CHAPTER 7

QUANTITATIVE LEVELS OF TOTAL LIPIDS AND HISTOCHEMICAL OBSERVATIONS ON LIPIDS, LIPASE, ESTERASE; GLUCOSE-6-PHOSPHATE, α-GLYCEROPHOSPHATE AND β-HYDROXYBUTYRATE DEHYDROGENASES IN THE GIZZARDS OF CERTAIN ADULT REPRESENTATIVE BIRDS

Importance of lipids as the major fuel for energy during long and sustained activity has been well established in the striated muscles of insects (Weis-Fogh, 1952; George & Bhakthan, 1960, 1963); fishes (Billinsky, 1963; George & Bokdawala, 1963; George, 1964); birds (George & Jyoti, 1955, 1957) and mammals (George & Jyoti, 1958) and muscular organs like vertebrate heart (Bing et al., 1954; George & Iype, 1963) and mammalian diaphragm (George & Susheela, 1961; Naik, 1969). Studies on the various enzymes which are of prime importance in fat metabolism in the skeletal muscles of vertebrates have been, by now, elucidated ('Avian Myology', George & Berger, 1966). Studies on the glycogen and fat in the developing pigeon gizzard (chapters 2 and 3) revealed certain fluctuations in the occurrence of lipids during post-natal development, while glycogen was found to be lesser in concentration when compared to lipids throughout the entire

period of the development and ultimately in the adult condition. But a comparative study on the presence and distribution of the metabolite like lipid and enzymes related to lipid metabolism in smooth muscles in general and gizzard in particular of birds is scanty. The present investigation was, therefore, undertaken with a view to obtain more information about the presence of lipid and related enzymes concerned with lipid metabolism in the gizzards of certain adult representative birds which show variations in the structural aspects and functional capacities with reference to the difference in their diet.

MATERIALS AND METHODS

All the birds studied herein were shot from the University campus in the early morning hours by means of an air rifle. They were grouped as given in chapter 6.

The gizzards of all the birds were separated, blotted well to remove their contents, blood and tissue fluids and fixed on a chuck of cryostat microtome maintained at -20°C. Sections of 12 μ thickness were cut and processed in the respective media as described in chapter 3 for the histochemical observations of lipids, lipase, esterase; glucose-6-phosphate,

α-glycerophosphate and β-hydroxybutyrate dehydrogenases (G6PDH, α GPDH and BDH).

For the quantitative estimation of total lipids, a known quantity of gizzard from an oven dried material was extracted in methanol-chloroform mixture (1:2 v/v). The amount of lipids is expressed in grams per 100 grams of wet tissue.

OBSERVATIONS

Lipids: (1-12; 1a-12a)

Though low, but an appreciable histochemical reactivity towards lipids could be observed in the gizzards of all types of birds studied herein. Moreover, both the anatomical entities of the gizzard viz., secretory tubules and smooth muscle fibres responded with equal intensity in all groups of birds under investigation except those of omnivores where a perceptible difference in the level of lipids in these two components of their gizzards could be observed, having a low level in the secretory tubules compared to the muscle fibres.

The quantitative data obtained on the total lipids in the gizzards of these representative birds is expressed

in grams per 100 grams of wet tissue as shown in Table I.

Lipase and esterase: (13 – 36, 13a – 36a).

A general feature of lipase and esterase distribution is that the activities of these two enzymes were either equal in both the components of the gizzard or slightly more in the muscle fibres. In the case of frugivores, both the enzymes recorded an almost equally moderate and identical intensity in both the components of the gizzards. But in the case of granivores, whereas the activity of lipase was quite similar in concentration to that noticed in frugivores; that of esterase was double this concentration. Amongst omnivores, in general, the concentrations of both lipase and esterase were similar in the same style and pattern as observed in the frugivore excepting crows, where the activities of both the enzymes, though equal, were nevertheless, slightly higher and in the case of fowl the level of lipase was slightly more than that of esterase. In nectar feeder like sun bird, the activity of lipase was slightly lower than that of esterase. With respect to carnivores and insectivores, all the members showed a slightly higher level of esterase in comparison to lipase excepting for

drongo (insectivore), where it was relatively slightly lower and equal in intensity to that of lipase, and shrike (carnivore) where the activities of the two enzymes were both equal and higher.

G6PDH, ŒGPDH and BDH: (37 - 72; 37a - 72a).

In general it may be noted at the very outset that both the components of the gizzard responded almost equally for all the three enzymes in the various groups of birds with slight variations. Amongst the carnivores and insectivores, excepting for drongo which showed a slightly lower and equal intensity of all the three enzymes and Indian robin and green bee-eater where ŒGPDH activity was slightly lower than the concentrations of the other two enzymes. Rest of all birds grouped under carnivores and insectivores showed an identical concentration of G6PDH, ŒGPDH and BDH. Similarly, both granivore as well as frugivore too responded in a similar pattern excepting for a slightly low level of activity of ŒGPDH in parakeet (frugivore). In comparison, the omnivores, in general, responded with a slightly low and equal intensity towards all the three enzymes excepting for fowl where ŒGPDH and BDH were slightly higher. Another

EXPLANATIONS FOR FIGURES

(Figures 1 - 12 Lipids in the mucosal tubules of gizzards of various birds) and

(Figures 1a - 12a Lipids in the smooth muscle fasciculi of gizzards of various birds).

Figs. 1 & 1a - Shrike

Figs. 2 & 2a - Kite

Figs. 3 & 3a - Crow

Figs. 4 & 4a - Fowl

Figs. 5 & 5a - Bee-eater

Figs. 6 & 6a - Drongo

Figs. 7 & 7a - Crow Pheasant

Figs. 8 & 8a - Bulbul

Figs. 9 & 9a - Babbler

Figs. 10 & 10a - Koel

Figs. 11 & 11a - Parakeet

Figs. 12 & 12a - Sunbird

EXPLANATIONS FOR FIGURES

(Figures 13 - 24 Lipase activity in the mucosal tubules of gizzards of various birds) and

(. Figures 13a - 24a Lipase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 13 & 13a - Shrike

Figs. 14 & 14a - Kite

Figs. 15 & 15a - Crow

Figs. 16 & 16a - Fowl

Figs. 17 & 17a - Bee-eater

Figs. 18 & 18a - Drongo

Figs. 19 & 19a - Crow Pheasant

Figs. 20 & 20a - Bulbul

Figs. 21 & 21a - Babbler

Figs. 22 & 22a - Koel

Figs. 23 & 23a - Parakeet

Figs. 24 & 24a - Sunbird

EXPLANATIONS FOR FIGURES

(Figures 25 - 36 Esterase activity in the mucosal tubules of gizzards of various birds) and

(Figures 25a - 36a Esterase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 25 & 25a - Shrike

Figs. 26 & 26a - Kite

Figs. 27 & 27a - Crow

Figs. 28 & 28a - Fowl

Figs. 29 & 29a - Bee-eater

Figs. 30 & 30a - Drongo

Figs. 31 & 31a - Crow Pheasant

Figs. 32 & 32a - Bulbul

Figs. 33 & 33a - Babbler

Figs. 34 & 34a - Koel

Figs. 35 & 35a - Parakeet

Figs. 36 & 36a - Sunbird

EXPLANATIONS FOR FIGURES

(Figures 37 - 48 Glucose-6-phosphate Dehydrogenase activity in the mucosltubules of gizzards of various birds) and

(Figures 37a - 48a Glucose-6-phosphate Dehydrogenase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 37 & 37a - Shrike

Figs. 38 & 38a - Kite

Figs. 39 & 39a - Crow

Figs. 40 & 40a - Fowl

Figs. 41 & 41a - Bee-eater

Figs. 42 & 42a - Drongo

Figs. 43 & 43a - Crow Pheasant

Figs. 44 & 44a - Bulbul

Figs. 45 & 45a - Babbler

Figs. 46 & 46a - Koel

Figs. 47 & 47a - Parakeet

Figs. 48 & 48a - Sunbird

EXPLANATIONS FOR FIGURES

(Figures 49 - 60 α- Glycerophosphate dehydrogenase activity in the mucosal tubules of gizzards of various birds) and (Figures 49a - 60a - Glycerophosphate dehydrogenase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 49 & 49a - Shrike

Figs. 50 & 50a - Kite

Figs. 51 & 51a - Crow

Figs. 52 & 52a - Fowl

Figs. 53 & 53a - Bee-eater

Figs. 54 & 54a - Drongo

Figs. 55 & 55a - Crow Pheasant

Figs. 56 & 56a - Bulbul

Figs. 57 & 57a - Babbler

Figs. 58 & 58a - Koel

Figs. 59 & 59a - Parakeet

Figs. 60 & 60a - Sunbird

EXPLANATIONS FOR FIGURES

(Figures 61 - 72 β-Hydroxybutyrate dehydrogenase activity in the mucosal tubules of gizzards of various birds)

and

(Figures 61a - 72a β-Hydroxybutyrate dehydrogenase activity in the smooth muscle fasciculi of gizzards of various birds)

