, it was detected in a form of a wide band, although it had comparatively high activity.

(3) In kidney, five bands (C_{1-2}, C_4, C_5, C_6 and C_7) were higher in BTC, PTC and ATC in this order, nevertheless, C_1–C_5 band activities were low in BTC; C_1–C_3 band activity showed almost no activity. Likewise on case of liver C_5 band activity was higher in ATC, PTC and BTC in this order. C_6 and C_7 band were distinctly separated.

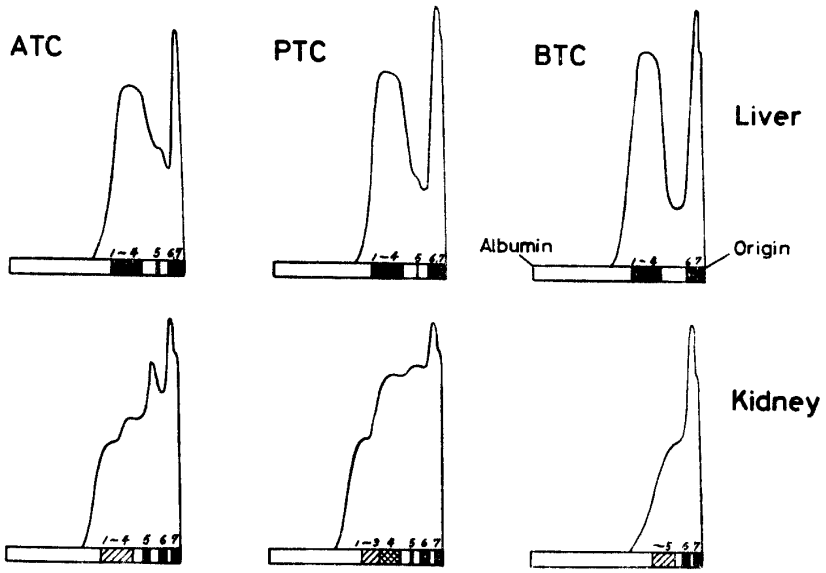

Fig. 2. Isoenzyme patterns of tissue ChE in dog.

(4) In heart, 5 bands (C_{1-3}, C_4, C_5, C_6 and C_7) were detected in each substrate, but C_5 band was not detected in BTC. C_6 and C_7 band were distinctly separated. Likewise in case of the other viscera C_5 band activity was higher in ATC, PTC and BTC in this order. Isoenzyme patterns in the three substrates were quite identical with those in other bands except C_5 band.

(5) In spleen, 4 bands (C_{1-4}, C_5, C_6 and C_7) were detected in each substrate. Against the observations in other viscera C_{1-4} band activity was remarkably low in each substrate C_5 and C_6 band activities were higher in ATC, PTC and BTC in this order. C_7 band activity was similar in ATC and PTC, but rather low in BTC.

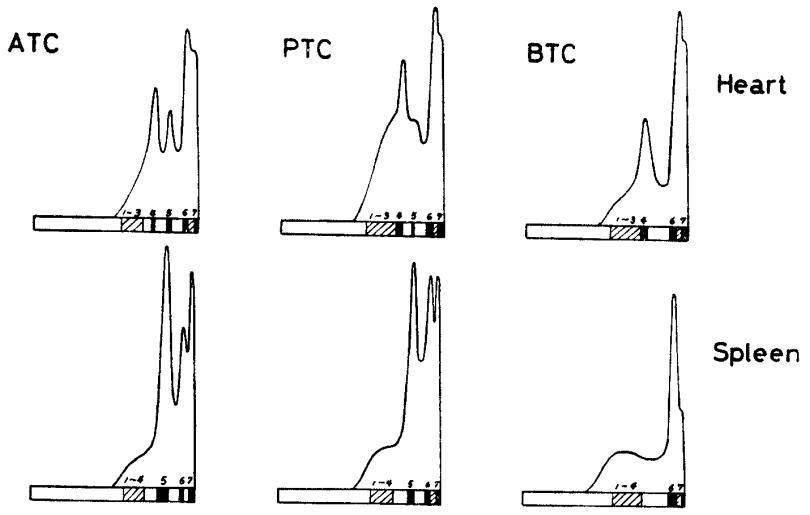

Fig. 3. Isoenzyme patterns of tissue ChE in dog.

(6) In pancreas, 4 bands (C_2, C_3, C_4 and C_{6-7}) were detected in each substrate. C_5, C_6 and C_7 bands were noted to be combined and were detected in the form of one band. Therefore, unlike in the case of the other viscera we could not compare pancreas activities in each substrate. Reflecting the high total pancreas ChE activity each fraction activity was remarkably high. C_4, C_3 and C_2 bands were distinctly separated, and activities were comparatively high.

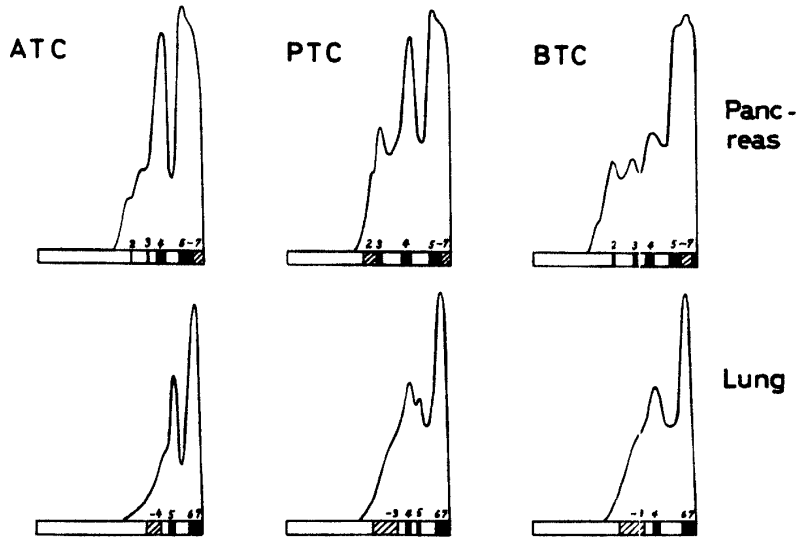

Fig. 4. Isoenzyme patterns of tissue ChE in dog.

