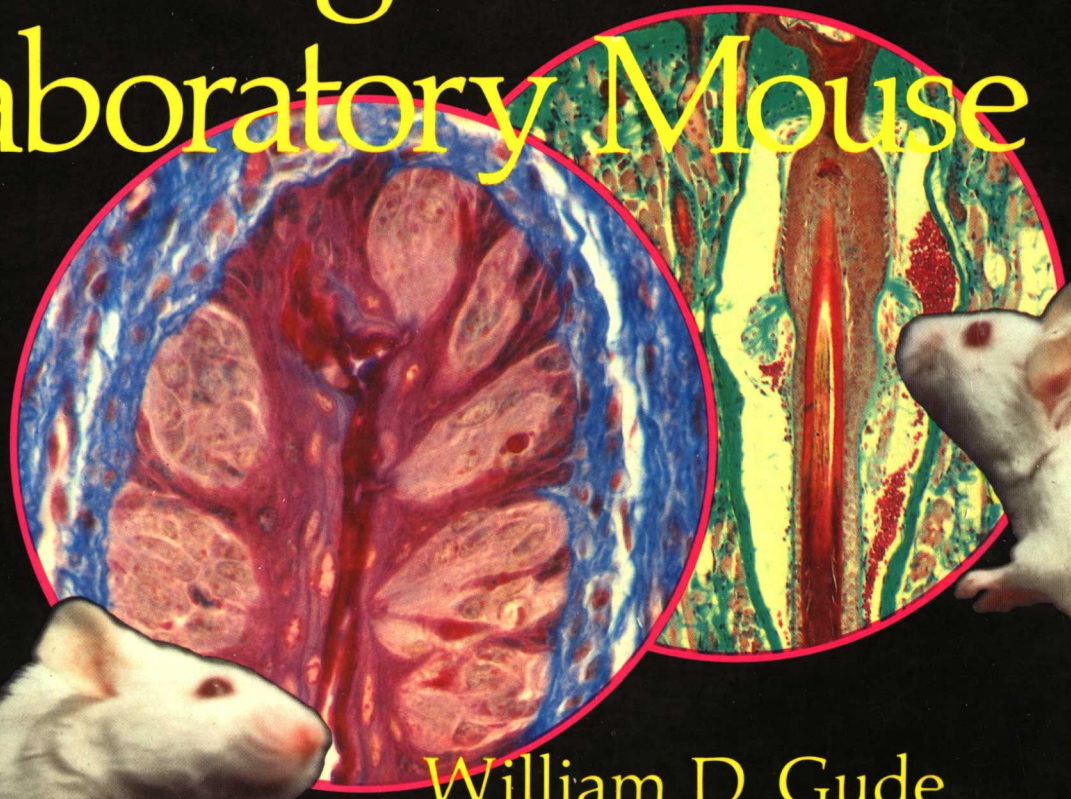
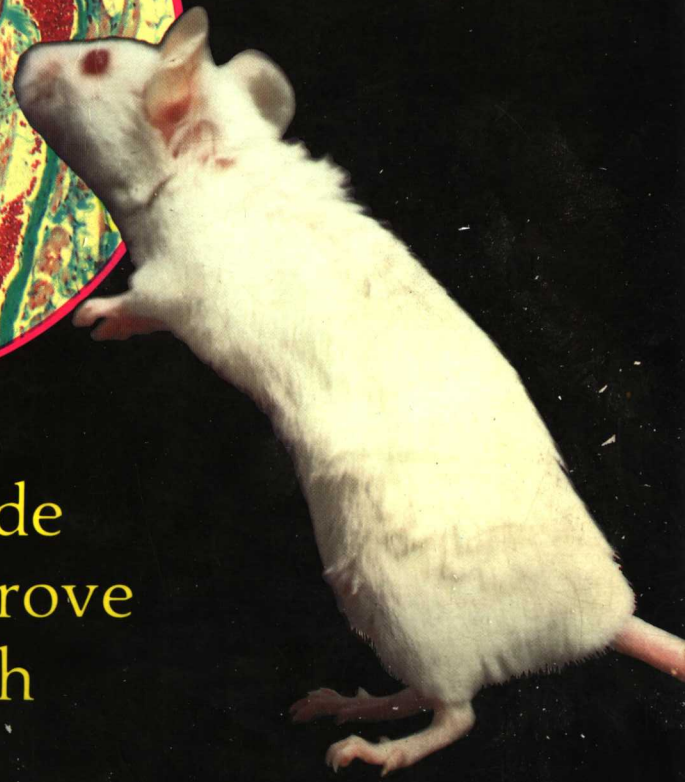
Histological Atlas of the Laboratory Mouse





