CHAPTER 4

RELATIONSHIP OF DIETARY PREFERENCES WITH PATTERNS OF DISTRIBUTION OF α-GLYCEROPHOSPHATE, LACTATE, SUCCINATE AND MALATE DEHYDROGENASES IN THE LIVER OF BIRDS BELONGING TO VARIOUS DIETARY GROUPS

The liver is considered as a most important organ in the vertebrate body as far as its metabolic activities are concerned. Almost all metabolic reactions concerned with the breakdown and synthesis of carbohydrates, lipids and proteins are known to occur in the liver. Being endowed with enzymic machinary for such metabolic activities, liver is the only organ that can effectively bring about interconversions of the three metabolites. This capacity helps liver ~~in~~ maintain~~ing~~ many of the circulating metabolites in the blood at a steady level. Apart from the metabolic activities liver also performs large number of other functions which require energy which is obtained by oxidizing various metabolites present within the organ itself. Extent of the oxidative metabolism prevalent in the livers of birds with different types of diet could be ~~envisaged from~~ obtained by a study of the intensity of ~~the~~ enzymes ~~like~~ such as α-GPDH, LDH, SDH and MDH. Histochemical methods have been employed

to understand the regional specializations in the hepatic lobules with regard to metabolic reactions. It has been shown that the liver exhibits regional specializations in performing various functions (Chapter II & III). The present study was planned to get more information regarding the metabolic adaptations of the liver with reference to dietary preferences of some selected birds.

MATERIAL AND METHODS

Birds representing groups with various dietary preferences viz., (1) Carnivores (2) Insectivores (3) Omnivores and (4) Graminivores were shot from their natural habitat around University Campus and were brought to the laboratory immediately. The birds thus collected are listed in (Chapter I, Table I).

The livers of these birds were quickly removed and small pieces of them were fixed on a cryostat microtome chuck to obtain fresh frozen sections. Sections of 12 μ thickness were cut and were incubated in the specific media to demonstrate localization of α-GPDH, LDH, SDH and MDH. The methods followed were those of Ogata and Mori (1964). The confirmation of

actual site of enzyme activity was carried out by comparing the sample section against control ones incubated in media devoid of the specific substrates.

OBSERVATIONS

GROUP I (Carnivores)

All the dehydrogenases demonstrated histochemically in the present study, were found localized in the parenchymal cells. The α-GPDH (Figs. G1 & G2), LDH (Figs. L1 & L2) and MDH (Figs. M1 & M2) were found to be uniformly distributed in the hepatic lobules of both Vulture and Kite. Though SDH (Figs. S1 & S2) was found in all the hepatocytes, fine granular formazan deposition representing enzyme activity was found slightly more in the hepatocytes situated near the portal spaces.

GROUP II (Insectivores)

In the insectivore livers, the reactivities of both α-GPDH (Figs. G3 & G8) and SDH (Figs. S3-S8) were seen to have close similarity, both being present more in the hepatocytes situated around portal areas. The LDH (Figs. L3-L8) and MDH (Figs. M3-M8) were more or less uniformly distributed in the liver lobules. However, in the liver of Tailor Bird (Figs. L6 & M6)

and Drongo (Figs. L8 & M8) these enzymes were found to be localized more within the periportal region.

GROUP III (Omnivores)

In this group of birds ~~also~~, all the dehydrogenases studied were found to be localized in the parenchymal cells. However, their intralobular distributions were found to vary. The α-GPDH (Figs. G9-G19) and SDH (Figs. S9-S19) with intense reactivity were localized in the periportal areas, with the exception of Crow (Figs. G15 & S15), Sparrow (Figs. G16 & S16) and Duck (Figs. G19 & S19) in which case a uniform distribution was seen ~~of α-GPDH and SDH was noticed~~. Both LDH (Figs. L9-L19) and MDH (Figs. M9-M19) were uniformly distributed in the hepatic lobules, with the exception of Crow (Fig. M15), Sparrow (Fig. M16), Barbet (Fig. M17) and Fowl (Fig. M18) where MDH was found in slightly higher concentration in the periportal areas.

GROUP IV (Frugivores and Graminivores)

Of the three birds studied within this group, the Parakeet exhibited higher α-GPDH reactivity (Fig. G20) in the periportal areas, whereas the Pigeon (Fig. G22) and the Dove (Fig. G21) had a uniform reactivity of the enzyme in all the parts of their liver lobules. LDH reactivity in all

the three birds (Figs. L20-L22) was uniformly distributed in all the areas of their liver lobules but in Parakeet (Fig. L20) the enzyme intensity was relatively higher than that in the other two birds. The SDH and MDH reactivity (Figs. S20-S22 and M20-M22), in general, was similar in the livers of all the three birds, with slightly higher concentrations in the periportal areas.

DISCUSSION

All the dehydrogenases studied presently viz., ∝-GPDH, LDH, SDH and MDH were quite active in the livers of all the birds selected for the investigation. This is only to be expected as these enzymes are part of the enzyme-complex that break down sugars and other metabolites to provide energy for the maintenance of endergonic processes.

Although, all these dehydrogenases are NAD-linked catalysts, LDH is extramitochondrial, SDH is purely mitochondrial and ∝-GPDH and MDH are found both within and outside the mitochondria (both these enzymes are concerned with the oxidation of mitochondrial and extra-mitochondrial $NADH_2$). The concentration of mitochondria,

in general, is ~~more~~ greater in the parenchymal cells that are situated nearer ~~to~~ the portal areas (Ratzlaff and Tyler, 1973) where these cells are the first to ~~get~~ become exposed to the blood loaded with oxygen and nutrients (Rappaport, 1963). The presence of more mitochondria in cells around the portal areas naturally results in a higher histochemical reactivity of the mitochondrial dehydrogenases in these cells. Thus the distribution of SDH is found to be greatest ~~more~~ in the cells around portal areas in all the birds studied.

Ratzlaff and Tyler (1973) also found that the SDH is highly concentrated around the portal areas in the liver of birds. On the other hand, LDH was found uniformly distributed ~~all over~~ throughout the hepatic lobules in all the birds studied. This is only as expected, since LDH is an extramitochondrial dehydrogenase.

Since Œ-GPDH and MDH are known to be mitochondrially and extramitochondrially localized, the higher concentrations of these two enzymes around periportal areas, would depend upon the presence of a higher reactivity of the mitochondrial variety of these enzymes in the liver of the birds concerned. Œ-GPDH is distributed uniformly in carnivores and graminivores but periportally in insectivores and omnivores. Extramitochondrial Œ-GPDH becomes highly active during

the conversion of dihydroxy acetone phosphate to glycerophosphate thereby reoxidizing $NADH_2$. α-glycerophosphate may be converted into glycerol which in turn is utilized for triglyceride formation. If the lipid intake is high (through insect food) then glycerol liberated from triglycerides is degraded to α-glycerophosphate and then to dihydroxy acetone phosphate which finally joins the Embden-Meyerhoff pathway. Mitochondrial α-GPDH catalyses the reaction involved in the conversion of α-glycerophosphate to dihydroxy acetone phosphate. Both extramitochondrial and mitochondrial α-GPDH together play an important role in the oxidation of extramitochondrial $NADH_2$. The conclusion that can be derived from the observation on α-GPDH distribution is that, in insectivores and omnivores the liver, with greater α-GPDH concentration in the mitochondria (because the distribution is more in periportal region), is adapted for utilizing more of lipids than carbohydrates.

The malate dehydrogenase (MDH), although found in all extramitochondrial and mitochondrial locations, was present in all hepatocytes of lobules in carnivores, a fact that denotes that the extramitochondrial MDH was the predominant one in the liver of carnivorous birds. In all other groups of birds the mitochondrial MDH

reactivity was more prominant as it was found slightly more in periportal areas. However, in few insectivores and omnivores the MDH was uniformly distributed. Lack of uniformity in distribution of MDH within the insectivore and omnivore groups could be due to heterogenous nature of these groups. Such heterogenous nature of omnivorous birds can also be seen with regard to the intralobular distribution of CC-GPDH. The presence of predominant mitochondrial or extramitochondrial CC-GPDH, MDH and LDH may indicate original metabolic adaptations of the livers. CC-GPDH is periportal in almost all omnivores except in Crow, Sparrow and Duck. In stenophagous birds (carnivores and graminivores) this enzyme was found uniformly distributed in hepatic lobules. Perhaps, the liver of Crow may be still possessing the original adaptation suited to a carrion feeder, while those of birds like Sparrow and Duck seem to have retained their graminivorous adaptations. Similarly, LDH and MDH were uniformly distributed in the livers of almost all insectivores except in Tailor Bird and Drongo where these enzymes have a periportal localization. Since MDH is periportal in graminivores, it could be reasonably assumed that Tailor Bird and Drongo may have been originally graminivores

and later became adapted exclusively for insect food nevertheless have retained original metabolic adaptation in their liver. This is also evident from the structure of gizzard of Drongo which is still muscular like that seen in other graminivores.

