SECTION I

STUDIES ON CERTAIN METABOLIC ASPECTS OF DEVELOPING PIGEON GIZZARD

CHAPTER 2

CARBOHYDRATE METABOLISM OF THE DEVELOPING PIGEON GIZZARD: A QUANTITATIVE EVALUATION OF THE METABOLITE, GLYCOGEN; AND HISTOCHEMICAL DISTRIBUTION OF THE ENZYMES ALDOLASE; LACTATE, SUCCINATE AND MALATE DEHYDROGENASES

Carbohydrate metabolism as the primary source of energy for the subservience of animal tissues is, by now, an established fact. Circulating blood glucose, or glycogen which is stored in tissues are the metabolites of choice which are consumed or broken down during the biochemical reactions characteristic of carbohydrate metabolism. Studies on glycolytic enzymes which could easily reveal the operation of the process of carbohydrate catabolism in animal tissues have been carried out by a number of workers in many adult vertebrate tissues (Mancini, 1948; Montagna, 1949; Bergman, 1960; Falin, 1961; Long, 1961; Markert and Masin, 1969; Rinaldo and Giunta, 1967; Ballard and Oliver, 1963; Penney and Cascarano, 1970; Goldberg and Wuntch,1967; Adams and Finnegan, 1965; Moyer et al., 1968). Significance of carbohydrate metabolism and the fluctuations in the amount of utilization of metabolites and the concentration of enzymes concerned with carbohydrate metabolism in different tissues including the regenerating ones have been reported by several workers (Roodyn, 1957; Brunngraber & Abbod,1960;

Needham, 1952; Frederickson & Gordon, 1958; Rossiter & Strickland, 1960; Schmidt, 1962, 1963b, 1964, 1966a; Chakko, 1967; Shah & Chakko, 1967, 1967b; Magon, 1970; Ramachandran, 1972; Shah & Ramachandran, 1970, 1972, 1973; Radhakrishnan, 1972).

Quite recently Grillo (1969) investigated histochemically and quantitatively the levels of glycogen and a few glycolytic and tricarboxylic acid cycle (TCA) enzymes in the gastro-intestinal tract of developing chick. Based on her studies, it was concluded that isocitrate, glucose-6-phosphate and lactate dehydrogenases could be histochemically demonstrated in the smooth muscles of stomach complex (proventriculus and gizzard) during its development. Though similar studies are carried out in a number of developing systems, such studies are, however, lacking in post-natally developing tissues. Since aldolase and lactate dehydrogenase are important enzymes which could denote the operation of glycolysis, a histochemical investigation of these two enzymes in the pigeon gizzard during its post-natal development was deemed worthwhile. Further, the two TCA cycle enzymes viz., SDH and MDH were also studied, not only to assess the importance of TCA cycle oxidation during the post-natal development of the

gizzard but also to understand the differences if any between the striated and smooth muscle fibres in the mode of utilization of carbohydrate. Moreover, for a complete understanding of the present line of investigation, a quantitative analysis of glycogen was also carried out in the post-natally developing pigeon gizzard at various intervals.

MATERIALS AND METHODS

Healthy young pigeons of different ages (in days) were collected from a well maintained aviary for the present study. They were sacrificed under mild anaesthesia at various periods of development. The birds used were of 1, 5, 10, 15, 20, 25 and 30 days old. Adult pigeons were also used for the present study. The gizzards 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 - 18 μ thickness were cut and processed as follows for the localization of various enzymes.

Aldolase: For the histochemical localization of aldolase the incubation medium contained the following ingredients.

Fructose-1-6-diphosphate, 0.02 M (substrate)	5 ml
Nitro Blue Tetrazolium (1 mg/ml)	5 ml
Arsenate-Hydrochloric acid buffer, (0.05 M, pH 7.6)	5 ml
Nicontinamide adenine dinucleotide (NAD)	2.5 mg

After incubating the sections for 20 minutes in the above medium, they were washed thoroughly in distilled water and fixed in 10% neutral formaline for one hour. They were then washed again in distilled water and mounted in glycerine jelly.

Lactate dehydrogenase (LDH): An incubation medium for the above mentioned enzyme contained the following ingredients as suggested by Ogata and Mori (1964).

Sodium lactate, 1 M (substrate)	4 ml
Nitro Blue Tetrazolium (5 mg/3 ml)	3 ml
Phosphate buffer, 0.1 M, pH 7.6	11 ml
Nicotinamide adenine dinucleotide (NAD)	2.5 mg
Sodium cyanide, 0.1 M	2 ml

After incubating the sections for 20 minutes, they were washed in distilled water and fixed in 10% formaline

for one hour. They were then washed in several changes of distilled water and mounted in glycerine jelly.

Succinate dehydrogenase (SDH): This enzyme was histochemically demonstrated according to the modified method of Nachlas et al. (1957). The incubation medium consisted of the following ingredients.

Sodium succinate, 0.2 M (substrate)	1 ml
Phosphate buffer, 0.2 M, pH 7.6	1 ml
Nitro Blue tetrazolium (1 mg/ml)	2 ml

The sections were incubated for 20 minutes in the above medium. They were then washed thoroughly in distilled water and fixed in 10% neutral formaline for one hour. After washing again in several changes of distilled water they were mounted in glycerine jelly.

Malate dehydrogenase (MDH): Histochemical demonstration of malate dehydrogenase was carried out according to the method of Ogata and Mori (1964). The incubation medium consisted of the following ingredients.

Malic acid (sodium salt), 1 M (substrate)	5 ml
Nitro Blue Tetrazolium (5 mg/3 ml)	3 ml
Phosphate buffer, 0.1 M, pH 7.4	10 ml
Nicotinamide adenine dinucleotide (NAD)	2.5 mg
Sodium cyanide, 0.1 M	2 ml

The sections were incubated in the above medium for 20 minutes. After incubation, they were fixed in 10% neutral formaline for one hour. They were then thoroughly washed in distilled water and mounted in glycerine jelly.

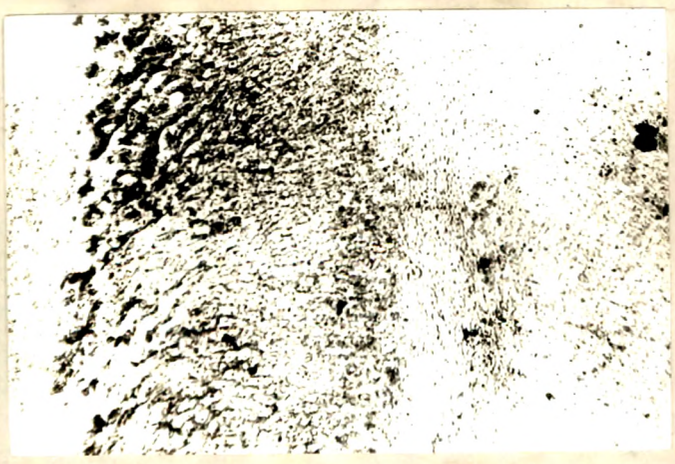
Controls: For controls, in each case of enzyme study, the sections were either incubated for specific length of time in the respective media devoid of specific substrates or heated to 80°C for 15 minutes to destroy the enzyme activity and then incubated in the respective media used for samples.

OBSERVATIONS

Aldolase : (Figs. 1 to 8; 1a to 8a)

On the day of hatching the aldolase activity was noticeable, though low in concentration, in both the smooth and muscle fibres as well as in the secretory tubules of the gizzard. During the successive stages of the post-natal development of the gizzard viz., on 5th, 10th and 15th days, there was a steady and gradual increase in the enzyme concentration in both the components of the gizzard which ultimately reached the highest level of expression on the 20th day and remained so on the 25th day also. A comparison at this stage (25th day) showed that the epithelial tubules

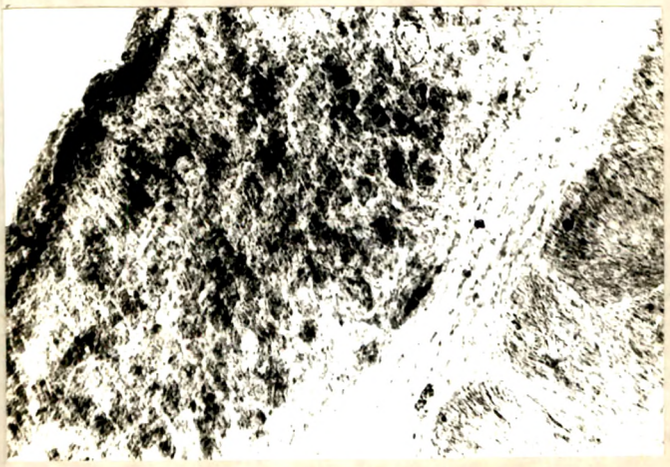
ALDOLASE ACTIVITY IN THE MUCOSAL TUBULES OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

