Endocrines And Ageing

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TABLE OF CONTENTS

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PREFACE

Studies by I. S. Edelman, T. H. Hamilton, B. W. O'Malley, J. D. Wilson and others have shown that the effect of hormones on cell function extends to control mechanisms at the level of the nuclear genome. Since nearly all of the current theories of ageing presume some effect of ageing on the nuclear genome, details of changes in endocrine function during ageing have come to be of particular interest. The articles contained within the present collection describe evidence for substantial changes in regulation of adrenal cortex, thyroid, and hypothalamus during mammalian ageing. Prominent within these articles are the studies of steriod intermediary metabolism done at the Worcester Foundation by L. P. Romanoff and other colleagues of Gregory Pincus. The unique events during ageing in adrenal function of Pacific salmon (O. H. Robertson et al.) provide an instructive contrast to the patterns of change found in mammals. Although there are major gaps of information about changes of endocrine function during ageing (e.g., concerning the pancreas, thyroid, gonad, hypothalamus and limbic system), enough is known to anticipate great returns from further research. Certainly, the field now has an authenticity which it lacked during many years of quiescence since the fabled attempts of Serge Voronoff to rejuvenate elderly men by providing them with grafts of monkeys' testicles.

Adrenal

HISTOLOGICAL CHANGES IN THE ORGANS AND TISSUES OF MIGRATING AND SPAWNING PACIFIC SALMON (GENUS *ONCORHYNCHUS*)[1]

O. H. ROBERTSON AND B. C. WEXLER

AMONG the many unusual features of the life cycle of Pacific salmon is the fact that they all appear to die of the same cause—universal death following the first spawning can be interpreted scarcely in any other way. Onset of the lethal process is frequently evident before spawning. Many Pacific salmon at full sexual maturity exhibit obvious degenerative changes, fungus infection, focal necrosis of the skin, loss of muscular strength and balance, but others reach this stage in apparently good condition. Following spawning, deterioration progresses rapidly, all fish are affected, and death ensues within a few days to two weeks. Some sexually ripe salmon die before spawning. That profound alterations are occurring in the tissues at this terminal period of the salmon's life can hardly be doubted. Indeed striking degenerative changes in certain organs were described by Greene (1) in 1926. But since his histological studies were limited in scope and did not include a number of body structures, a systematic microscopical examination of the salmon at successive stages in its sexual cycle seemed to be indicated.

[1] This work was aided by grant No. G-1374 from the National Science Foundation.

The present communication constitutes a continuation of our inquiry into the nature of the post-spawning death of the Pacific salmon. In previous papers, histological changes in the pituitary gland and the adrenal cortical tissue were described (2, 3, 4). Both glands were found to exhibit markedly increased activity (cellular proliferation and hypertrophy) during gonad maturation, followed at full sexual maturity by degeneration, more pronounced and constant in the pituitary than in the adrenal. The latter was characterized by extensive hyperplasia. Study of the other tissues and organs of the spawning salmon has revealed striking alterations in structure and cytology. These changes ranged from hypertrophy and hyperplasia on the one hand to atrophy and degeneration on the other.

MATERIALS AND METHODS

Sexually immature king salmon (*O. tschawytscha*) with infantile gonads were caught in the sea in Monterey Bay and in the open ocean just off Bolinas Bay. Those with gonads in varying stages of sexual maturation were secured at several stations on the Sacramento River. Sexually mature fish were taken at the spawning grounds of the same river.[2] Maturing and ripe blueback salmon (*O. nerka nerka*) were obtained at Entiat on the Columbia River.[3] Spawning kokanee (*O. nerka kennerlyi*) were caught in Donner Lake, California while immature kokanee were provided by the California Department of Fish and Game at their Lake Tahoe hatchery. A total of 104 king salmon, 6 blueback, and 12 kokanee were studied.[4]

All skin specimens were taken from the same general area just cephalad and lateral to the dorsal fin. Kidney specimens were taken from the mid-portion of the kidney. Histological methods used were described in a preceding publication (5). Additional special stains for certain tissues were employed; Heidenhain iron hematoxylin to bring out granules in glandular and granulosa cells of the stomach and intestine, iron hematoxylin azan for muscle fibers, Masson trichrome and Mallory technique for collagenous tissues, Gomori aldehyde fuchsin stain counterstained with Masson trichrome as employed by Abrams *et al.* (6) to bring out distinctive differences between the alpha and beta cells in the islets of Langerhans. Fat stains used were Sudan black B, Sudan orange and Oil Red 0.[5]