From the foregoing data it is evident that the mitochondrial localization of few dehydrogenases (α-GPDH and MDH) show some correlation with type of food ingested by the birds.

TABLE I

The histochemical distribution pattern of α-GPDH, LDH, SDH and MDH in the livers of birds belonging to various dietary groups

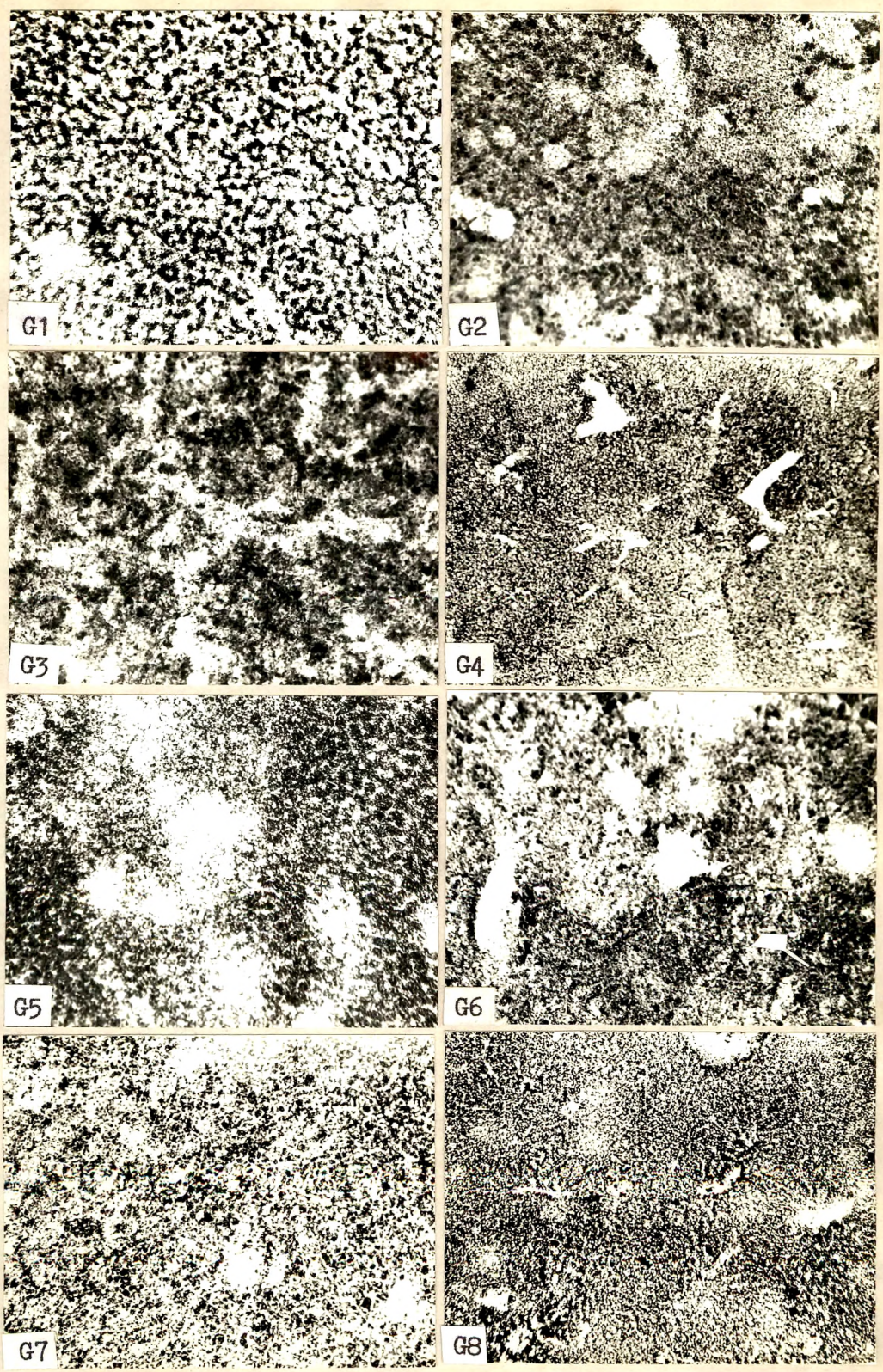
GROUP	α-GPDH M & E	LDH E	SDH M	MDH M & E
I. CARNIVORES	Uniform	Uniform	Periportal	Uniform
II. INSECTIVORES	Periportal	Uniform (periportal in Tailor Bird)	Periportal	Uniform (periportal in Tailor Bird)
III. OMNIVORES	Periportal (Uniform in Crow, Sparrow & Duck)	Uniform	Periportal	Uniform (periportal in Crow, Sparrow, Barbet & Fowl)
IV. FRUGIVORE (Parakeet) & GRAMINIVORES (Dove & Pigeon)	Uniform (periportal in Parakeet)	Uniform	Periportal	Periportal

M - Mitochondrial; E - Extramitochondrial

EXPLANATIONS TO FIGURES (CHAPTER 4)

Figs. 1 to 22. Photomicrographs of livers of birds showing α-GPDH activity.

GR. I.	Fig. G1.	Vulture (*G.bengalensis*)	125X
	Fig. G2.	Kite (*M.migrans*)	50X
GR. II.	Fig. G3.	Cattle Egret (*B.ibis*)	125X
	Fig. G4.	House Swift (*A.affinis*)	125X
	Fig. G5.	Bee-eater (*M.orientalis*)	50X
	Fig. G6.	Tailor Bird (*O.sutorius*)	50X
	Fig. G7.	Martin (*H.concolor*)	125X
	Fig. G8.	Drongo (*D.adsimilis*)	50X
GR.III.	Fig. G9.	Brahminy Myna (*S. pagodarum*)	125X
	Fig. G10.	Common Myna (*A.tristis*)	50X
	Fig. G11.	Jungle Babbler (*T.striatus*)	50X
	Fig. G12.	Indian Robin (*S.fulicato*)	125X
	Fig. G13.	Bulbul (*P.cafer*)	50X
	Fig. G14.	Koel (*E. scolopacea*)	50X
	Fig. G15.	House Crow (*C. splendens*)	50X
	Fig. G16.	House Sparrow (*P.domesticus*)	50X
	Fig. G17.	Barbet (*M.haemacephala*)	50X
	Fig. G18.	Fowl (*G.domesticus*)	50X
	Fig. G19.	Duck (*A.domesticus*)	50X
GR. IV.	Fig. G20.	Parakeet (*P.krameri*)	50X
	Fig. G21.	Little Brown Dove (*S.senegalensis*)	125X
	Fig. G22.	Blue Rock Pigeon (*C.livia*)	50X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G2
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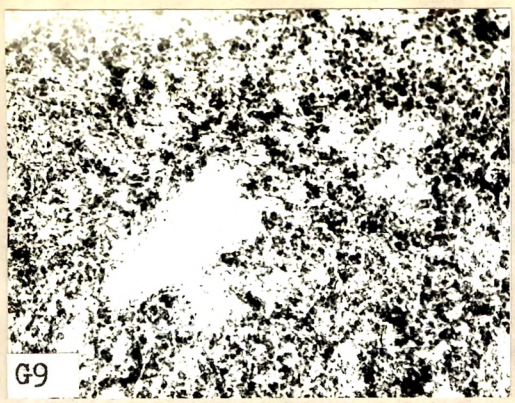
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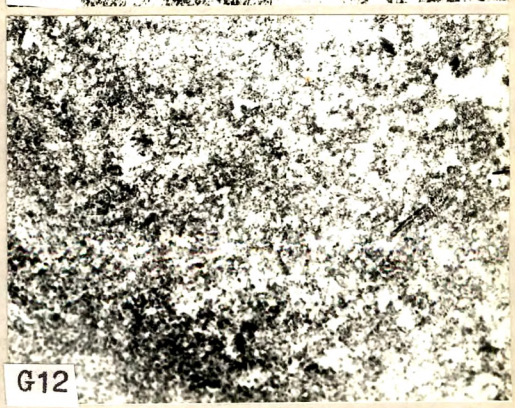
G10