William D. Gude
Gerald E. Cosgrove
Gerald P. Hirsch

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William D. Gude
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Gerald E. Cosgrove
San Diego Zoological Society
San Diego, California

and

Gerald P. Hirsch
Veterans Administration Wadsworth Hospital Center
Los Angeles, California

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Foreword

The Biology Division of the Oak Ridge National Laboratory was organized in 1946 for the purpose of studying the immediate and long-term implications of man's exposure to ionizing radiation. The program that developed concentrated on the basic mechanism of the effects in biological organisms from the genetic, biochemical, biophysical, and molecular biophysical points of view.

Most of its activities at the beginning concentrated on nonmammalian work (bacteria, fungi, *Drosophila*, plants, etc.) since no facilities to perform mammalian studies were available at that time. It became most obvious that specimens more closely related to mammalian tissue would likely yield more conclusive data to extrapolate these effects upon man.

At Oak Ridge, the first study was the Mammalian Genetics Study under William L. Russell designed to investigate the genetic changes produced by exposure to ionizing radiation and the implication for man. A couple of years later, this was followed by the study of somatic cell effects through the efforts of Jacob Furth, later by Arthur Upton, and finally by John Storer. In all of these assays, we concentrated on work with the mouse since more was known about its genetics than about the genetics of any other mammal. Studies could be conducted with very large numbers of mice, and mice proved to be especially suitable for cancer induction studies.

As this work progressed, we became convinced that a strong histology department was needed to prepare the tissues in a uniform manner and also to examine and interpret them. With the support of Dr. Furth at that time, we secured the services of William D. Gude, who organized this section of the Biology Division and whose dedicated management developed it into a central information source for histology work, not only for our Biology Division but also for this area of Tennessee, thus establishing its excellent reputation.

I am most pleased to see that Mr. Gude has assembled this work into a detailed atlas of the laboratory mouse. As essential as such information is to basic mammalian research, such a volume of complete data and illustrations is not always available in a single source, and I believe this volume will serve as an important handbook for researchers utilizing these species.

Alexander Hollaender
Associated Universities, Inc.
Council for Research Planning in Biological Sciences
1717 Massachusetts Avenue, N.W.
Washington, D.C. 20036

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We owe many thanks to Joyce Feezell and Jimmy Wesley for preparing excellent paraffin sections of the tissues and organs. Jimmy also prepared high-quality thin sections of methacrylate-embedded tissues. The photomicrographs were taken through a Zeiss Ultraphot II microscope using Kodak Professional Ektachrome Film 6118. The color film was processed by Color Technique, Inc., Chicago.

We want to express our gratitude to Mrs. Charlotte Rains for her diligence and patience in preparing the many typewritten copies of all sections of the manuscript.

We are grateful to Katharine Hummel for granting permission to copy descriptive material from the microscopic anatomy sections of Chapter 13, "Anatomy," of *Biology of the Laboratory Mouse*, 2nd ed., Blakiston Division, McGraw-Hill Book Co., New York, 1966.

For assistance with proofreading we are indebted to David A. Hambright and Craig Whitmire of the Technical Publications Department, Information Division, Oak Ridge National Laboratory.

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W. D. G.
G. E. C.
G. P. H.

