CHAPTER 6

PATTERN OF DISTRIBUTION OF HEPATIC CHOLINESTERASE IN THE BIRDS WITH VARIOUS DIETARY PREFERENCES

Both specific and nonspecific cholinesterases have been demonstrated in the livers of various mammals, such as guinea pigs, rats, cats and rabbits (Sawyer, 1945; Blaschko et al., 1947; Goutier-Pirotte and Goutier, 1956; Gerebtzoff, 1959; Bertrand, 1954; Koelle, 1951; Gerebtzoff, 1954). Sutherland (1964) used the histochemical technique for cholinesterases, to demonstrate nerve plexus in the livers of monkey, guinea pigs and rats. The functional significance of these enzymes is not yet clear. It is envisaged that plasma cholinesterase (ChE) is released from the hepatic cells (Augustinsson, 1948, 1950; Gajdos, 1950). Some metabolic functions are ascribed to cholinesterases in the liver (Gerebtzoff, 1959; Szendzikowski et al., 1961/62). The presence of cholinergic nerve plexus in the mammalian liver (Youssef and Saleh, 1961; Sutherland, 1964) and the fact that denervation causes a decrease in ChE activity in the rat liver (Shastin, 1962), suggests explains that the function of hepatic cholinesterase is to breakdown the acetylcholine secreted at the nerve endings.

Though a large number of studies on the activity of ChE have been carried out by different workers, all of them are on mammalian liver, only a few have been conducted on the avian liver. Recently Pilo (1969) using histochemical technique studied cholinesterases in the livers of a few birds, and Shah et al. (1974) explained the participation of nerves in the wound healing and repair in the pigeon liver.

While studying the histochemistry of livers of some birds with various dietary preferences, it has been found that most of the hepatic enzymes showed variations in their pattern of distribution as well as in their intensity. Gerebtzoff (1959) indicated a relationship of diet with the concentration and distribution pattern of cholinesterase in the rat liver. With the fact in view a comparative histochemical study on the avian livers was deemed worthwhile to gather information regarding possible relationship, if any, of cholinesterase activity with different dietary preferences of birds.

MATERIALS AND METHODS

Several birds representing carnivores (Group I), insectivores (Group II), omnivores (Group III) and frugivores and graminivores (Group IV) were shot and

collected from their natural environments, in and around the University Campus (list of birds selected for the present study with their dietary preference is given in Chapter I, Table I).

The livers from the birds were fixed in cold formol saline or 10% formaline ~~with~~ at pH 5-6 ~~on the acidic range (5-6 pH)~~, washed repeatedly with distilled water and sectioned on a cryostat microtome. The enzyme activity was demonstrated by employing the method of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957). Butyrylthiocholine iodide (Sigma Chemical Co., U.S.A.) was employed to demonstrate non-specific cholinesterase, (the enzyme is preferably termed butyrylthiocholinesterase-BuChE) and acetylthiocholine iodide (Sigma Chemical Co., U.S.A.) was used for the demonstration of specific cholinesterase (acetylcholin-esterase-AChE).

Control sections were treated with various concentrations of eserine sulphate ($3x10^{-5}$ was most effective) and incubated at 38°C as was done with sample sections.

OBSERVATIONS

Group I Carnivores ~~(carrion feeders)~~: (Figs. 1 & 2)

Both specific and nonspecific cholinesterases (AChE and BuChE) were found to be localized in the sinusoidal lining, particularly in the periportal regions (Table I). Compared to acetylcholinesterase (AChE) the butyrylcholinesterase (BuChE) reactivity, was relatively more intense in the livers of both the Vulture and Kite. The walls of major blood vessels were also enzyme positive (Figs. 1b, 2a & b).

Group II Insectivores:

The localization of the two enzymes ~~reactivity~~ was more or less with in the sinusoidal linings (Table I). Both AChE and BuChE in general showed a uniform distribution in all the parts of the lobules of the livers of insectivores. Tailor Bird was an exception to this general pattern, Its liver showed perilobular localization of both the cholinesterases, while centrolobular areas were devoid of the enzymes (Figs. 6A & B). Besides ? In addition ? the walls of portal blood vessels showed high enzyme reactivity.

Group III Omnivores:

Most of the birds of this group exhibited a periportal concentrations of both AChE and BuChE

reactivity in their livers (Figs. 9-18). To ~~this,~~ the Sparrow (Fig.16), Barbet (Fig. 17), Koel (Fig. 14) and Duck (Fig. 19) were the exceptions to this since the enzymes in these birds were more or less distributed evenly throughout all over the hepatic lobules. In these birds the walls of blood vessels (Fig. 16) and the walls of the central collecting veins (Fig. 14) also gave positive reactions for both ~~the~~ enzymes. As in the livers of Carnivores and insectivores the sinusoidal lining, by and large, was enzyme positive in most of the omnivorous birds (Table I).

Group IV Graminivores and Frugivores:

In all ~~the~~ three birds, viz., Parakeet (Frugivore), Dove and Pigeon (Graminivores) localization of both the cholinesterases was in the sinusoidal linings, blood vessel walls and linings of collecting veins (Table I). However, the Parakeet liver (Fig. 20) showed an almost uniform AChE and BuChE distributions within the hepatic lobules, while in the livers of both the graminivores (Dove and Pigeon, Figs. 21 & 22) a higher concentration of both AChE and BuChE in the periportal regions was discernible.

