were separated and compared with the esterases found so ubiquitously in other organs. The fact that we have also been able to separate and localize tyrosinase and phosphatase suggests that these analytic methods have general applicability in the study of the enzymatic composition of tissues.

In this investigation, the starch block was prepared according to the method of Smithies (*3*). The tissue was homogenized in an equal volume of water and centrifuged at 1700 *g* for 10 minutes. The supernatant liquid was adsorbed onto a strip of filter paper and inserted into the starch block as described by Smithies (*3*). Satisfactory separation of the proteins was achieved in 5 hours at a *p*H of 8.6 at room temperature using a 6-volt drop per centimeter of travel through the starch block. Migration occurred at about half this rate at 2°C. The starch blocks were sliced into two or more thin slabs, permitting several techniques to be applied to the same sample. When the short-chain esters of α-naphthol and naphthol AS acetate were used as substrates, the techniques described by Gomori (*4*) were employed. We propose the term *zymogram* to refer to strips in which the location of enzymes is demonstrated by histochemical methods.

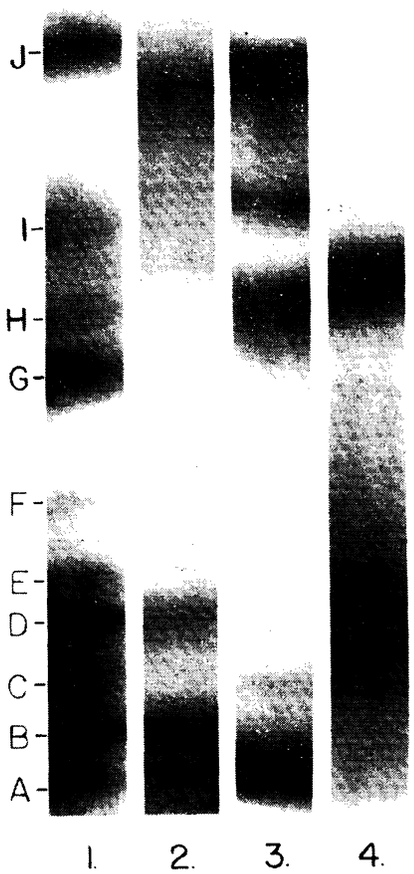

Fig. 1. Zymograms 1 and 2 are from normal adult mouse liver and were prepared using α-naphthyl butyrate as substrate; zymogram 2 was inhibited with eserine at $10^{-4}M$. Zymograms 3 and 4 are from adult mouse lung; these were prepared with different substrates—No. 3 with α-naphthyl butyrate and No. 4 with naphthol AS acetate. Note the inhibition of certain bands in No. 2; the different pattern of bands in lung (No. 3) and liver (No. 1); and the substrate specificity as seen in comparing No. 3 and No. 4.

The distinctive properties of tyrosinases from different species (*5*) were indicated by zymograms of aqueous extracts of mushrooms (*Psalliota*), potatoes, and mouse melanoma. These three tyrosinases were located at different characteristic positions on the starch slabs by their reaction with 3,4-dihydroxyphenylalanine to produce pink bands which later transformed to black melanin.

The separation of esterase-active proteins from mouse liver and other organs yielded a surprisingly intricate and reproducible spectrum of enzymes in the several hundred zymograms so far examined. Characteristically distinct zymograms were obtained from each organ tested (Fig. 1), seemingly reflecting the variety of functions of these organs, and suggesting that these several esterases are metabolically unique and not simply an artifact of the analytic procedures. Some esterases were found in several organs, while others, so far, have been found in only a single organ. However, nearly all the esterases found in any organ also occurred in liver.

Further support for the unique nature of these separated esterases was obtained by reacting the two halves of a starch block with different substrates. Zymograms made using α-naphthyl butyrate as substrate were conspicuously different from those in which naphthol AS acetate was used as substrate (Fig. 1). Certain differences in the distribution of esterases were observed by Gomori (*6*) when he compared α-naphthol esters with naphthol AS esters as substrates. Our results indicate that these differences were due to the substrate specificity of several esterases. To date, procedures using naphthol AS acetate have demonstrated only certain of the esterases that are demonstrated when α-naphthyl butyrate is used as substrate. The esterase in the G band is especially reactive with naphthyl AS acetate. Our results with these substrates demonstrate clearly that the substrate specificity of several of the esterases in the zymograms is not the same.