G11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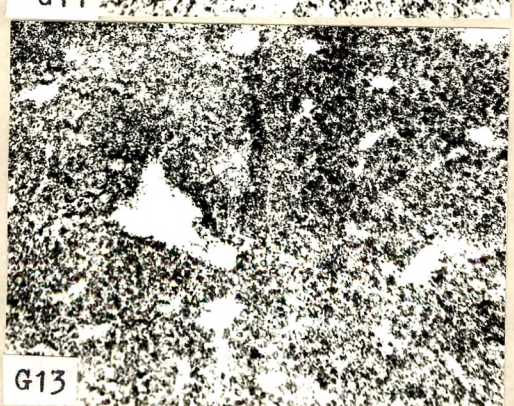
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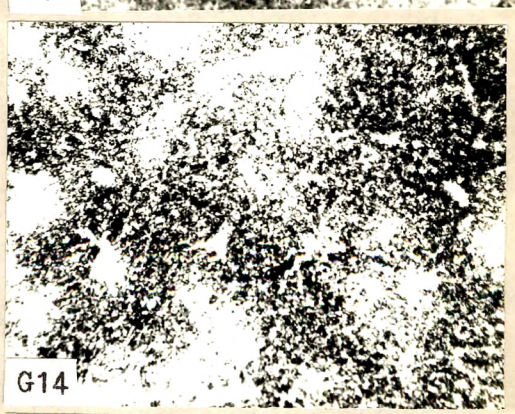
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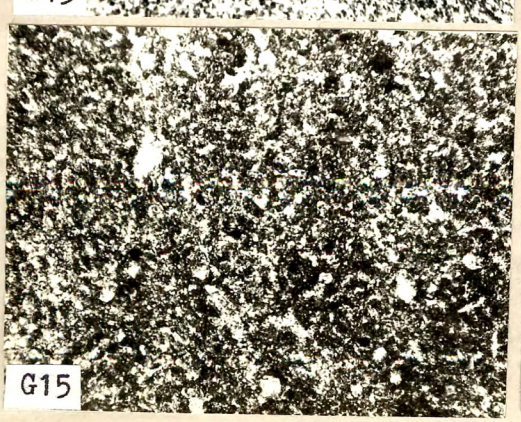
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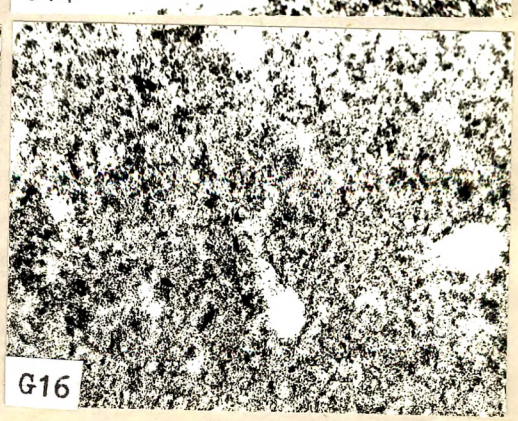
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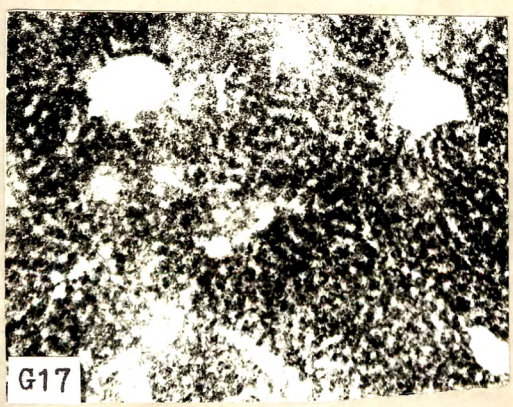
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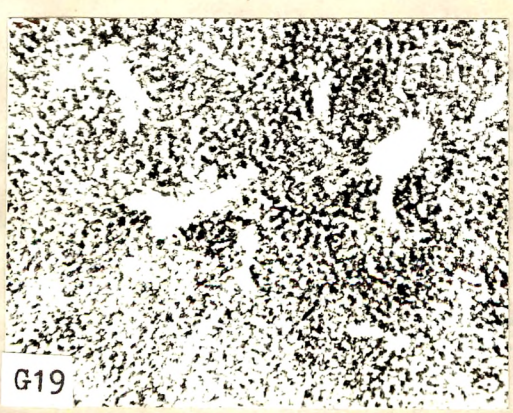
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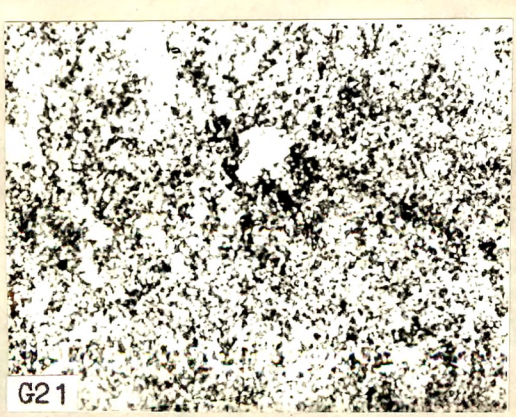
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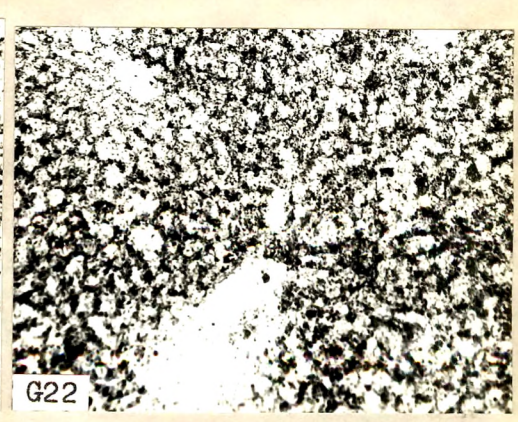
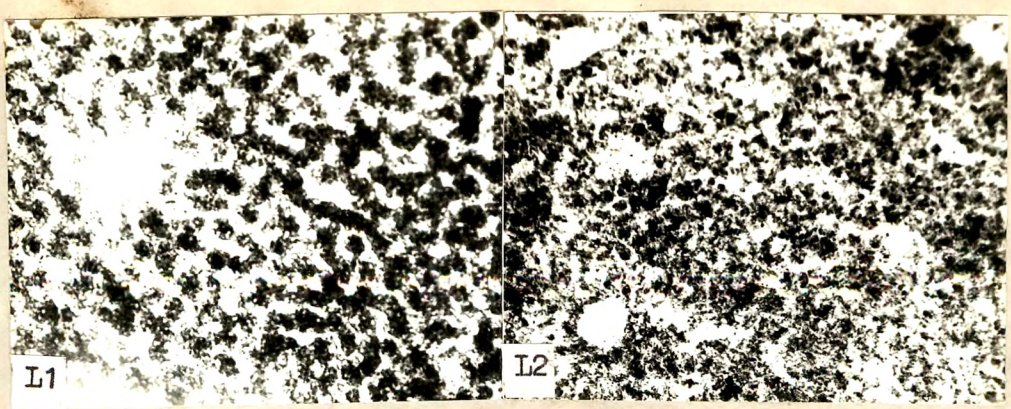

G22

EXPLANATIONS TO FIGURES (CHAPTER 4)

Figs. 1 to 22 Photomicrographs of livers of birds showing LDH activity.