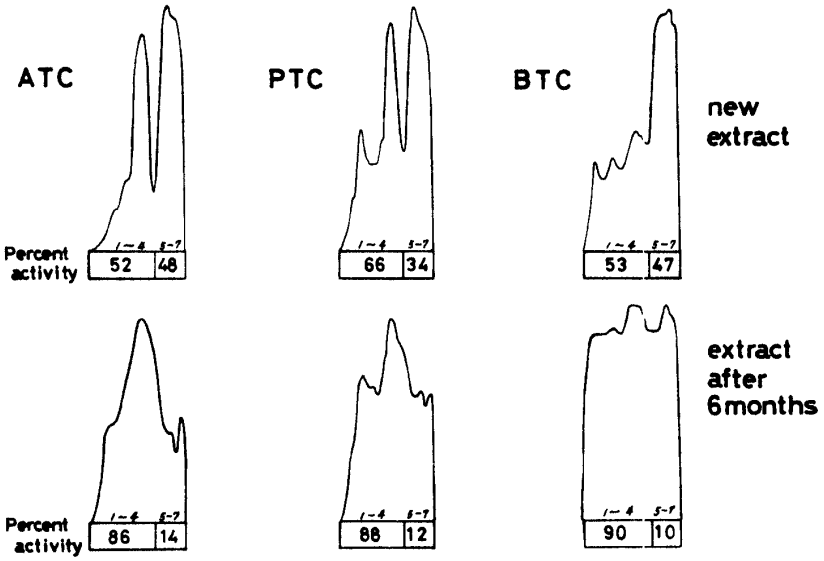

Fig. 5. Change of Isoenzyme patterns of Pancreas ChE by storage in dog.

(7) In lung, difference was noted among the three substrates. 3 bands (C_{1-4}, C_5 and C_{6-7}) were detected in ATC, 4 bands (C_{1-3}, C_4, C_5 and C_{6-7}) in PTC, 3 bands (C_{1-3}, C_4 and C_{6-7}) in BTC, while C_5 was hardly detected in BTC. Each band-activity was higher in ATC, PTC and BTC in this order.

2. Figs. 6–9 show isoenzyme patterns of T-ChE for the three substrates (BTC, PTC and ATC) in horses (illustrating serum in Fig. 6, liver and kidney in Fig. 7, heart and spleen in Fig. 8, pancreas and lung in Fig. 9).

(1) In serum, seven bands (C_1–C_7) were detected in the previous measurement, but in this measurement, C_1 and C_2 bands were not independent, respectively, owing to the indistinct separations of C_1 and C_2 bands. C_5 showed hardly any activity in BTC.

(2) In liver, 6 bands (C_1, C_{2-3}, C_4, C_5, C_6 and C_7) were detected and as to the number of bands the highest was noted in T-ChE of horse, closely resembling the one in the case of S-ChE. This

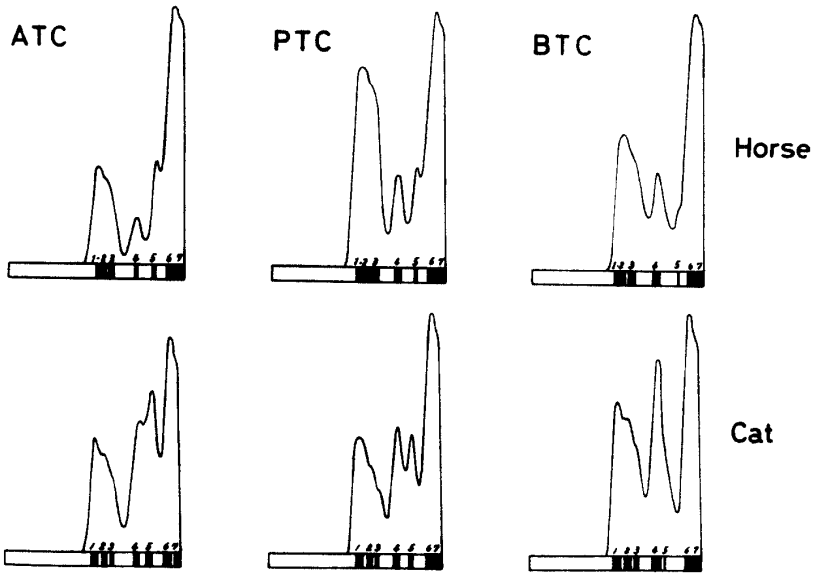

Fig. 6. Isoenzyme patterns of sera ChEs in horse and cat.

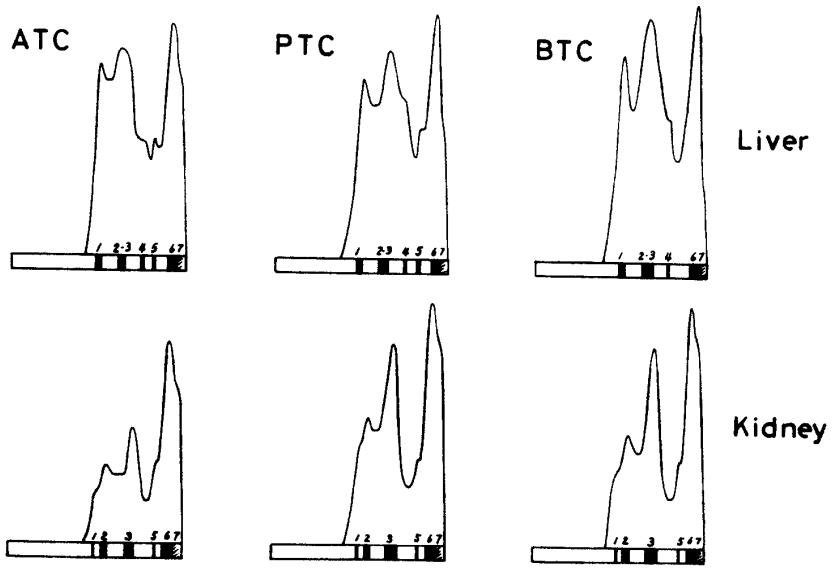

Fig. 7. Isoenzyme patterns of tissue ChE in horse.

differed from the case in dog liver in which the numbers of band were quite few, in spite of the high activity. Each band-activity was higher in BTC, PTC and ATC in this order, which was coincided with the suitability in substrate. C_1 band was detected individually, but C_2 and C_3 bands were not separated; C_5 band was detected in ATC and PTC however there was no clear difference noted between both the substrates.

(3) In kidney, 5 bands (C_1, C_2, C_3, C_5 and C_{6-7}) were detected, but no band was detected in each substrate. There was no clear difference noted among the three substrates due to the low activity in C_5 band. In comparison with the isoenzyme activity in BTC and PTC, the total isoenzyme activity in ATC was remarkably low; as may be seen on the following example that if activity in BTC was defined as 100, that in PTC would be 97 and that in ATC, 60.

(4) In heart, there was a difference among the three substrates. 2 bands (C_1 and C_6) were detected in BTC and PTC, but C_1 was not detected and C_6 band was detected only in ATC. C_2–C_5

and C_7 bands were not detected in each substrate.

