CHAPTER 3

LIPID METABOLISM IN THE DEVELOPING PIGEON GIZZARD: A QUANTITATIVE EVALUATION OF TOTAL LIPID CONTENT AND HISTOCHEMICAL DEMONSTRATION OF LIPIDS AND ASSOCIATED ENZYMES SUCH AS LIPASE, ESTERASE; GLUCOSE-6-PHOSPHATE, α-GLYCEROPHOSPHATE AND β-HYDROXYBUTYRATE DEHYDROGENASES

Lipids, being of high energy value, serve as important metabolites ~~source~~ for various energy linked reactions in animal tissues. The role of fat as the major fuel reserve for energy during long and sustained activity has been well established in the flight muscle of birds (George & Jyoti, 1955, 1957) and bats (George & Jyoti, 1958); fishes (George, 1962; Billinski, 1963) and muscular organs like vertebrate heart (Bing et al., 1954; George & Iype, 1963) and mammalian diaphragm (George & Susheela, 1961). George and Jyoti (1957) have shown that about 77% of muscle metabolism in pigeon during sustained activity is due to the oxidation of fat. Studies on different tissues, particularly those on skeletal muscle of various vertebrates, have proved the existence of glycolytic cycle, hexose monophosphate (HMP) shunt pathway and tricarboxylic acid (TCA) cycle as the energy yielding pathways. The shunt pathway is the major metabolic pathway operating quite actively in the adipose tissue and embryonic cells. The existence of this

pathway and its role in the metabolism of animal tissues have been clearly established by Cherurka (1957, 1958), Hoskins (1959) and Rossi et al. (1963). Glucose-6-phosphate dehydrogenase (G6PDH) is an enzyme which brings about the direct oxidation of glucose at the level of glucose-6-phosphate liberating abundant reduced co-enzyme II ($NADPH_2$) for the lipogenesis and also producing ribose sugar necessary for the synthesis of nucleic acids. Enzymes like α-glycerophosphate dehydrogenase (α-GPDH) and β-hydroxybutyrate dehydrogenase (BDH), the activities of which are related to lipid metabolism, have been studied by a number of workers in different tissues of vertebrates. α-GPDH catalyses the reversible reaction between α-glycerophosphate and dihydroxyacetone phosphate with co-enzyme I as an electron acceptor whereas BDH catalyses the reaction of fatty acid degradation with the synthesis of active acetate (Acetyl CoA). Studies of these enzymes together with lipids which could help in the understanding of the extent of lipid metabolism, in adult vertebrate tissues as well as in developing ones, received relatively little attention as far as the avian gizzard is concerned, especially during its post-natal development. Developmental process, as it calls for much cellular changes and organisation, intriguing metabolic changes

could be expected. Such changes might be of significance and interest especially during post-natal development as it is during this process that the tissues are undergoing fast changes in their attempt to acquire the adult pattern of structure as well as function. In this light, with an understanding already at hand regarding carbohydrate metabolism (chapter 2), it was thought pertinent to study both quantitatively as well as histochemically the distribution of lipids as well as histochemical demonstration and localization of enzymes such as lipase, esterase, G6PDH, αGPDH and BDH in the post-natally developing pigeon gizzard so that the role and extent of lipid metabolism could be understood. Besides, though such studies have been done extensively on skeletal and cardiac muscles, they are lacking in smooth muscle and hence the present study might be of importance and interest in an understanding of avian smooth muscle physiology, as gizzard is chiefly constituted of smooth muscles and heavily muscularised.

MATERIALS AND METHODS

Healthy young and adult pigeons from a well maintained aviary were used for the present study. Young ones of post hatching developing periods viz., 1, 5, 10, 15, 20, 25 and 30

days and adult pigeons were decapitated under mild anaesthesia and the gizzards were separated, blotted well to remove their contents, blood and other tissue fluids and fixed on a chuck of a cryostat microtome maintained at -20°C. Sections of 12 - 18 µ thickness were cut and processed as follows for the histochemical demonstration of lipids and different enzymes:

Lipids: For the histochemical demonstration of sudanophilic, neutral and acidic lipids, fresh frozen sections were fixed in 10% calcium formol for one hour. After fixation the sections were thoroughly washed in distilled water. Those sections which were to be stained for sudanophilic lipid were stained in Sudan Black B for 20 minutes and neutral lipids were stained by keeping the sections in Fettrot 7 B for 20 minutes (Pearse, 1960). Few sections were stained for 1 hour at 60°C in saturated Nile Blue sulphate as described by Cain (1947) for the localization of acidic lipids.

Lipase: For the histochemical demonstration of lipase activity the method of George and Ambadkar (1963) using 'Tween 85' (polyoxyethylene sorbitan trioleate) as the substrate was followed.

Esterase: Esterases were demonstrated according to the method of Burstone (1962) using Naphthol AS-D acetate as substrate and red violet LB as the diazonium salt.

Glucose-6-phosphate dehydrogenase: For the histochemical localization of this enzyme, fresh frozen sections of 12-18 μ thickness were cut and incubated for 20 minutes in a medium consisting of the following ingredients as suggested by Ogata & Mori (1964).

0.02 M Glucose-6-phosphate, disodium salt (substrate)	4 ml
Nitro Blue Tetrazolium (5 mg per 3 ml)	3 ml
Veronal Buffer, 0.1 M, pH 7.6	11 ml
Triphosphopyridine nucleotide (TPN)	8 mg

α-glycerophosphate dehydrogenase: The incubation medium for demonstration of this enzyme consisted of the following components as suggested by Ogata & Mori (1964).

α-glycerophosphate, sodium salt (substrate), 1 M	4 ml
Nitro Blue Tetrazolium (5 mg per 3 ml)	3 ml
Phosphate buffer, 0.1 M, pH 7.6	11 ml
Diphosphopyridine nucleotide (DPN)	2.5 mg
Sodium cyanide, 0.1 M	2 ml

β-hydroxybutyrate dehydrogenase: The incubation medium for demonstration of BDH consisted of the following ingredients as suggested by Ogata & Mori (1964).

β-hydroxybutyrate, sodium salt (substrate), 1 M	4 ml
Nitro Blue Tetrazolium (5 mg per 3 ml)	3 ml
Phosphate buffer, 0.1 M, pH 7.6	11 ml
Diphosphopyridine nucleotide (DPN)	2.5 mg
Sodium cyanide, 0.1 M	2 ml

The total lipids were quantitatively estimated after extracting the lipids from an oven dried material in methanol-chloroform (1:2 v/v) mixture. The amount of lipids is expressed in grams per 100 grams of wet tissue.

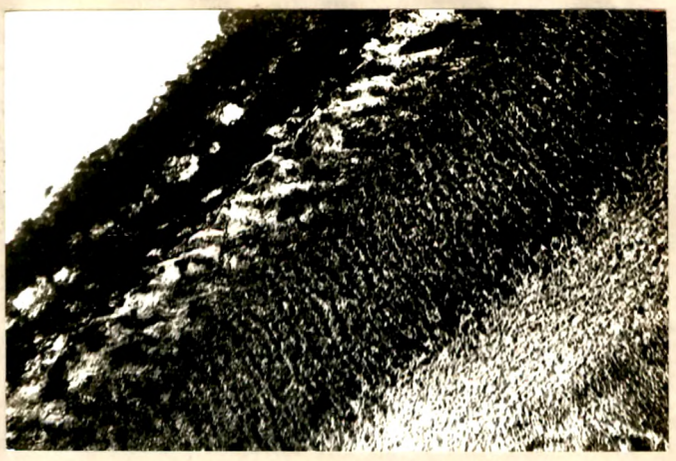
OBSERVATIONS

Lipids: (Figs. 1 to 16, 1a to 16a)

Both quantitative as well as histochemical studies showed variations in the lipid contents during the post-natal development of pigeon gizzard. From the first day after hatching till the 10th day both sudanophilic as well as acidic lipids were present in an appreciable amount in the secretory tubules of the tunica propria as well as in the muscle fibres. A slightly higher concentration of the metabolite was, however, noticed at the tips of the tubules. Sudanophilic lipids were found to register a sharp decline, hereafter, and reached the lowest level during the 15th day and remained so even on the 20th day of development. In

SUDANOPHILIC LIPID IN THE MUCOSAL TUBULES

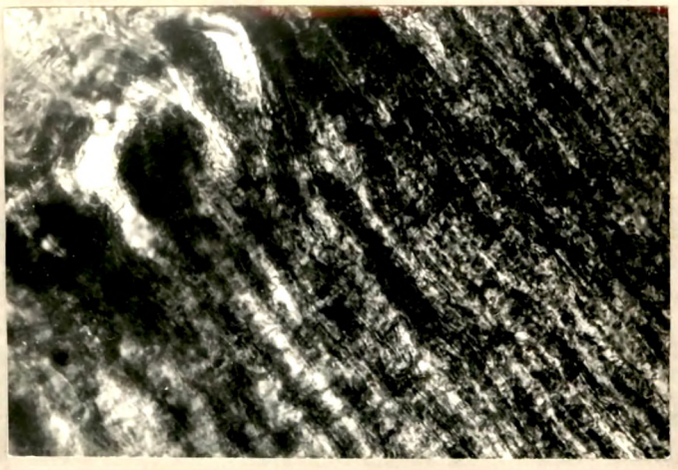
OF THE DEVELOPING PIGEON GIZZARD



200 μ

1 DAY OLD

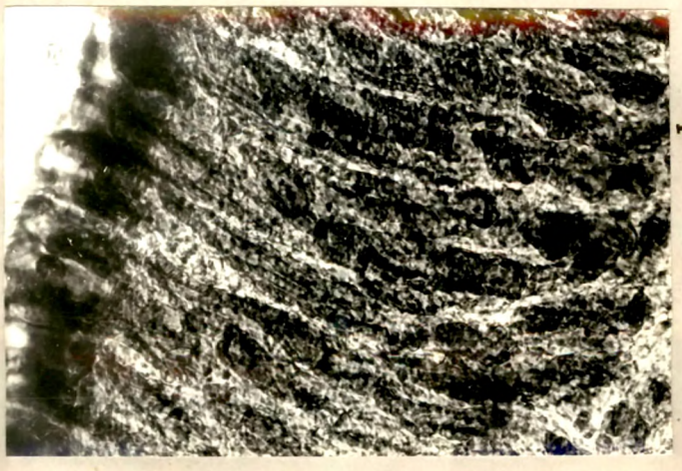
1



100 μ

5 DAY OLD

2

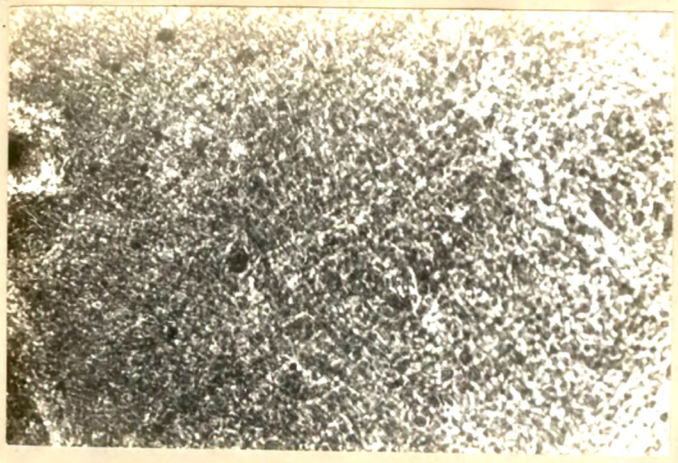


100 μ

10 DAY OLD

3

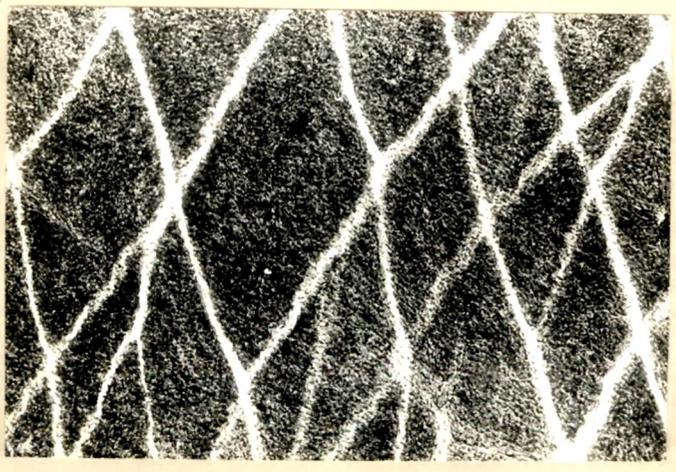
SUDANOPHILIC LIPID IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

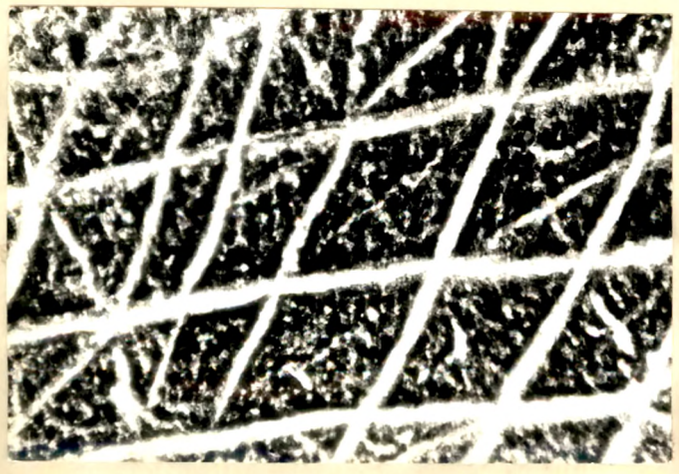
1a



200 μ

5 DAY OLD

2a



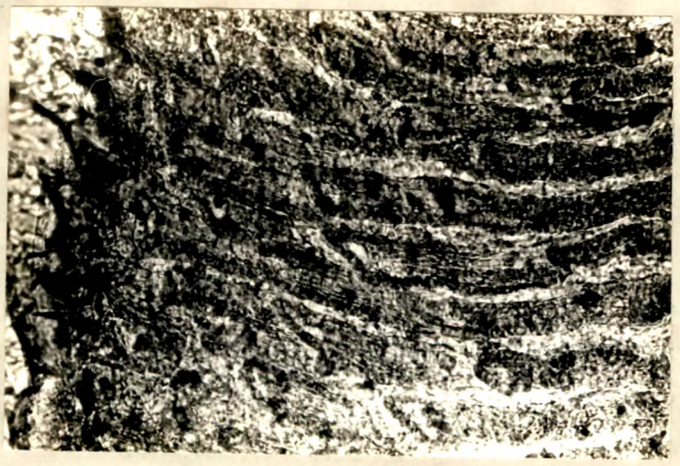
200 μ

10 DAY OLD

3a

SUDANOPHILIC LIPID IN THE MUCOSAL TUBULES

OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

4

100 μ

20 DAY OLD

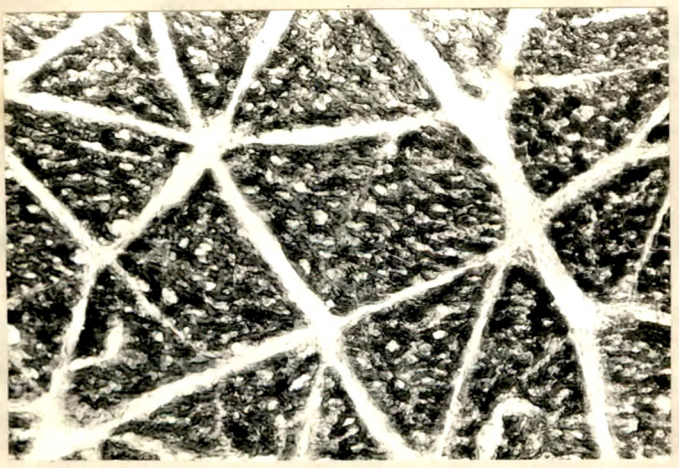
5

100 μ

25 DAY OLD

6

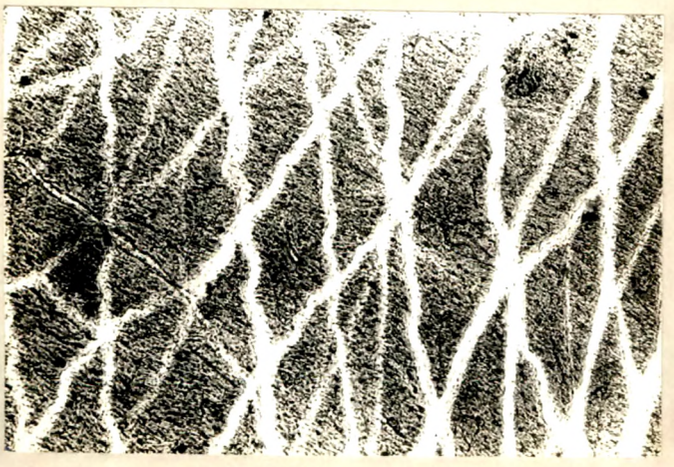
SUDANOPHILIC LIPID IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