GR. I.	Fig. L1.	Vulture (G.bengalensis)	125X
	Fig. L2.	Kite (M.migrans)	50X
GR. II.	Fig. L3.	Cattle Egret (B.ibis)	50X
	Fig. L4.	House Swift (A.affinis)	50X
	Fig. L5.	Bee-eater (M.orientalis)	50X
	Fig. L6.	Tailor Bird (O.sutorius)	50X
	Fig. L7.	Martin (H.concolor)	50X
	Fig. L8.	Drongo (D.adsimilis)	125X
GR.III.	Fig. L9.	Brahminy Myna (S. pagodarum)	50X
	Fig. L10.	Common Myna (A.tristis)	50X
	Fig. L11.	Jungle Babbler (T.striatus)	50X
	Fig. L12.	Indian Robin (S.fulicata)	50X
	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)	125X
	Fig. L18.	Fowl (G.domesticus)	50X
	Fig. L19.	Duck (A.domesticus)	125X
GR. IV.	Fig. L20.	Parakeet (P.krameri)	50X
	Fig. L21.	Little Brown Dove (S.senegalensis)	50X
	Fig. L22.	Blue Rock Pigeon (C.livia)	50X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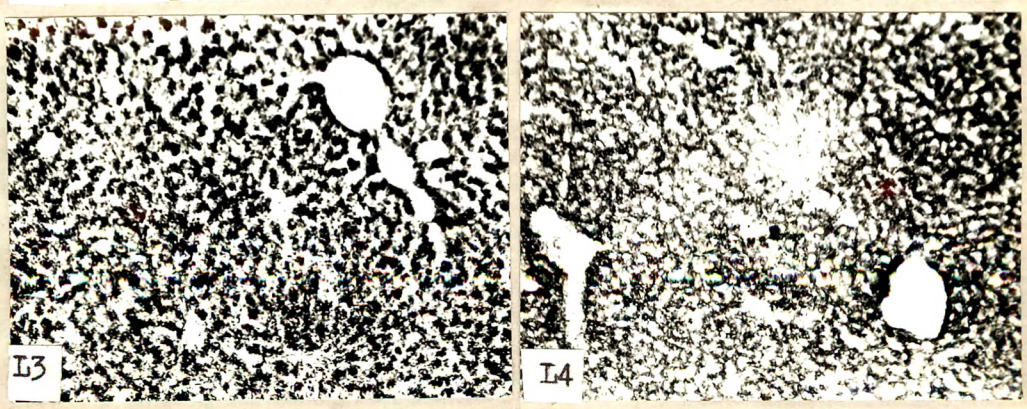
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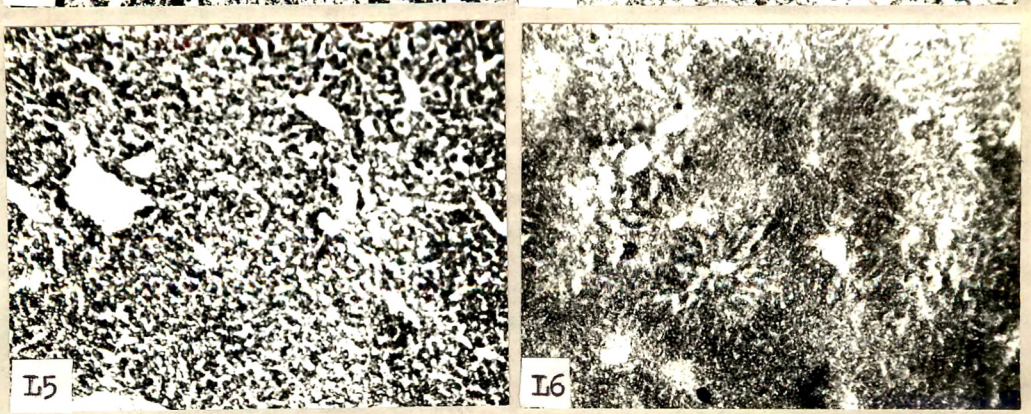
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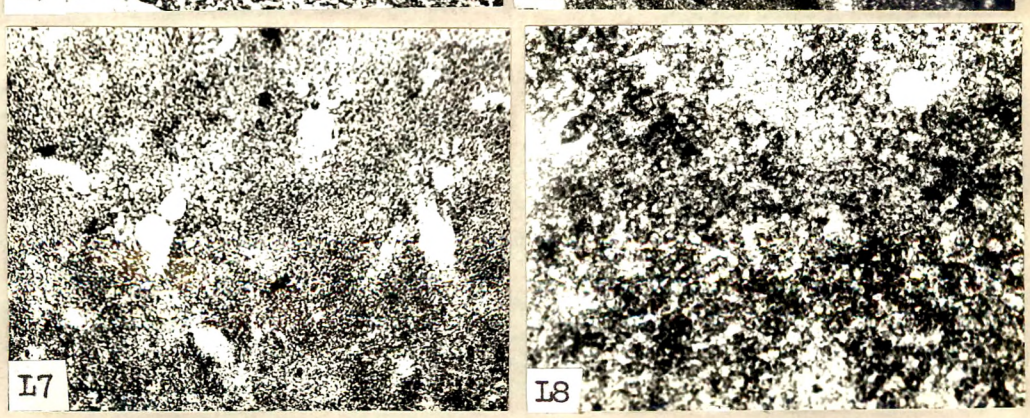
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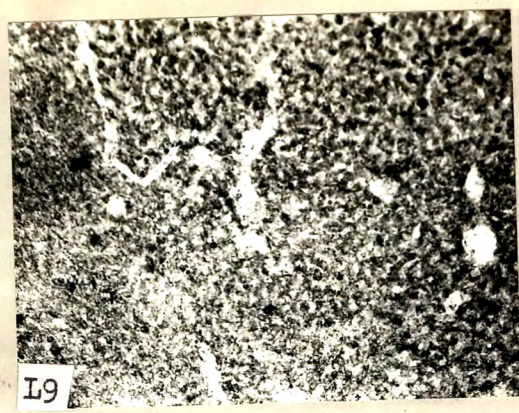
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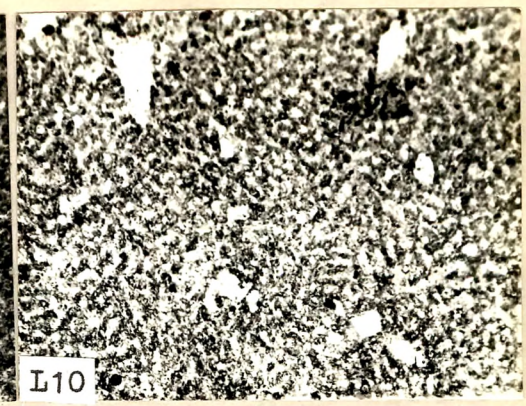
