Advances in Clinical Cytology

Leopold G. Koss

Dulcie V. Coleman

Butterworths

Advances in Clinical Cytology

Leopold G. Koss, MD

Professor and Chairman, Department of Pathology, Albert Einstein College of Medicine, Montefiore Hospital and Medical Center, Bronx, New York

Dulcie V. Coleman, MD

Senior Lecturer and Consultant in Clinical Cytology, Department of Experimental Pathology, St Mary's Hospital Medical School, London

Butterworths
London Boston Sydney Durban Wellington Toronto

First published 1981

ISBN 0 407 00174 3

British Library Cataloguing in Publication Data

Koss, Leopold George
Advances in clinical cytology.
1. Diagnosis, Cytologic
I. Title II. *Coleman,* Dulcie V
616.07'582 RB43 80-49874

ISBN 0-407-00174-3

Typeset and printed in England by Page Bros (Norwich) Ltd
Bound by Mansell (Bookbinders) Ltd., Witham

Advances in Clinical Cytology

Contributors

Robin O. Barnard, MD

Neuropathologist, The National Hospitals for Nervous Diseases, Maida Vale, London; Honorary Senior Lecturer (Neuropathology), St Thomas's Hospital Medical School, London

P. H. Bartels, PhD

Professor of Microbiology and Professor of Optical Sciences, University of Arizona, Tucson, Arizona

Dulcie V. Coleman, MD

Senior Lecturer and Consultant in Clinical Cytology, Department of Experimental Pathology, St Mary's Hospital Medical School, London

Wenancjusz Domagala, MD

Docent, Department of Electron Microscopy, Medical Academy, Szczecin; Visiting Scientist, Department of Pathology, Albert Einstein College of Medicine, Montefiore Hospital and Medical Center, Bronx, New York

Alex Ferenczy, MD

Associate Professor of Pathology and of Obstetrics and Gynecology, McGill University and the Jewish General Hospital, Montreal, Quebec

Leopold G. Koss, MD

Professor and Chairman, Department of Pathology, Albert Einstein College of Medicine, Montefiore Hospital and Medical Center, Bronx, New York

Torsten Löwhagen, MD

Associate Head, Department of Clinical Cytology, Institute of Tumour Pathology, Karolinska Hospital, Stockholm

A. B. Miller, MB, MRCP(Lond), FRCP(C), FFCM(UK)

Director, NCIC-Epidemiology Unit, Faculty of Medicine, University of Toronto, Ontario

Anne R. Morse, CMIAC

Chief Medical Laboratory Scientist in Cytology, Department of Experimental Pathology, St Mary's Hospital Medical School, London

Zuher M. Naib, MD

Professor of Pathology, Department of Pathology, Cytology Division, Emory University School of Medicine at Grady Memorial Hospital, Atlanta, Georgia

Bendicht U. Pauli, DVM

Associate Professor of Pathology, Department of Pathology, Rush Medical College and Rush-Presbyterian-St Luke's Medical Center, Chicago, Illinois

Ronald S. Weinstein, MD

Professor and Chairman, Department of Pathology, Rush Medical College and Rush-Presbyterian-St Luke's Medical Center, Chicago, Illinois

G. L. Wied, MD

Head, Section of Cytology, the Blum-Riese Professor of Obstetrics and Gynecology, and Professor of Pathology, University of Chicago, Chicago, Illinois

Jan-Silvester Willems, MD

Clinical Pathologist, Department of Clinical Cytology, Institute of Tumour Pathology, Karolinska Hospital, Stockholm

Lewis B. Woolner, MD

Professor of Pathology, Mayo Medical School; Consultant in Surgical Pathology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota

Preface

During the last 25 years an unprecedented explosion of research pertaining to cells of human and experimental origin has taken place. It has become apparent that the secrets of life, whether in health or in disease, are vested in mechanisms governing the function, replication, mutual relationships and death of cells. The tasks of unlocking the secrets of cell biology, once thought to be relatively simple, have been shown to be extremely complex and challenging even in this era of major technological progress. It proved much simpler to put a man on the moon than to understand a phenomenon as common as mitotic division.