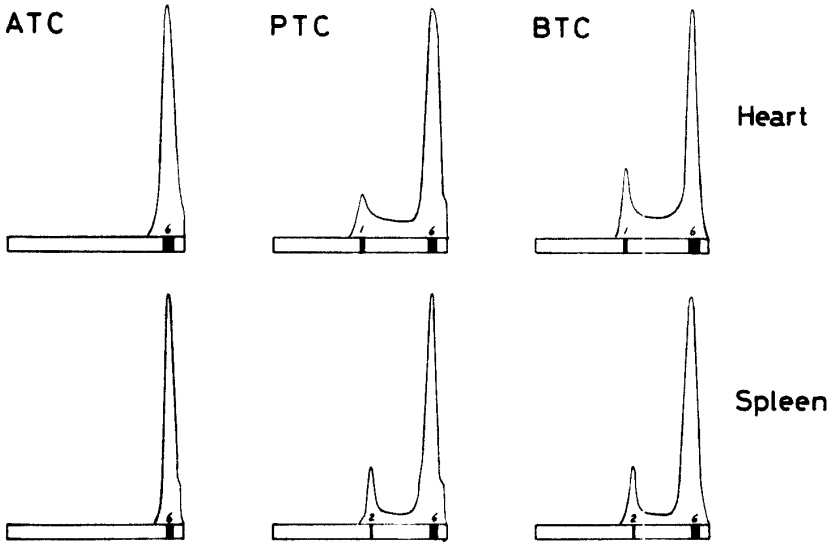

Fig. 8. Isoenzyme patterns of tissue ChE in horse.

(5) In spleen, likewise in heart 2 bands were detected in BTC and PTC, one band was detected only in ATC, but unlike in heart the bands (C_2 and C_6) were differed from the bands (C_1 and C_6).

(6) In pancreas, only C_6 band was detected in each substrate, and the number was least in all the viscera measured.

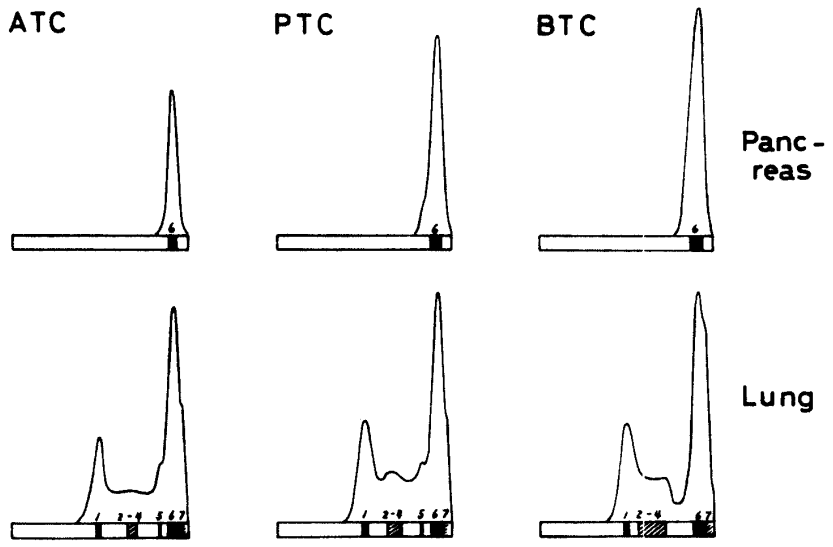

Fig. 9. Isoenzyme patterns of tissue ChE in horse.

(7) In lung, 4 bands (C_1, C_{2-4}, C_5 and C_{6-7}) were detected in each substrate. C_1 band was detected distinctly, but C_2–C_4 bands were combined and were detected in a form of one band. C_5 band was not detected in BTC. In the two substrates there was no clear difference between ATC and PTC both of which showing low activity.

3. Fig. 6 and Figs. 10–12 show isoenzyme patterns of T-ChE in the three substrates (BTC,

PTC and ATC) in cat. (illustrating serum in Fig. 6, liver and kidney in Fig. 10, heart and spleen in Fig. 11, pancreas and lung in Fig. 12).

(1) In serum, seven bands (C_1–C_7) were detected, which was almost coincided with the results obtained by the previous measurement excepting that the separation of C_6 from C_7 was indistinct. The C_5 band activity was higher in ATC, PTC and BTC in this order.

(2) In liver, 2 bands (C_{1-4}, C_{6-7}) were detected in each substrate, which was resembled closely liver ChE isoenzyme-patterns in dog, excepting the C_5 band.

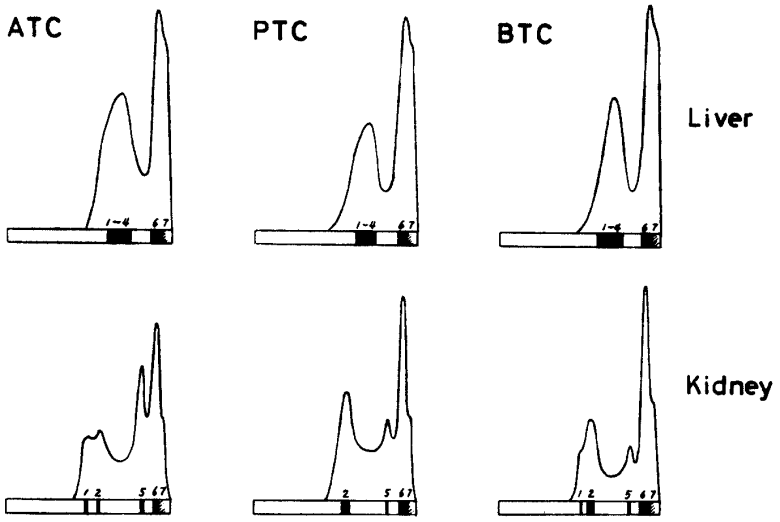

Fig. 10. Isoenzyme patterns of ChE in cat.

(3) In kidney, 4 bands (C_1, C_2, C_5 and C_{6-7}) were detected in PTC. C_5 band was detected distinctly and the activity was higher in ATC, PTC and BTC in this order.

(4) In heart, 5 bands (C_1, C_2, C_3, C_{4-5} and C_{6-7}) were detected in ATC and PTC, 4 bands (C_1, C_2, C_{3-4} and C_{6-7}) were detected in BTC. C_5 band activity was higher in ATC and PTC in this order, and C_5 band showed hardly any activity in BTC.