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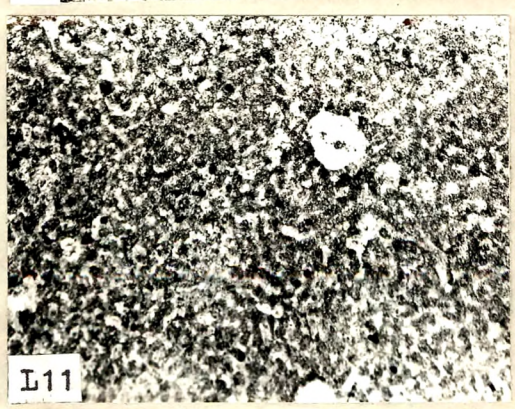
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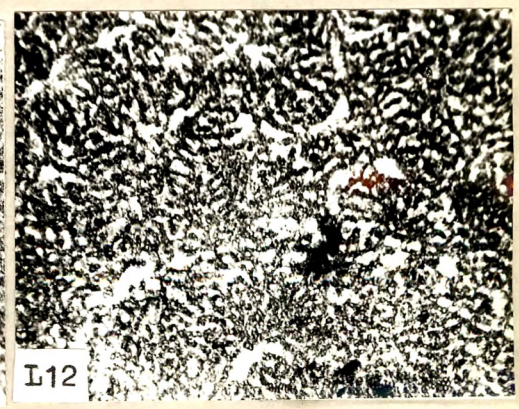
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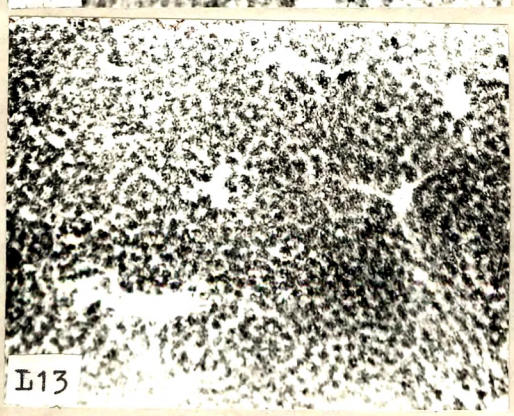
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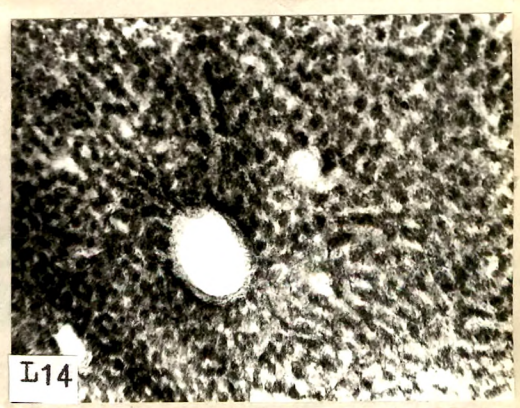
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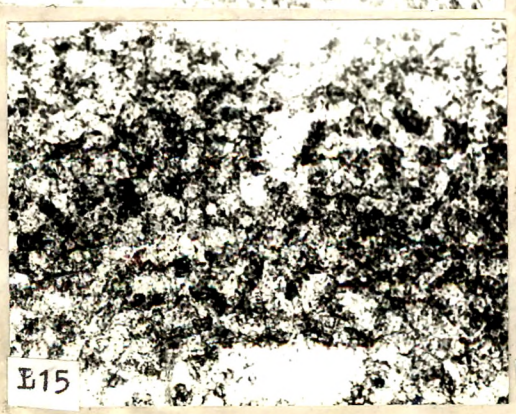
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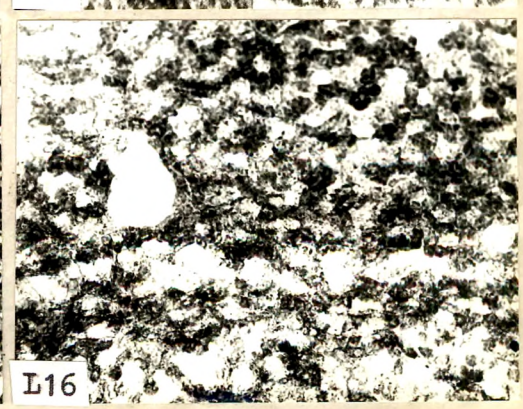
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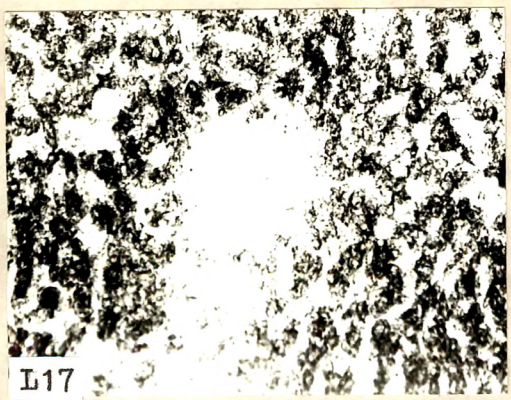
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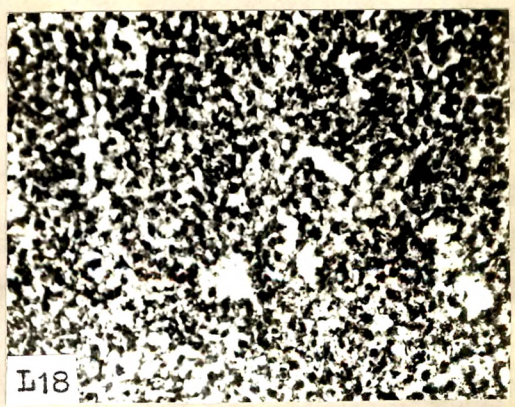
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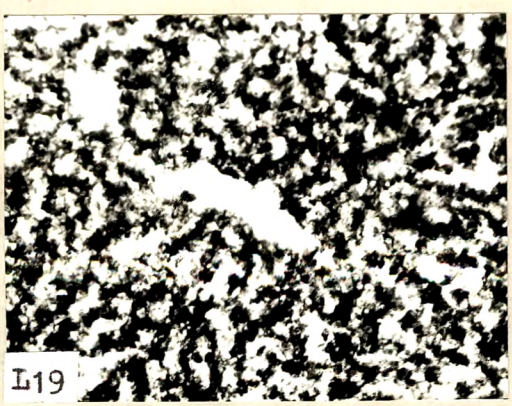
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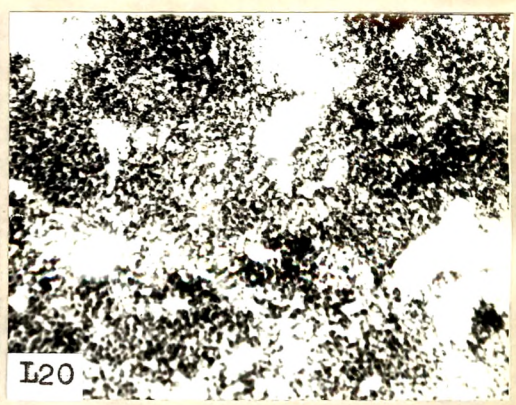
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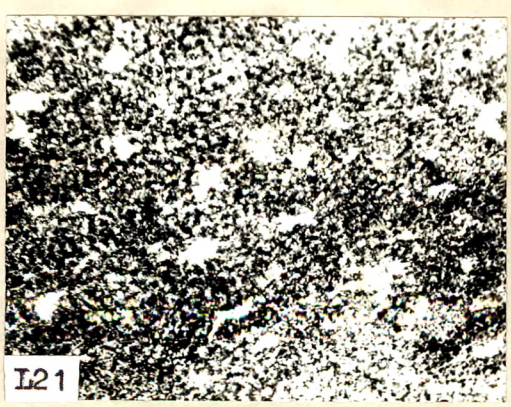
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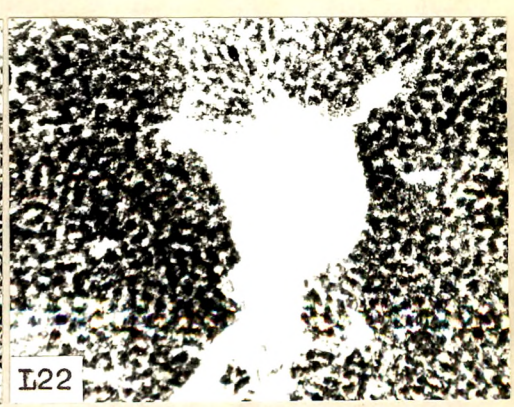
L22