DISCUSSION

Normally acetylcholinesterase which hydrolyzes acetylcholine is found in the nervous tissue (Augustinsson, 1950). Acetylcholinesterase can also hydrolyze propionylcholine but has little action on substrates containing larger fatty acyl groups or alcohols other than choline. Beside nervous tissues, cholinesterases are found in the erythrocytes, serum, liver and pancreas. These enzymes in the non nervous tissues hydrolyze esters of choline more rapidly than esters of other alcohols. And their hydrolytic activity increases as the fatty acid chain is lengthened (Fruton and Simmonds 1961).

The liver, being innervated by cholinergic nerves (Sutherland, 1964), would contain both specific (AChE) and nonspecific cholinesterases. Since, the localizations of both cholinesterases are similar, especially in the sinusoidal linings, one is tempted to state that there may be only one type of cholinesterase and that too a nonspecific type in the liver, the intensity of activity of which may vary when the substrates are different. As mentioned earlier, the liver cholinesterase hydrolyzes much more readily the esters of choline with fatty acids

of greater chain length (e.g. butyryl instead of acetyl). Accordingly, in the present study, the histochemical responses of the liver were found to vary when two substrates, namely Acetylthiocholine iodide and Butyryl-thiocholine iodide, were used. Since denervation of the liver resulted in a decreased ChE activity (Shastin, 1962) it is to be expected that the nonspecific cholinesterase also hydrolyzes acetylcholine secreted at the nerve endings. Sutherland (1964) is of the opinion that with the existing histochemical techniques it is not possible to distinguish between AChE and BuChE activities. In nervous tissue this fact poses no problem as the nerves contain both the enzymes, however, it is difficult to distinguish between the activities of both the types of cholinesterases in non nervous tissues like liver, and then to judge which of the enzymes is present in the organ. Perhaps, the liver in some species, may have only one type of cholinesterase, while that of some others may have both with more of nonspecific type.

on what basis is this statement made.

The presence of cholinesterases on the sinusoidal lining of almost all birds studied deserves some consideration. The parasympathetic nerve plexus of the liver is found to be located along the outer margin of

the liver cords (Sutherland, 1964). Since the nerve-net is ~~of~~ cholinergic in nature, acetylcholine could be expected to be secreted along the nerve net which in turn can explain the presence of acetylcholinesterase in these regions. If only the hydrolysis of acetylcholine is required at the lining of the sinusoids then only acetylcholinesterase need be present. Since a predominant reactivity of the nonspecific cholinesterase was also found in the avian liver, which could hydrolyze large number of choline esters; some additional function must be vested with ChE, other than hydrolysing acetylcholine. One reason, for the localization of ChE on the linings of sinusoids and terminal hepatic venules (central collecting veins), given was that they are in the process of being passed into the blood stream as the plasma ChE is believed to have an hepatic origin (Augustinsson,1950; and Gajdos, 1950).

From the distribution of cholinesterases in the livers of various groups of birds with different dietary habits, it is realized that all birds have highly active cholinesterases which are localized on the sinusoidal linings and walls of blood vessels. Intense reactivity

was found to be in the periportal regions of hepatic lobules. The vascular nerve plexus (nerve net work) that is present along the blood vessels in the liver, finally contributes to parenchymal nerve plexus along the sinusoids (Sutherland, 1964). This explains why cholinesterases are found along the blood vessels as well as on the linings of the sinusoids. The maximum periportal activity of the enzymes was found in graminivores followed by omnivores. Since only these birds consume large quantity of carbohydrates diet, some correlation between carbohydrate diet and cholinesterase activity could be derived. Bertrand (1954) observed cyclic variations in the intralobular localization of hepatic cholinesterase during feeding and fasting. He suggested that acetylcholine-cholinesterase system might be involved in the regulation of assimilation process. Acetylcholine usually influences the permeability of cell membrane and hence there is reason to believe that the uptake of metabolites by hepatocytes is facilitated through changes in permeability which may be under neural control. Mondon and Burton (1971) clearly showed that administration of acetylcholine or choline in the presence of insulin markedly enhanced the uptake of glucose by the liver from the perfusing medium and deposition of glycogen

in the liver. Cholinergic stimulation in the presence of insulin also resulted in the increased glucose uptake by the liver (Mondon and Burton, 1971). The effect of insulin with acetylcholine is on the glycogen synthesis in the liver by increasing the activity of glycogen synthetase rather than glucokinase. The stimulation of the vagus nerve increased the glycogen synthetase activity in the liver (Shimazu, 1967). Shimazu and Amakawa (1968a and 1968b) reported that the enzymes responsible for hepatic glycogen metabolism are under the influence of the autonomic nervous system. According to these workers sympathetic nerve stimulation increased the glycogenolytic enzyme like G-6-Pase and phosphorylase.

Since a fairly high activity of cholinesterase was also found in the livers of carnivores and insectivores, it could be reasoned that the same acetylcholine-cholinesterase system might also be involved in the assimilation of amino acids and lipids. By the virtue of their presence in wall of the blood vessels, the enzyme can influence a 'lipid clearing' effect in the portal areas and thereby could participate in the transport of lipids to and from the liver cells. In this regard the report that cholinesterase make lipid molecules ready

to be taken up by the cells by removing some moieties (Ballantyne and Burwell, 1965) is noteworthy. According to Szendzikowski et al. (1961/62) ~~that~~ about 50% of the lipolytic activity of rat aorta is due to nonspecific cholinesterase. Part of dietary fat is usually brought to the liver as phospholipid complex. Uptake of phospholipids is partly direct and partly after hydrolysis. The rat liver is found to have an enzyme that could split glycerophosphorylcholine named as "glycerylphosphoryl-choline diesterase" (Dawson, 1956). ~~enabling~~ the tissue to form glycerophosphate and choline from phospholipids like lecithins and cephalins (Lecithin and cephalin consist of glycerol to which are esterified two fatty acids and phosphoric acid, which in turn is attached to the organic base, choline-trimethylethanolamine). Phospholipids of plasma are derived from two sources: (i) ~~from~~ diet and (ii) ~~from~~ the liver itself. The plasma phospholipids are degraded and utilized exclusively by liver. The nonspecific cholinesterases, thus, may be assisting the uptake or release of lipids by the liver.

