Cell Separation

METHODS AND SELECTED APPLICATIONS

EDITED BY

THOMAS G. PRETLOW II AND THERESA P. PRETLOW

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Institute of Pathology
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Contributors

Numbers in parentheses indicate the pages on which the authors' contributions begin.

STEVEN J. ACKERMAN (15), *Departments of Immunology and Medicine, the Allergic Diseases Research Laboratory, Mayo Medical School, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905*

PAULETTE BERND[1] (1), *Department of Anatomy and Cell Biology, and Department of Psychiatry, Columbia University, New York, New York 10032*

MICHAEL G. BRATTAIN (235), *Bristol Laboratories, Syracuse, New York, and Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030*

EVERLY CONWAY DE MACARIO (273), *Laboratory Medicine Institute, Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201*

CHARLES B. DUGAN (273), *Laboratory Medicine Institute, Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201*

E. J. FIELD[2] (251), *Crossley House, Neurological Research Centre, 17 Brighton Grove, Newcastle upon Tyne NE4 5NS, England*

SHERMAN FONG (203), *Department of Basic and Clinical Research, Scripps Clinic and Research Foundation, La Jolla, California 92037*

MICHAEL D. GERSHON (1), *Department of Anatomy and Cell Biology, Columbia University College of Physicians and Surgeons, New York, New York 10032*

GERALD J. GLEICH (15), *Departments of Immunology and Medicine, the Allergic Diseases Research Laboratory, Mayo Medical School, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905*

[1]Present address: Department of Anatomy, Mount Sinai School of Medicine, New York, New York 10029.

[2]Present address: Department of Experimental Neuropathology, University of Newcastle, Newcastle, England.

ADELINE J. HACKETT (183), *Peralta Cancer Research Institute, Oakland, California 94609*

MIRIAM C. HANCOCK (183), *Peralta Cancer Research Institute, Oakland, California 94609, and Genetics Department, University of California, Berkeley, California 94720*

JAMES E. KLAUNIG (81), *Department of Pathology, Medical College of Ohio, Toledo, Ohio 43699*

Å LERNMARK (99), *Hagedorn Research Laboratory, DK-2820 Gentofte, Denmark*

DAVID A. LOEGERING (15), *Departments of Immunology and Medicine, the Allergic Diseases Research Laboratory, Mayo Medical School, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905*

ALBERTO J. L. MACARIO (273), *Laboratory Medicine Institute, Center for Laboratories and Research, New York State Department of Health, Albany, New York 12201*

MARVIN L. MEISTRICH (33), *Department of Experimental Radiotherapy, The University of Texas, M. D. Anderson Hospital and Tumor Institute at Houston, Houston, Texas 77030*

J. HØIRIIS NIELSEN (99), *Hagedorn Research Laboratory, DK-2820 Gentofte, Denmark*

ELADIO A. NUNEZ (1), *Department of Anatomy and Cell Biology, and Department of Psychiatry, Columbia University, New York, New York 10032*

CHARLES S. OWEN (127), *Department of Biochemistry, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107*

CHRIS D. PLATSOUCAS (145), *Memorial Sloan-Kettering Cancer Center, New York, New York 10021*

THERESA P. PRETLOW (63, 221), *Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106*

THOMAS G. PRETLOW II (63, 221), *Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106*

HELENE S. SMITH (183), *Peralta Cancer Research Institute, Oakland, California 94609*

MARTHA R. STAMPFER (183), *Donner Laboratory, Lawrence Berkeley Laboratory, Berkeley, California 94720*

GARY D. STONER (81), *Department of Pathology, Medical College of Ohio, Toledo, Ohio 43699*

HADASSAH TAMIR (1), *Division of Neuroscience, New York State Psychiatric Institute, New York, New York 10032*

TUOMO TIMONEN (93), *Department of Pathology, University of Helsinki, SF-00290 Helsinki 29, Finland*

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Preface

In 1975, we published a general review of methods of cell separation. Because of the interest in this review, we planned a sabbatical year to write a book with the same scope. Between the writing of the first review (1973–1974) and the attempt to write a book (1977–1978), the references to be cited increased from somewhat more than five hundred to somewhat more than seven thousand. Our bibliography pertinent to this methodology was expanding at a rate of two to four dozen articles weekly, and we were compelled to face the fact that it was no longer feasible for one or two authors to address this area adequately. The rapid growth in this area led us to plan this multivolume, multiauthored treatise.