(5) In spleen, 4 bands (C_{1-2}, C_{3-4}, C_6 and C_7) were detected in BTC, 5 bands (C_{1-3}, C_4, C_5, C_6 and

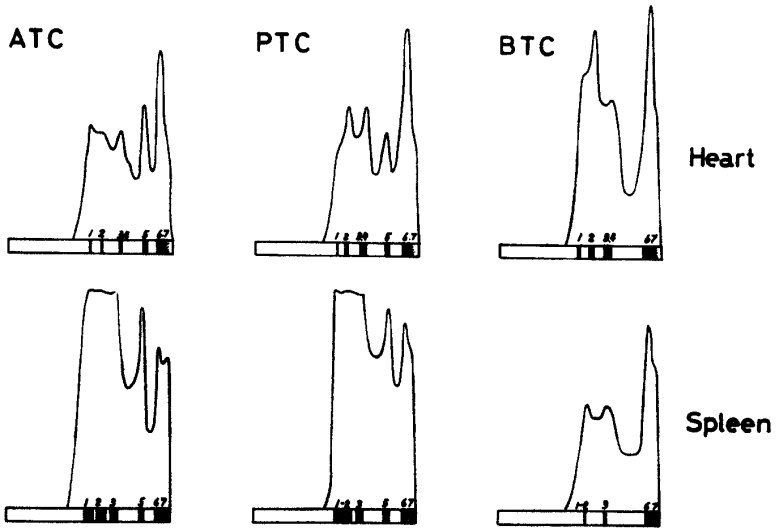

Fig. 11. Isoenzyme patterns of tissue ChE in cat.

C_7) were detected in PTC and ATC. Each isoenzyme pattern in substrates differed from those in other domestic animals, activity of the fraction with high mobility was higher than that with low mobility. This patterns was peculiar to cat. Namely, the activity got remarkably higher in ATC and PTC in this order, in accordance with the shifting from C_5 band to C_{1-3} band. Likewise in case of other domestic animals in BTC, C_{1-2} and C_{3-4} band activities were low in comparison with C_{6-7} band activity.

(6) In pancreas, 2 bands (C_4 and C_6) were detected in each substrate, but the activity was remarkably low.

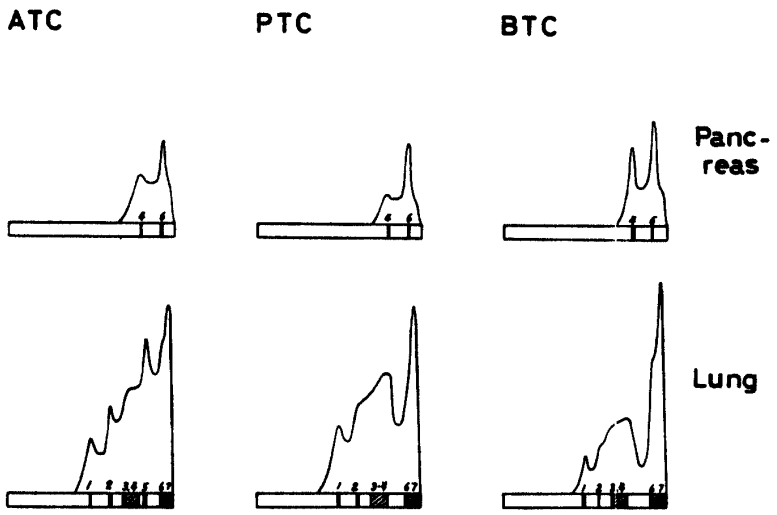

Fig. 12. Isoenzyme patterns of tissue ChE in cat.

(7) In lung, each fraction was distinctly separated in ATC, and 5 bands (C_1, C_2, C_{3-4}, C_5 and C_{6-7}) were detected, but in PTC and BTC only 4 bands (C_1, C_2, C_{3-4} and C_{6-7}) were detected.

4. Figs. 13–16 show isoenzyme patterns of T-ChE in three substrates (BTC, PTC and ATC) in pig (illustrating serum in Fig. 13, liver and kidney in Fig. 14, heart and spleen in Fig. 15, pancreas

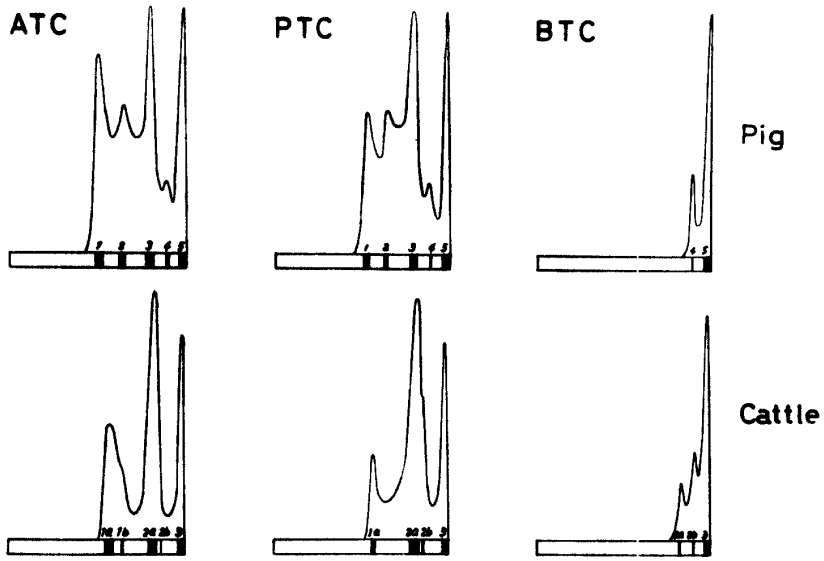

Fig. 13. Isoenzyme patterns of sera ChEs in pig and cattle.

and lung in Fig. 16).

(1) In serum, according to the numbers of band detected, isoenzyme patterns were split into 4 types therefore[13], in almost all the bands we measured isoenzyme patterns in the type IV, and then, 2 bands (C_4 and C_5) were detected in BTC, 5 bands (C_1 and C_5) were detected in ATC and PTC. Activity was higher in C_5, C_3, C_1, C_2 and C_4 in this order in ATC and PTC.

(2) In liver, 2 bands (C_{1-2} and C_5) were detected distinctly (in liver, kidney and heart, a new band with medium mobility between C_1 and C_2 in serum was detected). Unlike in case of serum the degree of substrate specificity of C_{1-2} band was not higher in ATC, PTC and BTC in this order but C_{1-2} activity in BTC was rather higher than those in ATC and PTC. C_5 band was detected along the side of the origin, showing the same degree of specificity for each substrate.

