Vet.Med.J.,Giza. Vol.43,No.4 .(1995): 429-448.

HISTOLOGICAL AND SOME HISTOCHEMICAL STUDIES ON THE UROPYGIAL GLAND OF YOUNG AND MATURE PIGEONS

By

EL-BARGEESY, G.A.; EL-SHAMMAA, M.A. and EL-GHARBAWY, S.M.

Department of Cytology and Histology, Fac. Vet. Med., Cairo Univ.

SUMMARY

A total of 20 pigeons at 3-6 weeks and 5-10 months of age were used to study the histological structure of the uropygial gland and its content of some carbohydrates, lipids and enzymes.

The uropygial gland is bilobed and situated in the integument dorsal to the free coccygeal vertebrae. It is a simple branched alveolar, holocrine gland. It is surrounded by a dense fibro-reticular capsule from which thick connective tissue septae extended into the interior part to separate the gland into two major lobes. Thin strands of connective tissue are present between the glandular alveoli. These alveoli are lined with a stratified epithelium containing cells of three shapes: the basal layers are squamous, the intermediate layers polyhedral, and the luminal surface is covered by transitional cells. The central cavity of each alveolus opens into a large cavity near the cranial end of each lobe, that in turn is connected to a duct lined with stratifiecd squamous epithelium. The lining epithelium of the duct is keratinized and opens onto the skin. The histological features of glands from young birds did not differ greatly from those of adults. The gland was studied histochemically for the distribution of PAS-positive and alcianophilic substances, lipids and glycogen granules. There were few reactive granules in the basal cells. The content of sudanphilic lipids was low in the basal cells, but increased gradually reaching the maximum in cells near the luminal surface. Enzyme histo-chemistry showed that the basal cells strongly reacted to SDH, GDH, ICDH, NAD, NADPH and AK pase, but these reactions decreased gradually in cells located near the lumen.

INTRODUCTION

Although a good deal of histological work has been done in recent years on glands which secrete triglycerides (e.g. sebaceous, mammary and harderian glands), the uropygial gland of birds (commonly called the "oil gland") has not received much recent attention. Only a few papers are available (Altmann, 1894; Bowen, 1926; Bhatia, 1943; Das and Ghosh. 1959; Mosallam, 1990 and Alhumead, 1992) dealing with morphological and histochemical studies of these glands. These studies lack specific data which might show exactly where and how the secretion is manufactured inside these holocrine glands. Some morphological studies of the uropygial gland (Cater and lawrie, 1950; 1951; Bradley and Grahame, 1960; Lucas and Stettenheim, 1972; Hodges,1974; Banks, 1981; Mosallam, 1990 and Alhumead, 1992) noted that this gland closely resembles the mammalian sebaceous gland, particularly in its holocrine mode of secretion, and

also that it is influenced by the secretion of other glands, particularly those which produce androgen hormones (Kar, 1947, 1949, Bhattachrjee and Ghosh, 1960 and Maiti, 1971). By histochemical investigation, Cater and Lawrie (1950, 1951) divided each lobe of the uropygial gland of chickens into an outer "sebaceous" zone and an inner" glycogen" zone. Ishida et al. (1973) also differentiated the gland into inner and outer zones based on their enzymo-hstochemical reactions. In contrast to this situation in the chicken, Bhattacharjee and Ghosh (1971) reprted that the gland of the pigeon has no well differentiated zones.

In this paper, we design the work to elucidate the overall microscopic structure of the uropygial gland in pigeons, and also histochemical studies of its specific secretory regions.

MATERIAL AND METHODS

Twenty male and female domestic pigeons were used in this work. They were divided into 2 groups; group 1 consisted of 10 young birds (aged 3-6 weeks); group 2 consisted of 10 mature birds (age 5-10 months).

For the histological examination, the birds were slaughtered and the glands dissected free. The glands were cut into small pieces and then immersed in various fixatives (10% buffered neutral formalin, Bouin's solution, susa and zenker's fluids). They were fixed, then washed, dehydrated and embedded in paraffin to obtain serial sections of 5-7 micrometer thickness. These sections were stained with haematoxylin and eosin (H & E), Crossmon's trichrome, Weigert's elastic stain, Gomori's reticulin method, and the periodic acid Schiff (PAS). Control sections for the PAS technique were treated with diastase for 30 minutes at 37°C. In addition, alcian blue (AB), Best's carmine stain and a combination PAS-AB method were used to specifically ident carbohydrates. For histochemical localization some enzymes and lipids, 10 fresh samples fr each of the two groups were cut using histocryotom, maintained at -20°C. Sections 10-15 micrometer thickness were stained wit saturated solution of sudan black B in 7 alcohol, and sudan IV to reveal lipids. Enzym reactions to identify succinic dehydrogen (SDH) Isocitrate dehydrogenase (ICD Nicotinamide adenine dinucleotide (NA reduced Nicotinamide adenine dinucleot phosphate (NADPH), Glutamic dehydrogen (GDH) and alkaline phosphatase (AK-pase) w eosin counter stain were also carried out on th sections.

The above mentioned staining methods w adopted from those described by Romies (194 Burstone (1962), Pearse (1972), Bancroft (19 and Drury and Wallington (1980).

RESULTS

Micromorphological findings:-

The uropygial gland of pigeons is a bilobed org lying in the hypodermis of the integum covering the dorsal surface of the free coccyg vertebrae. It is surrounded by a fairly thick a dense fibro-reticular capsule containing sm blood vascular channels (Fig. 1). The two lob are separated from each other by a thick interlob connective tissue septum, carrying large blo vessels engorged with blood (Fig. 2). There ar few elastic fibers in most parts of the capsule, well as around the blood vessels. From t capsule, thin strands of connective tissue exte inwards to demarcate the secretory units or alve (Fig. 3). Fine reticular fibers are visible in t inter-alveolar connective tissue strands, as well around the blood vessels.

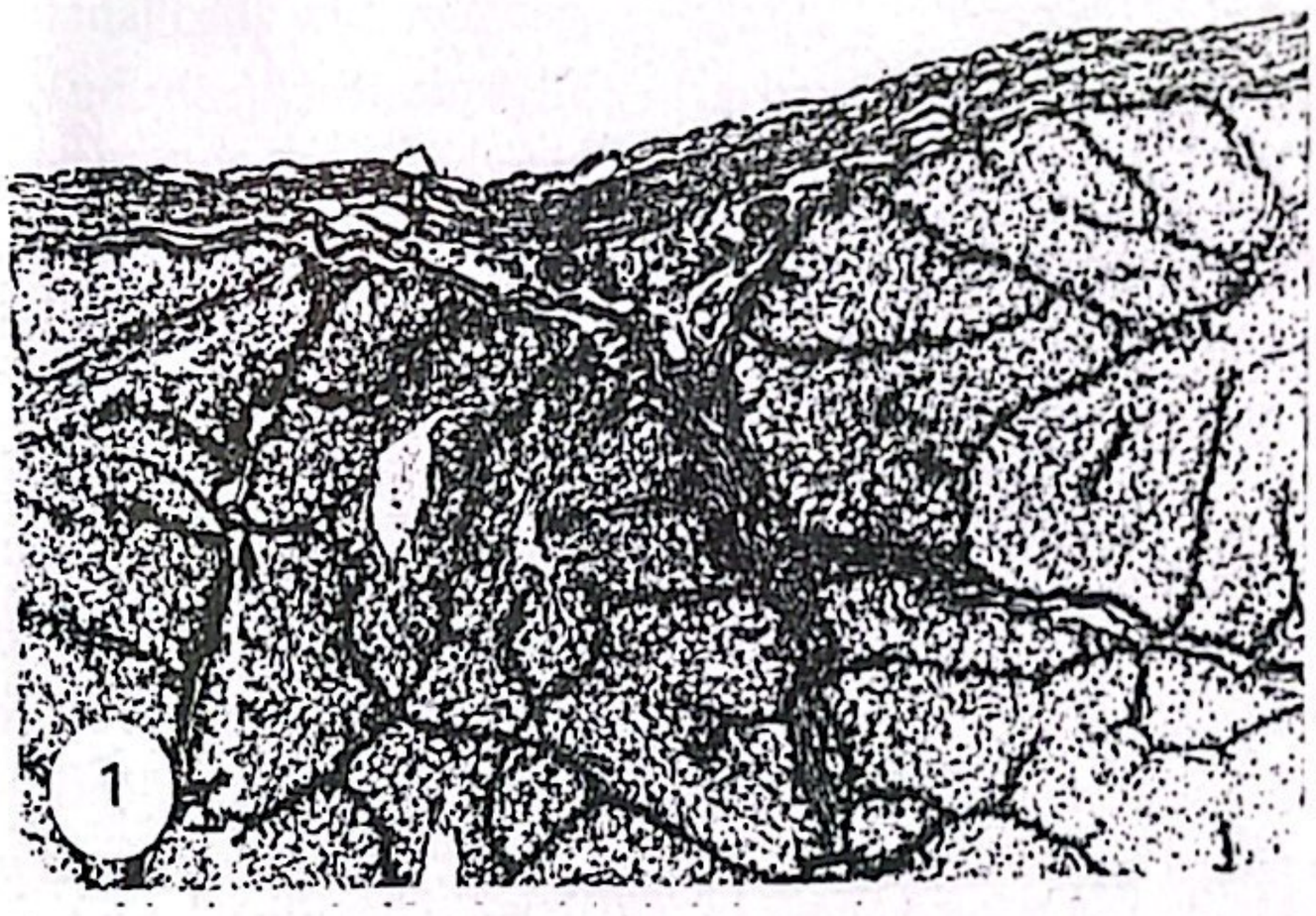

Fig. 1: The uropygial gland of mature pigeon showing fairly thick, dense fibroreticular capsule containing small blood vessels. H & E Stain. X 50.

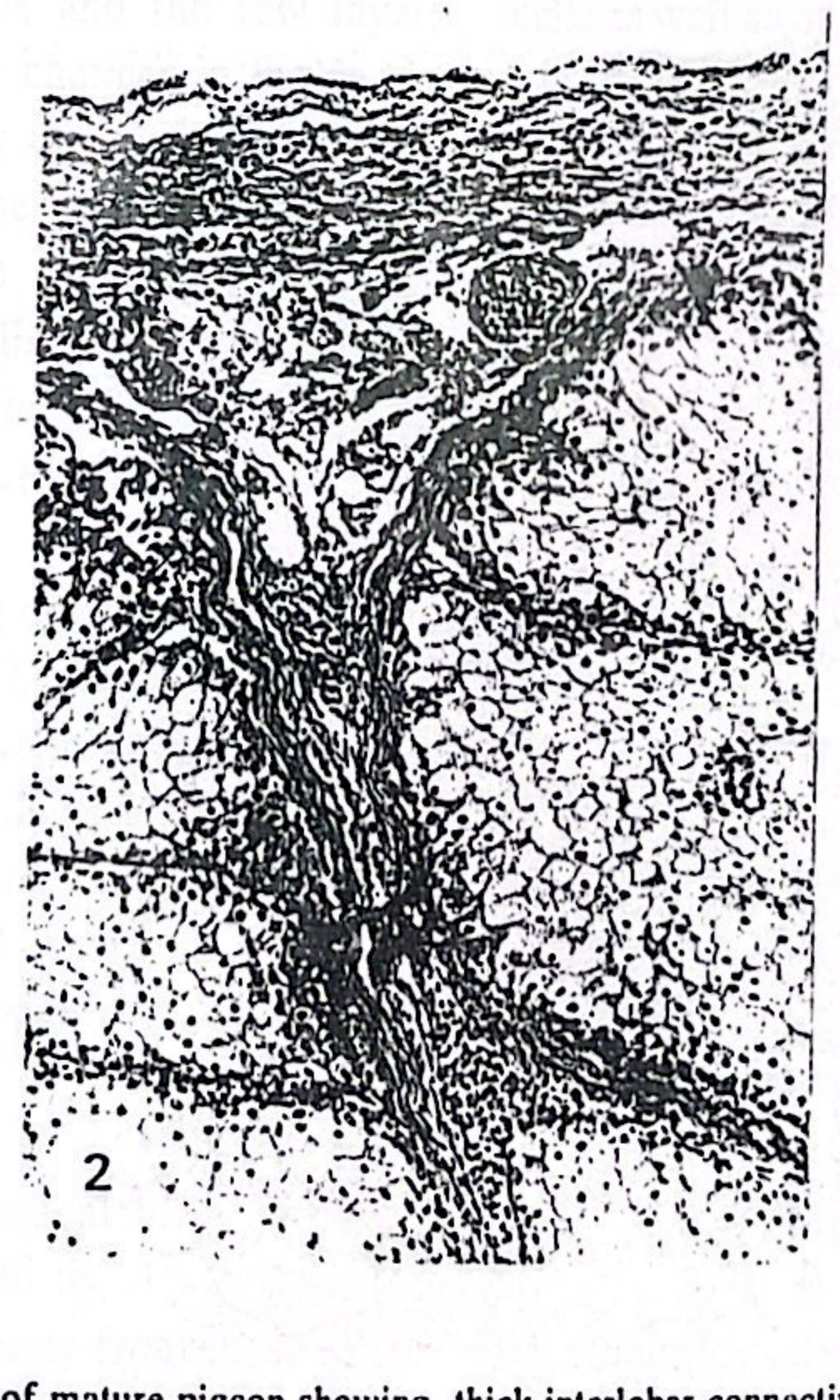

Fig. 2: The uropygial gland of mature pigeon showing thick interlobar connective tissue septum carrying more blood vessels engorged with blood extending from the capsule H & E stain X 130.

