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?

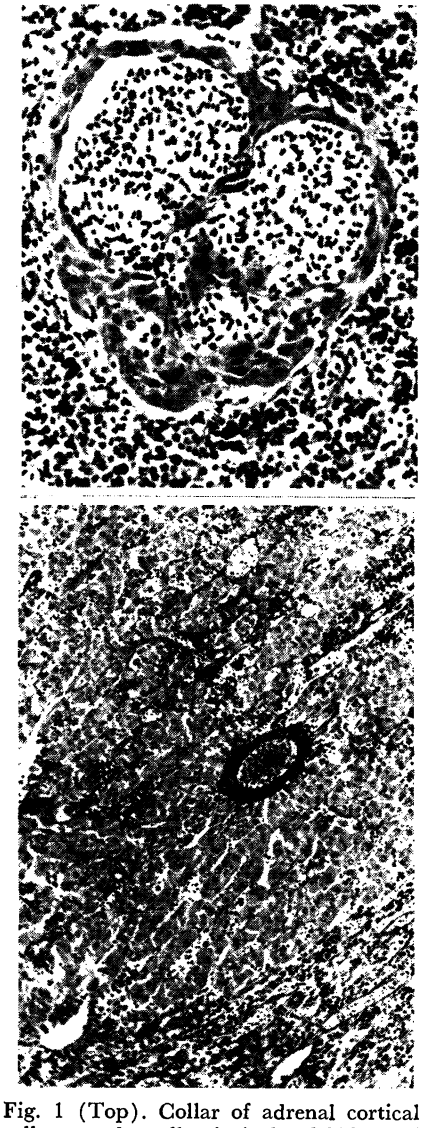

Fig. 1 (Top). Collar of adrenal cortical cells around small vein in head kidney of sexually immature king salmon at beginning of spawning migration (×200). Fig. 2 (Bottom). An area of extensive hyperplasia of adrenal cortical cells in head kidney of spawning king salmon (×85).

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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References and Notes

1. This study was aided by grants from the National Science Foundation and the American Philosophical Society.
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4. A detailed description of the degenerative changes found in the various organs and tissues of the spawning salmon and trout is in preparation.

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

Table 1. Comparison of untreated and extracted albumin.

	Untreated albumin	Extracted albumin
Sedimentation constant, S_w20*	4.3	4.3
Electrophoretic mobility, descending boundary, $\mu \times 10^5$	6.50	6.52
Optical rotation, $[\alpha]_D^{25}$	−55.7	−53.7

* Not extrapolated to zero concentration; albumin concentration approximately 0.7 g/100 ml.

by the method of Gordon (*3*). Analysis revealed that it contained 1.3 moles of long-chain fatty acid per mole of albumin (*4*). The human and bovine mercaptalbumin preparations may, of course, differ significantly in this respect; unfortunately none of the afore-mentioned human mercaptalbumin preparation is available for analysis. When preliminary attempts, in this laboratory, to prepare human serum albumin free of long-chain fatty acids by using mixed-bed resins proved unsuccessful (*5*), the method described in this report was developed.

The human serum albumin used in this study was a sample of fraction V, produced by fractionation of pooled blood plasma by the method of Cohn *et al.* (*6*). Stabilizers (N-acetyl tryptophane or sodium caprylate) or heavy metals had never been added to this material, and it had received no heat treatment (*7*).

A water solution of serum albumin was lyophilized, and the dried albumin was covered with a mixture of 5-percent glacial acetic acid (by volume) in isooctane. The acetic acid-isooctane mixture had been pretreated with anhydrous Na_2SO_4 to remove traces of water. The extraction was carried out without agitation, at 0°C, for 6 hours or more. The extraction mixture was then decanted and discarded, the albumin was washed with an aliquot of isooctane, and the extraction was repeated in identical fashion. The albumin was then washed twice with aliquots of isooctane and was subjected to a vacuum for several hours in order to remove remaining isooctane and acetic acid.

