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INTRODUCTION.

Succinic dehydrogenase is an important enzyme of the Krebs tricarboxylic acid cycle through which mainly glucose is oxidised. It is responsible for the oxidation of succinic acid to fumaric acid.

Comparative study on the distribution of this enzyme in liver of different mammals was carried out by Rutenburg et al (1). The activity of this enzyme in the liver of toad both during hibernation and non-hibernation was studied by Mukerji and Deb (2). The localization of succinic dehydrogenase in the liver of rat or rabbit and its variation was studied by Matsuyama (3). Succinic dehydrogenase activity in pelagic, nonpelagic and fresh water fishes was estimated biochemically (4).

It is expected that the activity of this important enzyme responsible for the oxidation of carbohydrate may vary in liver of different classes of animals even different species of the same class of vertebrate.

Considerable variation in blood glucose and liver glycogen in different classes of vertebrate has been studied in previous chapter (Chapter- IV.).

In the present investigation, comparative study of succinic dehydrogenase in liver of different classes of vertebrate was studied to have further information about the carbohydrate metabolism in them.
2. MATERIALS AND METHODS.

Specimens from each class of vertebrates, such as, Ophicephalus punctatus (Lata fish of carnivorous type), Girrhina marigala (Mrigal fish of herbivorous type), Bufo melanostictus (common toad), Calotes versicolor (garden lizard), Columba livia (domestic variety of rock pigeon), Rattus sp. (domestic variety of white rat) were collected from local animal dealer.

The Amphibian, Reptilian, Avian and Mammalian classes of animals were kept in the laboratory on normal diet for a couple of days prior to experimentation. These animals along with Piscine specimens were fasted overnight and killed next day. All these animals were male. A large number of animals of all these classes was collected throughout the year and experiments were repeated in the following year.

Some animals, like lata and mrigal fishes, toad and rat were killed by quick blow on the head, while lizard and pigeon were killed by decapitation. After killing the animals, pieces of liver were taken quickly and 15 fresh frozen sections were cut in freezing microtome, the sections were quickly dropped in the working solution and incubated for 2 hours.

Histochemical localization of succinic dehydrogenase in liver was carried out according to Nachlas et al (5).

Preparation of reagents.

1. Preparation of substrate solution.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of stock solution</th>
<th>Volume prepared</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Succinate.</td>
<td>0.2 M.</td>
<td>10 cc</td>
</tr>
<tr>
<td>Phosphate buffer</td>
<td>0.2 M</td>
<td>10 cc</td>
</tr>
<tr>
<td>(pH 7.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.33 M</td>
<td>0.2 cc</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>0.005 M</td>
<td>0.2 cc</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.6 M</td>
<td>2.0 cc</td>
</tr>
<tr>
<td>Aluminium chloride</td>
<td>0.01 M</td>
<td>0.8 cc</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>6.8 cc</td>
</tr>
</tbody>
</table>
The solution was prepared by mixing above ingredients and boiled vigorously, the volume was adjusted to 30 cc afterwards with boiled distilled water. It was kept in tightly glass stoppered bottle in refrigerator.

2. Preparation of working solution.

3 cc of the substrate solution was taken in a small reagent tube of approximately 5 cc capacity. To it, 1 cc of distilled water and 2 mg. of nitro blue tetrazolium was added. It was well stoppered and the temperature was raised to 37°C. prior to the experiment.

Method.

(1). 15 μ frozen sections of liver were cut and floated in the working solution.

(2). A limited number of sections were floated in the working solution and incubated for 2 hours. Sections were agitated from time to time to facilitate even reaction.

(3). After 2 hours substrate solution containing sections were poured into a large beaker full of water.

(4). Sections were mounted on clean glass slide with the help of pointed glass rod.

(5). They were blotted almost to dryness and mounted in glycerine jelly.

Result.

Blue colour indicates the sites of enzymatic activity.
3. Observations and Results.

(1). Lata fish.

Succinic dehydrogenase was found to be present uniformly in the hepatic cells of lata fish (Fig. 1). Concentration of the enzyme was found to be very small than all the other vertebrates studied.

(2). Mrigal fish.

In the hepatic cells of mrigal fish succinic dehydrogenase was observed to be slightly higher in concentration than in lata fish (Fig. 2).

(3). Toad.

Succinic dehydrogenase in the liver of toad was found to be present uniformly (Fig. 3). Concentration of the enzyme was more than in the above two types of fishes in July and August. This enzyme was found to decrease in hibernation.

(4). Lizard.

Fairly high amount of succinic dehydrogenase was present in the liver of the lizard (Fig. 4). Concentration of this enzyme in the hepatic cells was found to be more than in toad but less than in rat. Negligible amount of this enzyme was found to be present during hibernation.

(5). Pigeon.

High amount of succinic dehydrogenase was observed in the liver of pigeon (Fig. 5). Intensity of this enzyme in the hepatic cells were however less than in rat.

(6). Rat.

Highest amount of succinic dehydrogenase was found to be present in the rat than in the other vertebrates studied (Fig. 6).
DISCUSSION.

Succinic dehydrogenase is an essential enzyme whose activity takes part in oxidation of carbohydrate through Krebs cycle. Presence of higher amount of this enzyme in liver indicates high rate of carbohydrate oxidation.

It has been observed that varying amounts of glycogen and neutral lipid were present in the liver of the experimental animals. So, it may be suggested that the amount of succinic dehydrogenase present in the liver of different animals may be variable.