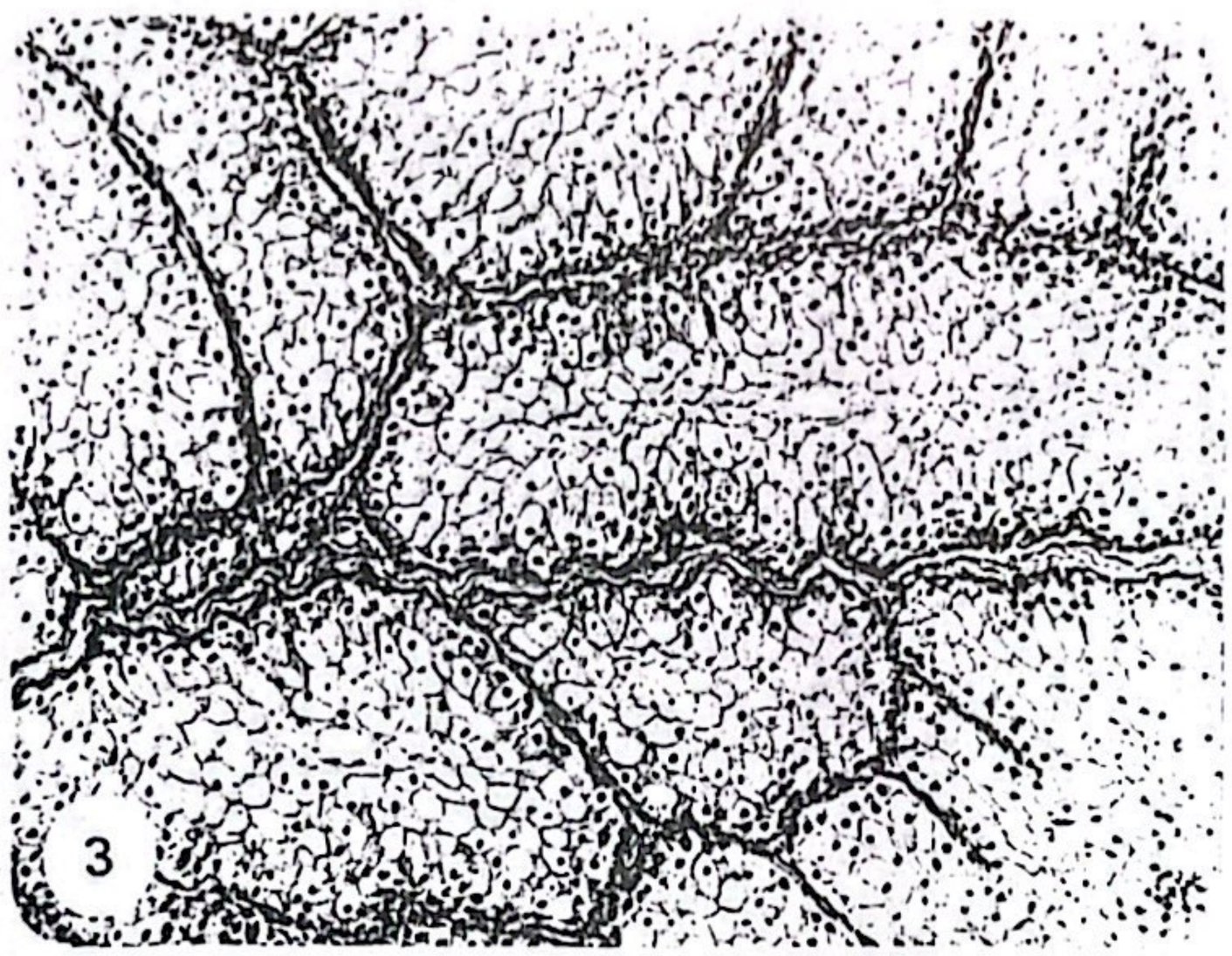

Fig. 3: The uropygial gland of mature pigeon showing thin strands of connective tissue extending between the secretory alveoli. H & E stain X 130.

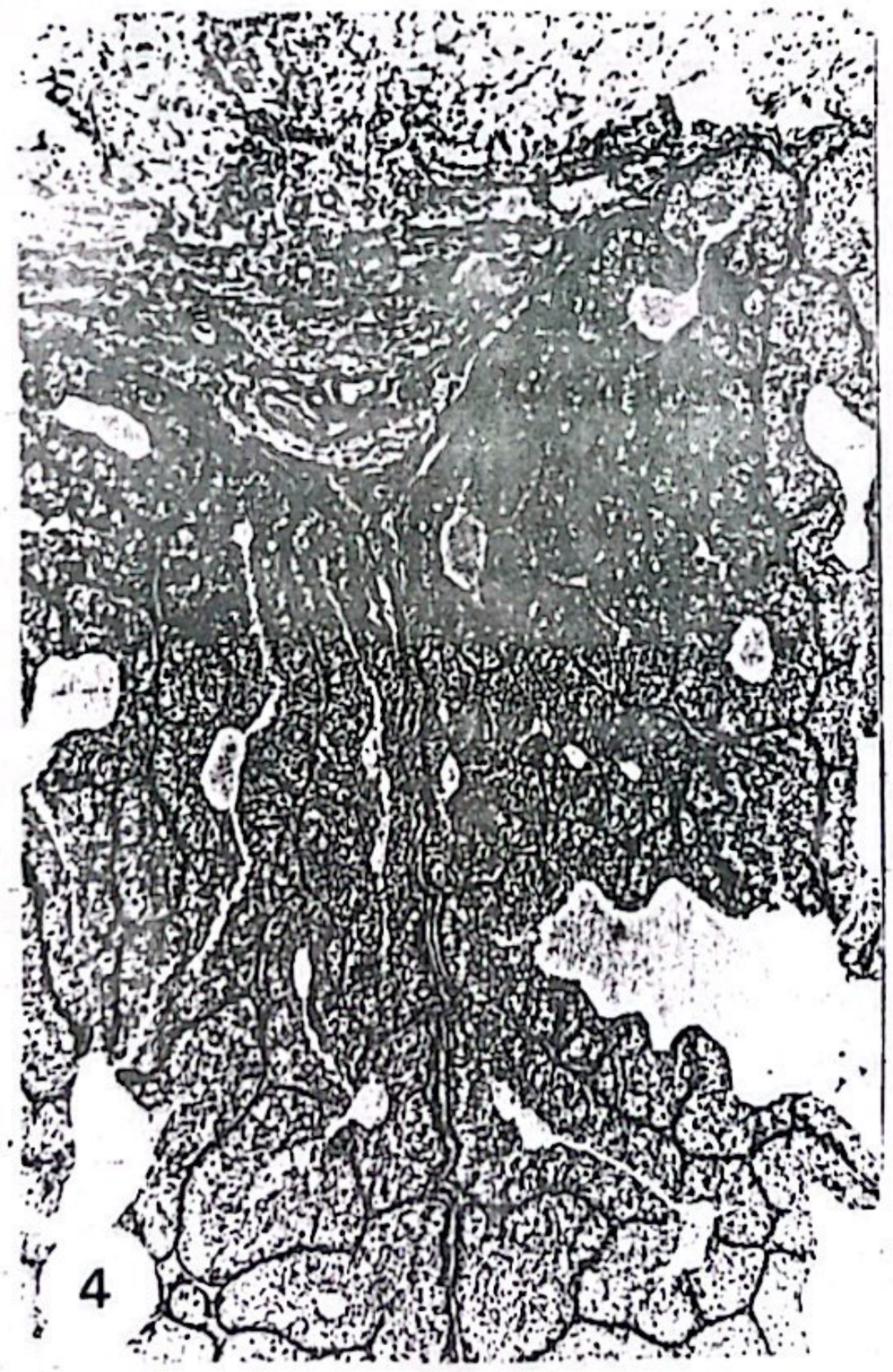

Fig. 4: The uropygial gland of mature pigeon showing different shapes and sizes of the alveoli. Notice the central cavity of each lobe near its cranial end H & E stain X 130.

There were three types of alveolar cells present, which appeared to be oriented in centripetal order. Along the periphery of the alveolus was a single layer of squamous or cuboidal cells with rounded, darkly stained nuclei and deeply basophilic cytoplasm that stained dark with eosin and acid fuchsin. These appeared to correspond to "indifferent cells" of the sebaceous gland. The rest of the alveolus was filled with a stratified epithelium composed mainly of spheroidal and polyhedral cells. These cells became progressively larger toward the center of the alveolus due to accumulation of fat droplets in their cytoplasm "Lipid-laden" cells (Fig. 5). The center of the alveolus was surrounded by a layer of 1-2 luminal cells, these were large disintegrating cells characterized by crumpled cell membranes and pyknotic nuclei. The cytoplasm of these cells had been converted into fat and they were in the process of being replaced by new cells. The peripheral cells and the few layers above neither showed any changes in the darkly stained cytoplasm nor any degenerative changes in the nuclei. Beyond these layers, however, the progressive degenerative changes were very marked in the different cells. The nuclei were in various stages of pyknosis and the cytoplasm was in the process of being converted into fat (Fig. 6).

The alveoli opened into the central cavity or sinus near the cranial end of each lobe (Fig. 7). These sinuses opened into ducts lined with stratified squamous epithelium. The ducts were keratinized before they opened into the thin elevated skin covering the gland. This skin was devoid of feathers around the two openings of the ducts (Fig. 8).

With regard to the histological features of the uropygial gland of young birds, it was apparent that these did not differ greatly from those of the mature ones. The shape and structure of the secretory units were the same at both ages. The only differences noted were in the level of enzyme reactions and the amount of secretory products in the lumina of the alveoli (Fig. 9).

Histochemical findings:

Carbohydrates (glycogen and glycoproteins):

The cytoplasm of the peripheral cells of the glandular units reacted weakly with PAS stain, and this reaction decreased gradually inwards, until it was absent in the cytoplasm of cells closest to the lumen. However, the cell boundaries and the luminal secretions were strongly positive as were the connective tissue fibers in the capsule and the inter-alveolar tissue (Fig. 10). There were fine granules in the basal cells that reacted positively with alcian blue stain. These decreased gradually until they disappeared in the luminal cell layers. The connective tisue stroma showed positive AB reactivity. Glycogen was demonstrated as very fine granules in the basal cells as well as, in the secretion.