Figs. 61 & 61a - Shrike

Figs. 62 & 62a - Kite

Figs. 63 & 63a - Crow

Figs. 64 & 64a - Fowl

Figs. 65 & 65a - Bee-eater

Figs. 66 & 66a - Drongo

Figs. 67 & 67a - Crow Pheasant

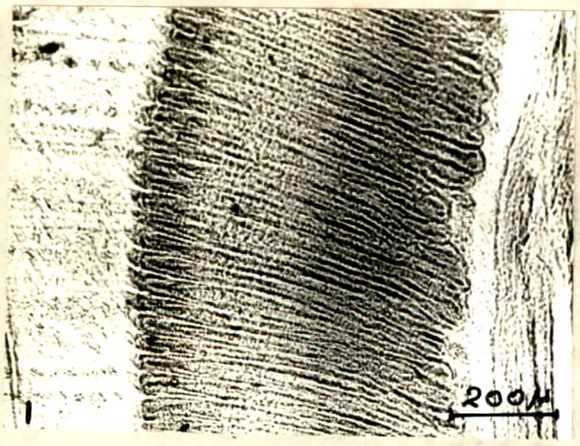
Figs. 68 & 68a - Bulbul

Figs. 69 & 69a - Babbler

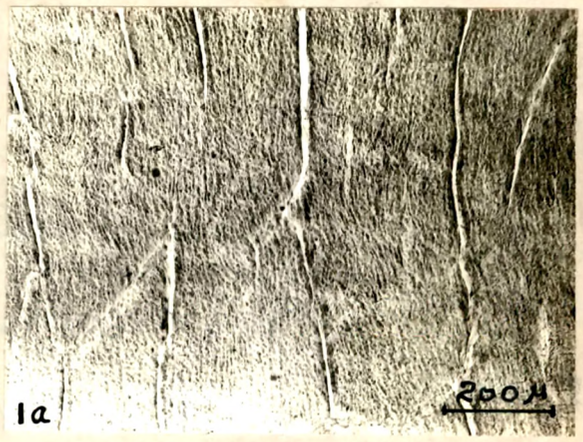
Figs. 70 & 70a - Koel

Figs. 71 & 71a - Parakeet

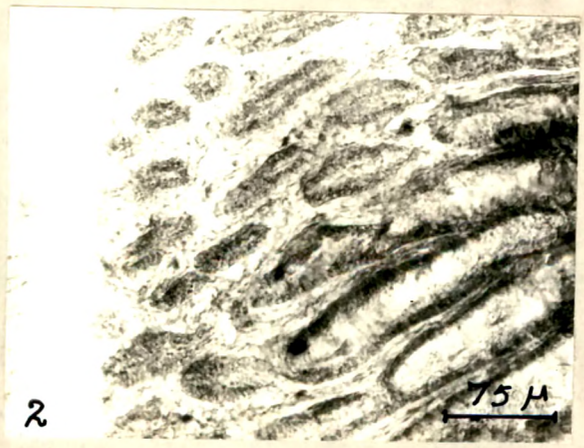
Figs. 72 & 72a - Sunbird



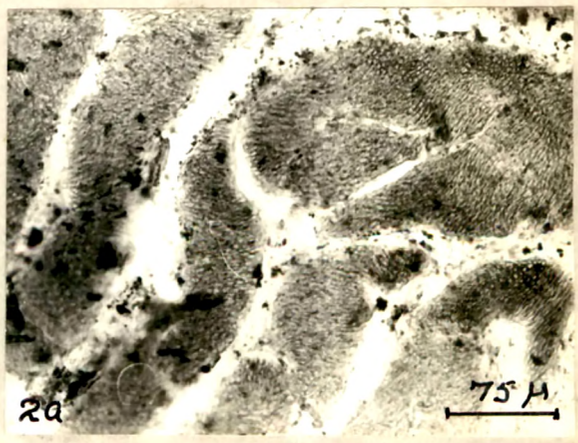
1



1a

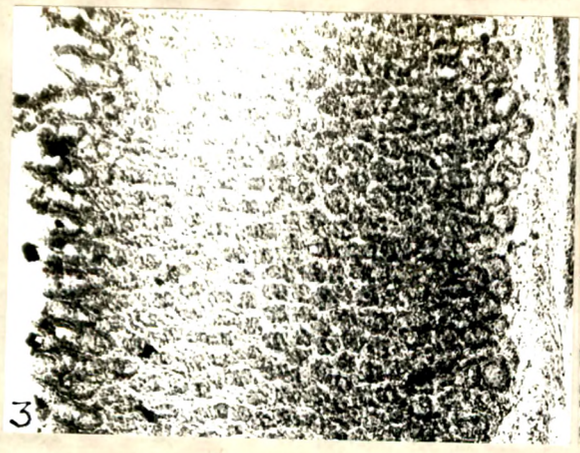


2



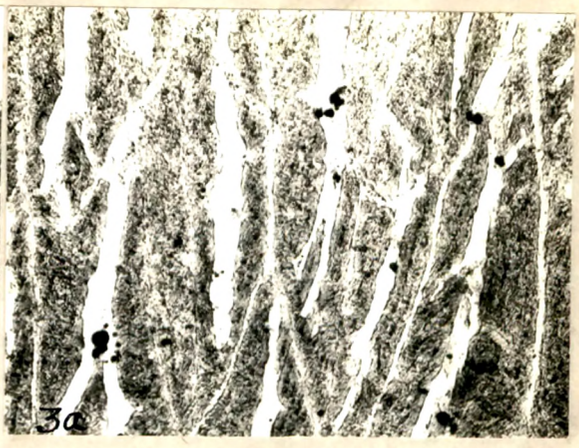
2a

100 μ

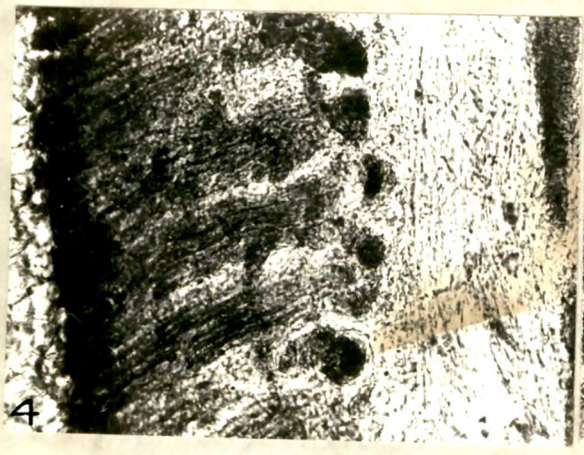


3

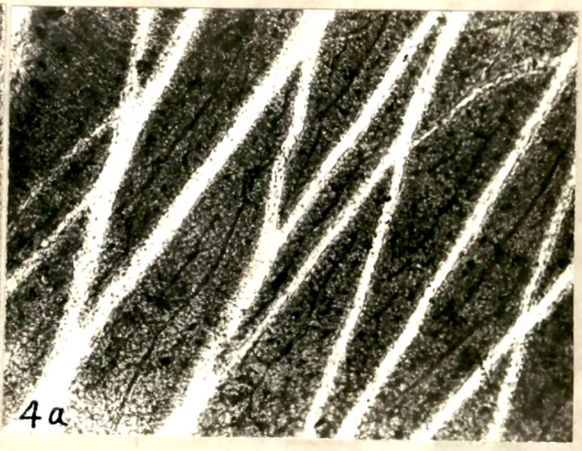
100 μ



3a



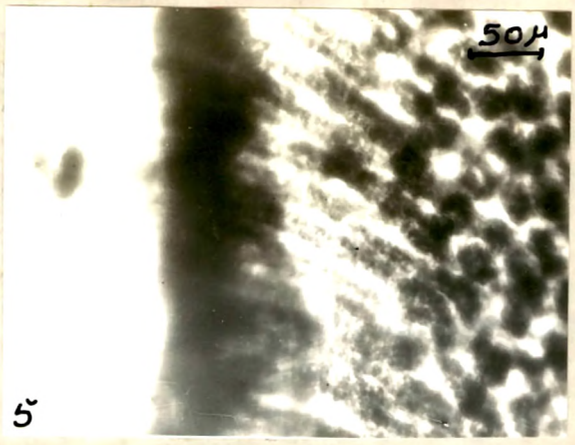
4



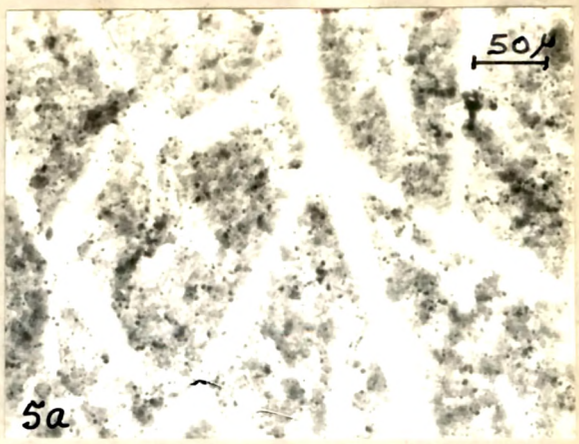
4a

100 μ

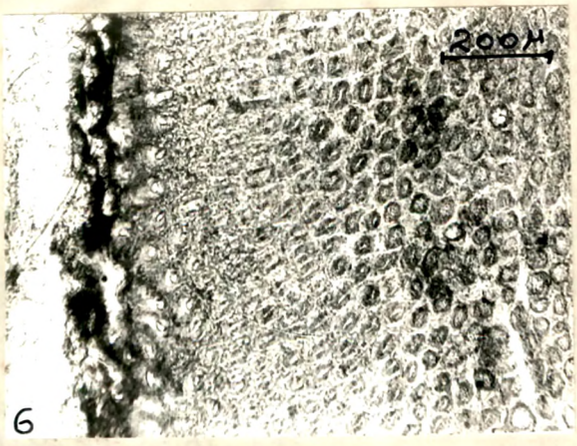
100 μ



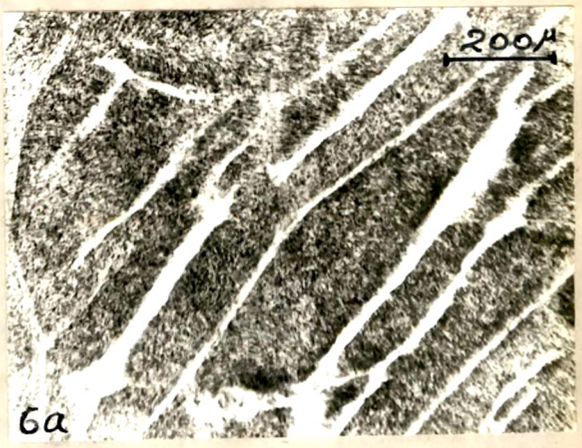
5



5a



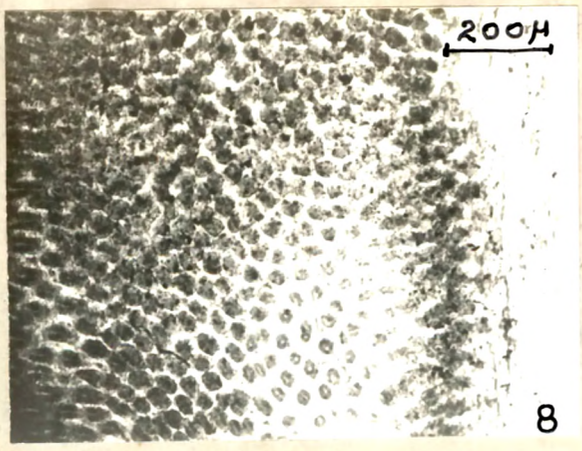
6

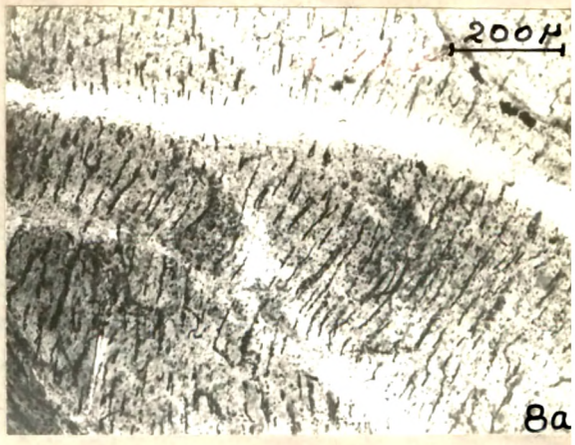


6a

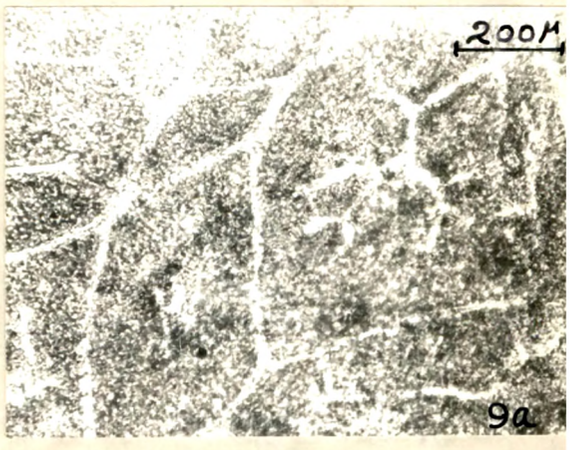
7

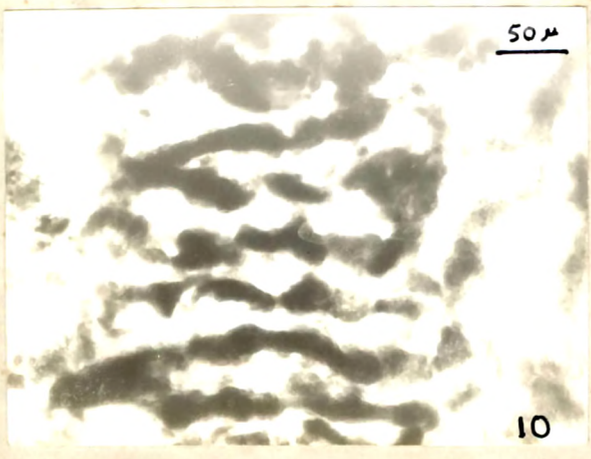
7a

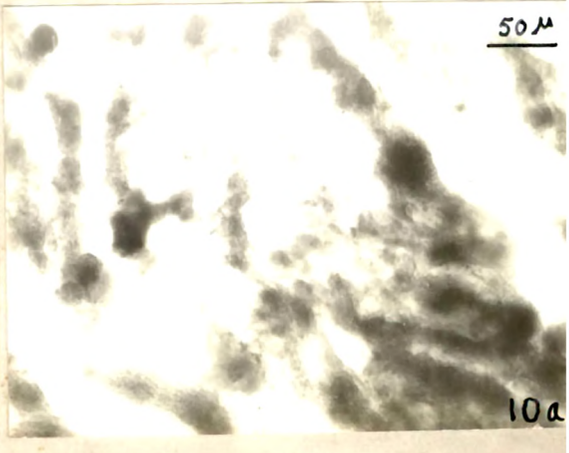

200μ
8


200μ
8a

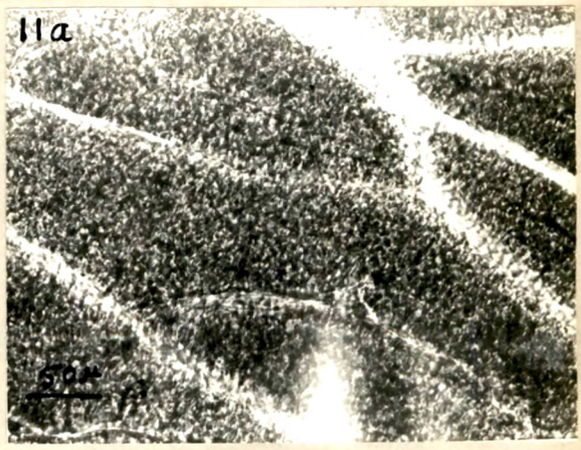

200μ
9


200μ
9a

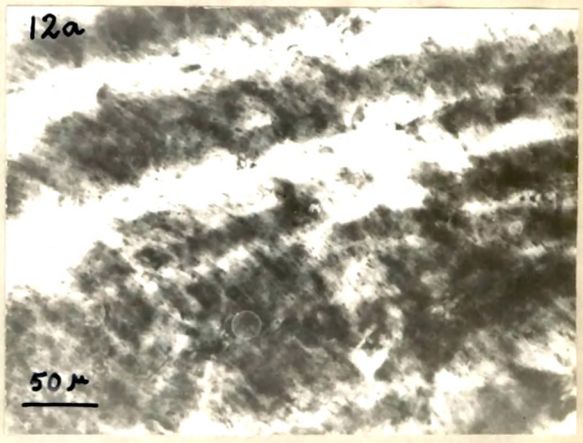

50μ
10

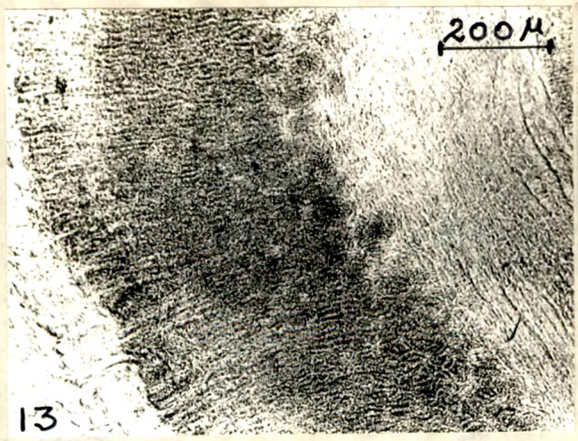

50μ
10a


11
50 μ

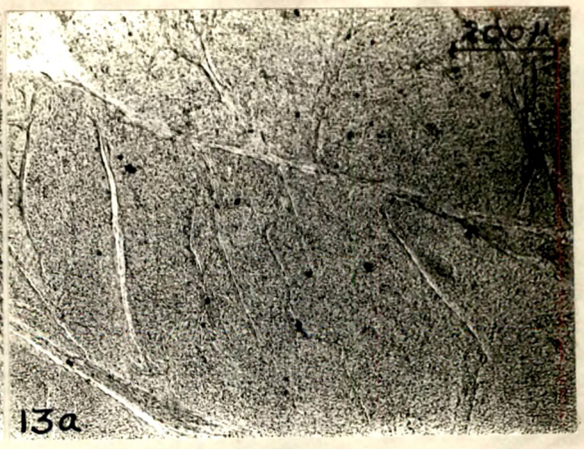

11a
50 μ


50 μ
12

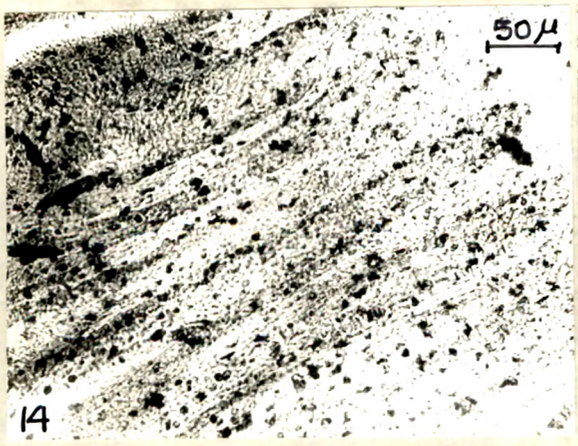

12a
50 μ



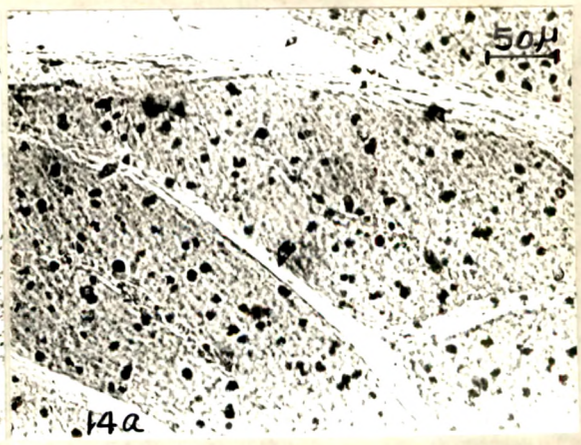
13



13a

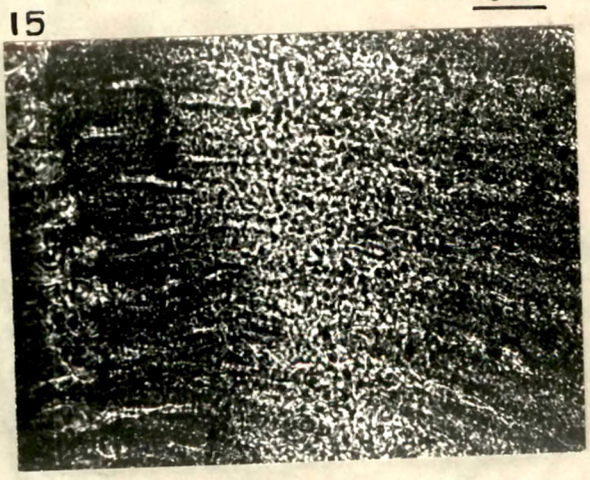


14



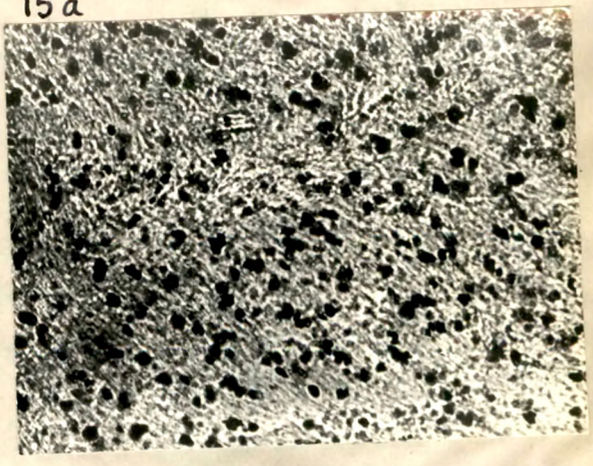
14a

15



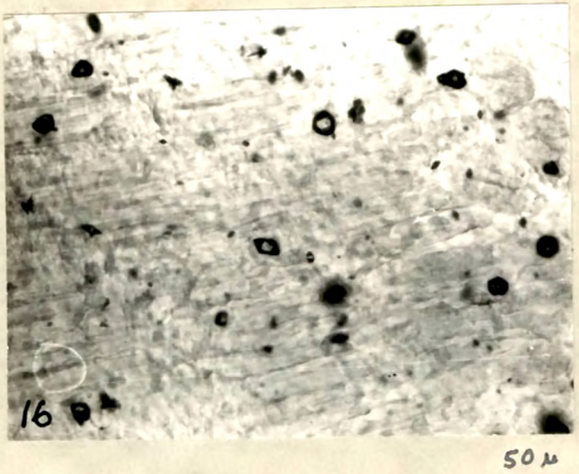
50 μ

15a



50 μ

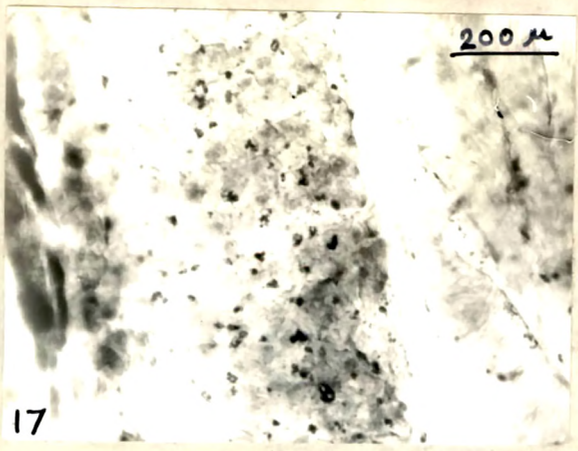
16



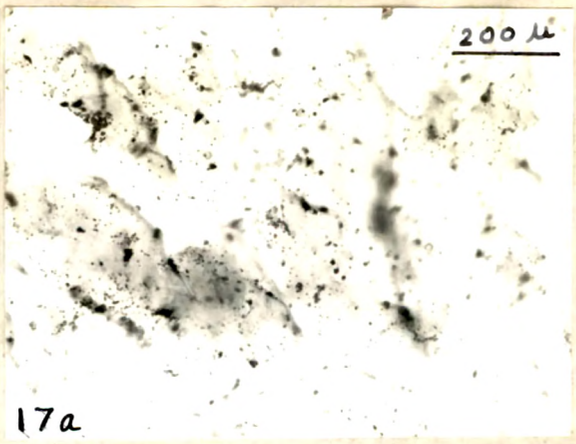
50 μ

16a

50 μ

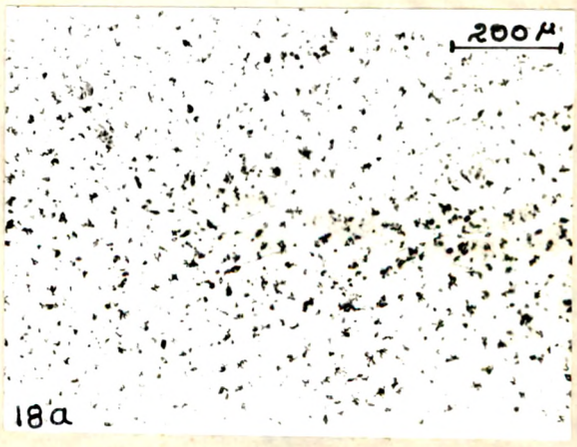


17



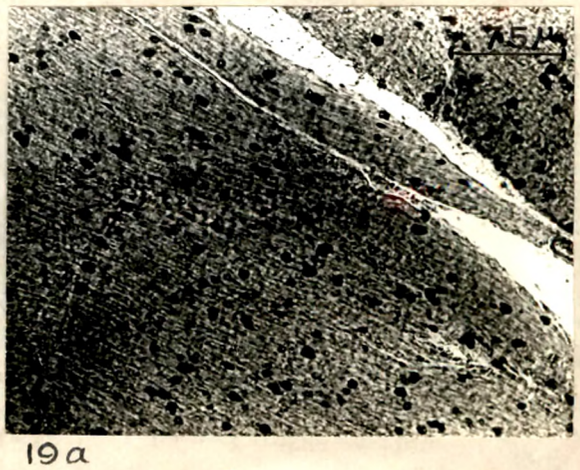
17a

18

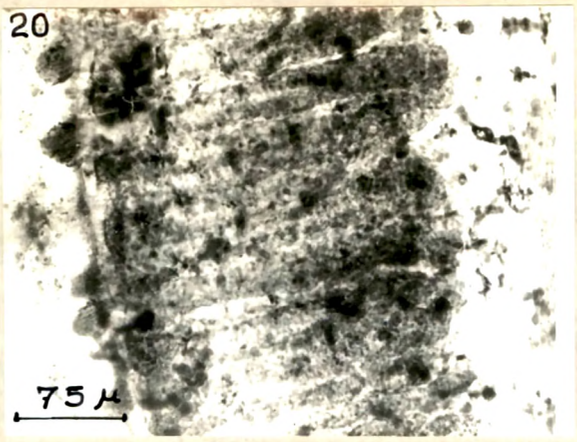


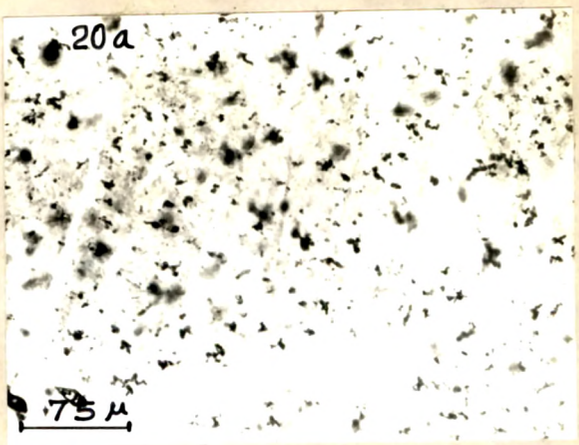
18a

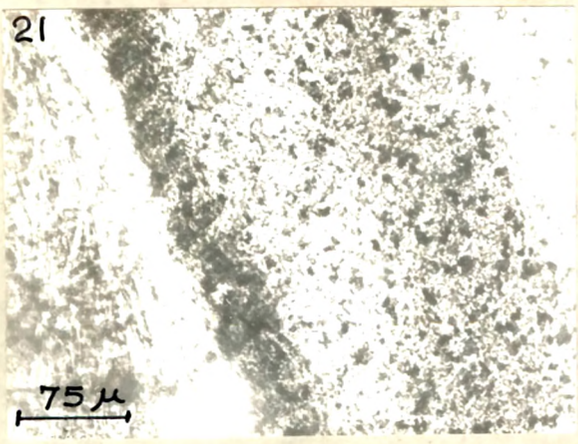
19



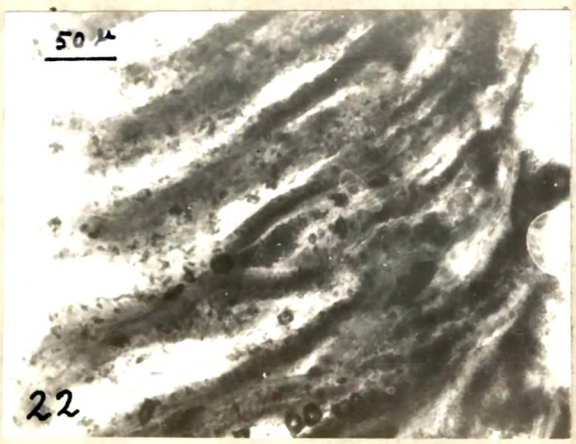
19a

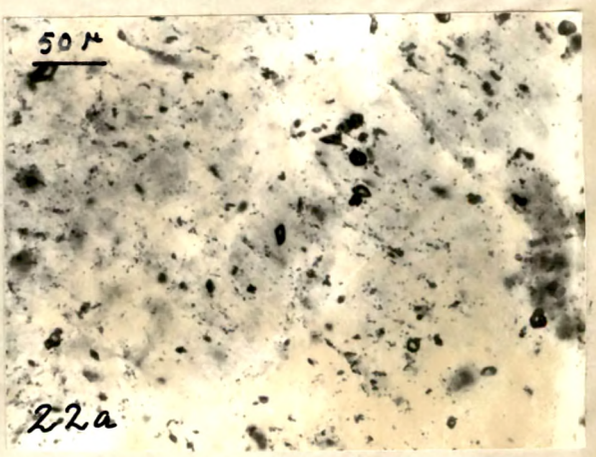

20
75 μ

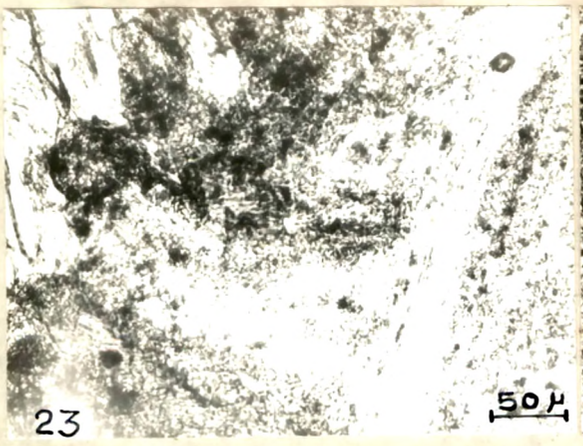

20a
75 μ

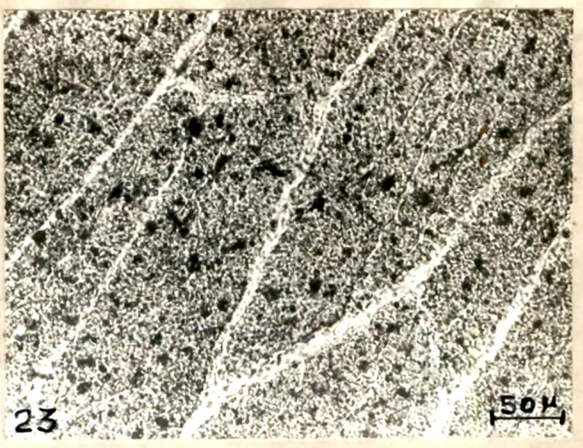

21
75 μ

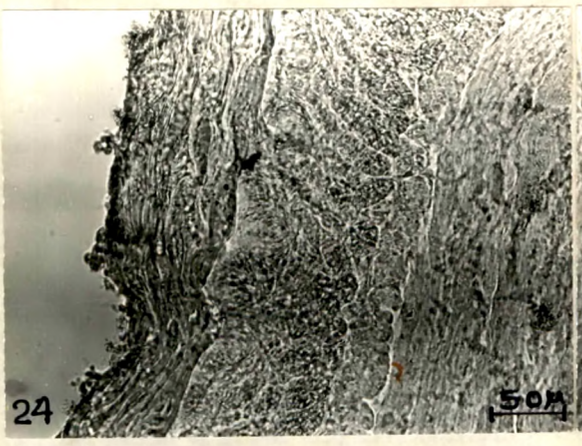

21a
75 μ

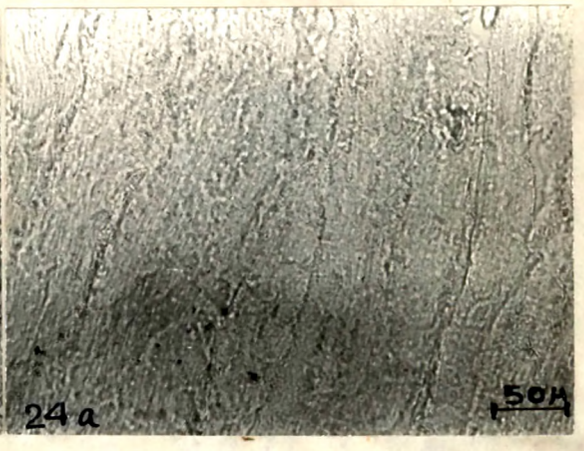

50 μ
22

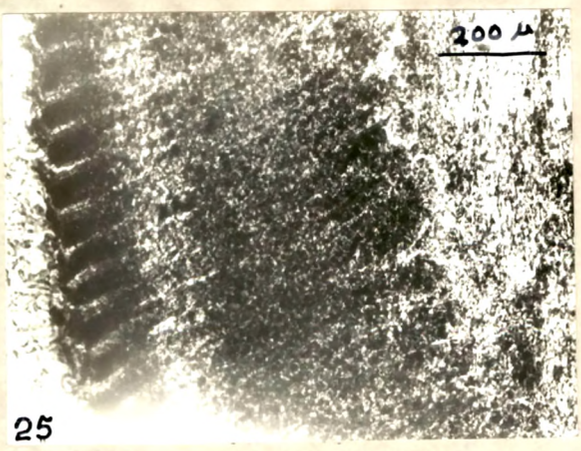

50 μ
22a


23
50μ

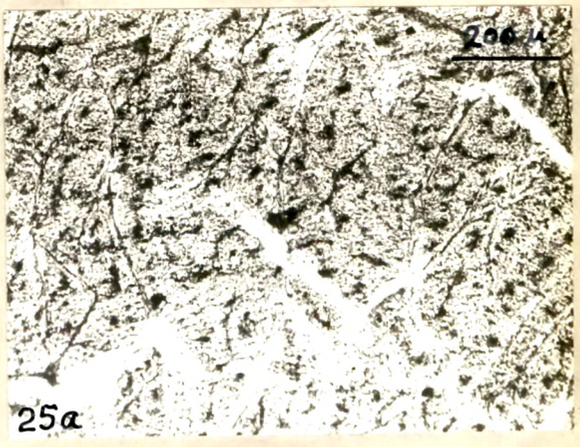

23
50μ


24
50μ