In the liver of lata fish a very high amount of neutral lipid along with lower amount of glycogen and negligible amount of succinic dehydrogenase was present in all the seasons. This indicates the possibility that the main pathway of carbohydrate oxidation in this class of vertebrate is not through the Kreb's tricarboxylic acid cycle. The important pathway of carbohydrate oxidation in these animals is possibly through the alternative, the hexose monophosphate shunt mechanism. Evidences have been presented in the previous chapters to show that possibly the neutral lipid present in the liver of fish was formed from carbohydrate. This also might be due to inactivity of the Kreb's cycle. The products of carbohydrate metabolism was not completely utilized through the tricarboxylic acid cycle so that neutral lipid was formed from these intermediate products. Similar results have recently been observed by Deb and Chakravarty, (unpublished). They have administered malonate, an inhibitor of succinic dehydrogenase to sugar fed rats. Due to inhibition of tricarboxylic acid cycle with this poison
there was appearance of neutral lipid in liver. A drop in the glycogen level and succinic dehydrogenase has also been observed. The malonate treated sugar fed rats can be favourably compared with lata fish so far as the distribution of cytoplasmic constituents like neutral lipid, plasmal, glycogen and succinic dehydrogenase are concerned. The hibernating animals like toad and lizard showed different type of distribution during hibernation and non-hibernation. In hibernation, the cytoplasmic constituents (neutral lipid, plasmal, glycogen and succinic dehydrogenase) in the liver of both the vertebrates resembled lata fish. So, it may be suggested that their metabolic pattern are similar. Okasaki (6) has observed accumulation of succinic acid in the liver of hibernating salamander, due to blockage in the enzyme succinic dehydrogenase. Deb and Mukherji (2) obtained previously similar type of distribution in hibernating toad.

In non-hibernation on the other hand, the distribution of cytoplasmic constituents resembled that of pigeon and rat indicating similar metabolic pathway in non-hibernating toad, lizard, pigeon and rat.

In the latter class of vertebrate higher amount of succinic dehydrogenase and glycogen with the absence of histochemically demonstrable neutral lipid was found to be present in the liver.

Comparative distribution of succinic dehydrogenase in six mammals was studied by Rutenburg et al (1). In the liver highest amount of enzymatic activity was observed by him. Livers of rat and guineapig showed highest enzymatic activity whereas, less activity was observed in the liver of the mouse, dog, hamster and least in the rabbit (1).

From the above observations, it may be suggested that the enzymatic activity in the liver of different mammals of different habits may be variable.
Similar variation in the distribution of this enzyme in the liver of different classes of animals of different habits and habitats was observed by us.

5. **SUMMARY**

Comparative distribution of succinic dehydrogenase in the liver of different classes of vertebrate was studied.

Amount of succinic dehydrogenase present in the liver of lata and mrigal fishes was lesser than in the toad, lizard, pigeon and rat.

Distribution of succinic dehydrogenase was higher in the liver of toad than in the fishes but less than in lizard. Fall of liver succinic dehydrogenase was observed in hibernating toad and lizard.

Concentration of the enzyme in the liver of pigeon was less than in rat. Liver of the rat was found to contain highest amount of this enzyme.
PLATE - I.

Explanation of Plates.

**Figure 1.** - Succinic dehydrogenase in the liver of lata fish. Lesser amount of enzymes is seen. x 96.

**Figure 2.** - Succinic dehydrogenase in the liver of mrigal fish. Enzymatic activity is more than lata fish. x 96.

**Figure 3.** - Succinic dehydrogenase in the liver of non-hibernating toad. More intensity of the enzyme is seen than lata and mrigal fishes but less than rat. x 96.

**Figure 4.** - Succinic dehydrogenase in the liver of non-hibernating lizard. Enzymatic activity is less than rat. x 96.

**Figure 5.** - Succinic dehydrogenase in the liver of pigeon, concentration of the enzymes are less than rat. x 96.

**Figure 6.** - Succinic dehydrogenase in the liver of rat. Highest enzymatic activity is seen. x 96.
7. REFERENCES

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5. Nachlas, M. M., Tsow, K-C., Sousa, E. De., Chang, C. S.,
   and Seligman, A. M. (1957).
   J. Histochem. Cytochem. 5 : 420.

The presence of glucose-6-phosphatase in the liver of mouse was correlated with the ability of this organ to liberate blood sugar (1). Extensive work has been done by large number of workers on the distribution of glucose-6-phosphatase in various tissues of mammals. Glucose-6-phosphatase was demonstrated to be very high in the liver of mice (2). High level of this enzyme was demonstrated by Fiore et al (3) in the liver of sheep. The absence of glucose-6-phosphatase activity in fetal liver and its appearance in the liver of new-born animals was demonstrated in the rat (Weber and Cantero) (4). From the biochemical study of Weber and Cantero (5) it was concluded that glucose-6-phosphatase activity was highest in liver than other tissues in the normal rat.

Nemeth (6) observed accumulation of liver glycogen in the late phase of gestation due to absence of glucose-6-phosphatase activity.

It has been suggested that the enzyme may be involved in the transport of glucose across cell membrane involving phosphorylation and dephosphorylation (7).

From the above review it is clear that glucose-6-phosphatase is directly concerned with the regulation of carbohydrate metabolism.

Extensive work on the distribution of glucose-6-phosphatase in mammals was
carried out by large number of workers. But little attention was paid on the distribution of this enzyme in the liver of lower classes of vertebrate. An attempt has been made in the present investigation to have an idea on the distribution of glucose-6-phosphatase in the liver of different classes of vertebrate and to establish correlations between this enzyme with each of blood sugar and the amount of glycogen present in the liver of different classes of vertebrate.

2. MATERIALS AND METHODS.

Specimens from each class of vertebrates, such as, Ophicephalus punctatus (Lata fish of carnivorous type), Gharina urigala (Urighal fish of herbivorous type), Bufo melanoatigus (common toad), Calotes versicolor (garden-lizard), Columba livia (domestic variety of rock pigeon), Rattus sp. (domestic variety of white rat) were collected from local animal dealer.

Treatment of the animals before the experiment and the method of killing was same as described in the beginning of this chapter.

After killing, small pieces of liver from the animals were taken out and frozen quickly. 15 /a sections were cut, which were dropped in incubating medium. The incubation was carried out at 32° C. for 15 minutes. The enzymatic activity was demonstrated according to Chiquoine (7).

