CHAPTER 5

RELATIONSHIP OF DIETARY PECULIARITIES WITH THE DISTRIBUTION PATTERN OF HISTOCHEMICALLY DEMONSTRABLE ALKALINE AND ACID PHOSPHATASES IN LIVERS OF CERTAIN REPRESENTATIVE BIRDS

Metabolic adaptations and the concentrations of enzymes in the liver are greatly influenced by the dietary preferences of the animals. Since avian members occupy a variety of niches, their feeding habits and diets vary considerably, and incidently some are exclusively carnivores, some are frugivores, some ~~others~~ graminivores, while quite a majority consume a mixed type of diet consisting of insects and grains. Such dietary variations lead to the intake of any one or two of the metabolites, viz., proteins, fat or carbohydrates, more than the others. Thus, the flesh or insect eating birds consume more proteins and fats than carbohydrates while the diet of graminivores contains more carbohydrates than protein and fat. Of all the metabolic enzymes in the liver, those concerning glycolytic, glycogenolytic and gluconeogenic reactions are likely to be influenced most by the variations in the type of food ingested by the birds, for the purpose of maintaining a steady level of glucose in the blood, irrespective of the type of metabolite available

through the food. In the liver, the enzymes responsible for phosphorylation and dephosphorylation of glucose and its intermediaries, especially the alkaline phosphatases of non-specific nature, could be expected to show adaptive changes in their pattern of distribution and/or concentration according to the high or low intake of carbohydrates due to diet variation. Such adaptive changes could then facilitate the uptake of glucose by hepatic cells, its ralease into the blood stream, its conversion into glycogen, synthesis of fat or protein from glucose or *vice versa* as the situation demands. The association of alkaline phosphatase with the transport of metabolites (Verzar and McDougall, 1936) and specifically with that of glucose (Anagnostopoulos and Matsudaira, 1958) has been quite well established.

Relatively little information is available regarding the histochemical patterns of distribution of enzymes in the liver of birds with reference to their food habits. The present study on the histochemical localization and distribution of non-specific phosphatases *viz.*, acid and alkaline phosphatases in the livers of representative birds having different dietary preferences was aimed at obtaining a basic information on the extent of the dietary influence on the pattern of distribution and localization of these two enzymes in the avian liver.

MATERIALS AND METHODS

According to diet preferences, the birds selected for the present study were classified into four groups viz., (I) Flesh eaters, (II) Insect eaters, (III) Omnivores, and (IV) Fruits and grain eaters. A few species of birds falling into each of these groups (Table I) were shot and collected during the morning hours from the university campus.

Immediately after shooting, the liver from each of the birds was excised and a piece from it was fixed on a cryostat microtome chuck for fresh frozen sections, while some pieces were weighed and digested in 30% KOH for the estimation of glycogen. For the histochemical localizations of alkaline and acid phosphatases, fresh frozen section of 12-18 μ thickness were cut and fixed in 10% cold neutral formalin for 15 to 20 minutes at 4°C. The sections, after fixation, were washed thoroughly with distilled water and kept in incubation medium prepared according to the azo dye coupling method of Burstone (1962) using Naphthol AS-MX phosphate and Naphthol AS-BI phosphate (Sigma Chemical Co., St.Louis, Mo., USA) as substrates for the alkaline and acid phosphatases respectively. Fast Blue B was used as the dye in both the cases. Suitable controls were employed for determining genuinity of the histochemical results. Glycogen was estimated from KOH digested material following the method of Seifter et al., (1950).

OBSERVATIONS

Alkaline Phosphatase

Group I (Carnivores) Figs. 1 & 2: The birds of this group represented by white backed Vulture and pariah kite (Table I, both belonging to Falconiformes), usually consume the flesh of animals. These carnivorous birds showed a moderate alkaline phosphatase activity in their livers and. The distribution pattern ~~of this enzyme~~ was ~~almost~~ quite similar in ~~livers of~~ both of the birds. The peribiliary (surrounding the bile canaliculi) areas showed a relatively high enzyme reactivity when compared to that in other areas of the liver lobules. The reactivity of the enzyme in fine granular form was also noticed in the portal areas of the liver lobules (peripotal distribution). The areas surrounding the central collecting veins (centrolobular region of the liver lobules) showed practically least enzyme reactivity. Of the two carnivorous birds studied, the Kite liver exhibited a comparatively high enzyme activity in the peribiliary region, however, in other parts the enzyme reactivity was similar in both of the birds.

Group II (Insectivores): Few birds which are insect eaters comprise this group. They are Cattle Egret (Ciconiformes), House Swift (Apodiformes), Tailor bird, Martin, Green Bee-eater and Drongo (Passeriformes).

Though all of them are obligatory insectivores, Cattle Egrets are also known to eat small lizards and frogs, besides insects and grubs. The intensity of alkaline phosphatase reactivity was found to be slightly ~~more~~ greater in the livers of these ~~insectivorous~~ birds when compared to that observed in the livers of flesh eating birds (Group I). Livers of all the birds of group II showed a peribiliary enzyme distribution with intensive reactivity (Figs. 3-8). In the liver of the Cattle Egret, the periportal areas were also found to have a higher enzyme activity.

Group III (Omnivores): Omnivores selected for the present study are represented by a number of Passeriformes (Myna, Babbler, Robin, Bulbul, Koel, Crow and Sparrow), one of Piciformes (Barbet), one of Galliformes (Fowl) and one representative of Anseriformes (domestic Duck). These birds with the exception of the duck are facultative insect eaters, though their gizzards are adapted for ~~treating~~ grains and seeds (Panicker, 1974). ~~The~~ Birds like the Crow and Fowl are also known to eat flesh and small animals like earthworms.

The livers of all these birds ~~recorded~~ revealed a histochemically demonstrable high activity of alkaline phosphatase(Table I). The enzyme was more or less restricted to the periportal

areas (Figs. 9-17). The peribiliary regions especially around the portal spaces also showed high enzyme reactivity (Figs. 9-17). The only exception, with regard to peribiliary distribution of the enzyme among the birds of this group was the Fowl and Duck, where the enzyme was seen distributed generally around the periportal areas (Figs. 18 & 19).