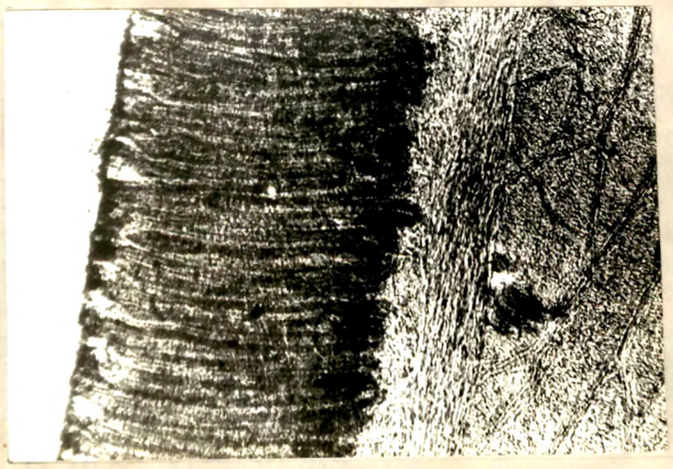
1



200 μ

5 DAY OLD

2



200 μ

10 DAY OLD

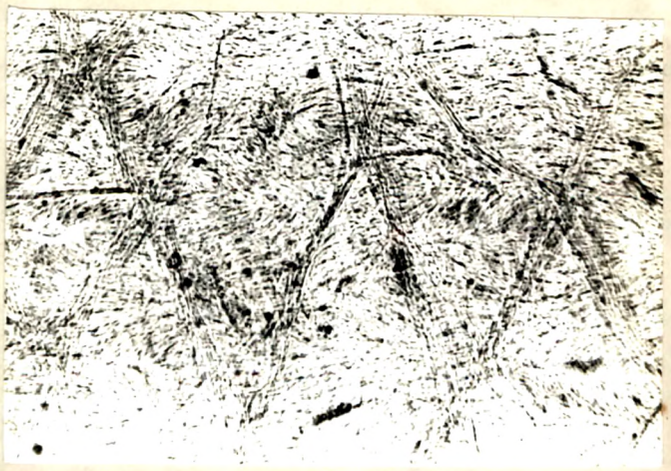
3

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

100 μ

1 DAY OLD

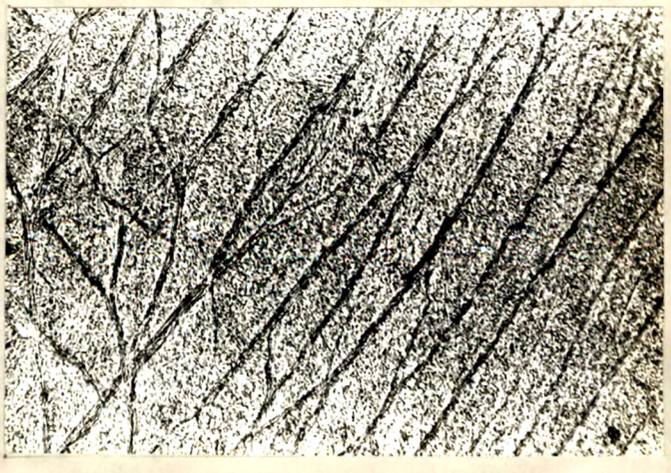
1a



200 μ

5 DAY OLD

2a

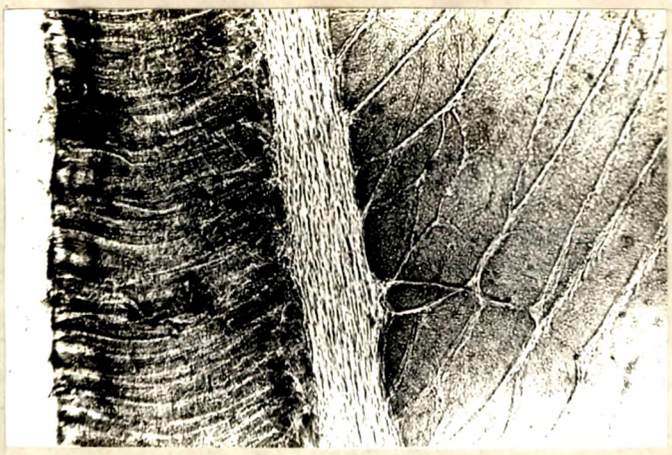


200 μ

10 DAY OLD

3a

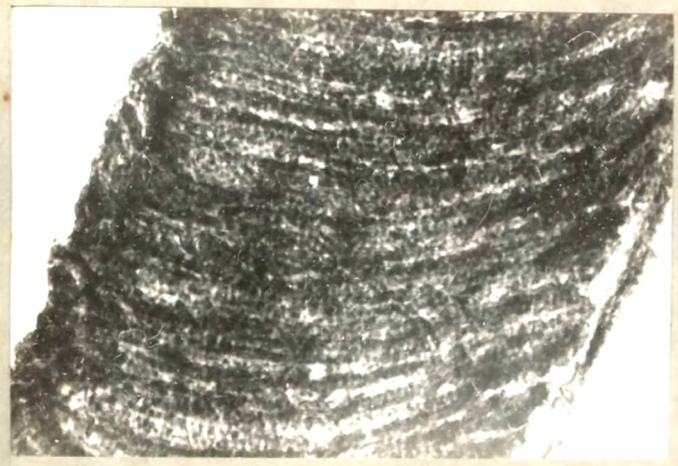
ALDOLASE ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



200 μ

15 DAY OLD

4



100 μ

20 DAY OLD

5

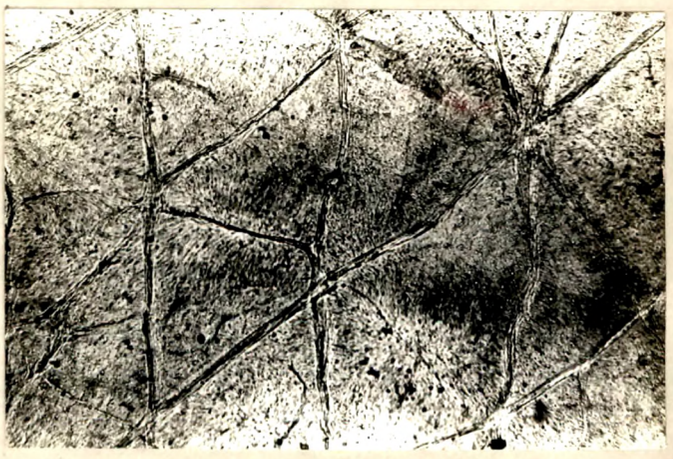
200 μ

25 DAY OLD

6

ALDOLASE ACTIVITY IN THE SMOOTH MUSCLE FIBRES

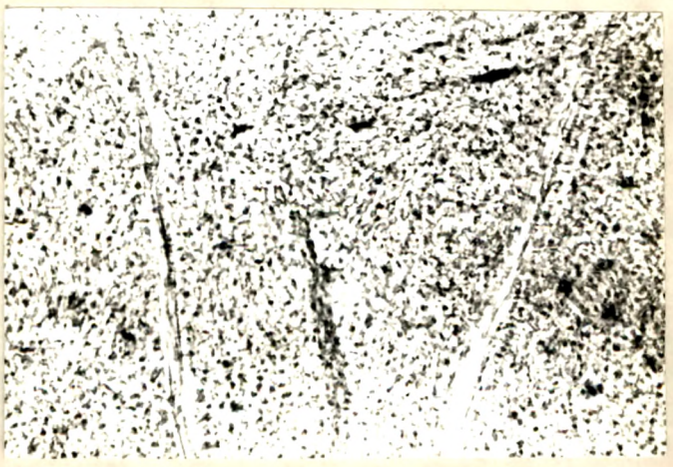
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

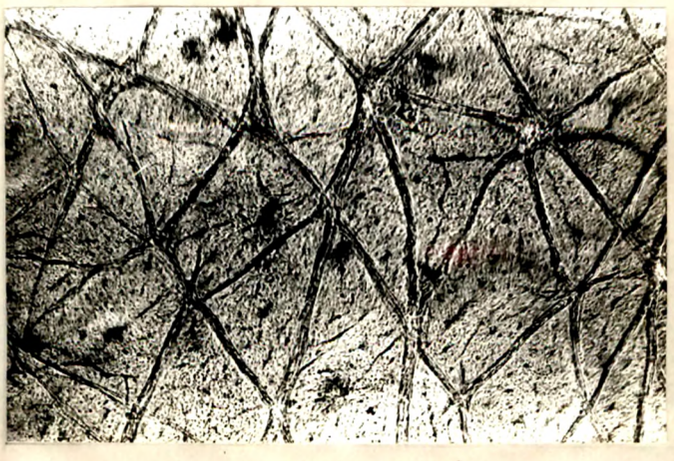
4a



100 μ

20 DAY OLD

5a



100 μ

25 DAY OLD

6a

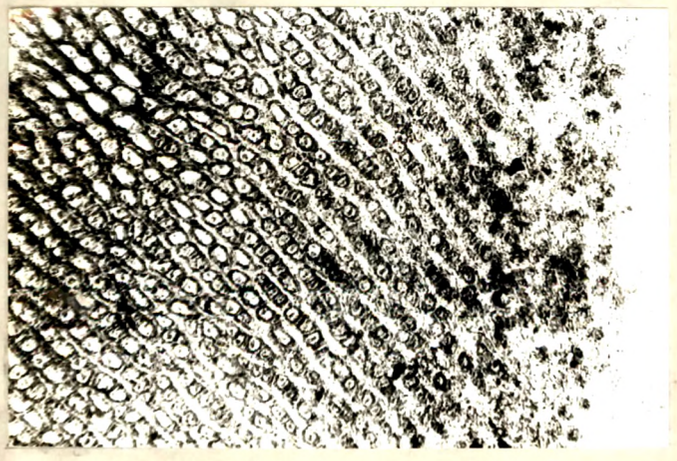
ALDOLASE ACTIVITY IN THE MUCOSAL TUBULES

OF THE DEVELOPING PIGEON GIZZARD

200 μ

30 DAY OLD

7



200 μ

ADULT

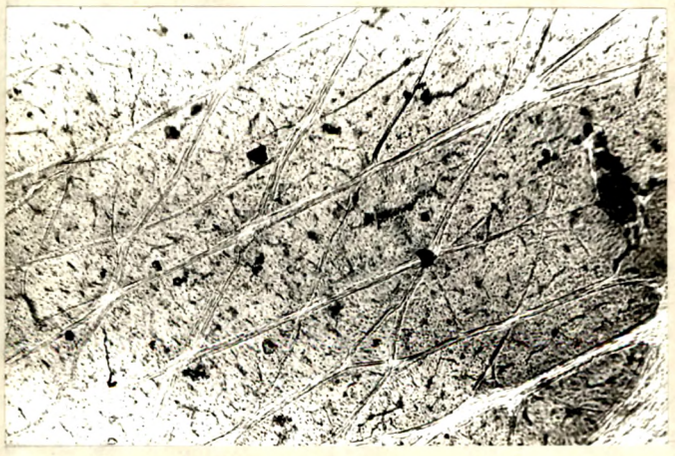
8

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

150 μ

30 DAY OLD

7a



100 μ

ADULT

8a

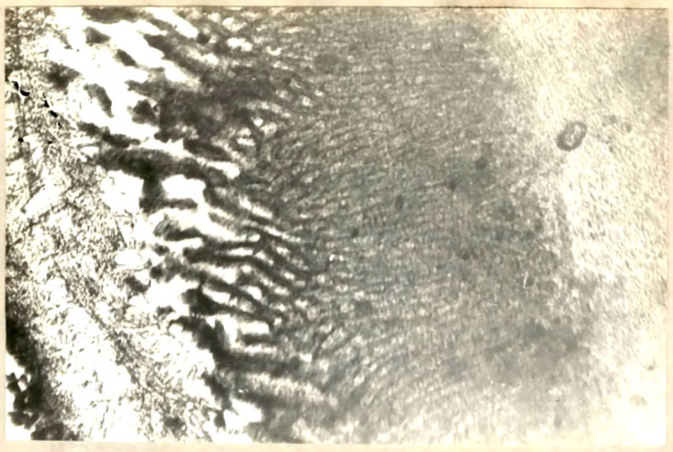
to be slightly more enzyme reactive than the muscle fibres. Hereafter, on 30th day, there was a slight drop in the aldolase activity in both the components of the gizzard and this level of enzyme activity was observed in the succeeding adult condition also. On an overall basis, excepting for a short period of gizzard development extending from days 5th to 15th when the enzyme activity was slightly more in the muscle fibres than in the secretory tubules, on all other periods of development, aldolase activity tended to be slightly more in the secretory tubules than in the muscle fibres.

