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HISTOPATHOLOGICAL OBSERVATIONS OF THE KIDNEY DURING AGING OF THE MALE ANNUAL FISH *NOTHOBRANCHIUS GUENTHERI*

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INTRODUCTION

THE ANNUAL fish *Nothobranchius guentheri* is currently being considered as a model system for studies in the biology of aging (Markofsky and Perlmutter, 1972, 1973; Markofsky, 1976) and for mosquito control in malarious areas of alternating rainy and dry seasons (Vanderplank, 1941; Bay, 1967; WHO, 1973; Scheel, 1975). Due to the importance of this fish in both "basic" and "applied" biology, it became necessary to perform detailed studies of histopathological changes with age in order to establish base-line data which could then be compared with experimental aging populations and with field populations introduced for mosquito control.

In a previous report we presented aging changes in the liver (Markofsky and Milstoc, 1979). In this study we describe the histopathological findings with age in the kidney.

MATERIALS AND METHODS

All fish used for study were male *Nothobranchius guentheri* (Class Pisces, Family Cyprinodontidae), an annual killifish from East Africa. The fish were purchased commercially in 1969 and their progeny maintained in our laboratory. Details of husbandry, longevity, growth, body composition and experimental design have already been published (Markofsky and Perlmutter, 1972, 1973; Markofsky, 1976). Six populations were born at different times between March 1969 and April 1970. On 29 June, 1970, each population was killed for study. Immediately prior to killing, within each population, individual fish were paired for body weight and length. One member of each pair was designated for body composition (Markofsky, 1976) and the other for histopathological study. In addition, 30 fish, most of which were part of the above aging study and which either died spontaneously or were moribund and killed, were investigated.

The fish which were to be killed were placed alive into 10% buffered neutral formalin. After fixation, a scalpel was used to perform a medial saggital section. Half of the fish was processed for routine histological examination. Serial saggital sections were prepared (about 4 sections on a slide) and every other slide stained with Hematoxylin and Eosin. If a fish was found dead, the ventral surface was opened with a scalpel and the fish placed into the fixative. Additional stains were performed where indicated (PAS, Trichrome, Wilder's reticulum, Fontana for melanin and Prussian blue for iron).

RESULTS

The kidney structure of *Nothobranchius guentheri* was generally similar to mammals except for the interstitium (2–3 months). In the young fish the glomeruli, tubules and interstitium appeared as a compact mass (Fig. 1). The glomeruli were almost avascular, filled with densely packed epithelial cells. Occasionally a cleft could be detected as evidence of the future Bowman's space. The tubules were lined with high columnar epithelial cells containing pale nuclei, uniformly located toward the basement membrane. Most of the tubules were in close proximity to each other with the interstitium formed by a limited number of

mononuclear cells. This interstitium was a mixture of predominantly lymphocytic cells and a small percentage of histiocytes.

With increasing age (5–7 months), Bowman's capsule and space became easily identifiable and the glomeruli tufts displayed more distinct capillaries. The entire kidney structure became less compact with increasing number of interstitial cells.

After 7 months certain histologic changes were noted. The organization of the parenchyma was loose, the glomeruli were enlarged, swollen, many with capsules thickened in varying degrees (Fig. 2). The tubules were slightly dilated (Fig. 3). The histiolymphocytic mixture was abundant with clumps of histiocytes phagocytizing increasing amounts of brownish-yellow pigment of lipochrome (Fig. 4).

In the more advanced age group (12–15 months) several pathologic entities were found. In some fish the tubules were markedly dilated, looking rigid in structure with rather low columnar cells (Fig. 5). Some of these sections also showed an increased number of polymorphonuclears and some eosinophils in the interstitium, a picture consistent with subacute interstitial pyelonephritis.