Preparation of reagents.

1. Conversion of Barium Salt of Glucose-6-phosphatase to Potassium Salt.

250 mg. of the barium salt was dissolved in 10 ml. of distilled water containing 2 drops of (2N)- HCl.

130 mg. of potassium sulphate was added to it. The solution was kept for two hours and stirred frequently. It was centrifuged and the
supernatant was tested for barium salt with a pinch of potassium sulphate. If no precipitation occurred, the solution was diluted up to 30 ml. with distilled water. The pH of the solution was adjusted to 6.7 with N-KOH.

2. Preparation of the Incubating Medium.

1 part of the above solution was diluted with 2 parts of 0.006 M-lead nitrate (0.2%). The solution was prepared and filtered just before use.

Method.
(1) 15 μ frozen sections of the liver were prepared after killing the animals and floated directly in the incubating medium kept at 32°C.
(2) They were incubated for 15 minutes at 32°C.
(3) Sections were washed in distilled water.
(4) They were then mounted on a clean slide with a drop of distilled water and dried.
(5) The slide was washed again in distilled water and dipped in dilute yellow ammonium sulphide (for 3-5 minutes).
(6) The sections were washed in distilled water and mounted in glycerine jelly.

Result.

Glucose-6-phosphatase activity was indicated by brownish black to black staining.

3. Observations and Results.

(1) Lata fish.

Histochemically demonstrable glucose-6-phosphatase was found to be present in the hepatic cells of this fish (fig. 1). Slight variation in the distribution of the enzymatic activity was observed. From the average result
it was found that the distribution of the glucose-6-phosphatase in the hepatic cells of lata fish was less than rat.

(2). **Mrigal fish.**

Distribution of glucose-6-phosphatase in the liver of mrigel fish was slightly less than the lata fish.

(3). **Toad.**

Histochemically demonstrable glucose-6-phosphatase was found to be low in the liver of August toad less than in other vertebrates (Fig. 2). In hibernation, a slight increase in the enzymatic activity was observed which was, of course lower than rat.

(4). **Lizard.**

Considerable amount of glucose-6-phosphatase was found to be present in the liver of lizard (Fig. 3). More enzymatic activity was observed in the liver of non-hibernating and hibernating lizard than in the case of toad.

(5). **Pigeon.**

Considerable amount of glucose-6-phosphatase was found to be present in the liver of the pigeon (Fig. 4). Enzymatic activity was found to be slightly less than rat.

(6). **Rat.**

Distribution of glucose-6-phosphatase in the parenchymal cells of the liver of rat was observed to be very intense, higher than other vertebrates studied (Fig. 5).
DISCUSSION

From the results supra, it was found that variable amount of glucose-6-phosphatase was present in the liver of different classes of vertebrate. The liver of rat, pigeon and lizard contained a very high amount of this enzyme while, the liver of mrigal fish contained a slightly lower amount than lata fish, and lowest activity having been observed in August toad.

Nemeth (6) has previously reported an inverse correlation between the distribution of glycogen in liver and the activity of glucose-6-phosphatase. When glycogen level was low glucose-6-phosphatase was high.

Variation in the distribution of liver glycogen has been observed in different classes of vertebrate. In lata fish, the glycogen level had already been observed to be very low, and a considerable amount of glucose-6-phosphatase was found to be present. The liver glycogen of August toad was very high and histochemically demonstrable glucose-6-phosphatase was found to be lowest. In hibernation, a fall in liver glycogen and a rise in the enzymatic activity have been observed. In all the above cases, the hypothesis of Nemeth (6) may be suitably applicable. But in the liver of lizard, pigeon and rat, a considerable amount of glucose-6-phosphatase was found to be present and the liver glycogen in them were also high.

Glucose-6-phosphatase is an enzyme that breaks down glucose-6-phosphate into glucose and phosphate. In the present experiment a positive correlation has been observed between the activity of this enzyme in the liver of different classes of vertebrate and the blood sugar level.
In rat, pigeon and lizard both the sugar level and the enzyme activity were high while both being intermediate in lata fish, and lowest in toad.

In rats made diabetic with alloxan an increase in blood sugar and fall of liver glycogen together with an increase in histochemically demonstrable glucose-6-phosphatase has been observed. (Deb and Chakravarty, unpublished).

5. SUMMARY.

Glucose-6-phosphatase was demonstrated histochemically in the liver of different classes of vertebrate and compared.

Liver of lata fish was found to contain more glucose-6-phosphatase than mrigal fish and toad.

High amount of glucose-6-phosphatase was observed in the non-hibernating and hibernating lizard which was more than toad.

Liver of rat was found to contain higher amount of glucose-6-phosphatase than other vertebrates studied.
Explanation of plates.

Figure 1. - Glucose-6-phosphatase in the liver of beta fish. Less than rat. x 96.

Figure 2. - Glucose-6-phosphatase in the liver of toad. Less than rat. x 96.

Figure 3. - Glucose-6-phosphatase in the liver of non-hibernating lizard. Enzymatic activity is more than toad but less than rat. x 96.

Figure 4. - Glucose-6-phosphatase in the liver of pigeon. Concentration of the enzyme is less than rat. x 96.

Figure 5. - Glucose-6-phosphatase in the liver of rat. Distribution of the enzyme is more than fish, toad lizard and pigeon. x 96.
FIGURES OF PLATE - II.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5
   J. Histochem. Cytochem. 1(6) : 429.

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INTRODUCTION

Esterase activity in the Mammalian liver was compared with that of the Amphibian (Necturus maculosus) by Glennor et al (1). He observed no similarity in the sites of enzymatic activity in two different classes of vertebrate. It has also been observed by Sake et al (2) that the enzymatic activity in the liver of the different species of birds are not identical.