Lactate dehydrogenase: (Figs. 9 to 16; 9a to 16a)

Unlike aldolase, LDH was found to have a slightly different pattern of activity during the post-natal development of pigeon gizzard. There was a difference in the pattern of LDH activity even in the two components of the gizzard during the first fifteen days of the development. In the muscle component there was an initial drop on day 5th and then a gradual increase while in the epithelial component, right from the day of hatching, there was a gradual and continuous increase without any drop as was in the case of aldolase. However, in both the components, after this minor disparity, LDH activity

LDH ACTIVITY IN THE MUCOSAL TUBULES

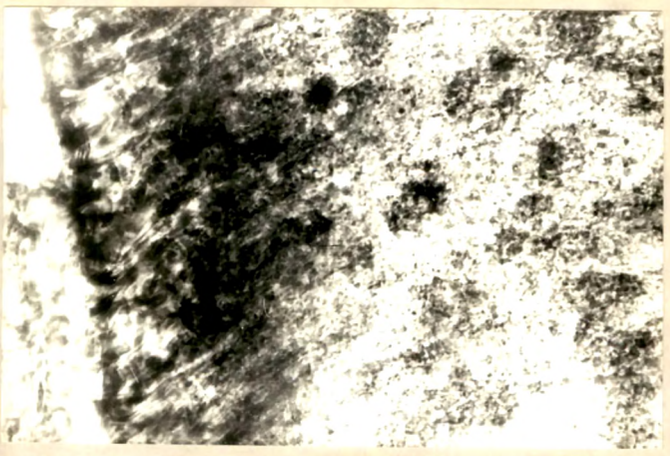
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

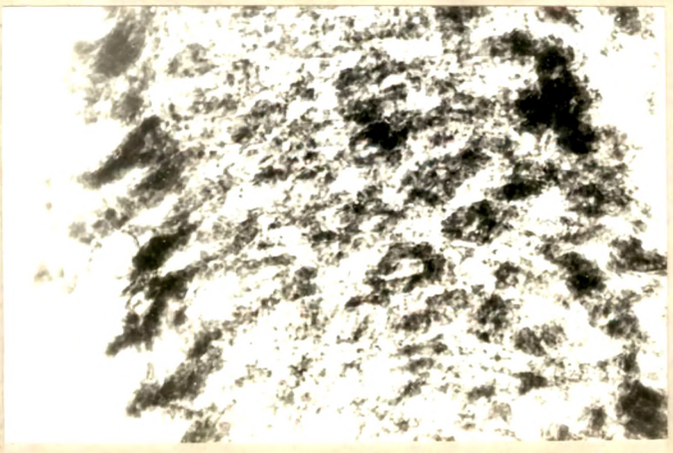
9



100 μ

5 DAY OLD

10



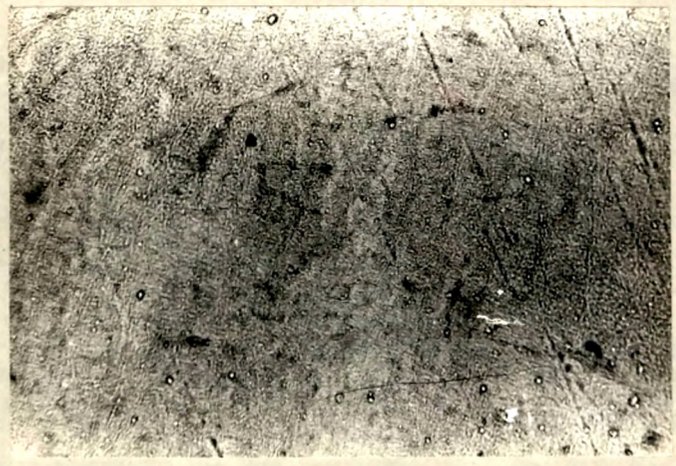
100 μ

10 DAY OLD

11

LDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

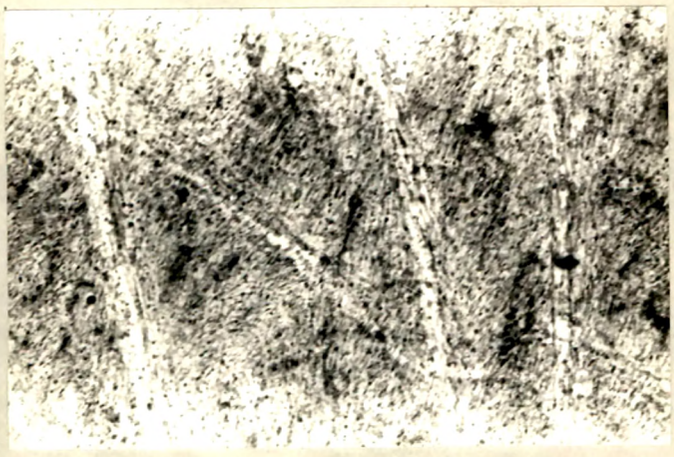
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

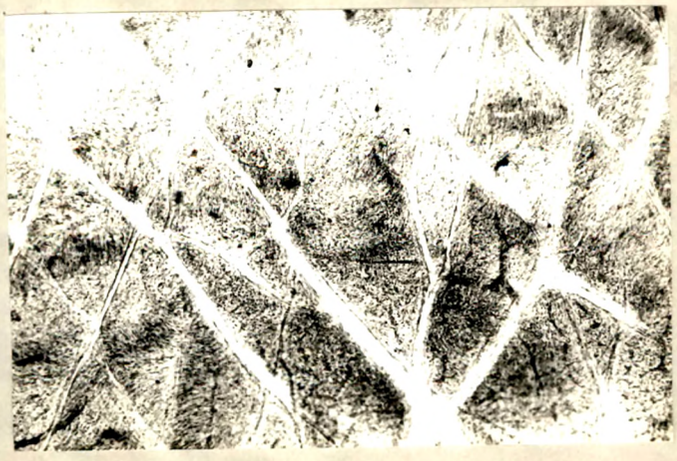
9a



200 μ

5 DAY OLD

10a



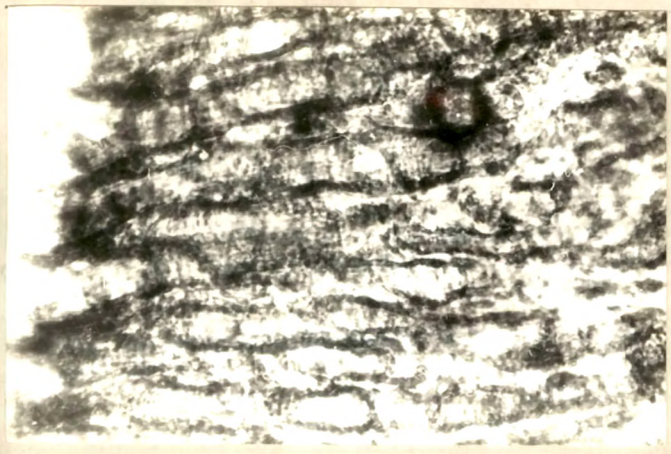
200 μ

10 DAY OLD

11a

LDH ACTIVITY IN THE MUCOSAL TUBULES

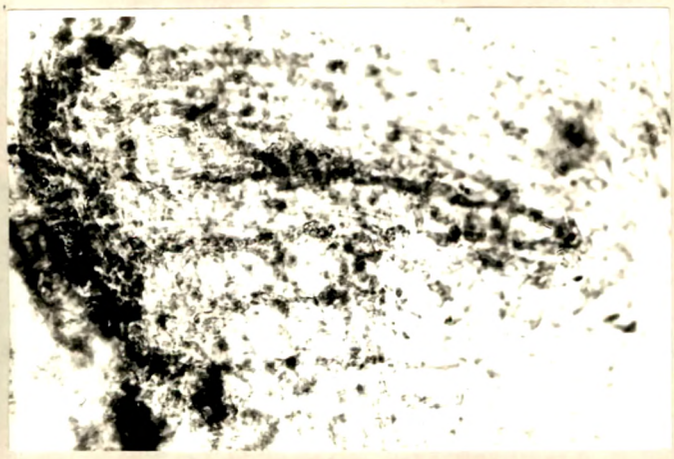
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

12



100 μ

20 DAY OLD

13

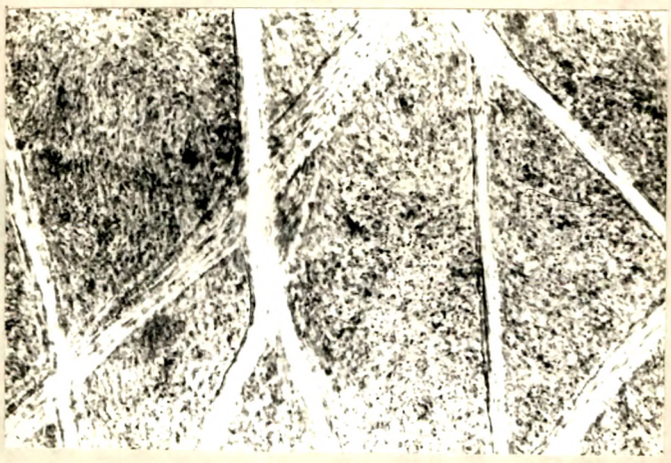
150 μ

25 DAY OLD

14

LDH ACTIVITY IN THE SMOOTHNMUSCLE FIBRES

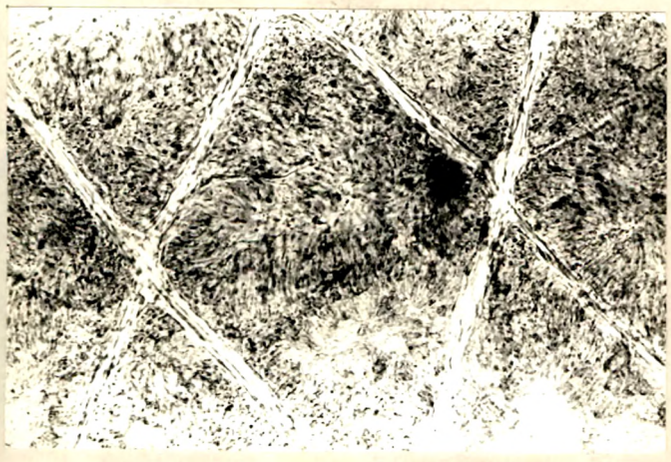
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

