CHAPTER 3

RELATIONSHIP OF DIETARY PREFERENCES WITH DISTRIBUTION PATTERN OF LIPASE, ESTERASE AND β-HYDROXYBUTYRATE DEHYDROGENASE IN THE LIVERS OF VARIOUS REPRESENTATIVE BIRDS

Most of the metabolic reactions taking place in the liver have certain relationship with the diet. Through the agency of various enzymes, the liver maintains steady levels of metabolites in the blood. Excess of any metabolite in the blood stream, in the post-digestion period, is taken up by the liver either to be oxidized or to be converted into storage products. From the studies on the distribution of fat in the livers of birds with different dietary preferences (Chapter 2) it is evident that some kind of division of labour is exhibited by the hepatocytes located at various areas of lobular units. Needless to say ~~that,~~ such regional adaptations are due to the differential distribution and concentration of enzymes in the liver. The mammalian liver has been shown to have specific enzymes localized in particular regions in the hepatic lobules, and the variations in the topographical distribution of enzymes in the hepatic hexagonal units (lobules) are found to be connected with supply of metabolites through the hepatic circulation (Wachstein, 1963). Such information is not available with regard to avian liver. Recently, Ratzlaff and Tyler (1973), using histochemical techniques, studied, on a comparative basis, the distribution pattern of certain enzymes in the livers of some birds. However, the distribution pattern of enzymes in the liver lobules of birds correlated with their dietary preferences are yet to be elucidated. This report, forming a part of a series of histochemical studies on the livers of birds with various dietary preferences, deals with observed variations in the distribution patterns of some of the enzymes concerned with lipid metabolism.

MATERIAL AND METHODS

Birds with different types of dietary preferences were shot from their natural habitats within the University Campus and were brought to the laboratory immediately. The species of birds collected were same as listed in Table I (Chapter 1). They represented four groups viz., Carnivores, Insectivores, Omnivores and Frugivores and Graminivores.

The livers from these birds were quickly removed, and small pieces were cut, blotted and 12 μ thick frozen sections were cut on a cryostat microtome. The sections were processed for histochemical demonstration of lipase, esterase and β-hydroxybutyrate dehydrogenase (BDH).

Lipase was demonstrated by the Gomori's method (Pearse, 1972) using 'Tween 85' (Sigma Chemical Co.,U.S.A.) as improved by George and Ambadkar (1963).

Nonspecific esterase was histochemically demonstrated using Burstone's azo dye technique (Burstone, 1962; Pearse, 1972). Naphthol As-D-Acetate (Sigma Chemical Co., U.S.A.) was used as the substrate.

The histochemical method used for the demonstration of BDH was that of Ogata and Mori (1964). The substrate used was β-hydroxybutyrate (Sigma Chemical Co., U.S.A.).

OBSERVATIONS

GROUP I (Carnivores)

Lipase: Lipase was not very active in the livers of both vulture and kite. The enzyme activity was seen as granules in the parenchymal cells and was uniformly distributed in all the cells of liver lobules (hexagonal units) (Figs. L1 & L2).

Esterase: Esterase (nonspecific) compared to lipase was more active in the livers of these two carnivore birds. The cells lining the sinusoids and parenchymal cells in portal areas showed relatively higher enzyme reactivity than observed in the cells of other areas of the liver (Figs. E1 & E2a). In the centrolobular areas the enzyme activity was confined only to the cells of sinusoidal linings (Fig. E2b).

BDH : Moderate level of ~~the~~ enzyme ~~activity~~ which was ~~localized~~ as fine granules, perhaps corresponding to the mitochondria was observed in all the liver cells. However, slightly higher activity ~~of the enzyme~~ was discernible in the cells around the portal areas (Figs. B1 & B2).

GROUP II (Insectivores)

Lipase: The livers of ~~insectivore~~ birds showed varied response to histochemical reactions for lipase with lowest enzyme reactivity seen in the liver of Cattle Egret (Fig. L3), highest in the Tailor Bird (Fig. L6) and moderate in the House Swift (Fig. L4), Bee Eater (Fig. L5), Martin (Fig. L7) and Drongo

(Fig. L8). However, in all the birds, the enzyme enjoyed an uniform distribution all over the hepatic lobules.

Esterase: In the livers of this group of birds the esterase (nonspecific) was relatively very active (Figs. E3-E8) and was confined to the parenchymal cells and sinusoidal linings. Cytoplasm of these cells stained intensively while nucleus showed no enzyme reactivity (Figs. E4b, E7b). The general pattern of enzyme distribution ~~in the livers of insectivores~~ was more or less uniform, unlike that observed in the carnivores (compare Figs. E1, E2 with E3a, E4a, E7a & E8).

BDH : All the insectivores studied had high BDH reactivity ~~in their livers~~. Like lipase and esterase the distribution of BDH in the hepatic lobules was more or less uniform (Figs. B3-B8). The activity of this enzyme was seen as fine granules (Fig. B5b) in the parenchymal cells.

GROUP III (Omnivores)

Lipase: Lipase was highly active in ~~the livers of~~ all omnivores (Figs. L9-L19). ~~The~~ distribution ~~of this enzyme~~ was uniform in the hepatic lobules.

Esterase: Esterase did not show high histochemical response as in the livers of birds of groups I & II. Localizations of esterase varied greatly in the livers of the birds belonging to this group. ~~Livers~~ of Brahminy Myna and Common Myna showed ~~the enzyme~~ activity in sinusoidal linings and Kupffer cells, while the parenchymal cells as such had very little ~~enzyme activity~~ (Figs. E9 & E10). House Crow liver showed ~~enzyme~~ localizations in the Kupffer cells and parenchymal cells (Fig. E15). The ~~enzyme~~ distribution in the livers of Barbet (Fig. E17), Koel (Fig. E14), Fowl (Fig. E18), Duck (Fig. E19) and Robin (Fig. E12a) was uniform and localized in the parenchymal cells. However, amongst these birds, Barbet, Koel and Fowl showed low enzyme reactivity ~~but~~ that in Robin and Duck was quite high. In Bulbul (Fig. E13) and Babbler (Fig. E11) the ~~enzyme~~ distribution was not uniform. The parenchymal cells in the portal areas showed higher enzyme reactivity compared ~~to~~ that observed in similar cells in the other areas of the liver lobules.

BDH : Except in the Koel (Fig. B14) ~~livers of all~~ the o~~ther birds~~ of this group showed only moderate ~~BDH~~ activity (Figs. B9-B13, B15-B18). In Koel enzyme concentration was higher and its distribution was more or less uniform. In livers of other omnivores, the enzyme was poorly localized in the cells around the portal areas (Figs. B9, B10, B12, B17, B18).

GROUP IV (Graminivores)

Lipase: Parakeet liver showed only moderate lipase activity (Fig. L20). ~~The localization was in~~ parenchymal and Kupffer cells. In the Dove and Pigeon ~~livers,~~ lipase was very active (Figs. L21 & L22). ~~In these birds also lipase activity~~ was found in parenchymal cells ~~as well as in~~ Kupffer cells (Fig. L22b).

Esterase: In the livers of frugivore and graminivore birds (Parakeet, Dove, Pigeon) ~~esterase distribution was uniform and the enzyme was localized in the parenchymal and Kupffer cells (Figs. E20, E21 & E22).~~ However, intensity ~~of the enzyme reactivity~~ in Parakeet was relatively moderate whereas it was quite high in the Dove and Pigeon ~~livers.~~

BDH : BDH activity in the Parakeet liver was moderate (Fig. B20) and was found to be more localized around the portal areas. Both Dove and Pigeon showed high BDH activity (Figs. B21 & B22) and the enzyme was uniformly distributed.

DISCUSSION

The enzyme ~~like~~ lipase, esterase and β-hydroxybutyrate dehydrogenase, participate in the metabolism of lipids. Lipase is said to act on glycerides of long chain fatty acids, while esterase acts readily on substrate such as ethyl butyrate but slowly on triglycerides. Some of the esterases can act on esters of cholesterol ~~also~~. β-hydroxybutyrate dehydrogenase (BDH) is a NAD linked specific dehydrogenase which converts β-hydroxybutyrate into acetoacetic acid, thereby participating in the hydrolysis of activated fatty acids. Since the reactions catalyzed by these three enzymes are reversible, they are concerned with both lysis and synthesis of lipids.