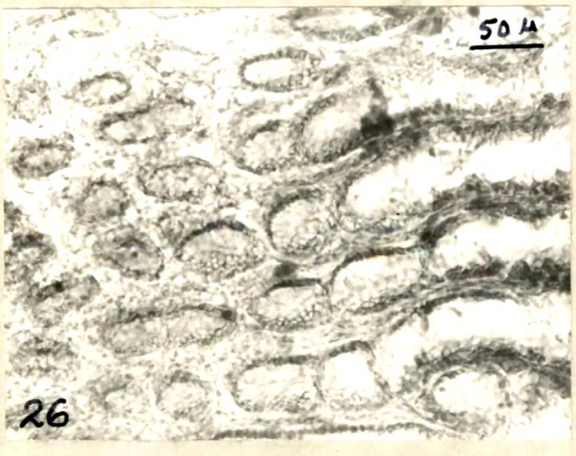

24 a
50μ



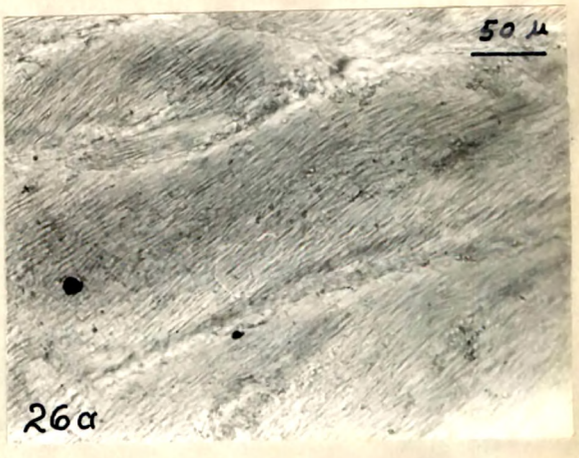
25



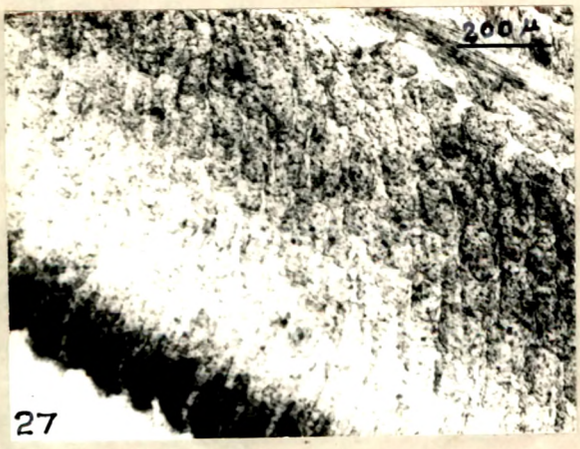
25a



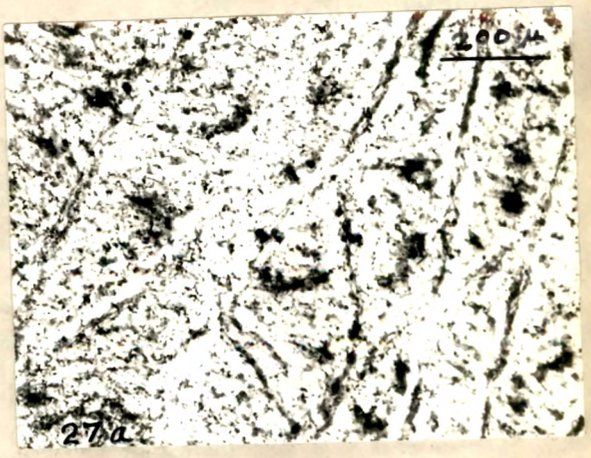
26



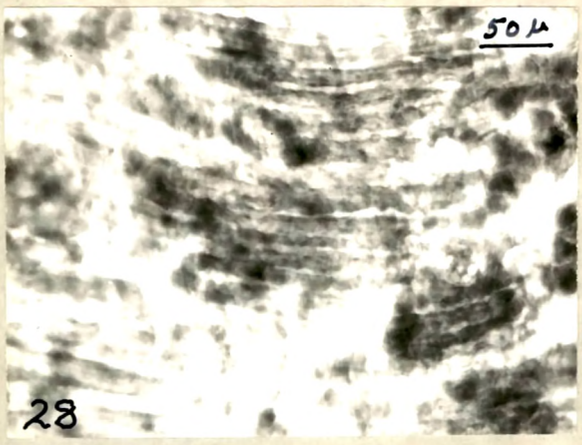
26a



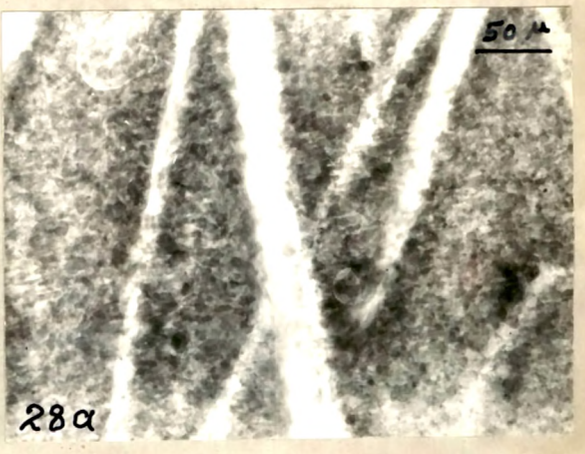
27



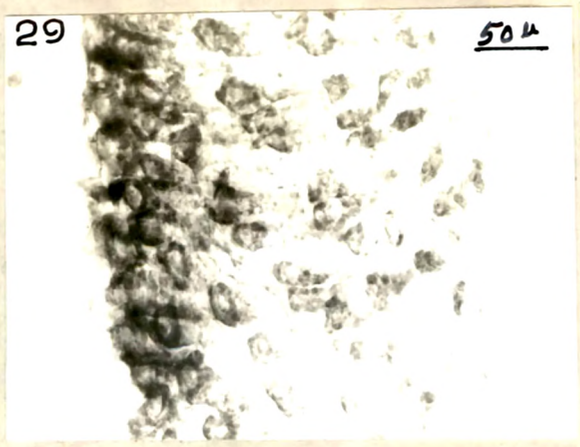
27a

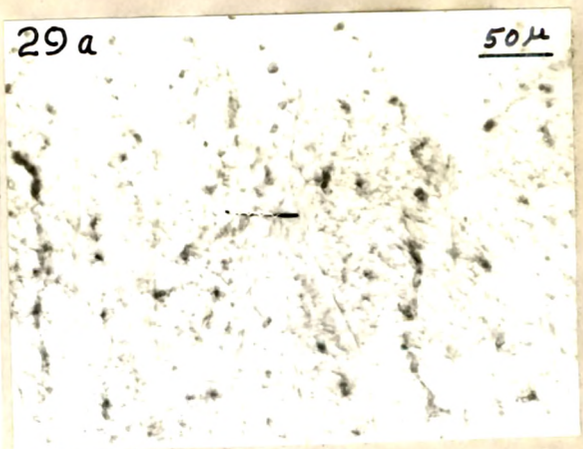


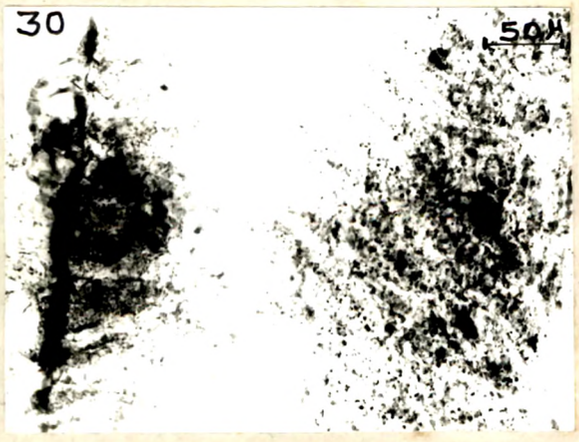
28

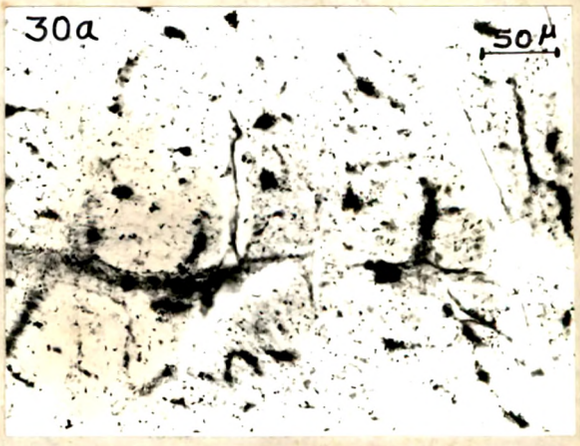


28a

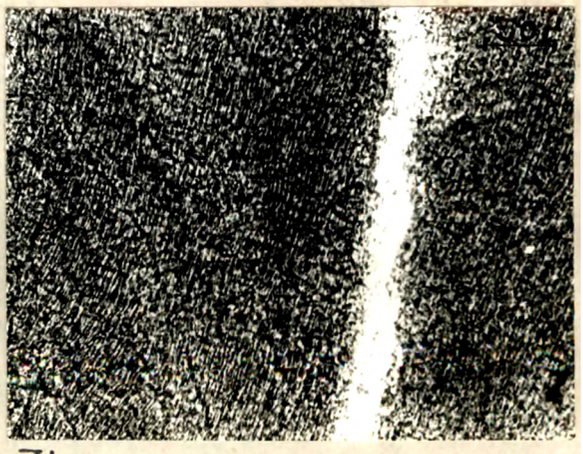




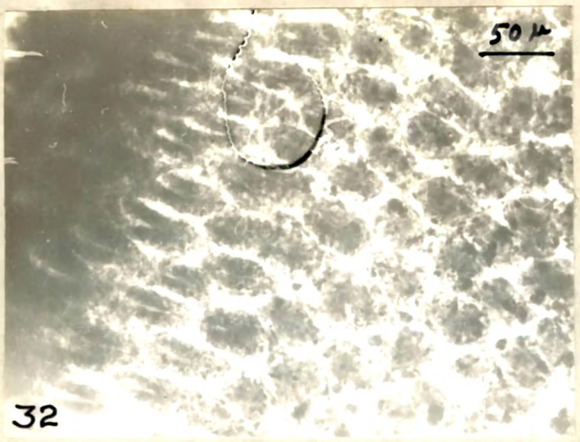




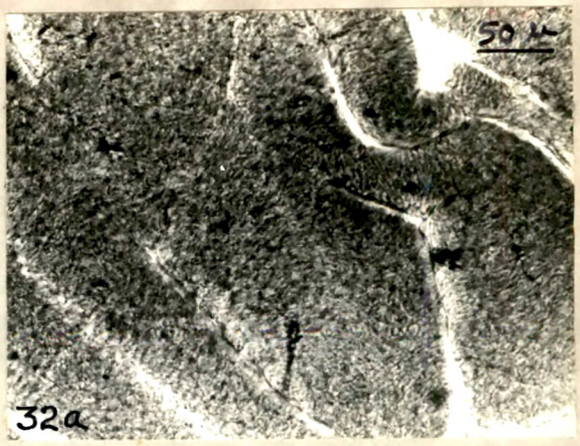
31



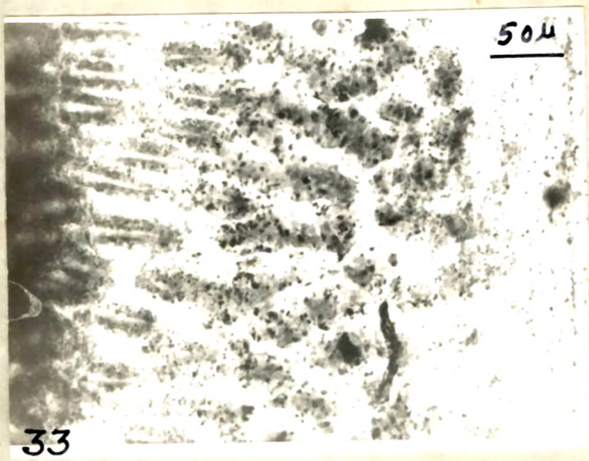
31a



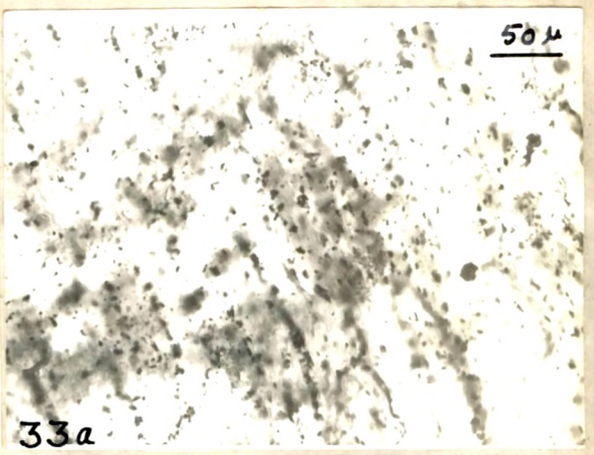
32



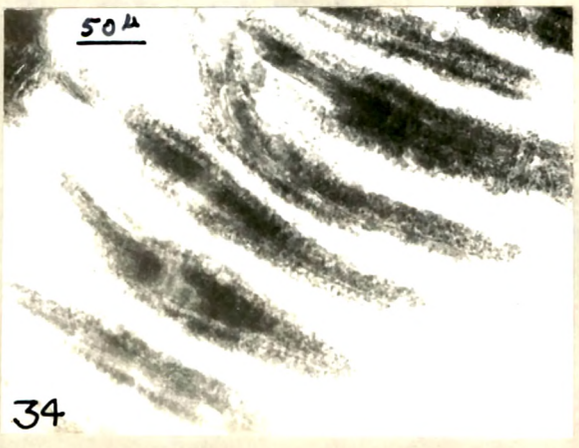
32a



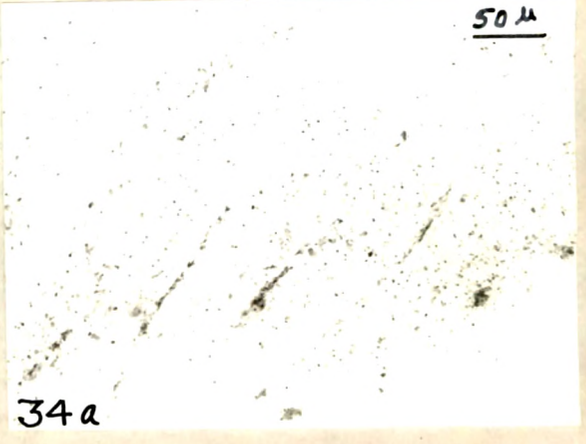
33



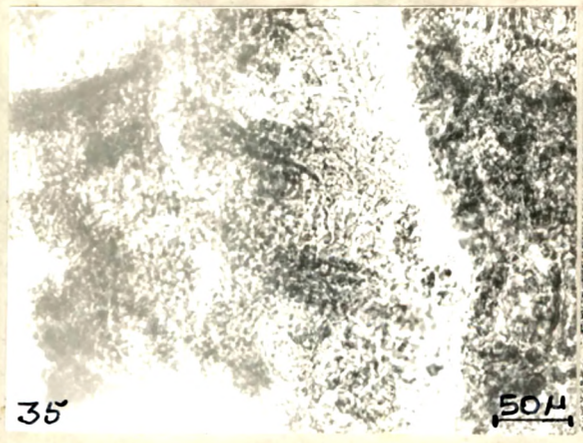
33a

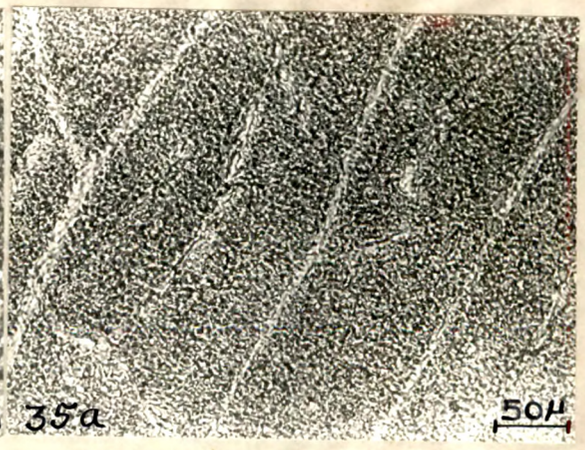


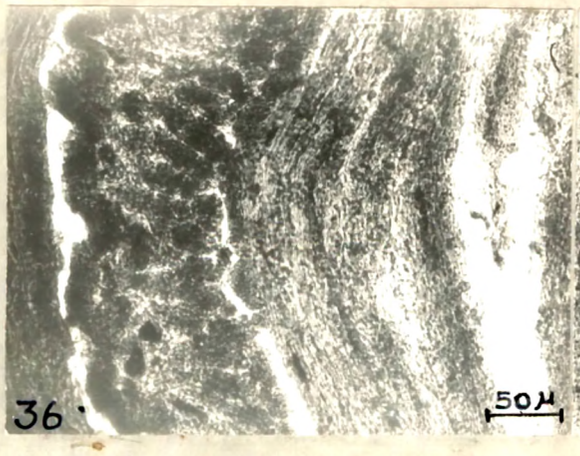
34

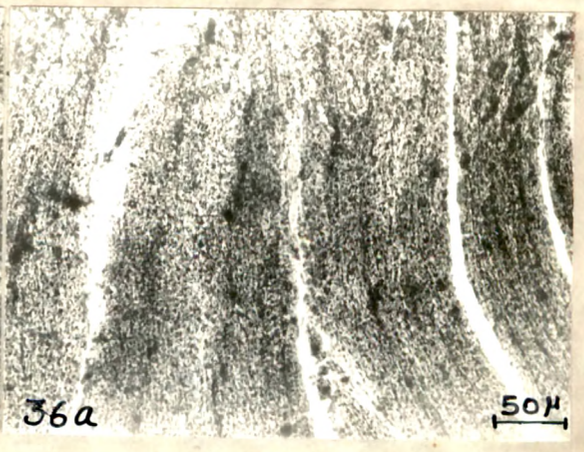


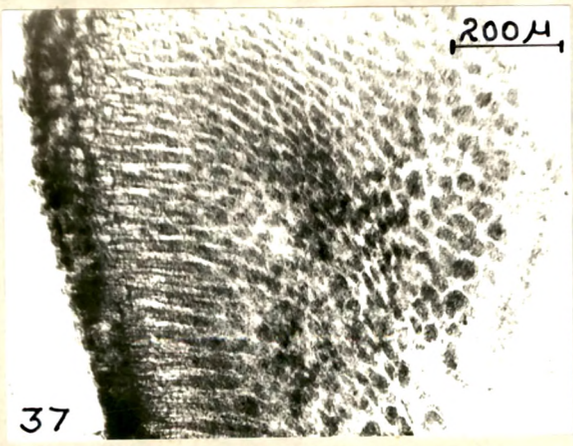
34a


35
50μ

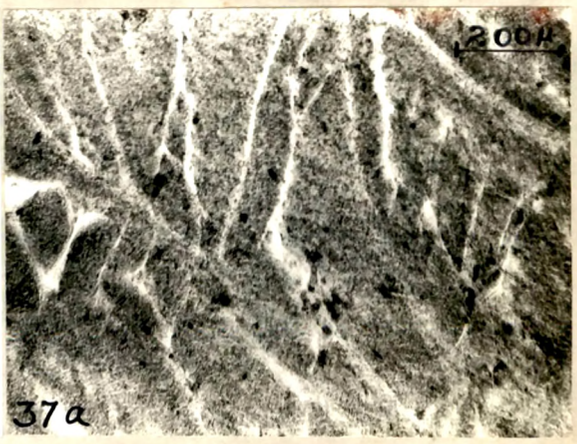

35a
50μ


36
50μ


36a
50μ

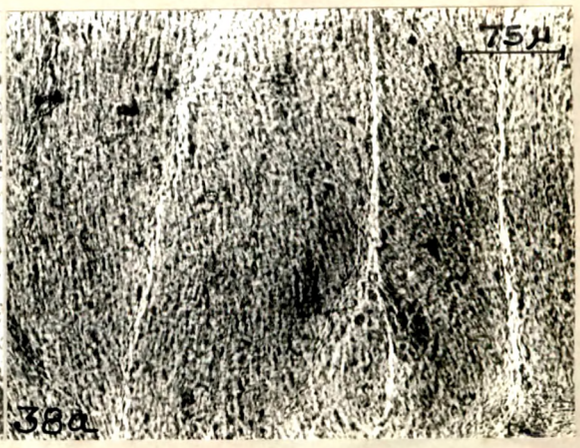


37



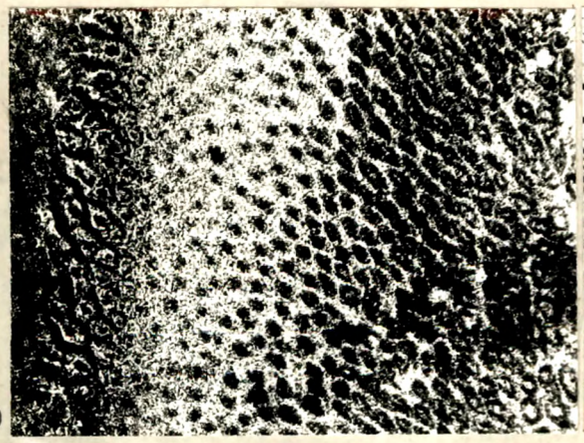
37a

38



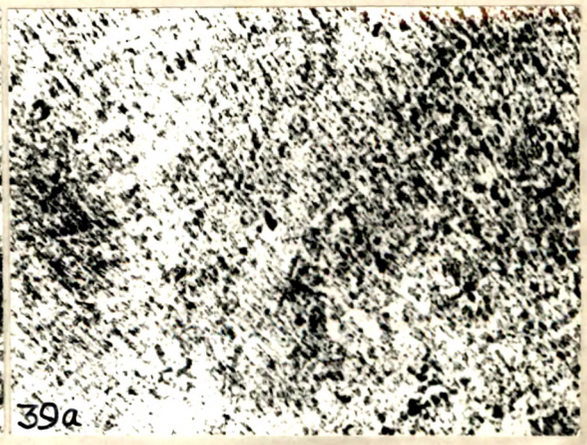
38a

100 μ

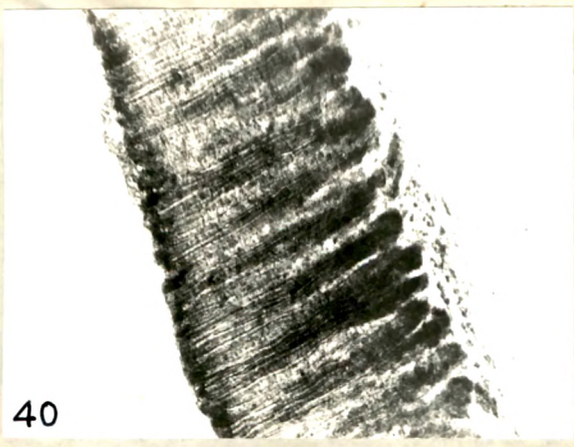


39

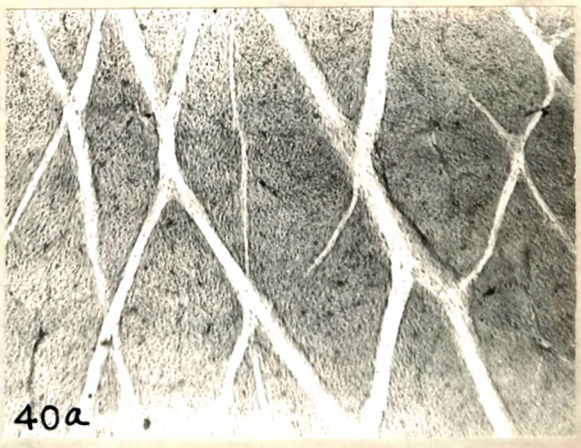
100 μ



39a



40

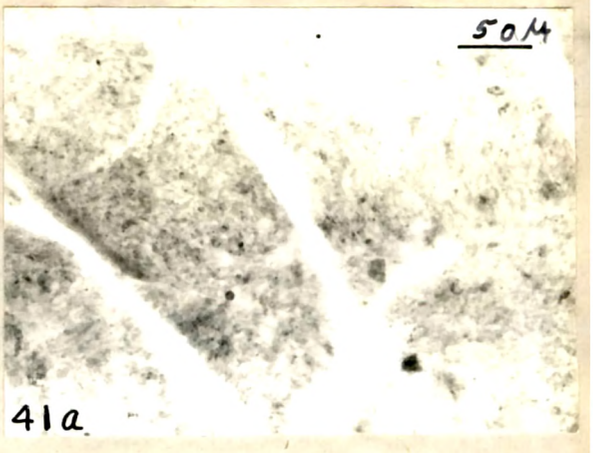


40a

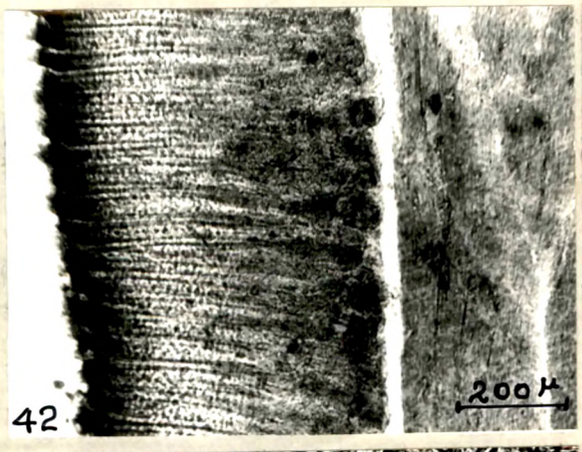
200μ

200μ

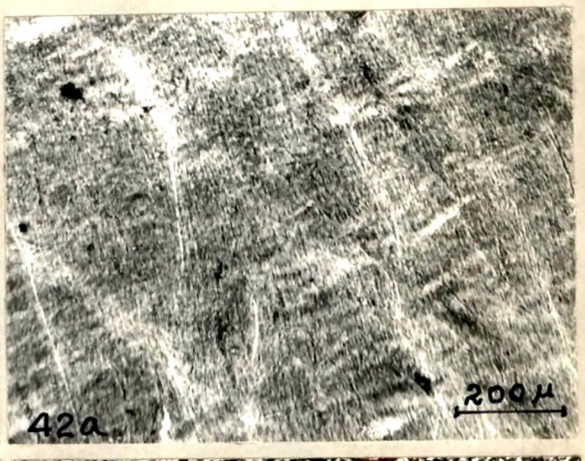
41



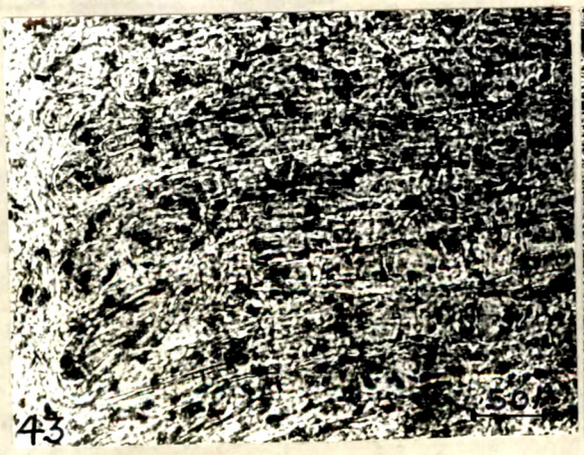
41a



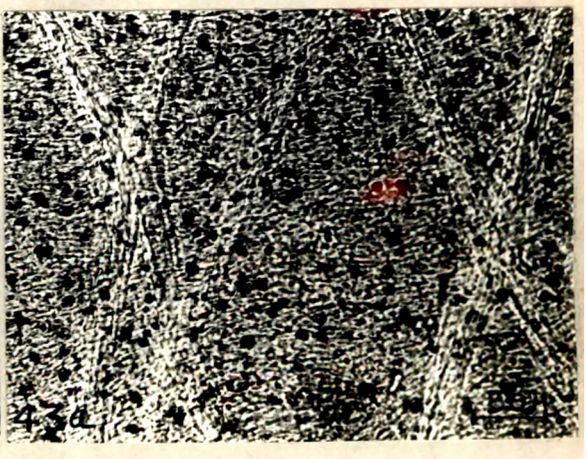
42



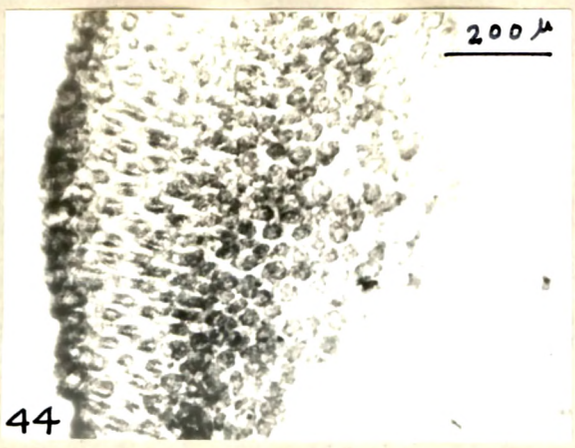
42a



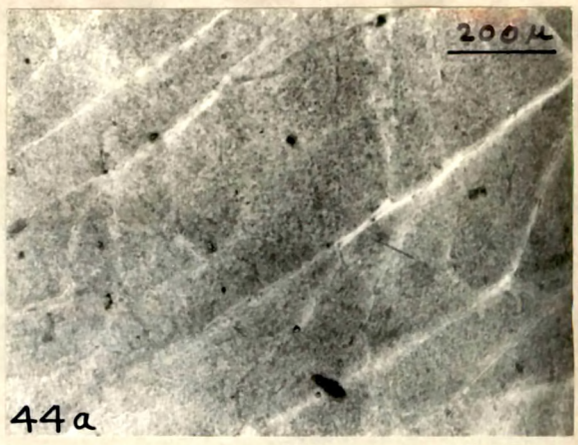
43



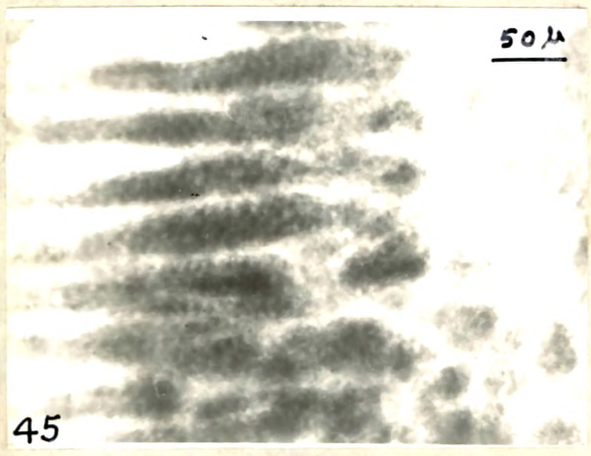
43a



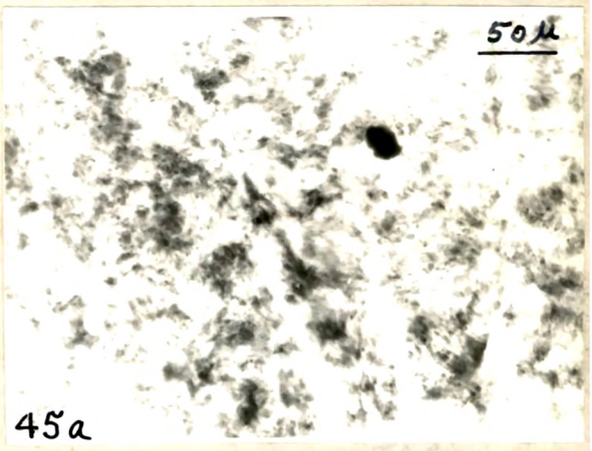
44



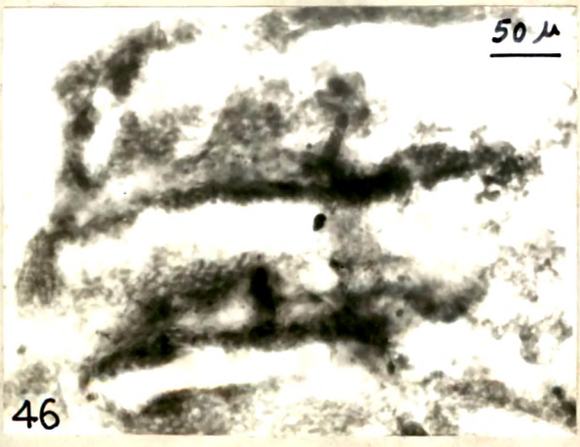
44a



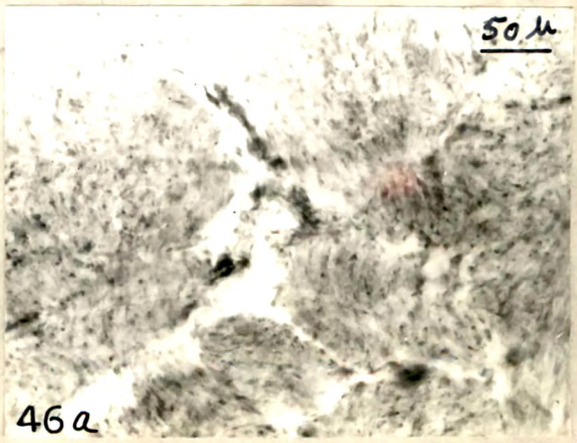
45



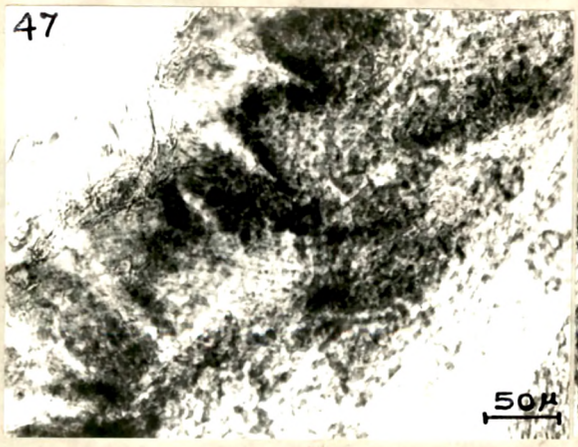
45a

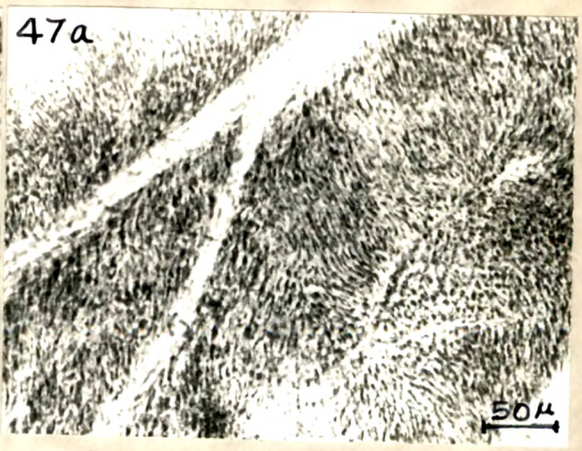


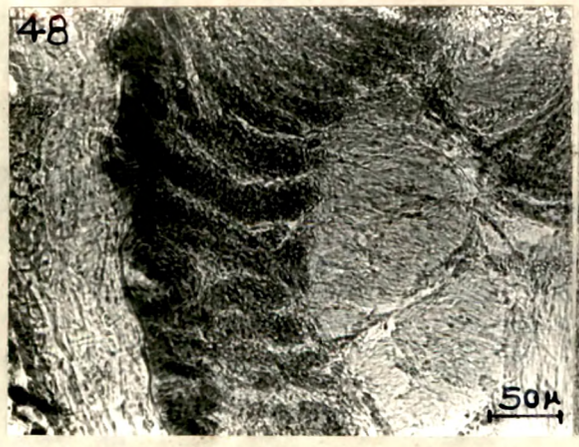
46

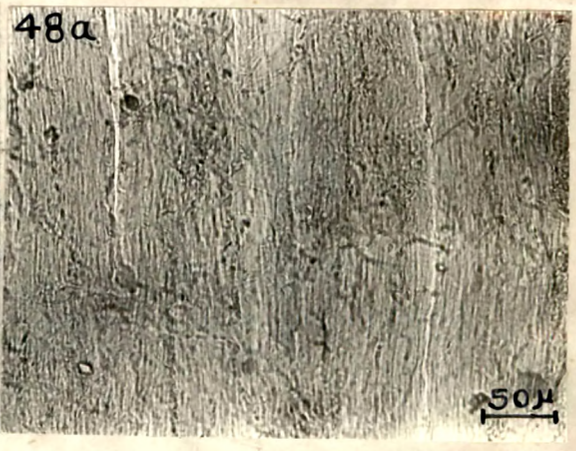


46a

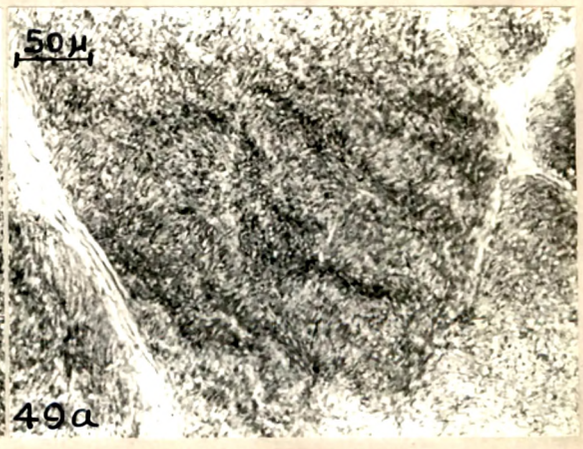

47
50μ


47a
50μ

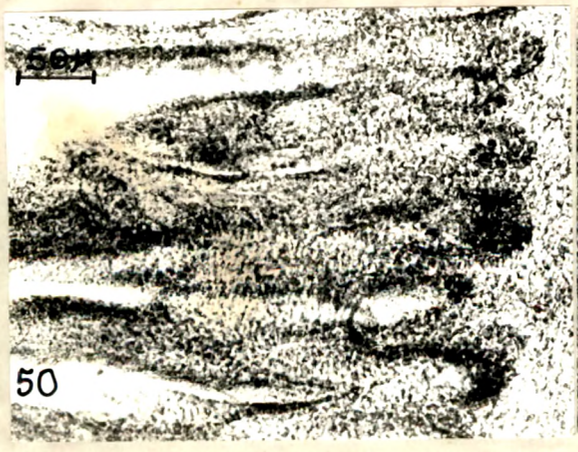

48
50μ


48a
50μ

49



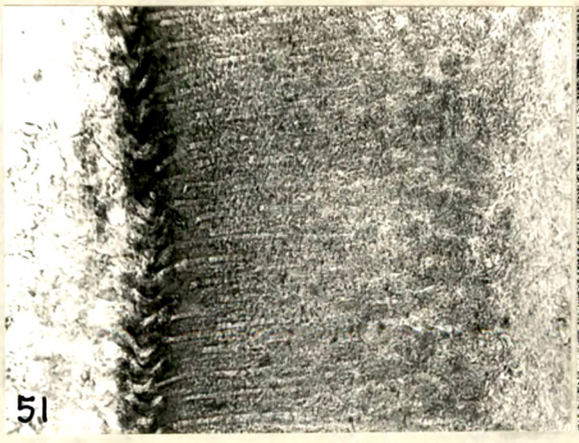
49a



50

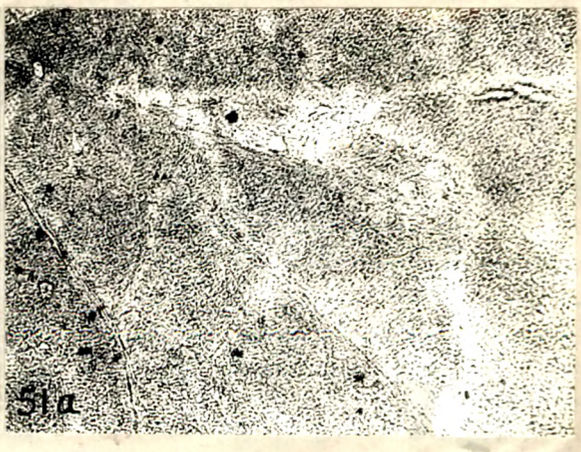
50a

200 μ

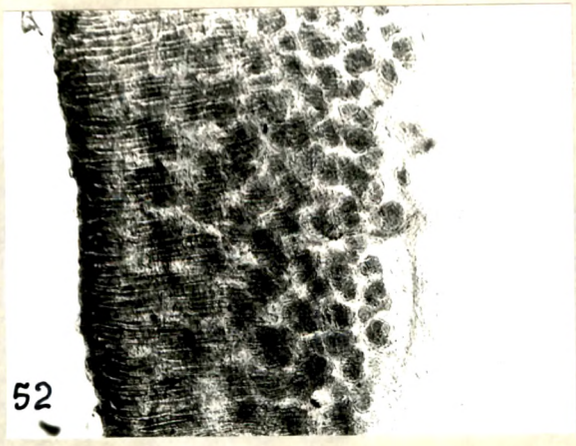


51

200 μ



51a



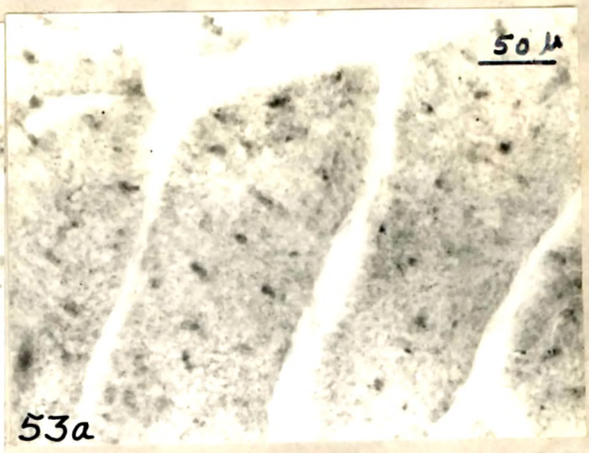
52