The histochemical distribution of esterase was compared in five mammals by Naehlas et al (3). He also observed variation in the distribution of this enzyme in the liver in different species of mammals.

In the present study the distribution of esterase in the liver of different classes of vertebrate was studied.

MATERIALS AND METHODS

Specimens from each class of vertebrates, such as, Ephippus punctatus (Lake fish of carnivorous type), Cirrhina mrigala (Mrigal fish of herbivorous type), Bufo melanostictus (common toad), Calotes versicolor (garden lizard), Columba livia (domestic variety of rock pigeon), Rattus sp. (domestic variety of white rat) were collected from local animal dealer.
Treatment of the animals before the experiment and the method of killing was same as described in the beginning of this Chapter.

After killing the animals small pieces of liver were taken out quickly and fixed in 10% cold Formol for 12 hours. 15 μ frozen sections were cut and mounted on clear slide with the help of a drop of water. The sections were dried and esterase activity was studied histochemically according to Malaty and Bourne (4) described below.

Preparation of reagents.

1) Naphthol-As-Acetate.

It was prepared according to Pearse (5) as follows:-

5 gms. of 2-hydroxy-3-naphthoic anilide was dissolved in 10 cc of dry pyridine and 20 cc of acetic anhydride was added to it. It was heated under reflux condenser for 1 hour, then poured into cold distilled water (nearly 250 cc). The pasty product was filtered off and dried. It was recrystallised from ethanol containing a little charcoal to obtain the acetate as a cream-coloured powder (m.p. 160°-161°C.)

2) Preparation of working solution of Substrate and diazo coupling.

Substrate solution was prepared freshly by dissolving enough of naphthol-As-acetate in 50% aqueous acetone to make 1% solution. To 1 cc of this solution 40 cc of distilled water was added with constant and vigorous shaking, 10 cc of M/5 phosphate buffer (pH 6.9 to 7) was then added followed by 40 mg. of diazo-compound (4-benzoylamino-2 : 5 dimethoxy-aniline). The solution was stirred thoroughly and filtered directly on to the sections.
**METHOD.**

(1) 15 µ prepared liver sections mounted on the slide were mashed in several changes of distilled water.

(2) The sections were dried.

(3) Slide was kept in a clean dry coupling jar and "substrate-azo coupling", prepared immediately before was filtered directly into it.

(4) Sections were incubated in the substrate medium for 2 hours at 37°C.

(5) They were then quickly washed in distilled water and mounted in glycerine jelly.

**Result.**

Steel blue colour indicated localization of esterase activity.

**OBSERVATIONS AND RESULTS.**

(1) **Lata fish.**

Esterase activity in the liver of lata fish was found to be very slight in comparison to rat. Distribution of esterase in the liver of this fish was similar throughout the year (Fig. 1).

(2) **Mrigal fish.**

Esterase activity in the liver of mrigal fish was found to be higher than lata fish but slightly less than rat (Fig. 2).

(3) **Toad.**

Very high amount of esterase was observed in the liver of toad during non-hibernation (Fig. 3). Slight fall in the distribution of
esterase in the liver of toad during hibernation was observed. Concentration of the enzyme in the liver of non-hibernating toad was similar to rat.

(4). L i z a r d .

Liver of the lizard was found to contain slight amount of esterase in comparison to toad, pigeon and rat (Fig. 4). Considerable variation in the distribution of this enzyme was observed in different animals of the same species. From the variable results it was found that the amount of esterase present in the liver of lizard was more than lata fish but less than toad, pigeon and rat in August. Enzymatic activity was found to be low in hibernation.

(5). P i g e o n .

Considerable amount of esterase was found to be present in the liver of pigeon (Fig. 5). In comparison to rat, lesser amount of this enzyme was present in the liver of this animal.

(6). R a t .

Distribution of esterase in the liver of rat was found to be highest. Enzyme was found to be distributed uniformly in the liver of this animal. Concentration of the enzyme was similar to toad.

D I S C U S S I O N .

It can be observed from the present set of experiments that the esterase content in the liver of lata fish was very slight in comparison to other vertebrates, but neutral lipid content in the liver of this fish was very high. On the other hand, neutral lipid content
of the mrigal fish was lower but considerable amount of esterase was present. It may be said that in the mrigal and lata fishes esterase and neutral lipid were found to be present in a reverse proportion.

Throughout the year similar lower amount of esterase and higher amount of neutral lipid were observed in the liver of lata fish.

Considerable amount of esterase was present in the liver of toad. Although a marked seasonal variation in the distribution of neutral lipid has been observed in this class of vertebrate but no such remarkable alteration in the distribution of esterase has been noted. In non-hibernating lizard, the esterase was lower than other vertebrates studied except lata fish.

No definite conclusion as regards the distribution of esterase in liver can be arrived at from the above results. It has been observed that in some vertebrate an inverse distribution as regards neutral lipid and the enzyme has been noted as in lata fish, pigeon and rat. But in hibernating toad, both of the cytoplasmic constituents were present in considerable amount. Mukherji and Deb (6) have also observed fairly high amount of esterase in the liver of hibernating toad. They have postulated that the enzyme was possibly required for the mobilization and utilization of fat at that period. The high amount of lipid observed by them to be present in non-hibernation have been suggested to be due to high rate of lipid turnover.

It can be suggested that the metabolic role of lipid present in the liver of lata fish might be different from that present in hibernating toad.
Distribution of esterase in the liver of Piscean, Amphibian, Reptilian, and Avian species was studied histochemically and compared with Mammalian species.

Enzymatic activity in the liver of lata fish was found to be very insignificant in comparison to rat. Liver of mrigal fish was found to contain higher amount of esterase than lata fish which was less than rat.

Liver of toad was found to contain high amount of esterase which was similar to rat. Slight fall was observed in hibernating toad.

Esterase activity was observed to be lower in the liver of non-hibernating lizard which was less than rat. Fall of activity of this enzyme was observed in hibernation.