Except for the variations in the intensity of histochemical reactivity, lipase was found to be more or less uniformly distributed in all the parenchymal cells of the hepatic lobules, irrespective of the type of food consumed by the birds. This suggests that the avian liver has a predominant role in lipid metabolism whether the diet contains large quantity of fat or not. Goodrich and Ball (1967) have reported that avian liver synthesizes more fat than the adipose tissue ~~does~~.

The nonspecific esterase showed differential patterns of distribution within and between various groups of birds. This is to be expected as there is a large number of nonspecific esterases. In carnivores, the parenchymal cells of periportal area showed higher concentrations of esterase, while in their centrolobular area only sinusoidal linings exhibited the enzyme reactivity. In insectivores and graminivores, the esterase was uniformly distributed all over the hepatic lobules. Its localization in insectivores was both in parenchymal cells as well as on the sinusoidal lining (see Fig. E7b) while in graminivores it was seen only in parenchymal cells (Figs. E21b, E22b).

In omnivores, the distribution pattern of esterase in their livers was observed to be a varied one. In some of these birds, the enzyme was localized in sinusoidal linings and occasionally in Kupffer cells (Mynas), in some others in parenchymal cells and Kupffer cells (Crow) while in others only in the parenchymal cells (Barbet, Koel, Fowl and Robin). Thus with respect to localization of the nonspecific esterase, the omnivores do not show any uniform pattern even though all of them are habituated to mixed diets. Such variations are only to be expected as these birds have become omnivores secondarily while some of them may still retain, to certain extent, their original basic physiological adaptation to one type of food. For example, Koel might have been originally a frugivore and had become euryphagous secondarily. However, their liver show almost similar distribution of esterase activity as that of the Parakeet (compare Figs. E14 with E20). This fact is all the more clear when we take into consideration the gizzard structure. Panicker (1974) reported that the gizzard of Koel is not at all muscular and is almost similar to that of Parakeet. Perhaps there may not be any necessity for bringing changes in the adaptations of liver and gizzard since the diet of Koel still mainly consists of fruits. This does not mean that all the omnivores have retained original stenophagous adaptations. The gizzard of Crow is bag like and is meant originally for fleshy food (Panicker, 1974). However, this carrion feeder leading an omnivores life, showed an esterase activity in the liver very different from that exhibited by either Vulture or Kite (compare Figs. E1 & E2 with E15). In this respect, it is difficult to derive any conclusion as to the effect of a particular diet on the enzyme distribution pattern in the liver. Perhaps a moderate shift in the diet may bring about changes in the physiological adaptations as in the liver, while only a drastic change of diet can bring about complete remodification of structural features, in digestive structures like gizzard. In other words, these birds, without undergoing much structural changes, have become oppertunistic feeders, eating whatever is edible and available in the surroundings during different seasons. Due to the physiological adaptations, especially in the liver, these birds could consume any type of food and occupy great many ecological niches forbidden to stenophagous birds.

Bhattacharya and Ghosh (1971) also came to the same conclusion after studying the amylase system in the digestive tract of birds. According to the data presented by them a typical graminivore liver produces large amount of amylase and secretes them into bile. The liver of a recently transformed omnivore like House Sparrow produces very little amylase, that of a carrion feeder changed to an omnivore (House Crow) produces slightly more quantity of amylase than that of House Sparrow, while the liver of insectivores like Cattle Egret do not produce the amylase. From this data they concluded that there is a correlation between food ingested and the ability to produce amylase by the liver.

In the carnivorous birds, the esterase was also found in the sinusoidal linings. An identical localization of cholinesterase was also found (Chapter 6) in the livers of these birds. Perhaps the nonspecific esterases could attack esters of choline too, because esterases are known to hydrolyze a variety of esters of fatty acids including those of cholesterol (Hofstee, 1952).

β-hydroxybutyrate dehydrogenase is a metabolic enzyme usually concerned with ketone formation by converting acetoacetic acid to β-hydroxybutyric acid. Being a NAD dependant dehydrogenase, BDH is usually seen associated with mitochondria. In the present histochemical investigations, the localization of BDH was found, as fine granules, in all the parenchymal cells of hepatic lobules. In the livers of omnivorous (passerine) birds the parenchymal cells around periportal areas showed more BDH activity which may be due to the presence of a greater number of mitochondria in the cells of those regions. BDH and esterase, on an over all basis were more active in the livers of insectivores and graminivores, where neutral fat is also found to be ~~more~~ (Table I). From these facts it could be surmised that the insectivores and graminivores might ~~be having~~ ~~quite~~ a higher rate of lipid metabolism though the source of lipids in the liver is different. In insectivores the hepatic lipid is mostly derived from the diet, while in graminivores the hepatic lipid is ~~mainly~~ synthesized *de novo* from carbohydrates.

The information gathered from the present study points out certain interesting facts. It is seen that in all birds studied, all the hepatic cells equally possess the lipase activity without showing regional differences in the activity in the lobules. The only influence the diet ~~can~~ exert on the hepatic lipase may be ~~on the~~ concentration than on distribution pattern. The esterase, on the other hand, showed variations in the distribution as well as in concentration according to the diet. The variation in the distribution on pattern of BDH, however, could be correlated with the population of mitochondria in hepatocytes. In majority of birds the hepatocytes around portal areas have large number of mitochondria which depict higher enzyme reactivity. The distribution of mitochondria itself may perhaps be ~~under the~~ influence ~~of~~ diets.

TABLE I

Intensity of histochemical reactivity of lipase, esterase, β-hydroxybutyrate dehydrogenase and neutral fat and lipid content of the livers of birds with various dietary preferences