TABLE I

Histochemical localization and distribution pattern of acetylcholinesterase (AChE) and Butyrylcholinesterase (BuChE) in the livers of various birds belonging to different dietary groups

GROUP		AChE	BuChE
GROUP I (CARNIVORES)	L.	Sinusoidal lining	Sinusoidal lining.
	D.	Periportal	Periportal
GROUP II (INSECTIVORES)	L.	Sinusoidal lining	Sinusoidal lining
	D.	Uniform (except in Tailor Bird where it is periportal)	Uniform (except in Tailor Bird where it is periportal)
GROUP III (OMNIVORES)	L.	Sinusoidal lining, Blood vessels, & lining of collecting vein	Sinusoidal lining, Blood vessels, & lining of collecting vein
	D.	Periportal (except in Sparrow, Barbet, Koel & Duck where it is uniform)	Periportal (except in Sparrow, Barbet, Koel & Duck where it is uniform)
GROUP IV (FRUGIVORES & GRAMI~~CARN~~NIVORES)	L.	Sinusoidal lining, blood vessels, & lining of collecting vein	Sinusoidal lining, blood vessels, & lining of collecting vein
	D.	Periportal	Periportal

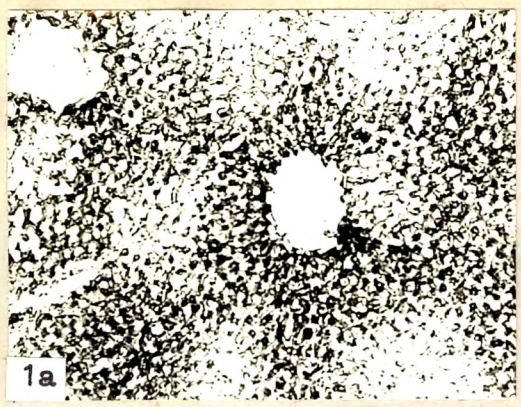
L - Localization; D - Distribution.

EXPLANATIONS TO FIGURES (CHAPTER 6)

Figs. 1a to 22a. Photomicrographs of the liver of birds showing Acetylcholinesterase activity. All photographs are of 50X magnification.

Figs. 1b to 22b. Photomicrographs of the liver of birds showing Butyrylcholinesterase activity. All photographs are of 50X magnification.