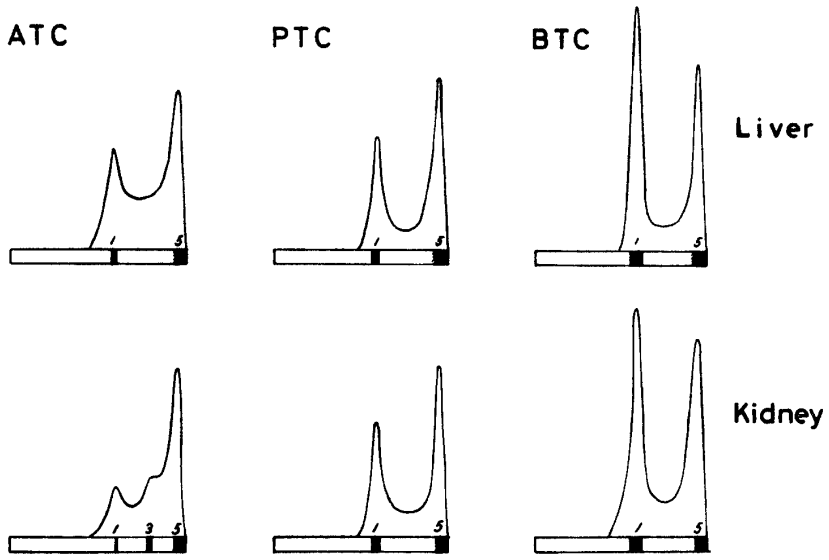

Fig. 14. Isoenzyme patterns of tissue ChE in pig.

(3) In kidney, 2 bands (C_{1-2} and C_5) were detected in BTC and PTC; 3 bands (C_{1-2}, C_5 and C_3) were detected in ATC; there was same difference among substrates in the number of band. But the C_{1-2} band activity was higher in BTC, PTC and ATC in this order and the decision of the most suitable substrate was noted to be difficult.

(4) In heart, 2 bands (C_{1-2} and C_4) were detected in BTC, 2 bands (C_3 and C_4) were detected in PTC, 3 bands (C_{1-2}, C_3 and C_4) were detected in ATC and C_5 band which was noted in the three substrates in serum was not detected in any one; and moreover, C_{1-2} was detected in BTC and ATC, but not in PTC. This fact was worthy of being noted.

(5) In spleen, 2 bands (C_1 and C_5) were detected in BTC, 3 bands (C_1, C_3 and C_5) were detected in PTC and ATC. Similar activities were noted in each substrate excepting C_2 band activity. C_3 band in serum was detected only in ATC and PTC, moreover, the activity was higher than those in others. C_3 band in viscera was not detected in BTC, and even in ATC and PTC it was detected only in kidney, heart and spleen, the activity being low.

(6) In pancreas, 2 bands (C_3 and C_4) were detected in each substrate. We could not recognize C_5 band which was detected in many other viscera.

(7) In lung, C_5 band was detected in BTC, 2 bands (C_4 and C_5) were detected in PTC and ATC. Likewise in C_5 band C_4 band showed high activity, and differed from C_4 band in serum having quite slight activity.

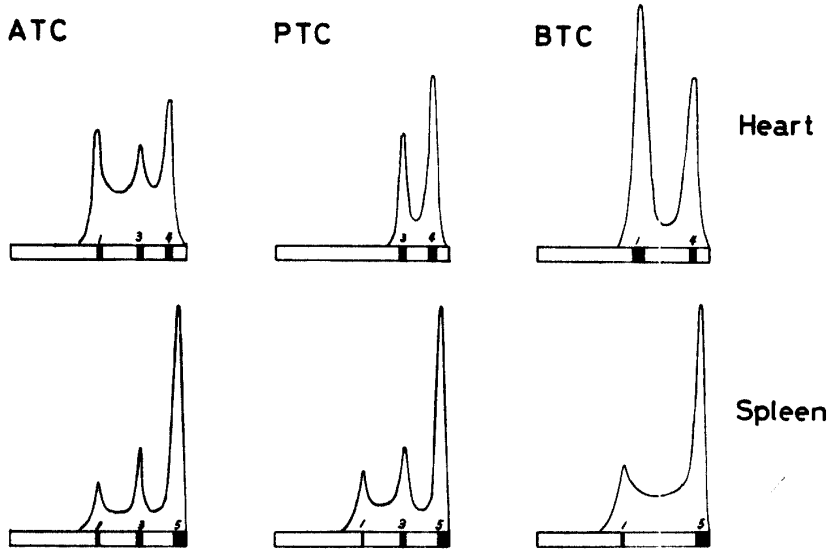

Fig. 15. Isoenzyme patterns of tissue ChE in pig.

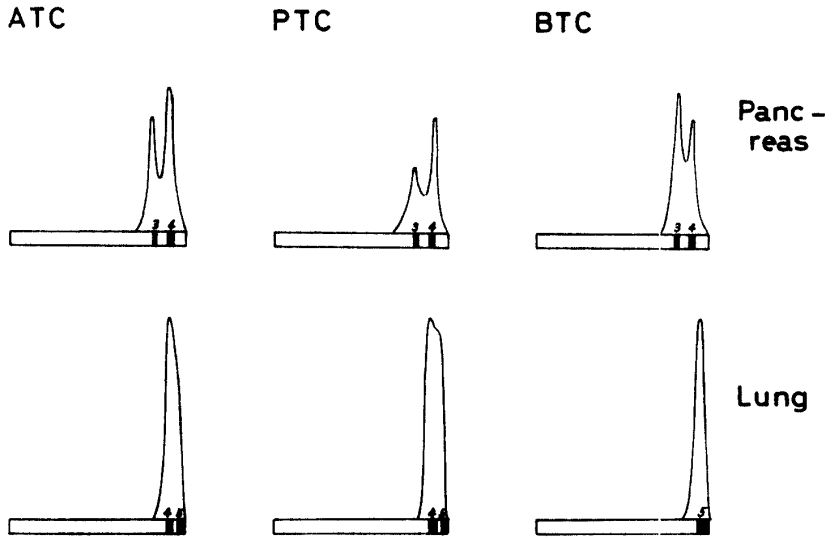

Fig. 16. Isoenzyme patterns of tissue ChE in pig.

5. Fig. 13 and Figs. 17–19 show isoenzyme patterns of T-ChE in the three substrates (BTC, PTC, and ATC) in cattle (illustrating serum in Fig. 13, liver and kidney in Fig. 17, heart and spleen in Fig. 18, pancreas and lung in Fig. 19).