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[2] To the following members of the California Department of Fish and Game we wish to express our sincere appreciation for their generous assistance in securing material for this investigation. Dr. Alex Calhoun, Mr. Leo Shapovalov, Mr. Jack C. Fraser, Mr. Allen F. Pollitt, Mr. Richard Hallock, Mr. William Van Woert, Mr. James A. Hinze, Mr. Donald P. Evins, Mr. Phillip Murray, Mr. John Riggs, and Mr. T. James Ready. We are grateful to Mr. John Pelnar, Coleman Station, U. S. Fish and Wildlife Service at Anderson, California, for his cooperation on repeated occasions in making available to us specimens of ripe and spent salmon.

[3] We wish to thank Mr. Roger E. Burrows, Director, Salmon Cultural Station, U. S. Fish and Wildlife Service, Entiat, Washington, for his kindness in providing a number of specimens of blueback salmon for this study.

[4] For their valuable assistance in securing specimens on the field trips we are greatly obliged to Dr. Sydney F. Thomas, Dr. Marcus A. Krupp, Dr. Cutting B. Favour, all of the Palo Alto Medical Research Foundation and to Mr. Satoshi Hane, Metabolic Unit, University of California Medical School, San Francisco.

[5] We are much indebted to Miss Janice Doron of the May Institute, Cincinnati and to Mr. John Daniels of the Department of Medicine, University of Chicago, for the excellent histological preparations on which this study is based.

Skin

The skin of the salmon resembles in general that of other fishes (7). Figure 1, skin of an immature sea salmon, shows a thin epidermal layer below which is an open reticular zone in which the scales lie. Next a thick laminated stratum compactum rests on fatty tissue and muscle. With sexual maturation the skin becomes much thicker (Fig. 2). This change involves all three principal zones. The epithelium shows very active proliferation of the germinal layer, characterized by tall columnar cells (Fig. 4) as compared with the much smaller germinal cells of the immature fish (Fig. 3). The reticular zone becomes solidified. The scales are deeply buried below the epithelium[6] and a 2 to 3 fold thickening of the stratum compactum takes place.

Comparative measurements of the skin of immature and spawning king salmon showed an average thickness of 420 μ for the former and 1370 μ for the latter. This difference might be accounted for partly by the larger size of the spawning salmon, however, immature and mature kokanee of similar size were found to have skin thicknesses of 213 μ and 816 μ respectively. The skins of male fish averaged about 25% thicker than those of the females. Increasing thickness of the skin appears to begin several months before spawning.

At the same time that active growth of the skin epithelium is occurring, degeneration of the scales is taking place. So-called scale absorption (Figs. 5 and 6) consists first in disappearance of peripheral circuli then loss of scale substance. Scales of females showed less marked absorption than did those of the males.

Stomach and Intestine

Greene (8) has described the anatomy and histology of the stomach and intestine of the normal immature king salmon and has reported on their marked atrophy in the spawning fish (1). Since no one, as far as we can find, has pictured these changes, we present the accompanying photographs which illustrate the most conspicuous alterations. In the spawning salmon the stomach is greatly contracted. Figures 7 and 8 show the difference in diameter of the empty stomach of a feeding sea salmon and a fully mature fish of similar size. Figures 9 and 10 reveal in more detail the marked changes that have taken place by the time of spawning. The villi have disappeared leaving smooth-surfaced folds of the mucosa. The tall epithelial cells of the sea salmon (Fig. 11) tend to become low columnar and in certain instances have shrunken to an almost cuboidal shape (Fig.

[6] It is almost impossible to secure scales from sexually mature salmon by scraping with a knife. We have found that a strip of skin placed in 2 per cent KOH for 48 to 72 hours becomes so digested that the scales are easily detached.