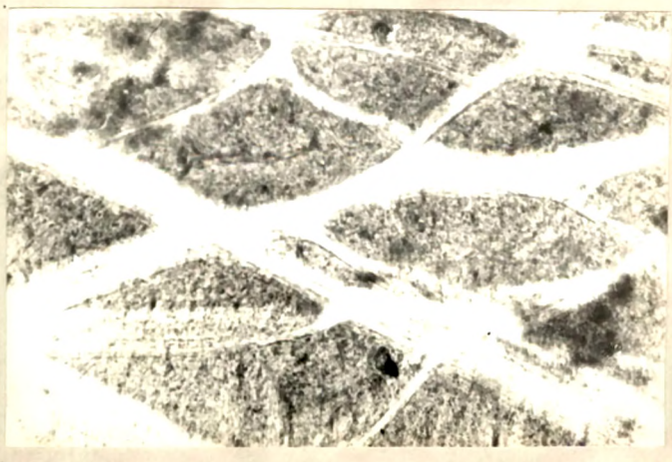
12a



100 μ

20 DAY OLD

13a

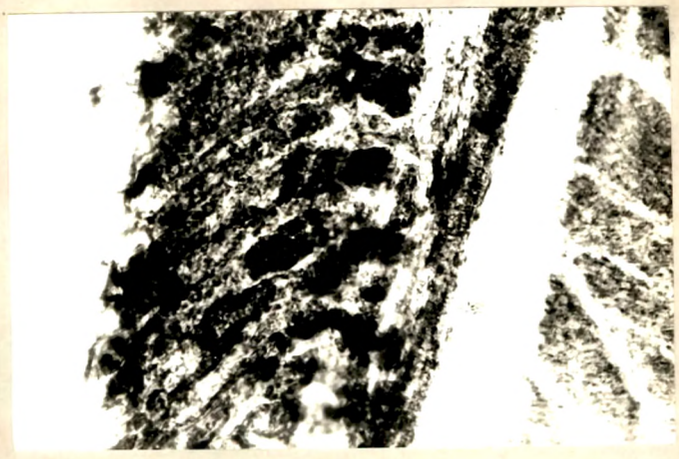


200 μ

25 DAY OLD

14a

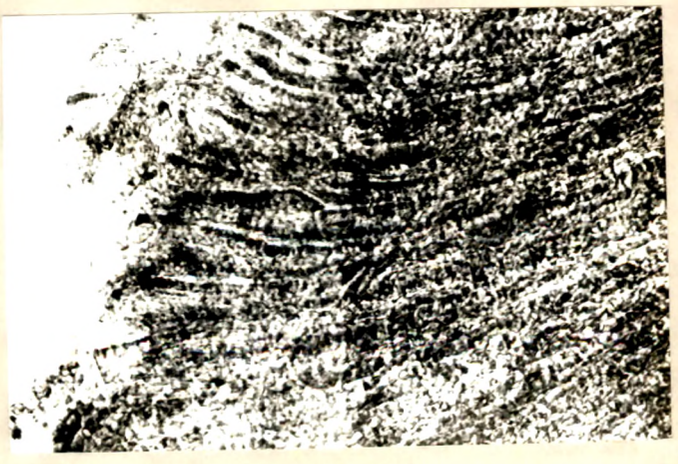
LDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

15

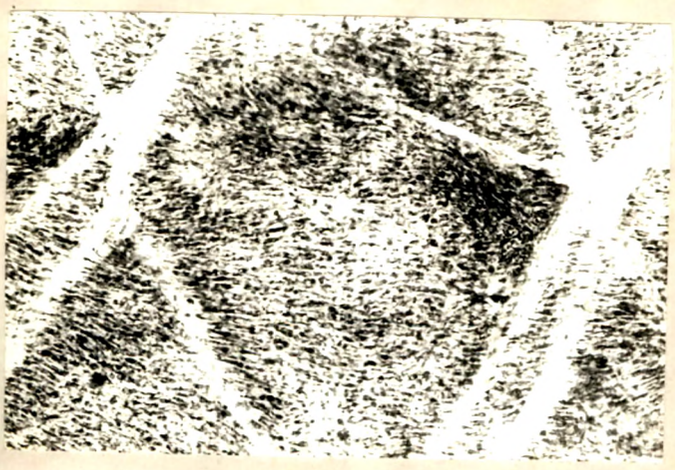


100 μ

ADULT

16

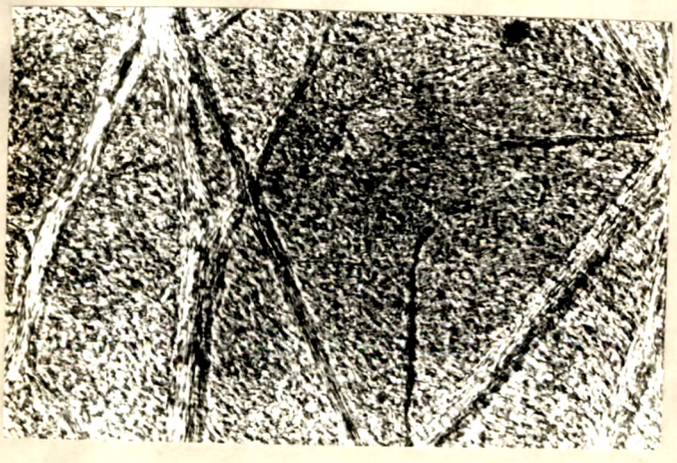
LDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

15a

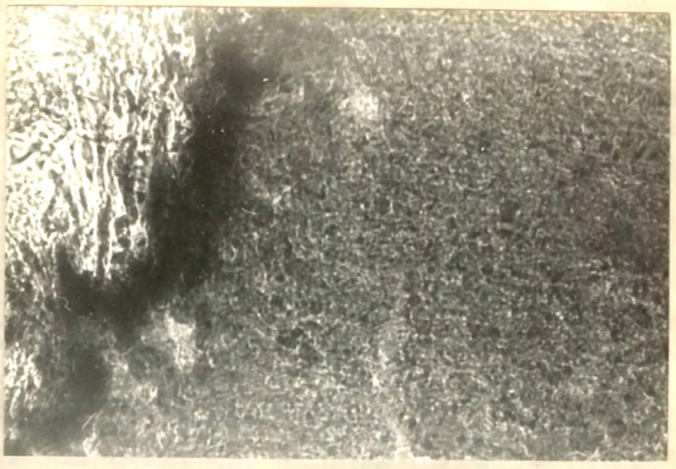


100 μ

ADULT

16a

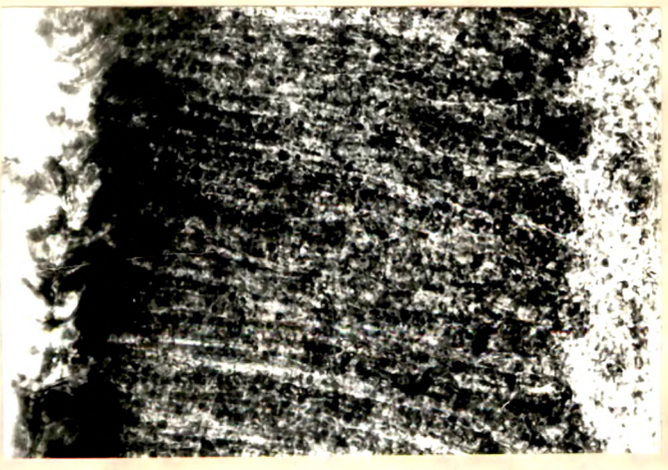
SDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

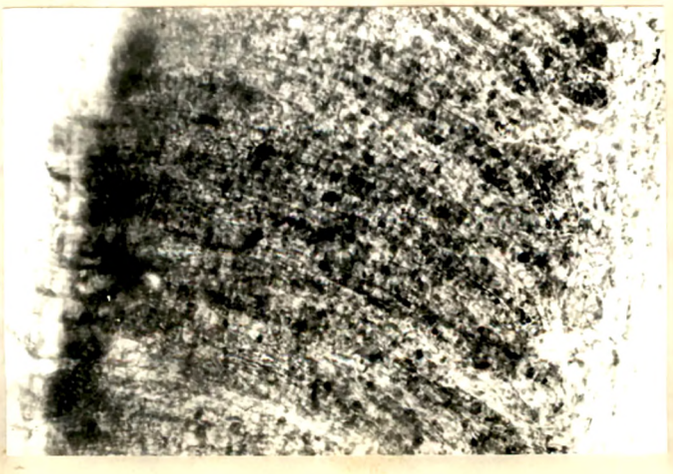
17



100 μ

5 DAY OLD

18



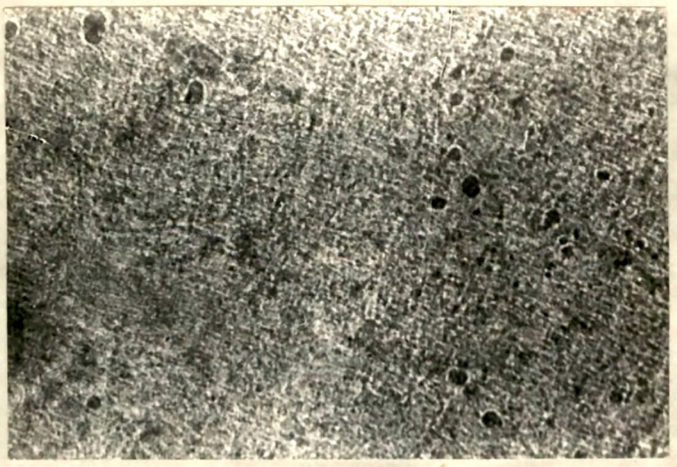
100 μ

10 DAY OLD

19

SDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

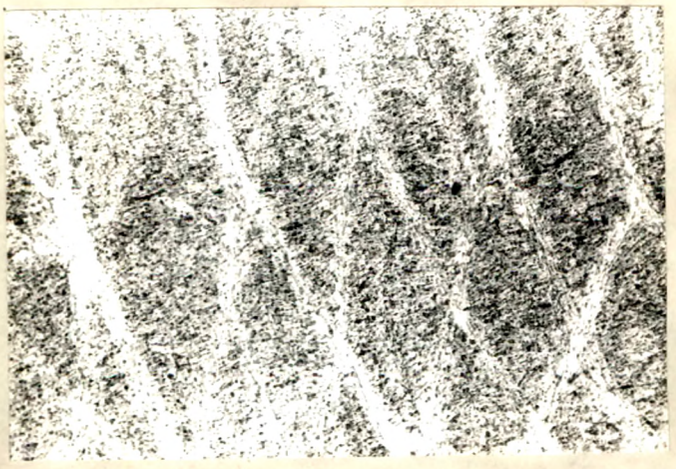
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