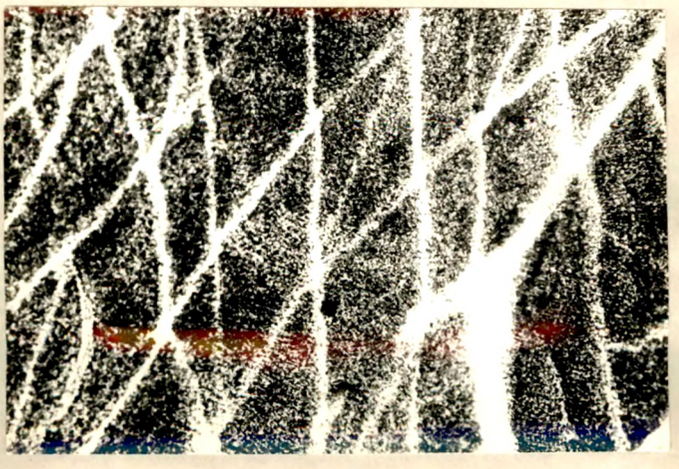
4 a



200 μ

20 DAY OLD

5 a

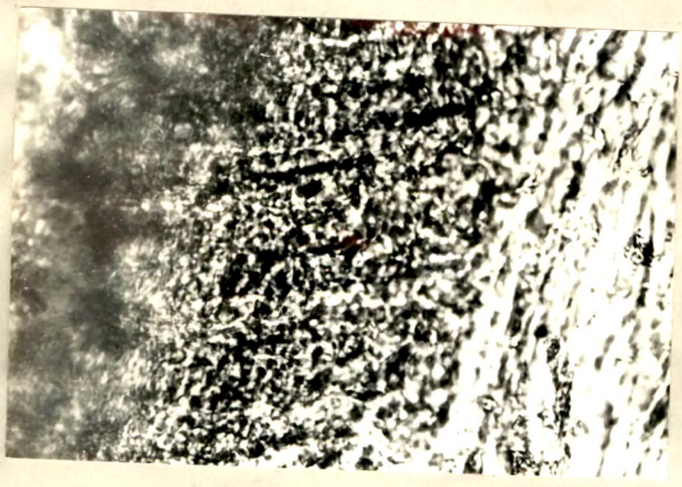


200 μ

25 DAY OLD

6 a

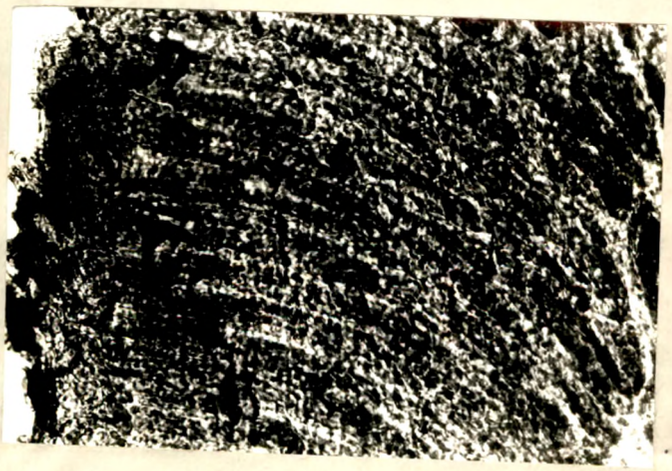
SUDANOPHILIC LIPID IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

7

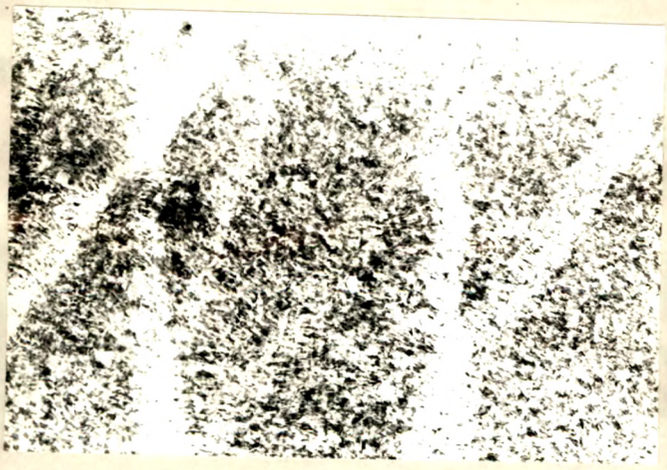


100 μ

ADULT

8

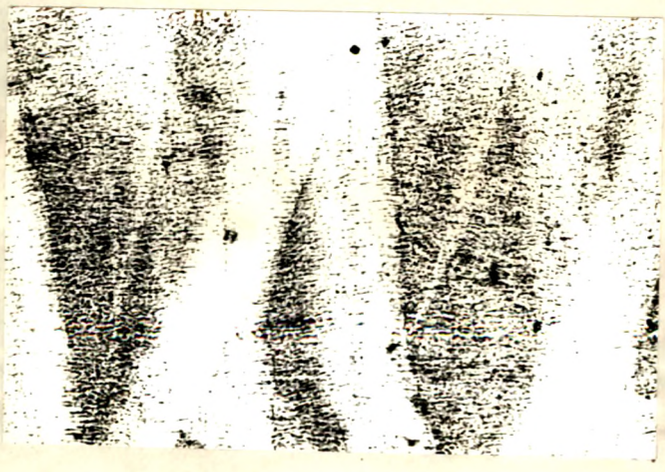
.SUDANOPHILIC LIPID IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



200. μ

30 DAY OLD

7a



200 μ

ADULT

8a

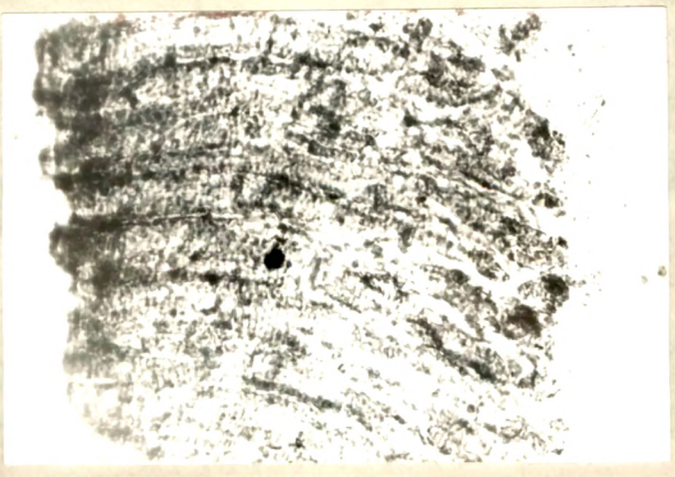
ACIDIC LIPID IN THE MUCOSAL TUBULES

OF THE DEVELOPING PIGEON GIZZARD

100 μ

1 DAY OLD

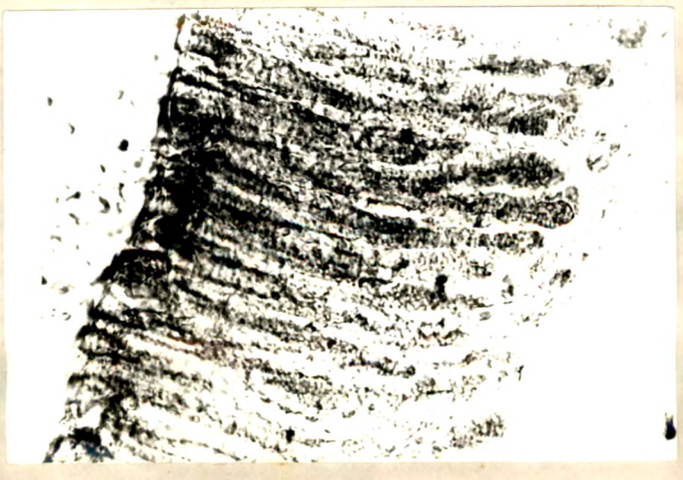
9



100 μ

5 DAY OLD

10



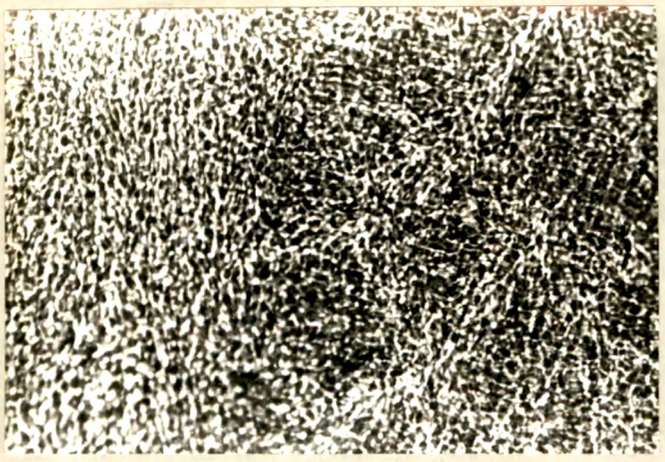
100 μ

10 DAY OLD

11

ACIDIC LIPID IN THE SMOOTH MUSCLE FIBRES

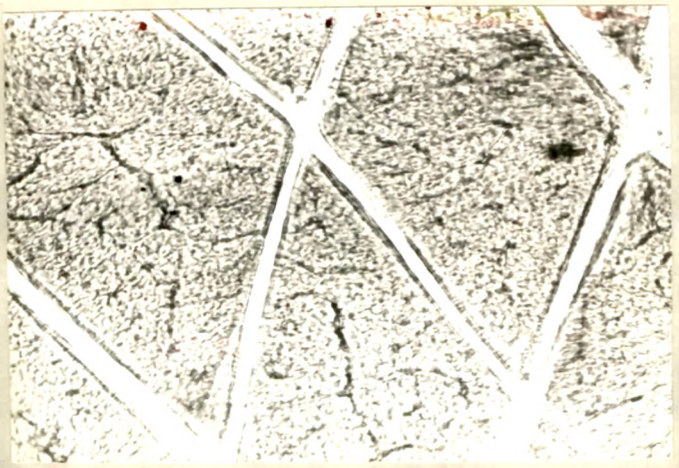
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

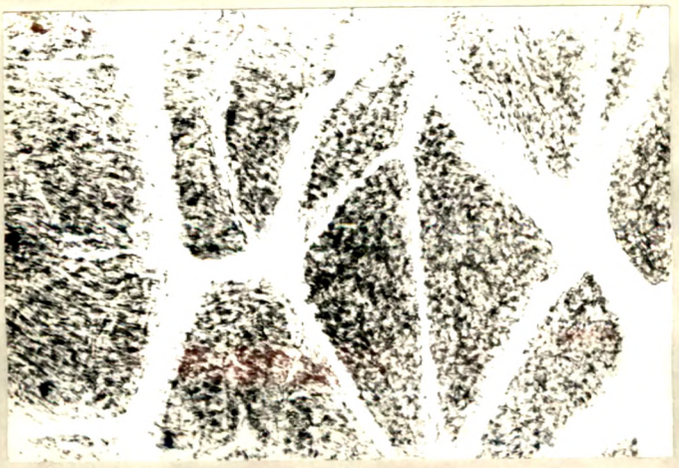
9a



200 μ

5 DAY OLD

10a



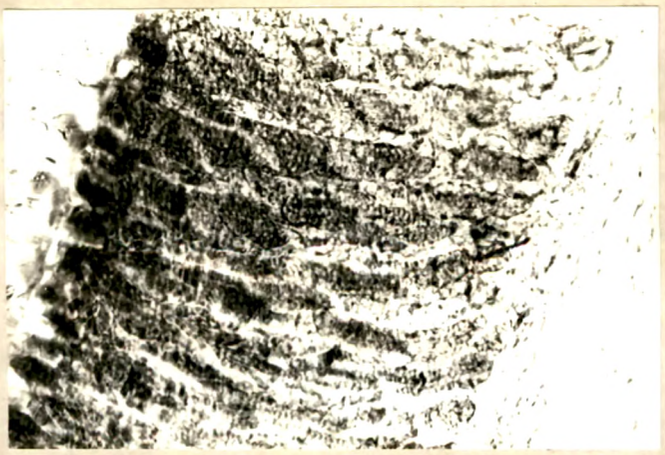
200 μ

10 DAY OLD

11a

ACIDIC LIPID IN THE MUCOSAL TUBULES

OF THE DEVELOPING PIGEON GIZZARD



100 μ

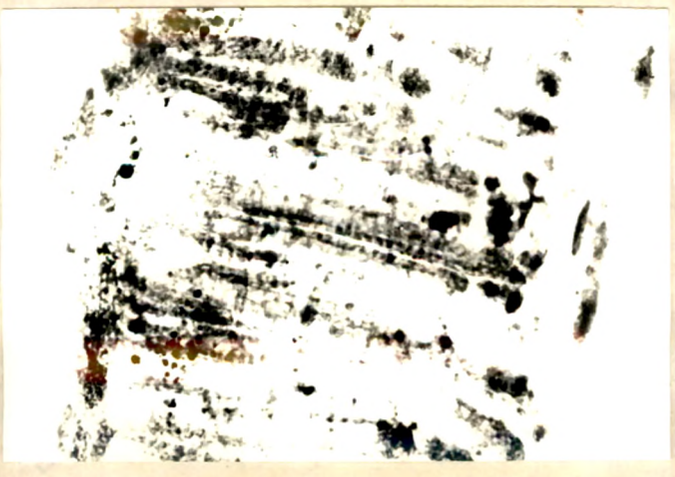
15 DAY OLD

12

100 μ

20 DAY OLD

13



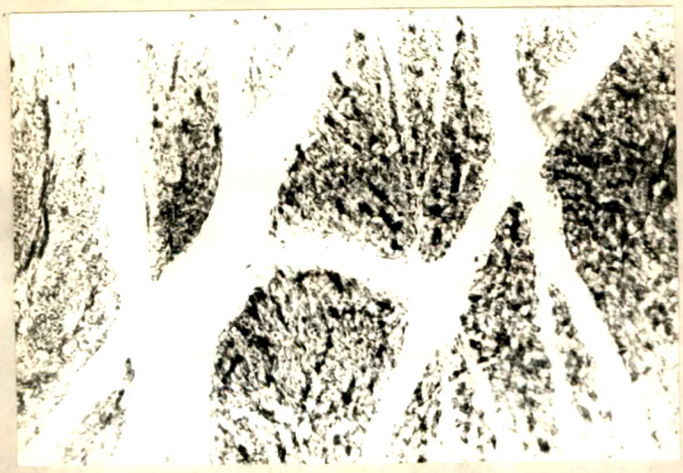
100 μ

25 DAY OLD

14

ACIDIC LIPID IN THE SMOOTH MUCSLE FIBRES

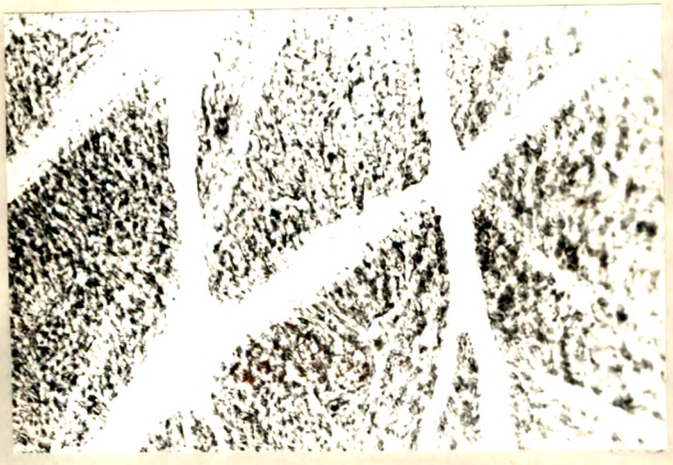
OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