Group IV (Frugivores and Graminivores): All the listed birds in this group consume invariably carbohydrate rich food. The Parakeet (Psittaciformes) is an exclusively fruit eater, the Dove and Pigeon (Columbiformes) are obligatory graminivores. Another reason for including all these birds in this group is absence of gall bladder in all of them.

As far as the intensity of the reactivity of alkaline phosphatase is concerned, the livers of all the three birds showed poor response to histochemical reactions and a uniform parenchymal localization (Figs. 20-22).

Acid Phosphatase:

Group I (Carnivores): In both Vulture and Kite, the acid phosphatase was low ~~in its activity~~. The

enzyme was selectively found to be most active in Kupffer cells, while the parenchymal cells showed little or no reactivity (Figs. 1' & 2').

Group II (Insectivores): With the exception of the Bee-Eater all the birds that were studied showed acid phosphatase reactivity in the parenchymal cells of their livers (Figs. 3',4',6',7' & 8'). In the Bee-Eater the enzyme was active only in Kupffer cells (Fig. 5') as was the case with the Carnivores.

Group III (Omnivores): The livers of all the birds of this group showed a more or less uniform parenchymal localization of acid phosphatase (Figs. 9'-19'), similar to that observed in insectivores except the Bee-Eater. However, a slightly greater concentration of the enzyme was found in the cells which were around the portal areas (Figs. 9',10',11',12',13',15' and 19'). On an overall basis, the livers of omnivores were found to have more acid phosphatases reactivity than that observed in the carnivores and insectivores.

Group IV (Frugivores and Graminivores): As mentioned earlier the birds represented in this group belonged to three different orders and the diet also

is very different. Obviously, the distribution pattern of acid phosphatase in their livers also showed variation. Strong uniform parenchymal localization of acid phosphatase was evident in the livers of Dove and Pigeon (Figs.21' & 22'), while the Parakeet liver showed two specific regions of the enzyme localization, one in the Kupffer cells and the other around periportal areas (Fig. 20').

DISCUSSION

Phosphatases are a group of enzymes that act on a variety of phosphate esters (Roche, 1950). These phosphatases are classified into phosphomonoesterases, phosphodiesterases and pyrophosphatases. The most widely distributed phosphomonoesterases are alkaline and acid phosphatases, the former having a pH optima around 9 while the latter has a pH optima near 5. Since these phosphatases hydrolyze a variety of phosphate esters, they are termed as non-specific phosphatases.

Because of their non specificity, it is difficult to ascertain the specific role played by either alkaline or acid phosphatases present in a particular tissue or cell. Usually, in such cases activity of these enzymes is correlated with the specialized function of that tissue

or cell. For example, the alkaline phosphatase found in the bone is believed to participate in bone formation or calcium phosphate deposition (Moog,1946). Similarly in the liver, the roles played by both these phosphatases can only be judged from the site of their activity. Before attempting a general discussion on the pattern of distribution of both alkaline and acid phosphatases and their activity, it would be pertinent to state that the purpose of this investigation is to find whether there is any semblance of unity in distribution pattern of these two enzymes in the liver of birds with reference to their different dietary preferences, keeping in mind that in all probability food may not be the only criteria that influence the pattern of the distribution of enzymes in various microanatomical parts of the liver.

The hepatic alkaline phosphatase was found chiefly in the peribiliary region in most of the birds studied (Table III) except in the group IV birds. This observation is most interesting as the birds of group IV do not have gall bladder. These birds usually consume carbohydrate rich food with little or no fat and hence

there is no need for the bile to be produced in large quantity and stored for immediate need. Thus the absence of gall bladder could easily be correlated with the diet of such birds. In other birds, where the gall bladder exists, the machinery concerned with the synthesis and secretion of bile components has to be very active and storage of bile for immediate requirement of large amount of bile is necessary. The non-specific alkaline phosphatases are believed to play important role in the transport of bile components from liver cells into bile canaliculi (Essner et al., 1958). This explains the exceptional preponderance of alkaline phosphatase in the peribiliary zones in the livers of the birds belonging to Group I, II and III.

Apart from peribiliary localizations, the livers of carnivores, insectivores and omnivores also showed the histochemical reactivity of alkaline phosphatase in the form of fine granules in the cells in the periportal areas of hepatic lobules. The periportal areas of hepatic lobules receive the portalvenous blood loaded with nutrients (Rappaport, 1963). This condition establishes a metabolically active zone around the portal areas (see Wachstein, 1963). Consequently phosphatases, concerned

with the transport of metabolites across the cell membranes (such as Glucose-6-phosphatase) as well as non specific alkaline phosphatases are found to be concentrated in this area (Schumacher, 1957; Wachstein, 1959). Even glycogen deposition takes place in this zone (Rappaport, 1963). The metabolic activities in the periportal areas is expected to be much more higher in the birds consuming a mixed diet (Omnivores, Group III) as the diet contains a large quantity of nitrogenous compounds, lipids and carbohydrates. The higher concentrations of alkaline phosphatase found in the periportal areas in the livers of birds of groups I, II and III, denotes, that the alkaline phosphatase in this area of their liver lobules, is involved in gluconeogenesis as well as the release of glucose into the blood stream. This contention derives support from the data of the glycogen content in the livers of birds (Table II). The graminivores have the highest glycogen content in their liver followed by omnivores, while carnivores and insectivores have the least. Moreover, Cardell et al., (1973) in their electron microscopic studies of rat liver observed significant quantities of glycogen in hepatocytes located around the portal tracts than in cells near central veins. The higher concentration when compared with?

of alkaline phosphatase in the hepatic cells situated near the portal areas then corresponded with the areas of glycogen deposition. The maintenance of blood glucose level, being the major activity of the liver, Glucogenic metabolites have to be converted into glucose in carnivores, and insectivores as well as in omnivores when the food is mainly consisting of insects. Hence, the alkaline phosphatase activity is kept high in the livers of these birds and that too in specific areas of the liver lobule (periportal areas).