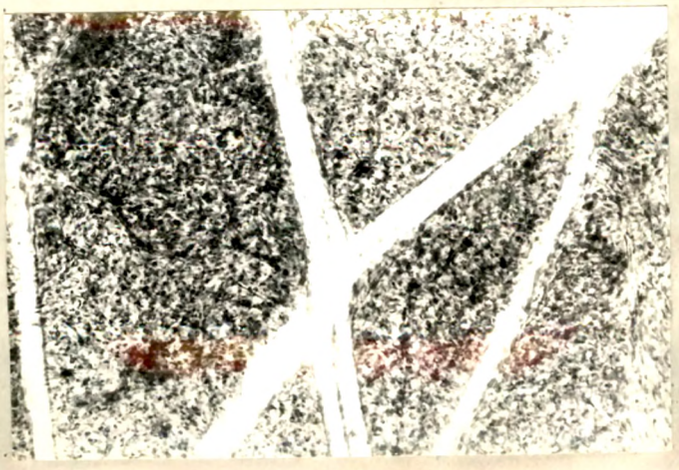
17a



200 μ

5 DAY OLD

18a

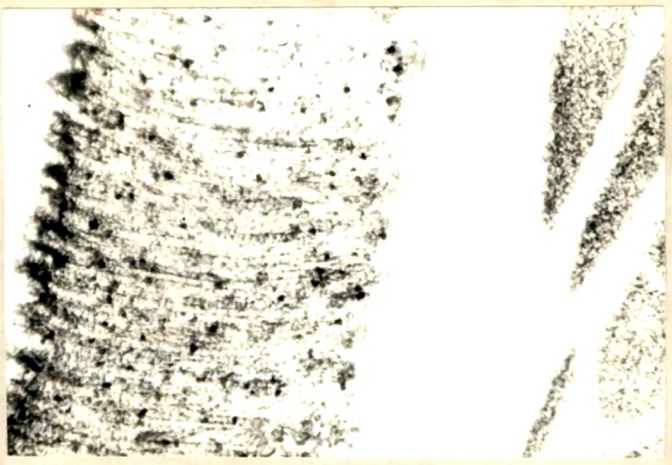


100 μ

10 DAY OLD

19a

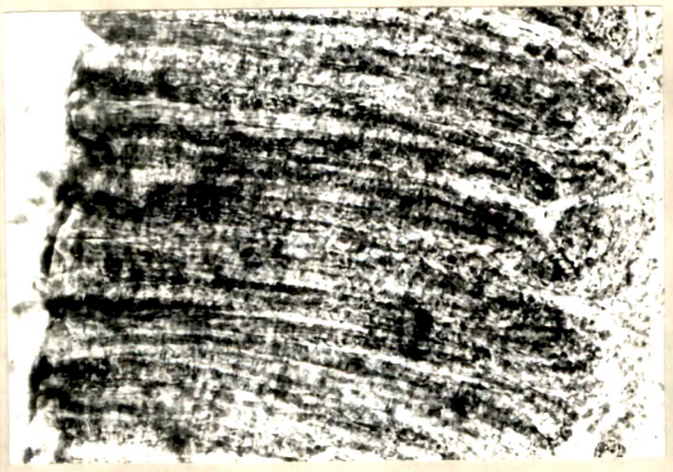
SDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



150 μ

15 DAY OLD

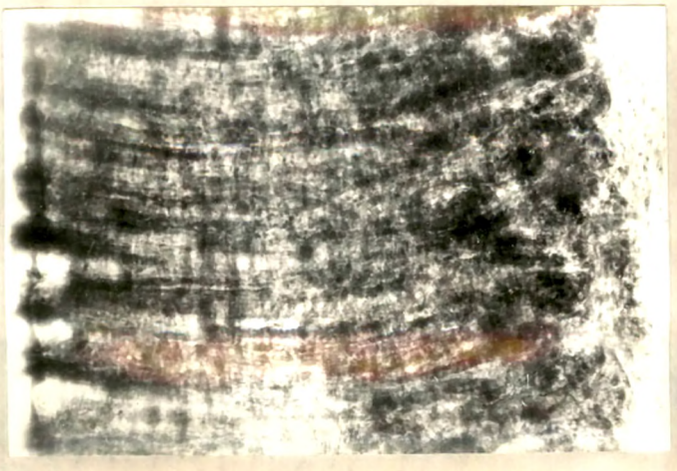
20



100 μ

20 DAY OLD

21

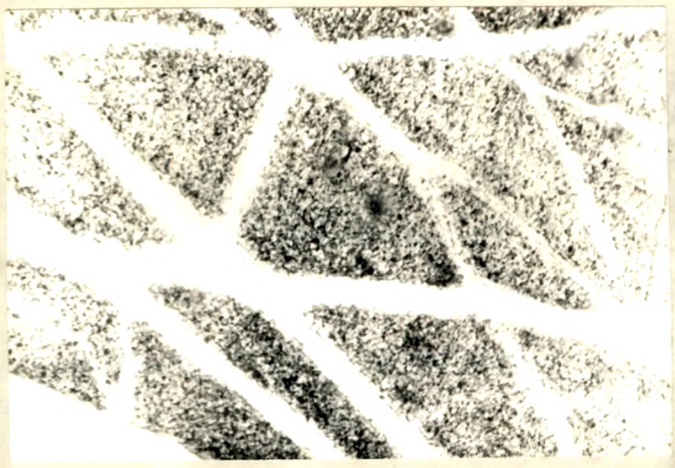


100 μ

25 DAY OLD

22

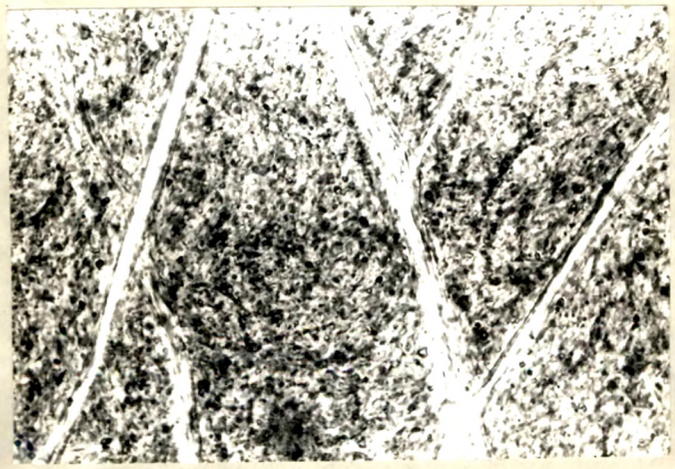
SDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



150 μ

15 DAY OLD

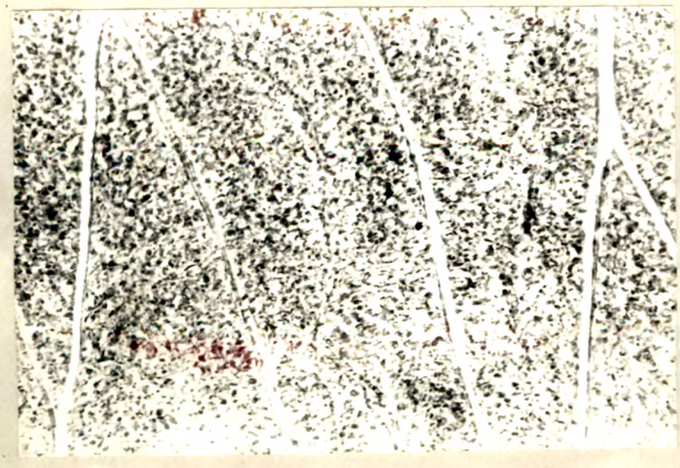
20a



100 μ

20 DAY OLD

21a

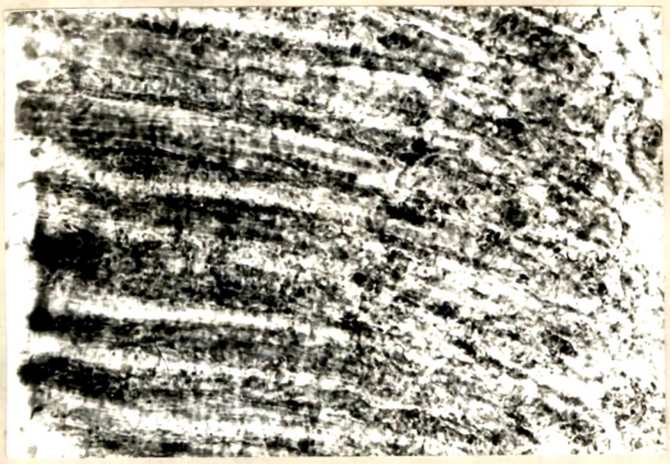


100 μ

25 DAY OLD

22a

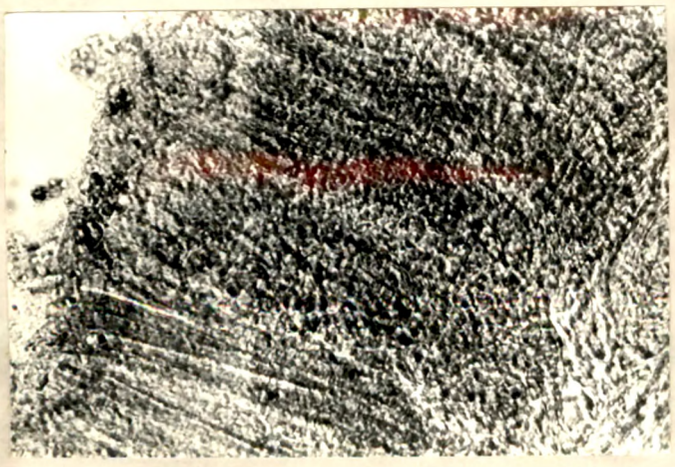
SDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

23

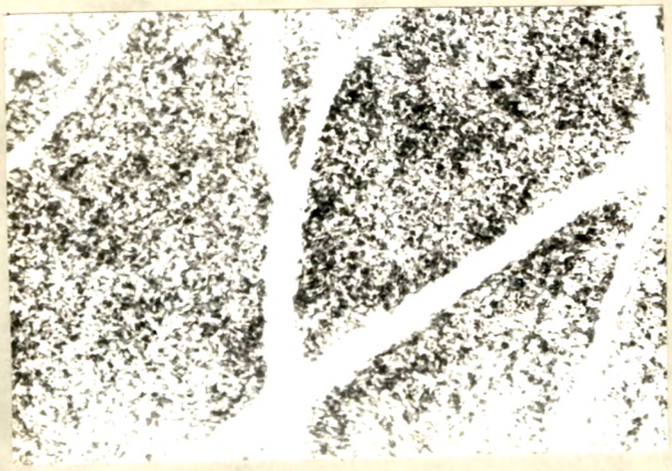


100 μ

ADULT

24

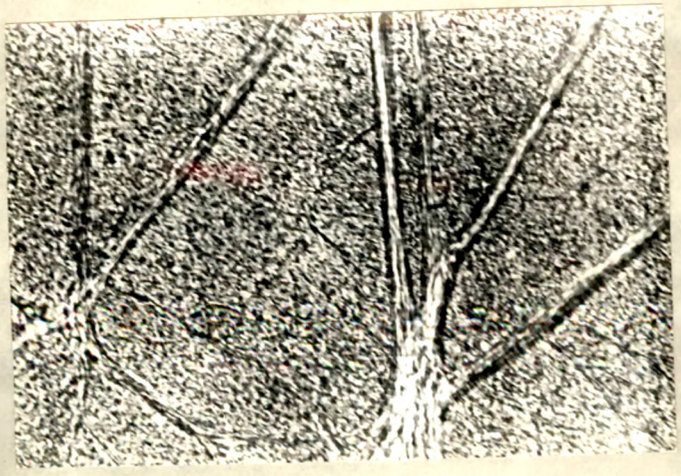
SDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

23a



100 μ

ADULT

24a

rose to a peak level on the 20th day and remained so even on the 25th day of development. This high level of enzyme activity was maintained on all the succeeding days of development and adult condition. An interesting point of observation to be noted is the activity of LDH in the muscle fibres on the day of hatching which was noticeable higher than that of the other three enzymes being investigated presently i.e., aldolase, SDH and MDH.