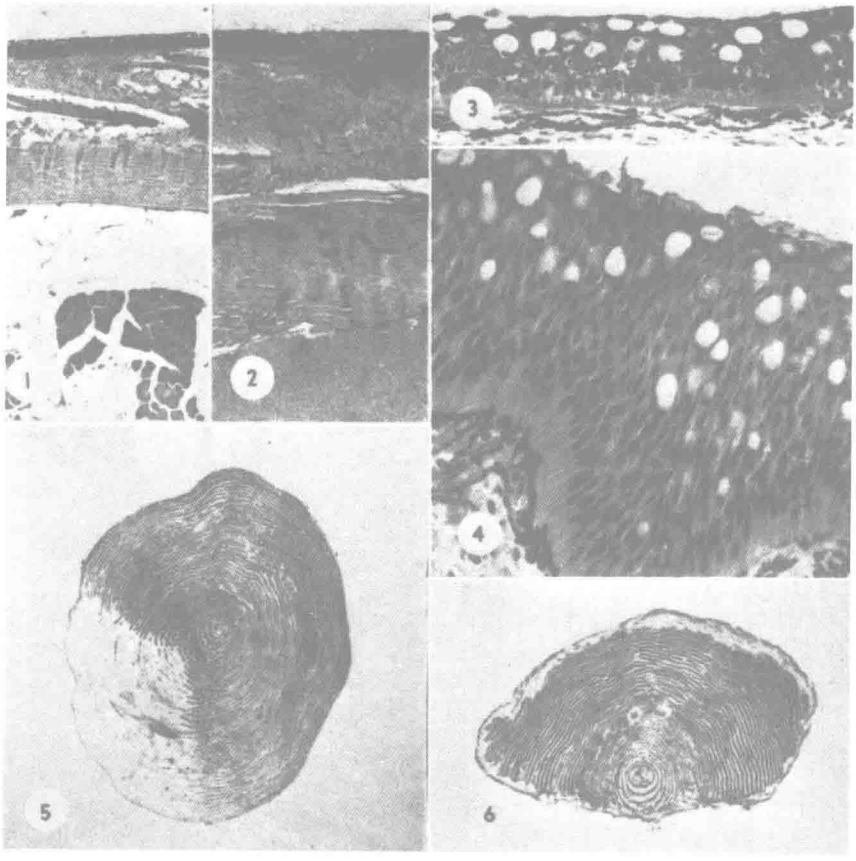

FIG. 1. Skin of immature male king salmon taken on spawning migration—length 30.5 inches. Scales are seen in the loose areolar tissue of the dermis. H. and E. ×40.

FIG. 2. Skin of spawning male king salmon—length 24 inches. H. and E. ×40

FIG. 3. Epidermis of immature salmon shown in Fig. 1. Cells of germinal layer are low columnar. H. and E. ×225.

FIG. 4. Epidermis of spawning salmon shown in Fig. 2. Shows marked proliferation. Germinal cells high columnar. H. and E. ×225.

FIG. 5. Scale of an immature kokanee. 4 years of age. Unstained ×13.5.

FIG. 6. Scale of spawning kokanee, showing marked absorption. Lower caudal ⅓ of scale has been lost and peripheral circuli of remaining portion are disappearing. Unstained ×16.

12). While the number of epithelial cells has shown a very great diminution they are still intact. The stratum compactum, a dense narrow band of collagenous tissue which lies near the outer margin of the mucosa is seen as a thickened convoluted structure (Fig. 10). The heavy circular muscle layer in the spawning salmon exhibited marked atrophy and in places fragmen-