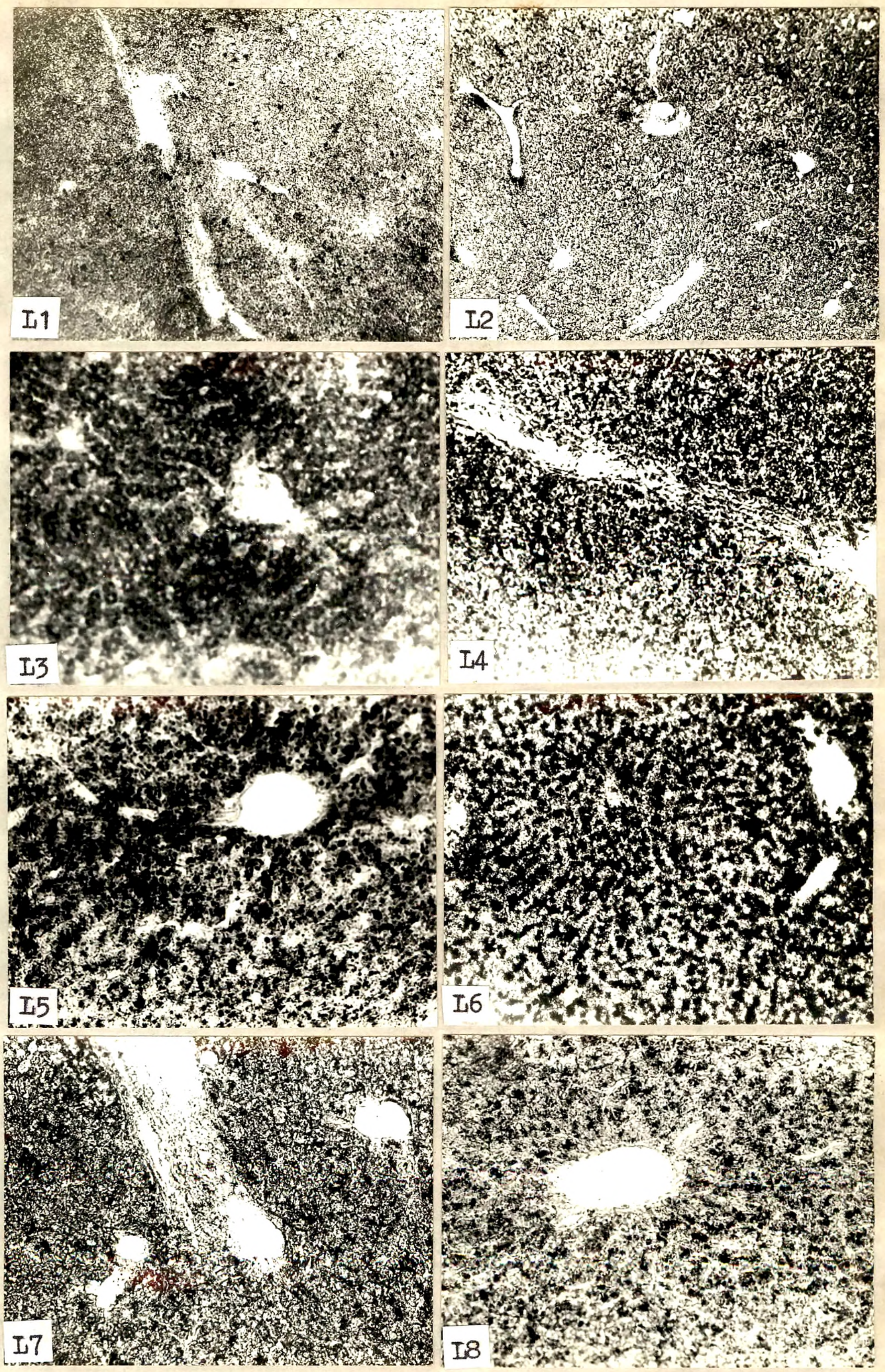
Sr. No.	Bird	Lipase	Esterase	BDH	Neutral fat	% lipid
GROUP I (CARNIVORES)						
1.	Vulture	+	++	++	++	10.56
2.	Kite	+	++	++	+++	12.32
GROUP II (INSECTIVORES)						
3.	Cattle Egret	+	+++	+++	+++	9.2~~0~~1
4.	House Swift	++	+++	+++	+++	12.58
5.	Bee-Eater	++	+++	+++	+++	15.40
6.	Tailor Bird	+++	+++	+++	+++	13.22
7.	Martin	++	+++	+++	+++	13.75
8.	Drongo	++	+++	+++	+++	11.28
GROUP III (OMNIVORES)						
9.	Brahminy Myna	+++	++	++	++	9.43
10.	Common Myna	+++	++	++	++	10.18
11.	Jungle Babbler	+++	++	++	++	9.06
12.	Indian Robin	+++	+++	++	++	11.03
13.	Bulbul	+++	++	++	++	
14.	Koel	+++	++	+++	++	8.13
15.	House Crow	+++	++	++	++	8.50
16.	House Sparrow	+++	++	++	++	9.76
17.	Barbet	+++	++	++	++	6.85
18.	Domestic Fowl	+++	+	++	++	11.20
19.	Duck	+++	+++	+++	+++	12.22
GROUP IV (~~OMNIVORES~~ Frugivores & Grain)						
20.	Parakeet	++	+++	++	+++	9.43
21.	Dove	+++	++++	+++	+++	9.23
22.	Pigeon	+++	++++	+++	+++	12.12

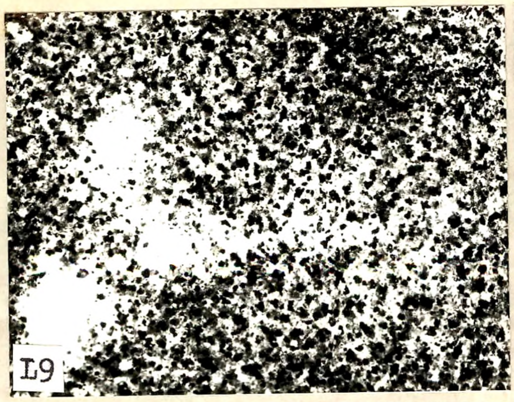
++ Moderate; +++ high; ++++ very high reactivity.

EXPLANATION TO FIGURES (CHAPTER 3)

Figs. L1 to L22. Photomicrographs of livers of birds showing Lipase activity.

GROUP I	Fig. L1.	Vulture (*G. bengalensis*)	50X
	Fig. L2.	Kite (*M. migrans*)	50X
GROUP II	Fig. L3.	Cattle Egret (*B. ibis*)	50X
	Fig. L4.	House Swift (*A. affinis*)	125X
	Fig. L5.	Bee-eater (*M. orientalis*)	125X
	Fig. L6.	Tailor Bird (*O. sutorius*)	125X
	Fig. L7.	Martin (*H. concolor*)	125X
	Fig. L8.	Drongo (*D. adsimilis*)	125X
GROUP III	Fig. L9.	Brahminy Myna (*S. pagodarum*)	125X
	Fig. L10.	Common Myna (*A. tristis*)	50X
	Fig. L11.	Jungle Babbler (*T. striatus*)	125X
	Fig. L12.	Indian Robin (*S. fulicata*)	125X
	Fig. L13.	Bulbul (*P. cafer*)	50X
	Fig. L14.	Koel (*E. scolopacea*)	50X
	Fig. L15.	House Crow (*C. splendens*)	125X
	Fig. L16.	House Sparrow (*P. domesticus*)	125X
	Fig. L17.	Barbet (*M. haemacephala*)	50X
	Fig. L18.	Fowl (*G. domesticus*)	50X
	Fig. L19.	Duck (*A. domesticus*)	50X
GROUP IV	Fig. L20.	Parakeet (*P. krameri*)	125X
	Fig. L21.	Dove (*S. senegalensis*)	50X
	Fig. L22a.	Pigeon (*C. livia*)	50X
	Fig. L22b.	Pigeon (*C. livia*)	125X