Other histologic sections of the fish revealed proliferating histiolymphocytic cells, invading and replacing most of the parenchyma. This neoplastic expansion showed an increased number of large cells in a multicentric nodular aggregation. The cells were irregular in size and shape, having a pale pinkish ill-defined cytoplasm, often containing lipochrome pigment. These cells were surrounded by small lymphocytes showing greater basophilia of the cytoplasm and pycknotic nuclei. The picture was consistent with nodular type of histiocytic lymphoma (Fig. 6).

Another tumor was diagnosed as tubular hypernephroma characterized by large, vacuolated cells and small nuclei. They were arranged in a cordlike pattern lining elongated spaces resembling the formation of tubules. The tumor was large, replacing most of the parenchyma (Fig. 7).

In one instance a very rare anomaly was noted. The kidney contained ectopic thyroid tissue which was also present in the vicinity of the pancreas, muscle and gills (Fig. 8).

DISCUSSION

During the last 9 yr, we have been performing studies in development (Markofsky and Matias, 1977; Matias and Markofsky, 1978), growth (Markofsky and Perlmutter, 1973), and aging (Markofsky, 1976; Markofsky and Perlmutter, 1972) in the annual fish *N. guentheri*. A detailed histopathological analysis was carried out in order to identify the sites of "normal" aging changes. Since *N. guentheri* has a relatively short lifespan both in nature (Turner, 1964; Bailey, 1972) and in the laboratory (Markofsky and Perlmutter, 1972) and since they exhibit many of the classical aging changes including some unique to themselves, they should prove to be a model system of choice for studies in the biology of aging.

As in mammals, the pathological changes take place in increasing number in rapport with age. The young fish show no kidney pathology. The dilation of the tubules in the older fish is a phenomenon commonly found in other species of fish and its cause is not yet clarified. In many instances it was considered to be due to back pressure of some lower part of the nephron in the course of its aging, while in other cases it appeared to be due to a process of hypertrophy (Smith and Jones, 1957). In any event, the epithelium does not become flattened and the tubules maintain their shape and function.