In approaching this work, it was our goal to select critical authors with considerable personal familiarity with the design and/or application of methods for the separation of cells. Rather than attempt comprehensive reviews, they were asked to address relatively finite subjects and to include sufficient references to direct those readers who want more information to the appropriate sources. We have attempted to address this work to a heterogeneous audience of experimental oncologists, hematologists, immunologists, cell biologists, endocrinologists, and others who are not already expert in the use of methods for cell separation. We are grateful that most of those invited to contribute to this work found the time to do so, and we hope that their critical, quantitative approaches to problems in cell separation will stimulate new investigators to examine critically many of the "accepted" methods for cell separation.

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Chapter 1

Separation of Parafollicular Cells from Thyroid Follicular Cells by Affinity Chromatography Using Thyroglobulin-Coupled Sepharose

PAULETTE BERND,[1] MICHAEL D. GERSHON, ELADIO A. NUNEZ, AND HADASSAH TAMIR

Department of Anatomy and Cell Biology and Department of Psychiatry, Columbia University, New York, New York

I. Thyroid Structure and Function

The thyroid parenchyma is comprised of two cell types, follicular and parafollicular cells (Fig. 1). These cells are enmeshed in a highly vascularized connective tissue stroma that contains, in addition to the usual cellular and fibrous components, a large number of mast cells (Melander *et al.*, 1971; Nunez and Gershon, 1973). Follicular cells are, in most species of mammal, the dominant parenchymal cell type and outnumber parafollicular cells by 9 to 1 in sheep (Bernd *et al.*, 1979a) and even more in humans (Tashjian *et al.*, 1974) and rats (Rohr and Hasler, 1968). Follicular cells aggregate to form hollow spheres or follicles, the lumen of which contains thyroglobulin (Whur *et al.*, 1969). Follicular cells present one face to the follicular lumen and one face to the basal lamina that surrounds each follicle. Parafollicular cells tend to be included within the basal laminae of follicles but they never reach the lumen (Lietz, 1971). Follicular and parafollicular cells thus are in extensive apposition to one another. Tight junctions between adjacent follicular cells prevent egress of the lumenal colloid to the intercellular space, and thus thyroglobulin rarely escapes from follicles or comes into contact with parafollicular cells.

[1]Present address: Department of Anatomy, Mount Sinai School of Medicine, New York, New York 10029.