L1
L2
L3
L4
L5
L6
L7
L8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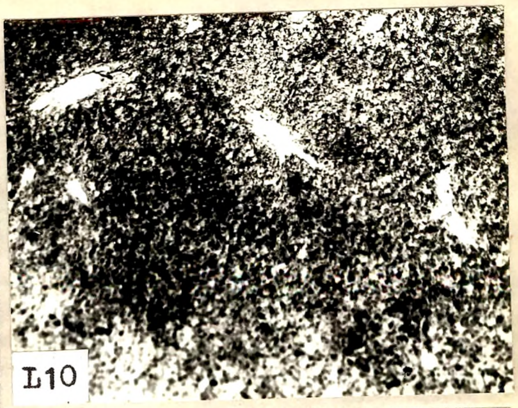
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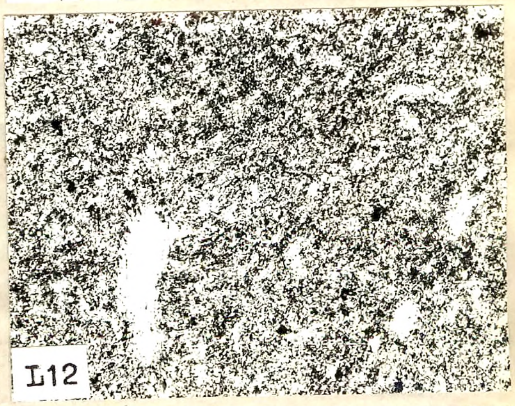
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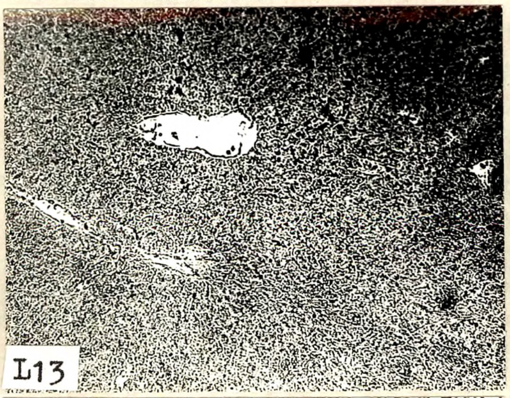
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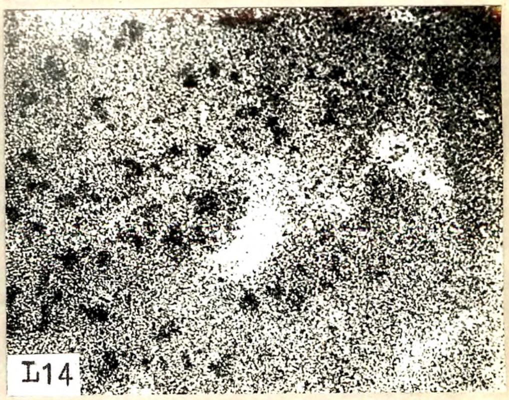
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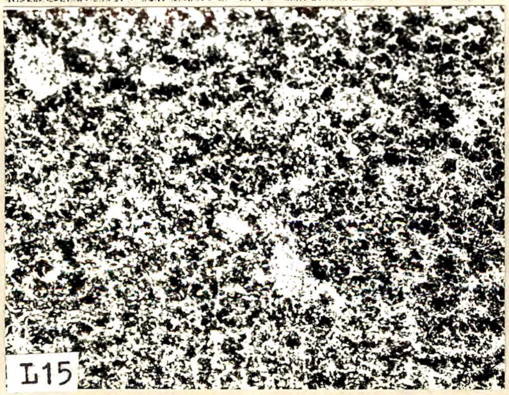
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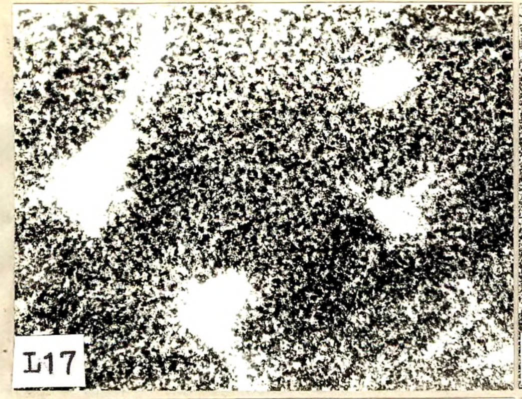
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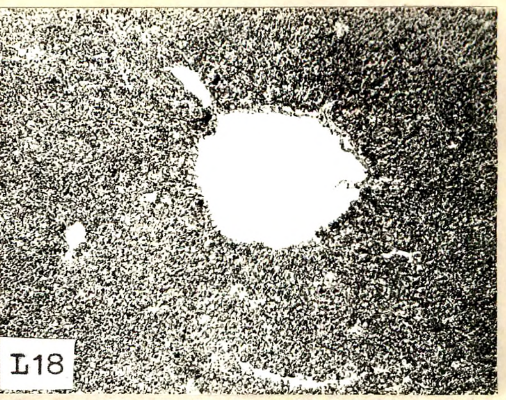
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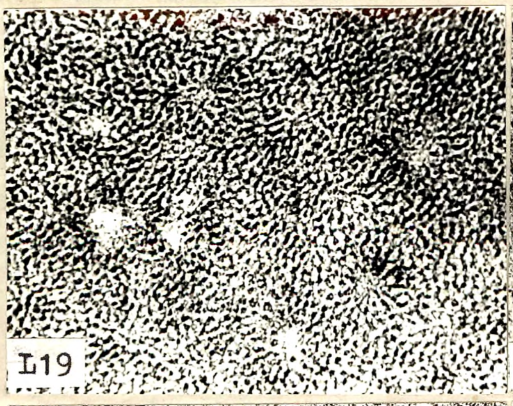
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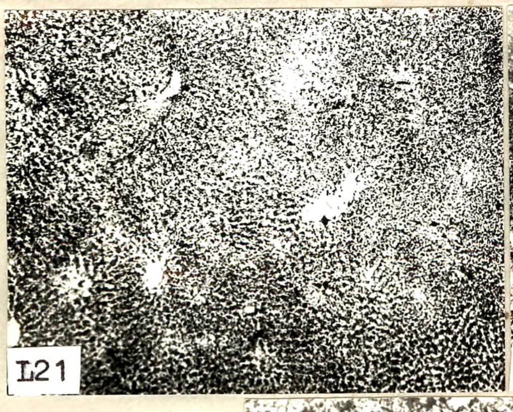

L21

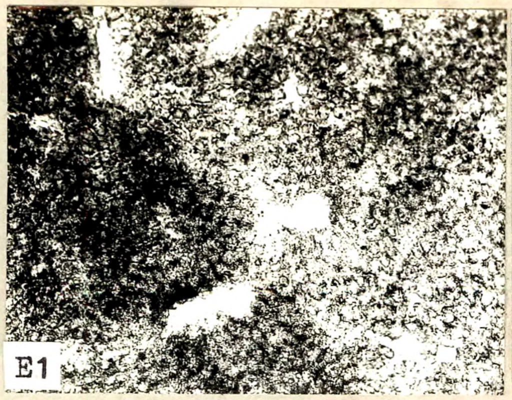

L22a


L22b

EXPLANATION TO FIGURES (CHAPTER 3)

Figs. E1 to E22. Photomicrographs of livers of birds of various groups showing esterase activity.

Fig.	E1	Vulture		50X
Fig.	E2	Kite	(E2a - 50X; E2b -	125X)
Fig.	E3	Cattle Egret	(E3a - 50X; E3b -	125X)
Fig.	E4	House Swift	(E4a - 50X; E4b -	125X)
Fig.	E5	Bee-Eater		50X
Fig.	E6	Tailor Bird	(E6a - 50X; E6b -	125X)
Fig.	E7	Martin	(E7a - 50X; E7b -	125X)
Fig.	E8	Drongo	(E8a - 50X; E8b -	125X)
Fig.	E9	Brahminy Myna		125X
Fig.	E10	Common Myna		125X
Fig.	E11	Jungle Babbler		125X
Fig.	E12	Indian Robin	(E12a - 50X;E12b -	125X)
Fig.	E13	Bulbul		50X
Fig.	E14	Koel		125X
Fig.	E15	House Crow		50X
Fig.	E16	House Sparrow		50X
Fig.	E17	Barbet		125X
Fig.	E18	Fowl		50X
Fig.	E19	Duck	(E19a - 50X; E19b -	125X)
Fig.	E20	Parakeet		125X
Fig.	E21	Dove	(E21a - 50X; E21b -	125X)
Fig.	E22	Pigeon	(E22a - 50X; E22b -	125X)



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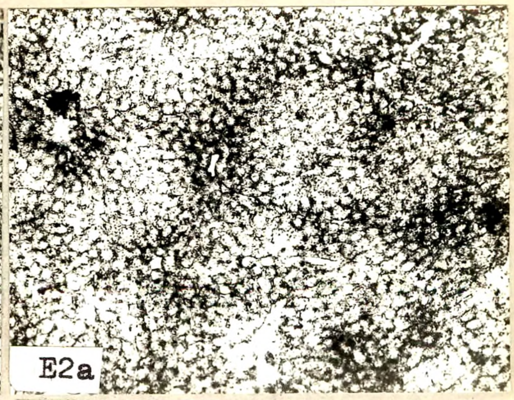

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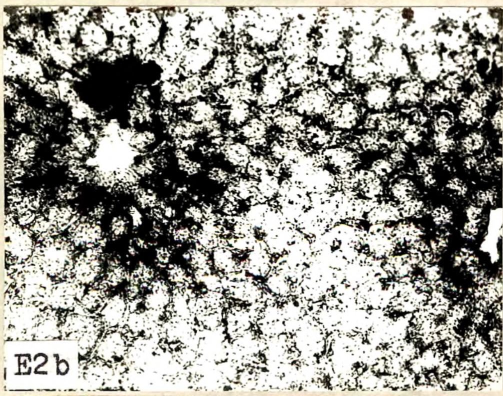

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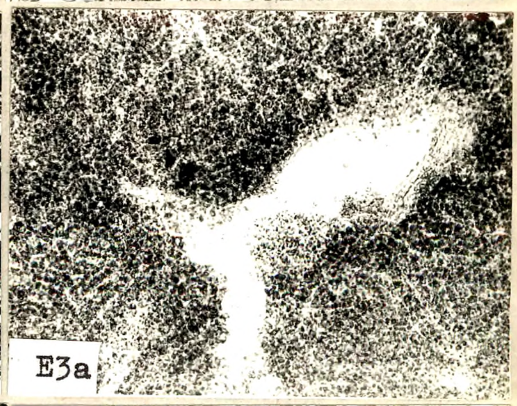