In young pigeons very little PAS positive reactivity was observed. In mature and young pigeons, the glandular units varied in their content of sudanophilic materials. The cytoplasm of the basal cells showed very weak reactivity, while the reaction was clearly evident and increased in strength in the successive layers toward the alveolar lumen (Fig. 11, 12, 13).

Succinic Dehydrogenase:

In mature birds, there was a strong reaction in the basal cells of the alveoli which decreased gradually in the successive superficial layers. The young birds showed only a weak reaction in the basal cells, which disappeared in more superficial layers (Fig. 14, 15).

Nicotinamide adenine dinucleotide:

The basal cells showed stronger positive reactivity than the other cell layers in mature pigeons (Fig. 16) However, there was moderate reactivity in all

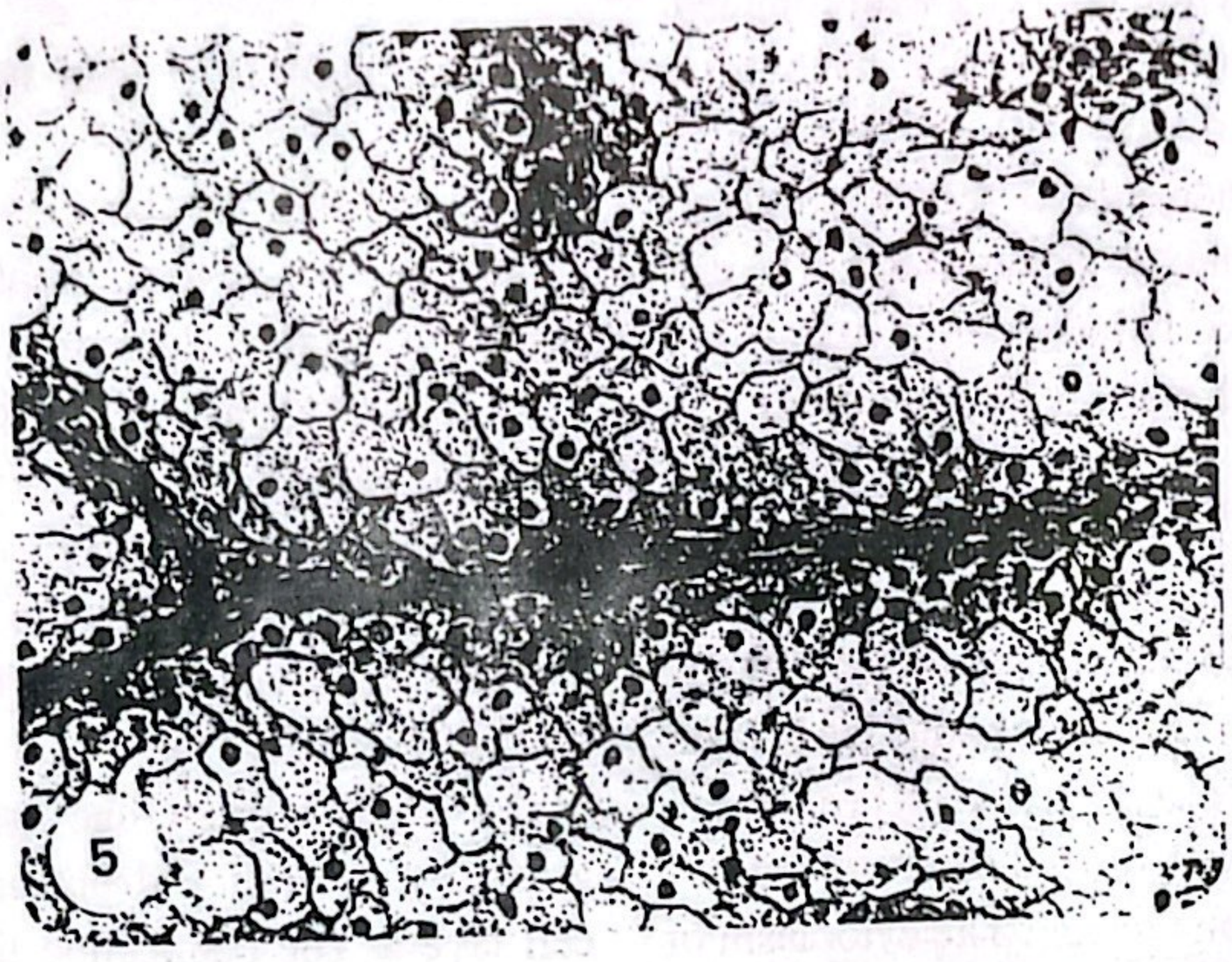

Fig. 5: The uropygial gland of mature pigeon showing the inter-alveolar thin strands of connective tissue. Notice: The basal squamous cells with flattened darkly stained nucleus and deeply basophilic cytoplasm, the intermediate large polyhedral cell layers (lipid laden cells). Crossmon's trichrome stain X 320.

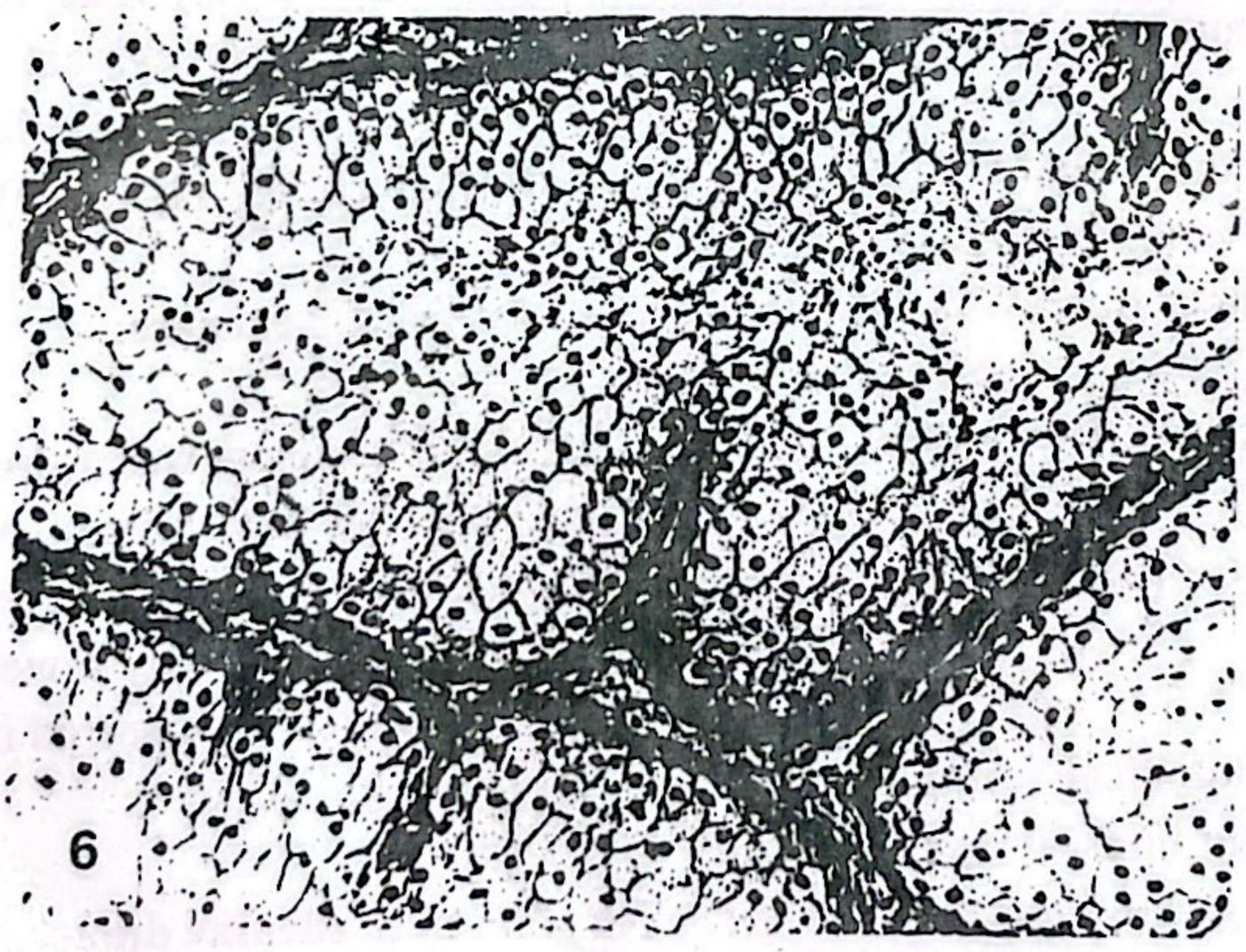

Fig. 6: The uropygial gland of mature pigeon showing the central region of the alveolus surrounded by 1-2 cell layers of disintegrated cells (crumpled cell membrane and pyknotic nuclei). H & E stain X 200.

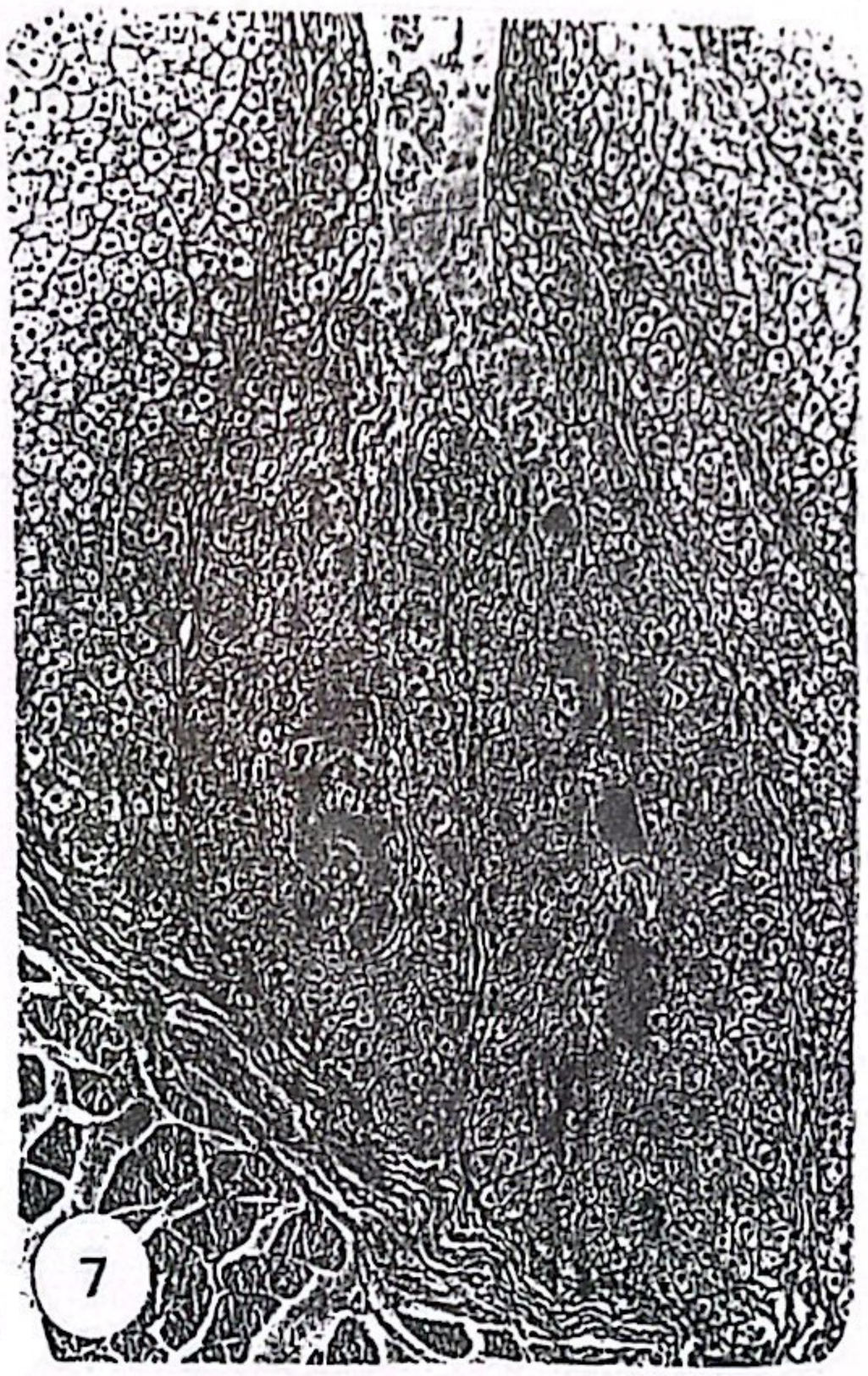

Fig. 7: The uropygial gland of mature pigeon showing the sebaceous like secretory alveolus that poured in central cavity of a lobe. H & E stain X 130.