Concentration of this enzyme in the liver of pigeon was less than rat. Very high amount of enzymatic activity was observed in rat.
Esterase in the liver of lata fish.
Less than rat. x 96.

Esterase in the liver of mrigal fish.
More than lata fish but less than rat. x 96.

Esterase in the liver of non-hibernating toad.
Distribution is similar to rat. x 96.

Esterase in the liver of non-hibernating lizard.
Less activity is seen than rat. x 96.

Esterase in the liver of pigeon.
Enzymatic activity is less than rat. x 96.

Esterase in the liver of rat.
Concentration of the enzyme is very high. x 96.
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6. Mukherji, M., and Deb, G.
The distribution of lipase was studied by Gomori (1) in the liver of various species of mammalia under normal and pathological condition. Histochemically demonstrable lipase was also studied in the liver of different species of Aves (2). Considerable variation in the results was observed by the above workers in the distribution of the above enzyme in the liver of different species within the same class of vertebrate.

From the above review, it may be expected that the distribution of the lipolytic enzyme in the liver of different classes of vertebrate may vary considerably.

In order to confirm the above concept, the distribution of lipase in the liver of different classes of vertebrate studied and compared.
2. MATERIALS AND METHODS.

Specimens from each class of vertebrates, such as, *Ophicephalus punctatus* (Lata fish of carnivorous type), *Cirrhina mrigala* (Mrigal fish of herbivorous type), *Bufo-melanostictus* (common toad), *Calotes versicolor* (garden lizard), *Columba livia* (domestic variety of rock pigeon), *Rattus sp.* (domestic variety of white rat) were collected from local animal dealer.

Treatment of the animals before the experiment and the method of killing was same as described in the beginning of this chapter.

Animals were killed and small pieces of liver were taken out quickly and 15 μ frozen sections were prepared. Distribution of lipase was studied according to George et al (3) described as below.

Preparation of reagents.

Preparation of substrate solution.

(a) 2 ml. of 5% Tween 80.
(b) 5 ml. of bicarbonate buffer pH 8.4.
(c) 2 ml. of 10% calcium chloride.
(d) 40 ml. of distilled water.

The above solutions were mixed and the mixture was incubated for 8-10 hours at 40°C to precipitate the free fatty acids. The substrate was filtered and thymol was added as preservative.

METHOD.

(1). Small pieces of liver were quickly taken out and 15 μ frozen sections were prepared and dropped in 6% Formol neutralized with Na₂HPO₄ maintained at 4°C for fixation. It was fixed for 4 hours. The
sections were washed in several changes of cold water for 1 hour and then in distilled water. They were then mounted on clean slide with 1% gelatin and dried. Sections were coated with thin layer of gelatin, the slide was kept in freezing chamber to solidify gelatin coating which then appeared white. Sections were then kept in 6% cold neutral formol for 1 hour to harden gelatin. It was washed in running water for 2 hours. The slide with the sections was washed in distilled water and incubated in substrate medium for 8-12 hours at 37°C.

(2). The sections were washed well in warm (40°C) distilled water.

(3). It was kept for 20 minutes in 1% lead nitrate.

(4). The slide containing the sections was washed in warm distilled water.

(5). Sections were washed in running water for 10 minutes.

(6). They were rinsed in distilled water.

(7). The sections were kept for few minutes (2-5) in 1% yellow ammonium sulfide.

(8). They were rinsed in 1% acetic acid.

(9). The sections were washed thoroughly in distilled water.

(10). Sections were mounted in glycerine jelly.

3. OBSERVATIONS AND RESULTS.

(1). Lata fish.

   No histochemically demonstrable lipase was found to be present in the hepatic cells of liver of lata fish.

(2). Mrigal fish.

   Histochemically demonstrable lipase was found to be absent in the liver of mrigal fish.
(3). Toad.

Slight amount of lipase was observed in the liver of the August toad which was less compared to rat (Fig. 1).

(4). Lizard.

Lipase was found to be present in a higher amount in the liver of August lizard (Fig. 2). Enzymatic activity was more than in non-hibernation toad but less than in rat.

(5). Pigeon.

Higher intensity in activity of the enzyme was observed in the liver of pigeon (Fig. 3). The reaction was observed to be more than in fish, toad and lizard but less than in rat.

(6). Rat.

Highest amount of lipase activity was observed in the liver of rat compared to other vertebrates studied (Fig. 4).

The reaction was more intense in the central area than in the periphery. The activity of lipase was also observed in the lining of the bile ducts.

4. DISCUSSION.

Highest amount of lipase was found to be present in the liver of rat. No lipase activity was observed in the hepatic cells of the lata fish and mrigal fish. Although in lata fish very high amount of neutral lipid was present but histochemically demonstrable lipase was not found. Positive reactions were only observed in the pancreatic glands (both fishes possess hepatopancreatic type of liver).
In elasmobranch fishes (liver and pancreas separate) Bal et al (4) observed little lipase activity in the liver and highest in the pancreas.

The liver of the toad was found to contain slight amount of lipase. Considerable amount of the enzyme was present in the liver cells of the lizard. Enzymatic activity in the liver of the pigeon was found to be lesser than in rat. It can be concluded that the highest amount of neutral lipid present in the liver of lata fish was accompanied by the absence of lipase indicating that the lipid present in the liver of this particular class of vertebrate has possibly a storage function and not metabolically active normally.

5. SUMMARY.

Distribution of lipase in the liver of different classes of vertebrate was studied histochemically and compared.

Histochemically demonstrable lipase was found to be absent in the liver of lata and mrigal fishes.

Liver of toad was found to contain slight amount of lipase which was less than in lizard.

Lipase was found to be present in higher amount in the liver of pigeon than the vertebrates studied except rat.
FLA35E - IV

Explanation of plates.