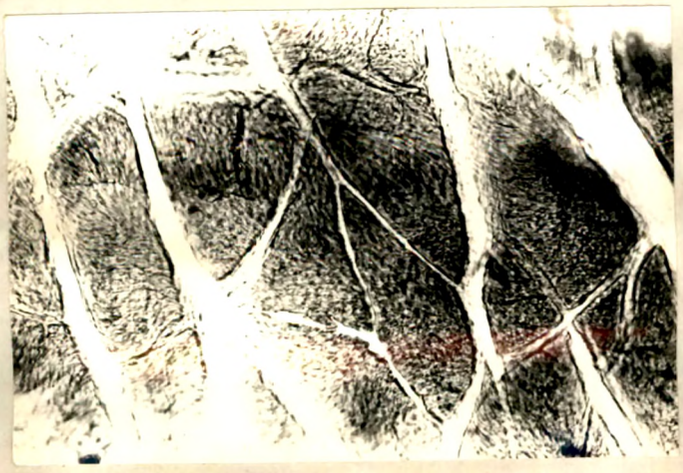
12a



200 μ

20 DAY OLD

13a

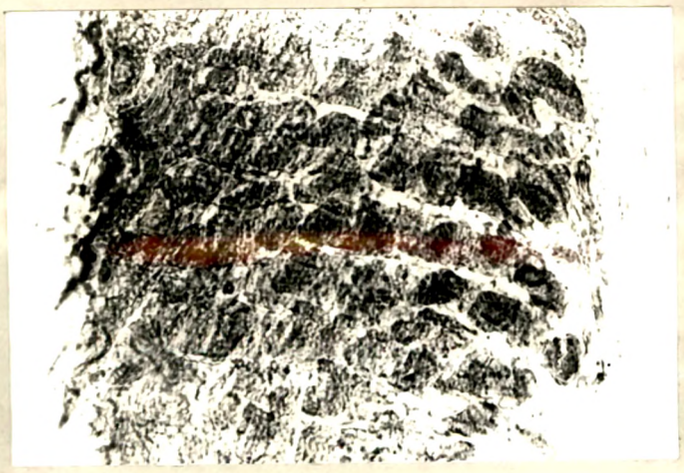


200 μ

25 DAY OLD

14a

ACIDIC LIPID IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

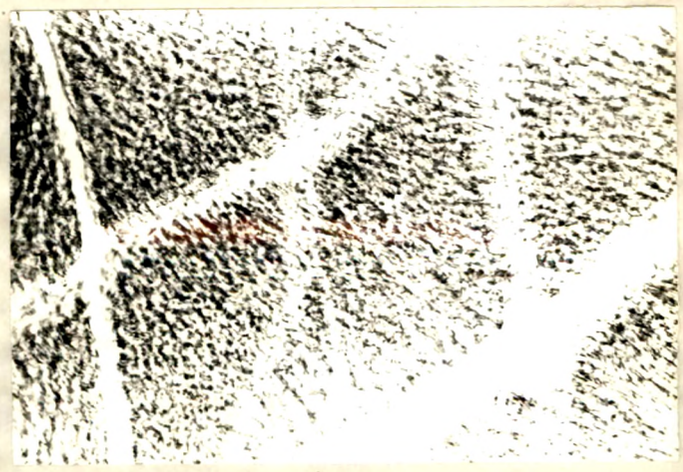
15

100 μ

ADULT

16

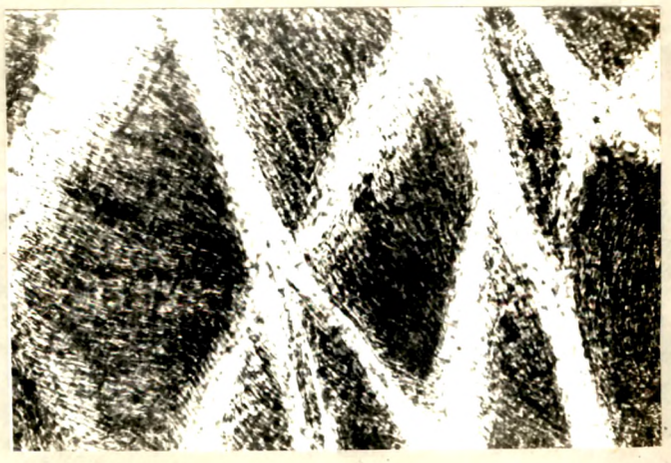
ACIDIC LIPID IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



200 μ

30 DAY OLD

15a



200 μ

ADULT

16a

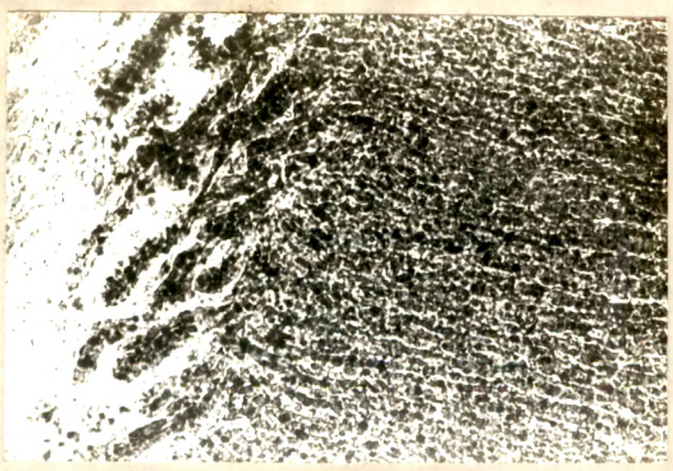
contrast, the acidic lipids were found to increase in concentration steadily and this steady increase continued through the later half of development, reaching the highest level in the adult gizzard. On the other hand, sudanophilic lipid, after the fall to the lowest level on the 15th through 20th days rose up quickly and attained the highest value on the 25th day which was maintained thereafter through 30th day to the adult condition. A comparison, however, revealed the highest level of acidic lipids to be slightly more in concentration than the corresponding highest level of sudanophilic lipid.

Lipase and esterase: (Figs. 17 to 32; 17a to 32a)

On the day of hatching, noticeable activities of these two enzymes could be seen in both the components of gizzard. Both lipase and esterase which showed similar concentrations in both the components of gizzard, found to register a constant and steady increase in their activities upto 15 days. The later half of development was, however, marked by a divergent pattern of concentrations of these two enzymes. Whereas the activity of lipase started declining from 15th day, that of esterase continued to show a considerable increase attaining its ultimate highest expression on the 30th day and remained so thereafter. On

LIPASE ACTIVITY IN THE MUCOSAL TUBULES

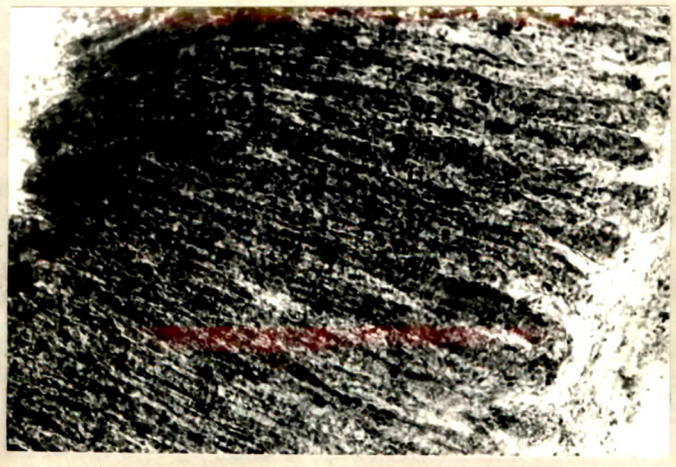
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

17



100 μ

5 DAY OLD

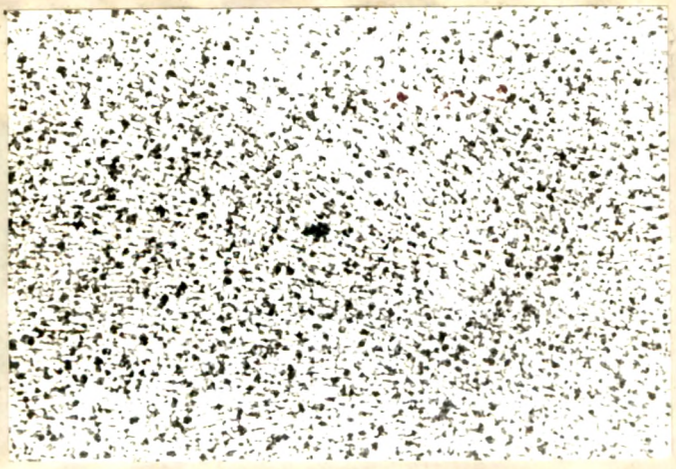
18

100 μ

10 DAY OLD

19

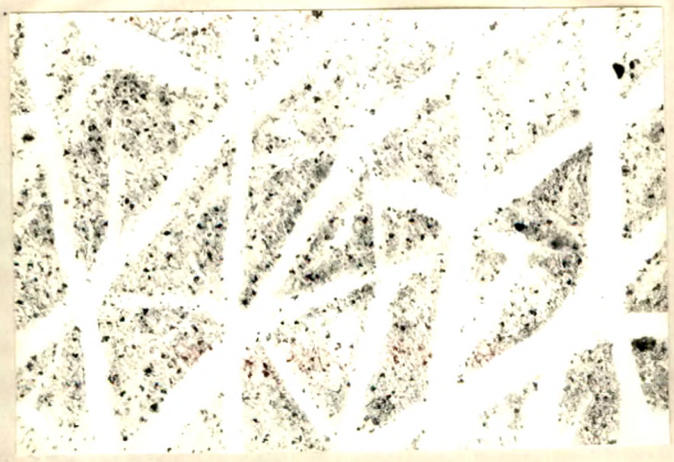
LIPASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

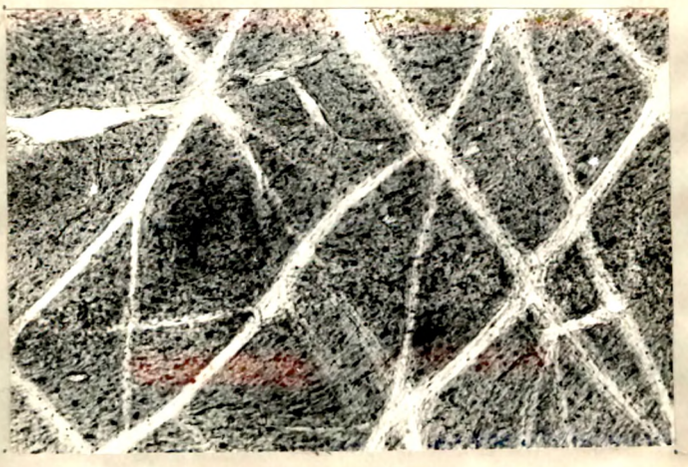
17a



200 μ

5 DAY OLD

18a

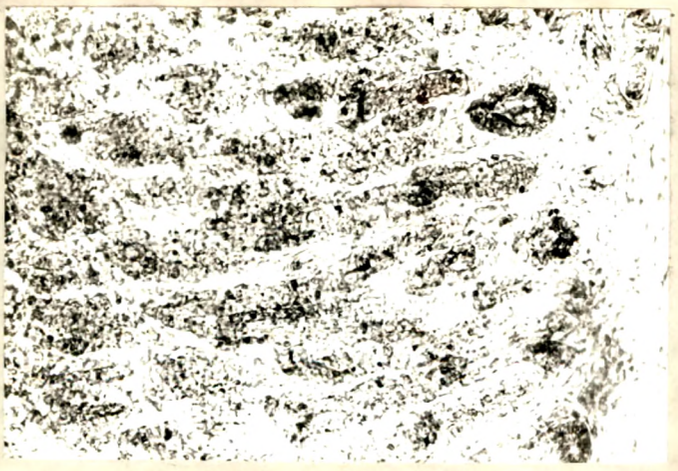


200 μ

10 DAY OLD

19a

LIPASE ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

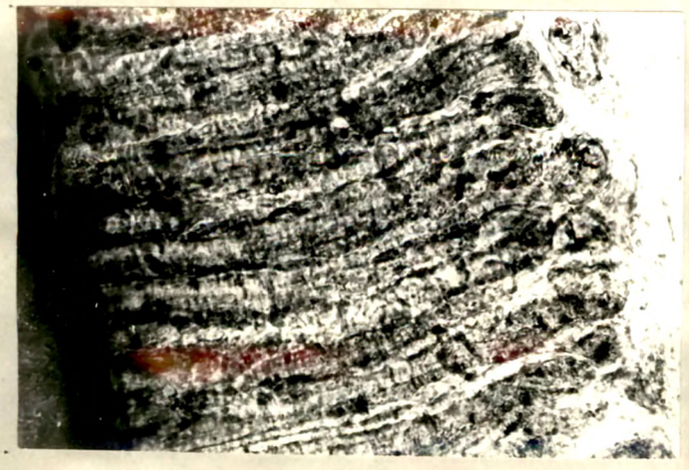
15 DAY OLD

20

100 μ

20 DAY OLD

21

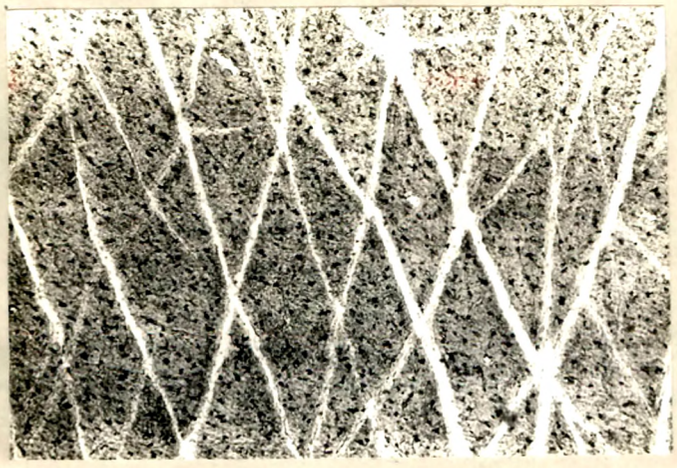


100 μ

25 DAY OLD

22

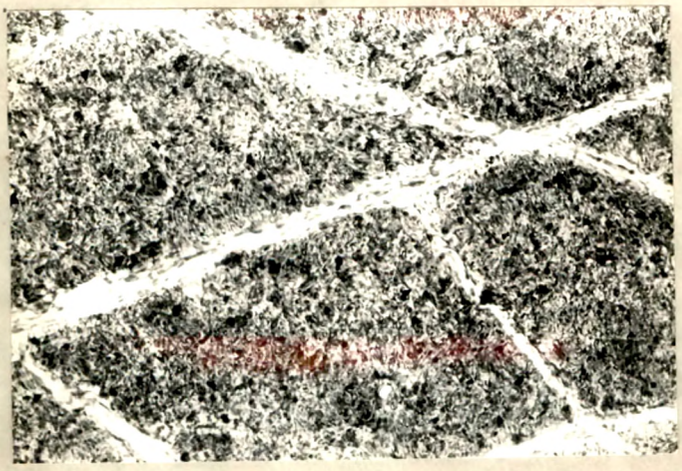
LIPASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

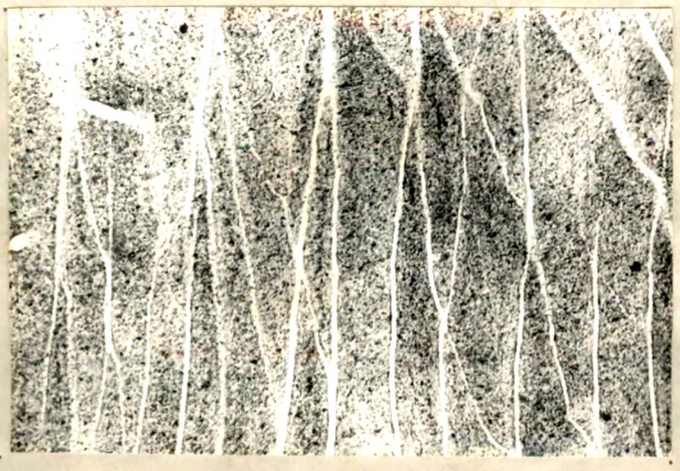
20a



100 μ

20 DAY OLD

21a



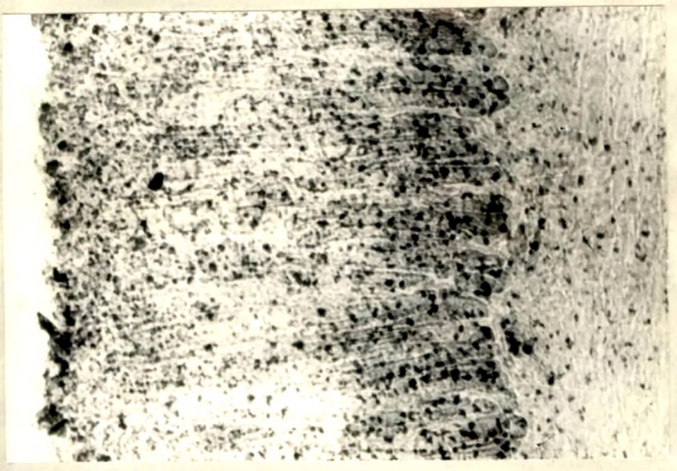
200 μ

25 DAY OLD

22a

LIPASE ACTIVITY IN THE MUCOSAL TUBULES

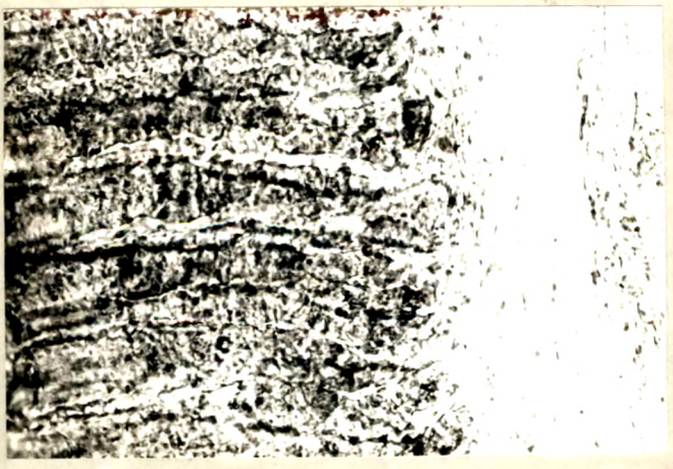
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