Acid phosphatase is considered to be a lysosomal enzyme and hydrolytic in nature. Therefore, the presence of this enzyme in the Kupffer cells is understandable. Ratzlaff and Tyler (1973) also found acid phosphatase in the Kupffer cells of avian liver. Both Vulture and Kite livers showed high acid phosphatase response in the Kupffer cells. As Wachstein (1963) opined, the degree of acid phosphatase in Kupffer cells reflected the functional state of reticuloendothelial cells in the liver. The activity of acid phosphatase in Kupffer cells increased in infections of following administration of bacterial toxins or hepatotoxins (Barka et al., 1961; Howard, 1959; Thorbecke et al., 1961; Novikoff, 1960; Wachstein and

and Meisel, 1959). Perhaps the carrion feeders like the Vulture and Kite might be ingesting bacteria or other germs through food or even toxins from decaying flesh and hence the Kupffer cells must be active to eliminate them.

In the livers of other birds (insectivores, omnivores, graminivores and frugivores) the acid phosphatase was found localized in parenchymal cells. The livers of the birds of group II and III have shown a periportal distribution, whereas those ~~but that~~ of the Group IV have shown a uniform distribution of the enzyme. The maximum activity of the enzyme was recorded in graminivores like Dove and Pigeon. Acid phosphatase is known to hydrolyze phosphorylcholine (for the formation of phospholipids) and phosphoproteins. One type of acid phosphatase splits the phosphatidic acid into diglycerides and phosphoric acid, the diglycerides are then combined with acyl CoA to form triglyceride, ~~and~~ another one is known to catalyze the reaction between glycerol and inorganic phosphates (Fruton and Simmonds, 1961; White et al., 1959). From the study of the distribution pattern of lipids in the livers of various birds (Chapter 2) it is realized that the livers of graminivores synthesize and

deposit more neutral fat (Triglycerides) than do those of birds of other groups. The presence of a high concentration of acid phosphatase in the liver of graminivores (Dove and Pigeon) then might be well correlated with triglyceride and phospholipid metabolism. In fact, Acid Pase is found to be associated with cells and tissues having abundant phospholipids (Hashimoto and Ogawa, 1963) as well as in phospholipid rich lysosomes (Ogawa et al., 1960; 1961).

In conclusion it could be stated that the distribution and concentration of alkaline and acid phosphatases in the livers of birds have some relationship with the type of food they ingest. If the food consists of flesh and insects, the alkaline phosphatase activity is found to be high in the liver and if the fruits and grains constitute the food, then the activity of acid phosphatase is high in the hepatic cells. Since omnivores consume both insects and grains, the liver of these birds contain active alkaline and acid phosphatases, exhibiting metabolic adaptability of their liver.

TABLE I

The type of diet taken by various birds and the organic constituents of the diet.

GROUP	BIRDS	DIET	MAJOR ORGANIC CONSTITUENTS OF THE DIET
GROUP I (CARNIVORES)			
1.	Vulture (G. bengalensis)	Flesh	Protein &
2.	Kite (M. migrans)		fat
GROUP II (INSECTIVORES)			
3.	Cattle Egret (B. ibis)		
4.	House Swift (A. affinis)		
5.	Bee-eater (M. orientalis)	Insects	Protein &
6.	Tailor Bird (O. sutorius)		fat
7.	Martin (H. concolor)		
8.	Drongo (D. adsimilis)		
GROUP III (OMNIVORES)			
9.	Brahminy Myna (S. pagodarum)		
10.	Common Myna (A. tristis)		
11.	Babbler (T. striatus)		
12.	Indian Robin (S. fulicata)	Insects,	Protein,
13.	Bulbul (P. cafer)	fruits,	fat, &
14.	Koel (E. scolopacea)	grains,	carbohydrates
15.	House Crow (C. splendens)	&	
16.	House Sparrow (P. domesticus)	flesh	
17.	Barbet (M. haemacephala)		
18.	Fowl (G. domesticus)		
19.	Duck (A. domesticus)		
GROUP IV (FRUGIVORES AND GRAMINIVORES)			
20.	Parakeet (P. krameri)	Fruits &	
21.	Dove (S. senegalensis)	grains	Carbohydrates
22.	Pigeon (C. livia)		

TABLE II

The intensity of alkaline and acid phosphatases, glycogen content and the presence or absence of gall bladder in the livers of birds with different dietary preferences

Sr. No.	Group	Birds	Alk. Pase	Acid Pase	Glycogen %*	Gall Bladder
1.	I	Vulture	++	+	0.07	P
2.		Kite	++	+	0.06	P
3.	II	Cattle Egret	+++	++	0.05	P
4.		Swift	+++	++	0.05	P
5.		Bee-Eater	+++	++	0.15	P
6.		Tailor Bird	+++	++	0.21	P
7.		Martin	+++	++	0.39	P
8.		Drongo	+++	++	0.21	P
9.	III	Brahminy Myna	++++	+++	2.03	P
10.		Common Myna	++++	+++	2.25	P
11.		Babbler	++++	+++	1.76	P
12.		Indian Robin	++++	+++	4.03	P
13.		Bulbul	++++	+++	1.10	P
14.		Koel	++++	+++	2.03	P
15.		Crow	++++	+++	1.39	P
16.		Sparrow	++++	+++	0.06	P
17.		Barbet	++++	+++	2.44	P
18.		Fowl	++++	+++	0.07	P
19.		Duck	+++	+++	2.08	P
20.	IV	Parakeet	+	+++	3.48	A
21.		Dove	+	++++	3.58	A
22.		Pigeon	+	++++	4.00	A

P - present; A - absent

*Average of values from five birds

++++ High; +++ Moderate; ++ Low; + Poor; reactivity of the enzyme.