Figure 1. - Lipase in the liver of toad.
Enzymatic concentration is seen to be less than rat. x 96.

Figure 2. - Lipase in the liver of lizard.
Distribution of enzyme is more than toad but less than rat. x 96.

Figure 3. - Lipase in the liver of pigeon.
Concentration of the enzyme is seen to be high but less than rat x 96.

Figure 4. - Lipase in the liver of rat.
Highest activity is seen. x 96.
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E. HISTOCHEMICAL STUDY ON THE DISTRIBUTION OF ACID PHOSPHATASE IN LIVER OF DIFFERENT CLASSES OF VERTEBRATE.

INTRODUCTION.

A number of workers have studied the distribution of acid phosphatase in the liver of different species of mammalia (1), (2) & (3). The distribution of acid phosphatase in the amphibian tissues was studied by Delson (4), Kind et al (5) and compared with mammals. They were found to be more or less similar in distribution.

Little work has been done to the knowledge of the author on the distribution of acid phosphatase in liver of fish, lizard and pigeon. So, an attempt was made to compare the distribution of acid phosphatase in the liver of different classes of vertebrate.

MATERIALS AND METHODS.

Specimens from each class of vertebrates, such as, Ophiophagus punctatus (Lata fish of carnivorous type), Girrhina urigale (Mrigal fish of herbivorous type), Bufo melanostictus (common toad), Calotes versicolor (garden lizard), Columba livia (domestic variety of rock pigeon), Rattus sp. (domestic variety of white rat) were collected from local animal dealer.

Treatment of the animals before the experiment and the method of killing was same as described in the beginning of this Chapter.
After killing the animals, small pieces of liver were taken out quickly and fixed in 4% cold Formol. It was kept in refrigerator for 12 hours. 15 μ frozen sections were cut and floated on distilled water. Sections were mounted on a clean slide. They were completely dried and reaction for the detection of acid phosphatase was carried out according to the improved method of Gomori (6) as follows.

Preparation of reagents:

1. Stock buffer.
   
   M/20 Sodium acetate- acetic acid buffer adjusted to pH 4.8 to 5 was prepared. To it 1.324 gm. of lead nitrate per 900 cc of buffer was added. This solution was kept in refrigerator.

2. Stock substrate.

   0.1 M or 3.15% solution of sodium beta-glycerophosphate was prepared and kept refrigerated.

3. Working solution.

   45 cc of stock buffer was added to 5 cc of stock substrate in a clean conical flask. The solution was mixed well and incubated at 37° C. for 1 hour. White precipitate formed was filtered off and clear solution was used as substrate medium for the experiment. Before using, it was checked for the desired pH.

Method.

(1). Small pieces of tissues were taken quickly and fixed in chilled 4% formol for 12 hours.

(2). 15 μ frozen sections were cut and mounted on clean slide with a drop of distilled water.
The sections were completely dried and washed in distilled water.

Sections were then incubated at 37° C. in substrate medium previously raised to that temperature for different periods of time (few minutes to several hours).

After desirable time of incubation sections were taken out from the substrate medium and washed in several changes of distilled water.

They were dipped in 3% acetic acid for 5 minutes.

Sections were then washed thoroughly in distilled water.

They were then treated with a dilute solution (1:10) of yellow ammonium sulfide for 5 minutes.

After thorough washing with distilled water, the sections were dehydrated and cleared and then mounted in following order -

(a). Acetone-water (1:1).
(b). Acetone absolute, 2 changes.
(c). Acetone-benzene (1:1).
(d). Benzene, 2 changes.
(e). Mounted in dammar.

Result.

Acid phosphatase activity was indicated by black or brown-black staining.

Observations and Results.

Reactions of acid phosphatase was carried out in section gradually increasing the period of incubation in the substrate solution and the first detectable enzymatic reaction was taken as on set of the reaction.
The distribution of acid phosphatase in the liver of the lata fish was observed in the cytoplasm, nuclei and also in the bile duct and central vein. Enzymatic activity was high within a short time of incubation. On set of the enzymatic reaction was observed within 2 to $2\frac{1}{2}$ hours (Fig. 1). A patchy distribution of the enzyme was frequently observed.

(2) Mrigal fish.

The liver of mrigal fish was found to contain lesser amount of acid phosphatase than lata fish. On set of the enzymatic reaction was observed within 4-5 hours (Fig. 2).

(3) Toad.

Sites of enzymatic reaction were observed in the cytoplasm and nucleus of the hepatic cells of the toad. Lesser amount of enzyme was found to be present in toad than both the fishes (Fig. 3). On set of the enzymatic reaction was observed 6-6$\frac{1}{2}$ hours in non-hibernating toad. In hibernation the time of incubation for on set of the enzymatic reaction has been decreased (2-2$\frac{1}{2}$ hours). So, enzymatic activity has been increased at this time.

(4) Lizard.

Enzymatic reaction in the liver of lizard in non-hibernation was observed to be very high in comparison to other vertebrates studied except rat. On set of enzymatic reaction was observed within 1-1$\frac{1}{2}$ hours (Fig. 4). In hibernation enzymatic activity has decreased slightly, on set of the reaction was observed after 2-2$\frac{1}{2}$ hours of incubation.
(5) **Pigeon.**

Sites of enzymatic activity were observed in the cytoplasm of the hepatic cells of pigeon. On set of the enzymatic reaction was observed within 4–4½ hours (Fig. 5). Lesser amount of enzymatic activity was observed in pigeon in comparison to other vertebrates studied except non-hibernating toad.

(6) **Rat.**

Highest amount of acid phosphatase activity amongst the other vertebrate studied was observed in the liver of rat (Fig. 6). On set of the enzymatic reaction was observed within 45 minutes to 1 hour.

**DISCUSSION.**

Acid phosphatase has been observed to be present in varying amounts in the liver of fish, toad, lizard, pigeon and rat. Liver of rat contained highest amount of acid phosphatase. Significant amount of the enzyme was observed in the liver of lizard. In the case of lata fish, patchy reaction was observed in the liver cells.