23



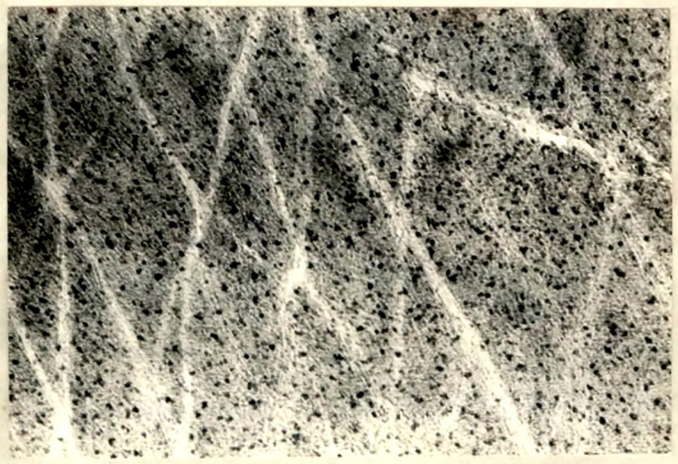
100 μ

ADUIT

24

LIPASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES

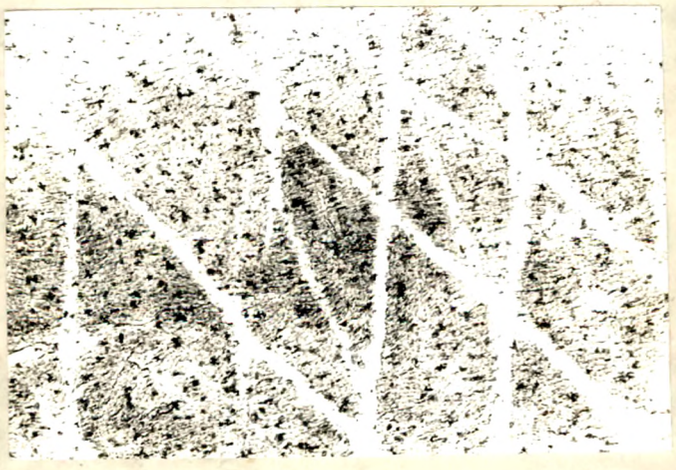
OF THE DEVELOPING PIGEON GIZZARD



200 μ

30 DAY OLD

23a

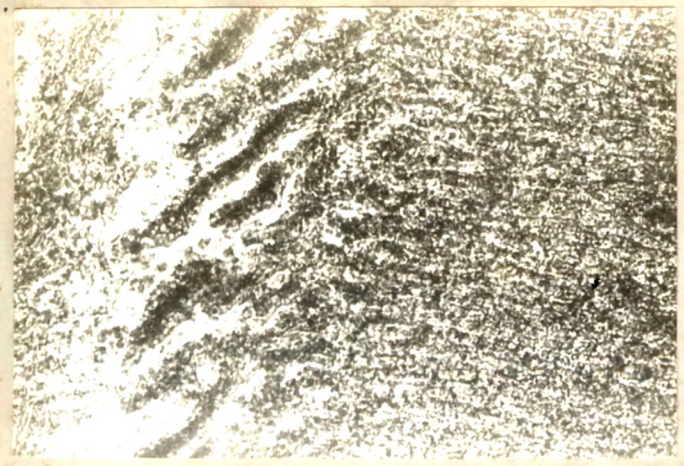


200 μ

ADULT

24a

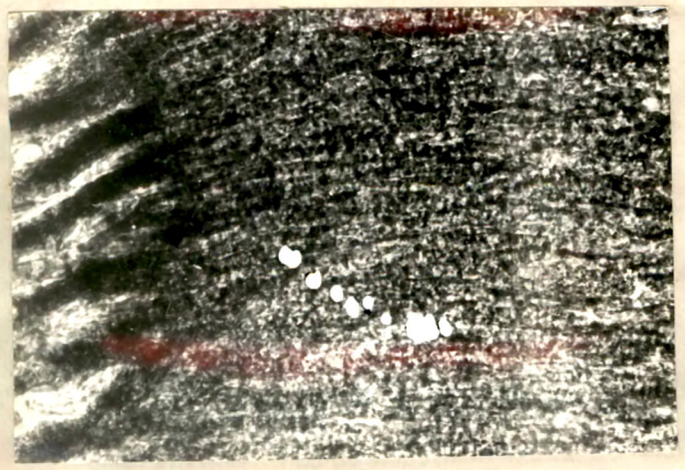
ESTERASE ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

25



100 μ

5 DAY OLD

26

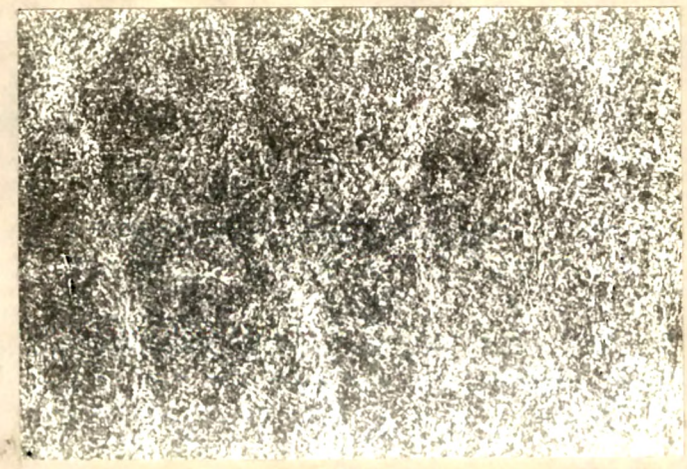
100 μ

10 DAY OLD

27

ESTERASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES

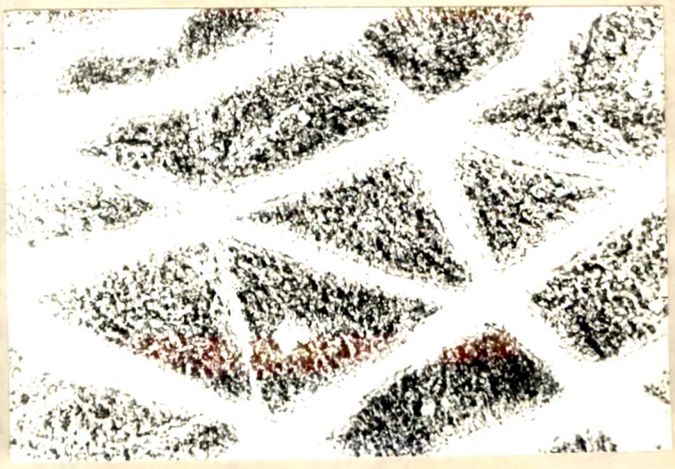
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

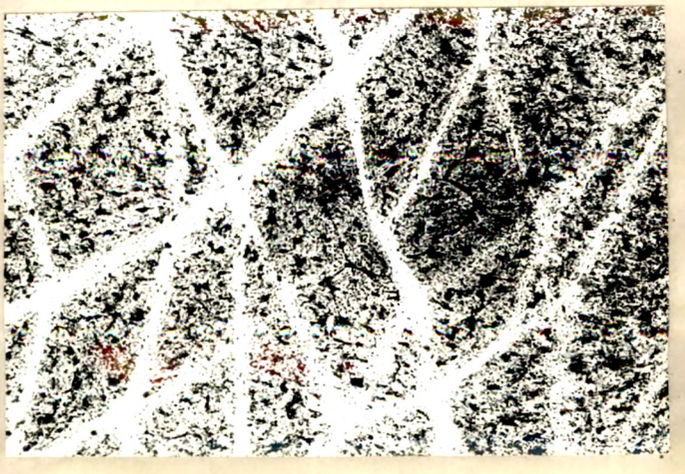
25a



150 μ

5 DAY OLD

26a



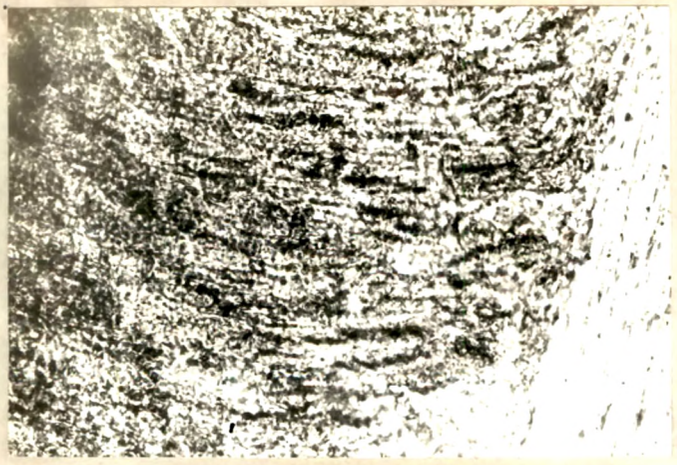
200 μ

10 DAY OLD

27a

ESTERASE ACTIVITY IN THE MUCOSAL TUBULES

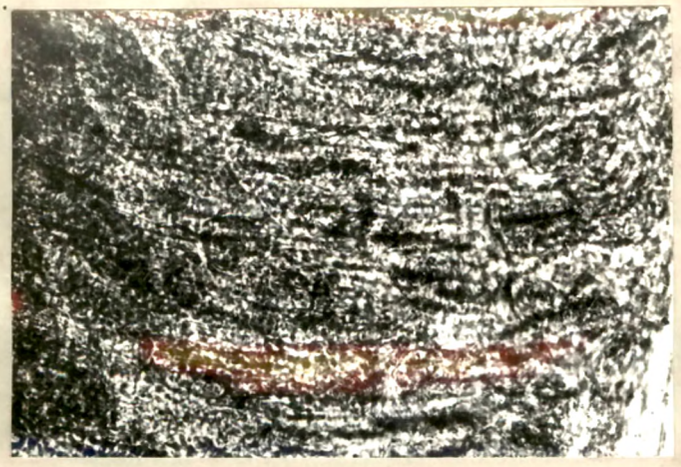
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

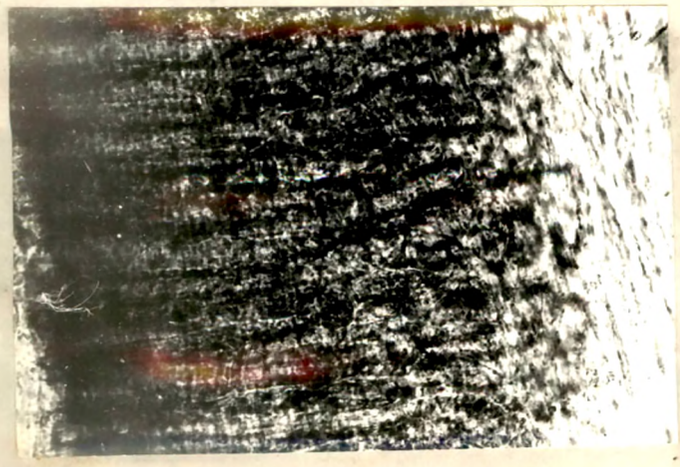
28



100 μ

20 DAY OLD

29

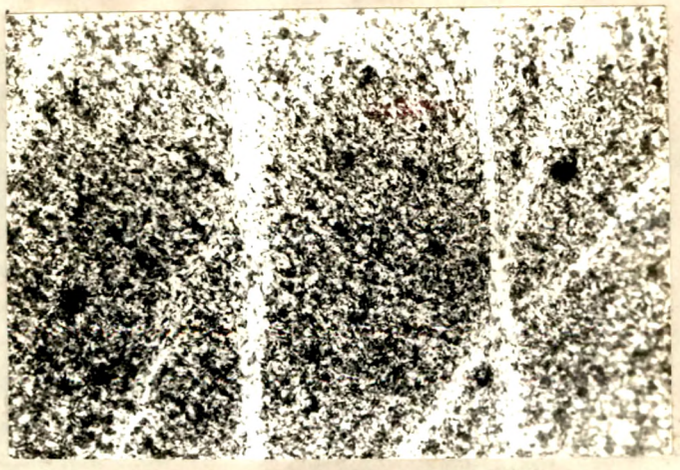


100 μ

25 DAY OLD

30

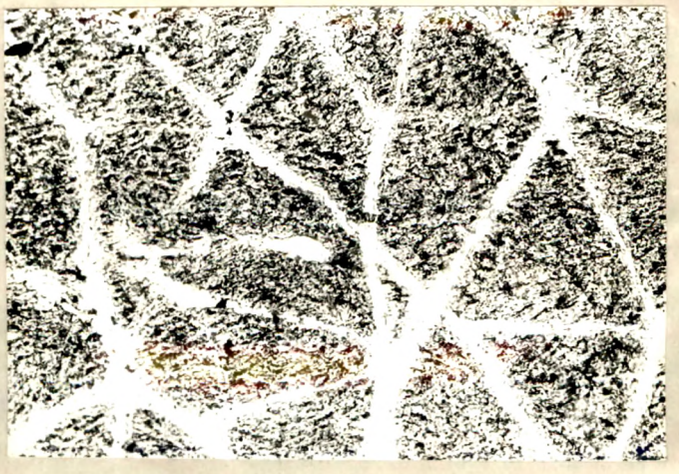
ESTERASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

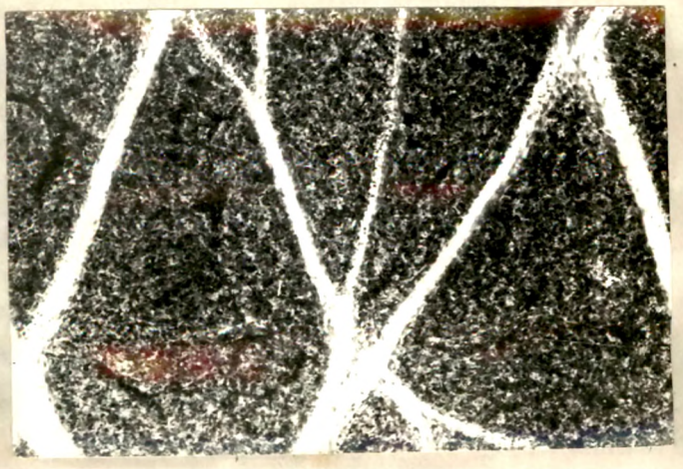
28a



200 μ

20 DAY OLD

29a



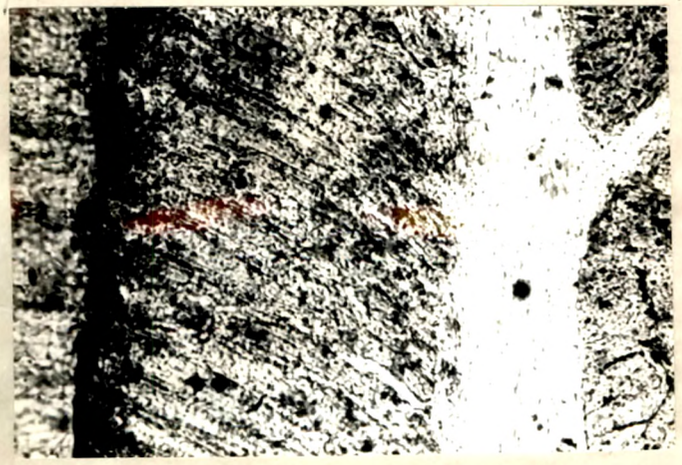
100 μ

25 DAY OLD

30a

ESTERASE ACTIVITY IN THE MUCOSAL TUBULES

OF THE DEVELOPING PIGEON GIZZARD



150 μ

30 DAY OLD

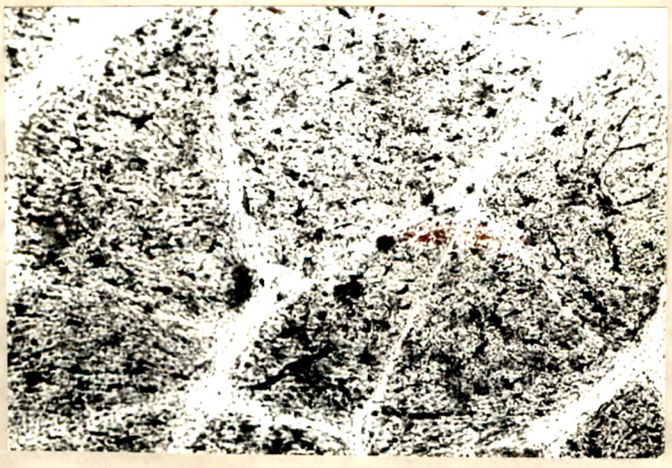
31

100 μ

ADULT

32

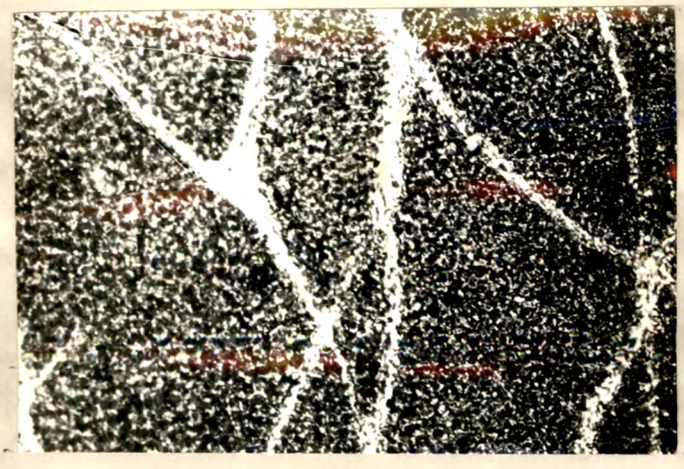
ESTERASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



150 μ

30 DAY OLD

31a



100 μ

ADULT

32a

the other hand, lipase activity decreased to a low level on the 30th day which was identical and equal to the one observed on the 1st day and this low level remained so even in the adult condition.