52a

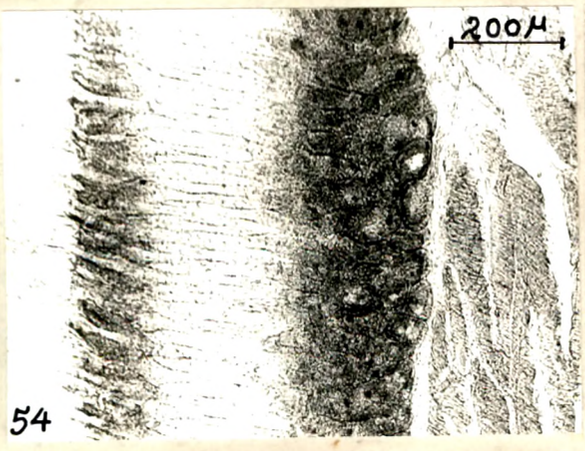
200 μ

200 μ

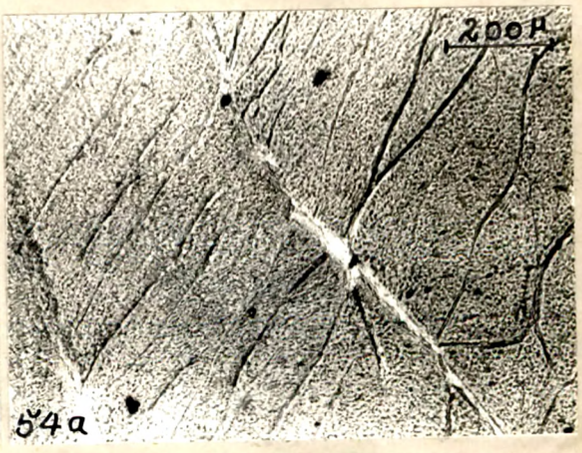
53



53a

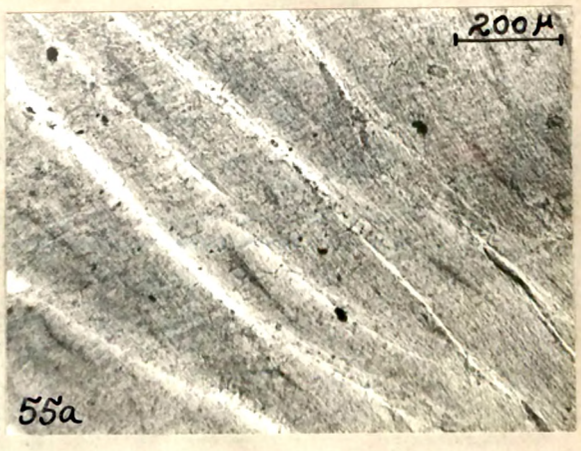


54

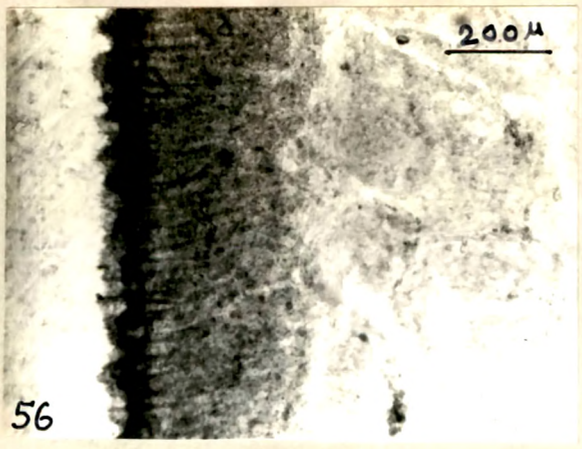


54a

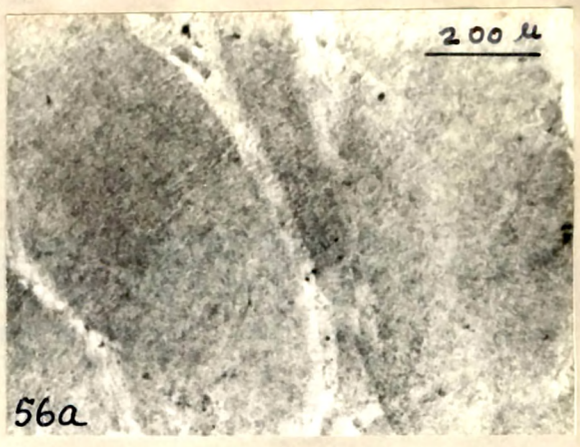
55



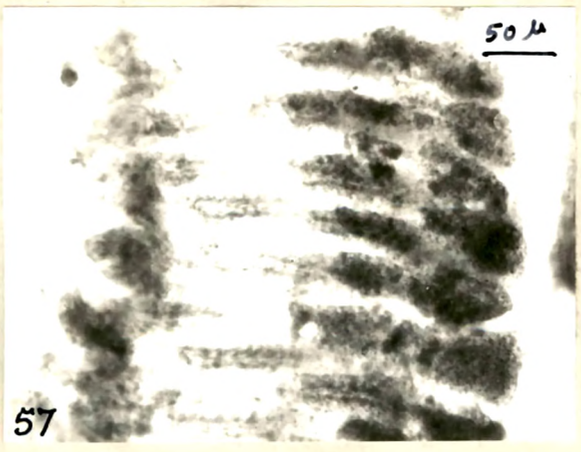
55a



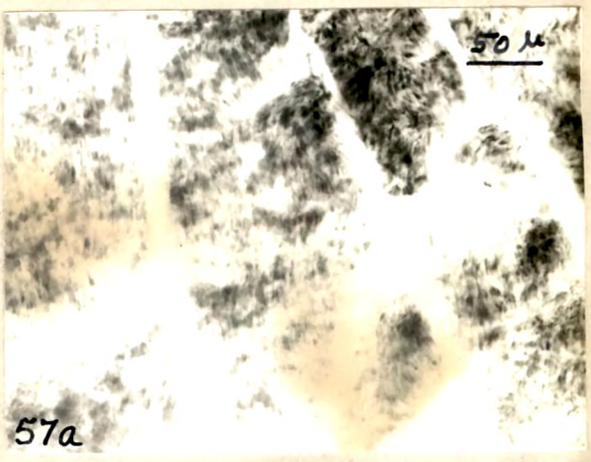
56



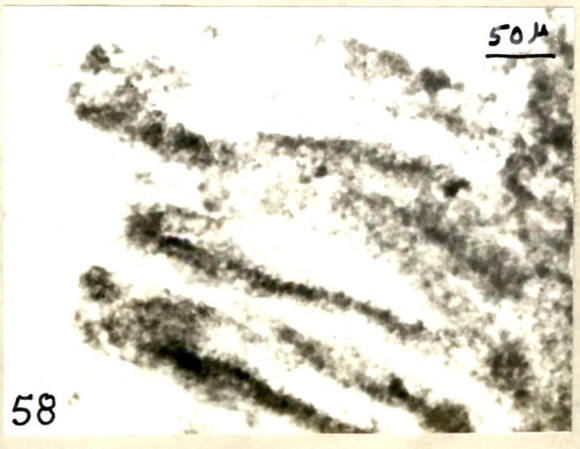
56a



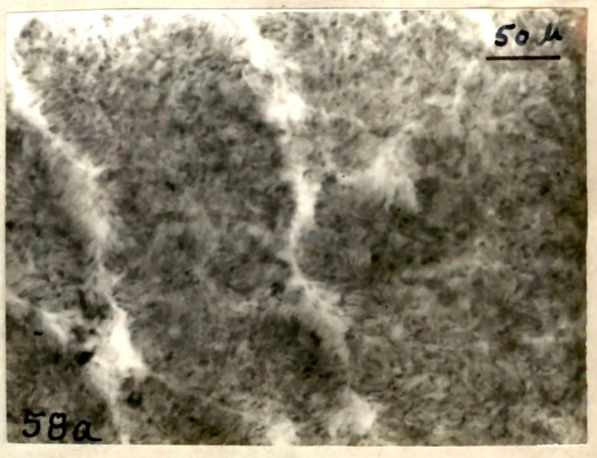
57



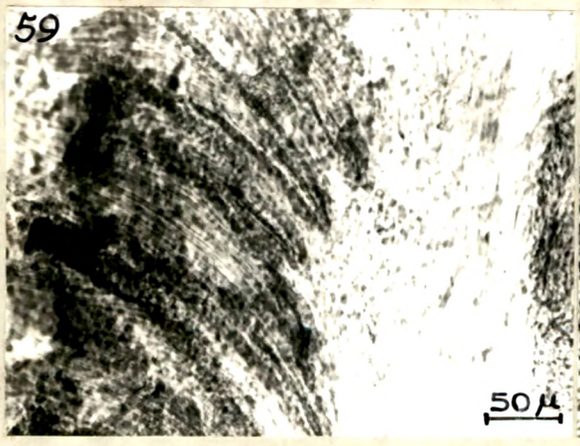
57a

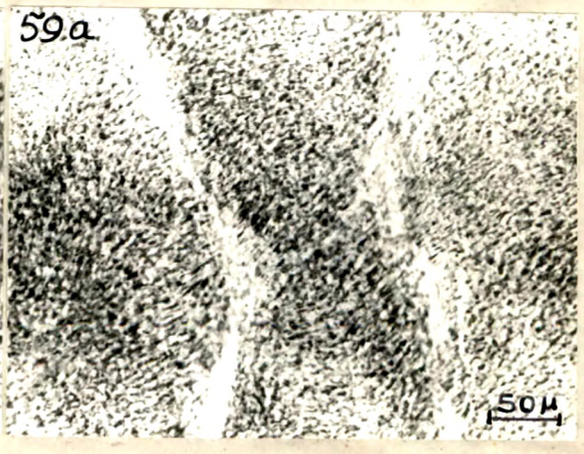


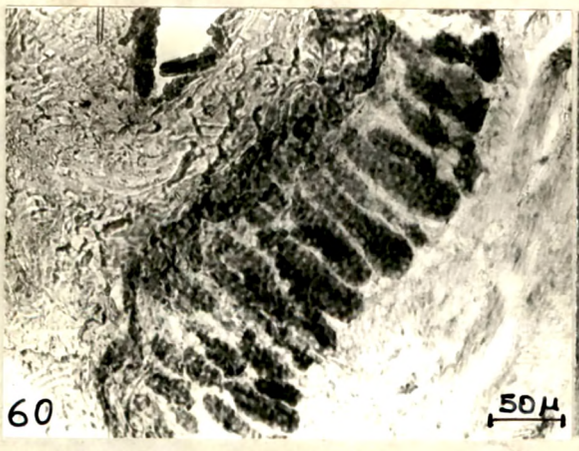
58

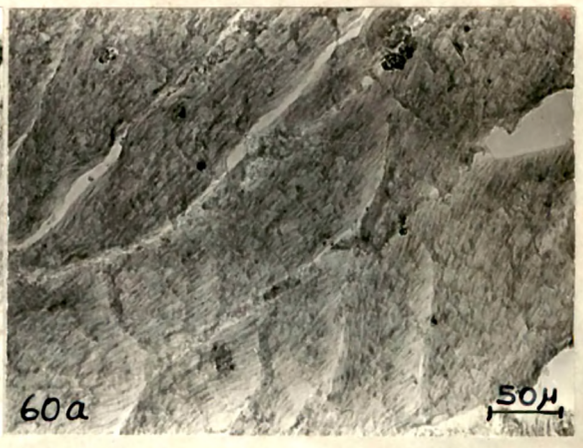


58a


59
50μ

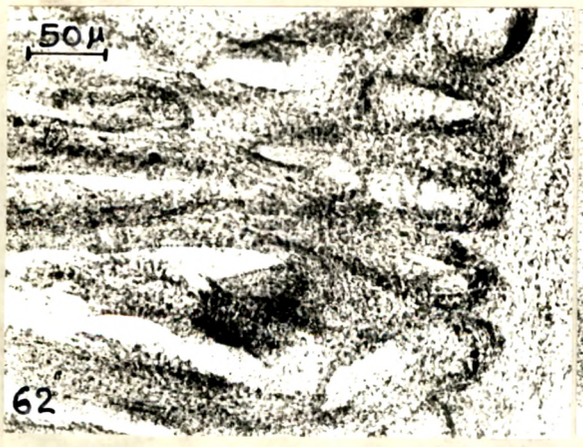

59a
50μ


60
50μ

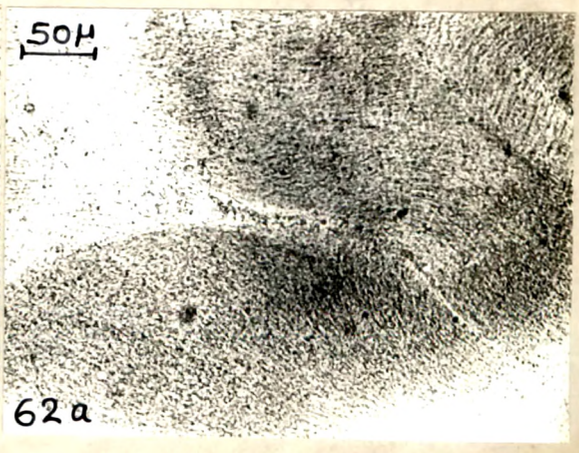

60a
50μ

61

61a

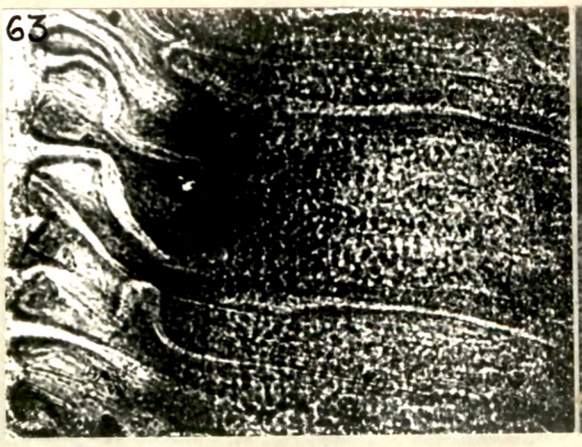


62

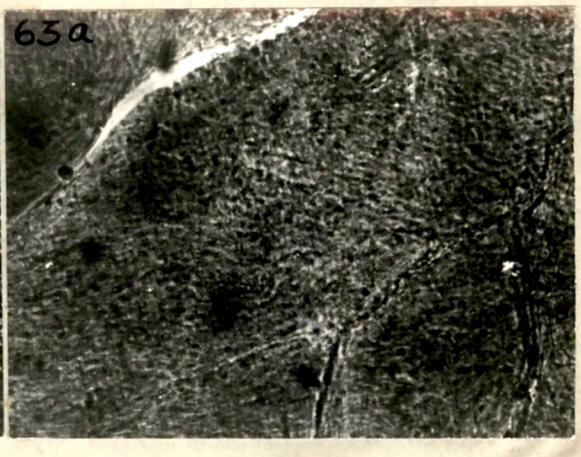


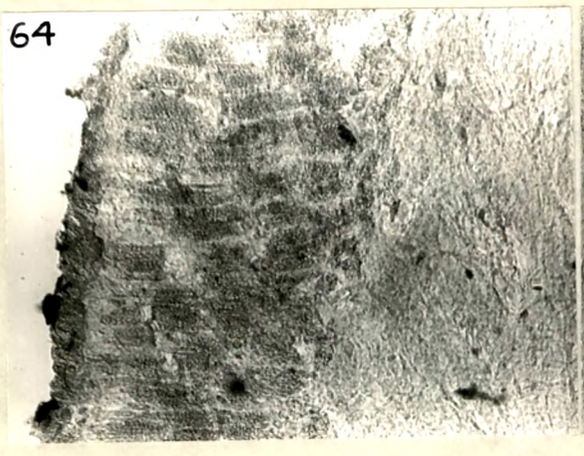
62a

100 μ



100 μ

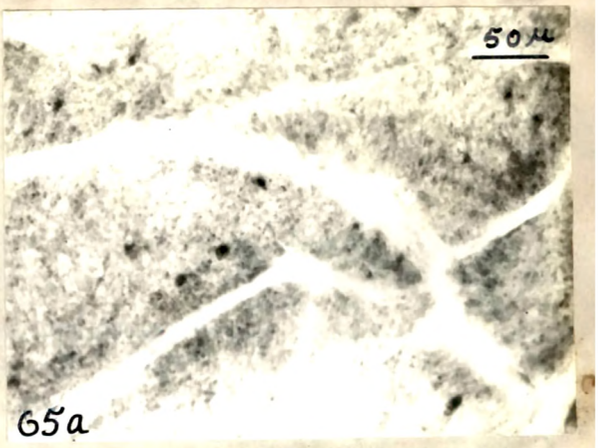




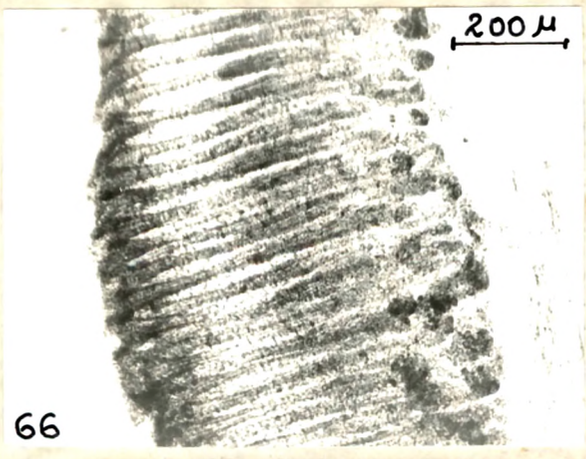
200 μ

200 μ

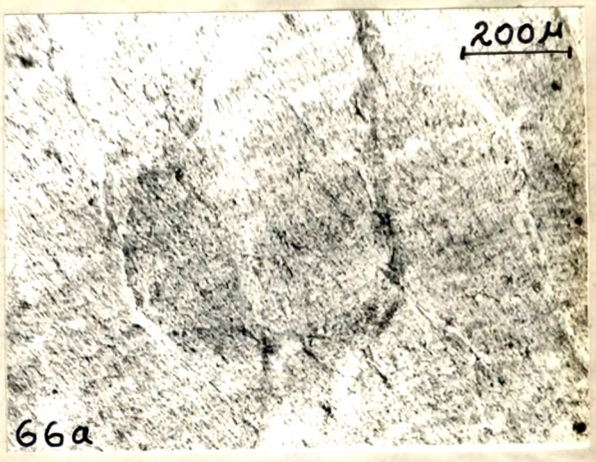
65



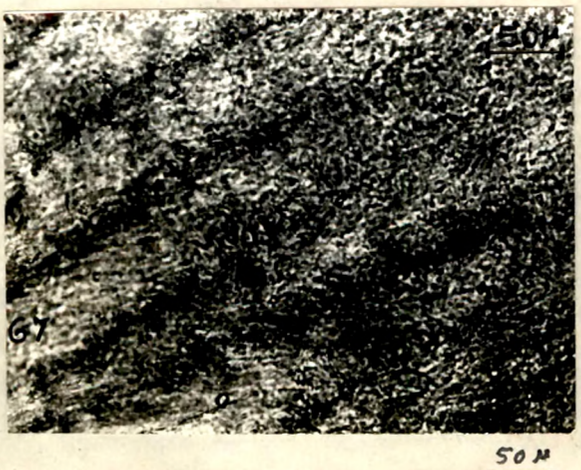
65a



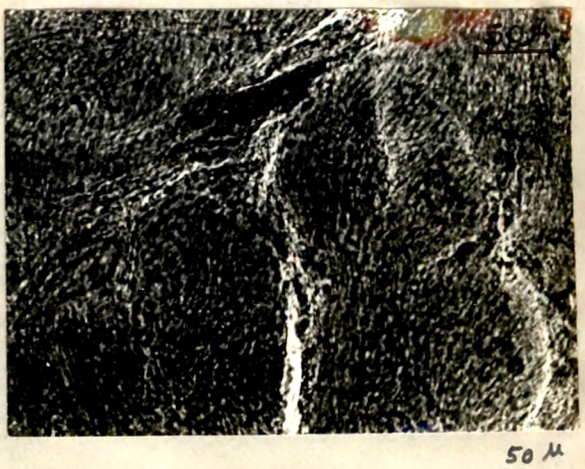
66



66a

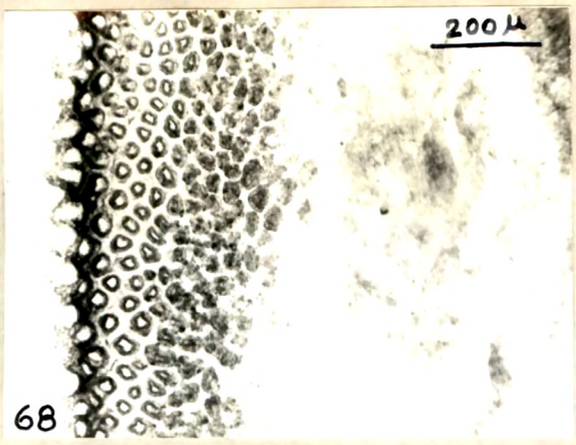


67

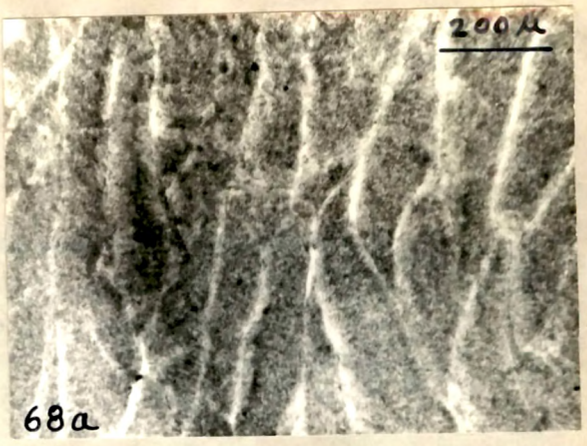


50 μ

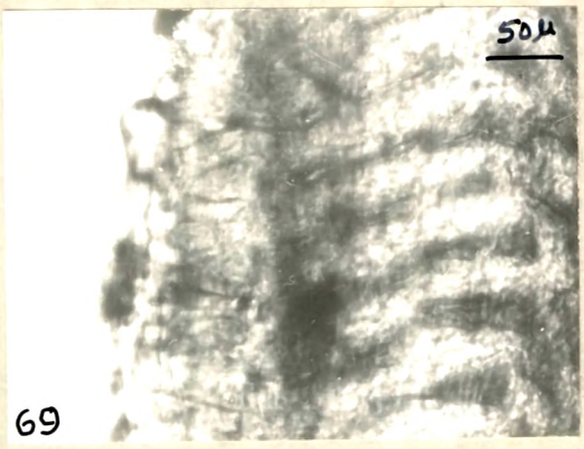
50 μ



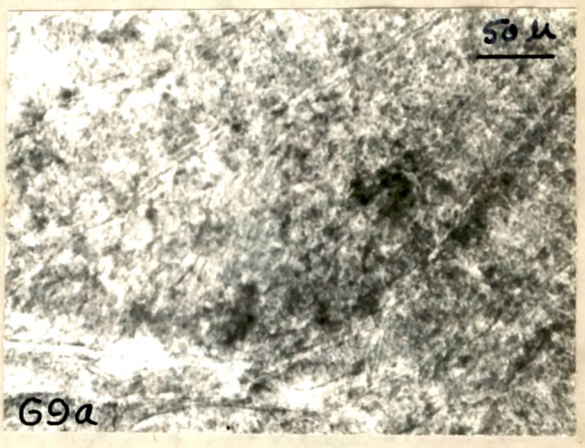
68



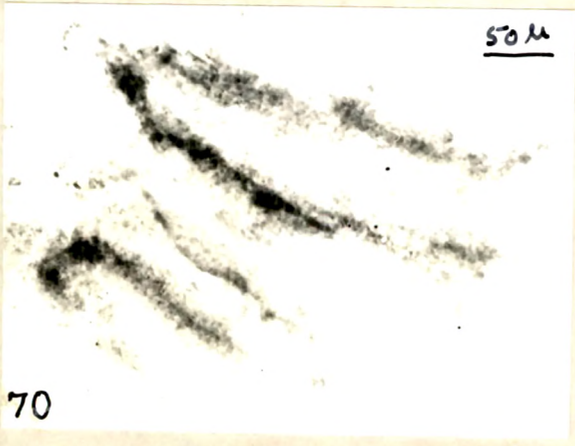
68a



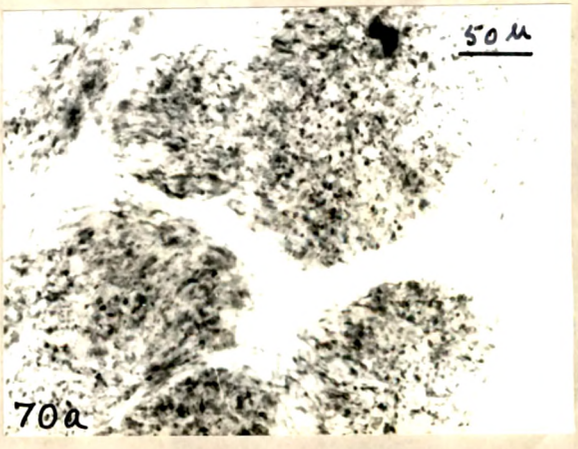
69



69a

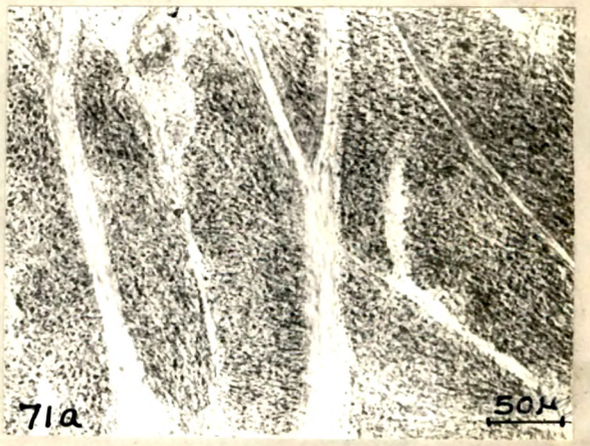


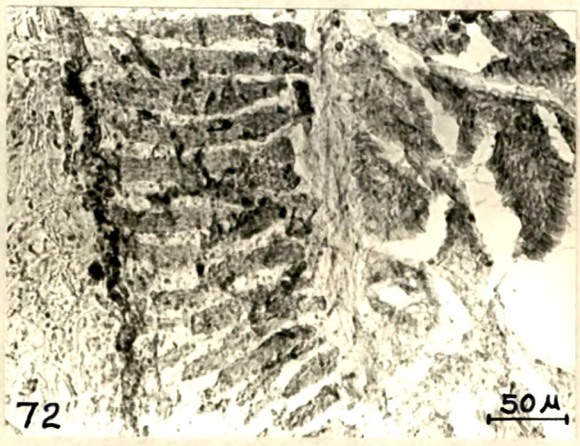
70



70a


71
50μ


71a
50μ


72
50μ

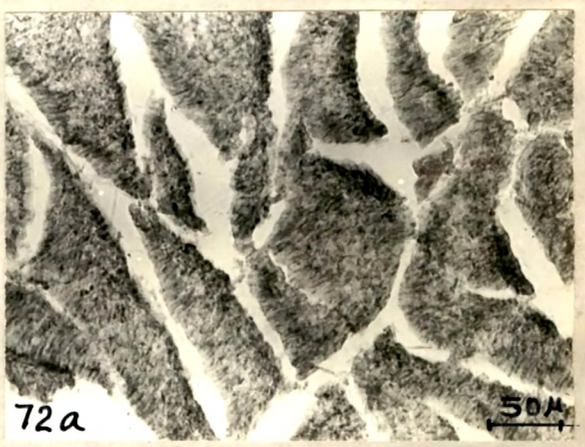

72a
50μ

TABLE I SHOWING THE LEVELS OF TOTAL LIPIDS EXPRESSED IN RELATION WITH BODY AND GIZZARD WEIGHTS OF ADULT REPRESENTATIVE BIRD*

Birds	Body weight	Gizzard weight	Amount of lipids (gm/100 gm wet tissue)
Blue rock pigeon	310	5.581	2.980
Shrike	40	1.163	4.132
Pariah kite	980	4.90	3.6986
Brahminy myna	31	0.9717	3.806
House crow	268	4.576	2.888
Jungle crow	407	5.225	3.149
Domestic fowl	1105	11.78	3.066
Indian robin	18	0.60	3.333
Green bee-eater	16	0.382	4.164
Drongo	45	1.483	3.935
Crow pheasent	215	3.873	4.387
Jungle babbler	48	1.1527	4.706
Redvented bulbul	40	1.273	4.114
Koel	220	3.991	3.685
Common myna	100	1.3669	3.321
House sparrow	16	0.264	4.916
Parakeet	118	2.387	2.987
Purple sunbird	07	0.193	2.2673

*Average of 5 birds

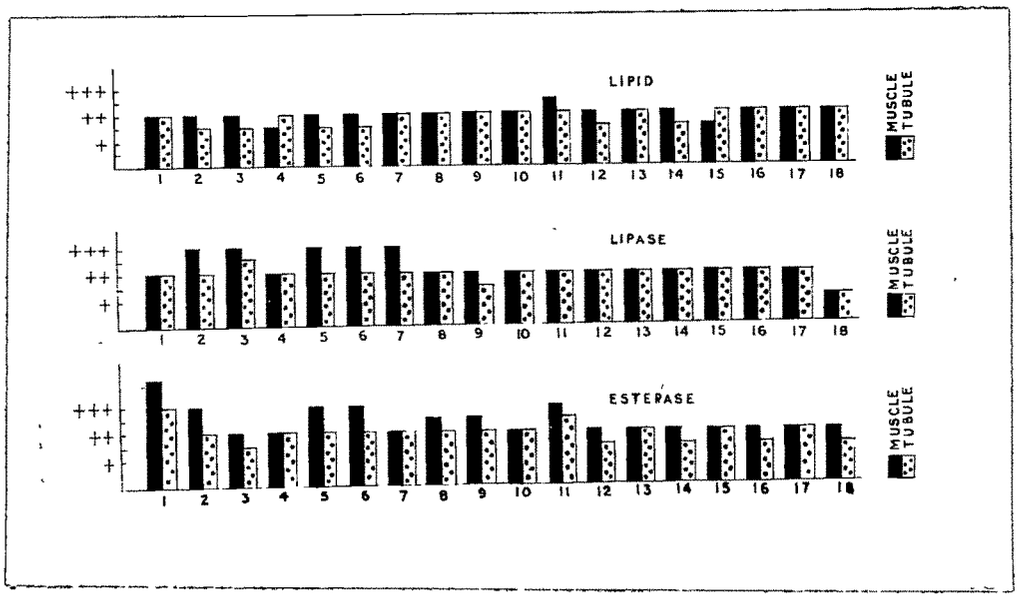


FIGURE 73