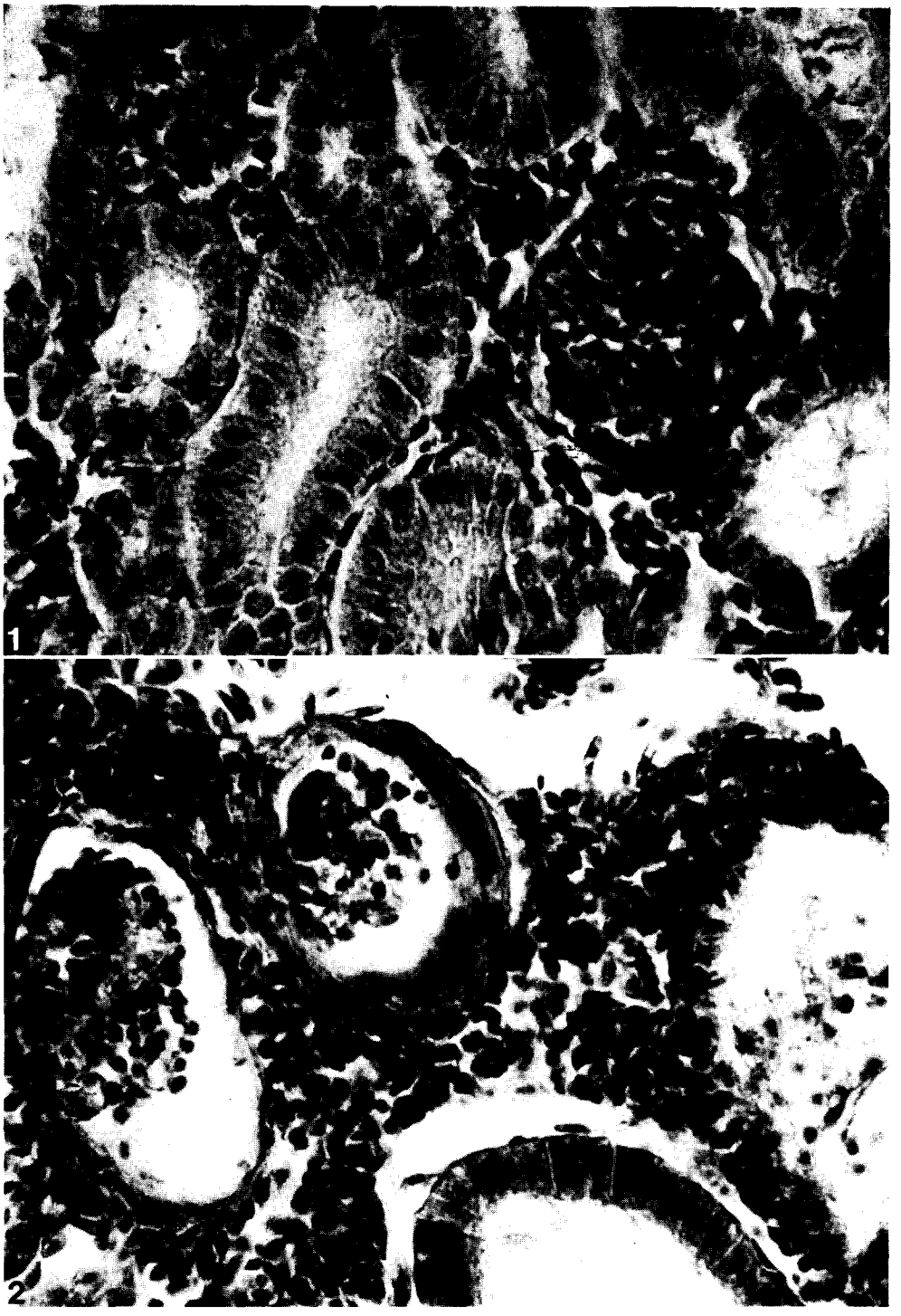

FIG. 1. Young fish kidney with dense glomerulus, crowded tubules and compact interstitium (H & E × 340).
FIG. 2. Enlarged glomeruli with thickened capsule (H & E × 340).