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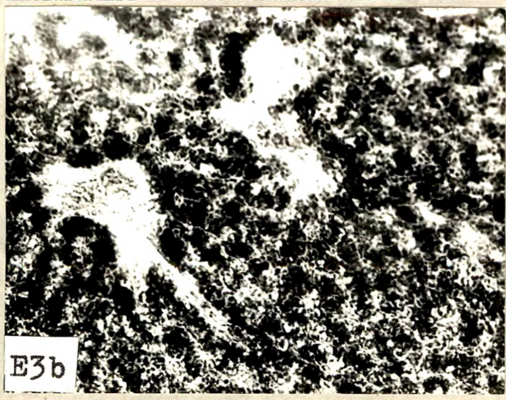

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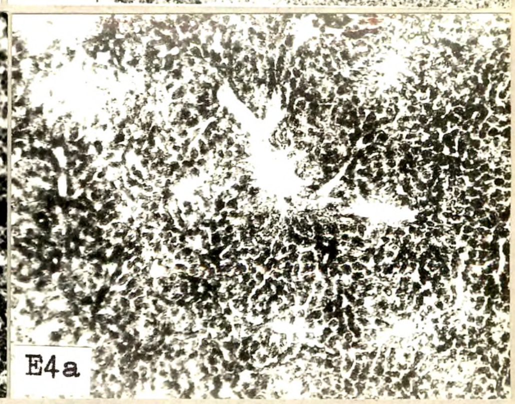

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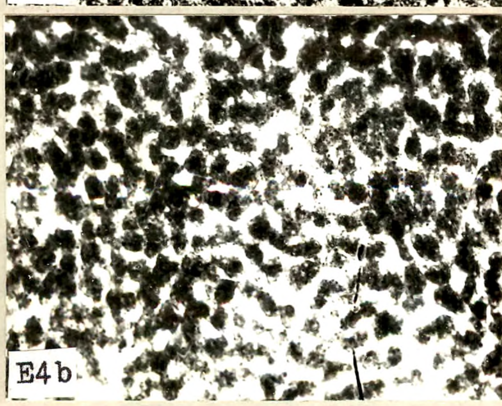

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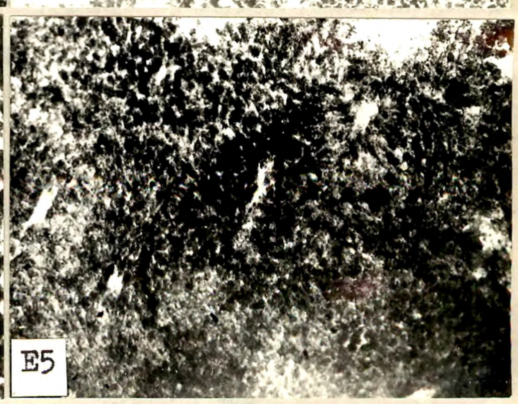