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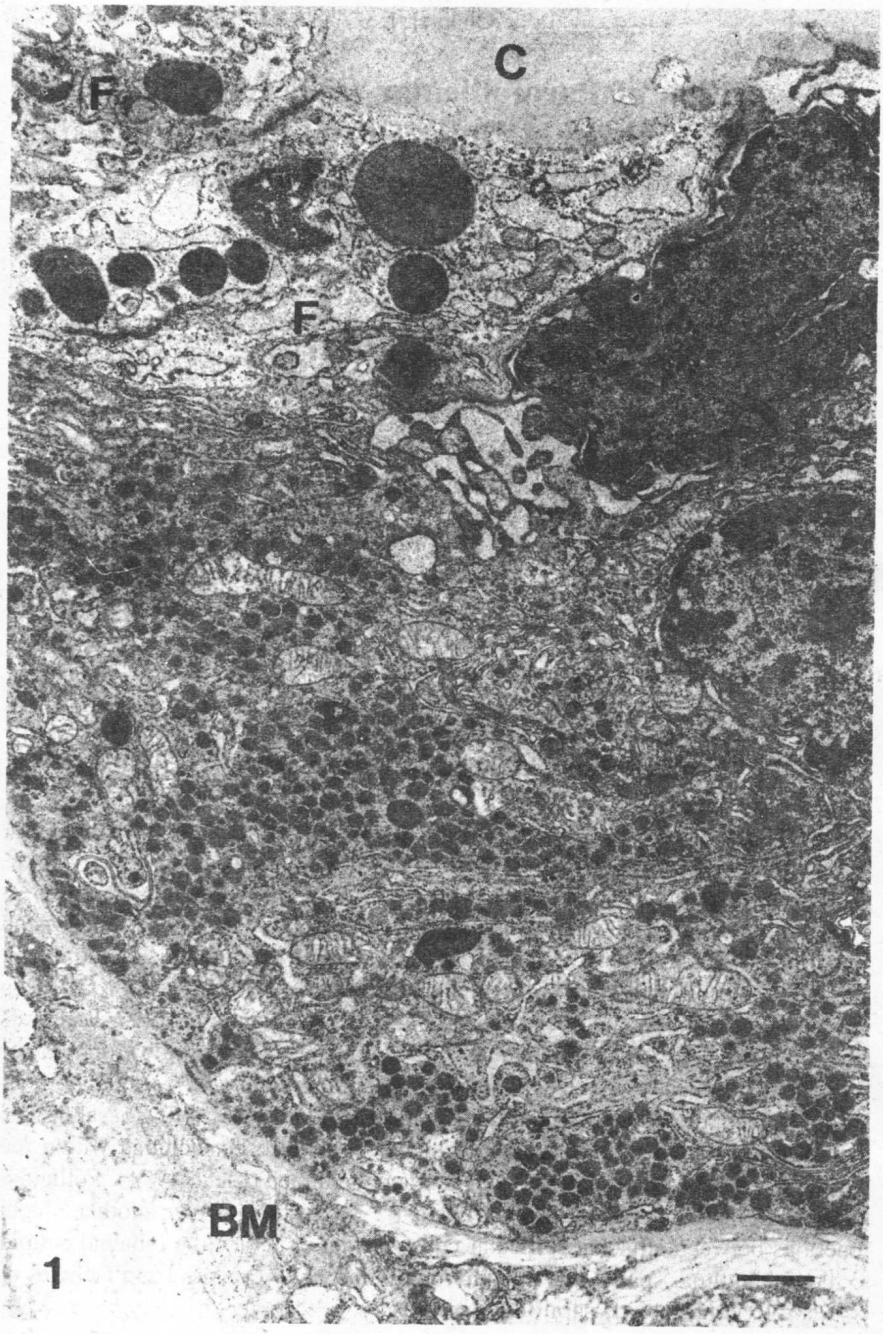

FIG. 1. Electron micrograph of the thyroid gland of the sheep. Note follicular cells (F) that border on the thyroglobulin-containing colloid (C). Parafollicular cells (P) lie next to follicular cells, within the basement membrane (BM) of the follicle. ×9580. Bar = 1 μm.

Thyroid stimulating hormone (TSH) exerts both acute and chronic effects on follicular cells. Sustained exposure to TSH causes follicular cell hypertrophy; however, within minutes of exposure to TSH follicular cells are stimulated to extend pseudopods into the colloid from their lumenal surface and to become intensely phagocytic (Wollman *et al.*, 1964; Wetzel *et al.*, 1965). Endocytosis of thyroglobulin is followed by lysosomal digestion of the protein that, in turn, releases thyroxine (T_4) and triiodothyroxine (T_3). These thyroid hormones can thus diffuse out of follicular cells to reach the circulation. In contrast to follicular cells, parafollicular cells are not induced to become phagocytic by TSH (Isler *et al.*, 1960).