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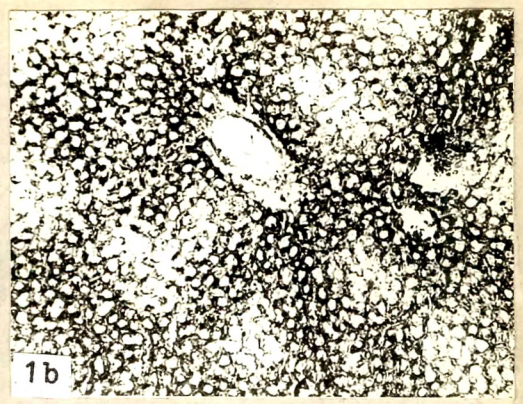
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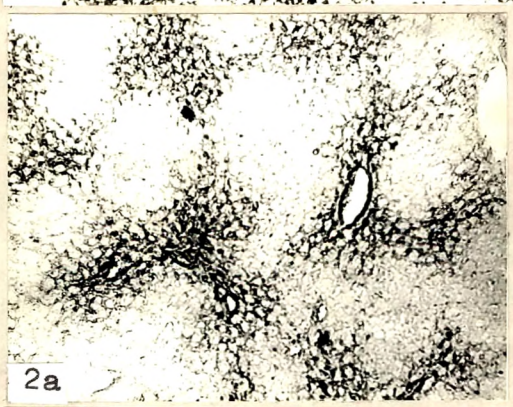
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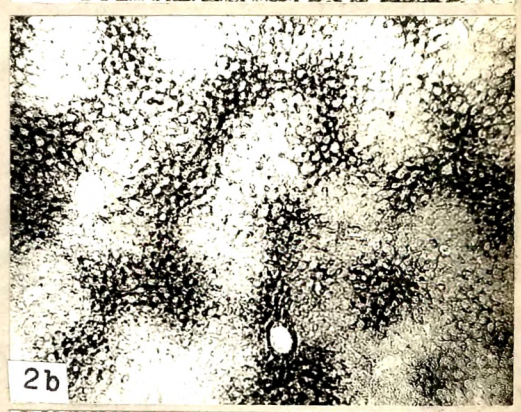
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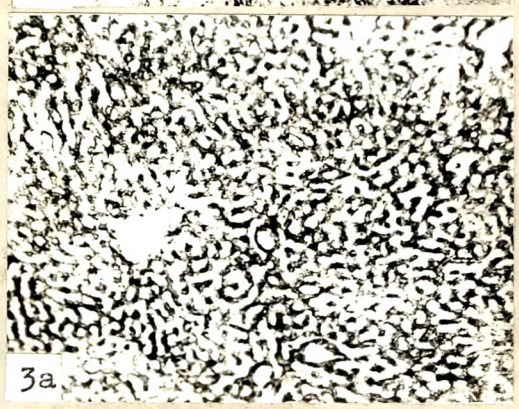
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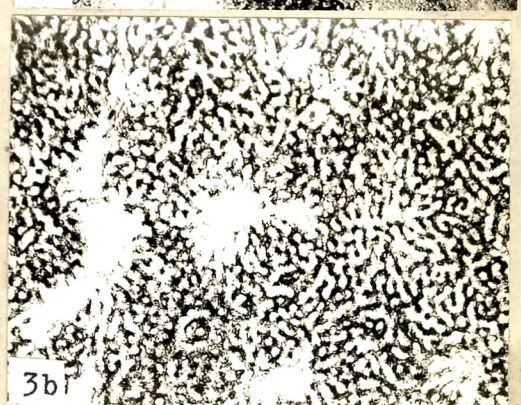
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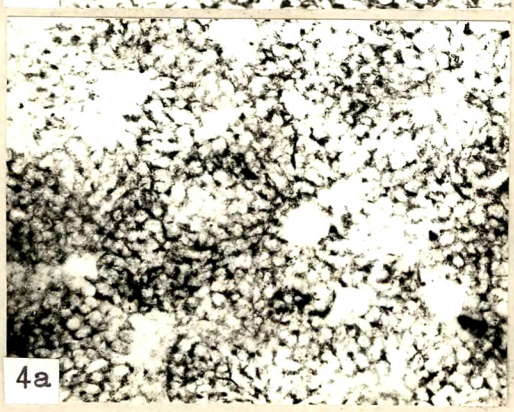
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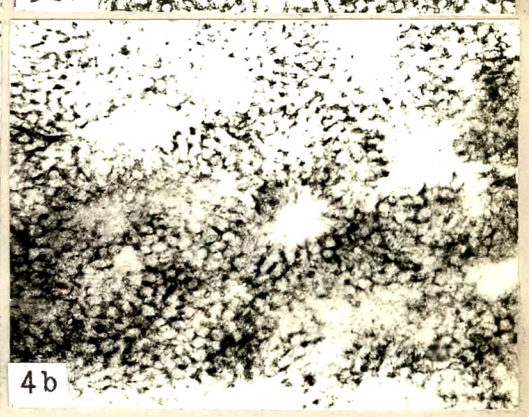
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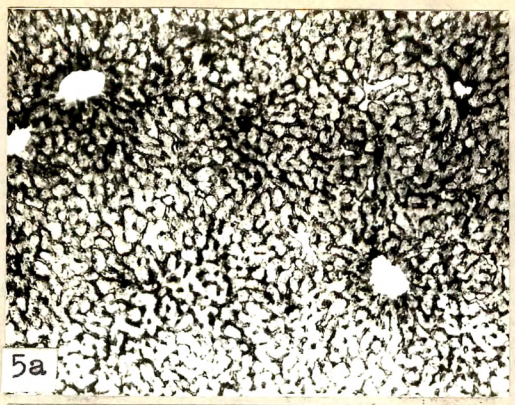
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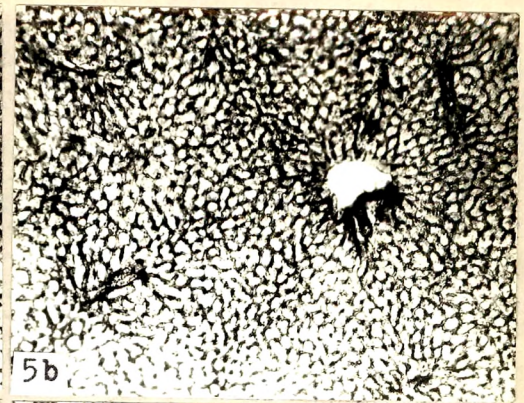
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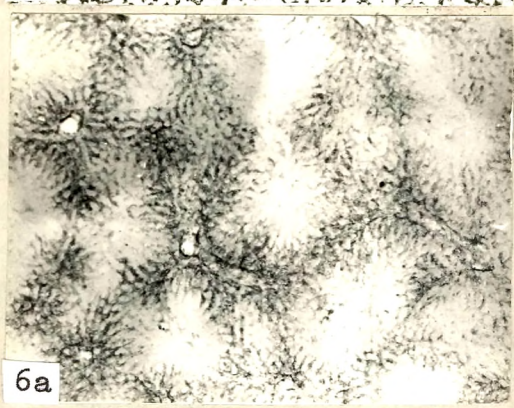
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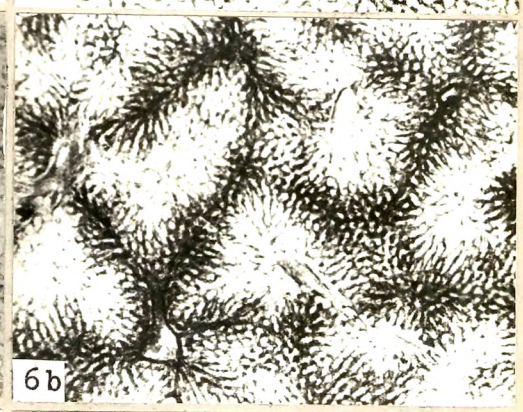