Succinate dehydrogenase: (Figs. 17 to 24; 17a to 24a)

All throughout the periods of post-natal development of gizzard, SDH activity remained higher in the muscle fibres than in the secretory tubules. However, the enzyme concentration in the two components was found to be different. The enzyme activity which was quite appreciable on the day of hatching in the muscle component remained so even on the 5th day. But on the 10th day a sudden increase in its activity, almost two-fold, was noticed. This high level of SDH activity in the muscle fibres reappeared on the 20th day of development, after a slight decrease in activity on the 15th day. After the 25th day there was again a slight drop in SDH activity and this level was maintained all throughout the rest of the development period and adult condition. In the epithelial tubules the enzyme

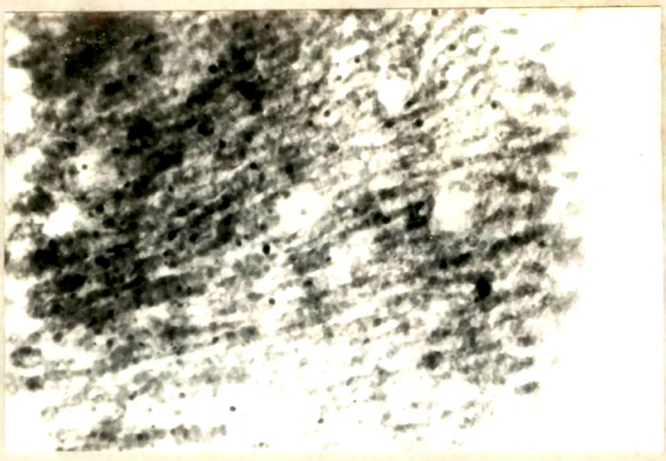
activity which was slightly less than that observed in the muscle layer, increased slowly and reached, on the 10th day, a level twice the one observed on the 1st day. However, on a comparative basis, the muscle fibres were slightly more enzyme reactive than the epithelial tubules at this stage. Hereafter, the SDH activity in tubules dropped a little and then rose sharply again to register a two fold increase on the 20th day. At this period the intensity of enzyme activity was equal in both the components. But in the secretory tubules, unlike in the muscle fibres, there was a sharp fall in the SDH activity on the 25th day, almost to one half of that noticed on the 20th day. This reduced level was the characteristic feature of the rest of the phases of gizzard development and also in the adult state.

Malate dehydrogenase: (Figs. 25 to 32; 25a to 32a)

Of all the four enzymes presently investigated viz., aldolase LDH, SDH and MDH, the activity of MDH was found to be comparatively lower. Whereas a more or less steady level of MDH activity was observable in the muscle fibres, a fluctuation in the levels of the enzyme activity was characteristic feature in the epithelial (tubule) layer. In the muscle fibres, the MDH activity which was moderate

MDH ACTIVITY IN THE MUCOSAL TUBULES

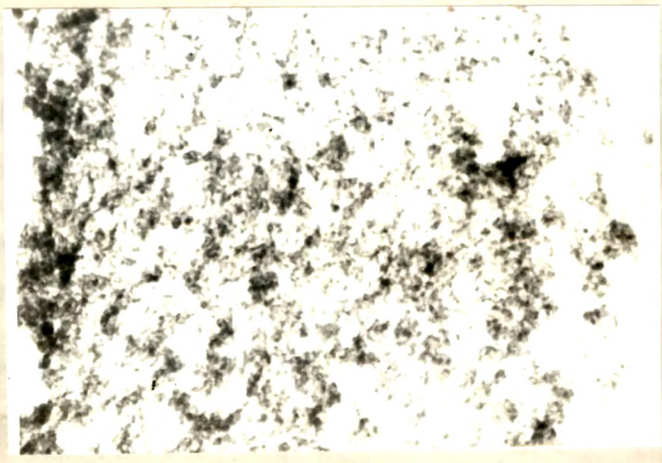
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