At the other end of this spectrum, within the same time period, considerable progress has been made in the assessment of cell morphology for the diagnosis of human diseases such as cancer and precancerous states of various organs, viral infections and other abnormalities too numerous to name.

The communications between the complex world of cell biology and the world of clinical cytology are tenuous at best. Few cell biologists understand or care about the messages that are inscribed in the morphology of cells and few clinical cytologists (or cytopathologists as they are called today) know much about the progress made in the laboratories of cell biology. Yet improved communications may prove beneficial to both groups. The basic research of today may have major diagnostic or prognostic implications for human disease tomorrow. The understanding of some of the problems of cell morphology may assist in targeting future research efforts. Opening extensive channels of communication between the two groups may require much time, understanding and mutual tolerance.

This volume represents a modest attempt to bring together selected chapters of applied cell research and selected chapters pertaining to progress in clinical cytology. We were fortunate indeed in having been able to secure a number of distinguished contributors who share our faith in this enterprise and its goals. Should the response of the readers be favorable, it is our plan to continue presenting, from time to time, additional volumes of *Advances in Clinical Cytology* and thus trace a modest pathway of understanding between the cell biologists and the cytopathologists.

Leopold G. Koss
Dulcie V. Coleman

Contents

1
The Ultrastructural Dynamics of Endometrial Hyperplasia and Neoplasia

Alex Ferenczy

Introduction

The relationship of endometrial hyperplasia to the development of endometrial carcinoma represents one of the major problems in the pathogenesis of endometrial neoplasia. Many retro– and prospective clinical–pathological studies[1–9] have appeared in the past three decades to determine the natural history of lesions which may precede endometrial carcinoma. Unfortunately, all of them suffer from major pitfalls in their design and statistical analysis, and consequently our knowledge and understanding of these presumed precursors are fragmentary. Also, from a morphological point of view, the distinction between hyperplasia and early, well differentiated carcinoma remains one of the most difficult diagnostic dilemmas confronting the pathologist. This difficulty stems partly from the inconspicuous cytological changes which frequently accompany malignant transformation in the endometrium and partly from the complex histological appearance of the endometrial mucosa. As a result, the commonly used criteria to define preinvasive endometrial neoplasms appear to be too subjective and indecision has resulted in a variety of terms, including 'adenomatous hyperplasia', 'atypical adenomatous hyperplasia', 'dysplasia', 'anaplasia' and 'carcinoma in situ', being used to describe these changes. To compound the problem, the clinical implications of these terms are different to different investigators. Since the biological significance of presumed precursors of endometrial carcinoma remains highly controversial, more objective morphological criteria are needed to gain insight into their natural history. Studies of the endometrial morphologic altera-

tions mediated by sex-steroid hormones may provide insight into our basic understanding of the relationship between endometrial hyperplasia–neoplasia and abnormal hormonal environment. In a similar vein, ultrastructure may be helpful in the investigation of the effects of exogenous progestogens used for the treatment of endometrial hyperplasia and carcinoma.

A series of comparative scanning and transmission electron microscope studies[10–15] were carried out to test these assumptions in normal, hyperplastic and neoplastic endometrium. The specific aim was to correlate ultrastructure, Feulgen–DNA microspectrocytophotometry[16–17] and short-term *in vitro* historadioautography using tritiated thymidine[18–19] with histology to define objective light microscopic criteria for distinguishing early, well differentiated endometrial carcinoma from hyperplasia. In addition, the dynamics of cellular activity as determined by ultrastructure[10–15] and histochemistry [20–22] as well as hormone–receptor binding measurements[23–27] were correlated with hormonal stimuli[28]. Finally, the regressive structural changes produced by pharmacological doses of progestogens were studied in relation to the possible mechanisms of action of these substances on hyperplastic and neoplastic endometrial tissues. The following discussion is a review of our knowledge of the pathology of endometrial hyperplasia and neoplasia, and is based on study of endometria of over 300 women.