TABLE III

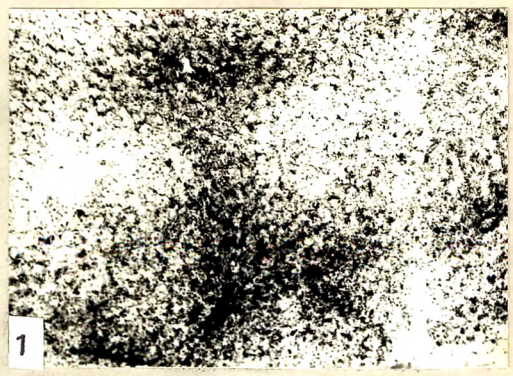
The distribution pattern of histochemically demonstrable alkaline and acid phosphatases in the liver of majority of birds in each dietary group

GROUPS	ALKALINE PHOSPHATASE	ACID PHOSPHATASE
GROUP I (CARNIVORES)	Peribiliary Periportal	Histiocytic (in (Kupffer cells)
GROUP II (INSECTIVORES)	Strongly Peribiliary Periportal	Histiocytic and parenchymal Periportal
GROUP III (OMNIVORES)	Strongly Peribiliary Periportal	Parenchymal Periportal
GROUP IV (FRUGIVORES & GRAMINIVORES)	Parenchymal Uniform	Parenchymal Uniform

EXPLANATION TO FIGURES (CHAPTER 5)

Figs. 1 to 22. Photomicrograph of liver of birds showing ALKALINE PHOSPHATASE activity. All photographs are of 50X magnification.