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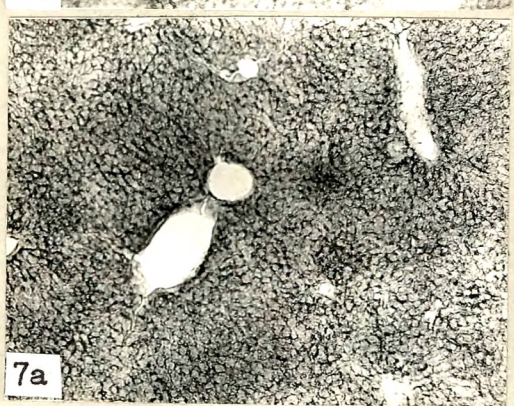
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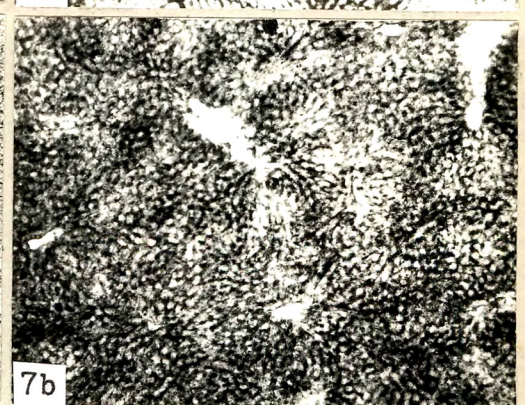
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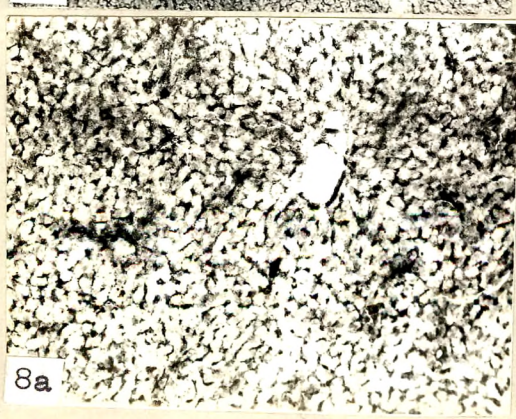
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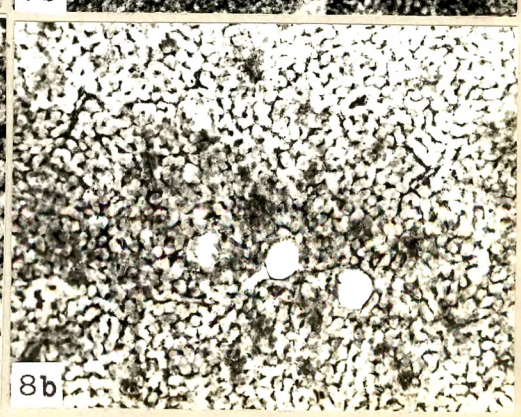
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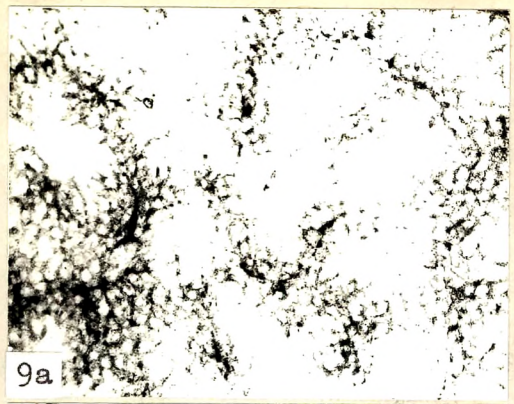
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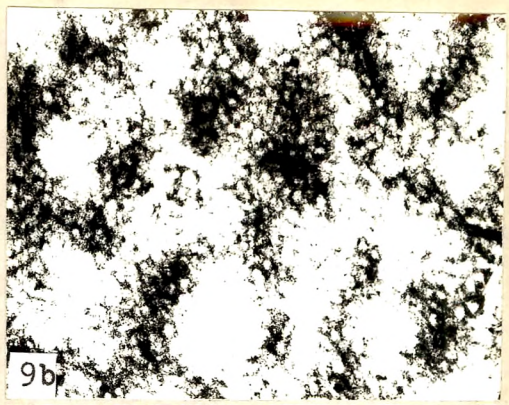
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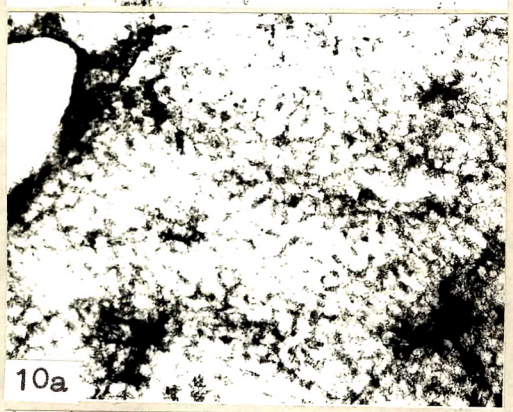
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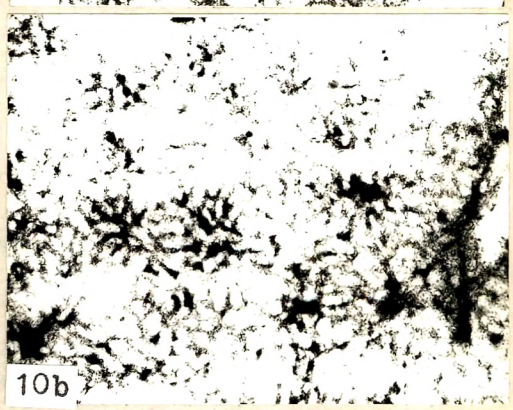
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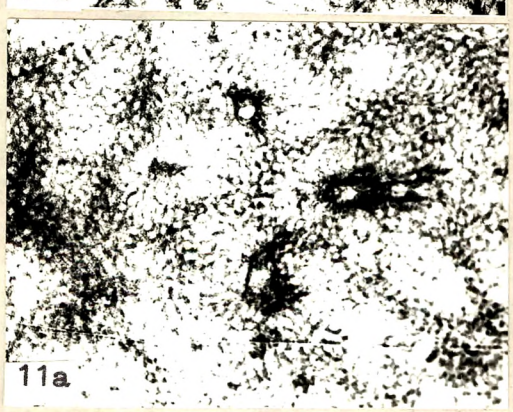
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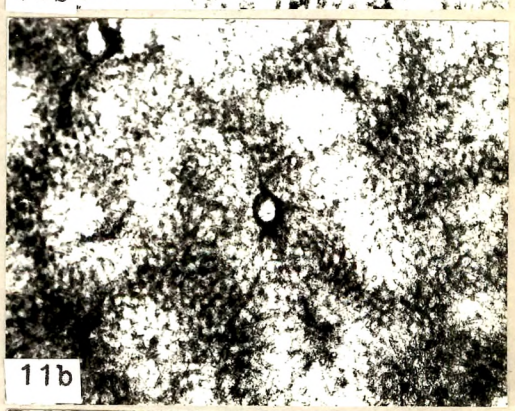