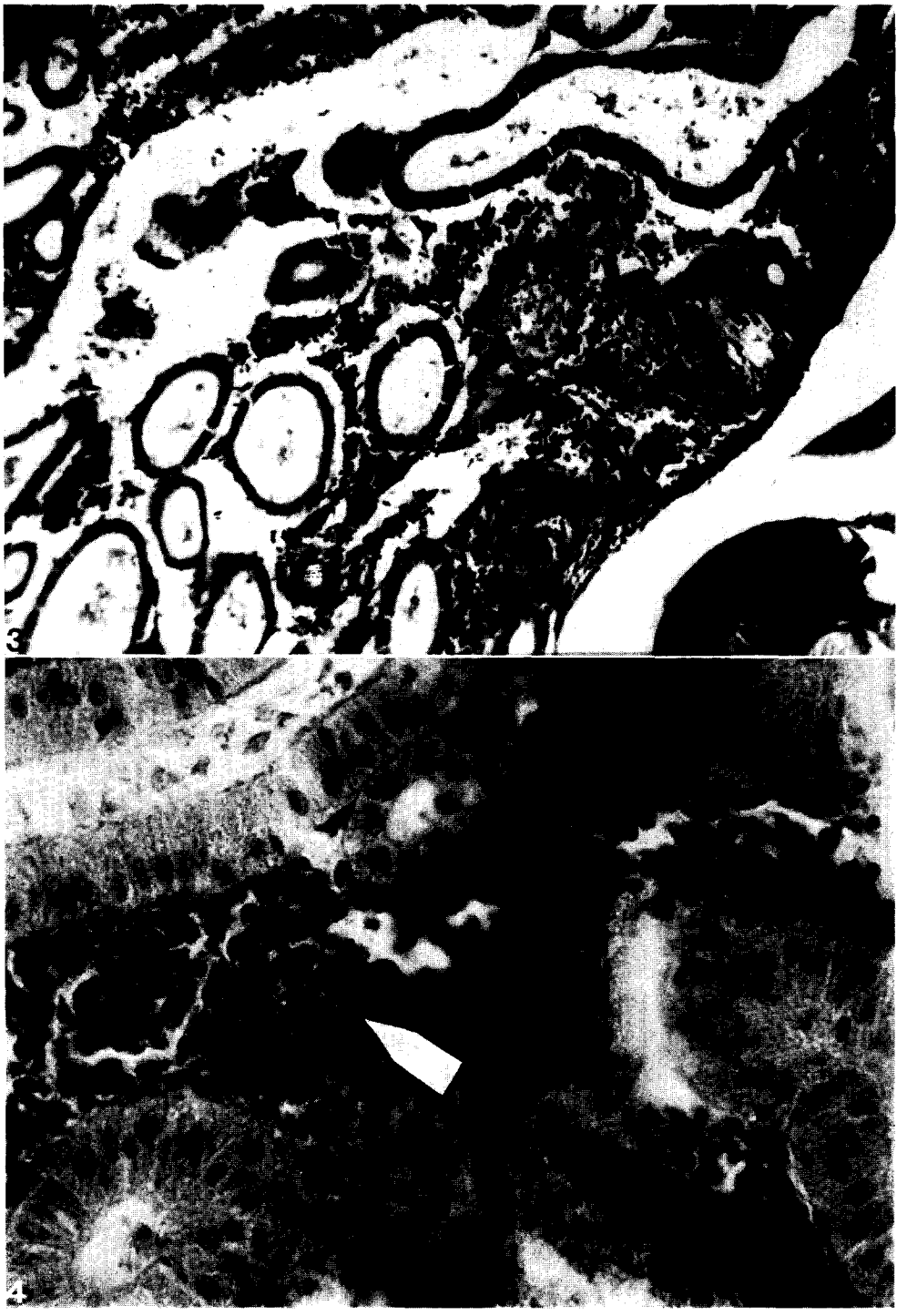

FIG. 3. Loosely arranged slightly dilated tubules (H & E × 85).
FIG. 4. Clumps of histiocytes with phagocytized lipochrome (H & E × 340).