(1) In serum, 3 bands (C_1, C_2 and C_3) were detected in the VII report[13], serum amounts to be used were exchanged 0.1 ml for 0.2 ml, and the substrate concentration was exchanged 1×10^{-3} M/l (5 folds of Km value) for 5×10^{-3} M/l (26 folds of Km value) in this measurement; consequently, there was a trace of bands with low mobility left near C_1 and C_2 band; therefore, for convenient sake, we divided C_1 band into 2 bands (C_{1a} and C_{1b}) and C_2 band into 2 bands (C_{2a} and C_{2b}) (mobility of a is more than that of b, a and b were fit C_1 and C_2 band respectively). In this way, we divided 3 bands (C_{2a}, C_{2b} and C_3) in BTC, 4 bands (C_1, C_{2a}, C_{2b} and C_3) and 5 bands (C_{1a}, C_{1b}, C_{2a}, C_{2b} and C_3) in ATC. Isoenzyme activities were higher in C_2, C_3 and C_1 in this order in PTC and ATC; in C_3 and C_2 in this order in BTC. This was coincided with the previous report[14] excepting BTC.

(2) In liver, only C_3 band was detected in BTC, 2 bands (C_{2b} and C_3) were detected in PTC and ATC. C_{2a} showed overwhelming higher activity than C_{2b} in C_2 of serum. But in C_2 band of liver, C_{2a} was not detected and there was a trace of C_{2b} band detected and C_{2b} activity was higher in ATC, PTC and BTC in this order.

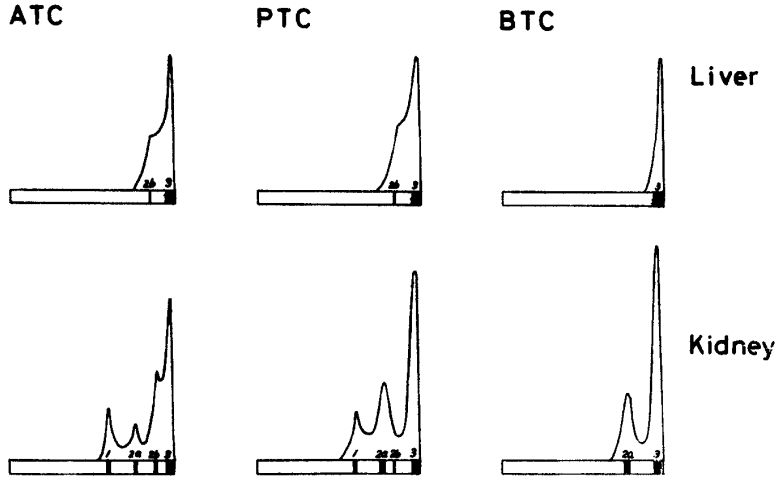

Fig. 17. Isoenzyme patterns of tissue ChE in cattle.

(3) In kidney, 2 bands (C_{2a} and C_3) were detected in BTC, 4 bands (C_{1a}, C_{2a}, C_{2b} and C_3) were detected in ATC and PTC. C_{2a} activity was higher in ATC and PTC in this order.

(4) In heart, likewise in serum 5 bands (C_{1a}, C_{1b}, C_{2a}, C_{2b} and C_3) were detected in each substrate. While we could not detect C_{1a} and C_{1b} in PTC, in heart we could detect these bands. Hereafter, heart showed almost all bands through all viscera. C_{2b} band activity was higher in ATC, PTC and BTC in this order.

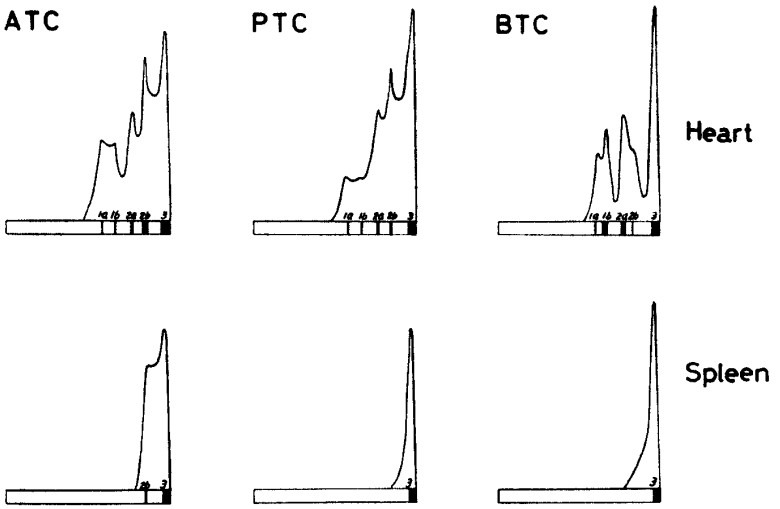

Fig. 18. Isoenzyme patterns of tissue ChE in cattle.

(5) In spleen, only C_3 band was detected in BTC and PTC, C_3 band and a trace of C_{2b} band were detected in ATC, in either case band-activities were low.

(6) In pancreas, 2 bands (C_{2b} and C_3) were detected in ATC and BTC, 3 band (C_{2b}, C_3 and C_{2a}) were detected in PTC.

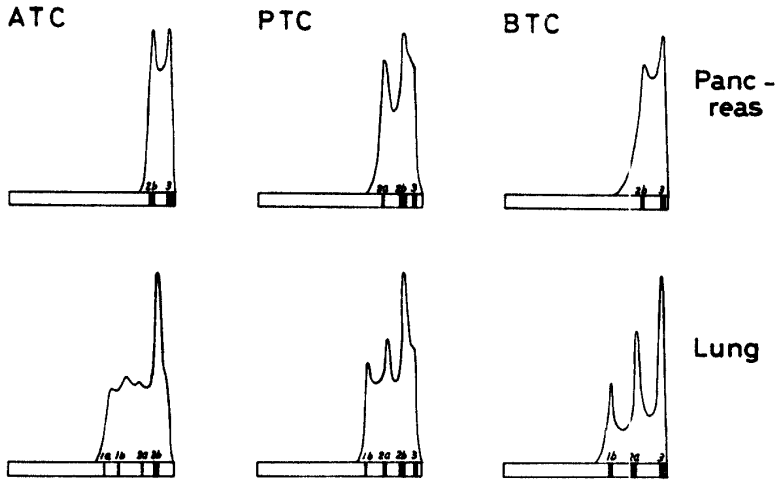

Fig. 19. Isoenzyme patterns of tissue ChE in cattle.

(7) In lung, 3 bands (C_{1b}, C_{2a} and C_3) were detected in BTC, 4 bands (C_{1b}, C_{2a}, C_{2b} and C_3) in ATC. C_{2b} and C_2 band activities were noted to be quite different in each substrate; in short, C_{2b} band showed no activity in BTC but showed high activity in PTC and ATC; C_3 band showed the highest activity in BTC in the three substrates. Eventually, this showed that there were some substrate specificities of isoenzyme in lung and kidney. Each isoenzyme band in viscera of pig and cattle which was distinctly detected regardless of the number and the degree of the activity value noted in comparison with those of dog, horse and cat, was not combined and not wide in band-width, too.