Contents

Introduction

The purpose of this book is to provide investigators and students with an atlas of tissues of mice, commonly used experimental animals in laboratory studies. Many strains of mice are available for research and clinical laboratory studies, but histological differences between strains are usually minor. We have taken samples from six strains presently used in the Biology Division, Oak Ridge National Laboratory: BALB/c, RF, C3H, C57BL, $BC3F_1$/Cum, and $B6D2F_1$/Jax. Both males and females between the ages of 3 and 10 months were used. They were killed with ether and the dissected tissues were placed in an appropriate fixative immediately upon removal from the mouse. The choice of fixative depended on the stain to follow and included 10% neutral buffered formalin, Bouin's fixative, Zenker-formol (Helly's fluid), formal-mercuric chloride, and Williams's fixative (formulas given in the Fixatives section). Many of the sections pictured in this book were stained with hematoxylin and eosin (H & E). In addition, various special stains were used to illustrate specific structures or features. For example, mucus present in goblet cells of the intestine was stained brilliant red with periodic acid–Schiff reagent (PAS). Neurons and nerve ganglia stained black or dark brown with silver, as in the Bodian stain, are outstanding against a colorless background. Procedures for obtaining the color combinations of many other tissues are given in the Appendix for those who wish to reproduce them for their own investigative research.

With the development of embedding materials other than paraffin (methacrylate and epon) and the availability of microtomes that section tissues 1–2 μm thick, it is now practical to obtain very thin sections of cells in which fine cytoplasmic and nuclear structures can be identified that would be very difficult to see in a 5-μm-thick section.

We believe this atlas may also be used as a supplement to standard histology texts. For more detailed descriptions of tissues and organs characteristic of the mouse see *Biology of the Laboratory Mouse* (see Recommended Reading).

Nervous System
(Figures 1–19)

Irritability and conductivity are two characteristics of the nervous system that require specialized cells and tissues. Anatomically, the nervous system is divided into the central nervous system (CNS), including the brain and spinal cord, and the peripheral nervous system (PNS), which includes all other nerve elements in the organism (peripheral nerves, ganglia, etc.). Specialized cells that do not conduct nerve impulses but contribute supporting tissue to the nervous system comprise the neuroglia. Nervous tissue also may be divided into gray and white matter. Nerve cell bodies, dendrites, and unmyelinated portions of axis cylinders comprise gray matter. White matter consists primarily of myelinated nerve fibers, which give it a glistening white appearance. Neuroglial cells are present in both gray and white matter.

The functional, impulse-conducting unit of nervous tissue is the neuron. Its basic structure consists of a large globular nucleus within which are a few chromatin granules and usually one large nucleolus. The cytoplasm or perikaryon surrounding the nucleus contains abundant, irregular masses of basophilic material rich in ribonucleic acid also known as Nissl or tigroid substance. Following silver staining numerous fine threads of neurofibrils may be identified in the cytoplasm.

All neurons have one process extending from the cell body at the axon hillock, the axon or axis cylinder. Impulses travel away from the cell body down the axons, which decrease in diameter as the distance from the cell body increases. Collateral branches may be given off at intervals. Other processes, dendrites, are direct extensions of the cell body and they connect with axons of functionally related neurons at synapses or junctions. Impulses travel along dendrites toward the cell body from the synapsis.

The axis cylinder in peripheral nerves is covered by a neurolemma or Schwann's sheath. In nerves having a myelin sheath covering the axon the neurolemma forms the outermost covering. Nuclei of Schwann cells may be identified alongside the axis cylinder. A node of Ranvier occurs at the junction of two merging segments of myelin sheath.

Neurons may be further categorized by the number of processes extending from the cell body. Those having only one axon are unipolar neurons. Bipolar neurons have an axon and one dendrite extending from the cell body at opposite poles; multipolar neurons are determined by the number and arrangement of dendrites. Ganglia are the chief paths of communication between the CNS and outer ganglionic plexuses. Their nerve cell bodies are usually large and spherical with a predominant nucleolus and abundant chromatin in the cytoplasm.

Neuroglia or specialized interstitial tissue of the nervous system includes ependyma, a type of cuboidal or low columnar epithelium that lines the ventricles of the brain and spinal canal, and satellite cells of peripheral ganglia. Three types of neuroglia are identified: astrocytes (fibrous and protoplasmic), oliogodendroglia, and microglia. Protoplasmic astrocytes, which are found chiefly in gray matter, exhibit processes, many of which are attached to blood vessel walls and to the pia mater. Fibrous astrocytes, more commonly found in white matter of the brain between nerve fibers, have a larger elongated nucleus than the protoplasmic type, but they also send out processes that attach to blood vessel walls.