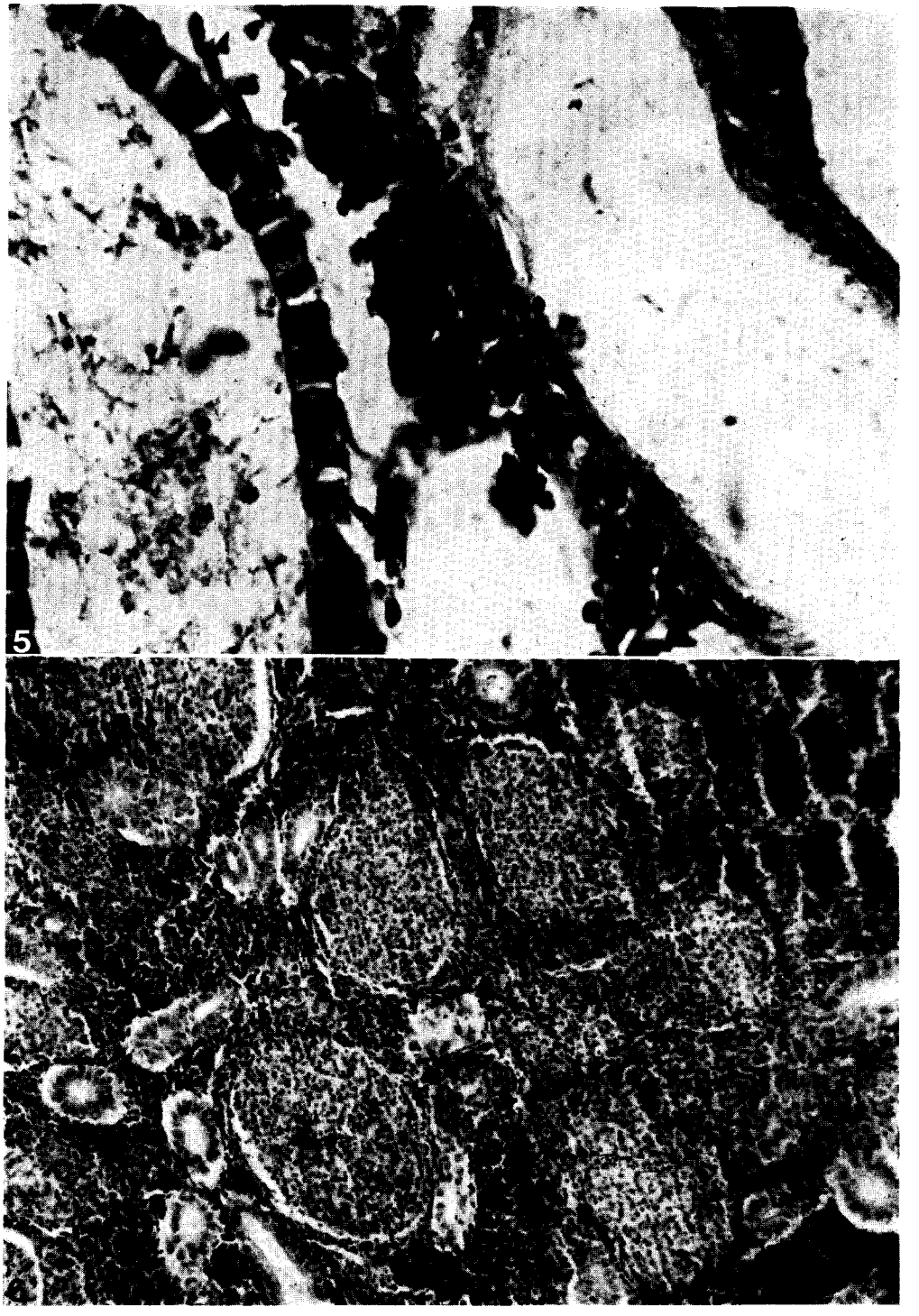

FIG. 5. Markedly dilated fibrotic tubules, rigid in structure (H & E × 340).
FIG. 6. Nodular histiocytic lymphoma (H & E × 85).