Discussion

Concerning the numbers of band and isoenzyme activities in domestic animals a considerable difference was noted between the T-ChE isoenzyme patterns and the S-ChE isoenzyme patterns. Compared with the mobility of isoenzyme, the mobility of T-ChE isoenzyme was almost wholly coincided with any of the S-ChE isoenzyme, especially, C_6 bands in dog, horse and cat were distinctly detected, showing high activity through all the viscera as in case of serum. In C_1–C_4 bands, we recognized the fractions equal to these bands in most viscera in dog and cat though they were indistinct, showing low activities. But these bands in horse were distinct, but there were some lacking in bands. Namely, through all of these viscera in the three parts, heart, spleen and pancreas, we noted C_6 band; but in heart only C_1, in spleen only C_2 and in pancreas only C_6; in a word, a considerable difference was noted not only among domestic animals but in viscera of the same domestic animals. In the liver ChE, C_1–C_4 bands in both dog and cat were not separated but combined and were detected in a form of a band, showing comparatively high activity. Three bands (C_1, C_{2-3} and C_4) in horse were separated. In the domestic animals (dog, horse and cat) in which S–ChE isoenzyme was noted to be similar a considerable difference was noted even in liver which was suspected of influencing S-ChE. C_5 band was noted commonly through the 3 domestic animals (dog, horse and cat) and the activity was higher in ATC, PTC and BTC in this order. This fraction was clearly differed from other fractions in substrate specificity, and was slightly recognized in lung of horse, in heart and spleen of cat, while this was observed in almost all the viscera of dog. C_5 fraction had a high possibility of being true ChE especially due to the fact that the activity was high

in cerebram. S-ChE isoenzyme patterns of pig and cattle were remarkably differed from those of dog, horse and cat; moreover, there was quite a difference between pig and cattle in T-ChE isoenzyme patterns, too. For example, though liver ChE of cattle showed higher activity than other ChEs, the number of band was noted to be quite few (2 bands), contrary to the fact that in ATC, it showed the number of band equal to that in serum in kidney, heart and lung; moreover, C_{2a}, one of the chief elements was not detected. Accordingly, we suspected that the influence of liver ChE on S-ChE in cattle was negligible. In pig the number of band in the respective T-ChE was few; and C_3, chief element of S-ChE (C_3) turned similarly to be a chief one only in pancreas; C_1, C_4 and C_5 band activities were high in each viscus except pancreas; moreover, T-ChE in which C_5 was a chief element were numerous (liver, kidney, spleen and lung) and these viscera showed hardly C_3 fraction; hence there was the possibility that C_5 and C_3 fractions got supply mainly from other viscera or tissues. If there were visceral specificity of S-ChE in pig, pancreas might be the supply source of chief element (C_3) and this might also be supported fairly by Augustinsson's[1] theory that pancreas is the supply source of S-ChE in pig. C_2 and C_4 of S-ChE in pig are sometimes detected but they are not to be detected, sometimes while in T-ChE the medium type between C_1 and C_2 was detected, viscera with C_4 band are generally in possession of C_3 or of C_5 but do not have both at the same time. Consequently, we suspect that C_2 is made out of C_1 but C_4 is made neither out of C_3 nor of C_5 in T-ChE of the type having C_4 in S-ChE but is synthesized somewhere else. But we did not ascertain whether C_4 is genetically different from C_3 and C_5. In cattle we could not obtain the views supporting the visceral specificity of S-ChE but suspection remained that ChE of liver, heart and lung was firmly related with S-ChE due to a lot of bands detected in comparison with other viscera. C_{2a} activity of most of the T-ChE in cattle was higher ATC, PTC and BTC in this order, and in case of C_{2a} similar tendency was noted in S-ChE. We suspected that this as well as C_5 fraction in dog was under the influence of true ChE.

There are a lot of reports. Of the difference between S-ChE and T-ChE in human. It has been assumed that there is not any visceral specificity in S-ChE of human. Judging from the nature for the inhibitor, Liddell *et al.*[10] reported that S-ChE and T-ChE are put under the control of same gene, but differing from human in domestic animals. We can hardly affirm that there is not any visceral specificity. Basing on the results of the examination of each visceral isoenzyme in human by cellogel electrophoresis Kurose[6] reported that Che 1 (within α_2–β globulin) and Che 2 (within γ-globulin) were detected, and in this case Che 1 was similar to Che 2 of S-ChE, but Che 2 was the one peculiar to the tissue itself on account of the fact that it does not exist in S-ChE. By DEAE chromatograph, Svensmark[20] divided liver ChE of human into three fractions, and the asserted that one of the three is identical with that of S-ChE, but the others are to be assumed to have been synthesized in liver as a sort of precursor of S-ChE as it is devoid of all acid.

In electrophoresis in polyacrylamide gels of human S-ChE, 4 fractions (C_1–C_4) have been detected, here, C_4 having the smallest mobility is the chief element, having the highest activity, while that of C_1 is the lowest. LaMotta *et al.*[7] suspected that the small amount of C_1, C_2, C_3 was the medium product in the formation of C_4 fraction. Oppositely Saeed[18] suggested that C_1–C_3 were a sort of resolution product of C_4 and were synthesized owing to the deavage of peptide chain brought by the action of proteinase.

In electrophoresis of polyacrylamide gels in S-ChE and T-ChE made by authors in domestic animals, for example, in dog, 7 bands were detected. In this case, as C_6 fraction was assumed to be a chief element, if it was to be looked upon as C_4 fraction in human were naturally regarded as C_1–C_3 in dog. (C_5 was omitted as it was suspected to be true ChE). And then, in T-ChE, C_1–C_4

in liver were not separated and detected in a form of a wide band, in other viscera, the number of band was few in comparison with that in S-ChE and their activities were low. Therefore, T-ChE are assumed to be on a course of development, if S-ChE were regarded as the final product. We suppose that relevancy lies in Saeed's[18] theory rather than LaMotta *et al.*[7] owing to the noted fact that C_1–C_4 shows low activity and are not differentiated yet, and it may be probable that C_1 and C_2 are wanting. However, fractions in serum of pig and cattle showing highest activities hardly show similarly high activity in viscera. From these items, we assumed that each element in pig and cattle is under a process of production which is from that in human.