G6PDH, αC GPDH and BDH: (Figs.33 to 56; 33a to 56a)

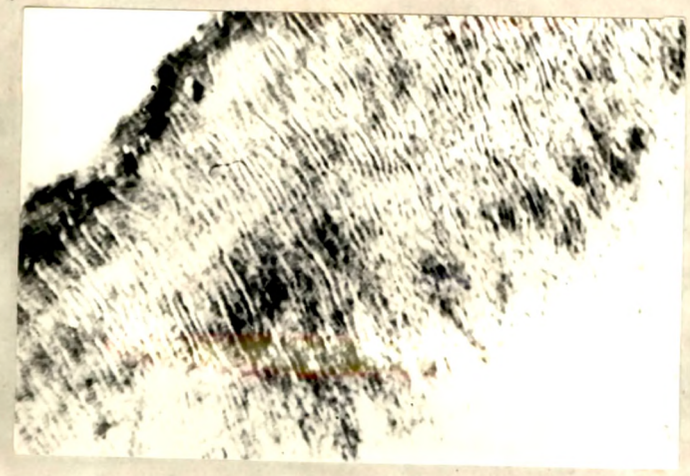
Of the three enzymes, the activity of G6PDH was moderate, that of αC GPDH was highest and BDH the least on the 1st day of post-natal development. Though a more or less equal concentration of both G6PDH and BDH could be noted in the two anatomical parts of the gizzard, that of αC GPDH was noticeably higher in the smooth muscle fibres than in the secretory tubules. During the first fifteen days of development, this disparity in αC GPDH activity was reduced greatly by a gradual increase of the enzyme activity in the epithelial tubules whereas in the smooth muscle fibres, it remained at the same high level. Ultimately with initial high level of αC GPDH on the day of hatching, it reached to its peak of activity on the 20th day of development whereas the enzyme concentration tended to be equal in both the components of the gizzard. There was a slight decrease in αC GPDH activity on the 30th day and this level of enzyme concentration, very much similar to the one observed during the first fifteen days, was maintained in the adult gizzard too. The activity of BDH

G6PDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD

100 μ

1 DAY OLD

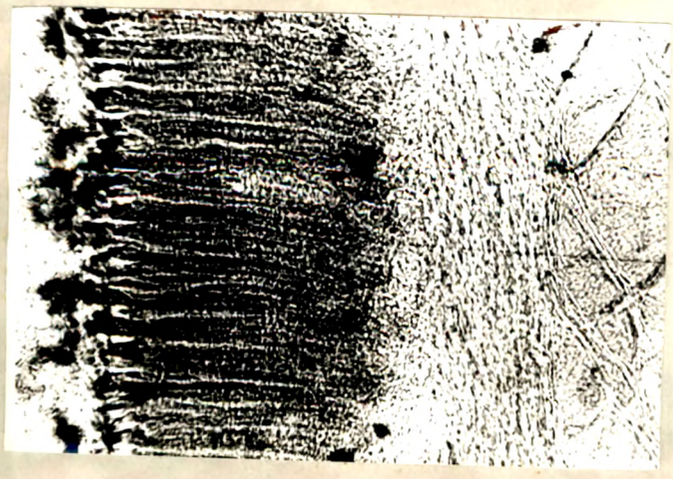
33



200 μ

5 DAY OLD

34



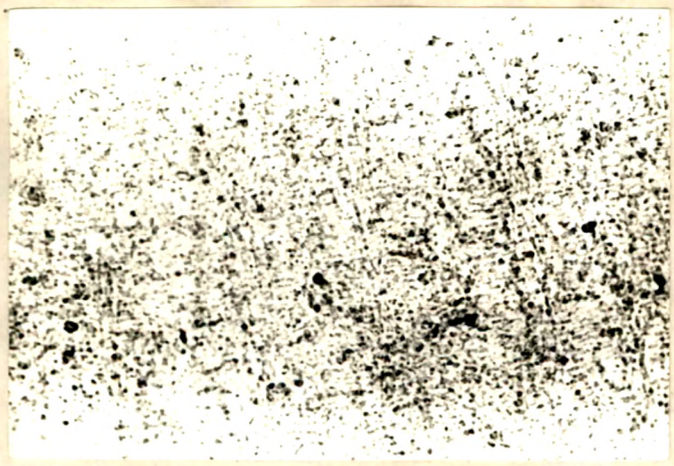
200 μ

10 DAY OLD

35

G6PDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

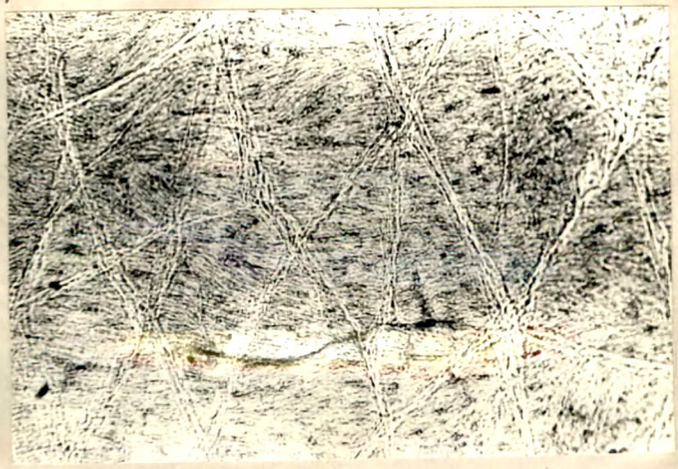
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

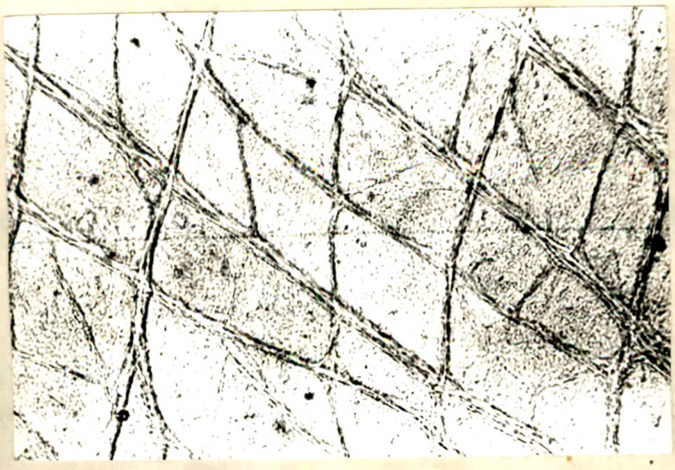
33a



150 μ

5 DAY OLD

34a



200 μ

10 DAY OLD

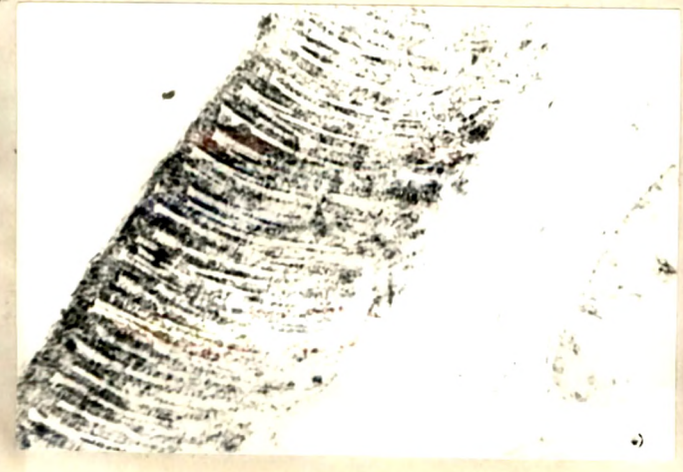
35a

G6PDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD

200 μ

15 DAY OLD

36



200 μ

20 DAY OLD

37

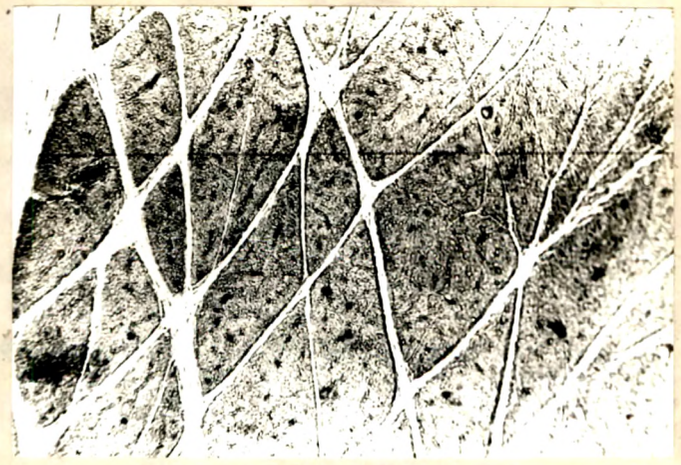
200 μ

25 DAY OLD

38

G6PDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

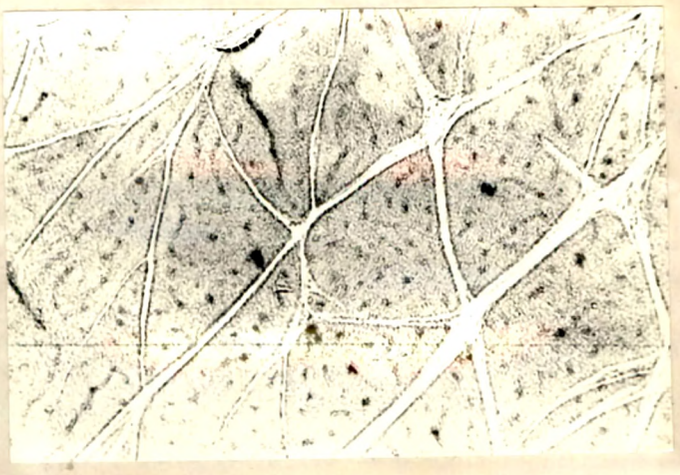
OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

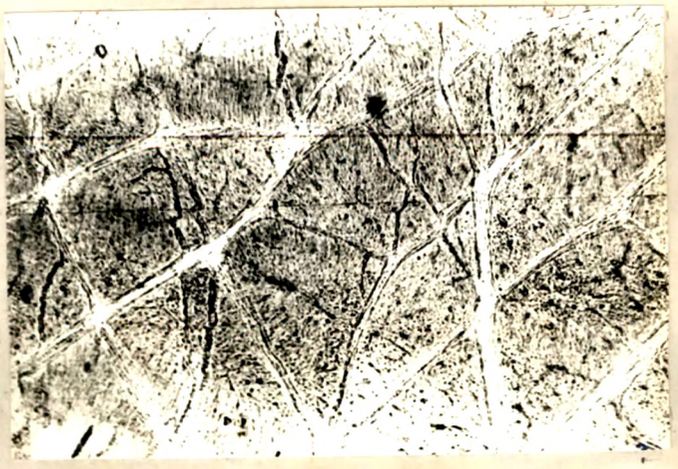
36a



200 μ

20 DAY OLD

37a

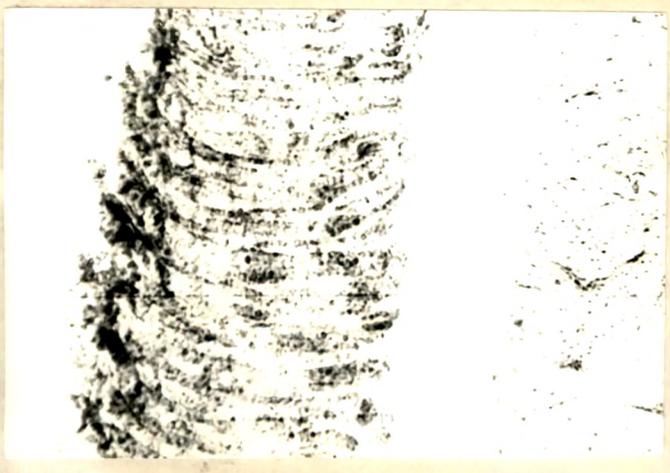


200 μ

25 DAY OLD

38a

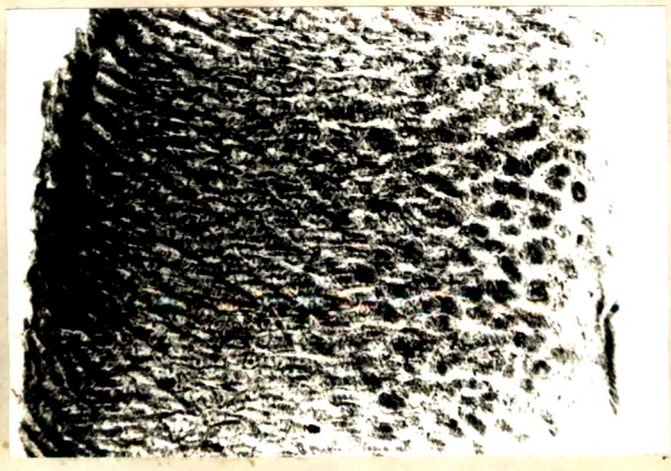
G6PDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