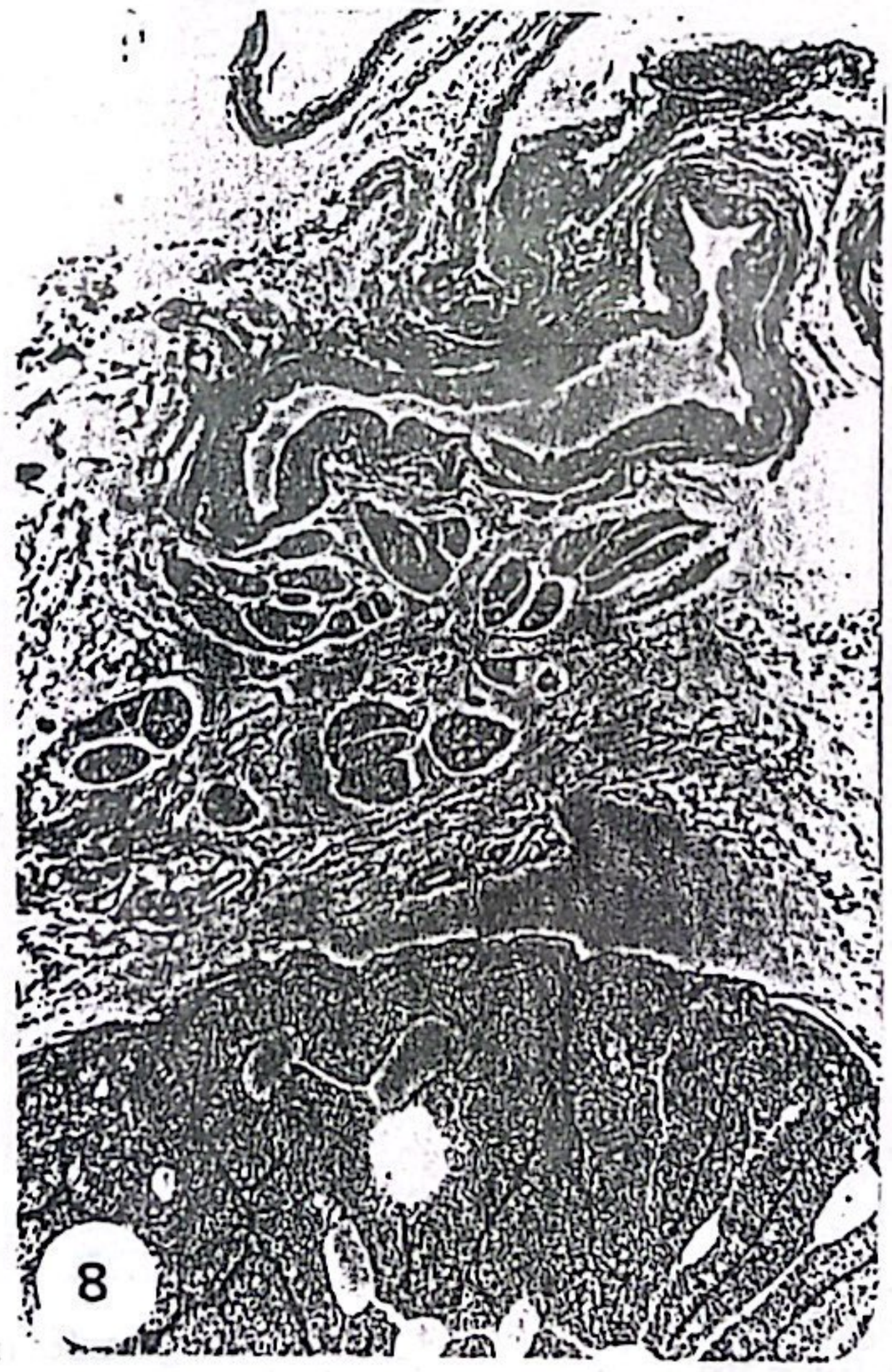

Fig. 8: The uropygial gland of mature pigeon showing the excretory duct lined with stratified squamous keratinized epithelium under the thin elevated skin. H & E stain X 50.

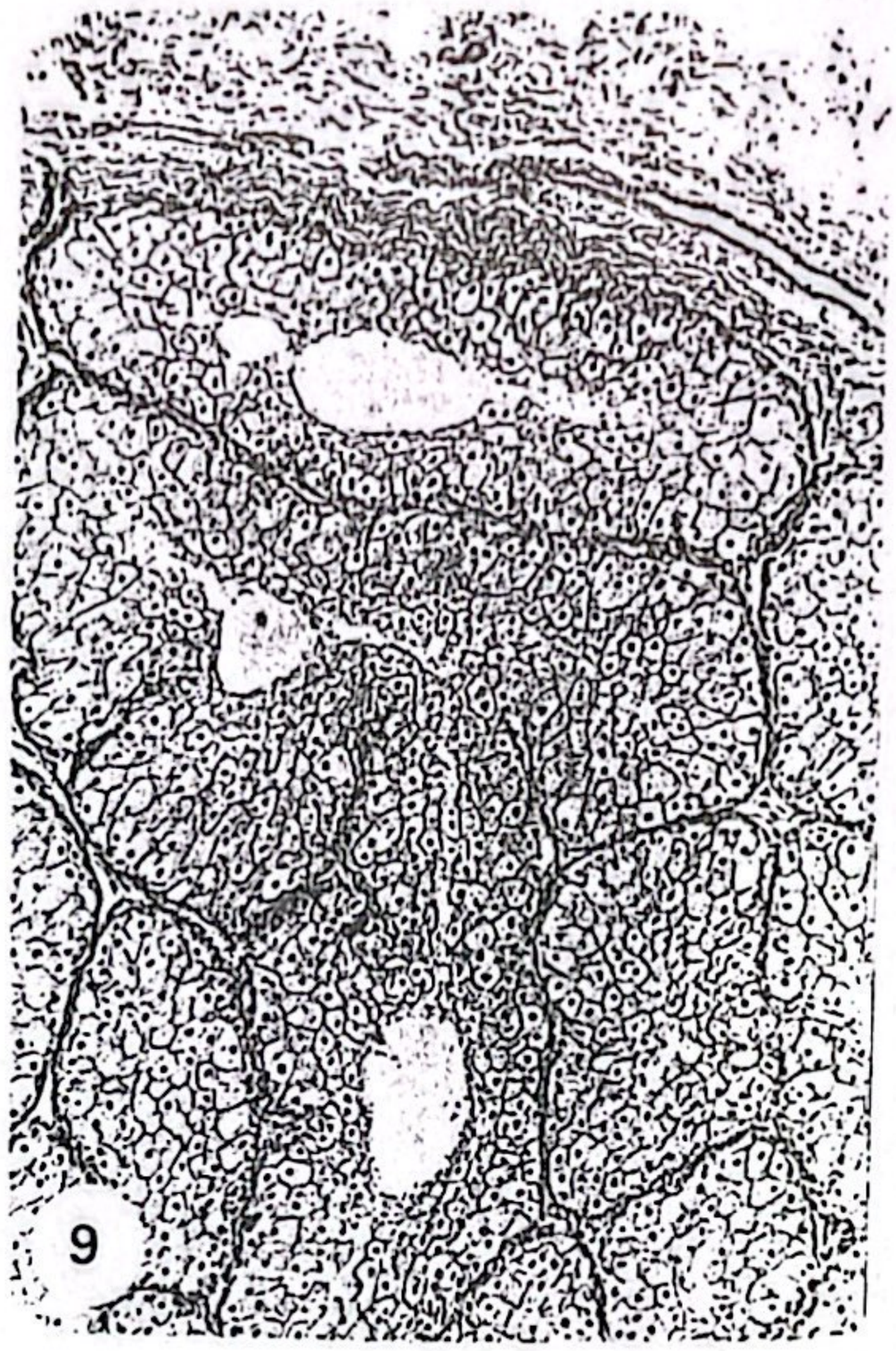

Fig. 9: The uropygial gland of young pigeon showing the same structure of that described in mature ones. Notice: Less secretory products in the lumen of the alveoli H & E stain X 130.

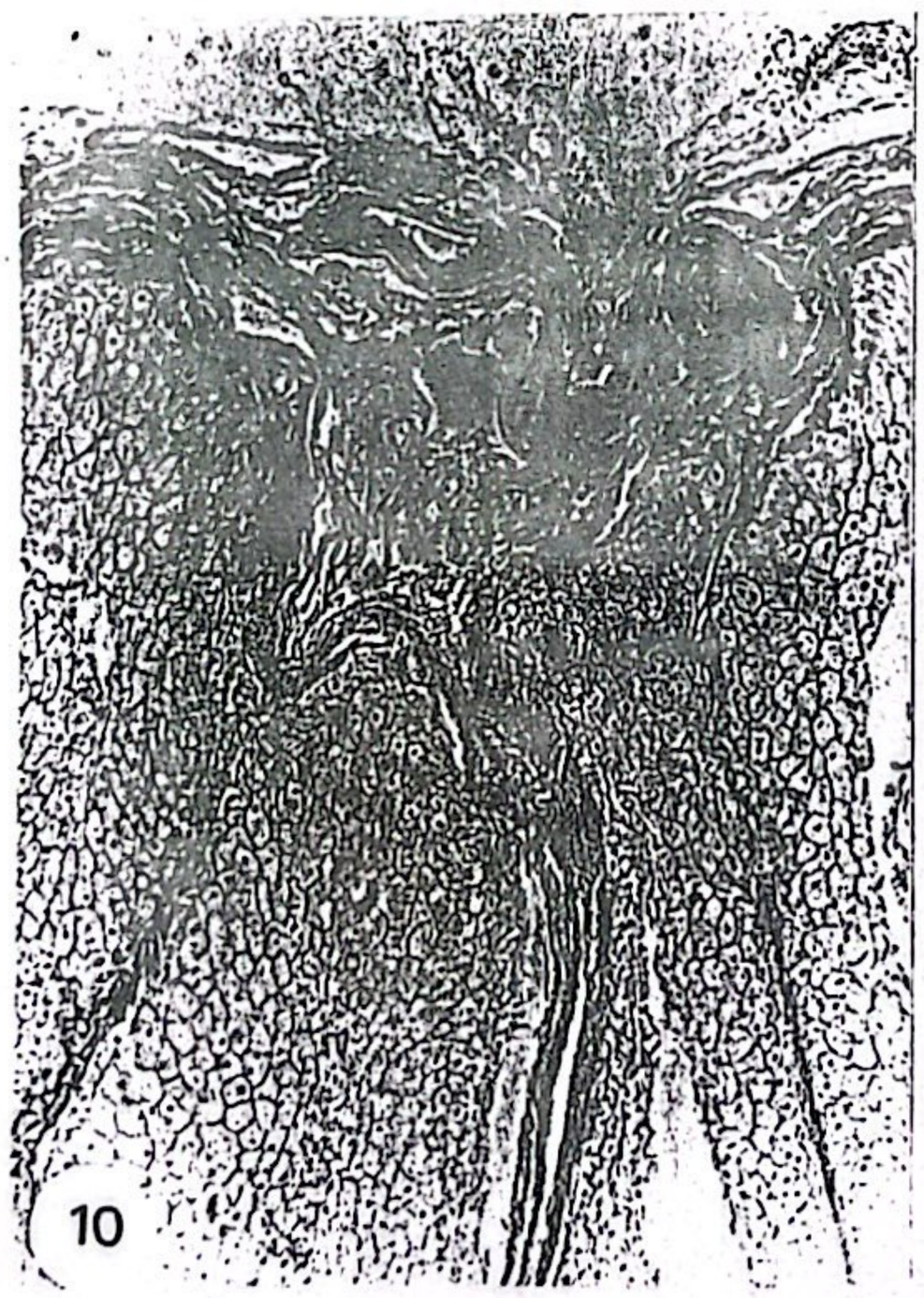

Fig. 10: The uropygial gland of mature pigeon showing finer PAS + vely reacted granules in the basal cells which decreased gradually in successive layers Notice: strong reactivity in the connective tissue stroma PAS stain X 130.