Oliogodendroglia resemble astrocytes but are much smaller cells and have only a few processes with few branches. They are associated with supporting nerve fibers rather than blood vessels, and are called "satellite" cells when found adjacent to nerve cell bodies. Microglia have a small nucleus and a small amount of cytoplasm. They are scattered throughout the brain and spinal cord.

The meninges, connective tissue coverings of the brain and spinal cord, include (1) the outermost dura mater, a dense and tough material next to the bone, (2) the arachnoid, a thin network of loose connective tissue devoid of blood vessels and closely adherent to (3) the pia mater, which is the innermost thin membrane that contains blood vessels. The dura mater, arachnoid, and pia mater together form the leptomeninges.

The choroid plexuses found in the roof of the third and fourth ventricles and in part of the walls of the lateral ventricles are lined by specialized epithelial cells that differ from ependymal cells. Cuboidal cells of the choroid plexus are arranged in a single layer and many blood vessels occupy its folds. The choroid plexus is the source of cerebrospinal fluid.

Special Sense Organs
(Figures 20–31)

EAR

The ear may be divided into three parts: external ear, middle ear, and inner ear. The pinna and external auditory meatus comprise the external ear. The middle ear consists of the tympanic membrane, bony ossicles, and the auditory or eustachian tube, connecting the ear with the oropharynx. Included in the inner ear are the bony labyrinth and spiral cochlea, which contains the organ of Corti.

The outer third of the external ear is composed of elastic cartilage covered by hairy skin in which sebaceous and ceruminous glands are distributed.

Simple squamous epithelium lines the tympanic cavity of the middle ear, which contains the three bony ossicles: stapes, malleus, and incus. The tympanic membrane is an oval, semitransparent membrane that is composed of two layers of collagenous fibers and fibroblasts similar to a tendon plus epithelium on the inner and outer surfaces. It divides the middle ear from the inner ear.

Both elastic and hyaline cartilage are found in the auditory tube from its beginning to its terminus at the oropharynx. It is lined by mucous membrane with many folds. At the bony tympanic portion the epithelium is low, ciliated columnar but as the tube approaches the cartilaginous oropharynx the epithelium becomes pseudostratified with tall columnar cells, many bearing cilia.

The inner ear is divided into a bony labyrinth containing a clear fluid, perilymph, lined by endothelium, and a membranous labyrinth that contains endolymph. Within the bony portion are the vestibule, three semicircular canals filled with perilymph, and the cochlea. Enclosed in the membranous labyrinth are the utricle, utriculosaccular duct, the saccule, endolymphatic sac and duct, three membranous semicircular canals containing endolymph, and the cochlear duct (scala media). In each semicircular canal are cristae—ridges lined by an epithelium of tall columnar cells of two types, hair cells and sustentacular cells. In each utricle and saccule are maculae similar to the cristae with both hair and supporting cell types. In each macula are tiny crystals of a mixture of calcium carbonate and protein that are called otoliths. The cristae and maculae are predominantly involved with maintaining equilibrium.

ORGAN OF CORTI

On the floor of the cochlear duct is the organ of Corti, resting on a basement membrane of low cuboidal cells. This

structure extends from the bony spiral lamina to the spiral ligament, and consists primarily of hair cells and supporting cells. One particular structure is the tectorial membrane, a thin gelatinous membrane in intimate contact with the cilia of hair cells. Sound waves transmitted to the organ of Corti by the endolymph of the cochlear duct from the perilymph of the scala vestibuli activate the hair cells. Nerve fibers around the bases of the hair cells receive the stimulus and transfer it to nerves in the spinal ganglion and finally, by way of bipolar cells, to the cochlear division of the acoustic nerve.

Many strains of mice are affected by mutants involving the ear structures. Malformed cristae and absence of otoliths produce conditions resulting in imbalance. Other strains are born with defects that eventually produce deafness owing to degenerating hair cells and a malformed organ of Corti. Waltzing mice, twirlers, and shaker mice fit into this category.