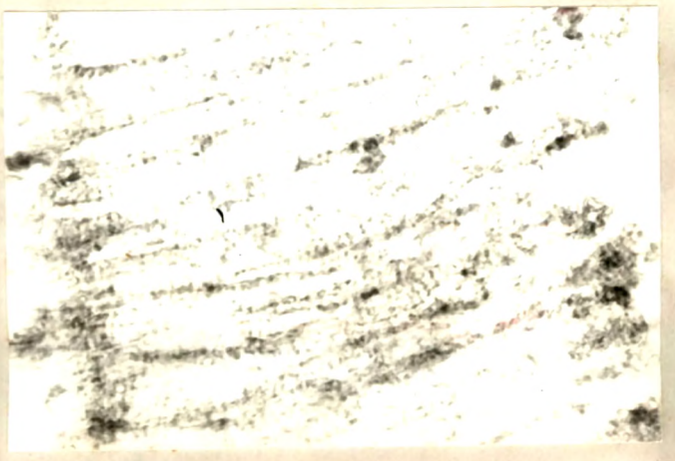
25



100 μ

5 DAY OLD

26



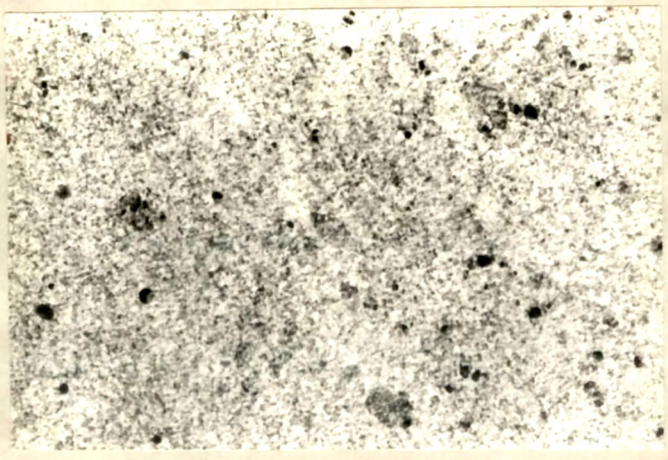
100 μ

10 DAY OLD

27

MDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

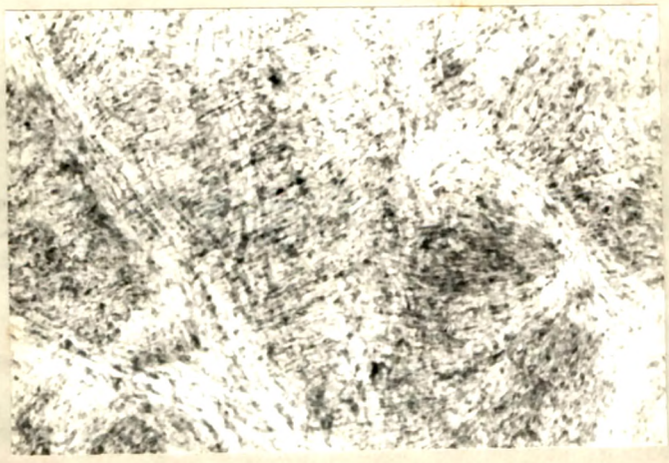
OF THE DEVELOPING PIGEON GIZZARD



100 μ

1 DAY OLD

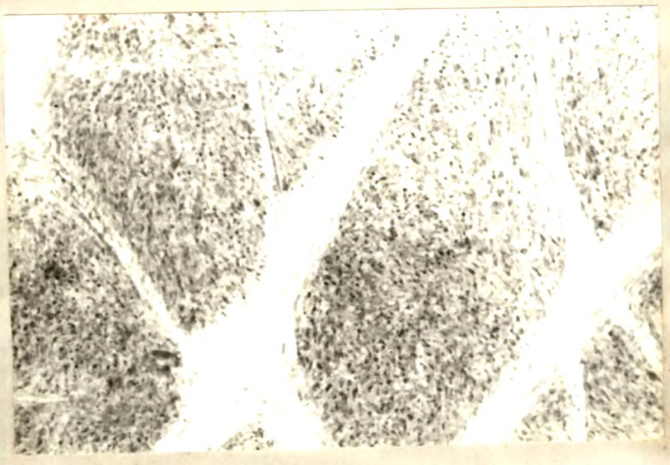
25a



100 μ

5 DAY OLD

26a



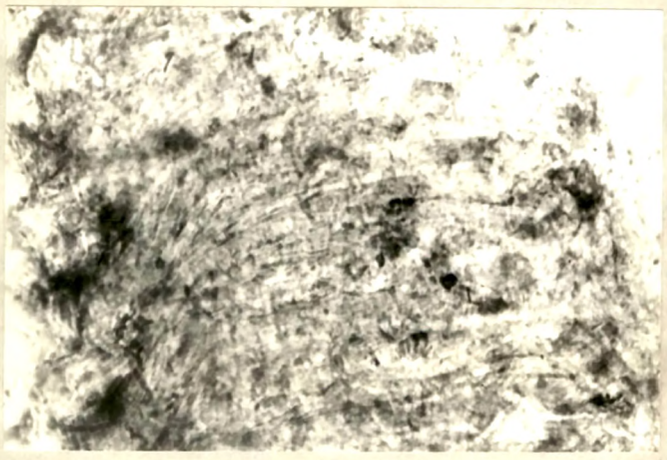
100 μ

10 DAY OLD

27a

MDH ACTIVITY IN THE MUCOSAL TUBULES

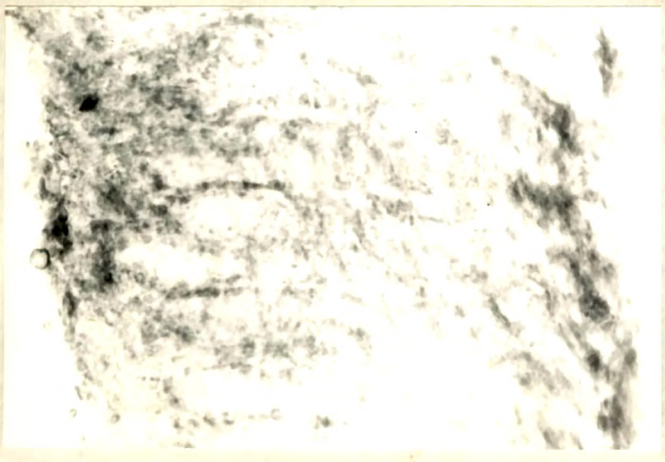
OF THE DEVELOPING PIGEON GIZZARD



150 μ

15 DAY OLD

28



100 μ

20 DAY OLD

29

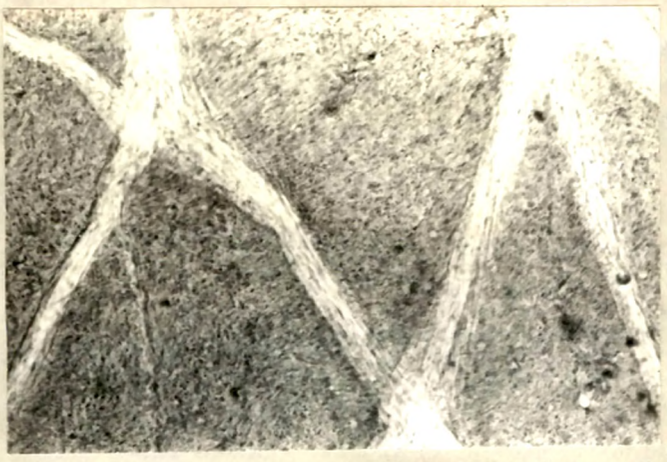
100 μ

25 DAY OLD

30

MDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

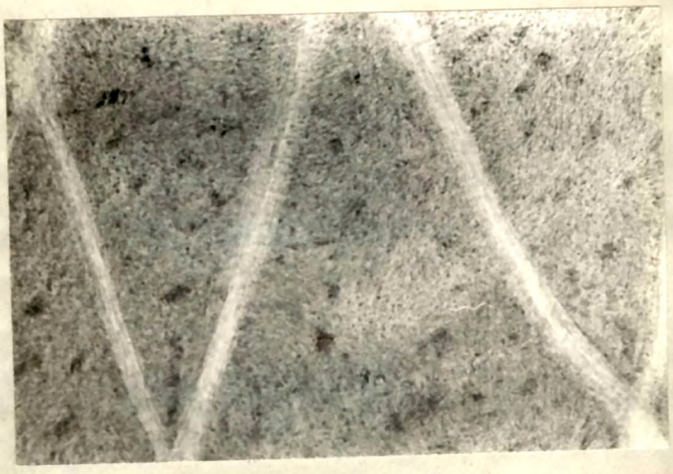
OF THE DEVELOPING PIGEON GIZZARD



100 μ

15 DAY OLD

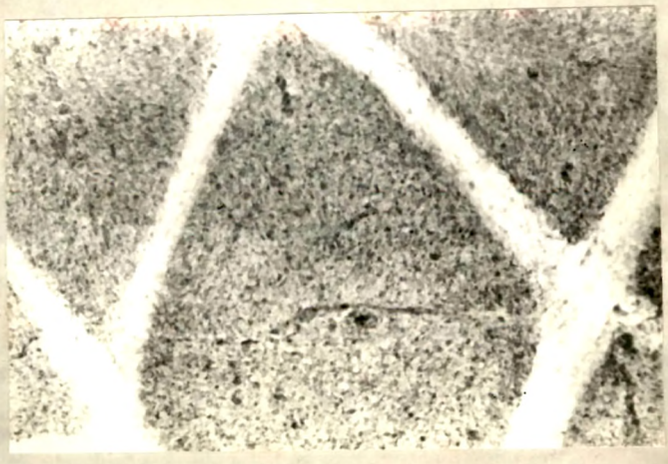
28a



100 μ

20 DAY OLD

29a

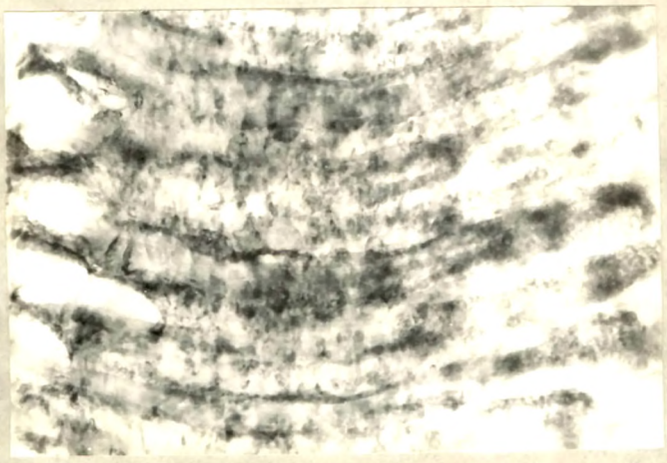


100 μ

25 DAY OLD

30a

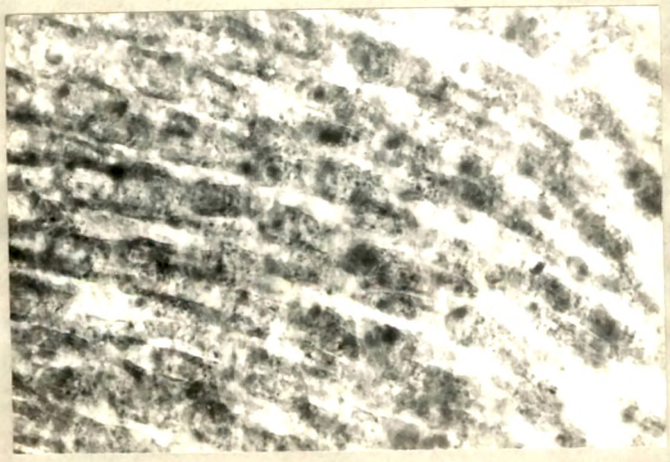
MDH ACTIVITY IN THE MUCOSAL TUBULES
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

31



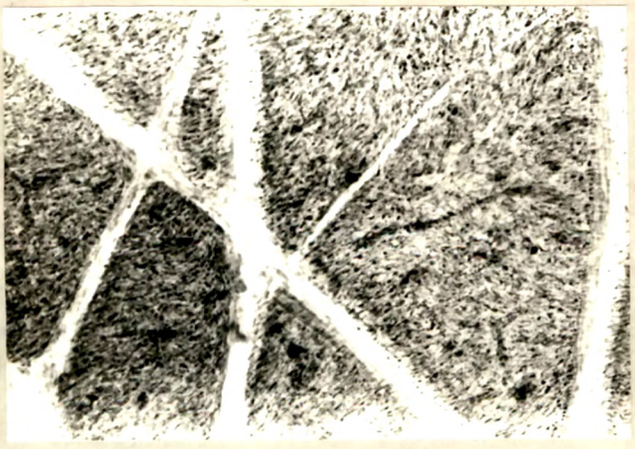
100 μ

ADULT

32

MDH ACTIVITY IN THE SMOOTH MUSCLE FIBRES

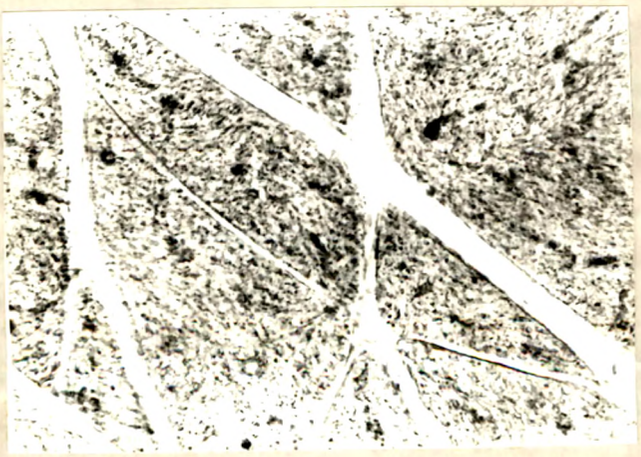
OF THE DEVELOPING PIGEON GIZZARD



100 μ

30 DAY OLD

31a



100 μ

ADULT

32a

on the day of hatching as well as on the 5th day, rose to a maximum level on the 10th day and was maintained uptil the 25th day, excepting for a slight short lasting decrease on the 15th day. Hereafter, the enzyme activity, though registering a slight drop, nevertheless, maintained almost the same intensity of activity not only throughout the remaining periods of development of gizzard but also in the adult gizzard muscles. But in the epithelial tubules MDH activity commencing from a low level on the 1st day, rose gradually to the maximal level on the 10th day to be followed immediately by a reduction to half the level on the 15th day. However, its activity again climbed up to the maximal level on the 20th day, quite similar to the one observed on the 10th day. The enzyme activity was again halved on the 25th day which remained so upto the 30th day, but later increased to the same high level, as noted on the 20th day, in the adult pigeon gizzard.

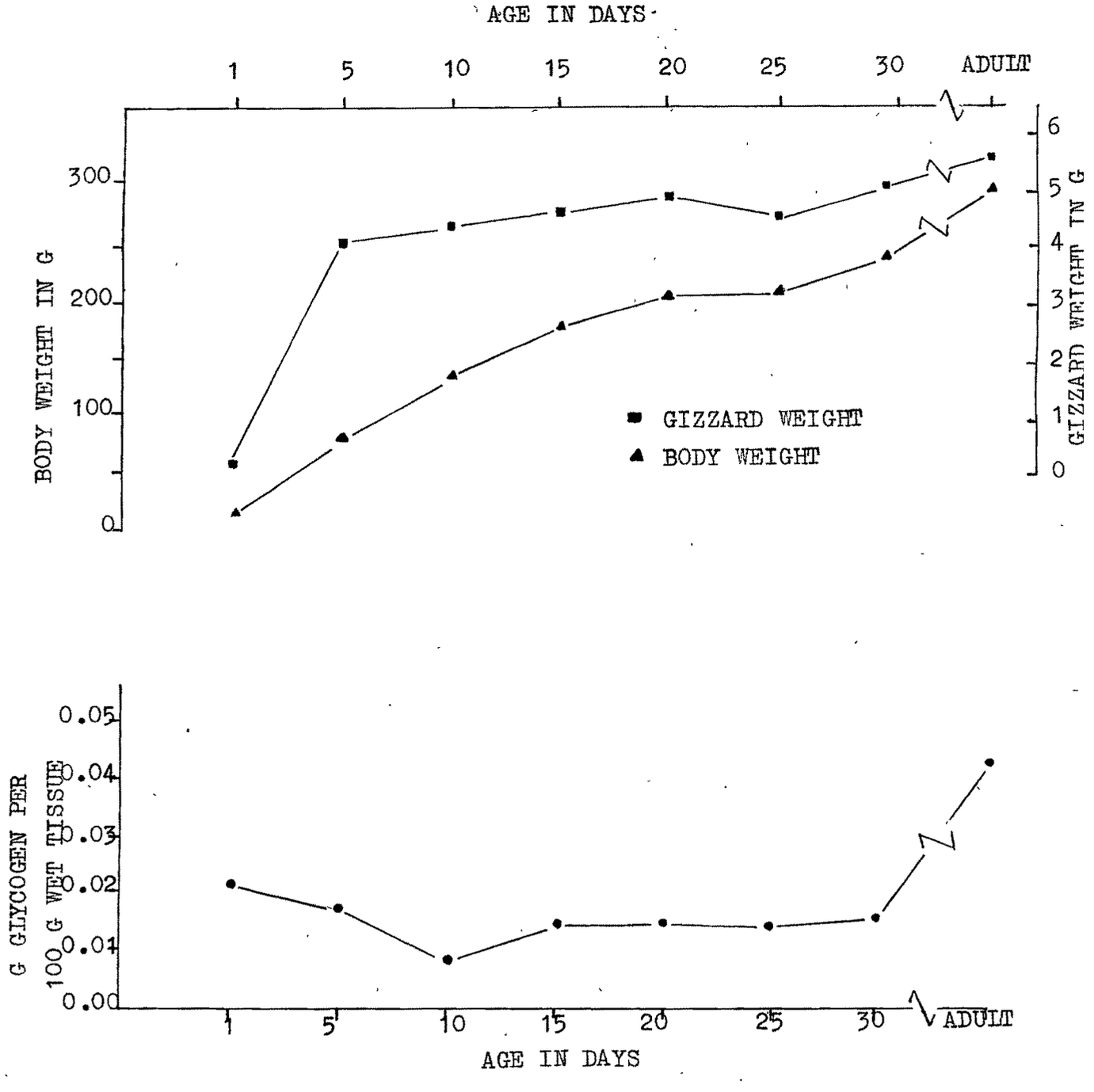
Glycogen: It has been observed from the quantitative study that from the day of hatching till 10th day there was a slow decline of glycogen content. An almost steady level of glycogen content was maintained during the successive periods i.e., 15th, 20th, 25th and 30 days while an increased glycogen content was the feature in the adult condition.