The peripheral lobules of rat's liver are strongly positive in acid phosphatase reaction. Varying amounts of enzyme were observed to be distributed in the liver of toad, lizard and pigeon. A great variation in the distribution of acid phosphatase was also observed in the liver of normal dog, cat and rat by Gomori (1).

Kind and Macchi (5) studied the enzyme in the liver of frog and found to be comparable with the results of mammals where the liver exhibits
the greatest enzymatic activity. Although we have also observed highest enzymatic activity in mammals as observed by Kind and Macchi (5) but lowest activity was detected in toad by us. In hibernation, an increase in enzymatic activity has been observed in toad but a fall was noted in lizard. Mukherji and Deb (unpublished) have also observed an increase in acid phosphatase in the liver of toad during hibernation.

The function of acid phosphatase in liver is not definitely clear. From the present set of experiments no correlation could be obtained between the activity of this enzyme and the presence of other cytoplasmic constituents in the liver of different classes of vertebrate. No definite conclusion can be obtained about the metabolic role of this enzyme from the above observations.

SUMMARY

Activity of the acid phosphatase in the liver of different animals, such as lata and mrigal fishes, toad, lizard, pigeon and rat was studied histochemically.

Highest amount of acid phosphatase was found to be present in the liver of rat while in the liver of non-hibernating toad was observed lowest amount of this enzyme which was found to increase in hibernation.

Next to rat higher enzymatic activity was observed in the liver of non-hibernating lizard which was observed to fall in hibernation.

Lesser amount of the enzyme was also observed in the liver of pigeon.

Liver of mrigal fish was found to contain lesser amount of acid phosphatase than lata fish.
Explanation of plates.

Figure 1. - Acid phosphatase in the liver of late fish. Reaction (on set) after 2 hours of incubation. x 96.

Figure 2. - Acid phosphatase in the liver of mrigal fish. Reaction (on set) after 4 hours of incubation. x 96.

Figure 3. - Acid phosphatase in the liver of non-hibernating toad. Reaction (on set) after 6 hours of incubation (counterstain with eosin). x 96.

Figure 4. - Acid phosphatase in the liver of non-hibernating lizard. Reaction (on set) after 1 hour of incubation. x 96.

Figure 5. - Acid phosphatase in the liver of pigeon. Reaction (on set) after 4 hours of incubation. x 96.

Figure 6. - Acid phosphatase in the liver of rat. Reaction (on set) after 45 minutes of incubation. x 96.
Figures of Plate V.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6
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F. HISTOCHEMICAL STUDY ON THE DISTRIBUTION OF ALKALINE PHOSPHATASE IN LIVER OF DIFFERENT CLASSES OF VERTEBRATE.

INTRODUCTION.

Liver of rat contains considerable amount of alkaline phosphatase while, the liver of Guinea pig was found not to contain any detectable enzyme activity (Gomori) (1). It has been observed by Bouchilloux (2) that the liver of some Selachian and teleostean fishes scarcely contained any alkaline phosphatase, whereas in other teleost fishes considerable amount of alkaline phosphatase was present.

Histochemically detectable alkaline phosphatase was studied in the liver of different species of Aves and Reptiles and a great variation in the distribution of the enzyme was observed (3).

From the above review, it may be suggested that the distribution of alkaline phosphatase in the liver of different vertebrates may vary widely. In the present study the distribution of alkaline phosphatase activity in the liver of different classes of animals was studied and compared.

MATERIALS AND METHODS.

Specimens from each class of vertebrates, such as, Ophicephalus punctatus (Lata fish of carnivorous type), Cirrhina urigala (Mrigal fish of herbivorous type), Bufo-melanostictus (common toad), Calotes versicolor (garden lizard), Columba livia (domestic variety of rock pigeon), Rattus sp. (domestic variety of white rat) were collected from local animal dealer.
Treatment of the animals before the experiment and the method of killing was same as described in the beginning of this chapter.

After killing the animals, small pieces of livers were taken quickly and fixed in cold acetone. It was kept in refrigerator for 24 hours.

Alkaline phosphatase reaction was carried out according to the method of Gomori (4) as described below.

**Preparation of reagents:**

Substrate solution was prepared by mixing the following ingredients.

- 2% Sodium beta-glycerophosphate ------------ 10 cc
- 2% Sodium diethyl barbiturate ------------ 10 cc
- 2% Calcium chloride ---------------------- 2 cc
- 2% Magnesium sulfate ---------------------- 2 cc
- Distilled water -------------------------- 20 cc

The pH of the solution was adjusted to 9 - 9.5 with 0.1 (N) HCl.

**METHOD:**

(1) Acetone-fixed liver pieces were dehydrated in two changes of acetone of 12 hours each and cleared in acetone-petroleum ether (1:1) for half an hour followed by two changes of petroleum ether of half an hour each. Tissues were embedded in paraffin at 52°C. for 1½ hours in vacuum.

(2) 6 μm paraffin sections were prepared. They were deparaffinised and hydrated to water as usual. The sections were then incubated at 37°C.
in substrate medium previously raised to that temperature for different periods of time (5 minutes to several hours).

(3). The sections were then washed in several changes of water. In case of very short time of incubation, the slides were dipped in cold water to stop the reaction instantaneously after desired time of incubation.

(4). They were then treated with 2% cobalt nitrate solution for 3-5 minutes.

(5). They were washed thoroughly in tap water followed by distilled water.

(6). The sections were then dipped in yellow ammonium sulfide solution (a few drops in 50 cc of distilled water) for a few minutes (3-5 minutes).

(7). They were washed thoroughly in water, dehydrated in alcohol, cleaned in xylene and mounted in Canada balsam.

Result.

Alkaline phosphatase activity was indicated by black or brownish black staining.

Observations and Results.