GROUP I.	Fig. 1.	Vulture (*G. bengalensis*)
	Fig. 2.	Kite (*M. migrans*)
GROUP II.	Fig. 3.	Cattle Egret (*B. ibis*)
	Fig. 4.	House Swift (*A. affinis*)
	Fig. 5.	Bee-eater (*M. orientalis*)
	Fig. 6.	Tailor Bird (*O. sutorius*)
	Fig. 7.	Martin (*H. concolor*)
	Fig. 8.	Drongo (*D. adsimilis*)
GROUP III.	Fig. 9.	Brahminy Myna (*S. pagodarum*)
	Fig.10.	Common Myna (*A. tristis*)
	Fig.11.	Jungle Babbler (*T. striatus*)
	Fig.12.	Indian Robin (*S. fulicata*)
	Fig.13.	Bulbul (*P. cafer*)
	Fig.14.	Koel (*E. scolopacea*)
	Fig.15.	House Crow (*C. splendens*)
	Fig.16.	House Sparrow (*P. domesticus*)
	Fig.17.	Barbet (*M. haemacephala*)
	Fig.18.	Fowl (*G. domesticus*)
	Fig.19.	Duck (*A. domesticus*)
GROUP IV.	Fig.20.	Parakeet (*P. krameri*)
	Fig.21.	Little Brown Dove (*S.senegalensis*)
	Fig.22.	Blue Rock Pigeon (*C. livia*)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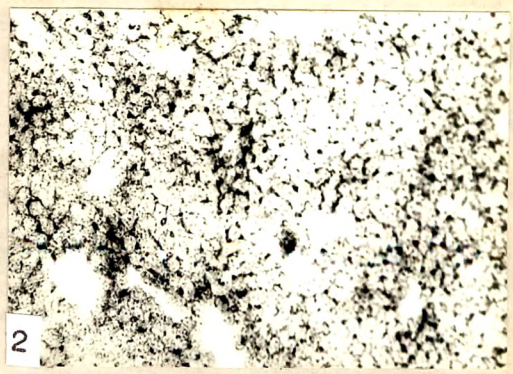
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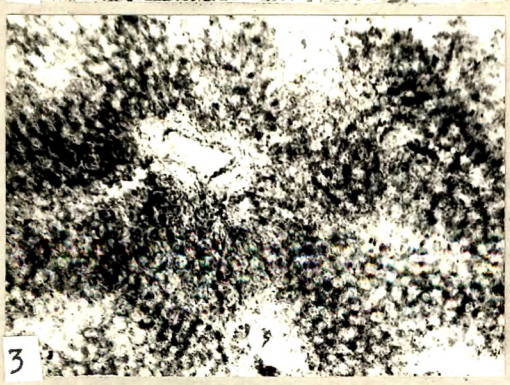
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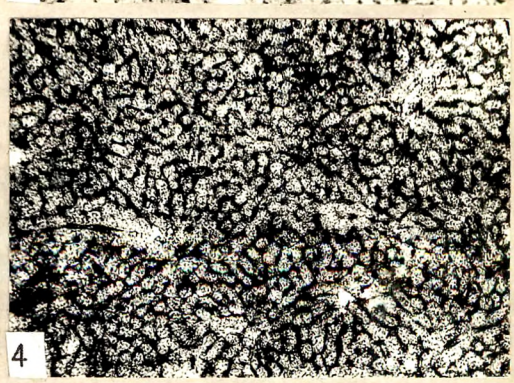
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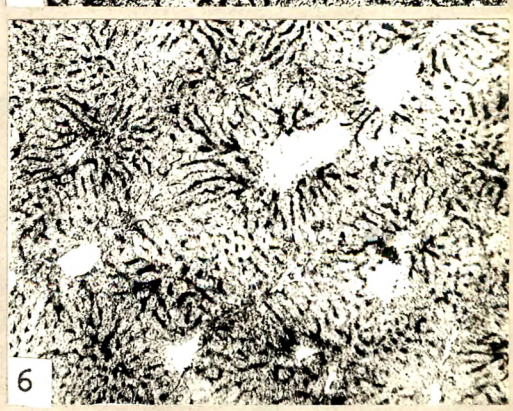
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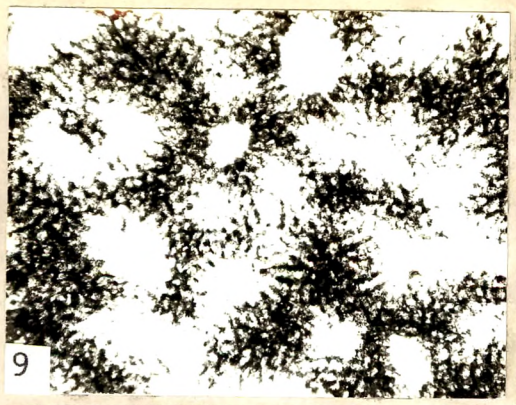