Finally, the fact that eserine, a known cholinesterase inhibitor, selectively inhibits, for example, the G and H bands (Fig. 1), leads us to suggest that many, if not all, of the bands shown in the α-naphthyl butyrate esterase zymogram are enzymatically distinct.

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References and Notes

1. This investigation was aided by grants from the American Cancer Society and the Michigan Memorial Phoenix Project No. 56.
2. We thank H. Eldon Sutton for his helpful suggestions in the use of the starch gels in electrophoresis and T. M. Oelrich for help in constructing the electrophoresis apparatus.
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14 March 1957

Pituitary Degeneration and Adrenal Tissue Hyperplasia in Spawning Pacific Salmon

In a study of the nature of the death of the Pacific salmons, which occurs regularly following their initial spawning, our attention was drawn to the possible role of the pituitary gland in this process by a series of changes characterizing sexual maturation and subsequent deterioration in these fishes (*1*). The fully mature male and, to a lesser extent, the female salmon exhibit a marked overgrowth of bone and cartilage of the head and vertebral column which produces a hooking of the jaws and a hump back, evidence of greatly increased activity of the pituitary other than the production of gonad-stimulating hormones. At the same time, degenerative changes are occurring—absorption of the scales and focal necrosis of the skin. Following extrusion of sex products, disintegration of the integument increases, and the fish loses its muscular power and balance and dies within a week or two. That such degeneration and death can be hastened by the injection of salmon pituitary glands was observed by R. E. Burrows in the course of experiments on producing accelerated sexual maturation in blueback salmon (*2*). A. P. Rinfret, who, with S. Hane, showed that the salmon pituitary elaborates ACTH (*3*), suggested to us the possibility that the degenerative changes might be caused by intolerable concentrations of a catabolic hormone or hormones secreted by the fish's adrenals under the stimulus of large amounts of adrenocorticotrophic hormones.

Pituitaries and adrenal tissues were secured from several species of the genus *Oncorhynchus*, including king salmon (*O. tschawytscha*), blueback (*O. Nerka*

nerka), and kokanee (*O. Nerka kennerlyi*), during various stages of their life-cycle. The morphology of the pituitary of the salmon, in common with that of other fishes, differs in many respects from that of the mammalian gland. While the salmon pituitary has no separate neural lobe, the dorsal region of the gland presents a cellular picture which corresponds to the anterior lobe of the mammal. Neural elements, accompanied by fine reticular tissue, penetrate and branch through the dorsal region or lobe (called by some the "transitional lobe") and the ventral part of the gland, often called the "intermediate lobe." In addition to these two distinct regions, there is a follicular area situated anterior to the dorsal lobe.

As sexual development proceeds, the basophiles and acidophiles of the dorsal lobe increase in number; the follicles of the anterior region enlarge, and some of them contain a colloidlike material. As maturity approaches, the basophiles predominate, and the gland gives the appearance of intense secretory activity. With full maturity and spawning, a marked change in the histologic picture takes place; degeneration is evident throughout the gland. First of all, there has been a great increase in connective tissue. This is especially pronounced in the lower lobe and separates the cells into clumps of varying size. Within the fibrous recticulum are shells or ghosts of nuclei without surrounding cytoplasm or contained chromatin. The cells of the dorsal region are markedly vacuolated, especially the basophiles, and exhibit pycnosis and cytolysis. The same is true of the anterior and ventral lobes. The follicles of the anterior lobe expand to huge, thin-walled bullae. Is this a picture of exhaustion following great secretory activity, or is it part of a general catabolic process?