II. Parafollicular Cells and Serotonin

Parafollicular cells produce, store, and secrete calcitonin (Lietz, 1971). In several mammalian species, they also co-store the biogenic amine, serotonin (5-hydroxytryptamine; 5-HT; Paasonen, 1958). Not every mammal has 5-HT-storing parafollicular cells; those that do include sheep, horse, goat, bat, and callithricid primate (Atack *et al.*, 1972; Falck and Owman, 1968: Machado, 1976; Nunez and Gershon, 1980; Paasonen, 1958; Solcia and Sampietro, 1968). Other mammals, that do not as adults have parafollicular cells that contain high levels of 5-HT, have 5-HT-rich cells during embryonic life and their adult parafollicular cells retain the ability to synthesize 5-HT from 5-hydroxytryptophan (Pearse, 1966a,b; Gershon and Ross, 1966a,b; Gershon and Nunez, 1970; Gershon *et al.*, 1971). The serotonergic properties of parafollicular cells are of interest because, in addition to whatever role they play in the physiology of the thyroid, they are shared with neurons. Like neurons as well, parafollicular cells are of neurectodermal origin (LeDouarin *et al.*, 1974). Parafollicular cells originate from the neural crest and, in a manner that is reminiscent of enteric neurons, associate with endodermally derived parenchymal cells (follicular cells; Fisher and Dussault, 1974) and a mesenchymal stroma. The enteric nervous system is also a site where 5-HT-storing cells, enteric serotonergic neurons, develop (Gershon, 1977). The neuron-like properties of endocrine parafollicular cells have prompted Fujita to classify them as paraneurons (1977). The neuron-like properties of parafollicular cells further suggest that, especially in species that have a 5-HT-rich parafollicular cell population, this cell type may be valuable as a model system for the examination of neuronally relevant serotonergic mechanisms. Serotonergic neurons themselves are not easy to study. In the brain they are inaccessible and mixed with many other neurons of different types (Gershon, 1977). In the gut serotonergic neurons are more accessible but they are not very numerous (Gershon, 1977). It follows, therefore, that parafollicular cells might be exploited to learn more about a neuronally relevant serotonergic cell system

than can be learned from directly studying either central or peripheral serotonergic neurons. In an analogous manner, the neurectodermally derived endocrine cells (or paraneurons) of the adrenal medulla have been extremely useful in providing insights into catecholaminergic mechanisms (Douglas, 1968).

III. Obtaining Parafollicular Cells for Study

In contrast to the adrenal medulla, parafollicular cells cannot be separated from nonneurectodermally derived parts of the gland by simple dissection. In order to accomplish this separation the intrinsic architecture of thyroid follicles has to be disrupted. This can be accomplished by dissociating the thyroid gland into its component cell types. Once this dissociation has been done, the resulting mixture of cells must be separated according to a scheme that will provide a cell population that has been substantially enriched in parafollicular cells. The challenge in this case is to obtain a cell that is a relatively small minority of the total cell population of the gland.

The task of obtaining an enriched population of parafollicular cells is made easier by an appropriate choice of animal. Parafollicular cells constitute a 10-fold higher proportion of the parenchymal cell population in sheep than they do in rat. Sheep parafollicular cells, moreover, are rich in 5-HT and the sheep thyroid gland is a large one, yielding substantial amounts of tissue for experimentation. It is also economical, as it is provided with minimal charge by local slaughterhouses.

IV. Serotonin Binding Protein

Serotonergic neurons are known to contain a specific serotonin binding protein (SBP; Tamir and Huang, 1974; Tamir *et al.*, 1976; Jonakait *et al.*, 1977). This protein binds 5-HT with high affinity ($K_{D1} \cong 10^{-10}$ *M*; $K_{D2} \cong 10^{-8}$ *M*) and appears to be contained within synaptic vesicles (Tamir and Gershon, 1979). It has been postulated that SBP is a storage protein for 5-HT (Bernd *et al.*, 1979b; Tamir and Rapport, 1978), serving to decrease intravesicular osmotic pressure by forming macromolecular aggregates with 5-HT. Such a role could be instrumental in preventing osmotic swelling of vesicles in response to the very high concentration of 5-HT inside of synaptic vesicles. In order to test the hypothesis that parafollicular cells are good models for the study of the cellular biology of a neurally relevant serotonergic cell, we wished to determine whether SBP is present in sheep parafollicular cells.