Fig. 11: The uropygial gland of mature pigeon showing few sudanophilic material in the basal cells of alveoli while the successive cell layers showing large amounts of these materials Sudan black B-stain X 130.

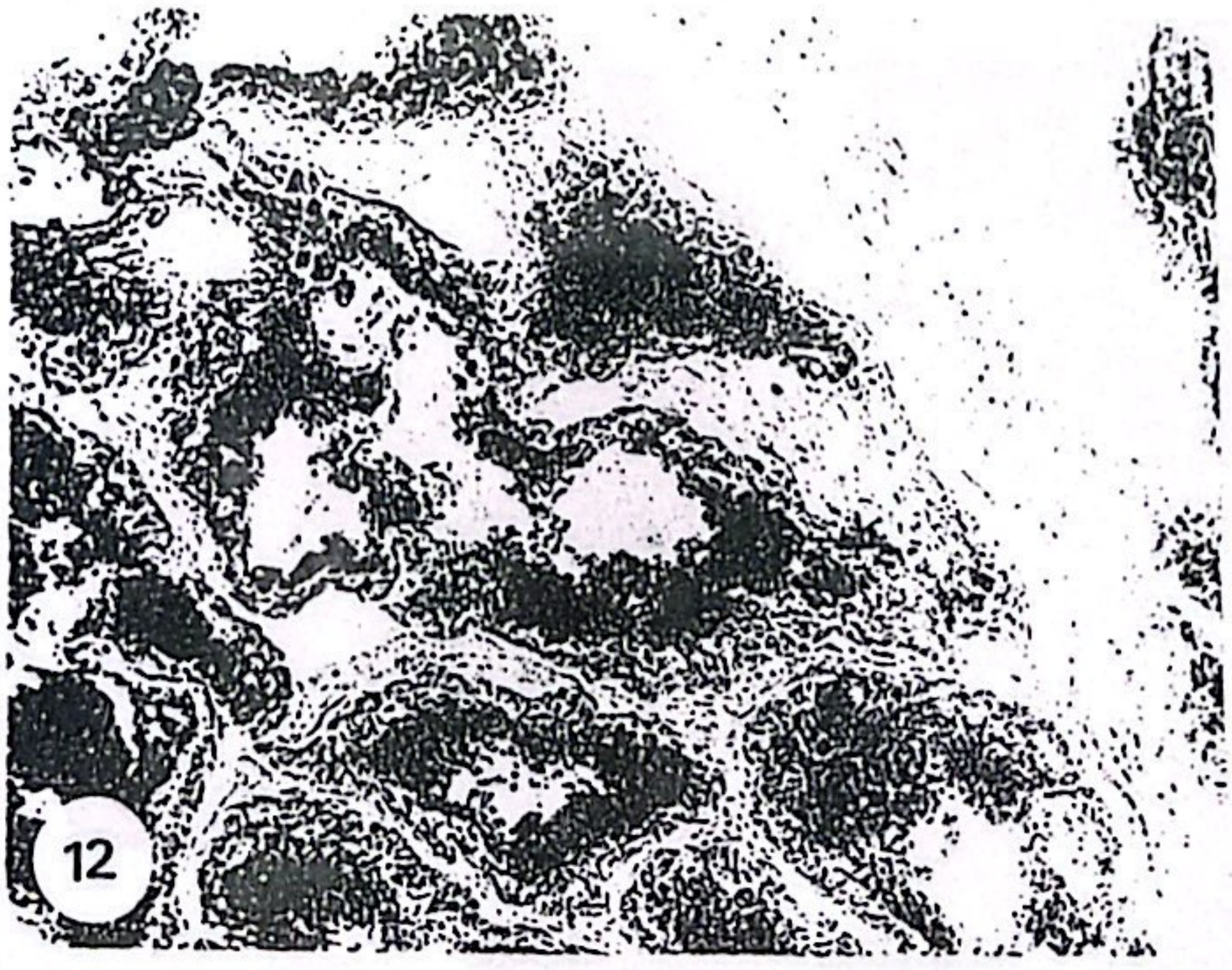

Fig. 12: The uropygial gland of mature pigeon showing weak + ve reactivity to Sudan IV in the basal cells but the reactivity increased in the successive cell layers Sudan IV stain. X 130.

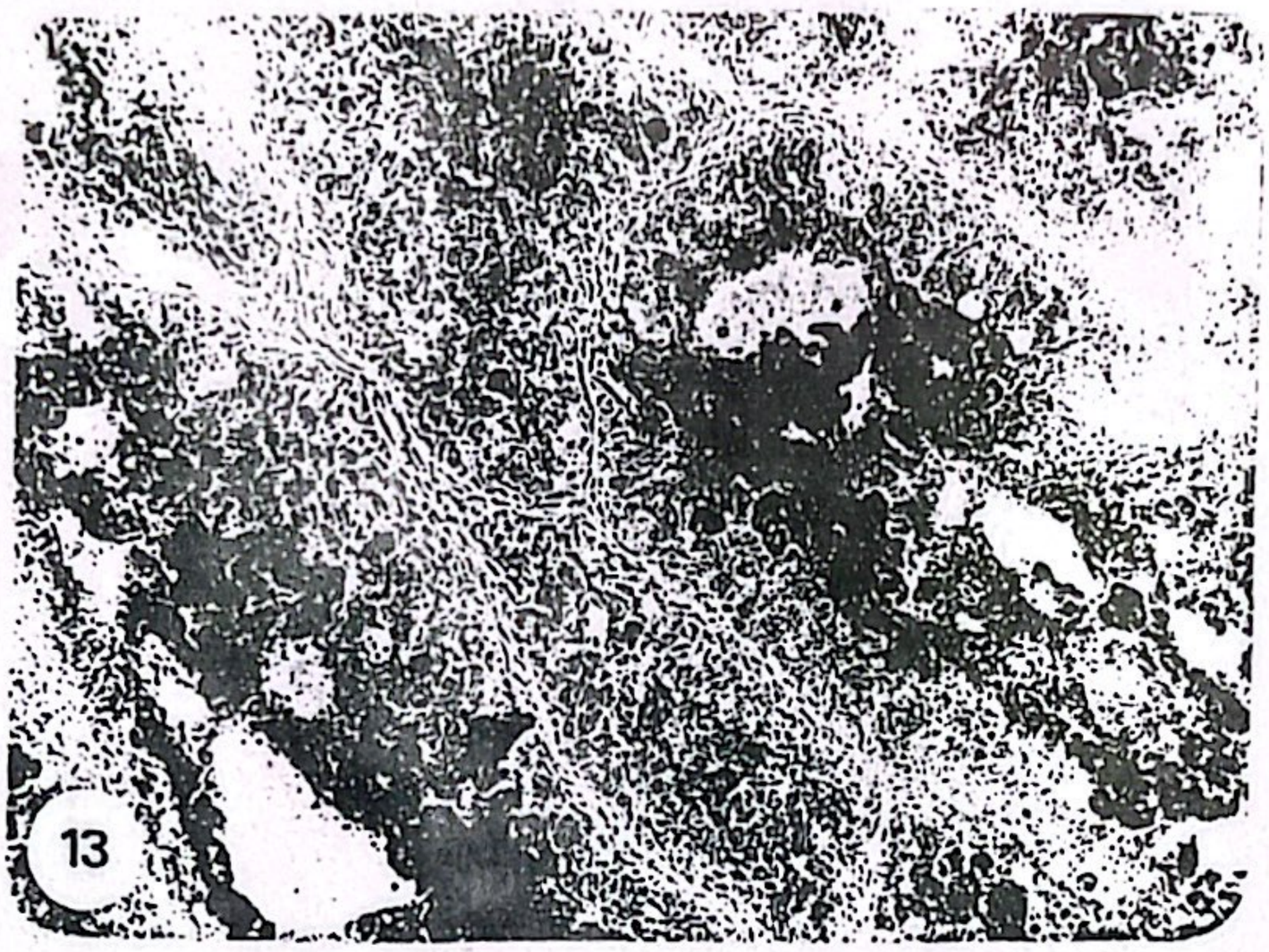

Fig. 13: The uropygial gland of young pigeon showing that, not all the alveoli have the same reactivity, the most reactive cells are the luminal cell layers. Sudan IV stain. X 130.

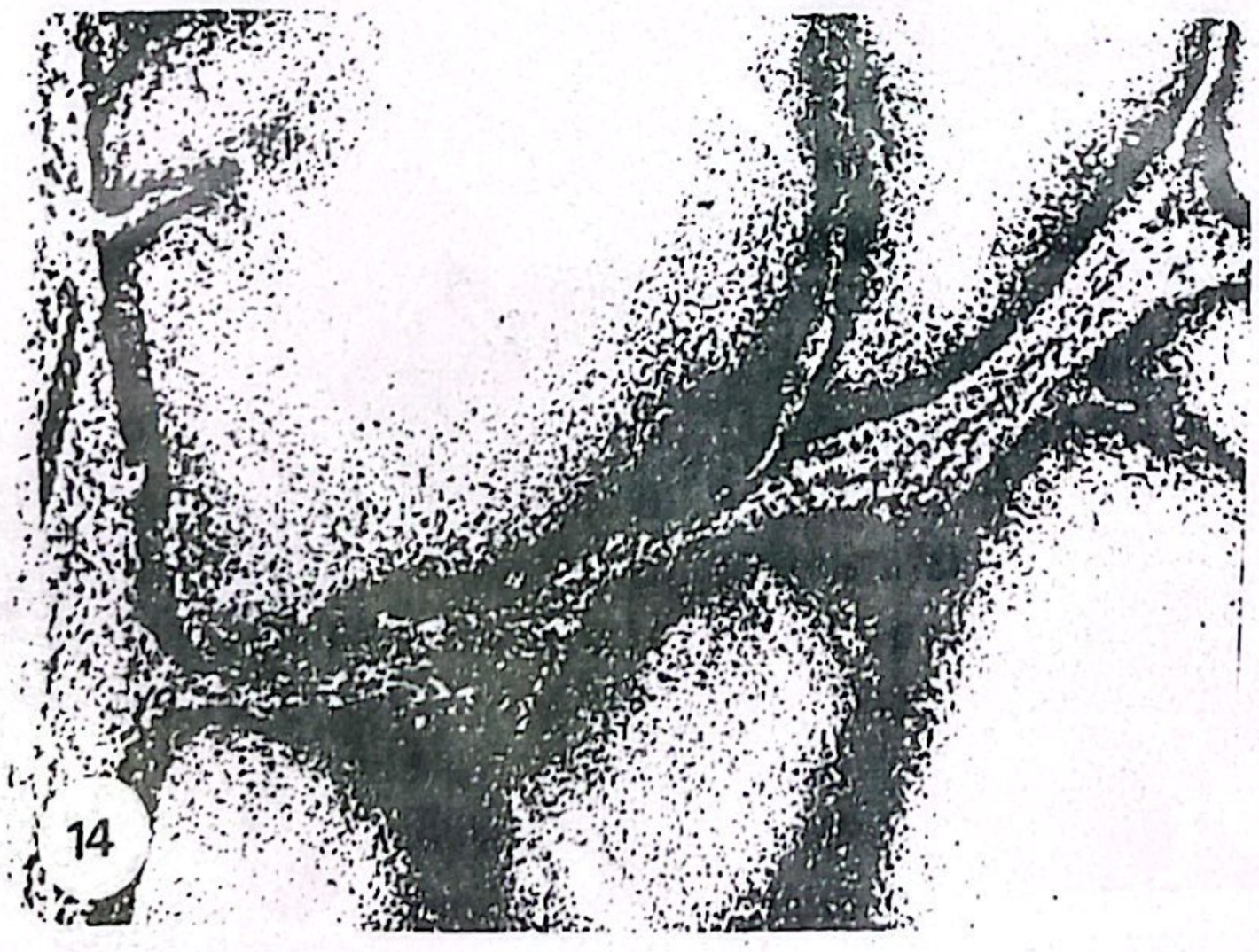

Fig. 14: Succinic dehydrogenase reactivity in the glandular alveoli of the uropygial gland of mature pigeon, being strong in the basal cells and decreased gradually toward the lumen. X 130.