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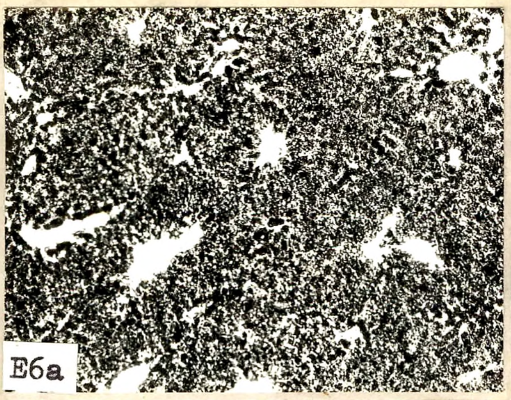
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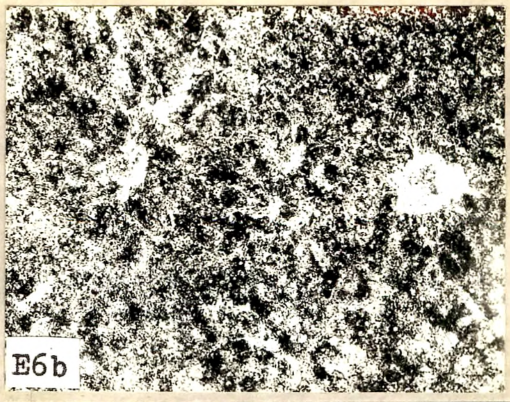
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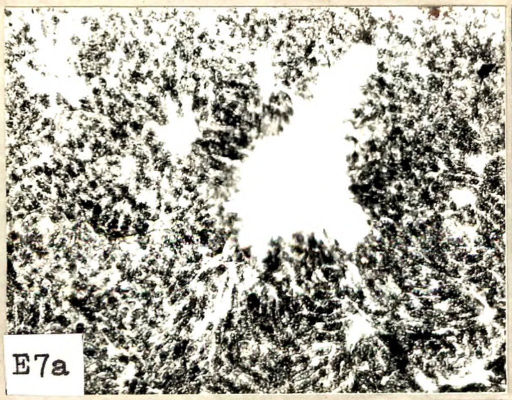
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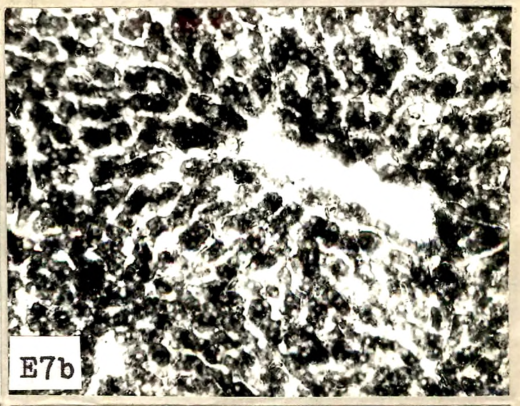
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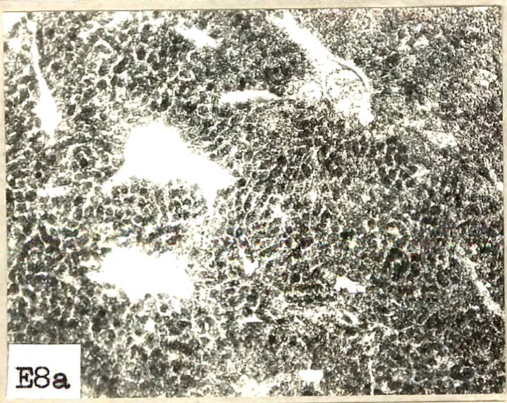
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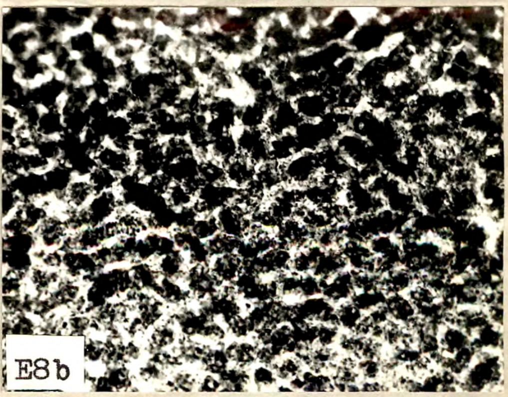
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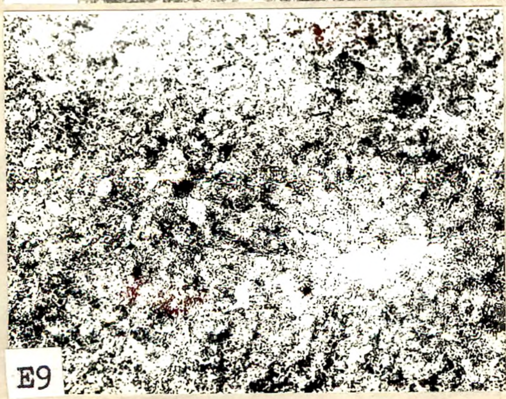
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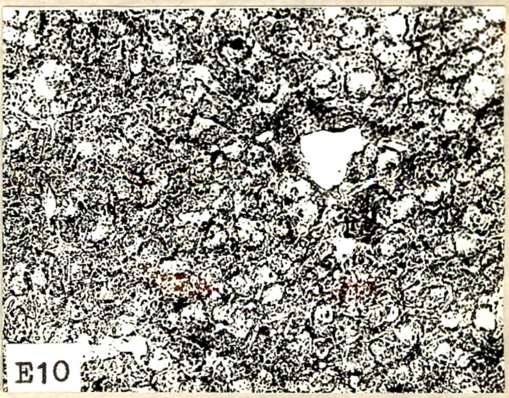
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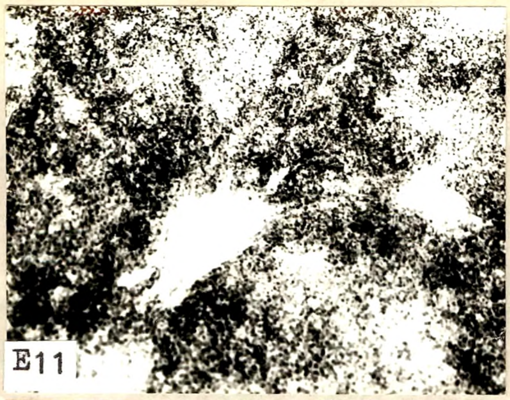
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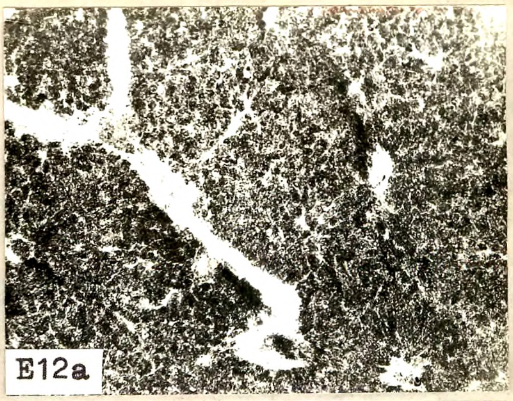
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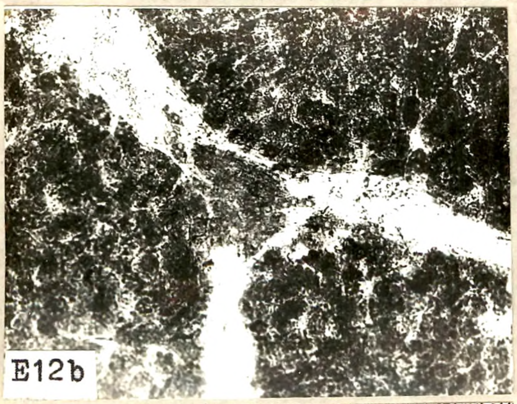