EYE

The principal structures of the eye consist of three layers constituting the wall of the eye: the supporting layer, middle layer, and retinal layer. The sclera or "white" of the eye is the primary supporting structure of dense connective tissue. The cornea, forming the bulging anterior portion of the eye, functions primarily as a transparent tissue transmitting light rays through the lens to the retina. However, in the rodent it also provides support, covering about half of the surface of the eyeball, and is considered a part of this layer.

The vascular middle layer or uvea provides nourishment to ocular structures and contains smooth muscle. It includes the pigmented iris and choroid, the ciliary process and ciliary body, and the lens, which is characteristically spherical in the rodent, filling almost all of the eyeball.

The retinal layer is divided into two layers; the outermost is pigmented and the inner or nervous portion contains photoreceptors, cones, and, in the mouse, a few rods.

The jellylike vitreous humor fills the interior of the eye between the lens and retina. The anterior and posterior chambers are filled with aqueous humor, secreted by the ciliary processes.

CORNEA

The cornea is a transparent avascular structure consisting of four identifiable strata. A stratified squamous epithelium covers the cornea, resting upon a basement membrane. Below is a substantia propria of specialized, dense connective tissue containing a few flattened fibroblasts dispersed among collagen fibers bound together by an amorphous cement substance. Behind this layer is Descemet's membrane, a band of refractile, homogeneous, elastic substance adjacent to a very thin squamous epithelium.

The choroid lying internal to the sclera is heavily pigmented and contains elastic fibers in addition to a single layer of capillaries.

The nervous portion of the retina consists of nine layers made up of cell bodies of many neurons and ganglia in addition to the rod and cone special photoreceptors.

The spherical lens lies just behind the pupil between the anterior and posterior chambers of the eye. This transparent structure contains a highly refractive capsule that coats the outer surface of the lens epithelium of flat cuboidal cells. Lens

fibers constitute the substance of the lens, forming elongated prisms. There is no epithelium in the capsule covering the posterior portion.

Mutations resulting in a wide variety of eye defects—such as microophthalmia, cataracts, and abnormal lens formation and development—are common findings in several strains of mice. Incomplete retinal development has resulted in either abnormally short rods or absence of rods.

HARDERIAN GLAND

Partially surrounding the eyeball posteriorly is this tubuloalveolar gland. It is covered by a thin capsule whose strands divide the gland into lobules. The pyramidal epithelial cells contain a nucleus with two or more nucleoli lying along the base and lipid-containing cytoplasm, which, in prepared sections, appears vacuolated. The lamina propria of fibrous connective tissue contains melanocytes filled with pigment. A single excretory duct is present. The gland produces an oily secretion for lubricating the surface of the eye.

LACRIMAL GLAND

Paired tubuloalveolar lacrimal glands lie in two areas, extraorbital and intraorbital. The extraorbital gland is below and in front of the ear. The intraorbital gland is located where the excretory duct opens into the conjunctival sac. The pyramidal secretory cells have spherical nuclei lying near the base and intensely basophilic cytoplasm. Myoepithelial cells are present between the epithelial cells and the basement membrane. The serous secretion moistens and lubricates the surface of the eyeball and the eyelids.

Endocrine System
(Figures 32–43)

The endocrine glands include the pituitary, thyroid, parathyroid, adrenal, pineal, ovary, placenta, testis, and islets of Langerhans in the pancreas. Endocrine features of the ovary and testis will be discussed under the reproductive systems of the male and female.

PITUITARY GLAND

The pituitary gland (hypophysis) lies within the bony sella turcica of the floor of the skull, the dorsal basisphenoid bone. It consists of a pars distalis or anterior lobe, pars intermedia or intermediate lobe, and pars nervosa (neurohypophysis) or posterior lobe.

The anterior lobe is very vascular with many capillaries. Histologically, three types of cells may be identified: (1) Acidophils contain small, round nuclei and eosinophilic cytoplasm and are characterized by elaborate growth and the production of lactogenic hormones. (2) Agranular chromophobes whose cytoplasm does not stain may be stem cells. (3) Basophils may be divided into two classes: beta basophils, whose granules are aldehyde-fuchsin-positive and secrete thyrotrophic hormone, and delta basophils, whose granules are aldehyde-fuchsin-negative but stain positive with periodic acid-Schiff (red) and produce gonadotrophins, follicle-stimulating hormone (FSH), and luteinizing hormone (LH).