Reaction of the alkaline phosphatase was carried out in liver by gradually increasing the period of incubation in the substrate solution and the first detectable enzymatic reaction was taken as onset of the reaction.
(1). **Lata fish.**

No enzymatic reaction was observed in the liver of this fish. Cytoplasm of the hepatic cells was devoid of alkaline phosphatase even after hours of incubation while nuclei of the cells were highly stained.

(2). **Mrigal fish.**

Fair amount of alkaline phosphatase was found to be present in the cytoplasm and nuclei of the hepatic cells of this fish. On set of the enzymatic reaction was observed within 12 - 13 hours of incubation (Fig. 1).

(3). **Toad.**

A very high enzymatic activity was found to be localized in the hepatic cell of toad mainly at the periphery of the cells. The enzymatic activity appeared in the non-hibernating toad, within 15 - 20 minutes of incubation (Fig. 2). After 30 minutes of incubation a high enzymatic activity was found to be distributed in the cytoplasm of the hepatic cells.

Alkaline phosphatase activity in the liver of hibernating toad was found to decrease. On set of the enzymatic reaction was observed within 2 - 2½ hours of incubation.

(4). **Lizard.**

The distribution of the enzyme in the liver of lizard was observed to be similar to that of toad. Enzymatic activity was first observed at the periphery of each hepatic cells which increased after longer period of incubation and found to be distributed throughout the cytoplasm and nucleus. On set of the enzymatic reaction was observed within 20 - 25 minutes of incubation in non-hibernating lizard (Fig. 3).
(5) Pigeon.

Alkaline phosphatase activity was observed in the cytoplasm of the hepatic cells of the pigeon. Distribution of the enzyme in the liver was found to be similar to that of the rat. On set of the enzymatic reaction was observed within 12-13 hours of incubation (Fig. 4) when both nucleus and cytoplasm were stained.

(6) Rat.

Alkaline phosphatase was found to be distributed both in the cytoplasm and nucleus of the liver cells of rat. On set of the enzymatic reaction was observed in 12-12½ hour (Fig. 5). Alkaline phosphatase in the liver of rat was observed to be lesser than toad and lizard.

DISCUSSION.

From the above histochemical results considerable varying quantum of alkaline phosphatase activity has been observed in the liver of different classes of vertebrate.

According to Danielli (5), tissues requiring lesser time of incubation for the on set of enzymatic reaction is said to contain higher concentration of enzymes. Keeping in view the opinion of Danielli the study was resorted to, and it was observed that the highest amount of enzyme was found to be present in the liver of experimental animals when lesser time of incubation was employed for the on set of the reaction.

Maximum amount of alkaline phosphatase was observed in the liver of non-hibernating toad and lizard, lesser amount of the enzyme was detected in the liver of pigeon, rat and mrigal fish. Histochemically
detectable alkaline phosphatase was absent in the liver cells of lata fish and glycogen content in the liver was also lowest in this vertebrate.

Similarly no detectable alkaline phosphate was observed in the liver of selachian fish (*Scyllium canicula*) and teleostean fishes (*Mugil ohelo*, *Zeus faber*) (2). On the contrary, although considerable amount of glycogen was present in guineapig's liver but alkaline phosphatase was found to be absent (Somori) (1). Liver of rat and pigeon contain considerable amount of glycogen but low alkaline phosphatase activity. When alkaline phosphatase is absent or low in the liver of any animal which utilises carbohydrate efficiently, it becomes difficult to relate this enzyme with carbohydrate metabolism.

Bouchilloux (2) reported that fish phosphatases behave very much like mammalian phosphatases as regards the effect of the common activator and inhibitors. With the same pH value and same time period of incubation (12 hours) the liver of rat, pigeon and mrigal fish did not indicate any enzymatic activity. So, it may be said that the liver of the lata fish does not contain histochemically demonstrable alkaline phosphatase.

Highest amount of glycogen and alkaline phosphatase was found to be present in the liver of the August toad, and a fall in both has been noted in hibernation. The liver of lizard contain a considerable amount of both the cytoplasmic constituents during non-hibernation. In hibernation a fall in glycogen but not in the enzyme has been recorded. Mukherji and Deb (6) have also observed considerable amount of alkaline phosphatase in the liver of non-hibernating toad which decreased in hibernation.

From the above observation, it may be suggested that presence of alkaline phosphatase may not be always correlated with the presence of
glycogen in liver of the vertebrates studied. Histochemically detectable alkaline phosphatase was detected in the liver of some species of Aves and Reptilia by Arvy et al (3). Considerable difference in the enzymatic activity was observed by him and he also suggested that alkaline phosphatase activity does not seem to be correlated with the content of glycogen and R. N. A.

**SUMMARY.**

Distribution of Alkaline phosphatase was studied in the liver of Piscean, Amphibian, Reptilian, Avian and Mammalian species by means of histochemical method.

In the liver of lata fish no histochemically demonstrable alkaline phosphatase was observed. Liver of non-hibernating toad and lizard was found to contain highest amount of this enzyme while the liver of mrigal fish, pigeon and rat was found to contain similar lesser amount of it. Slight fall of alkaline phosphatase was observed only in the hibernating toad.
PLATE- 6.

Explanation of plates.

Figure 1. - Alkaline phosphatase in the liver of mrigal fish. Reaction (on set) after 12 hours of incubation. x 96.

Figure 2. - Alkaline phosphatase in the liver of non-hibernating toad. Reaction (on set) after 15 minutes of incubation. x 96.

Figure 3. - Alkaline phosphatase in the liver of non-hibernating lizard. Reaction (on set) after 20 minutes of incubation. x 96.

Figure 4. - Alkaline phosphatase in the liver of pigeon. Reaction (on set) after 12 hours of incubation. x 96.

Figure 5. - Alkaline phosphatase in the liver of rat. Reaction (on set) after 12 hours of incubation. x 96.
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