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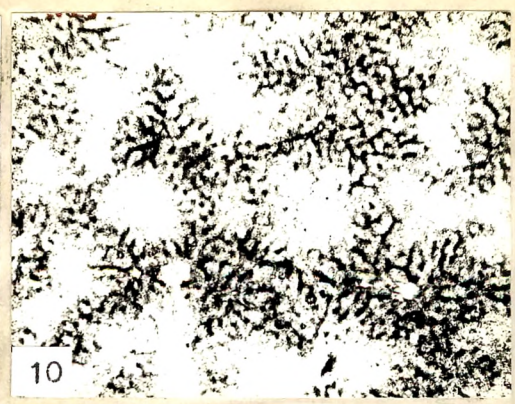
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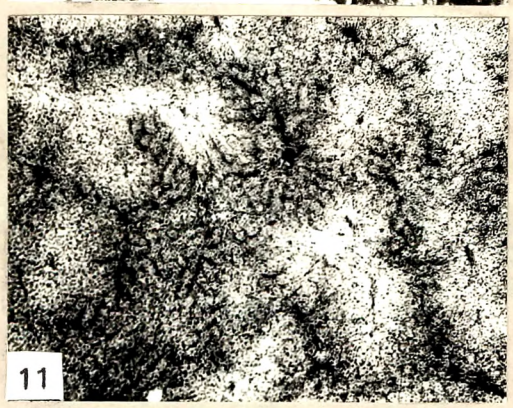
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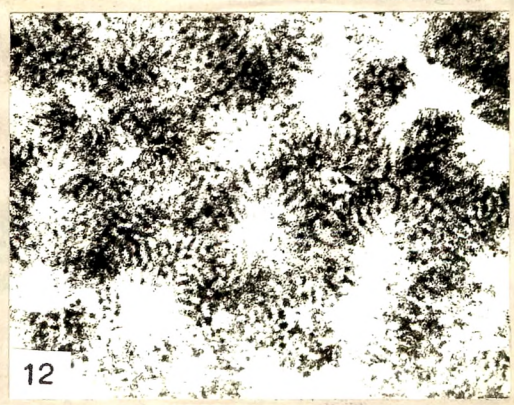
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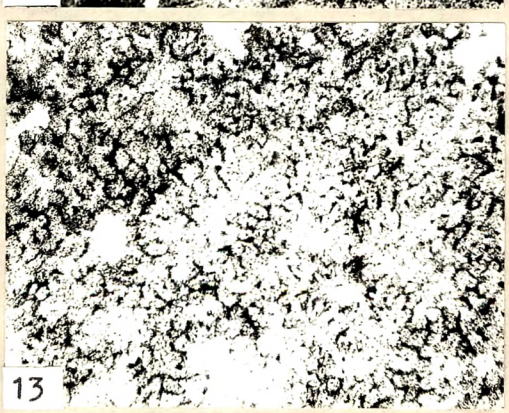
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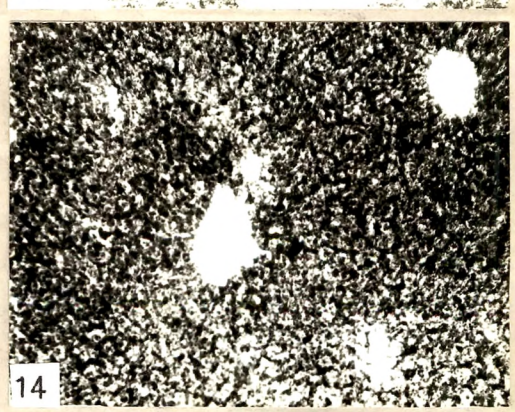
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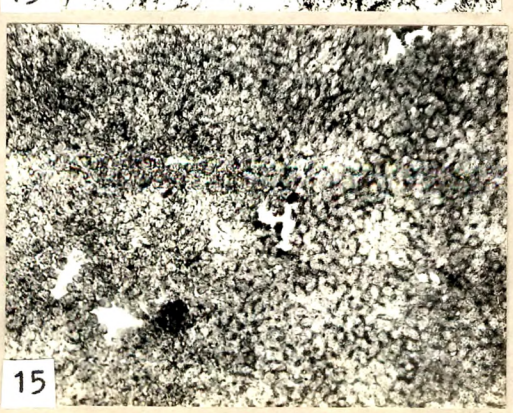
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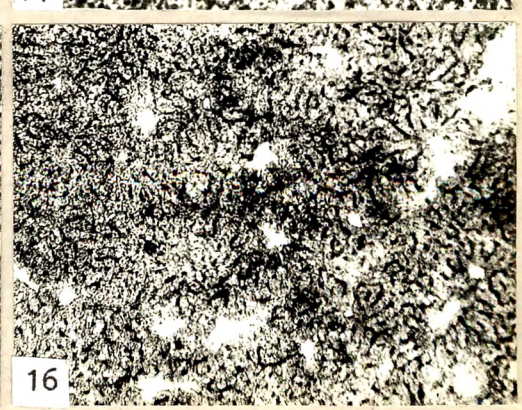
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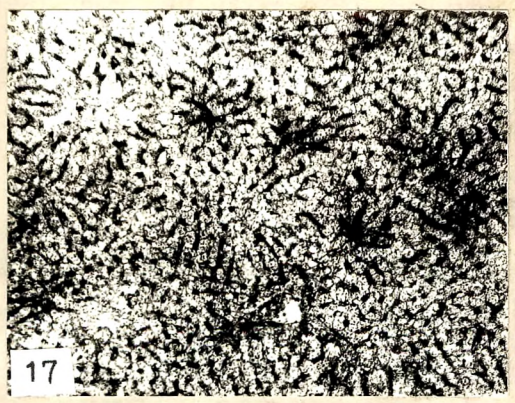
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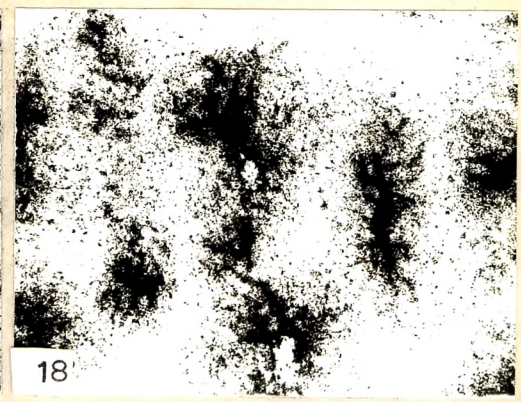
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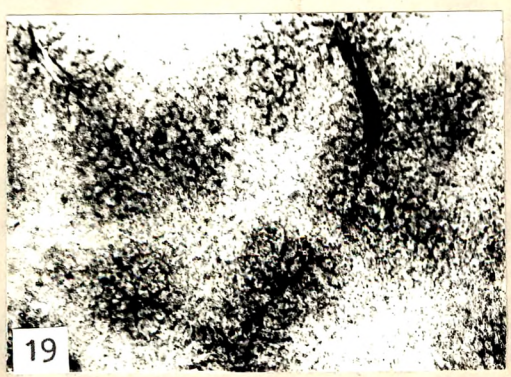
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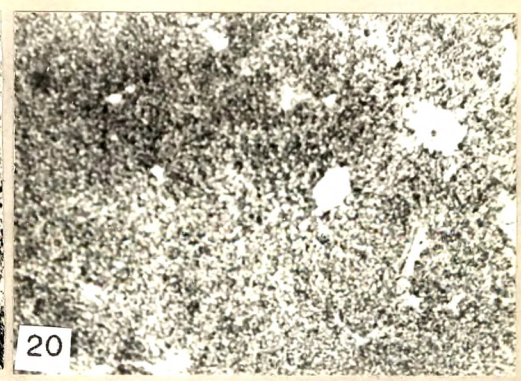
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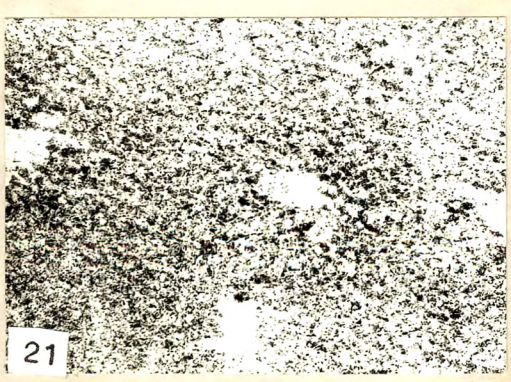
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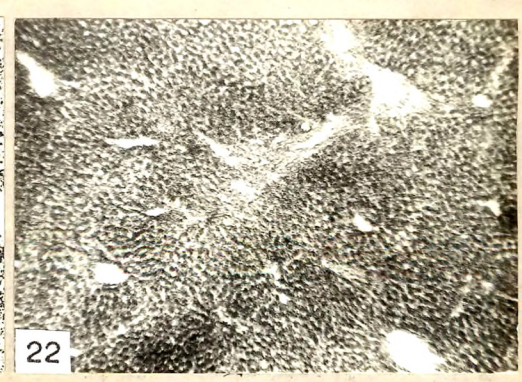
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EXPLANATIONS TO FIGURES (CHAPTER 5)