Graphical representation of the changes in lipids, lipase, and esterase distribution pattern in the gizzards of adult representative birds

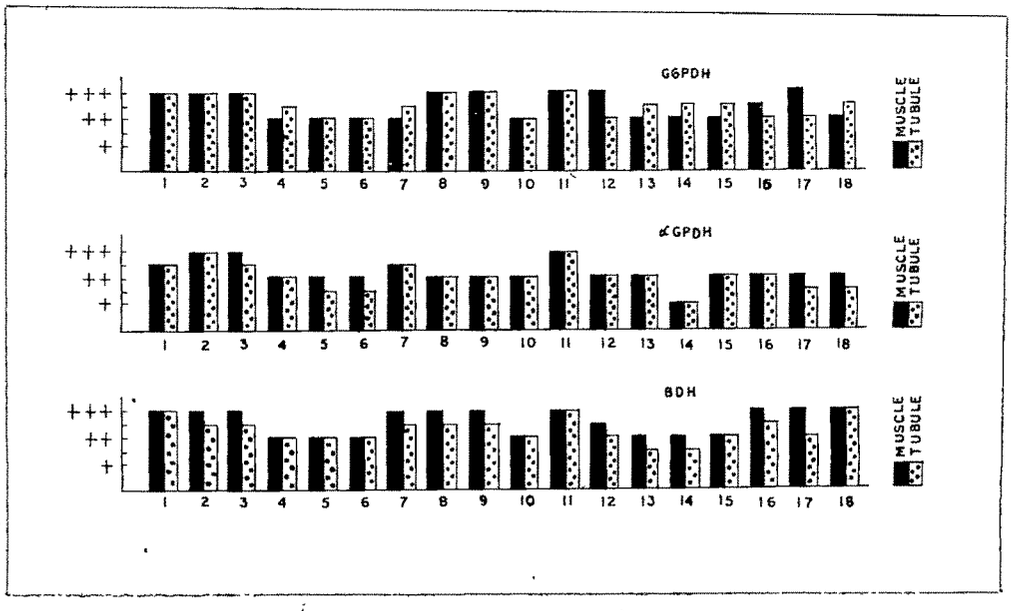


FIGURE 74

Graphical representatinn of the changes in G6PDH, αGPDH and BDH distribution pattern in the gizzards of adult representative birds

noticeable feature was the activity of G6PDH which in brahminy myna and fowl seems to be slightly more in the secretory tubules than in the muscle fibres of their gizzards.

Histochemically observed concentrations of the metabolite and enzymes in the gizzards of various adult representative birds are diagrammatically represented in figs. 73 and 74.

DISCUSSION

The present histochemical and quantitative studies on lipids in the gizzards of various adult representative birds have revealed the fact that there is an appreciable concentration of this metabolite in both the secretory tubules as well as the smooth muscle fibres of the gizzard. In general, the gizzards of all the birds studied herein are found to respond more intensely towards lipid staining in comparison to glycogen. Lipid splitting enzymes lipase and esterase elicited a stronger responses in the case of granivores and carnivores and nectar feeder at the bottom.

It is well established from the biochemical and histochemical observations on red and white skeletal

muscles of vertebrates that the red narrow fibres are suitably well adapted for aerobic metabolism while white broad fibres are better adapted for anaerobic metabolism (George & Berger, 1966). It is also known that the predominant metabolite in the red muscle fibres is fat whereas in the white muscle fibres it is glycogen. It was shown by Weinhouse et al. (1950) that pigeon breast muscle is capable of metabolizing fat. George and Jyoti (1957) studied relative reduction in the fat and glycogen content in an exercised pigeon pectoralis muscle and estimated that over 70% of the energy expended is derived from fat. In the present study a comparatively higher content of fat than glycogen might, probably, be indicative of the former being metabolized more efficiently for energy yield than the latter by the gizzards of the adult birds.