EXPLANATIONS TO FIGURES (CHAPTER 4)

Figs. 1 to 22. Photomicrographs of livers of birds showing SDH activity.

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GR. II.	Fig. S3.	Cattle Egret (*B.ibis*)	125X
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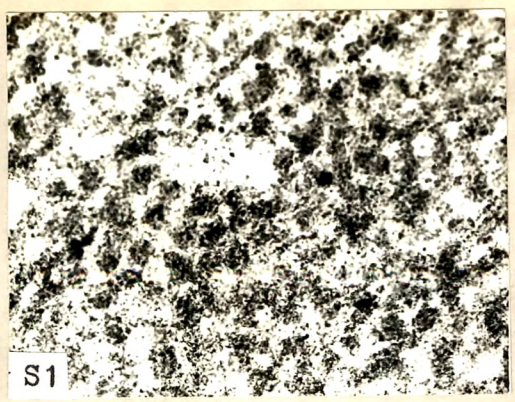
GR. IV.	Fig. S20.	Parakeet (*P.krameri*)	50X
	Fig. S21.	Little Brown Dove (*S.senegalensis*)	50X
	Fig. S22.	Blue Rock Pigeon (*C.livia*)	50X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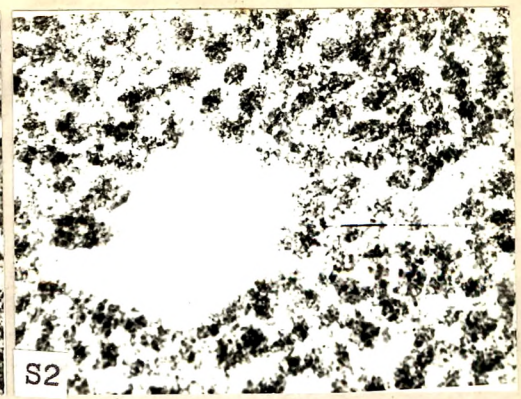
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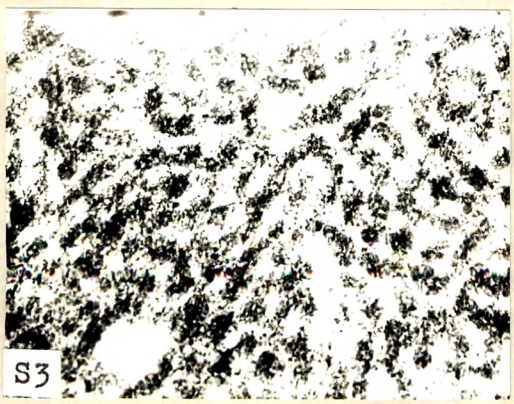
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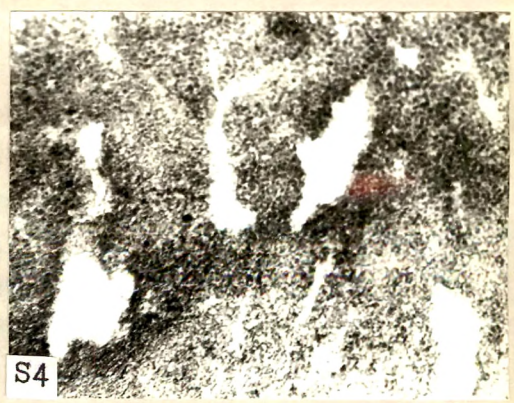
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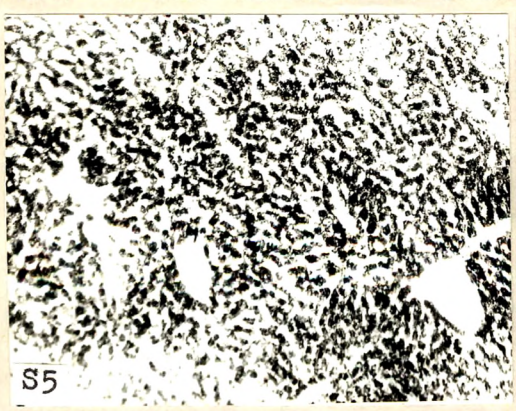
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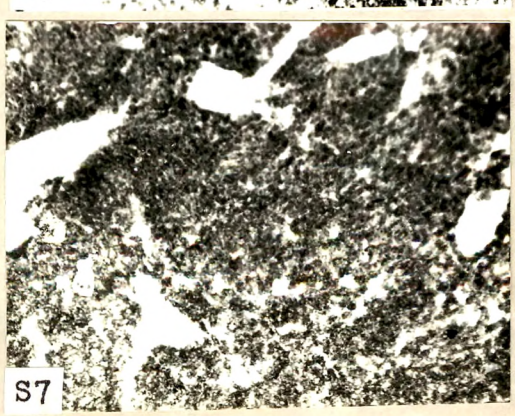
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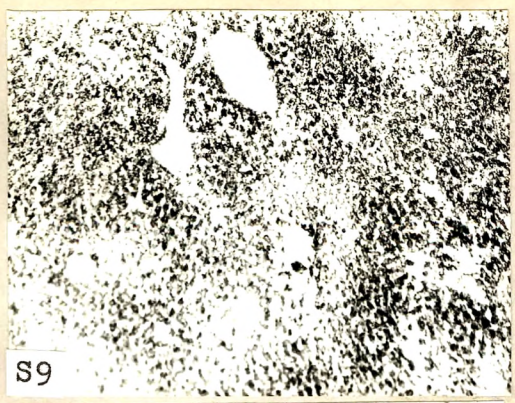
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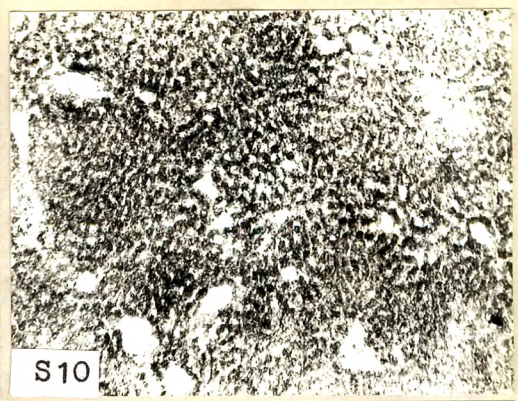
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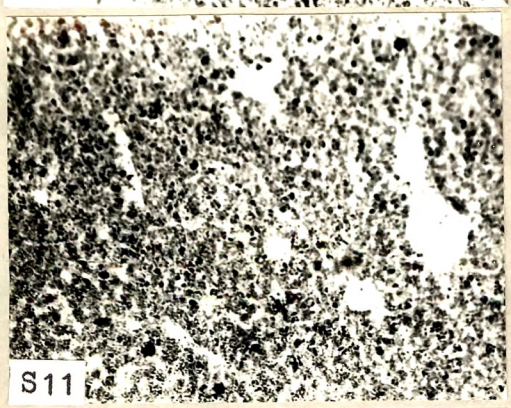
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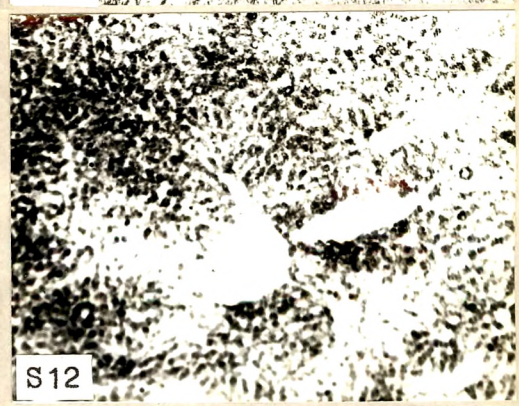
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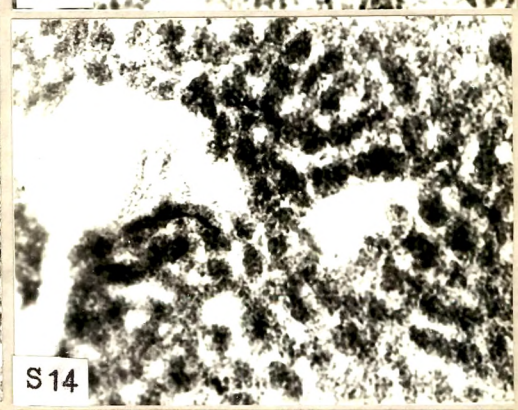
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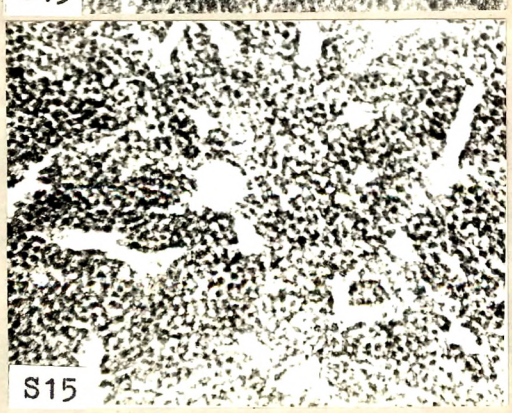
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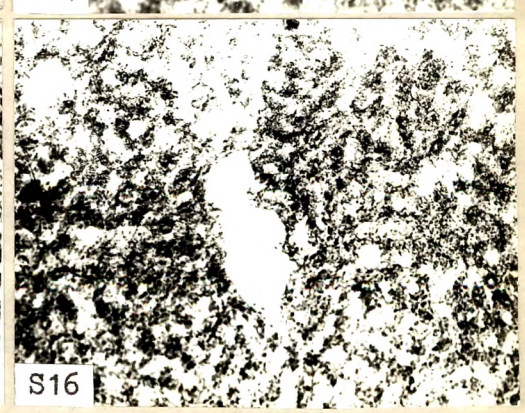
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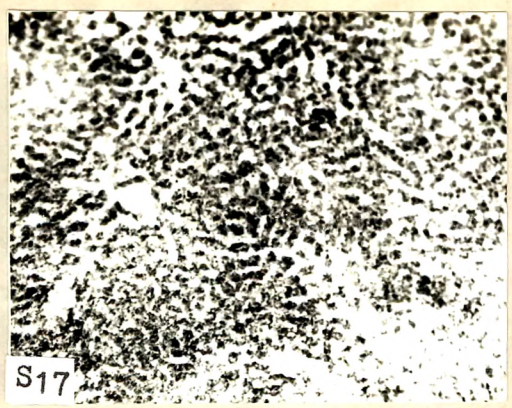
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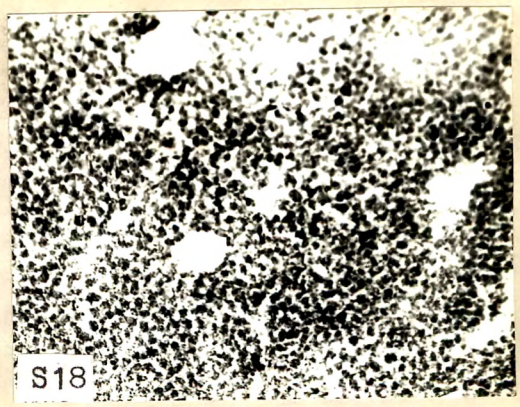
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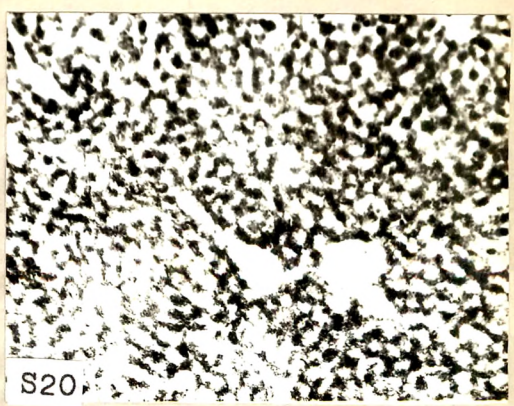
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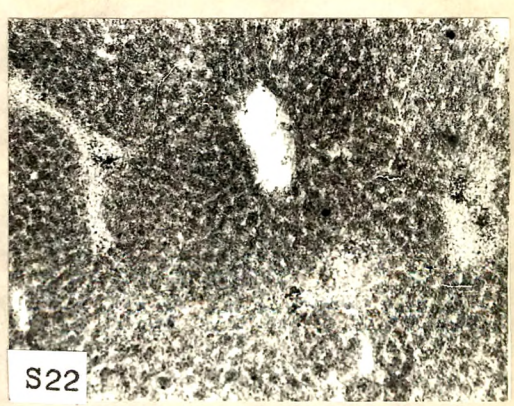
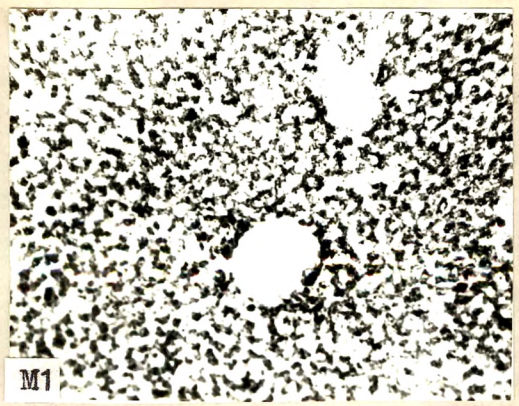