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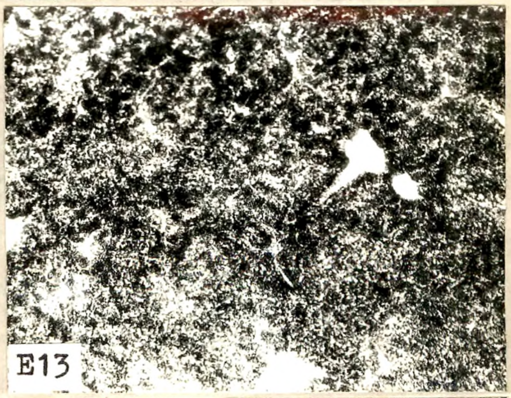
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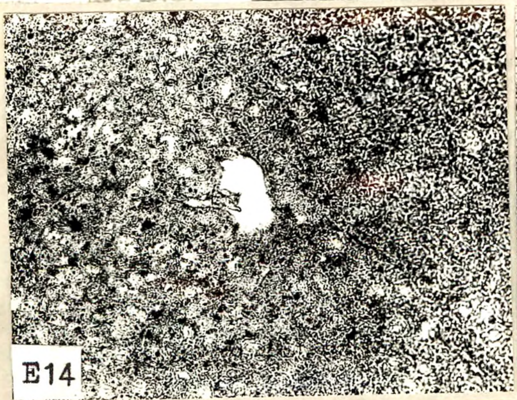
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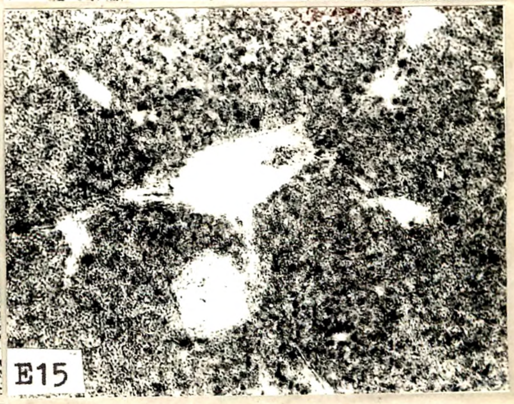
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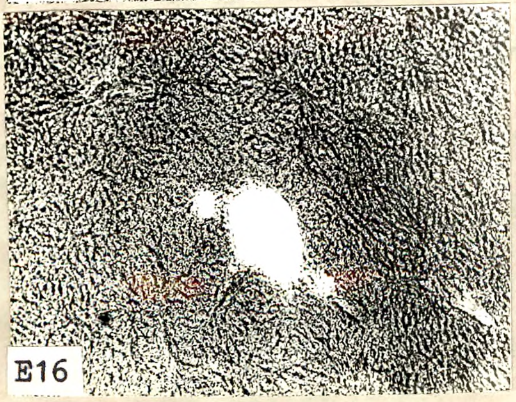
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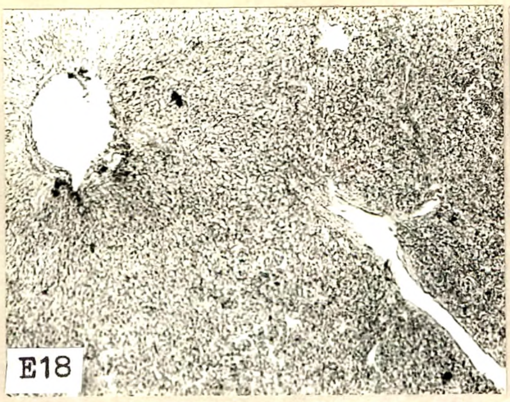
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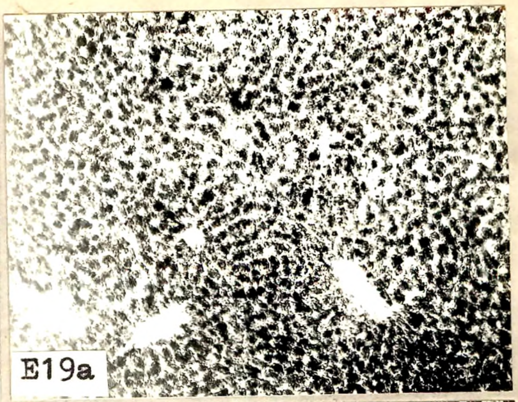
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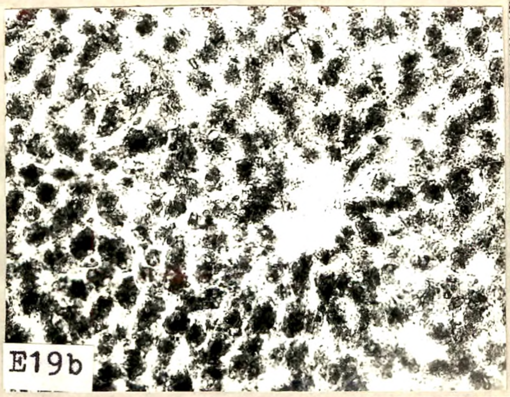
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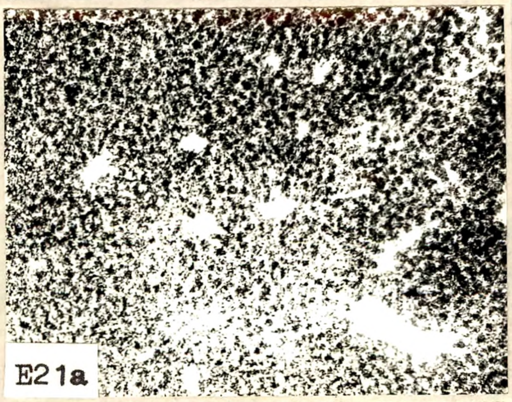
E20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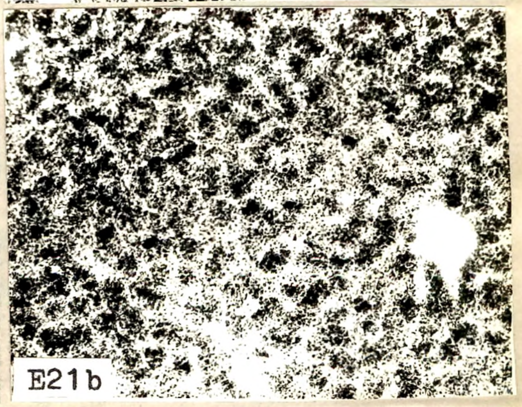
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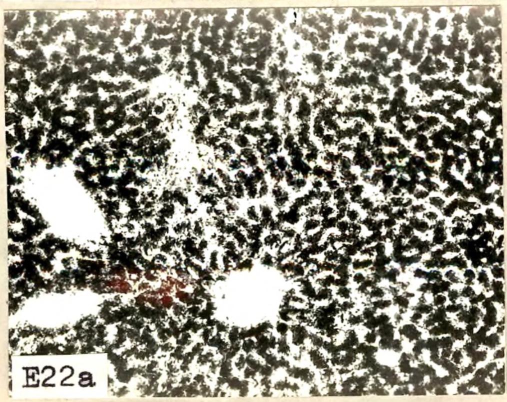
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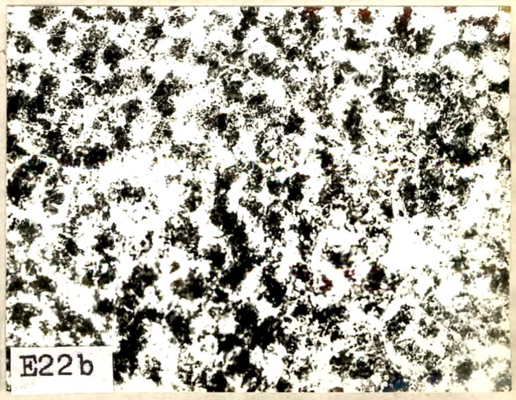
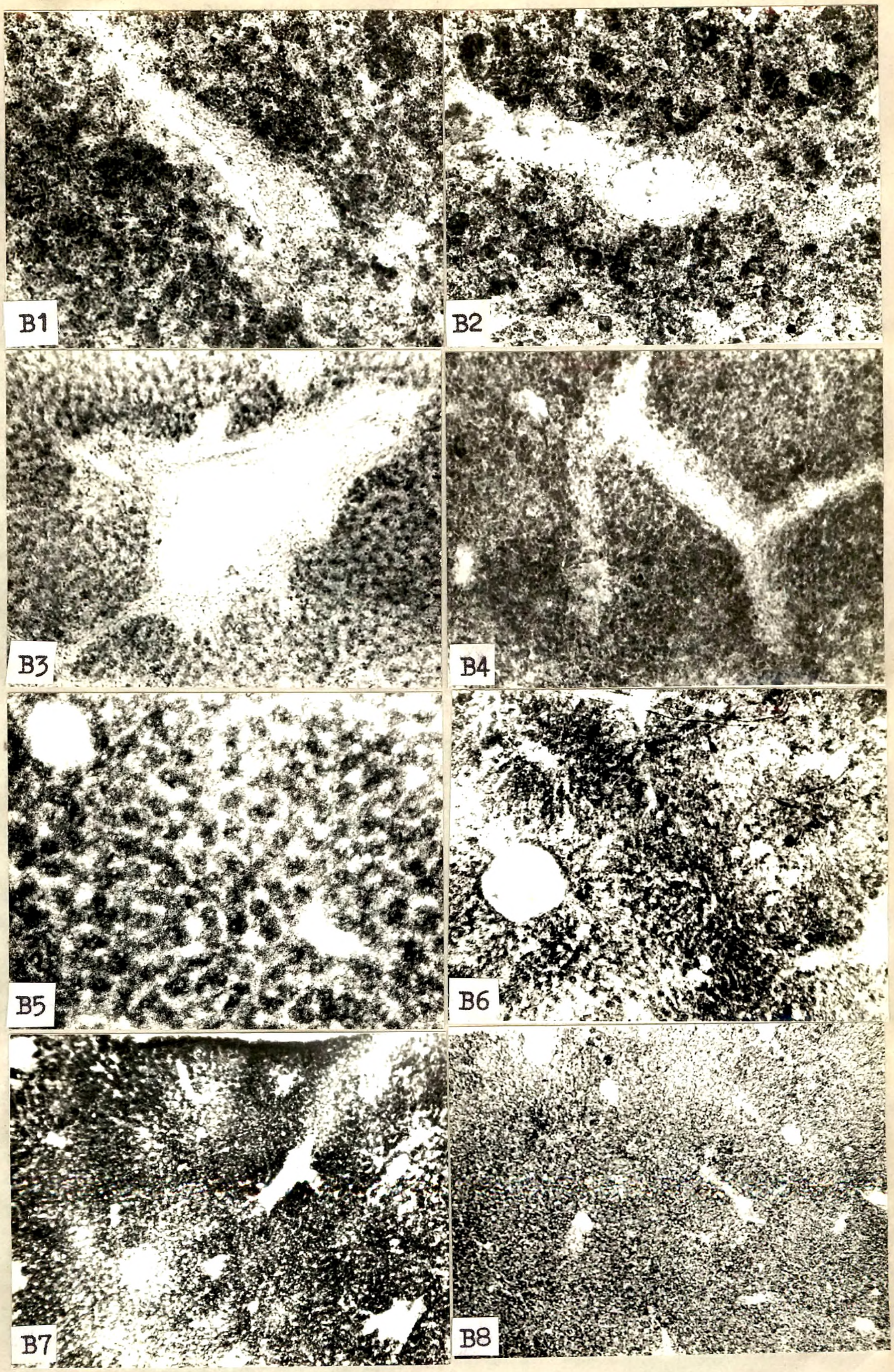
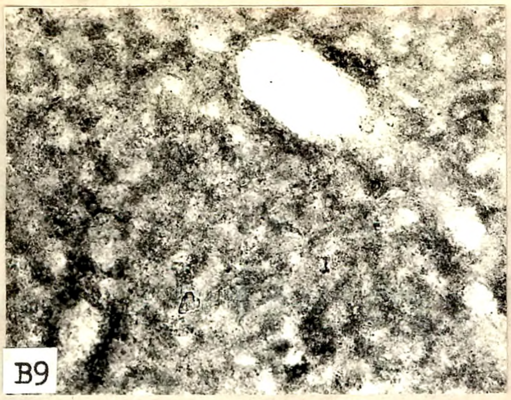