Holmes and Masters[4] measured T-ChE isoenzyme in wide range in guinea pig and detected 5 bands in liver, but no band in serum. Isoenzyme patterns of animals including human remarkably differ among serum and viscera. LaMotta[8] made an explanation of multiplicity, describing that the various isoenzymes in S-ChE of human were produced by polymerization of common subunit with some reservation. Boutins and Brodeur[2] recognized that it was impossible to be produced in vivo while it was otherwise in vitro. In author's measurement, making use of the difference of activity ratio of each element. There is a certain possibility of transforming isoenzyme with some reservation of electrophoresis (lengthening or shortening of electrophoresis time, etc.).

Ogita[17] suggested that by regarding the element with mobility smaller than a chief one, as abnormal one in human, abnormal elements in S-ChE were to be looked upon as the embelishment enzyme synthesized by the action of neoraminidase which were carried into blood with the physiologic pathological transmission on the basis of hereditary backing. But in T-ChE of domestic animals, there are certainly various possibilities including the case that C_7 having smaller mobility than C_6, chief element, shows hardly any activity or that it shows high one, it requires further examination. And next, as to the source of S-ChE, especially of ChE activity of dog, it was noted to be remarkably high in pancreas, and, the problem how S-ChE was influenced by it would not be made clean until clinical cases related to pancreas disease were clarified and clinical experiment was carried out hereafter.

Goutier and Goutier-Pirotte[3] reported that there is certainly a possibility of pancreas ChE being synthesized in mitochondria in pancreas, suggesting that T-ChE were synthesized in each tissue, receiving some action in the process of reaching blood and turned into S-ChE as final product.

In the above mentioned, hereafter we should examine the transformation process from T-ChE to S-ChE.

Summary

The measurements of T-ChE isoenzyme with Disc electrophoresis were carried out in domestic animals, and T-ChE isoenzyme was examined in comparison with S-ChE isoenzyme. The obtained results were summarized as follows.

1. Isoenzyme of S-ChE was distinctly detected. That of T-ChE was characteristically detected in a form of wide band of complex of a few isoenzyme with the difference among the respective domestic animals and viscera of the same ones having no settled tendency.

2. In the number of isoenzyme fraction, the relation between T-ChE and S-ChE was as follows.

$$\text{T-ChE} \leqq \text{S-ChE}$$

We obtained the view that suggested the existence of the visceral specificity in pig.

3. C_5 fraction with different substrate specificities in S-ChE isoenzyme of dog, horse and cat

existed in most viscera in a form of C_5 fraction of T-ChE isoenzyme in dog, and existed in some viscera in horse and cat showing the highest suitability of substrate in ATC, PTC and BTC in this order.

4. In dog, reflecting the high activity pancreas ChE showed largest number of isoenzyme bands through all T-ChE giving a large influence on S-ChE.

References

1) Augustinsson, K. B.: Electrophoresis studies on blood plasma esterase. III. Conclusions. *Acta Chem. Scand.*, **13**, 1097–1105 (1959)
2) Boutin, D. and Brodeur, J.: Human serum pseudocholinesterase. molecular weight estimation of a subunit structure. *Can. J. Physiol. Pharmacol.*, **49**, 777–779 (1971)
3) Goutier, R. et Goutier-Pirotte, M.: Localization intracellulaire des cholinesterases. II. Pancreas et suc pancreatique de chien. *Biochim. Biophys. Acta.*, **16**, 558–565 (1955)
4) Holmes, R. S. and Masters, C. J.: The developmental multiplicity and isoenzyme status of avian esterases. *Biochim. Biophys. Acta.*, **132**, 379–399 (1967)
5) Juul, P.: Human plasma cholinesterase isoenzymes. *Clin. Chim. Acta*, **19**, 205–213 (1968)
6) Kurose, K.: Studies on plasma cholinesterase. *Physico. Chemi. Biol.*, **16**, 51–67 (1971)
7) LaMotta, R. V., McComb, R. B. and Wetstone, H. J.: Isozyme of serum cholinesterase. A new polymerization sequence. *Canad. J. Phys. Pharma.*, **43**, 313–318 (1965)
8) LaMotta, R. V., McComb, R. B., Noll, Jr. C. R., Wetstone, H. J. and Reinfrank, R. F.: Multiple forms of serum cholinesterase. *Arch. Biochem. Biophys.*, **124**, 299–305 (1968)
9) LaMotta, R. V. and Woronick, C. L.: Molecular heterogeneity of human serum cholinesterase. *Chin. Chem.*, **17**, 135–144 (1971)
10) Liddell, J., Newman, G. E. and Brown, D. F.: A pseudocholinesterase variant in human tissues. *Nature*, **198**, 1090–1091 (1963)
11) Morizono, M. and Kaba, H.: Clinical studies on serum cholinesterase in domestic animals. I. Measuring method of serum cholinesterase activity in domestic animals. *Bull. Fac. Agr. Kagoshima Univ.*, **27**, 115–121 (1977)
12) Morizono, M. and Kaba, H.: Clinical studies on serum cholinesterase in domestic animals. IV. The method for detection of serum cholinesterase isoenzyme in domestic animals. *Bull. Fac. Agr. Kagoshima Univ.*, **27**, 131–139 (1977)
13) Morizono, M. and Omae, K.: Clinical studies on serum cholinesterase in domestic animals. VII. The measurement of zymogram of serum cholinesterase isoenzyme in normal domestic animals by the authors' detecting method. *Bull. Fac. Agr. Kagoshima Univ.*, **30**, 185–191 (1980)
14) Morizono, M. and Akinaga, Y.: Method in determination of tissue cholinesterase activities in domestic animals. *Mem. Fac. Agr. Kagoshima Univ.*, **17**, 201–217 (1981)
15) Myers, D. K.: Studies on cholinesterase. 9 specifies variation in the specificity patterns of the pseudocholinesterases. *Biochem. J.*, **55**, 67–79 (1953)
16) Nakamura, S.: Disc electrophoresis. *Jap. J. Clin. Path.*, **No. 11**, 77–93 (1968)
17) Ogita, Z.: Genobiochemical studies on serum cholinesterase. *Physico. Chemi. Biol.*, **15**, 1, 91–102 (1971)
18) Saeed, S. A., Chadwick, G. R. and Mill, P. J.: Action of proteases on human plasma cholinesterase isoenzymes. *B. B. A.*, **229**, 186–192 (1971)
19) Svensmark, O.: Enzymatic and molecular properties of cholinesterase in human liver. *Acta Physiol. Scand.*, **59**, 378–389 (1963)
20) Svensmark, O.: Molecular properties of cholinesterase. *Acta Physiol. Scand.*, **64**, Suppl. 245, 1–74 (1965)