S22

EXPLANATIONS TO FIGURES (CHAPTER 4)

Figs. 1 to 22. Photomicrographs of livers of birds showing MDH activity.

GR. I.	Fig. M1.	Vulture (*G.bengalensis*)	50X
	Fig. M2.	Kite (*M. migrans*)	50X
GR. II.	Fig. M3.	Cattle Egret (*B.ibis*)	125X
	Fig. M4.	House Swift (*A.affinis*)	50X
	Fig. M5.	Bee-eater (*M.orientalis*)	50X
	Fig. M6.	Tailor Bird (*O.sutorius*)	50X
	Fig. M7.	Martin (*H.concolor*)	50X
	Fig. M8.	Drongo (*D.adsimilis*)	50X
GR. III.	Fig. M9.	Brahminy Myna (*S.pagodarum*)	50X
	Fig. M10.	Common Myna (*A.tristis*)	50X
	Fig. M11.	Jungle Babbler (*T.striatus*)	50X
	Fig. M12.	Indian Robin (*S.fulicata*)	50X
	Fig. M13.	Bulbul (*P.cafer*)	50X
	Fig. M14.	Koel (*E. scolopacea*)	50X
	Fig. M15.	House Crow (*C.splendens*)	50X
	Fig. M16.	House Sparrow (*P.domesticus*)	50X
	Fig. M17.	Barbet (*M. haemacephala*)	50X
	Fig. M18.	Fowl (*G.domesticus*)	50X
	Fig. M19.	Duck (*A.domesticus*)	50X
Fig. IV.	Fig. M20.	Parakeet (*P.krameri*)	50X
	Fig. M21.	Little Brown Dove (*S.senegalensis*)	50X
	Fig. M22.	Blue Rock Pigeon (*C.livia*)	50X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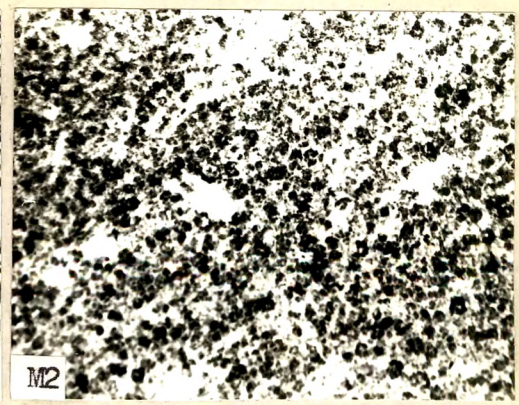
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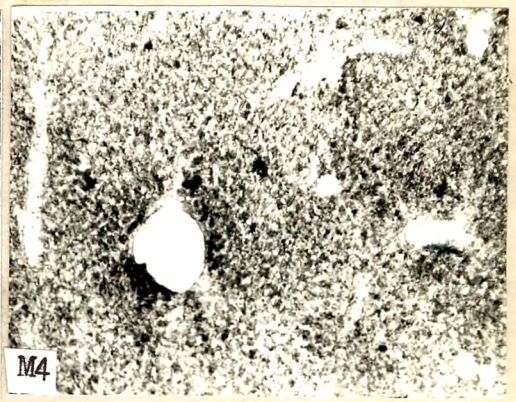
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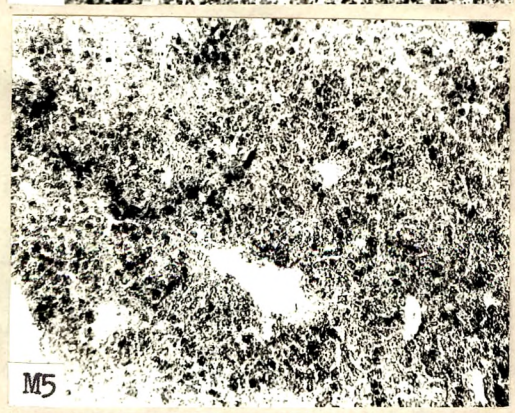
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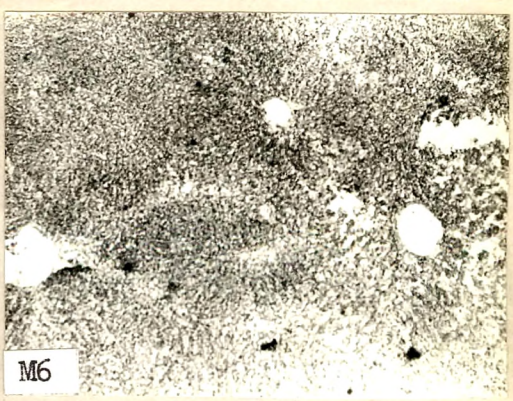
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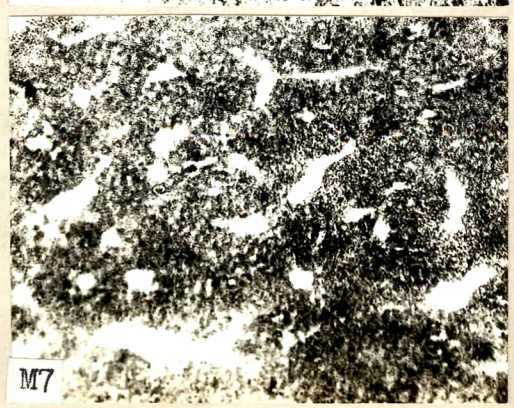
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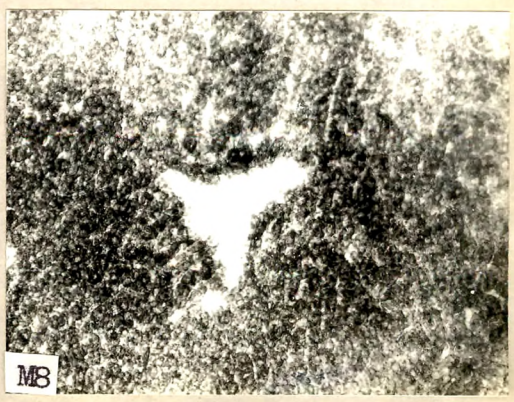
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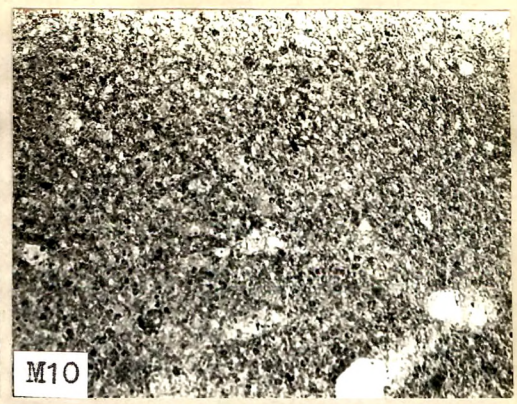
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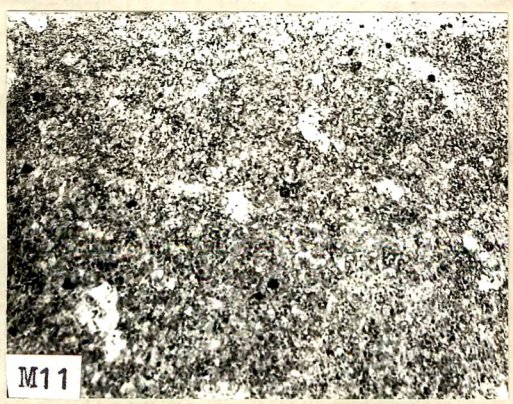
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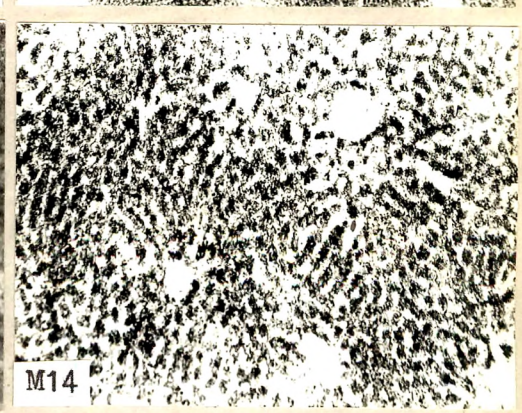
M12