200 μ

30 DAY OLD

39



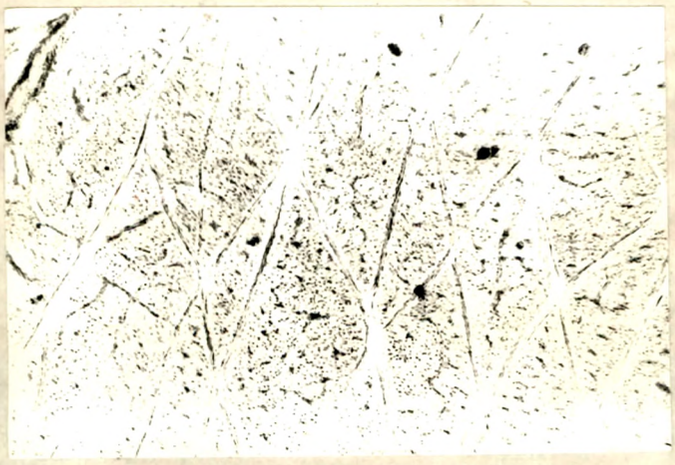
100 μ

ADULT

40

G6PDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

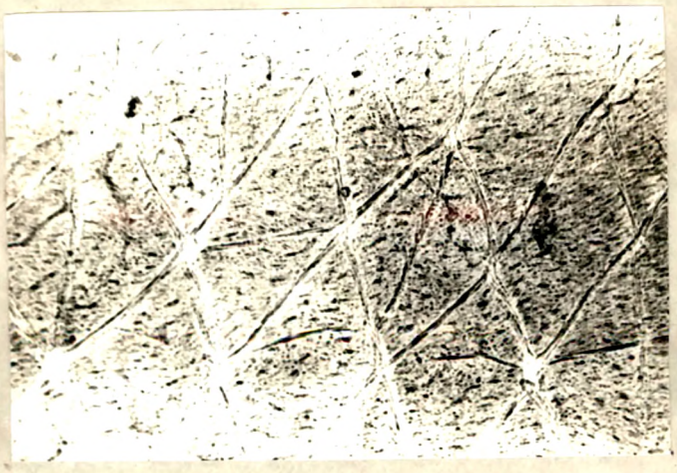
OF THE DEVELOPING PIGEON GIZZARD



150 μ

30 DAY OLD

39a

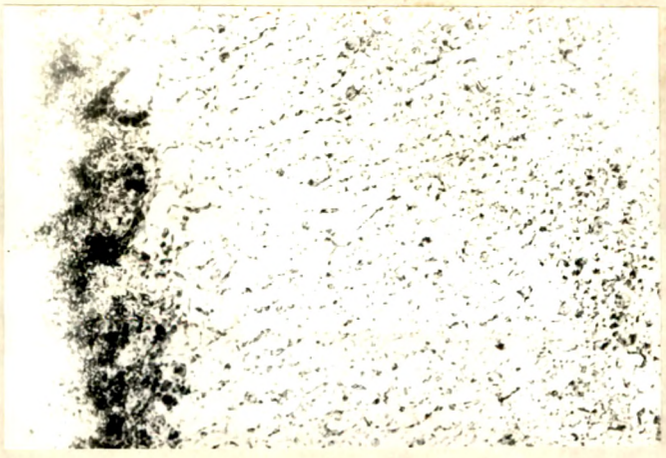


200 μ

ADULT

40a

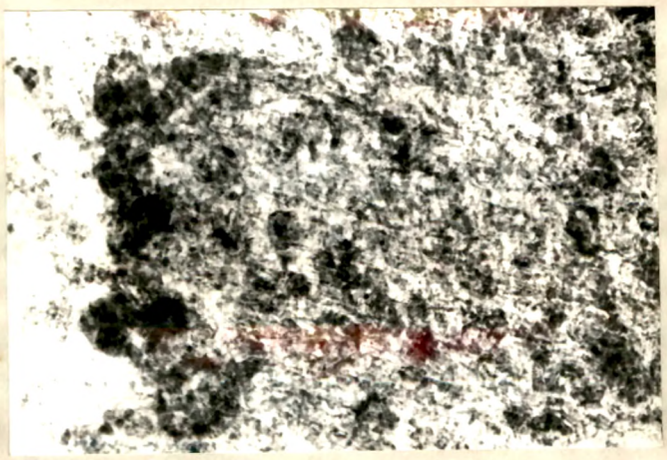
αC GPDH ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



200 μ

1 DAY OLD

41



200 μ

5 DAY OLD

42

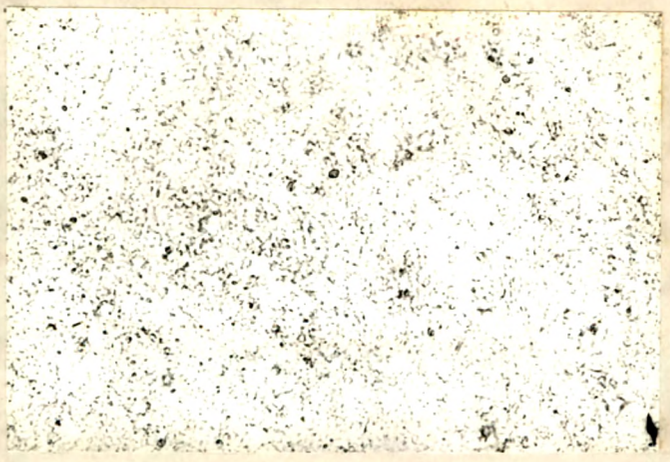
200 μ

10 DAY OLD

43

αC GPDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

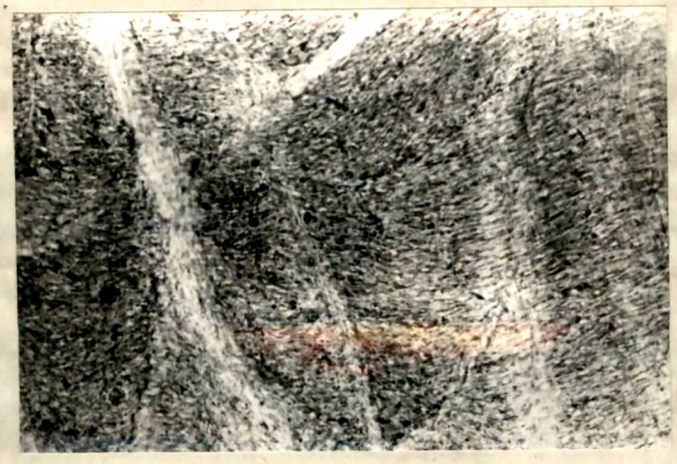
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

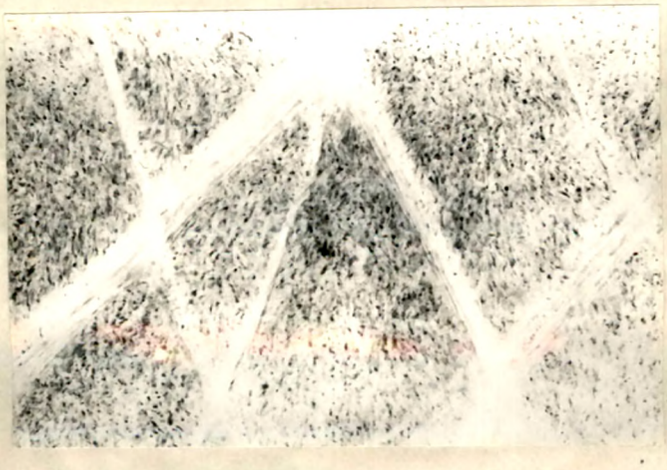
41a



100 μ

5 DAY OLD

42a

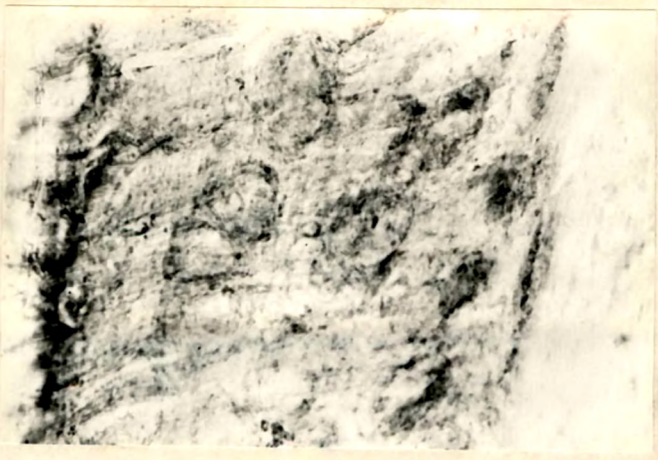


100 μ

10 DAY OLD

43a

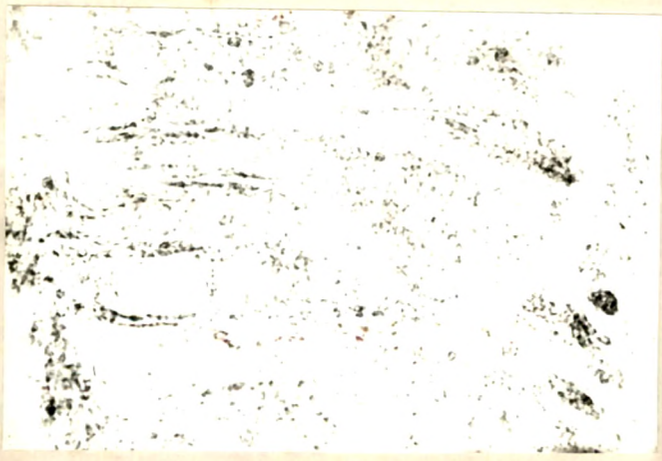
αC GPDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

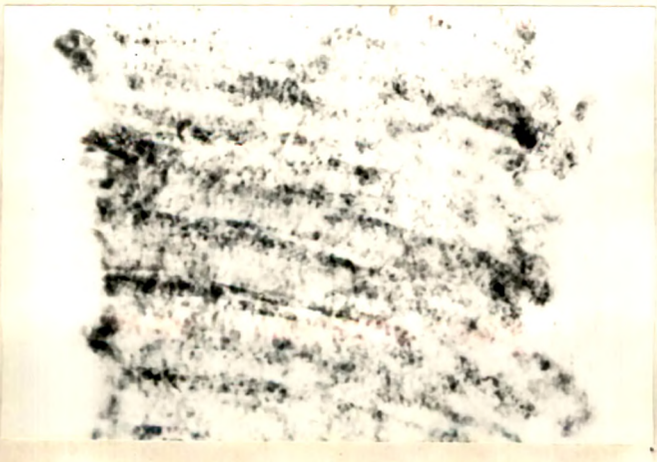
44



200 μ

20 DAY OLD

45

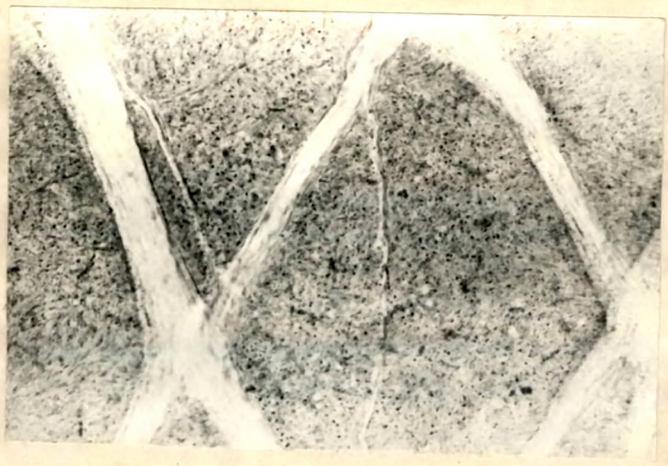


200 μ

25 DAY OLD

46

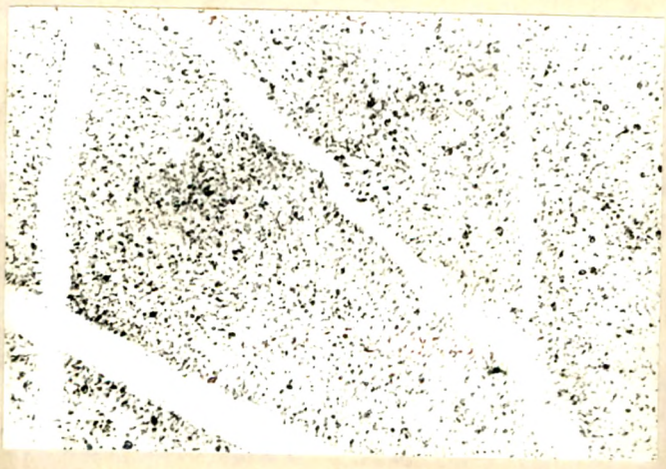
αGPDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

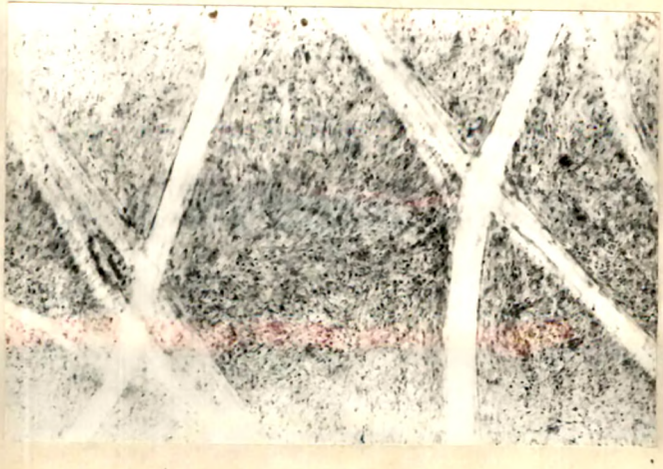
44a



100 μ

20 DAY OLD

45a



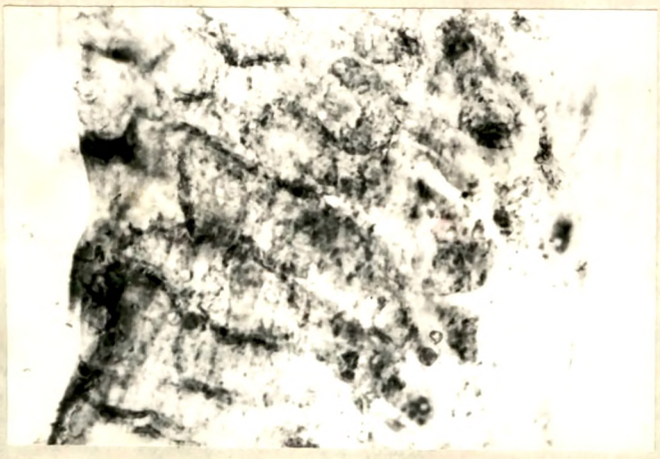
100 μ

25 DAY OLD

46a

α GPDH ACTIVITY IN THE MUCOSAL TUBULES

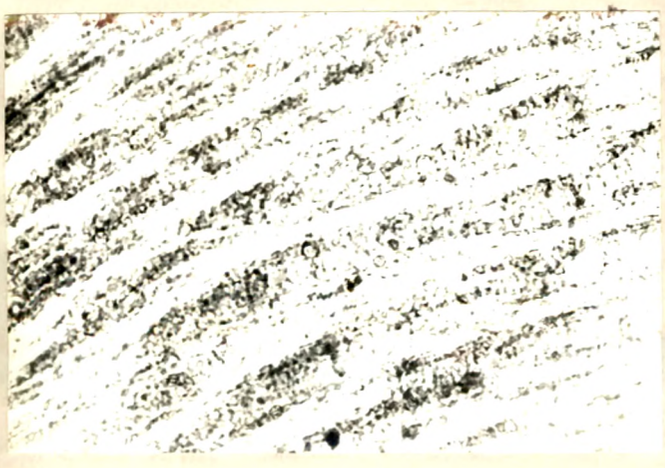
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

47



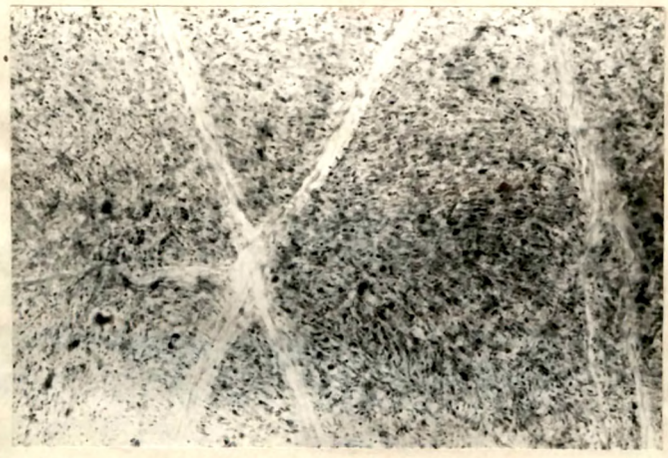
100 μ

ADULT

48

αC GPDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

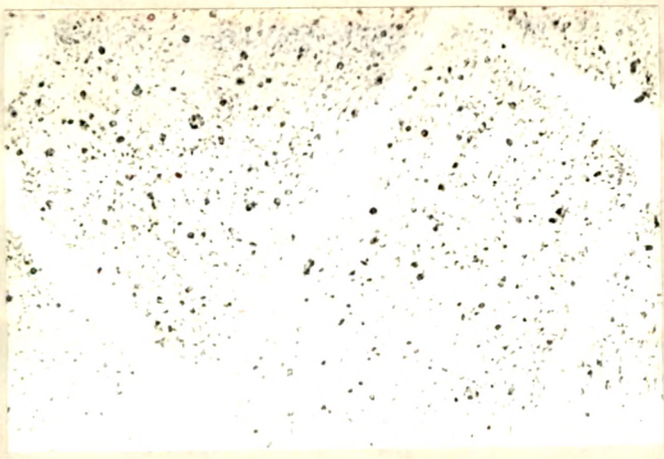
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