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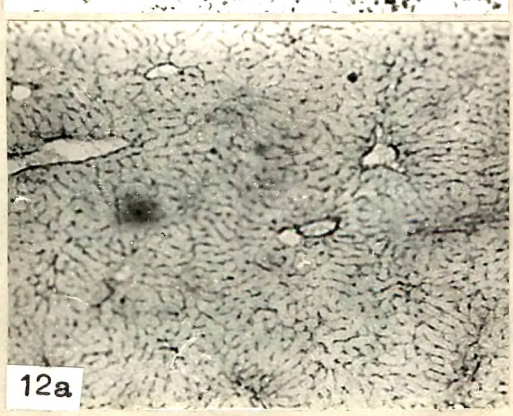
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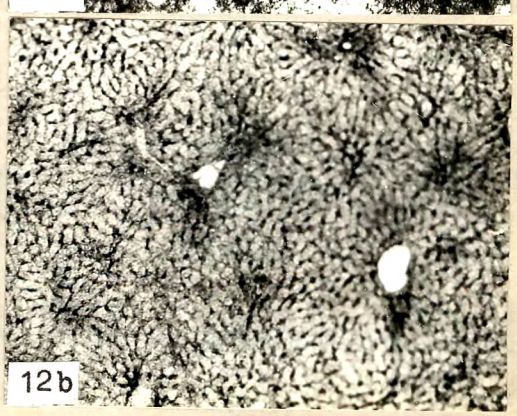
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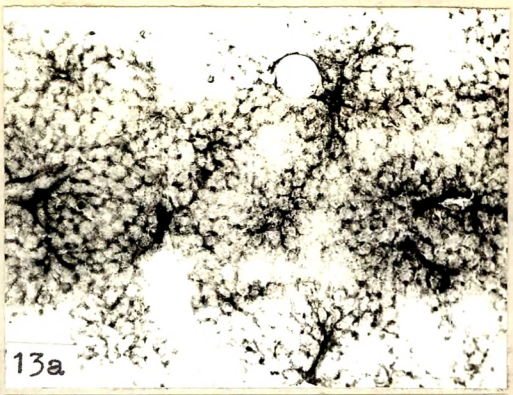
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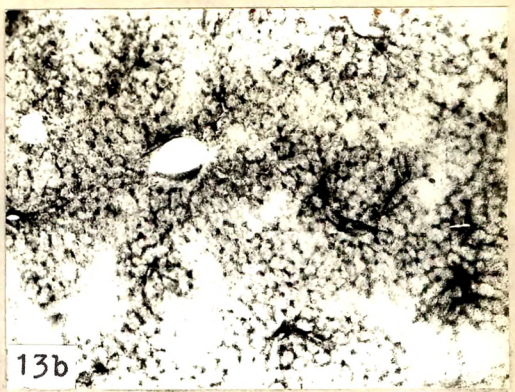
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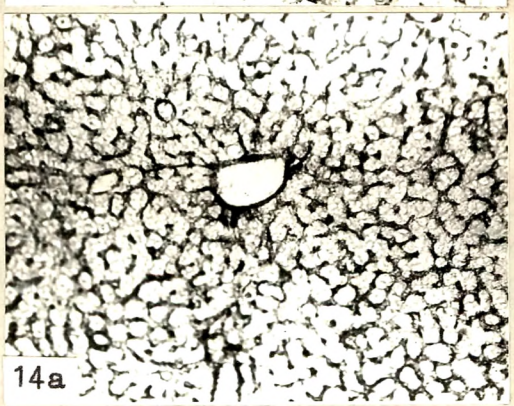
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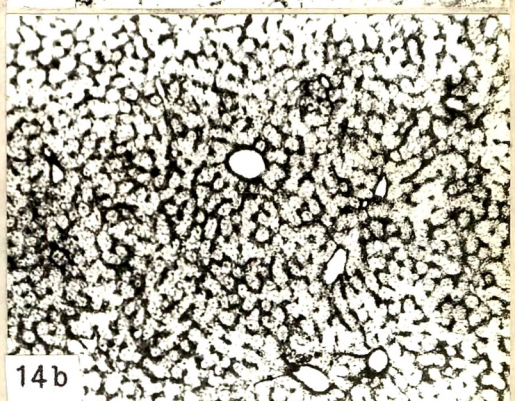
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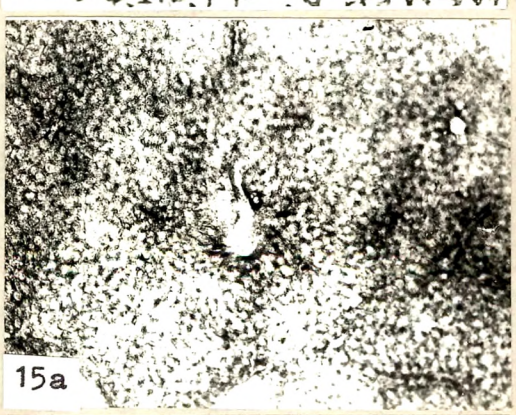
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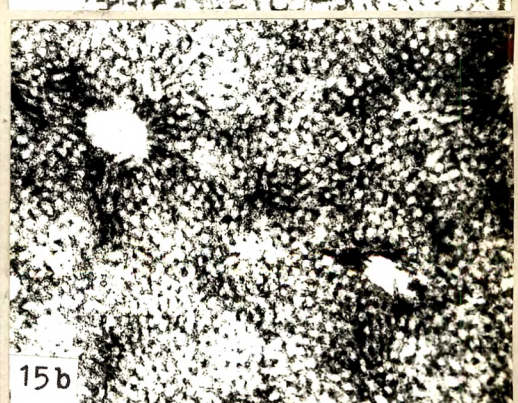
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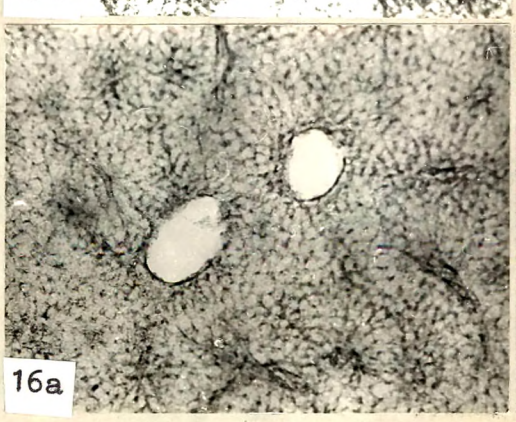
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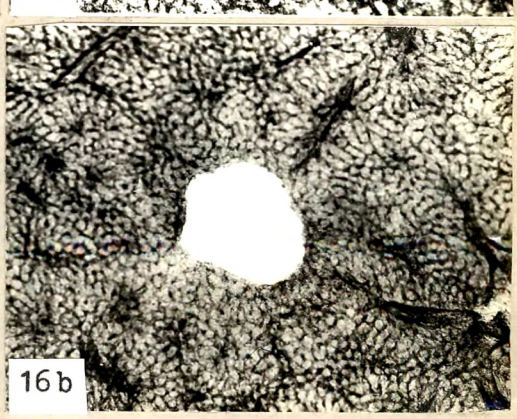
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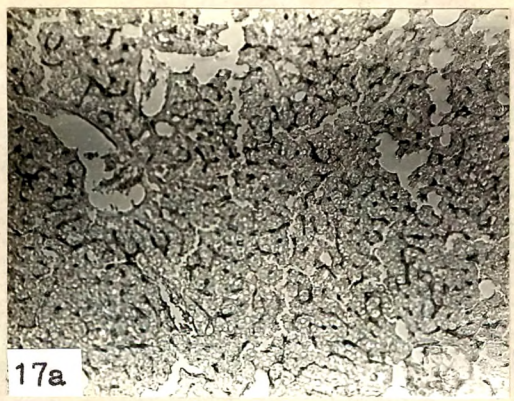