Figs. 1 to 22. Photomicrographs of liver of birds showing ACID PHOSPHATASE activity. All photomicrographs are of 50X magnification.

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	Fig. 2.	Kite (*M. migrans*)
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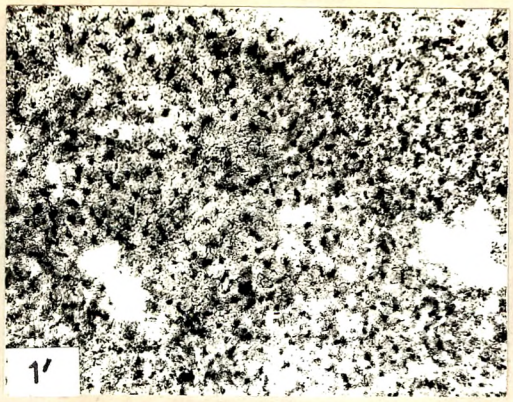
	Fig.10.	Common Myna (*A. tristis*)
	Fig.11.	Jungle Babbler (*T. striatus*)
	Fig.12.	Indian Robin (*S. fulicata*)
	Fig.13.	Bulbul (*P.cafer*)
	Fig.14.	Koel (*E. scolopacea*)
	Fig.15.	House Crow (*C. splendens*)
	Fig.16.	House Sparrow (*P. domesticus*)
	Fig.17.	Barbet (*M. haemacephala*)
	Fig.18.	Fowl (*G. domesticus*)
	Fig.19.	Duck (*A. domesticus*)
GROUP IV.	Fig.20.	Parakeet (*P. krameri*)
	Fig.21.	Little Brown Dove (*S.senegalensis*)
	Fig. .22.	Blue Rock Pigeon (*C. livia*)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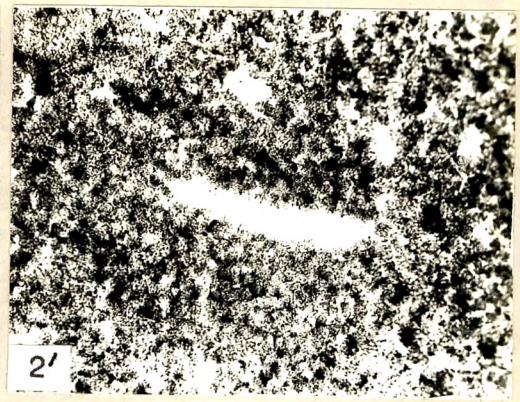
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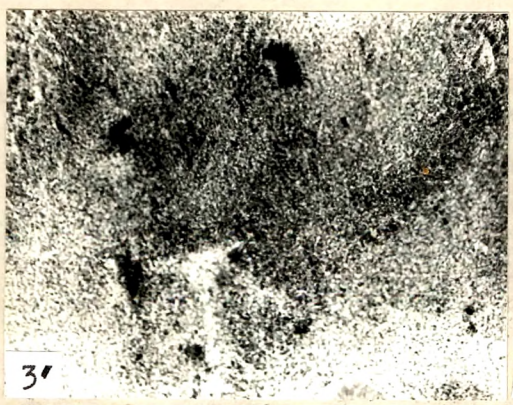
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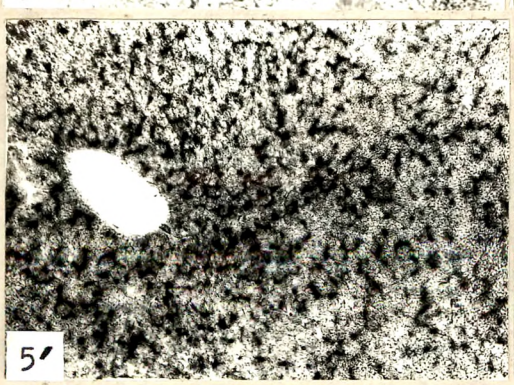
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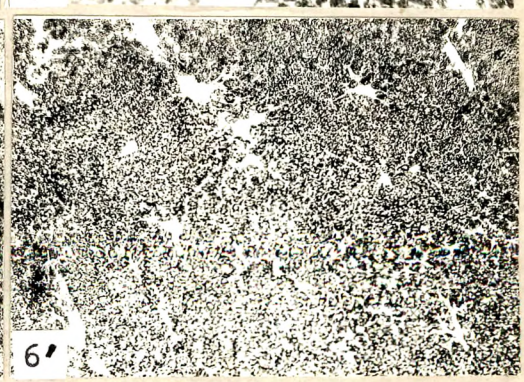
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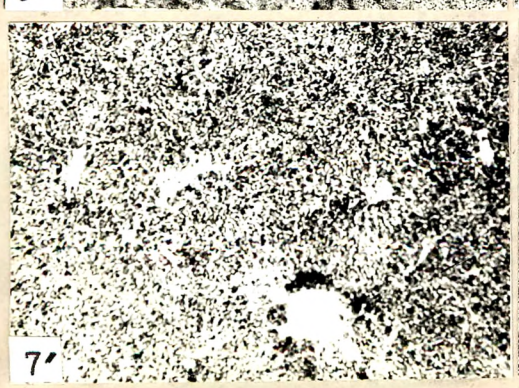