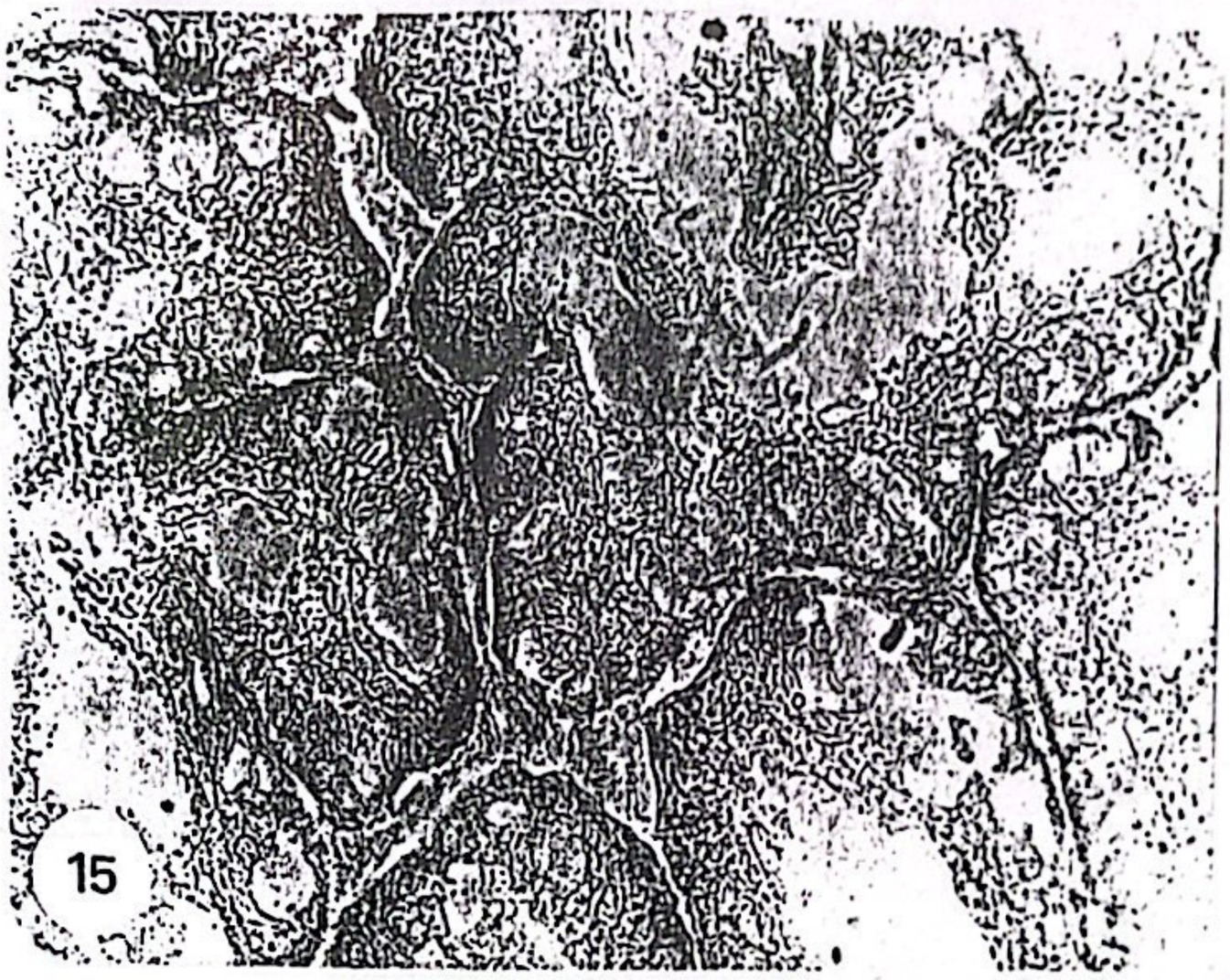

Fig. 15: Succinic dehydrogenase reactivity in the glandular alveoli of young pigeon, being moderate in the basal cells and very weak in the successive cell layers. X 130.

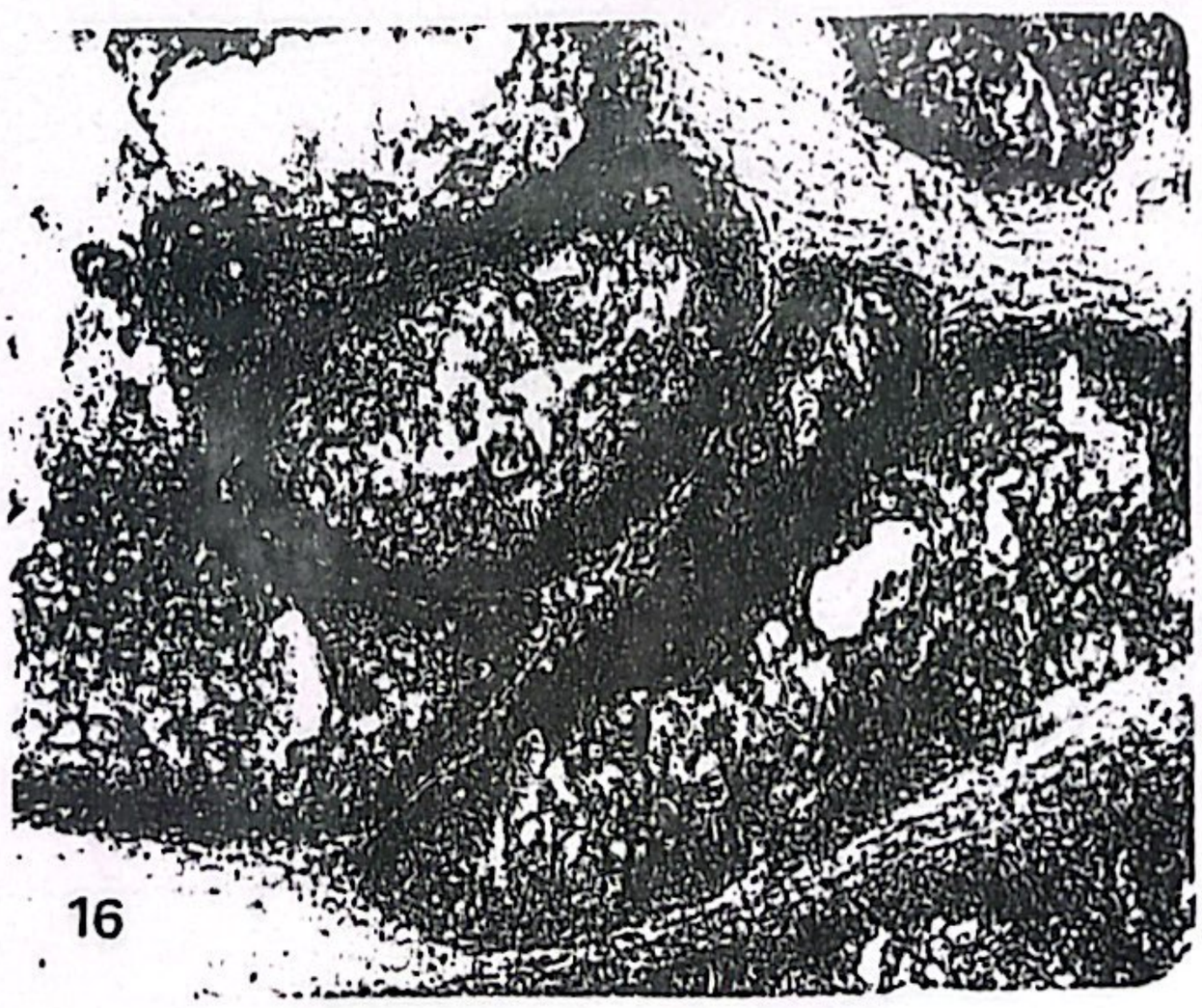

Fig. 16: Nicotinamide adenine dinucleotide reactivity in the glandular alveoli of mature, pigeon, the basal cells were strongly reactive than the successive cell layers. X 130.

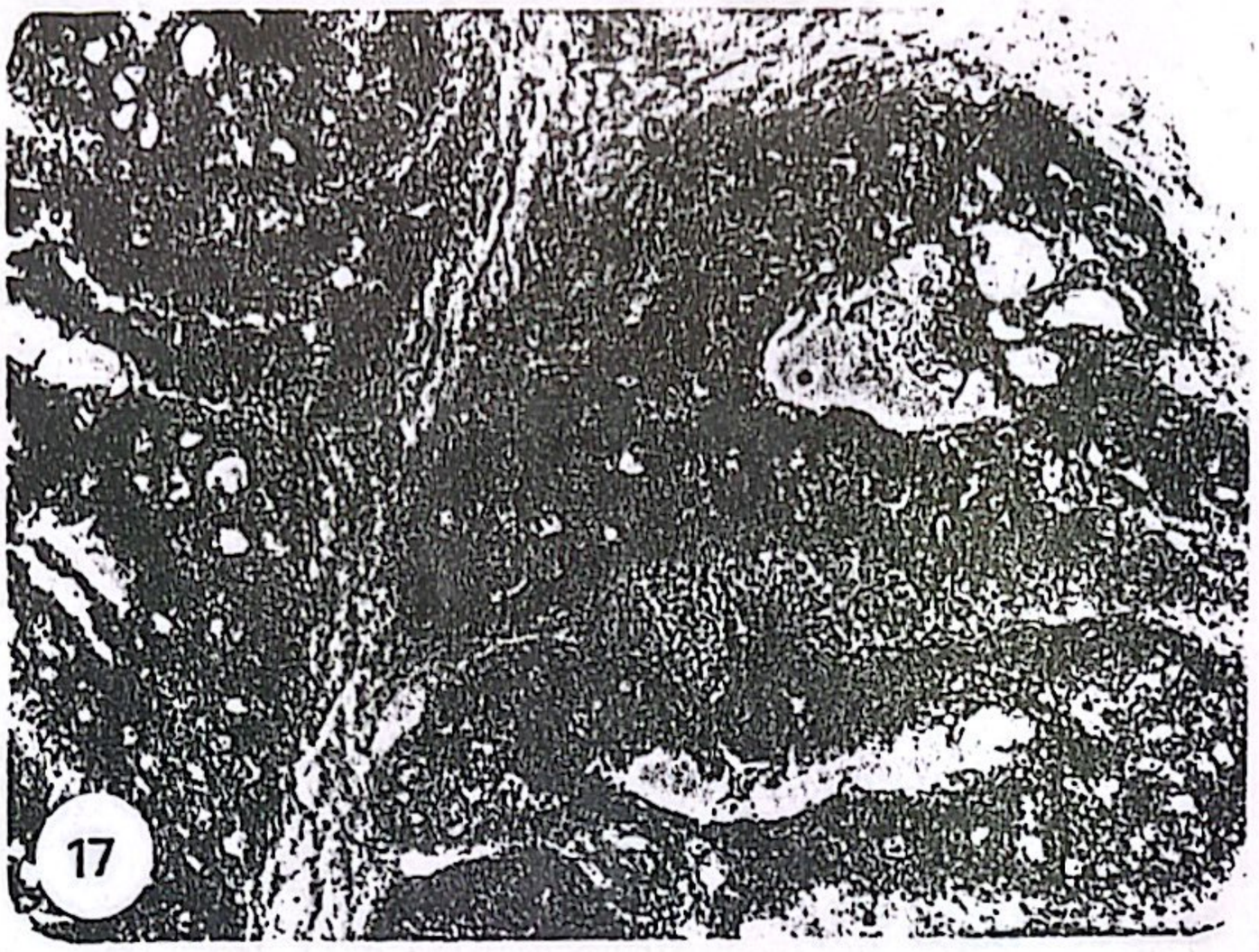

Fig. 17: Nicotinamide adenine dinucleotide reactivity in the glandular alveoli of young pigeon. The reaction was moderate in all cell layers. X 130.