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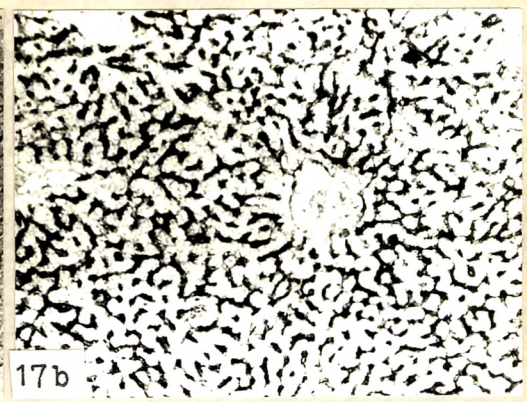

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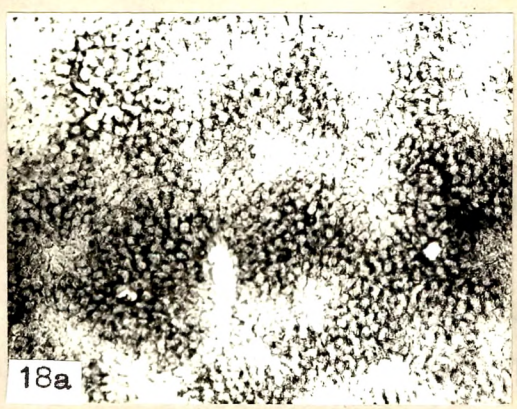