Endometrial sampling was performed either by biopsy or immediately after transabdominal hysterectomy. Endometrial tissues were examined with the electron microscope with the following histologic diagnoses: cyclic, normal proliferative endometrium (71 cases: in all these cases previous and/or subsequent ovulations have been documented); secretory endometrium (43 cases); anovulatory, persistent proliferative endometrium (15 cases); cystic glandular hyperplasia (38 cases); adenomatous hyperplasia without cytologic atypia (40 cases); adenomatous hyperplasia with cytologic atypia (atypical or severe adenomatous hyperplasia; 25 cases); carcinoma in situ (6 cases); and invasive adenocarcinoma: well differentiated (21 cases); moderately differentiated (24 cases); and poorly differentiated (13 cases). Seven patients with adenomatous hyperplasia with atypia and 7 patients with well differentiated to moderately differentiated invasive adenocarcinoma were given weekly intramuscular injections of 1 g of medroxyprogesterone acetate. Endometrial sampling in these patients was performed by endometrial biopsy at 4, 8, 32 and 90 weeks after starting therapy.

Endometrial tissue blocks were divided into three portions: one for

light microscopy, fixed in Bouin's solution; one for transmission electron microscopy, fixed in 3% glutaraldehyde in 0.1 M cacodylate buffered at pH 7.2; and one for scanning electron microscopy, fixed in 3% glutaraldehyde in Sorensen's phosphate buffer at a pH of 7.3. Speciments for transmission electron microscopy were postfixed in 1% osmium tetroxide and embedded in Epon 812. Thin sections were stained with 2% uranyl acetate and Reynold's lead citrate, and examined in a Hitachi H.S. 8.2 electron microscope at 50 kV. Samples for scanning electron microscopy were dehydrated in ethyl alcohol and amyl acetate of ascending concentrations from 50 to 100%, critical point dried, coated with a 200 Å thick film of gold–palladium and examined with a JSM-U-3 scanning electron microscope at an accelerating voltage of 25 kV using a constant tilt angle of 45°.

Normal cyclic endometrium

Since disease represents exaggeration or changes of normal biological processes, a thorough knowledge and understanding of normal endometrial events is essential for the comparative study of endometrium in pathologic states.