M13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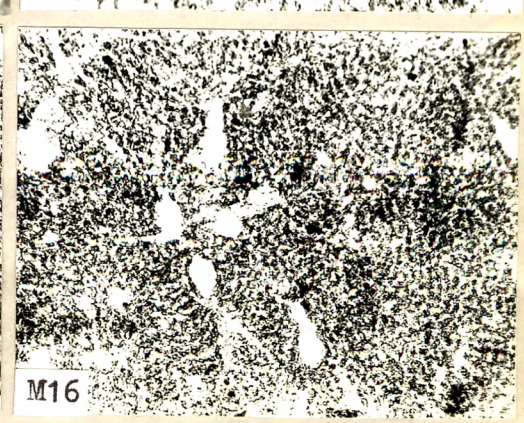
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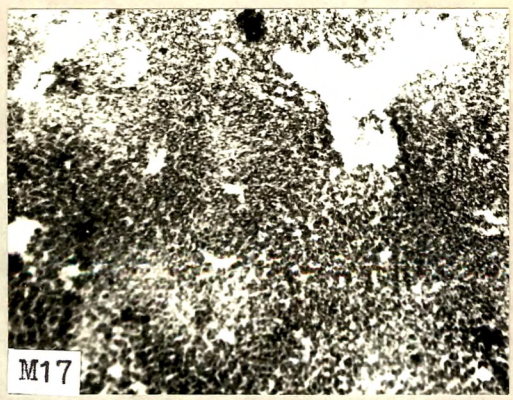
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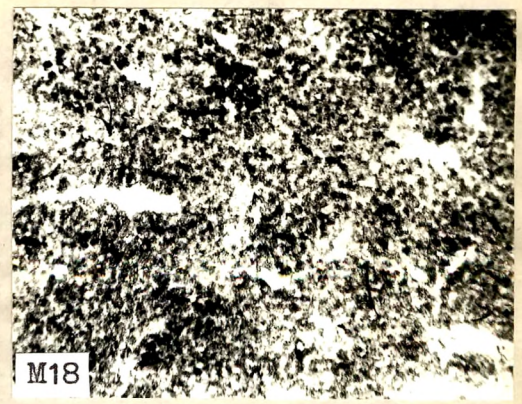
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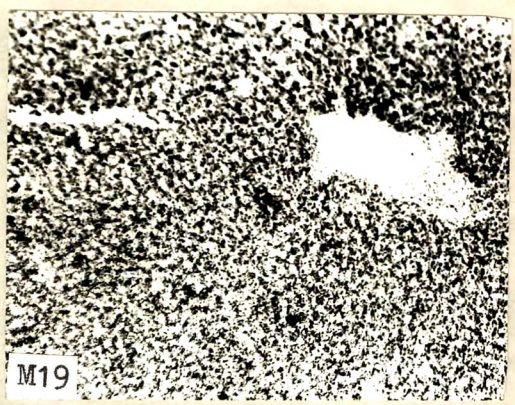
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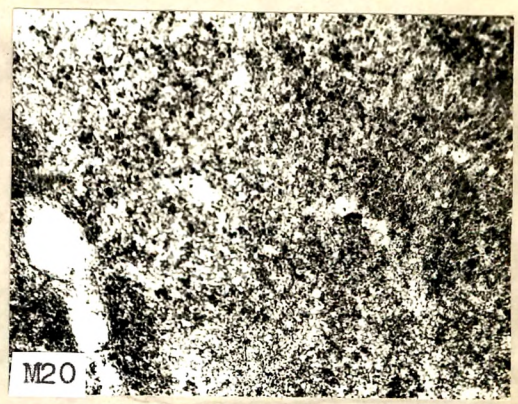
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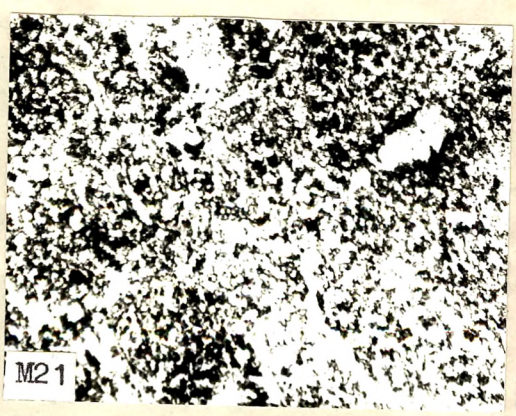
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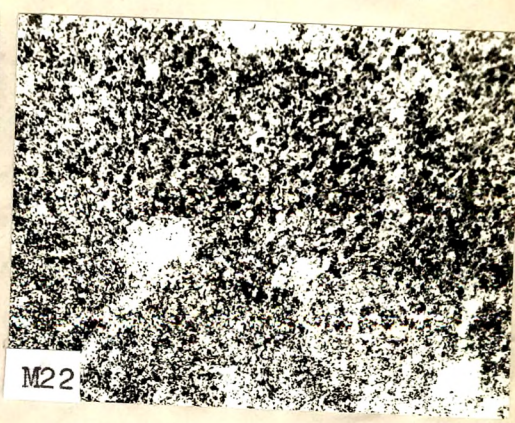
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