47a



100 μ

ADULT

48a

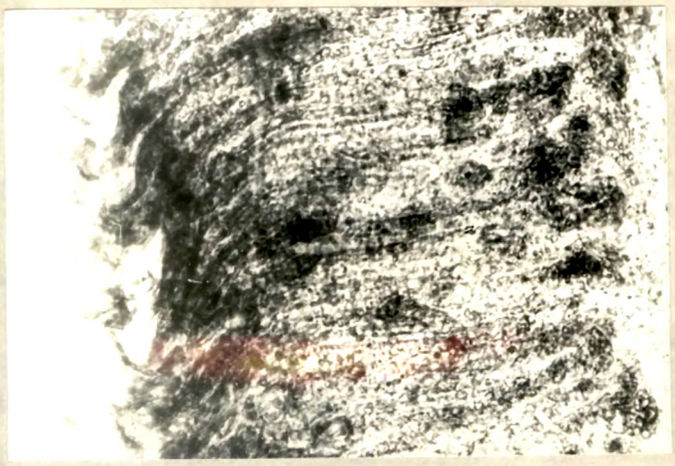
BDH ACTIVITY IN THE MUCOSAL TUBULES

OF THE DEVELOPING PIGEON GIZZARD

100 μ

1 DAY OLD

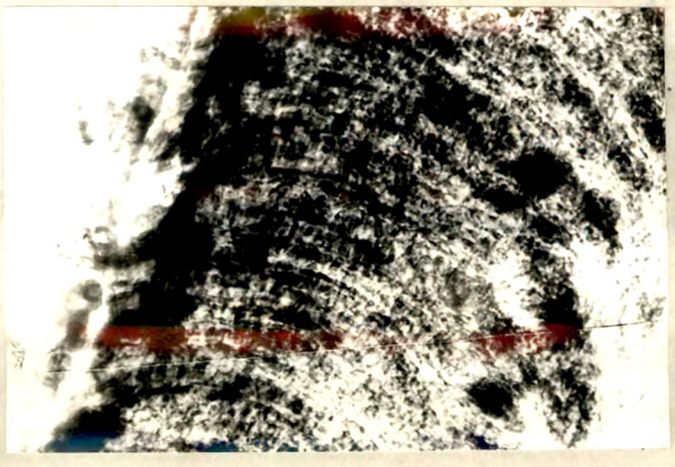
49



100 μ

5 DAY OLD

50



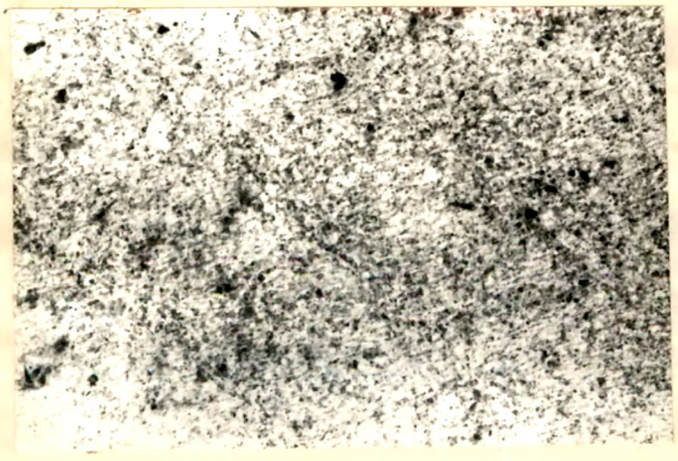
100 μ

10 DAY OLD

51

BDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

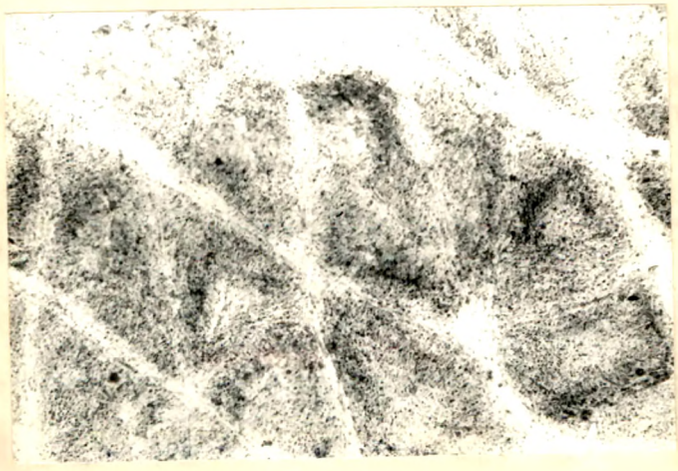
OF THE DEVELOPING PIGEON GIZZARD



200 μ

1 DAY OLD

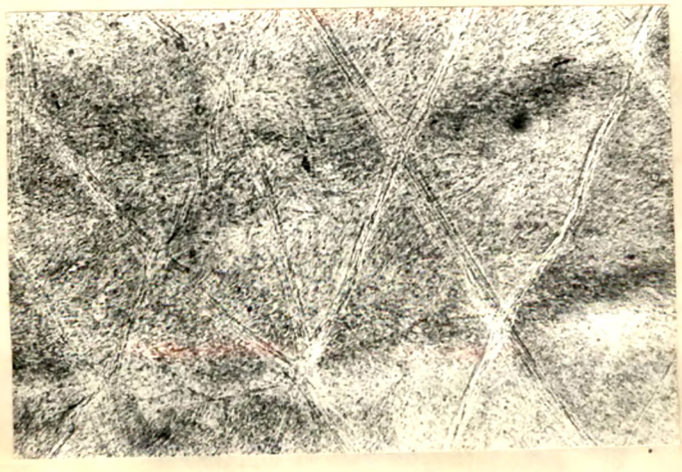
49a



200 μ

5 DAY OLD

50a



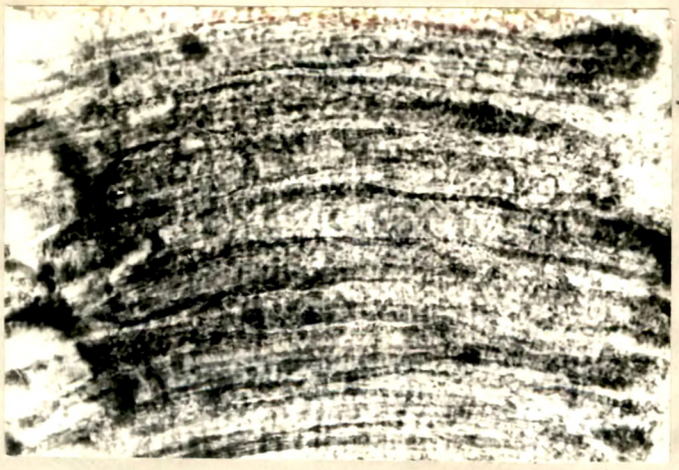
200 μ

10 DAY OLD

51a

BDH ACTIVITY IN THE MUCOSAL TUBULES

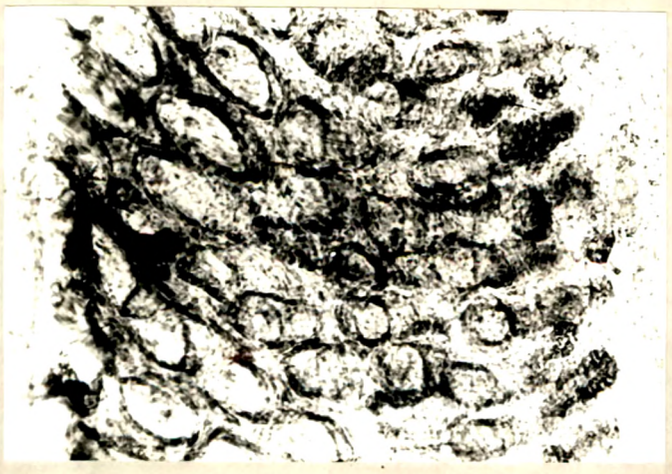
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

52



100 μ

20 DAY OLD

53

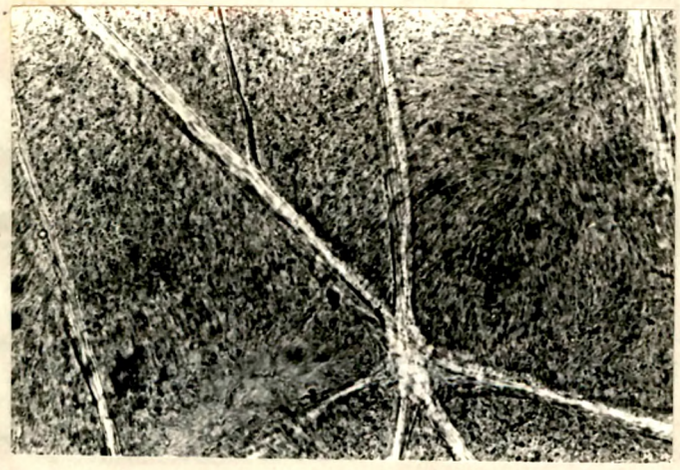
100 μ

25 DAY OLD

54

BDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

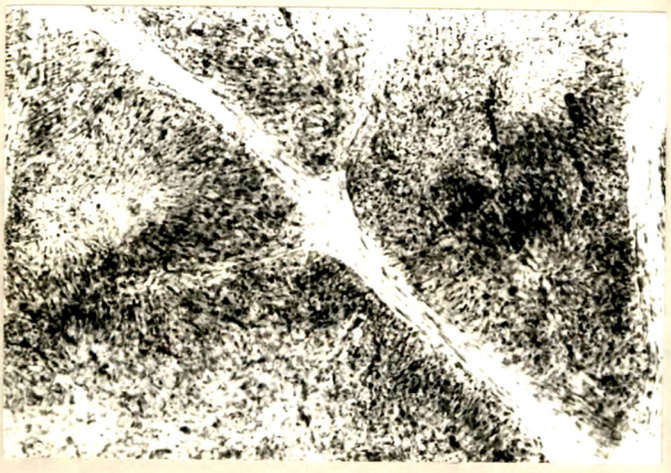
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

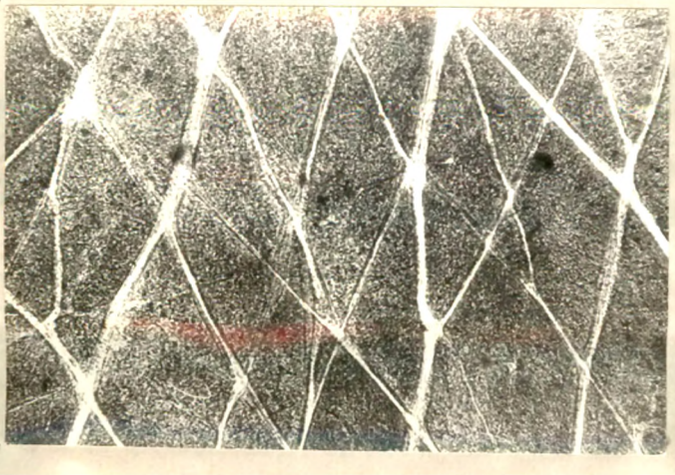
52a



100 μ

20 DAY OLD

53a

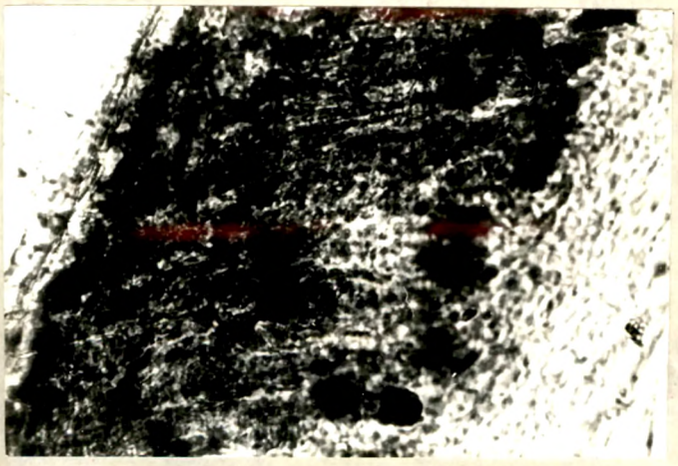


100 μ

25 DAY OLD

54a

BDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

55

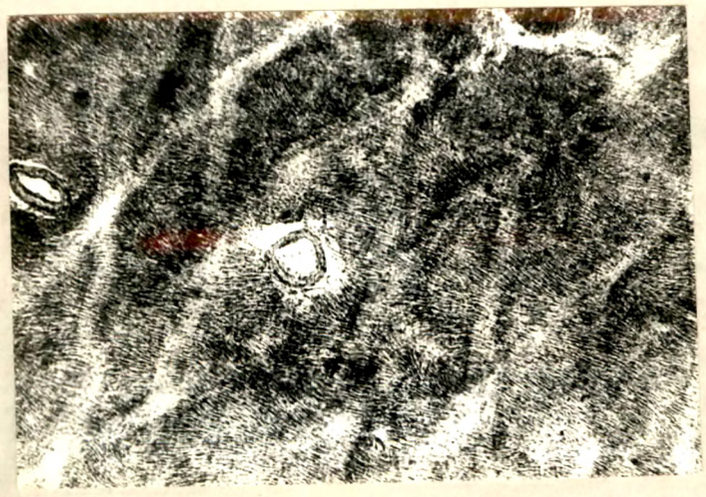
100 μ

ADULT

56

BDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

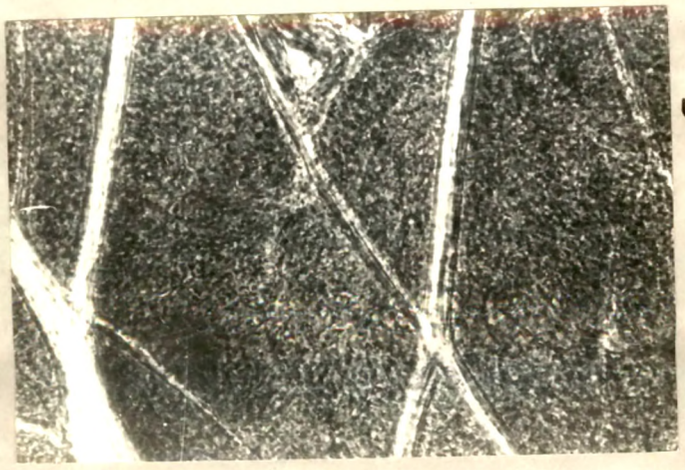
OF THE DEVELOPING PIGEON GIZZARD



200 μ

30 DAY OLD

55a



100 μ

ADULT

56a

which was low on the first day showed only a gradual and little increase during the first half of gizzard development. But the second half of the development was marked by a very high activity of BDH in both the muscle fibres and tubules. Even though the entire second half of development had a very high BDH activity it was slightly more during the period between 25th and 30th days than the one preceding (20th day) and the one following (adult condition).

Moderate activity of G6PDH, on the other hand, rose slowly and attained the peak level on the 15th day with the smooth muscles being slightly more enzyme reactive than the epithelial tubules. There was a sudden and sharp decline in G6PDH activity immediately after and this decreased level was observed on 20th through 25th day of development. However, a slight increase in the enzyme activity was registered on the 30th day and this level was characteristic of the adult gizzard too.