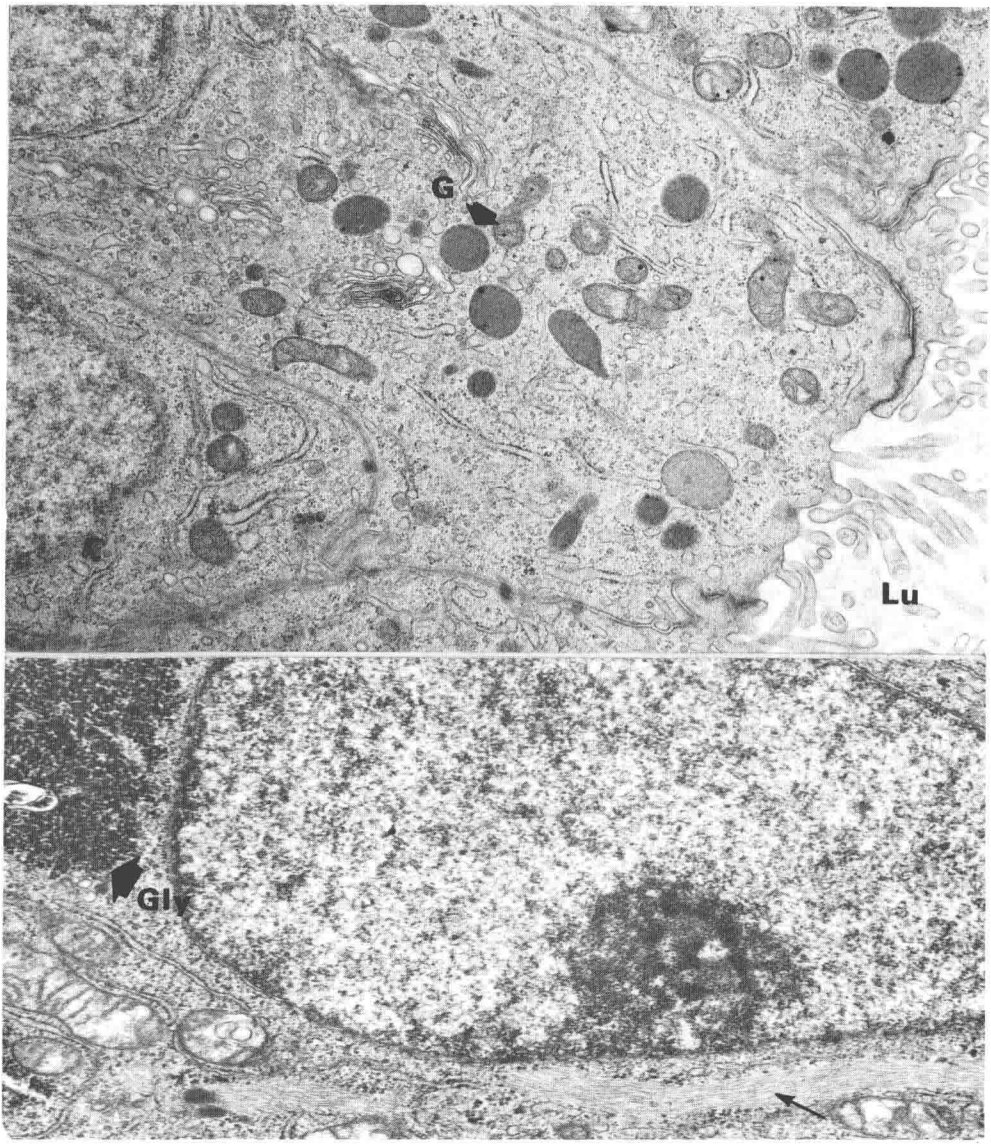
The cyclic variations in blood levels of estradiol 17β (E_2) and progesterone (P) are regarded to be responsible for the spectacular and dynamic changes observed in the endometrium during the menstrual cycle. Endometrial growth in response to estrogenic stimulation is a basic prerequisite for the regulation of physiological processes which later, under the influence of P, are programmed for the synthesis and secretion of glycoprotein-rich cellular products. These are produced for the nutrition of migrating gametes and implanting blastocyst. The different stages of cyclic modifications of human endometrium including postmenstrual regeneration, proliferation, secretory differentiation, regression and degeneration have been documented by light[5] and electron microscopy[12, 29–31] as well as histochemistry [33], historadioautography[34, 35] and ultracytochemistry[36]. The results of these studies support the concept that, in the endometrium, cyclic morphologic and physiologic changes and release of ovarian sex-steroids are inter-related. Because of this interplay, the endometrium is considered among the most sensitive indicators of the hypothalamo–pituitary–ovarian endocrine axis and evaluation of the status of the endometrium is an essential part of the investigation of the infertile patient.

Steroid and biochemical studies demonstrated that E_2 molecules

stimulate DNA-dependent RNA synthesis in target cells[37], resulting in the production of several specific enzymes. Some of these enzymes are involved in the premitotic and mitotic phases of the cell cycle, resulting in increased mitotic activity, which in turn leads to epithelial, stromal and vascular growth of the endometrium. *In vitro* historadioautographic studies[35] demonstrated a higher degree of incorporation of labelled DNA precursor, tritiated thymidine, into the nuclei of gland cells as well as stromal cells and endothelial lining of the endometrial vessels of proliferative compared with secretory endometrium. The newly synthesized nuclear DNA is programmed for subsequent mitotic activity, a feature which is prominent during the preovulatory phase of the menstrual cycle. The findings support the view that E_2 stimulates the proliferative potential of the endometrium. Other important proteins thought to be produced by E_2 are receptors for E_2 itself as well as for progesterone[27]. E_2 receptor and P receptor concentrations increase both in the blood and in the endometrium during the proliferative phase of the cycle.