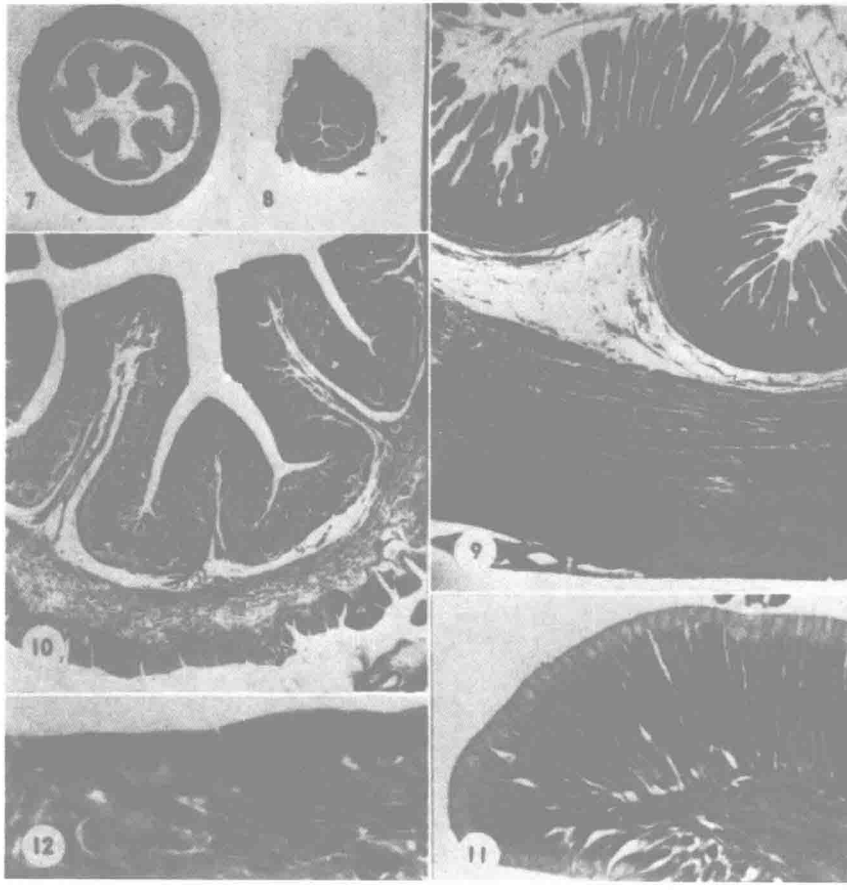

FIG. 7. Cross section of stomach of an immature, sea, male king salmon. 26 inches in length in 4th year of age. Iron, hematoxylin, azan, ×2.5.

FIG. 8. Cross section of stomach of spawning female king salmon 24 inches long, in 4th year of age. Iron, hematoxylin, azan, ×2.5.

FIG. 9. Higher magnification of an area of stomach of sea salmon shown in Fig. 7. Note numerous tall villi and thick muscle layer. Iron, hematoxylin, azan, ×22.

FIG. 10. Higher magnification of stomach of spawning salmon shown in Fig. 8. Note loss of villi and thinning of muscle coat. Iron, hematoxylin, azan, ×22.

FIG. 11. Epithelial cells lining villi in stomach of sea salmon shown in Fig. 8. H. and E. ×400.

FIG. 12. Epithelial cell layer covering surface of mucosal folds shown in Fig. 9. H. and E. ×400.

tation of the muscle fibers. Comparative measurements of this muscle layer in sea and sexually mature fish gave an average thickness of 1510 μ for the former and 344 μ for the latter. The external or longitudinal muscle, scarcely visible in Figure 9, has become a much thickened zone in the

spawning fish (Fig. 10) marked by rugae. Its composition is largely hypertrophied plexuses of Auerbach, blood vessels and newly-deposited collagenous tissue: Scattered individual muscle fibers are seen, some of them degenerating, but it is difficult to ascertain whether or not they are diminished in number.

These changes in the stomach of the migrating salmon begin some time before spawning. While marked contraction accompanying starvation is always present from the time the salmon leaves the sea, significant atrophy has not been observed until 2–3 months before full sexual maturity. During the mid-stage of migration the eosinophilic granular cells of the mucosa are present in greater abundance than previously and are heavily granulated.[7] At spawning these cells are present in even greater numbers but are less heavily granulated. Similar degranulation is characteristic of the cells of the gastric glands.

The changes occurring in the intestine and pyloric cecae of the spawning salmon are in general like those of the stomach. In addition, degeneration and desquamation of the epithelium was found in some fishes, the mucosa being replaced by a dense mass of cells, polymorphonuclear, mononuclear and multinucleated giant cells.

Liver

The principal differences between the livers of fishes (including salmonids) and mammals is, (1) the absence of any definite lobulation in the former and, (2) two-layered cords of cells instead of a single layer characteristic of the mammalian liver (12). During transition from the sea to the spawning grounds the liver of the salmon undergoes marked changes. Before the beginning of the spawning migration the liver cells accumulate a large store of fat (Fig. 14). Prior to this time the liver shows little or no fat deposition as judged by staining methods (Fig. 13).[8] The amount of fat in the liver diminishes gradually as the fish ascends the river and by the time of spawning it can no longer be detected by fat stains.[9] The first evidence of degenerative change in this organ was found in salmon with well-developed gonads, circa 6 weeks to 2 months before spawning. This consisted chiefly of alterations in the nuclei which were irregular in shape and size; the chromatin was coarse and consisted of granules rather than

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[7] Greene (9) believes that these cells may be a source of lipase. Bolton (10) considers the granulosa cells to be mast cells. However, Weinreb and Bilstead's (11) histochemical studies of these cells indicated that they were not mast cells.