Fig. 18: Iso-citrate dehydrogenase reactivity in the glandular alveoli of mature pigeon, being strong in all cell layers. X 130.

cell layers of the glandular units of young birds Fig. 17).

Iso-citrate dehydrogenase:

The reaction was strong in all cell layers of secretory units of mature birds (Fig. 18), and moderately reactive in the young ones (Fig. 19).

Reduced Nicotinamide adenine dinucleotide phosphate:

In mature birds, the basal and intermediate cell layers showed strong reacitivity, but only limited enzyme reactivity was observed in the luminal cells of the alveoli (Fig. 20). Reactivity was moderate in all layers of young birds (Fig. 21).

Glutamic dehydrogenase:

The reactivity was strong in the basal cells and moderate in the successive superficial cell layers of mature birds (Fig. 22). The young birds showed moderate reactivity in all cell layers.

Alkaline phosphatase:

Clear positively reacted granules were demonstrated in the basal cells, and these granules decreased gradually in the successive layers (Fig. 23).

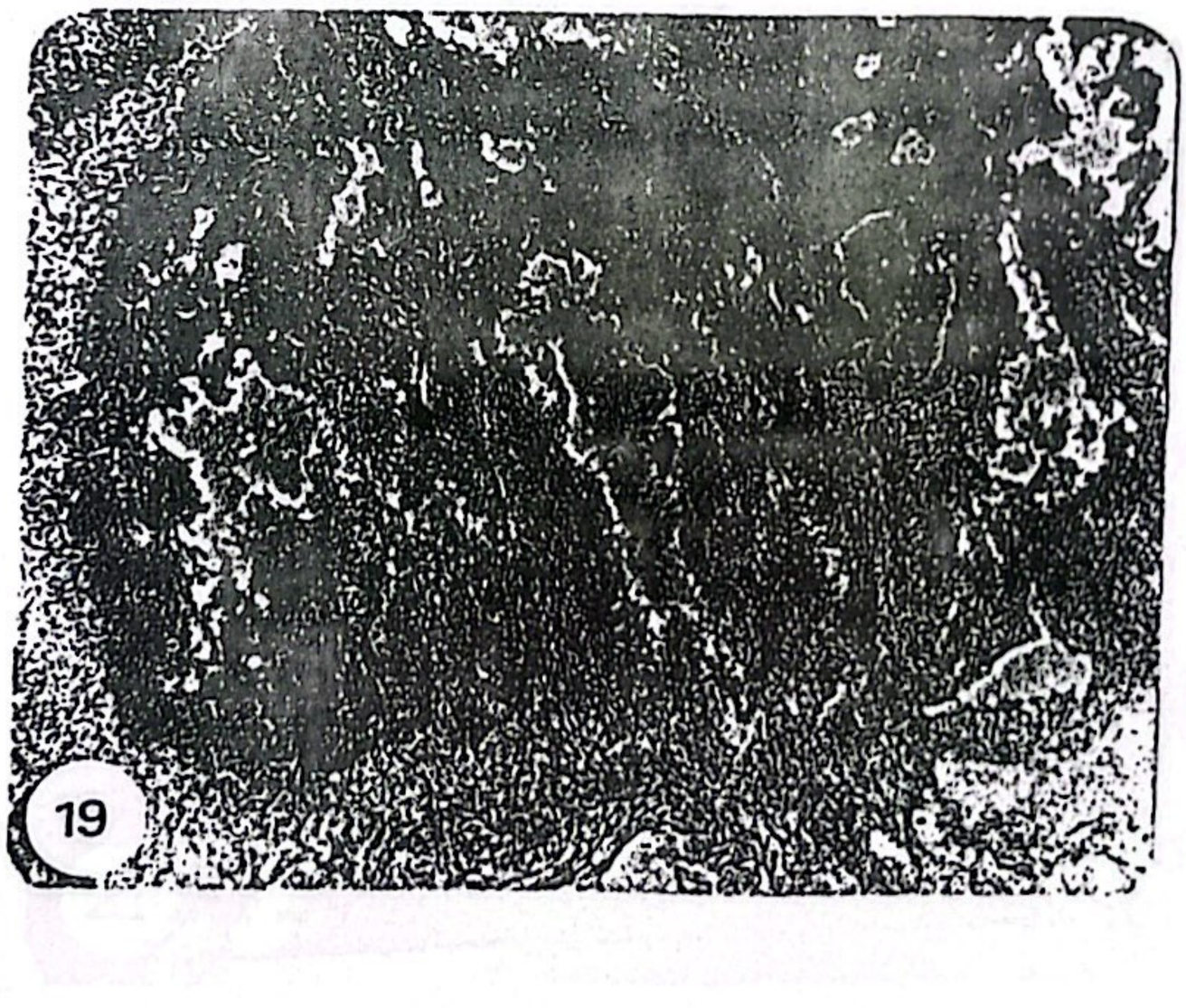

Fig. 19: Iso-citrate dehydrogenase reactivity in the glandular alveoli of young pigeon, being moderate in all cell layers. X 130.

Fig. 20: Reduced nicotinamide adenine dinucleotide phosphate reactivity in the glandular alveoli of mature pigeon showing strong reaction in all cell layers. X 130.

Fig. 21: Reduced nicotinamide adenine dinucleotide phosphate reactivity in the glandular alveoli of young pigeon showing moderate reactivities x 130.

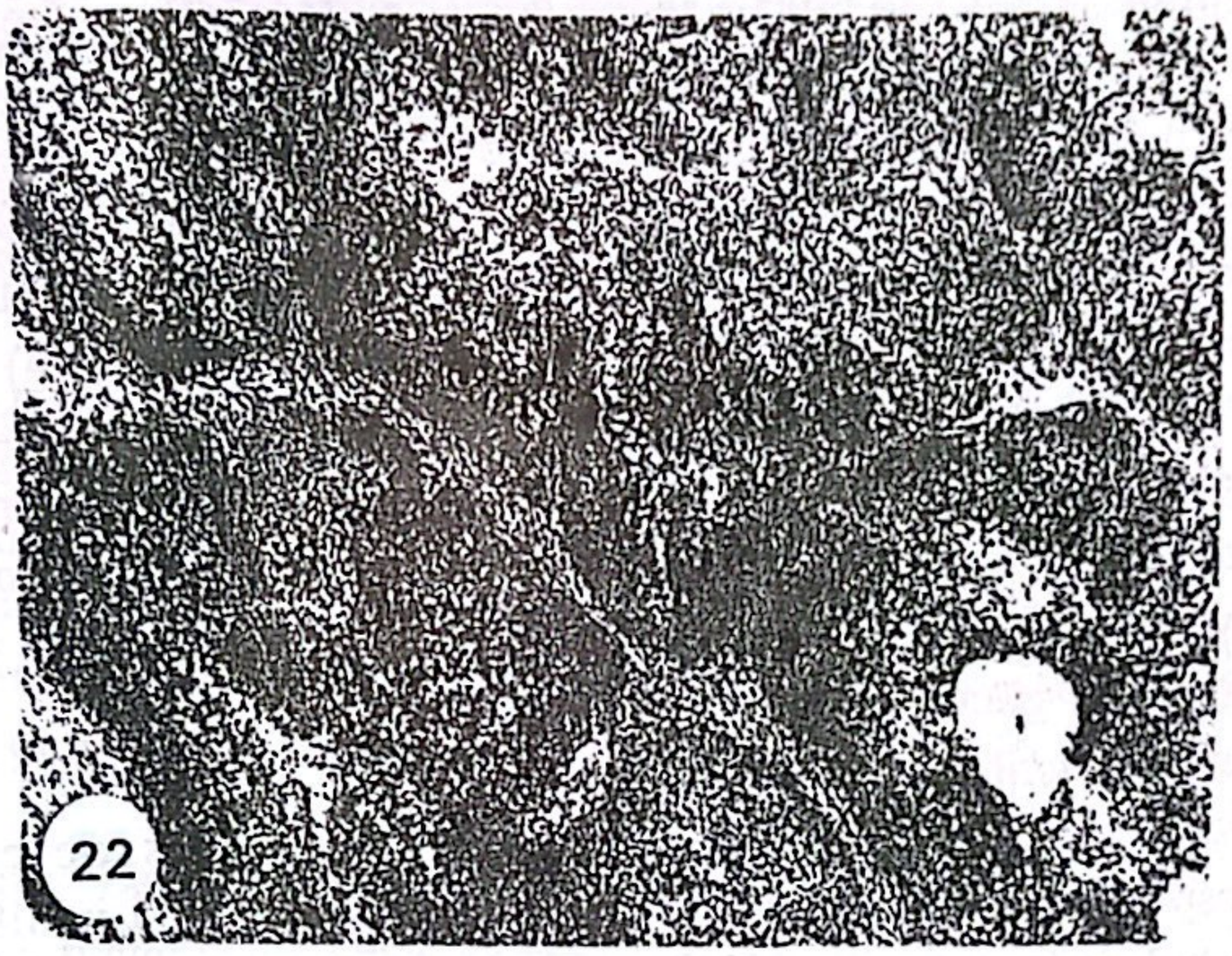

Fig. 22: Glutamic dehydrogenase reactivity in the glandular alveoli of mature pigeon, being strong basally and moderate in the successive superficial cell layers. X 130.