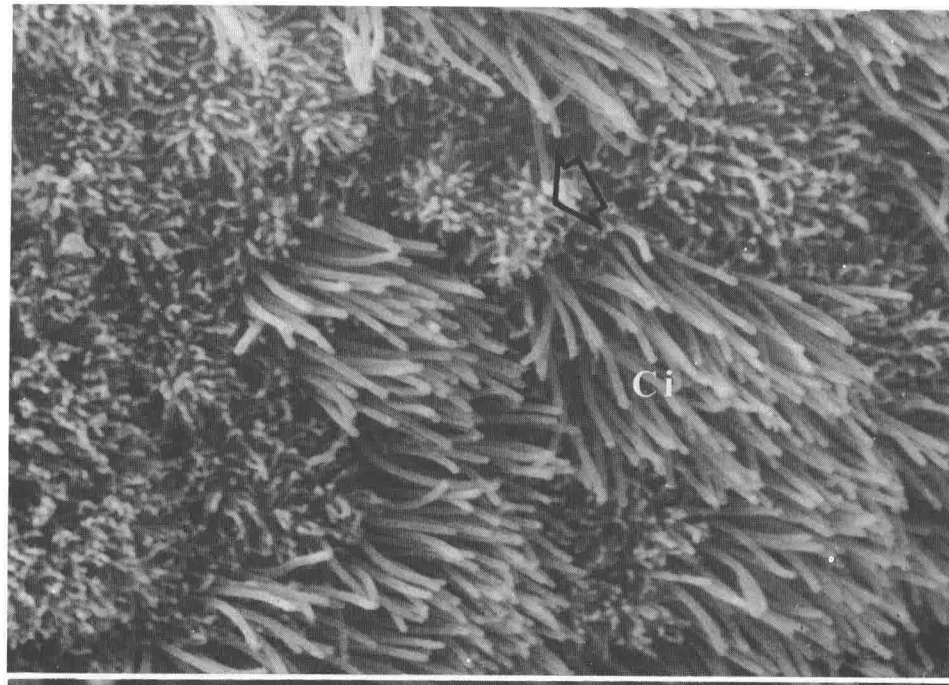
At the electron microscope level, estrogenic influence on the endometrium is chiefly confined to the cytoplasm of gland-lining epithelium and is expressed by an increase in free ribosomes and granular endoplasmic reticulum–glycogen–Golgi–ATP-rich mitochondria complexes[12, 31, 32]. These organelles contain the protein and glycogen matrix, enzymes and energy, respectively, necessary for carbohydrate metabolism and production of glycoproteinaceous endometrial fluid during the postovulatory period. (*Figure 1.1*). Changes in organellar constitution are initiated by E_2-mediated DNA-dependent RNA polymerase activity, which in turn stimulates transcription of messenger RNAs and their translation into cytoplasmic proteins[37]. Also, an accumulation of acid phosphatase-rich primary lysosomes of Golgi origin is observed, the function of which is related to regression of postovulatory endometrium and its menstrual breakdown[30, 36]. Additionally, there is an

Figure 1.1 Proliferative endometrium. →
(a) Gland cells with well developed supranuclear Golgi (G) associated with membrane-bound primary lysosomes (arrow), mitochondria and free and bound ribosomes. Surface microvilli project into glandular lumen (Lu). (× 16,000 reduced to 85%).
(b) Subnuclear glycogen granules (Gly) in close association with mitochondria, granular endoplasmic reticulum and bundles of perinuclear microfilaments (arrow). These features are characteristic of gland cells of the late proliferative phase of the cycle. (× 25,000 reduced to 85%)