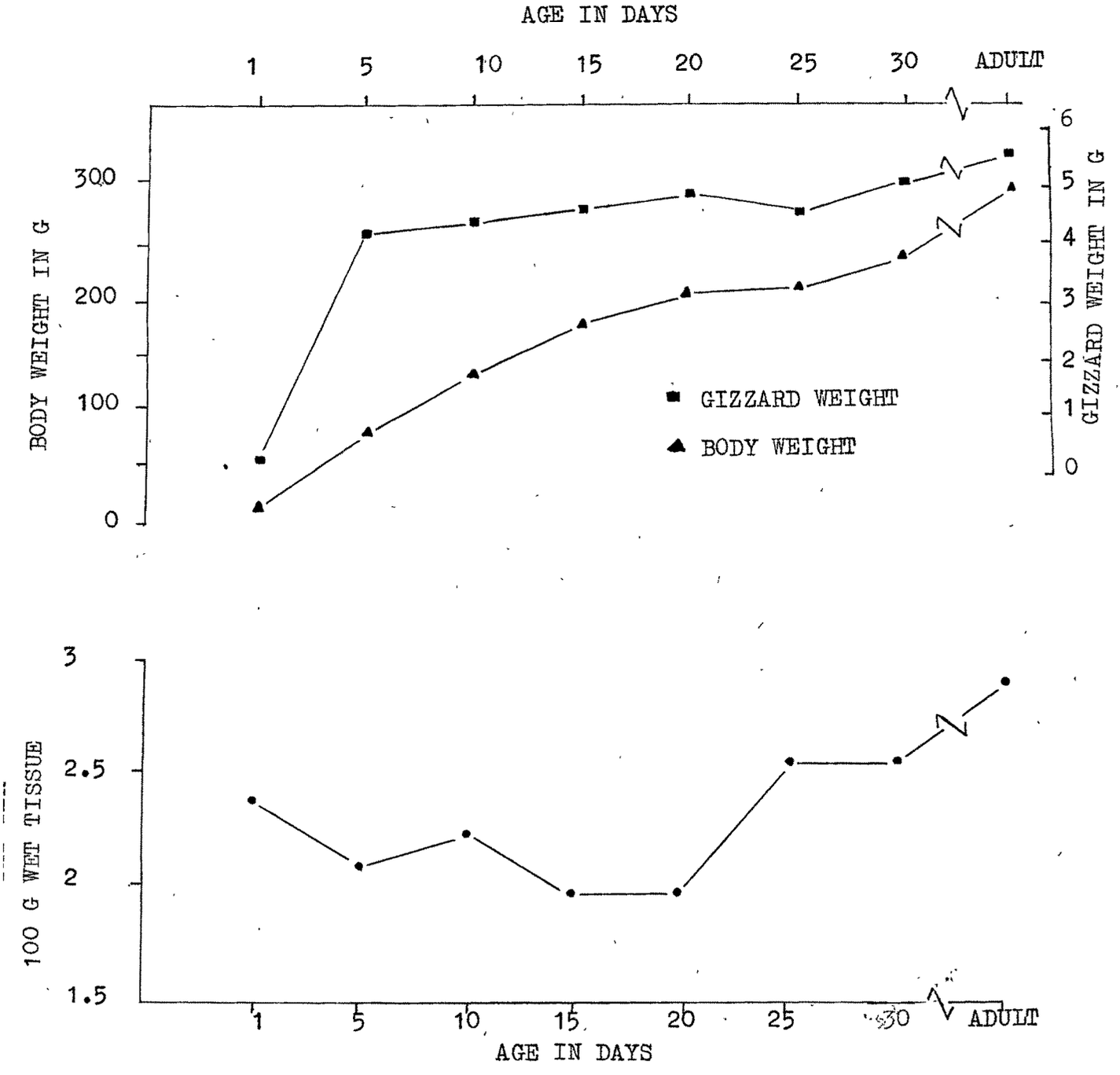
Quantitative total lipid level: On the day of hatching till the 10th day the lipid content was higher. But on the 15th and 20th days there was a decrease in its content. During the successive periods of development viz., 25th and 30th days and adult condition there was a gradual and progressive

TABLE I : LEVELS OF TOTAL LIPIDS EXPRESSED IN RELATION WITH BODY WEIGHT AND GIZZARD WEIGHT DURING DIFFERENT DAYS OF POST NATAL DEVELOPMENT OF PIGEON GIZZARD*

Age in days	Body weight	Gizzard weight	Amount of lipids (gm/100 gm wet tissue)
1	13.5	0.408	2.352
5	85	4.155	2.103
10	135	4.368	2.264
15	181	4.513	1.957
20	203	4.731	1.976
25	212	4.984	2.578
30	245	5.174	2.637
Adult	310	5.581	2.980

*Average of 5 birds

Fig. 57. Percentage of total lipids expressed in relation to body and gizzard weights during different days of post-natal development of pigeon gizzard



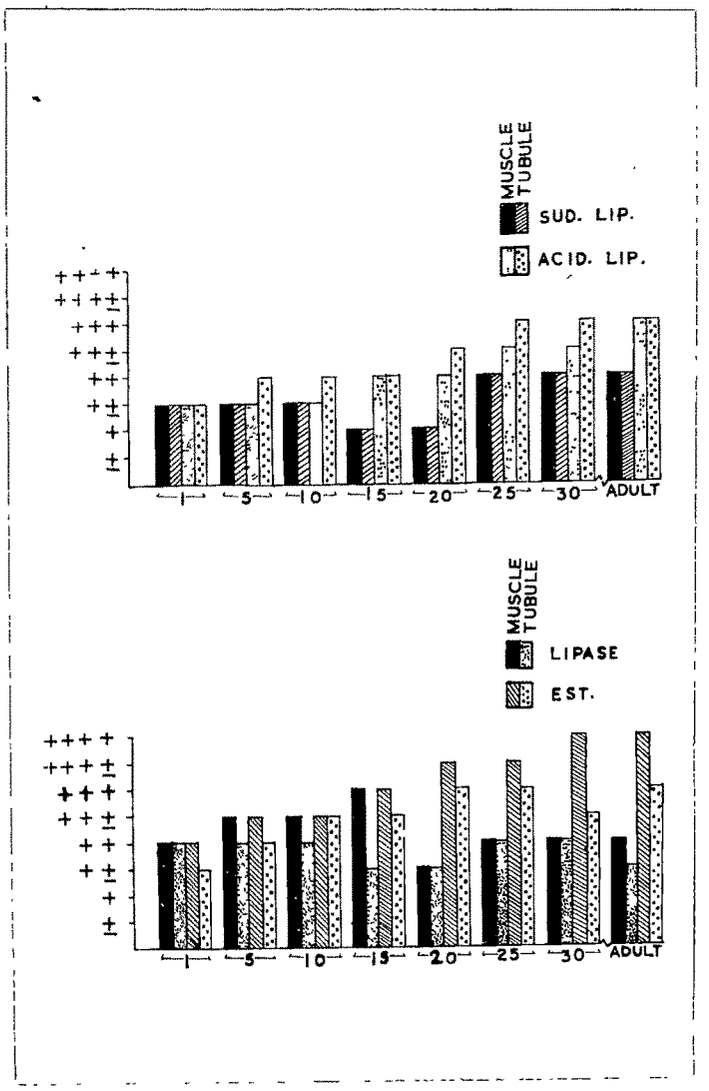


FIG. 58

Graphical representation of the changes in the lipids, lipase and esterase distribution pattern during the various days of gizzard development

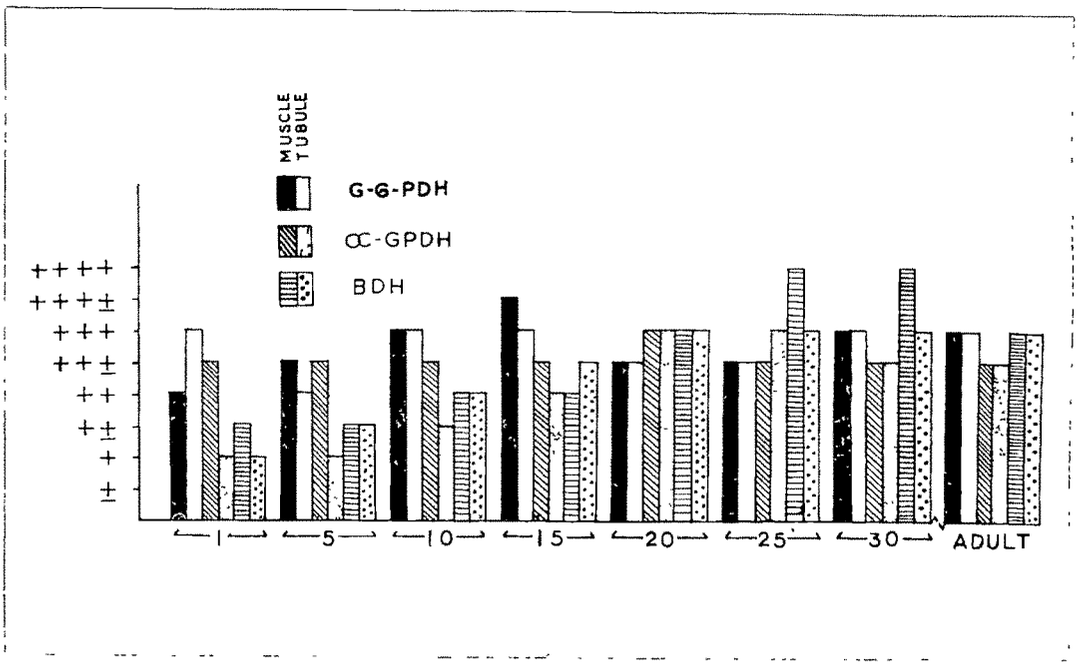


FIG. 59

Graphical representation of the changes in the G6PDH, α GPDH and BDH distribution pattern during the various days of gizzard development

increase in the lipid content which was in a comparative basis more than the one observed during the initial stages of development.

The quantitative data obtained from the present study on total lipids is presented in table I; fig. 57.

Histochemically observed concentrations of the lipids and associated enzymes viz., lipase, esterase, G6PDH, αGPDH and BDH during the process of post-natal development are represented diagramatically in figures 58 & 59.

DISCUSSION

Unlike carbohydrate metabolism, which was noted to be a continuous process all throughout the various phases of the post-natal development of the pigeon gizzard, lipid utilization appears to be of a significant nature only during the second half of the development (i.e., after first 15 days). It is now clear that the initial five days of the development of pigeon gizzard are dependant on carbohydrate metabolism (chapter 2). However, during the same period, very low level incidence of lipolysis also appears to be apparent when viewed in the light of the gradual increase in activities of both lipase and esterase observed in the present study. Though no

apparent decrease in the lipid content could be observed in either of the two components of the gizzard histochemically, the data on the quantitative analysis indicate a small but gradual diminution of lipid. It is interesting that at the same time the activity of G6PDH also shows a gradual increase which could probably be indicative of a possible simultaneous synthesis of lipids, though on a low scale, thus offsetting the low level utilization of lipids. Again, the complete oxidation of lipids through TCA cycle seems to be rather unlikely by the presently observed low level of BDH and those of SDH and MDH (chapter 2). In the light of these observations the higher incidence of αC GPDH during this initial stages is rather significant and by its purported role in αC-glycerophosphate based catalysis could safely be presumed to be of importance in diverting and utilizing the available glycerol moieties through the Embden-Meyerhof Pathway (EMP). Such an αC GPDH mediated lipid oxidation has been suggested by Ramachandran (1972) in the regenerating tail of the lizard, Mabuya carinata. It may, thus, be inferred that the pigeon gizzard during the initial days of development, though dependent on carbohydrate metabolism, also utilizes lipids through the glycolytic pathway.

The period between 10th and 20th days of development appear to be marked by an increased rate of depletion of lipids as recorded both by the histochemical as well as quantitative studies. These studies reveal the lowest levels of lipids on the 15th to 20th days. This is in correlation with the highly increased levels of both lipase and esterase observed at about the same period. Keeping these two evidences in mind, it could be safely presumed that there is an increased rate of lipolysis of both long chain as well as short chain fatty acids. Further significant observations in this direction are the increased activity of BDH (present chapter) and the maximum level of activities of SDH and MDH (chapter 2) during the period between 10th and 25th days of post-natal development. αC GPDH, another enzyme under investigation, which was already in a moderately high level of activity right from the first day, also registered the maximal level on the 20th day. Thus these observations positively indicate a highly active metabolic phase and interestingly enough this active period appears to be in good correlation with both the anatomical development of the organ marked by an increase in its size and the attainment of full functional competence, denoted by the extensive contractions of its smooth muscle complex. The understanding gained by the studies on carbohydrate metabolism when reflected on the

present observations tends to lend credulence and validity to the fact that carbohydrate metabolism fails to satisfy the energy necessities of the fast developing pigeon gizzard at this stage and is hence supplemented by active participation of lipids and their complete catabolism and oxidation via the TCA cycle so as to liberate the maximum amount of energy. Concurrently observed maximal activity of αGPDH also appears to be playing a significant role during this phase of lipid utilization by its possible participation in the oxidation of glyceride moieties through glycolytic pathway. At the same time the increasing activity of G6PDH and the attainment of its highest level on the 15th day when there was a sharp decline in the lipid content, though slightly puzzling, is understandable and tallies well with the gradual increase of acidic lipids noticed simultaneously. Thus it appears that the sudanophilic lipids are being catabolised actively on the one hand and the acidic lipids are being anabolised gradually on the other during the 10th to 15th days of development. However, this anabolic process appears to experience a temporary setback in the phase of active catabolic processes at work during the 20th and 25th days as observed by the decline of activity of G6PDH during these days. It is also a distinct possibility, that the presence of G6PDH at this stage might also involve a low level of synthesis of lipids, but the lipids so formed

are being immediately catabolized and exhausted as the breakdown reactions are of greater importance during this period. A good incidence of G6PDH in the mucosal tubules observed during the first 15 days can well be associated with a good rate of protein synthesis to be expected in these cells as they are not only engaged actively in the constant generation of new cells but also in the synthesis and secretion of keratinized layer during the first half of the post-natal development of the pigeon gizzard. It could be noted in this connection that Grillo (1969), based on her histochemical studies on the developing chicken gastro-intestinal tract, has conclusively proved the participation of G6PDH in the development of the epithelial and muscle cells of both the stomach and intestine as early as 6th day of development in ovo.

With the completion of the initial spurt of muscular activity of the gizzard coinciding with the first feeding experiments by young one during the 20th through 25th days of post-natal development the metabolic necessities also seem to be very much reduced as could be visualized by the gradual increase of lipids shown by the histochemical as well as quantitative studies till the maximum level was registered in the adult gizzard. The concomitant increase in the activity of G6PDH noticed from the 25th day onwards

is rather self explanatory and indicative of a phase of stepped up lipid biogenesis. It is interesting to note in this connection the works of Abraham and Chaikoff (1959), Abraham et al., (1954), Glock and McLean (1954) and Levy (1961) who showed high G6PDH activity and that of Wise and Ball (1964) who stressed the role of malic enzyme in fatty acid synthesis. Further significant observations are those 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 and Anderson-Cedergren (1961) who have reported very high protein synthesis in conjunction with the high G6PDH activity in the sarcotubular fraction of the frog skeletal muscle. Moreover, the importance of $NADPH_2$ generated through shunt pathway in the synthesis of fatty acids in animal tissues has been shown by Siperstein (1958) and Shah and Ramachandran (1973).

The reduced levels of lipase (present chapter) and SDH and MDH (chapter 2) from the 25th day onwards are further indications of a low level of lipid catabolism. These observations coupled with the increasing levels of glycogen (chapter 2) and lipid (present chapter) are definite indications of a reduced level of metabolism from the 25th day of development onwards. From this period there appears to

be a gradual conditioning of the level and state of metabolism in the gizzard mainly to settle down towards the characteristic adult pattern of metabolism. Though there is a low toned lipid catabolism from the 25th day onwards, the maximal increased activities of both esterase and BDH are rather indicative of the contrary. An interesting probability, in the wake of these observations, is the possible utilization of fatty acids made available through the rich blood supply. Apparently it is quite probable that though there is still an active process of lipid catabolism, the synthetic process, however, far outweighs the degree of lipid utilization. This aspect appears to gain significance not only from the fact that the gizzard, as it is chiefly made up of smooth muscles, has a poor capacity for storage of metabolites but also from the fact that as its functional process involves continuous sustained contractions, the ready and instant supply of fatty acids through blood might serve as very useful and economical. Moreover, this could also explain the high activity of BDH and the optimal activities of SDH and MDH (chapter 2) and G6PDH (present chapter) noticeable during this final phase of the gizzard development. An important observation in this connection is the report that the energy for the relatively slow but sustained contractions of the smooth

muscle is derived from the oxidation of fatty acids (White et al., 1964). The persistant high activity of αC GPDH from the 25th day onwards is in good correlation with the observed increase in lipid contents, especially the acidic lipids. The increase of phospholipids, which is nothing but acidic lipids, during the post-natal development of pigeon gizzard is rather to be expected as it is important in the laying down of the structural frame work of the proliferating and developing cellular elements of pigeon gizzard. In this regard the observation that the phospholipids content of the gizzard is about 2.85% is noteworthy (Bloor, 1936). The presently observed αC GPDH activity and the increase in phospholipid observed during the final stages of gizzard development together with the active process of glycolysis (chapter 2) are indicative of the glycerophosphate based formation of diglycerides and triglycerides for the elaboration of phospholipid molecules. Such an association of αC GPDH and phospholipid synthesis has been stressed by a number of workers (Rossiter et al., 1957; Kennedy, 1953, 1954, 1957a,b; Kornberg & Pricer, 1953; Ramachandran, 1972). The moderately high activity of αC GPDH all throughout the development observed herein might be presumed to play an important role either in the formation of glycerides or in the

oxidation of these moieties through the glycolytic pathway and even in the diversion of glycerides towards gluconeogenesis by its reversible reactions as per necessity as suggested by Ramachandran (1972) in the regenerating tail of the lizard, Mabuya carinata.

On the whole the highlights of the present line of investigations undertaken in the post-natal development of pigeon gizzard appear to be (1) a continuous process of phospholipogenesis and (2) an active phase of lipid utilization between 15th and 25th days of development and (3) the significant possibility of the supply of fatty acids through the circulating blood for the energy purpose for gizzard functioning.