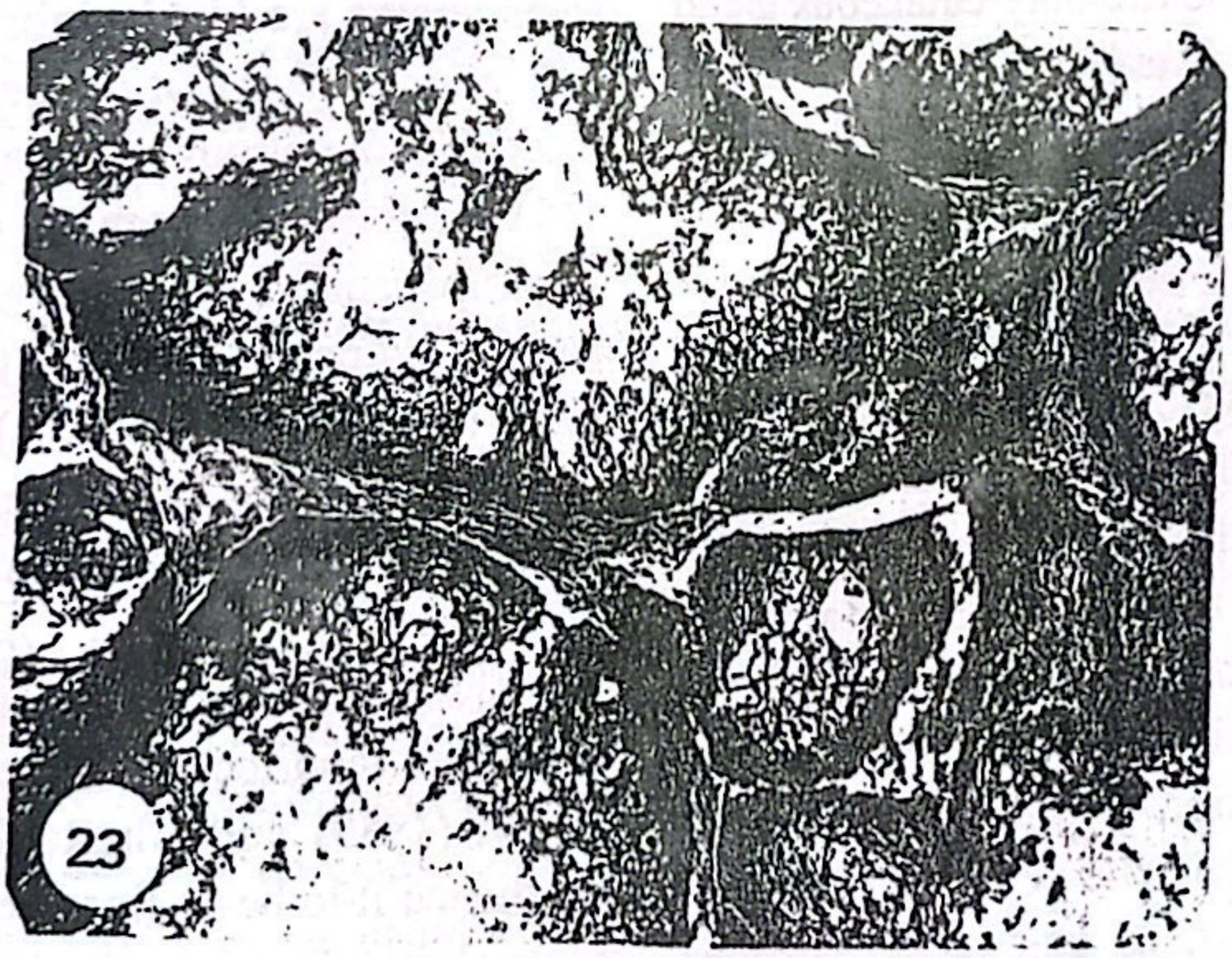

Fig. 21: Clear alkaline phosphatase positively reacted granules in the basal cells of the glandular alveoli of mature pigeon. Notice gradual decrease of reactive granules in the sucessive suprficial cell layers. Ak. pase with eosin counter stain. X 130.

DISCUSSION

Our study revealed that the gland is bilobed. This finding disagree with those of Crisp (1860) and Alhumead (1992) who described it as two separate glands. The latter author described the gland of pigeons as having a "flask-shape" with the two glands separated at their bases but fused at the neck to form a duct. We did not find this to be the case. There is one gland with two distinct lobes, but they are connected to form an organ of heart shape. In its overall structure the uropygial gland of the pigeon resembled that of the mammalian sebaceous gland, there was considerable structural similarity between the two organs, particularly in the holocrine mode of release of their respective secretions. In this respect the present study is in agreement with the results of Cater and Lawrie, (1950, 1951), Das and Ghosh, (1959), Kanwar, (1961), Maiti and Ghosh, (1969), Ishida et al., (1973), Hodges, (1974) and Banks, (1981) who have generally accepted this homology in other avian species.

The uropygial gland is more popularly known as the "oil gland" and it is the only cutaneous gland that occurs in birds. It is especially well developed in aquatic species (Hodges, 1974 and Banks, 1981) where its secretions serve to waterproof the feathers. The highly developed gland of pigeons is probably associated with a requirement for an oily secretion to render the feathers impermeable to water loss. It has been noted that after removal of the uropygial gland, the plumage of most species of birds becomes dry and brittle, and even in non aquatic species, the secretions are necessary to maintain normal flexibility and function of the feather coat. Extirpation of the uropygial gland (Hou, 1928, 1931); Knowles et al., (1935) and Kar, (1947) from young and adult chickens caused a disturbance of feather growth and an impairment of general health and resulted in rickets in young birds.

There is some disagreement in the literature about the location and even the presence of the gland in various species. We found it to be located on the dorsal surface of the free coccygeal vertebrae which agrees with the descriptions of Crisp (1860), Das and Ghosh (1959) and Alhumead (1992) and Alhumead (1992). The last author said that it is present between the two central feathers of the tail, but Hou (1928) found it on the levator ani muscle. On the other-hand, the well-known text by Sisson and Grossman (1975) states that there is no gland in pigeons. Alhumead (1992) reported this to be true in about 4% of their samples.

All of the pigeons we examined did have a uropygial gland, and in all cases tite gland was apparently functional and active. We speculate the absence of this gland as described in some sources to be due to oversight, or possibly there are strain variations among pigeons in which the gland is small and inconspicuous. The latter suggestion was supported by the "absence" of this gland in 4% of Alhumead's (1992) samples. Pigeons have been domesticated for thousands of years and some strains are highly inbred, it is conceivable that a genetic mutation resulting in a small, inconspicuous uropygial gland may have arisen and been consenued in many domesticated strains. A comparison of domestic and wild stock might clarify this point. The fact that most, if not all, birds (even flightless ones) have a functional uropygial gland is evidence that its presence is important for more than were water proofing.

Our observations regarding the capsule coincide with that reported by Elder (1954), Das and Ghosh (1959) and Bhattacharyya (1972) who mentioned it to be of fibro-reticular struture with some elastic fibers enveloping each lobe, the two lobes together are further enveloped by another thin connective tissue capsule. However, Crsip (1860), Hou (1928), Maiti and Ghosh (1969, 1972) and Alhumead (1992) who maintained

there were two separate glands, argued that each gland is surrounded by its own fibro-vacular capsule. The second and the last author added that there are some elastic fibers pesent especially on the lateral aspect of each gland and around the blood vessels. Alhumead (1992) found no smooth muscle in the capsule which we confirm is the case from this study. It should be noted that in 1928 Hou, did find muscular structures but did not describe its type.

We did not observe mast cells in the capsule. This is in contrast to Alhumead (1992) who found them and interpreted their occurrence as important to prevent coagulation of blood in this area. This would be important since this area is more exposed to bruising or trauma during flying.

In agreement with Zamaoska (1975) and Alhumead (1992) we found many blood vascular channels in the capsule, as well as, in the septum between the two lobes. These authors interpreted their occurrence as necessary to regulate the blood stream of the gland, as the area in which it is located, it is usually subjected to different external stimuli and pressures during flying. We agree with Hou (1928), Bhattacharyya (1972) and Alhumead (1992) in the occurrence of small septae of fine inter-alveolar connective tissue. Paris (1913) reported elastic fibers in these septae and in 1954, Elder added that there were some smooth muscle fibers which increased at the point of branching of the glandular alveoli, as well as, around the blood vessels.

The present study broadly coincides with those of Das and Ghosh (1959), Kanwar (1961), Maiti and Ghosh (1969, 1972), Bhattacharyya (1972) and alhumead (1992) in that, the gland is entirely filled with secretory alveoli of different shape and sizes, containing cavities in their centers, which emptied in one large cavity in the center of the lobe near its cranial end. On the other-hand, Crisp (1860), Paris (1913), Hou (1928) and Menon et al. (1981) showed glandular tubules extending cranio-caudally to open individually in the central cavity of the gland.

The secretory alveoli are lined with stratified epithelium that can be differentiated into three types of cells. This was also noted by Bhattacharyya (1972), Menon et al. (1981) and Alhumead (1992). Like these investigators, we were able to distinguish them to squamous basal cells, intermediate polyhedral cells (1-3 layers), and transitional cells (3-5 layers) in which the degenerative changes begin. Contrary to this, Kanwar (1961) described only 2 types of cells, the indifferent cells and the large alveolar cells. There is general agreement that the secretion in all species is formed from the degeneration of cells as they approach the lumen (Elder, 1954 and Alhumead, 1992).

With regard to the duct system, several authors described two separate excretory ducts, each one emerged from one lobe and opened separately in a pyramidal skin elevation. This observation was made by Paris (1913), How (1928) and Das and Ghosh (1959); but Alhumead (1992) reported only one common papilla carrying two ducts which carried secretions from both glands. On the other-hand, in 1954 Elder showed as many as 1-9 ducts, all of which opened to the outside via a structure resembling a papilla.

Regarding the presence of mucopolysaccharides, we observed fine granules in the basal cells. Moderately reactive materials were demonstrated by Alhumead (1992), which is in general agreement with our observations. on the other-hand, Das and Ghosh (1959) did not find any PAS positive material.

In our study the intermediate cells showed weak positive reactivity, but Kanwar (1961) and Bhattacharyya (1972) both described mucopolysaccharides which were not glycogen. In, 1992 Alhumead demonstrated polysaccharide

substances, as well as, glycogen in the intermediate cell layers. Mosallam (1990) stated that in ducks, the glandular cells of the outer third of the tubules contained very little of such substances, but in the middle third, the peripheral and intermediate cells showed small and large strongly reactive granules, PAS positive materials were also demonstrated in the inner third, but only in the peripheral cells. The luminal cell layers in this study did not show any positive reactivity, a finding which is supported by the observation of Kanwar (1961). Alhumead (1992) found a large amount of acid mucopolysacchariedes and very little glycogen, but Das and Ghosh (1959) and Bhattacharyya (1972) claimed that these cells do contain small amounts of mucopolysaccharides which are not glycogen. According to Cater and Lawrie (1950, 1951), the PAS positive materials, in the presence of acid phosphatase and non specific esterase, could be utilized by the cells of the uropygial glands of ducks as a precursor of lipids. In 1990, Mosallam revealed staining of some cells in the glandular tubules with AB pH 2.5 and indicated that these cells secreted acid mucopolysaccharides. Furthermore, the high content of alcianophilic materials in the lumina of the glandular tubules and the ducts suggested that they constituted products formed as part of the secretion. In agreement with Alhumead (1992) very fine granules of glycogen were observed in the basal cells only.

Regarding lipid distribution, there is weak reactivity in the basal cells but the reaction increased gradulally in the successive superficial cell layers and becoming more intense toward the lumen. Das and Ghosh (1959), Bhattacharyya (1972) and Menon et al. (1981) described very little of such material, but much greater amounts were demonstrated by Hou (1928) and Alhumead (1992). The intermediate cells showed strong reactivity with Sudan III and IV (Bhaattacharyya, 1972) containing more neutral fats but lesser unsaturated ones. Large amounts of sudanophilic substances (neutral fat) were observed by Das and Ghosh (1959), Kanwar (1961), Bhattacharyya (1972) and Menon et al. (1981). On the other-hand, lesser amounts were demonstrated by Hou (1928). The lumina of glands in Hou's study contained neutral lipids, proteins and carbohydrates, and he reported protein bodies with incomplete sudanophilic lipid ensheathment in the glandular cells. His conclusion concerning the formation of secretion was that the secretory droplets develop from minute lipoproteinaceous granules in the basal cells. Hsu (1934) discussed the role of the Golgi apparatus in the formation of these fat droplets in many organs (such as the mammalian sebaceous gland) and proved that the remnant of the Golgi apparatus remains connected to these fat droplets of sudanophilic material giving them the black envelope.

Our demonstration of the distribution of oxidative enzyme together with phosphatase enzymes indicates that the respective cells in which these exist are capable of carrying out many metabolic functions. This was particularly noticeable in the basal and intermediate cell layers. The demonstration of SDH together with G6P. DH and LDH might suggest the existence of different metabolic pathways in these parts, especially the glycolytic pathway, the Kreb's cycle and the hexose mono-phosphate shunt pathway pentose-shunt (Mosallam 1990). The pentose cycle is involved in the manufacture of ribose for the synthesis of nucleic acids, and it also provides quantities of reduced NADP for lipid synthesis (Flatt and Ball, 1966). The idea said that the normal functioning uropygial gland in the fowl is regulated to a great extent by the gonadal hormones was supported by many authors (Smith, 1942; Seyle, 1943, Kar, 1947 & 1949 and Maiti, 1971). Since the gonadal hormones are steroid in nature and NADPH produced through, G6PDH is utilized for the hydroxylation of steroid metabolism (Ishida et al., 1973). This might

xplain the noticeable amount of NADPH in the present study.

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