If fat is to be utilized for energy, it has to be, first of all, broken down to fatty acids and glycerol so that the fatty acids can readily undergo further oxidation. George & Talesara (1962) demonstrated that the pigeon breast muscle is capable of direct oxidation of triglycerides and therefore suggested that fats will have to be acted upon first by the muscle lipase. Muscle lipase capable of hydrolyzing glycerides of long chain

fatty acids has been localized in the mitochondria of skeletal muscles of fishes (Bokdawala & George, 1964). In a review on muscle lipase George (1964) has suggested that another lipase which acts on short chain fatty acids is present in the microsomes. The function of microsomal lipase has been attributed to the esterification of fatty acids and perhaps also to effect the splitting up of fat into fatty acids and glycerols thereby rendering the products of hydrolysis suitable for oxidation by mitochondria. A moderate activity of both these fat splitting enzymes noted in the present study, thus, could be taken as a clear cut indication of both long as well as short chain fatty acids being actively catabolized in the adult avian gizzards.

The glycolytic pathway and tricarboxylic acid (TCA) cycle are the important routes whereby the organic food stuffs are completely metabolized. However, the hexosemonophosphate (HMP) shunt pathway is also another useful one which serves not only as a supplimentary pathway but also aids in the biosynthesis of lipids. The operation of such a pathway whereby glucose at the level of glucose-6-phosphate is directed towards the formation of pentose

sugar and reduced $NADPH_2$ could well be visualized by the presence of G6PDH. The main feature of this shunt pathway is the production of $NADPH_2$ which is required for the synthesis of lipids. A type of synergistic relationship between the HMP shunt and lipid biogenesis could, thus, be perceived by which the shunt pathway provides $NADPH_2$ for the process of lipid synthesis which in turn regnerates NADP for the continued operation of shunt pathway. The activities of G6PDH, GGPDH and BDH were found to be noticeably high in the gizzards of all the groups of birds studied, though variations in their concentrations could be observed when the birds were taken individually for recording the activity. Both the mucosal tubules as well as the smooth muscle fibres depicted pronounced G6PDH activity in all the birds used in the present study. Nene and George (1965) in certain vertebrate muscles, Cherian (1967) in the pigeon breast muscle, Bokdawala and George (1967) in the fish skeletal muscle and Shah and Ramachandran (1972) in the regenerating caudal muscles of reptiles have also reported high G6PDH activity. The presently noted high activity of G6PDH is in perfect accordance with the presence of lipids in the adult avian gizzard. It is also worthwhile to note in