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

Age in days	Body weight	Gizzard weight	Amoung of glycogen (gm/100 gm wet tissue)
1	13.5	0.408	0.0220
5	85	4.155	0.0205
10	135	4.368	0.0080
15	181	4.513	0.0154
20	203	4.731	0.0138
25	212	4.984	0.0141
30	245	5.174	0.0157
Adult	310	5.581	0.0430

*Average of 5 birds

Fig. 33. Percentage of glycogen expressed in relation to body and gizzard weights during different days of post-natal development of pigeon gizzard



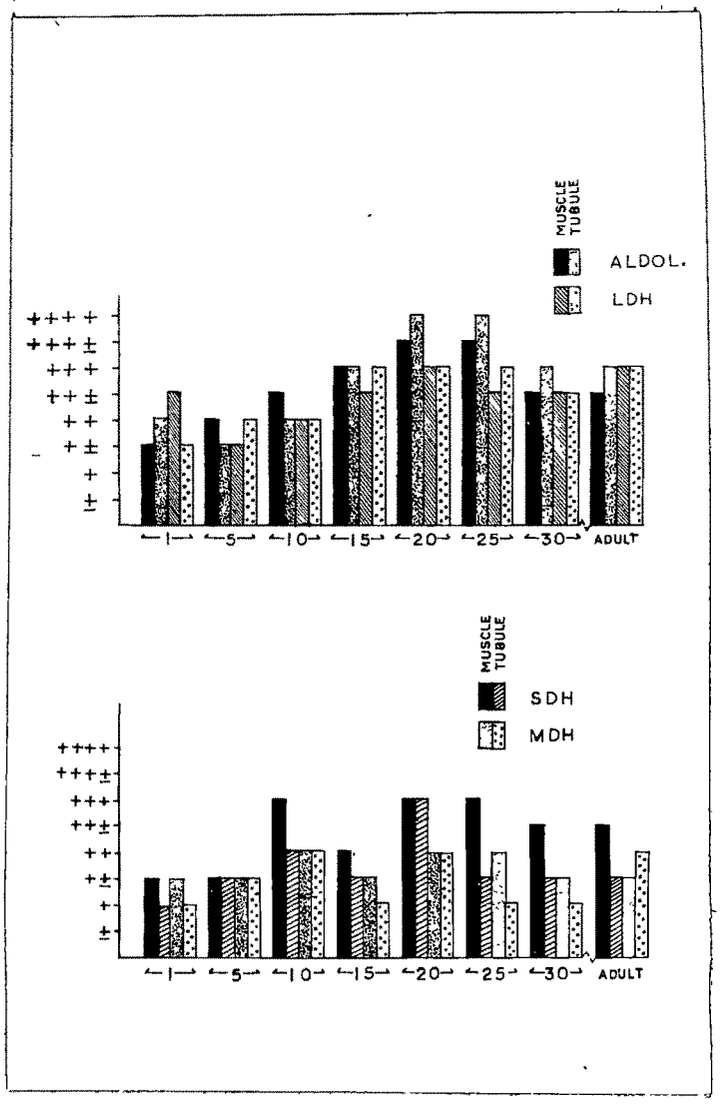


FIG. 34

Graphical representation of the changes in the aldolase, LDH, SDH and MDH distribution pattern during the various days of gizzard development

The data obtained from the quantitative estimation of glycogen has been represented in table I and figure 33.

The histochemically observed concentrations of all the four enzymes viz., aldolase, LDH, SDH and MDH during the process of post-natal development are represented diagrammatically in figure 34.

DISCUSSION

The present observations on aldolase, LDH, SDH and MDH tend to indicate the fact that the pigeon gizzard, during the early days of development is completely subservient on glycolytic metabolism. This appears to be well reflected in the moderately high activity of LDH and aldolase and weaker activities of SDH and MDH noted in both the components of the gizzard on the 1st day after hatching. The distribution of these enzymes noted herein seems to be well in conformity with the findings of Grillo (1969) in the developing gastro-intestinal tract of chick wherein LDH was found to be the most active of all enzymes. The presence of LDH as indicative of anaerobic glycolysis as suggested by Pearse (1960) is noteworthy here. Aldolase and LDH as indicators of glycolytic metabolism are well established by studies on various vertebrate tissues (George

& Talesara, 1961, in pigeon pectoralis muscle; Long, 1961, in striated muscle; Roodyn, 1955, 1959, in the nuclei of liver cells; Duve et al., 1962, in liver and other tissue homogenates; Magon, 1970; Shah & Ramachandran, 1970, 1972; Ramachandran, 1972, in different tissues of normal and regenerating lacertilian tail). In this wake, the constant and gradual increase in activities of both the above mentioned enzymes all throughout the course of post-natal development of gizzard are rather self explanatory and indicative of a continuous process of anaerobic glycolytic metabolism. The concurrent slow but gradual decline in the glycogen content of the gizzard during the first 10 days is in good correlation with the suggestion of a predominant carbohydrate metabolism during the initial days of development as mentioned earlier. However, the utilization of glucose supplied through blood is also a distinct possibility and cannot be overruled. This is quite apparent not only from the very low values of glycogen content observed in the pigeon gizzard but also by the very small decline of glycogen content during the first 5 days of development. The meagre glycogen content, and the negligible amount of decrease of the same coupled with the unchanged weak activities of SDH and MDH and negligible utilization of lipid (chapter 3) together with the steady

increase of aldolase and LDH are noteworthy here and apparently suggest the utilization of glucose. It may be safely persumed herefrom that during the first 10 days the metabolic necessities of the pigeon gizzard are only of a low order and hence could be easily met with by the mere degradation of glucose through the anaerobic glycolytic pathway.

However, on or around 10th day of post-natal development the metabolic activities appear to be very much stepped up as could be inferred from the increased levels of all the four enzymes under investigation. It is also interesting to note the sharp decline of glycogen with maximal levels of SDH and MDH activities in the wake of no apparent lipid catabolism (chapter 3) at this stage of gizzard development. This accelerated metabolic activity appears to be in good correlation with the concomitant spurt in the morphological and physiological development of the organ marked by an increased rate of proliferation and differentiation of both the smooth muscle fibres as well as the mucosal epithelium. During this hectic phase of structural development of the organ directed mainly at the attainment of its functional competence, the glucose utilization through the glycolytic pathway fails to keep pace and hence the developing gizzard calls upon the glycogen

reserve to meet the increased energy requirements, is well indicated not only by the decreased glycogen content but also by the increased activities of SDH and MDH, the two TCA cycle enzymes. In the absence of any signs of lipid catabolism (chapter 3) at this period, the peak levels of both SDH and MDH are rather significant and tend to indicate the possibility of complete oxidation of glycogen through the TCA cycle so as to liberate maximum amount of energy. This increased metabolic activity at this period gains further validity by the observation of fragmented and partially digested grains in the gizzard of the young ones as they are fed so by the parents which need to be ground by the gizzard where muscles are subjected to considerable activity.

The latter half of the post-natal development of the pigeon gizzard, extending from 15th to 25th days, is marked by a slow and gradual increase of glycogen content and coincidently, this period when the gizzard, now having attained its functional endowment, has commenced full scale activity, as the pigeon young ones have started with the feeding experiments on solid grains. Even as the glycogen content is registering a slow rise, both the glycolytic enzymes, aldolase and LDH continue to show increasing

activities. At the same time the two TCA cycle enzymes, SDH and MDH, after a slight decrease on the 15th day, once again attained the highest level of activities on the 20th day and remained so on the 25th day of development. Simultaneous decrease of lipid content and increase of associated lipolytic enzymes (chapter 3) when viewed in the light of the present observations tend to show a period of active lipid catabolism and a gradual glycogen anabolism. It may be assumed that at this phase of high activity of the gizzard, the extra energy necessities are being successfully met by the utilization of lipids (chapter 3),at the same time the organ is also involved in a slow replenishment of the depleted glycogen through gluconeogenesis. However, with the presently noted high activities of glycolytic enzymes, the possibility of utilization of a continuous supply of blood glucose cannot be overruled. Comparatively high activities of aldolase and LDH in the epithelial tubules between 15th and 25th days of development together with the observed low levels of glycogen in the gizzard as a whole are highly noteworthy and signify the ability of the tubules to utilize glycogen or glucose for the energy requirements for both the synthesis of keratin as well as for an active cellular proliferation. Such association of glycogen and aldolase

in keratin synthesis and cellular proliferation has been attributed by Montagna and Ellis (1958) and Bradfield (1951) in the skin of mammals and Shah and Ramachandran (1972), Ramachandran (1972) and Radhakrishnan (1972) in the skin of normal and regenerating tail of the lizard, Mabuya carinata. It is interesting to note in this connection the suggestion of Shah and Chakko (1967) that the glycogen through phosphorylase activity in the beta and alpha cells of the new generation (formed before ecdysis) provide necessary materials for the synthesis of keratin in the skin of the lizard, Hemidactylus flaviviridis.

The period between the 25th and 30th days which could be considered as one where final and finer changes of development, differentiation and maturation aimed at the change over to adult pattern of gizzard are at work, is marked by a great increase in glycogen content and slightly reduced level of all the four enzymes under study. Thus it seems from the above observations that with the completion of the process of post-natal development and the attainment of the adult structural and functional state, the metabolic necessities of the pigeon gizzard also come down. The highest level of glycogen content obtained on the 30th day is an indication that the final

growth phase might be marked by a high rate of gluconeogenesis. But with a similar, though slightly reduced, nevertheless high, activity of aldolase and LDH from 30th day to adult state, the incidence of anaerobic glycolysis as an energy yielder still appears to be a certainty. Increased levels of lipids and slightly lower levels of associated enzymes have also been observed at this stage (chapter 3). It is pertinent to note here similar observations of increased glycogen content and reduced glycolytic enzymes during the growth phase of the regenerating tails of lizards (Magon, 1970; Ramachandran, 1972; Radhakrishnan, 1972).

A probable interesting aspect that is brought out by the present observations is the fact that, excepting for a small period of high energy requirement the rest of the periods of post-natal development of pigeon gizzard are dependent on blood glucose for the continuous and smooth functioning of the carbohydrate metabolism.