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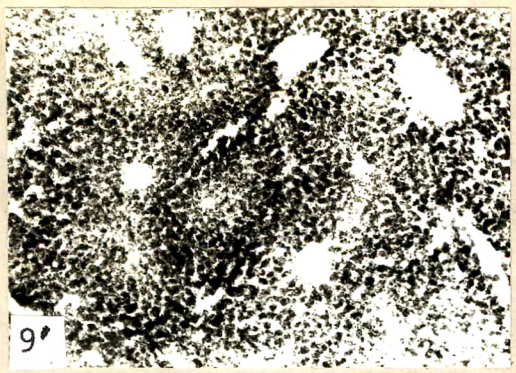
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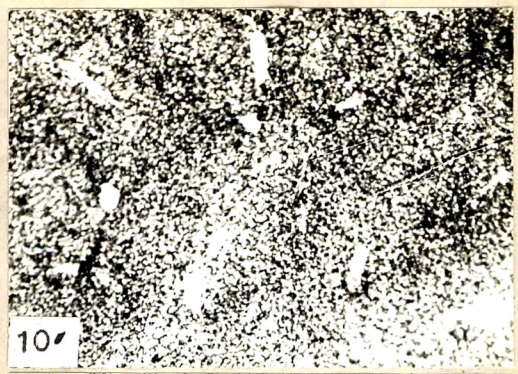
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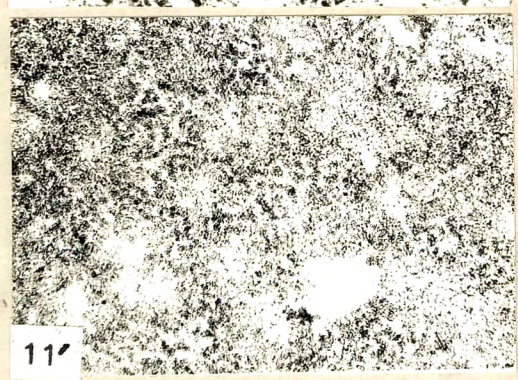
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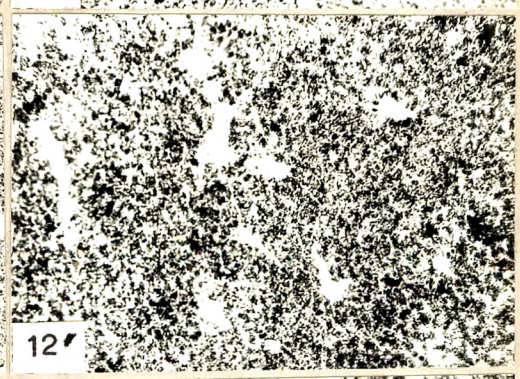
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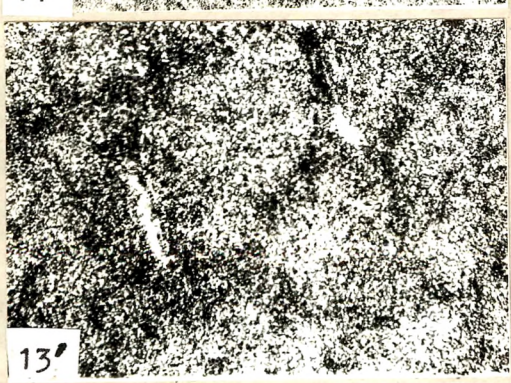
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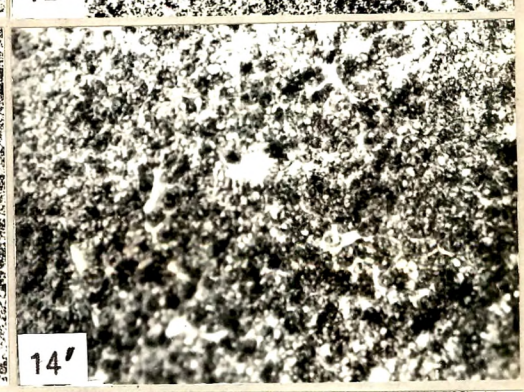
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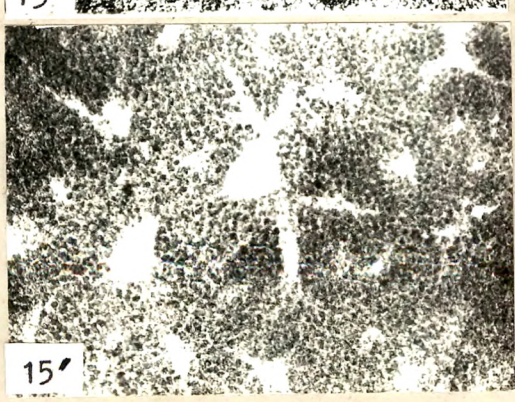
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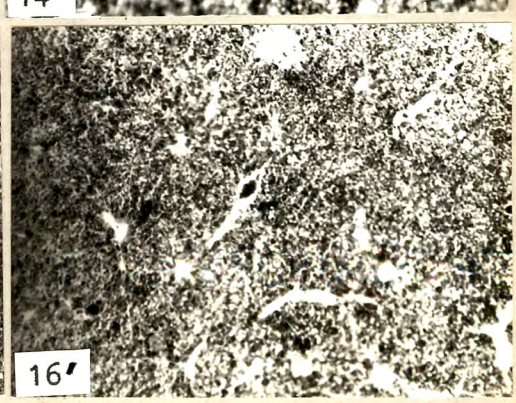
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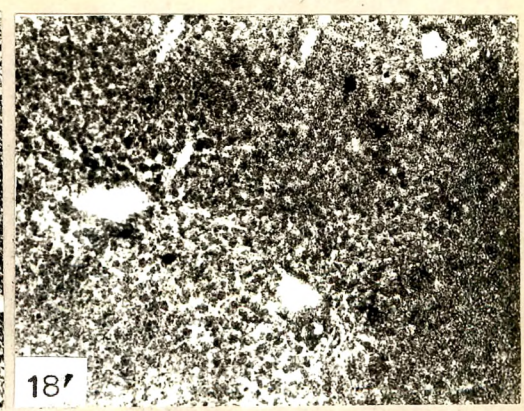
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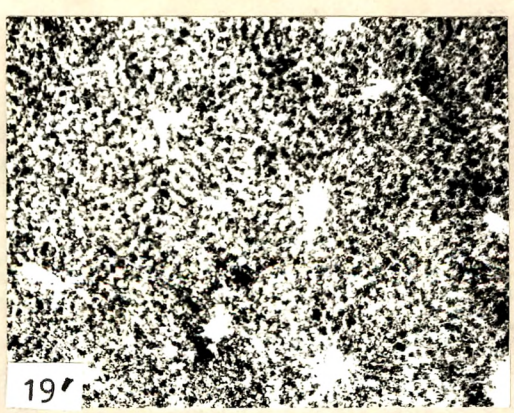
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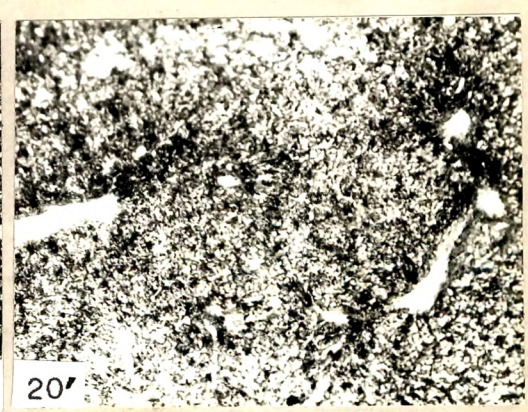
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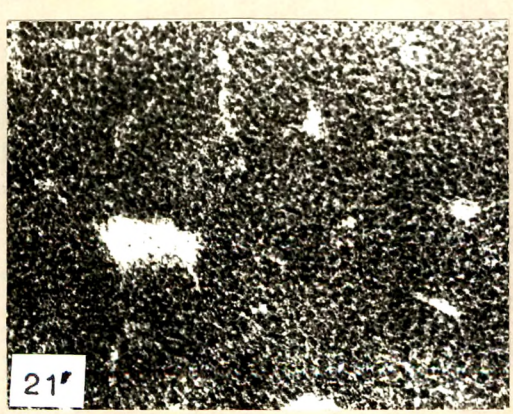
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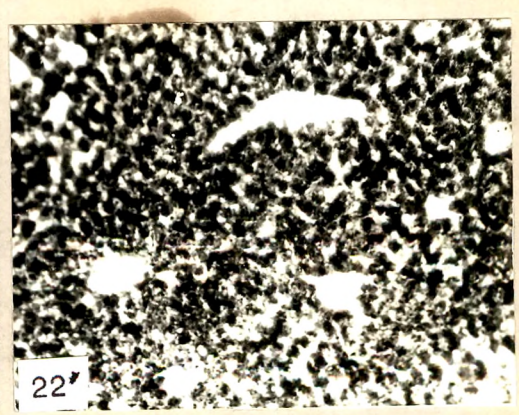
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