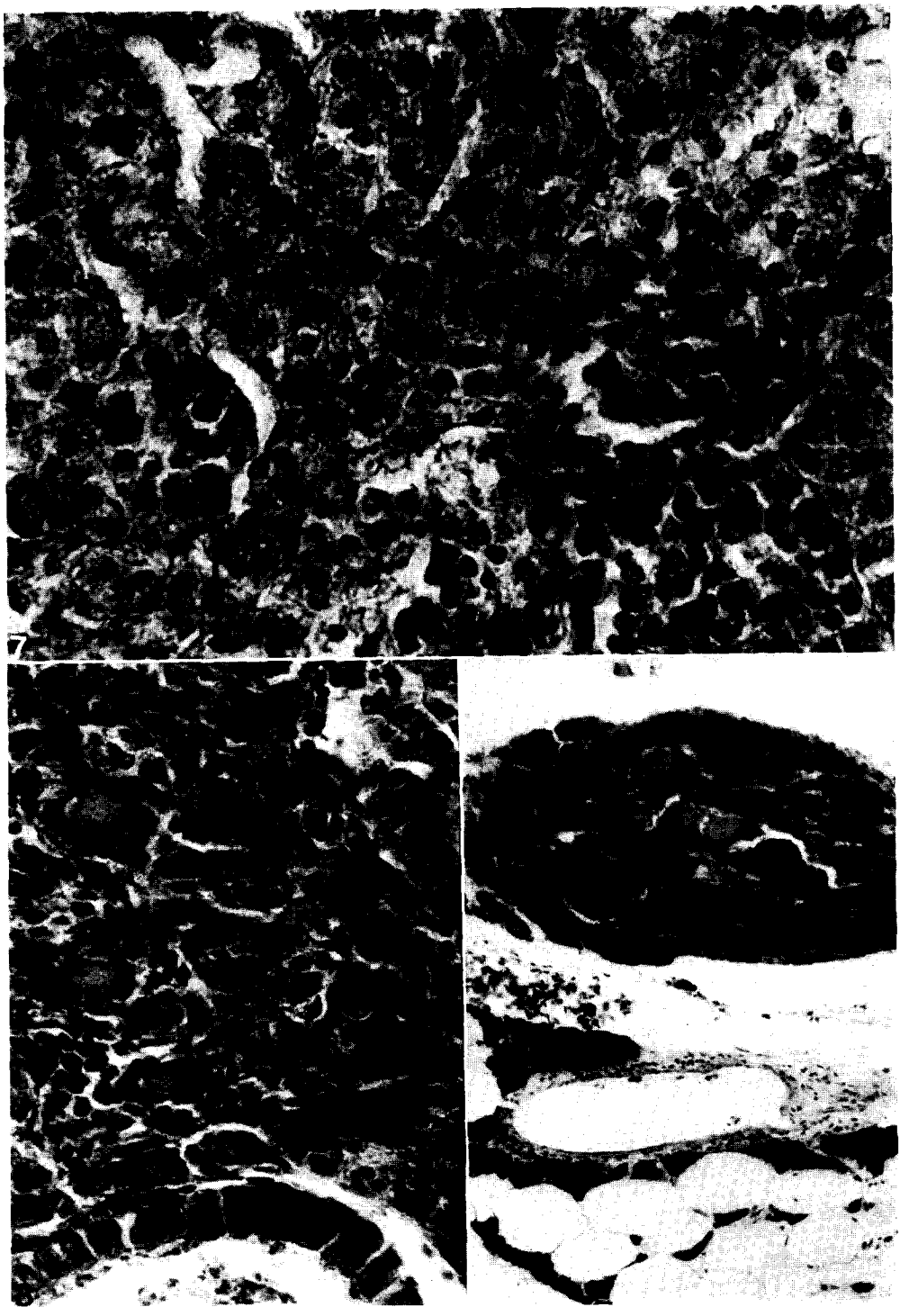

FIG. 7. Tubular pattern of hypernephroma. Note also typical clear cells (H & E × 85).

FIG. 8. Ectopic thyroid follicles found in the kidney (left), seen also in the vicinity of the pancreas (right).

Thickening of Bowman's capsule becomes more prominent with age and there were occasionally minimal and focal sclerotic changes. Unlike other species, true nephroscelerosis and tubular degeneration were not observed (Robertson and Wexler, 1960, 1962).

The accumulation of lipochrome with age was a common finding. Special stains ruled out the presence of iron or melanin pigment.

Due to the lymphoid structure of the kidney interstitium in fishes, it seems that the predominant renal tumors are primary lymphosarcomas. In some species a thymic origin of these tumors has been suggested (Dawe, 1969; Dunbar, 1969; Warren, 1969). We did not find a pure lymphocytic type of sarcoma. Because the interstitium contains a number of histiocytes, the tumors observed were considered to originate from these cells, a large percentage of which continued to maintain their phagocytic property, demonstrated by the presence of lipochrome in their cytoplasm. The etiologic factors of malignant neoplasia in fishes are speculative, but there is a prominent age relationship (Warren, 1969).

The presence of ectopic thyroid follicles was previously observed in the senescent Amazon molly and they were considered to be due to a spontaneous growth in old age (Woodhead *et al.*, 1977). Although the follicles were spread within the body, the histological appearance ruled out an invasive neoplasia.

SUMMARY

Aging changes were studied in the kidney of the male annual fish, *Nothobranchius guentheri*. The aging changes included tubule dilation, thickening of the Bowman's capsule, lipochrome in histiocytes, malignant tumors and ectopic thyroid tissue.

Since these fish are short-lived in both nature and the laboratory, they should provide an interesting model for studies in the biology of aging.

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