[8] Fat storage would appear to depend largely on the kind of food taken. Just before migration, salmon feed actively on fat-rich pilchards and anchovies. The varying kinds of food found in the stomachs of salmon off the California coast during different seasons of the year has been reported by Merkel (13).

[9] This may not be true of salmon spawning in short coastal streams. We know of no studies on the fat content of the livers of such fishes.

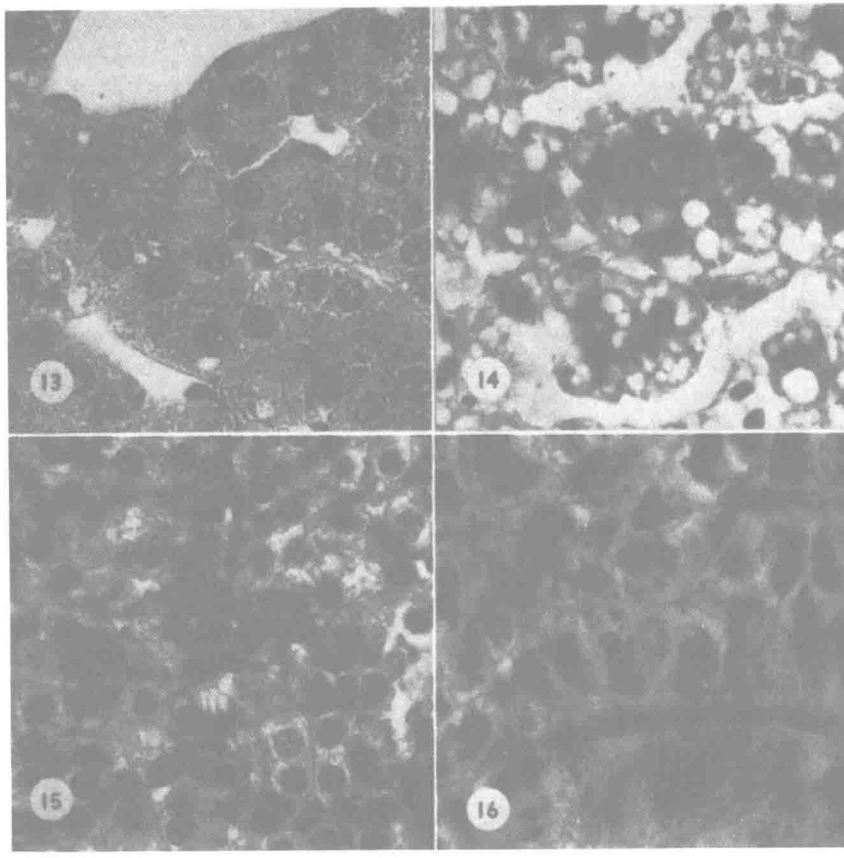

FIG. 13. Liver of sexually immature sea king salmon. H. and E. ×900.

FIG. 14. Liver of sexually immature king salmon at beginning of spawning migration. Liver cells loaded with fat droplets of varying size. H. and E. ×900. Presence of fat corroborated by stain with Sudan orange.

FIG. 15. Liver of male king salmon with well-developed testes. Would probably spawn within one to two months. Shows beginning degeneration of cells. H. and E. ×900.

FIG. 16. Liver of spent kokanee taken from Donner Lake. Marked generalized degeneration of hepatic cells. H. and E. ×900.

threads. The size of the nuclei was unchanged. However the cells had become somewhat smaller giving the appearance of a greater number of nuclei per unit area of liver substance. In addition the cytoplasm of many cells exhibited a moth-eaten aspect and here and there nuclei were disintegrating (Fig. 15).

With full sexual maturity and spawning cellular degeneration had become pronounced. The amount of cytoplasm was diminished and what remained was irregularly distributed in small masses giving the cell a