this connection the work of Abraham and Chaikoff (1959), Glock and McLean (1954), Abraham et al. (1954), and Levy (1961) who showed high G6PDH activity and the significant observations of Beaconsfield and Carpi (1964a) and Beaconsfield and Reading (1964b) who have shown that the level of G6PDH runs parallel with increase in the nucleic acid synthesis and that of Muscatello & Anderson-Cedergren (1961) who reported very high protein synthesis in conjunction with high G6PDH activity in the sarcotubular fraction of the frog skeletal muscle.

The observation noted herein revealed a high activity of both α GPDH and BDH in both the components of the gizzard. A prominent α-GPDH activity corresponds well with the similar activity of aldolase (chapter 6) and could be suggestive of the continual operation of glycolytic pathway and the prominent role of α -GPDH in it. A highly active α-GPDH in the gizzard tissue is suggestive of a significant α-glycerophosphate based metabolism as suggested by Ramachandran (1972) in the normal and regenerating reptilian tail. The operation of glycolysis as evidenced by the presence of aldolase (chapter 6) readily yields a continuous supply of dihydroxyacetone phosphate, an intermediary product which

is also the principal substrate for α-GPDH catalysis forming α-glycerophosphate, an important substrate much needed for the synthesis of triglycerides and phospholipids (Kornberg & Price, 1953; Rossiter et al., 1957; Kennedy, 1953, 1954, 1957a, 1957b). Kennedy (1957a & b) has not only identified α-GPDH as one of the most important enzymes in phospholipogenesis but also emphasized the glycolytic pathway as the most potent source of α-glycerophosphate. The increased activity of BDH noted herein is indicative of an active lipid catabolism and its oxidation via TCA cycle. The presently observed moderate activities of lipase and esterase and considerable activities of SDH and MDH (chapter 6) in the adult gizzards lend further support to the above contention. It is also pertinent to note here a similar high BDH activity in the pigeon