The intermediate lobe is separated from the anterior lobe by a cleft lined by cuboidal epithelium. Cells in this lobe have intensely staining oval nuclei and light basophilic cytoplasm. The hormone elaborated by this lobe is melanin-stimulating hormone (MSH).

The posterior lobe is filled with nonmyelinated nerve fibers, neuroglial cells (pituicytes), and connective tissue supporting capillaries. Two hormones stored and released from the pars nervosa are oxytocin and vasopressin.

THYROID GLAND

The characteristic arrangement of various-sized spherical follicles containing eosinophilic colloid identifies the thyroid gland. The follicles are lined by simple cuboidal epithelium. Increases in the height of these cells as well as changes in the staining quality and amount of colloid materials indicate levels of secretory activity. Such changes occur as an aging process in some strains of mice in which tall columnar follicle cells decrease in cell size in young mice, and increase in follicle size in mature mice. Senile changes include increased

fibrous connective tissue between follicles and merging of follicles to form very large structures with flattened epithelium. The follicular cells produce the colloid substance, which contains thyroxin and triiodothyronine.

"Ultimobranchial bodies," "parafollicular cells," and C cells are synonymous terms for lightly staining cells distributed throughout the thyroid connective tissue adjacent to but outside the follicular cells. These cells are producers of thyrocalcitonin, a hormone that acts in collaboration with the parathyroid gland in maintaining calcium homeostasis in body tissues and blood. Parafollicular cells have been described in most mammals, including the mouse.

PARATHYROID GLANDS

The parathyroid gland, consisting of two or more lobes, is embedded within the thyroid gland but separated from it by a capsule of connective tissue. The cells form masses of densely packed groups interspersed with capillaries or sinusoids. At least two cell types can be identified. These chief cells consist of the dark, actively secreting cells and the light, inactive cells. A third type (similar to the oxyphil cell) has been described by some investigators.

PINEAL GLAND

The pineal gland lies on the dorsal surface of the brain at the junction of the cerebral cortex and cerebellum. A very thin capsule surrounds the gland and merges with the choroid plexus. Two types of cells can be identified: cells that originate from neuroectodermal cells and form parenchymal cells of the body of the gland, and neuroglial cells. Parenchymal cells are spherical with slightly basophilic cytoplasm. Neuroglial cells support the parenchymal cells. Mesenchymal cells in the pia mater covering give rise to connective tissue of the capsule, which provides incomplete partitions that divide the body into lobules.

ADRENAL GLAND

The adrenal glands are paired, each gland located adjacent to the anterior pole of the kidney. The adrenals are larger in the female and occasionally show strain differences. A fibrous connective tissue capsule surrounds the gland, which is divided into a cortex and medulla. In most mammals the cortical division can be subdivided into three zones of epithelial cells, but in mice only two zones are clearly seen. The subcapsular zona glomerulosa consists of small cells with large nuclei arranged in the form of arches, with capillaries between the cells. Beneath the glomerulosa is the wide zona fasciculata, which consists of columns of cells separated by connective tissue. These cells are large with vesicular nuclei and contain lipid material, appearing foamy in sections when lipid is removed in processing. The zona reticularis identified in most mammals is rarely seen in the mouse. However, a zone between the cortex and medulla is identified as the X zone in males and females before sexual maturity. The zone disappears in sexually mature males and with the first pregnancy in the female. It has been observed to persist in virgin females for varying lengths of time and in castrate males. In aging mice of certain strains (RFM, BALB/c, etc.) large foamy

cells with brown pigment form in the cortical zone adjacent to the medulla. The pigment is similar to ceroid in the ovary and is referred to as "brown degeneration." In some strains of mice the change is more pronounced.

Clusters of polyhedral cells that produce adrenaline and noradrenaline form the medulla. Two cell types, the sites of origin of these hormones, have an affinity for potassium bichromate, and brown cytoplasmic granules can be seen following treatment with this chemical—the result of the chromaffin reaction.