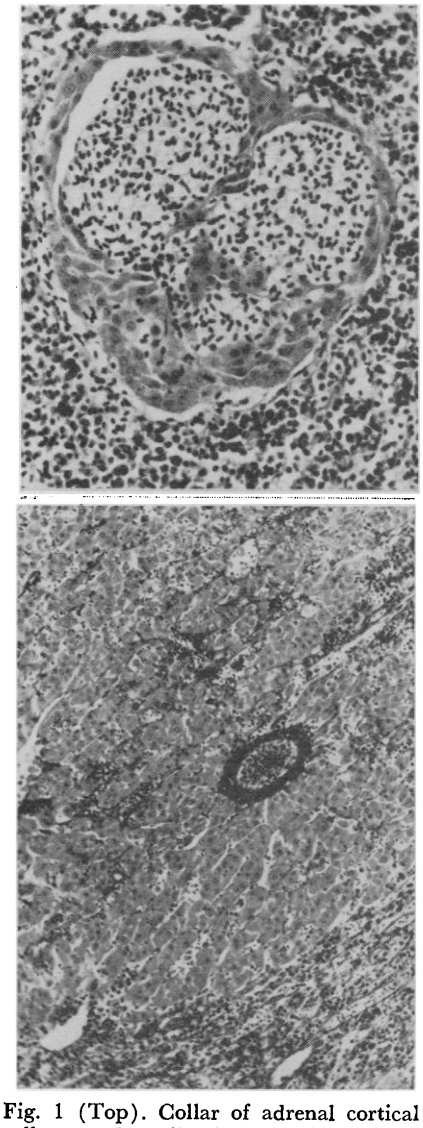

Fig. 1 (Top). Collar of adrenal cortical cells around small vein in head kidney of sexually immature king salmon at beginning of spawning migration (×200). Fig. 2 (Bottom). An area of extensive hyperplasia of adrenal cortical cells in head kidney of spawning king salmon (×85).

In contrast to the picture in the salmon, the pituitary glands of fully mature rainbow trout (which spawn repeatedly) usually show these degenerative changes to a slight or moderate degree at most, or not at all.

The adrenal cortical tissue in salmon and trout, as with other teleost fishes, is found to be diffusely distributed through the cephalic portion of the kidney, called the "head kidney," and is intimately associated with the cardinal vein and its branches, often forming collars around the veins, as is shown in Fig. 1. The cells of this cortical tissue are of a single type and, in the sexually immature stage, exhibit a certain resemblance to the cells of the glomerulosa of the mammalian adrenal cortex. As the salmon's gonads mature, the cortical tissue increases in amount and the cells take on more of the appearance of the fasciculata zone in the mammal. By the time of full sexual maturity, tremendous hyperplasia of the adrenal tissue has occurred. The cells radiate outward from the veins in great masses of a more or less lobulated character, displacing the blood-forming tissue of the head kidney (Fig. 2). These foci of cortical tissue are so large that they are easily visible to the naked eye as pale areas in unstained thick sections of Bouin's solution-fixed tissue. The cells contain relatively large quantities of cytoplasm, in which fine granules are evenly dispersed. (The granules do not differentiate well with osmic acid.) In some spawning fish, degeneration of these cells is occurring—shrunken cytoplasm, pycnosis, and cytolysis—which tends to be more marked in fish near death. This final phase in the changing morphology of the adrenal cortical cells of the salmon suggests a possible analogy to the zona reticularis of the mammalian adrenal gland.

The degenerative changes observed in the pituitaries of the spawning salmon resemble, in certain respects, those that occur in the pituitary glands of senescent mammals, including man—for example, increase in connective tissue, vacuolization of the basophiles, in particular, and diminished number of cells, which is the result, in the salmon, of visible cytolysis. In addition, study of the other internal organs and tissues of the salmon at full sexual maturity has revealed widespread degeneration of a degree which would seem to be incompatible with continued life (*4*). Is the marked hyperplasia and presumptive correspondingly increased secretory activity of the adrenal cortical tissue causally related to the rapid and extensive deterioration of body structures? It should be possible to secure experimental evidence on this point.

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2. Personal communication from R. E. Burrows, director of the Salmon Cultural Laboratory, U.S. Fish and Wildlife Service, Entiat, Washington.
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8 April 1957

Preparation of Human Serum Albumin Free of Long-Chain Fatty Acids

It is well known that serum albumin binds fatty acid anions strongly and that both crystalline preparations of albumin and those prepared by routine fractionation contain small but significant amounts of long-chain fatty acids (*1*). The only preparation of albumin which has been reported to be free of fatty acids was that of Dintzis (*2*); this was a preparation of recrystallized human serum mercaptalbumin which was "deionized" by passage through a mixed-bed ion-exchange resin. A sample of recrystallized bovine mercaptalbumin, deionized in identical fashion, was analyzed for unesterified fatty acid content