E22b

EXPLANATION TO FIGURES (CHAPTER 3)

Figs. B1 to B22. Photomicrographs of livers of birds showing BDH activity.

GROUP I	Fig. B1.	Vulture (G. bengalensis)	125X
	Fig. B2.	Kite (M. migrans)	125X
GROUP II	Fig. B3.	Cattle Egret (B. ibis)	50X
	Fig. B4.	House Swift (A. affinis)	125X
	Fig. B5.	Bee-eater (M. orientalis)	125X
	Fig. B6.	Tailor Bird (O. sutorius)	50X
	Fig. B7.	Martin (H. concolor)	50X
	Fig. B8.	Drongo (D. adsimilis)	50X
GROUP III	Fig. B9.	Brahminy Myna (S. pagodarum)	125X
	Fig. B10.	Common Myna (A. tristis)	50X
	Fig. B11.	Jungle Babbler (T. striatus)	50X
	Fig. B12.	Indian Robin (S. fulicata)	50X
	Fig. B13.	Bulbul (P. cafer)	50X
	Fig. B14.	Koel (E. scolopacea)	50X
	Fig. B15.	House Crow (C. splendens)	125X
	Fig. B16.	House Sparrow (P. domesticus)	50X
	Fig. B17.	Barbet (M. haemacephala)	50X
	Fig. B18.	Fowl (G. domesticus)	50X
	Fig. B19.	Duck (A. domesticus)	50X
GROUP IV	Fig. B20.	Parakeet (P. krameri)	50X
	Fig. B21.	Dove (S. senegalensis)	50X
	Fig. B22.	Pigeon (C. livia)	50X


B1
B2
B3
B4
B5
B6
B7
B8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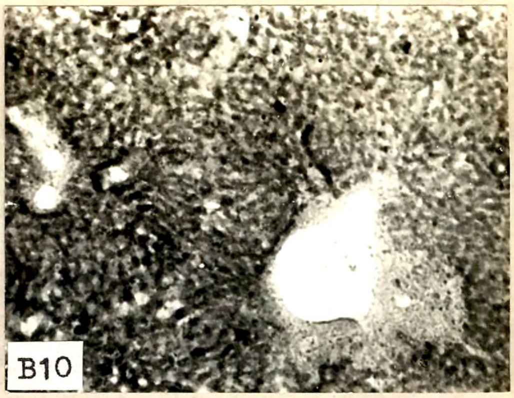
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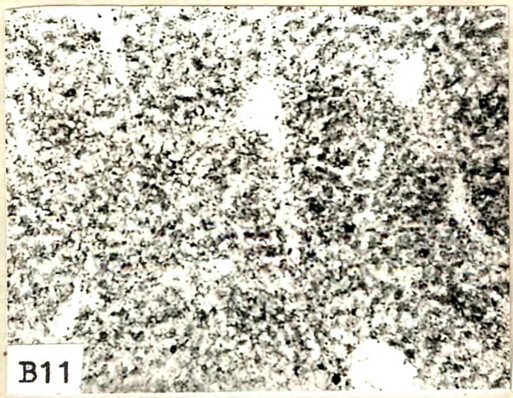
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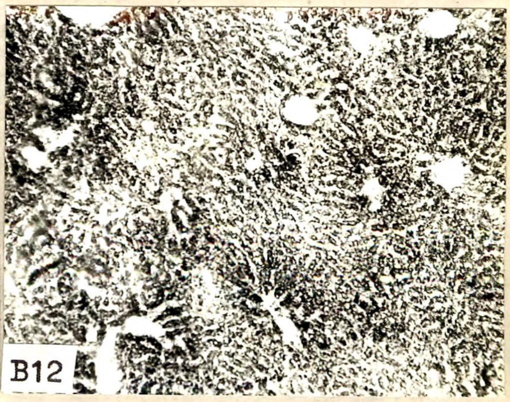
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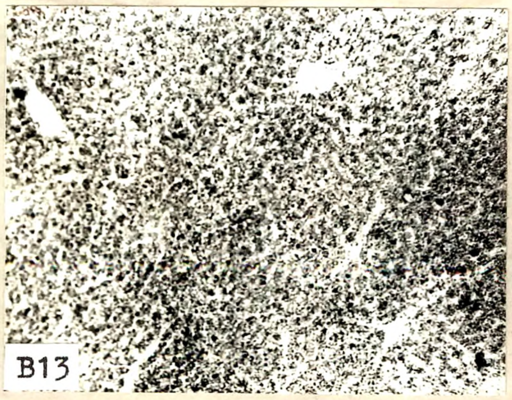
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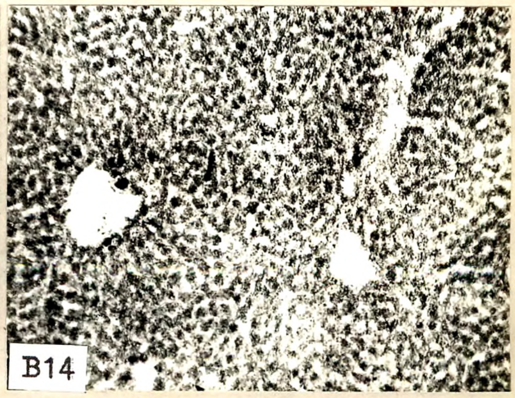
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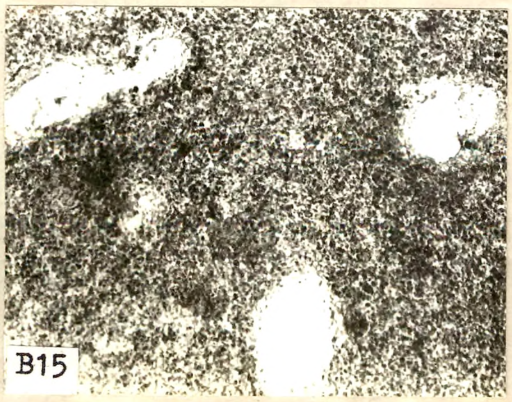
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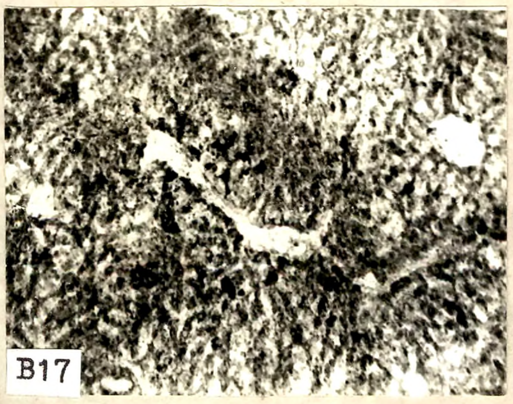
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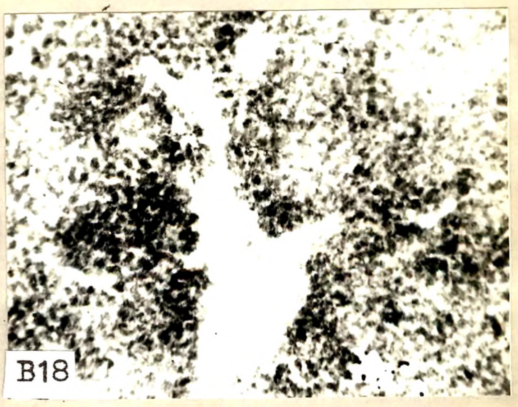
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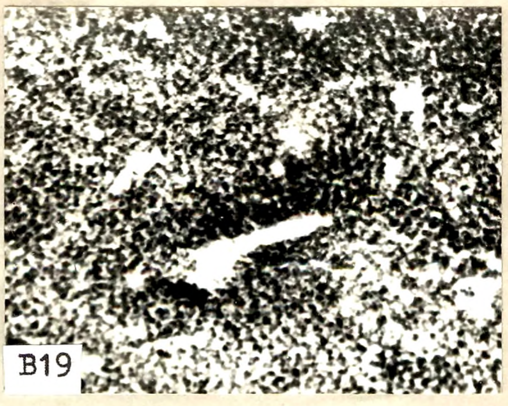
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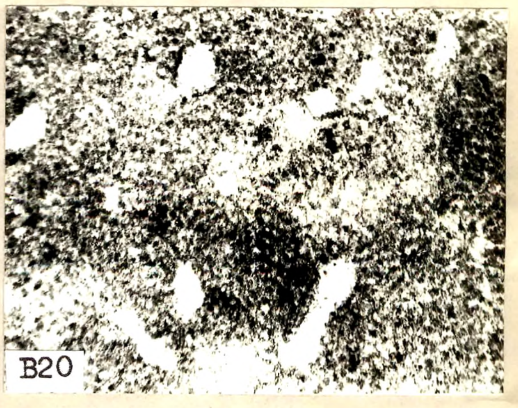
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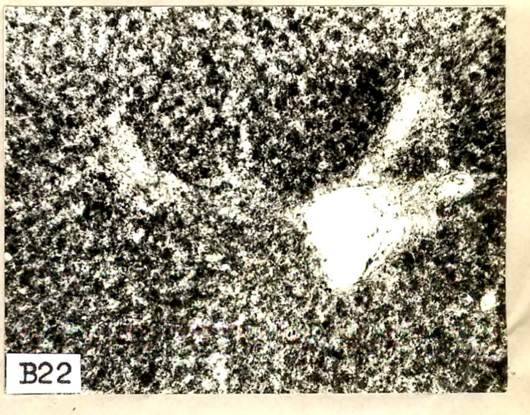
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