When the resulting albumin preparation was dissolved in water, a turbid solution was obtained. The turbidity is caused, it is believed, by the presence of isooctane that has not been removed by the vacuum distillation. It could be made to disappear by any one of the following procedures: by dialysis against water; by allowing the solution to stand from 12 to 24 hours at room temperature or from 3 to 4 days at 1°C; or by application of a vacuum to the solution for a short period. The procedure selected was exhaustive dialysis of the albumin solution against distilled water. Acetic acid which remained was removed concomitantly during this dialysis; this was demonstrated by the disappearance of tracer amounts of C^{14}-labeled acetate that had been added prior to dialysis. The albumin preparation was then lyophilized and stored at −10°C.

Analysis of the serum albumin before extraction, by the method of Gordon, revealed that it contained 1.8 moles of long-chain fatty acid per mole of protein. After extraction, it contained only 0.02 mole of fatty acid per mole of albumin by the method of Gordon, and 0.10 mole of fatty acid per mole of albumin by the method of Dole (*8*).

The extracted, fatty acid-free albumin has been extensively compared with the untreated albumin to determine whether denaturation may have occurred during the extraction procedure. Some of the data obtained are presented in Table 1. Ultracentrifugal analysis was performed in a Spinco model E ultracentrifuge. Electrophoretic analysis was performed in barbital buffer, *p*H 8.6, ionic strength 0.1, in an Aminco model B electrophoresis apparatus. Optical rotation was measured in a Brinkmann polarimeter in which photocells from Rudolph and Sons were used. There was no detectable difference between the two albumin preparations by any of these analytic techniques.

The extracted albumin is also immunologically identical with the untreated albumin. This has been demonstrated by the agar diffusion method (*9*), with rabbit antiserum against recrystallized human serum mercaptalbumin. The "reaction of identity" was obtained at the junction of the precipitin lines for the two preparations. Finally, the two albumin preparations react identically with methyl orange anions. This has been demonstrated by a quantitative study of the binding of methyl orange anions to serum albumin by the method of equilibrium dialysis. Studies were performed with the untreated albumin, with the extracted albumin, and with a preparation of the extracted albumin to which 0.9 mole of oleic acid and 0.9 mole of palmitic acid had been added per mole of protein. The binding curves for all three preparations were identical, within the limits of error of the measurements. The accumulated evidence thus indicates that the albumin is not significantly altered during the extraction procedure.

In experiments designed to study the binding of small molecules or ions to serum albumin, an albumin preparation from which all tightly bound small molecules and ions have been removed should be used. The method described here was, in fact, developed in the course of quantitative studies of the interaction of human serum albumin with long-chain fatty acid anions. This method, which may be of general interest in investigations of the chemistry of albumin, may therefore prove to be of particular value in preparing albumin for use in a wide variety of binding studies.

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2. H. Dintzis, Ph.D. dissertation, Harvard University (1953).
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4. This was a sample of a three-times recrystallized bovine mercaptalbumin preparation deionized by Dintzis, and was provided through the courtesy of George Scatchard of the Massachusetts Institute of Technology.
5. It is recognized that the conditions of the resin treatment of albumin in this laboratory may have differed significantly from those used by Dintzis, and that this may be responsible for the lack of success in using this method.
6. E. J. Cohn *et al.*, *J. Am. Chem. Soc.* 68, 459 (1946).
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10. I wish to thank Robert S. Gordon, Jr., for invaluable advice and assistance.

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Sensitivity of the Skin to Changes in Rate of Intermittent Mechanical Stimuli

We have recently reported data on comparative speed of response of the eye and the ear, shown by measuring difference limens (DL) for intermittent stimuli (*1–3*). This paper (*4*) reports extension of such measurements to rate discrimination by the human skin and compares these with difference limens on flutter—that is, rate discrimination for intermittent white noise. The skin may be considered to be the phylogenetic antecedent of the ear, and v. Békésy's remarkable success in demonstrating the skin as a dimensional model of the cochlea points up the similarity of the two sensory processes (*5, 6*). It is shown in this report that data on rate discrimination suggest a further similarity between the skin and the ear.

The procedure was to measure differ-