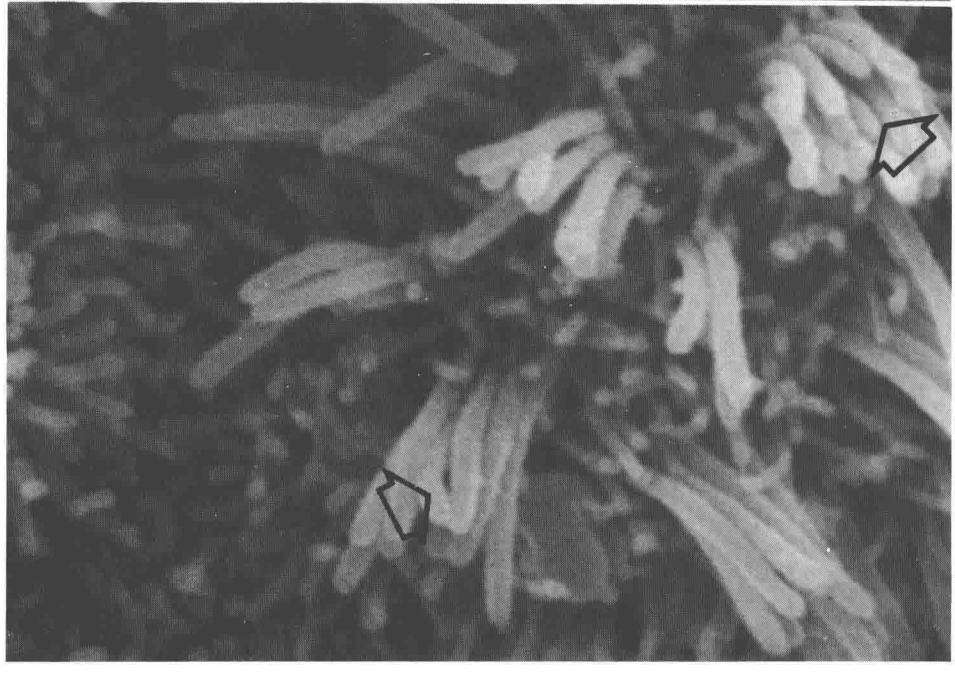


(a)

(b)



(a)



(b)

increase in lipid bodies and microfilaments (*Figure 1.1(b)*). The latter presumably help to support the cytoplasmic substance of gland-lining cells. Finally, E_2 stimulates the formation of new cilia and surface microvilli in endometrial gland and surface epithelial cells[12,30]. Because ciliated cells disappear in the glands during the progestational, luteal phase of the cycle as well as in ovariectomized women or animals, but reappear when exogenous estrogens are administered, *endometrial ciliogenesis is regarded as one of the most sensitive indicators of estrogenic stimulation*[12,14]. The fine structural organization and development of endometrial cilia are identical with their Fallopian tube counterparts[12], and their function appears to be to promote secretory fluid circulation over the surface of cells. The persistence of ciliated cells in the surface epithelial cells of the endometrium, and in particular around glandular openings during the postovulatory phase of the cycle[11,12], support this contention.

About 50–60 ciliary shafts averaging 0.2 μm in width and 4–5 μm in length project as a tuft from a single cell (*Figure 1.2(a)*). The mature ciliary apparatus consists of a basal body and a ciliary shaft or cilium (*Figure 1.3(a, b)*). The basal body is composed of nine sets of three tubules embedded in an amorphous matrix. The basal body often is continuous with a cross-striated rootlet. This is made of closely spaced, longitudinally arranged fibres with regularly spaced collateral fibres (*Figure 1.3(b)*). The basal bodies and ciliary rootlets are likely to provide assembly sites for the ciliary shafts and coordination of interciliary activity, respectively[38]. The ciliary shaft has nine sets of peripheral double tubules surrounding a central pair of tubules (*Figure 1.3(a), inset*). The tubules are enveloped in an extension of the cell membrane. Ciliary motion in the endometrium consists of a straight-armed effective stroke, followed by a curling return stroke in all respects similar to ciliary motion occurring in other 'fixed' ciliated cells of the body[39]. Such a form of beat is considered to promote secretory fluid circulation over the surface of cells. Recent biochemical and kinetic studies[39] have demonstrated that adenosine triphosphate (ATP) is the energy source for motility in cilia and that cilia beat when the microtubules, powered

← *Figure 1.2 Proliferative endometrium.*
(a) Scanning electron microscopy of surface epithelium containing mature ciliated cells (Ci) and non-ciliated cells; the latter have microvillous promontories (arrow). (× 6,000)
(b) Numerous short ciliary buds (arrows) characterize early ciliogenesis. Hair-like surface microvilli are abundant. (× 23,000)