breast muscle and fish skeletal muscle (Cherian, 1967 and Bokdawala & George, 1967 respectively) where lipid catabolism is known to be high. A similar observation on α-GPDH and lipids are made by Shah & Magon (1969) and Shah & Chakko (1967) respectively in the regenerating tail tissues of the wall lizard, Hemidactylus flaviviridis. However, the very high activity of α-GPDH noted in the present study may also aid in the glycerol utilization

through glycolytic pathway or even in the gluconeogenesis as per time and need of the organ. Again, another aspect which has to be kept in mind is the possible utilization of fatty acids from the blood stream in the light of the herein observed not so high but uniform content of lipid in the gizzards.

Finally, in general, it could be noted that though all types of gizzards are capable of lipid catabolism, the extent of its utilization appears to show a gradation as per the type of food ingested by the various groups of birds. In this respect the gizzards of granivores and carnivores appear to be more dominant in that order as their food consists of grains and flesh respectively which are comparatively much harder and tough, thus demanding active muscular action. From the figures and table it becomes clear that the granivores catabolize more of short chain fatty acids and carnivores both long as well as short chains equally well, nevertheless both the groups have equal level of lipid anabolism (in the light of equal G6PDH activity). In contrast, the frugivore and nectar feeder seem to be at the opposite end in the grade of lipid metabolism keeping in good conformity with the soft

nature of their food; the gizzards of these groups having a greater tendency to utilize circulating free fatty acids as could be visualized from the higher activities of lipase and esterase but low level of muscle lipids. The omnivores and insectivores in that order, tend to take up an intermediary position between these two extremes as the texture of the food materials consumed by them also appear to be of an intermediary nature to those of granivores and carnivores on one hand and the frugivores and nectar feeder on the other. It may also be noted here that this pattern of grading the gizzards of various groups of adult birds appears to gain further validity when taken together with ~~them~~ the degree of carbohydrate metabolism (chapter 6).