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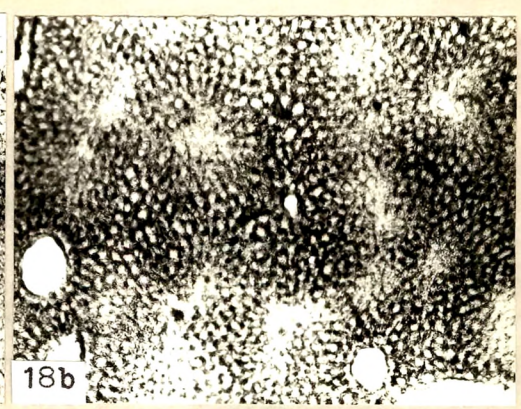

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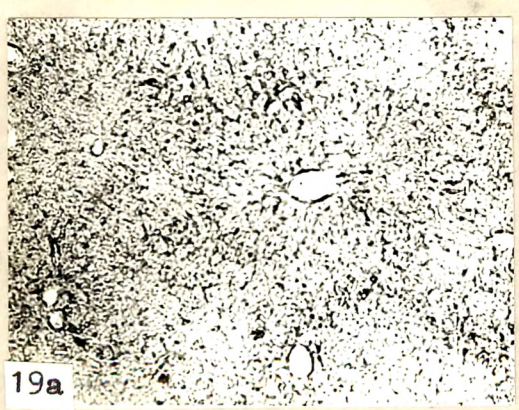

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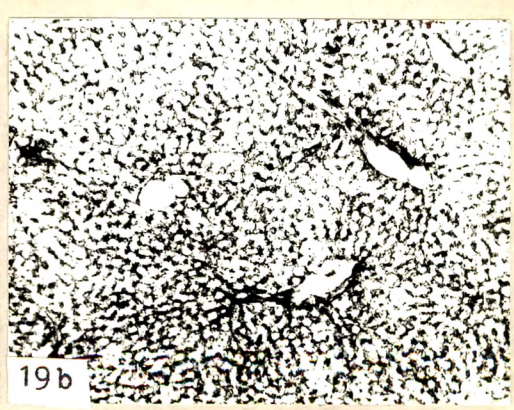

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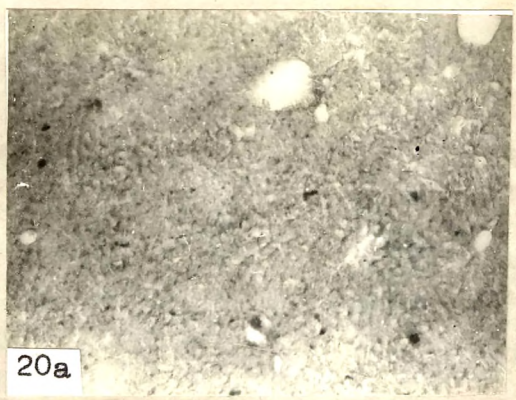

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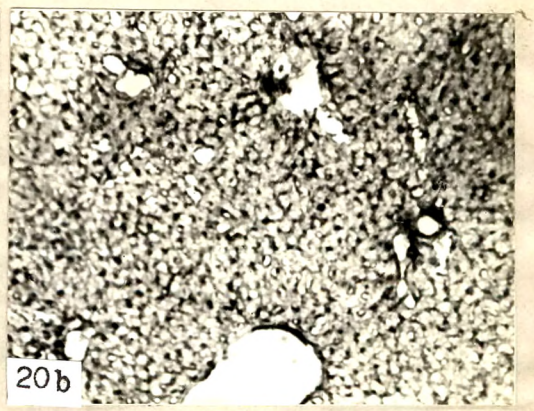

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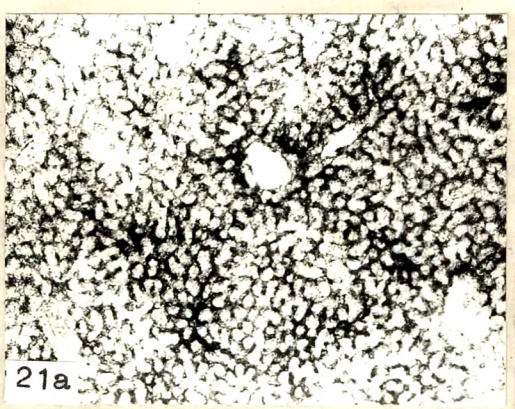

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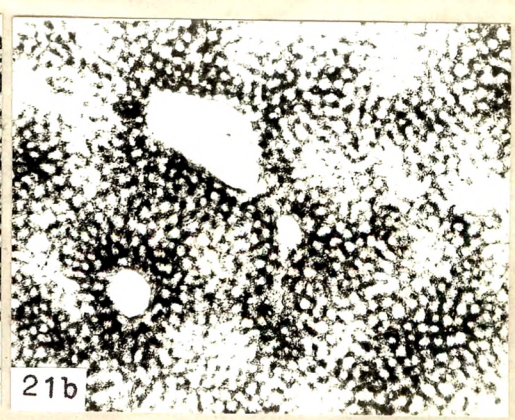

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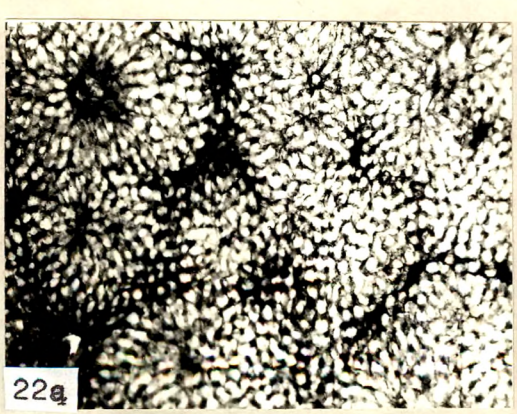

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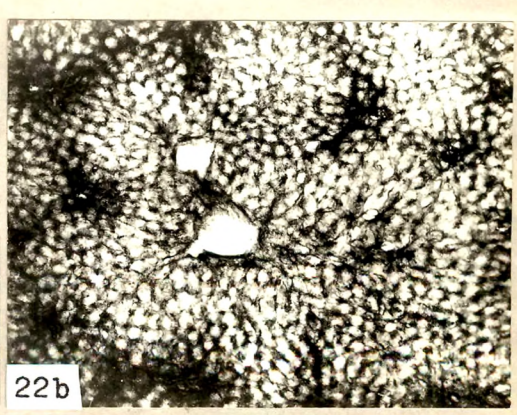

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