

# METHODS IN CELL BIOLOGY

## B

### VOLUME 21

*Normal Human Tissue and Cell Culture  
Endocrine, Urogenital, and  
Gastrointestinal Systems*

*Edited by*

CURTIS C. HARRIS

BENJAMIN F. TRUMP

GARY D. STONER

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*Prepared under the Auspices of the American Society for Cell Biology*

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*Normal Human Tissue and Cell Culture*  
*B. Endocrine, Urogenital, and*  
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# PREFACE

One of the central problems in biomedical research is the extrapolation of data from experimental animals to the human situation. This is especially a problem in studies of chronic diseases such as atherosclerosis, diabetes, and cancer. Extrapolation is made difficult by variation among species as well as variation among individuals within a species. Such variation may be wide, particularly in outbred populations such as humans. Finally, within a single individual one may find variations in response to exogenous agents among different tissues and within a tissue among different cell types.

The use of cultured human tissues and cells for investigations in biomedical research can be a logical bridge between experimental animals and the beneficiary of our research, humans. Volumes 21A and 21B of *Methods in Cell Biology* describe the current state of methodology in culturing human tissues and cells. The organization is on the basis of organ systems and both explant and cell culture approaches are included. A common strategy in this field of research is initially to develop the methodology with cells and tissues from experimental animals and then to adapt this methodology for use with human cells and tissues. Thus far, some investigations have been conducted only in cells and tissues from experimental animals. Studies with human material are still at an early stage of development. A few chapters are included that reflect the progression in methodology from experimental animals to humans. In general, this progression has been easier than most of us would have predicted.

The primary objective of these methodologic studies is to provide experimental tools for investigating the pathogenesis of human disease. In fact, as noted in many of the chapters, cultured human cells and tissues are already being used in conjunction with sophisticated methods to investigate a wide variety of important problems in biomedical research. The purpose of these volumes is to further encourage future investigations.

CURTIS C. HARRIS  
BENJAMIN F. TRUMP  
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## Chapter 1

# *Studies of Human Endometrium in Organ Culture*

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## I. Introduction

Approximately 37,000 new cases of endometrial carcinoma will be diagnosed in the United States this year. Thus, endometrial carcinoma is now the most prevalent cancer of the female genital tract in the United States, but only limited insight into the etiology of this disease has been elucidated by epidemiological studies. Consequently, we have attempted to learn more about the pathogenesis of endometrial cancer by studying this target tissue directly as organ cultures *in vitro*.

Efforts to study structurally intact endometrial tissue are not new. As early as the 1920s, attempts were made to maintain endometrial tissue *in vitro*. These investigations used menstrual effluent or tissue from endometrial curettings and evaluated the morphology of cellular outgrowths from explants maintained for up to 1 month on plasma clots. Randall, Stein, and Stuermer were the first group to systematically investigate the cytodynamic properties of human endometrial tissue derived from biopsies. In their first study, Randall *et al.* (1950) maintained tissue fragments immersed in fluid media for up to 35 days. Parameters investigated for their effect on culture maintenance included gas composition, pH, and temperature. Other studies (Stuermer and Stein, 1950) utilized the hanging drop and roller tube culture techniques. Later studies (Stuermer and Stein, 1952) evaluated variations in biochemical properties of the tissue including respiration, anaerobic and aerobic glycolysis, and representative enzyme pathways as a function of the menstrual cycle. Ehrmann *et al.* (1961) maintained endometrial fragments in organ culture on serum-agar plates. Although their cultures often evidenced extensive stromal necrosis, the epithelium was well maintained, in one case for as long as 12 weeks. Morphologic evidence of secretory activity was observed in the epithelium of the cultured tissue in the absence of supplemental hormones, as well as in the presence of estrogen, progesterone, or both hormones. Hughes *et al.* (1969) placed endometrial organ cultures on filter membranes supported by stainless-steel grids in organ culture dishes. Using fluid medium supplemented with serum, they observed biochemical and morphologic responses to estrogen and progesterone in endometrial cultures maintained for up to 18 days *in vitro*. Specifically, the morphology of cultures was better preserved when media were supplemented with both estrogen and progesterone at near physiologic levels as compared to media without both hormones. More recent studies by a number of investigators have dealt largely with the variations of macromolecular synthesis and hormone receptor levels as functions of the menstrual cycle state. In these studies tissue generally has been maintained *in vitro* for short periods of time to characterize biochemical properties or observe hormonal effects. These observations usually were made with short-term culture, typically within 1 or 2 weeks.

The ultimate goal of this project is to gain a greater understanding of the



process of chemical carcinogenesis in human endometrium. One possible avenue for accomplishing this goal is to observe long-term toxic and carcinogenic effects of chemicals on this tissue. Such observations require that methods be developed for the long-term maintenance of this tissue *in vitro* as organ cultures. Furthermore, such methods should preserve, as far as possible, the morphologic and biochemical properties of the endometrium. In this chapter we describe such an *in vitro* model designed for long-term maintenance of human endometrial explants in organ culture.

## II. Materials and Methods

### A. Obtainment of Human Endometrial Tissue

The use of human tissue for experimental studies requires far more clinical judgment than would be required for use of animal tissues. Scientific investigations with human tissue must not compromise the clinical care of the patient or impair a complete and accurate pathological diagnosis. Our studies have utilized endometrial tissue obtained from hysterectomy specimens. Effort has been made to select carefully a small amount of tissue for experiments, in order not to interfere with pathological diagnosis. For this reason we have developed a detailed procedure for evaluating patients and their tissue prior to use in our studies. Obtainment of tissue from other human organs poses problems because operative specimens generally are removed for intrinsic disease. Obtainment of human endometrial tissue is in a different category because there are other gynecologic disorders in addition to endometrial disease that necessitate hysterectomy. Nonetheless, the procedures which have been utilized to obtain human endometrial tissue may benefit investigators who wish to use this or other human tissues.

Our tissue obtainment procedure requires a judgment to be made by the patient, the physician, and the laboratory investigator. Patients admitted for elective hysterectomy undergo preliminary screening to eliminate individuals who cannot freely offer informed consent (mentally incompetent persons, minors, prisoners, acutely ill patients, etc.) and those whose clinical history suggests the presence of intrinsic endometrial disease. The remaining patients are informed about the objectives of the project, the method of specimen collection, and the possible benefit to humanity. They are informed fully that no direct benefit will accrue to them as individuals. Possible risks related to their participation are outlined. Only those patients who freely offer their informed consent are considered as donors. (In fact, no patient so informed has refused to participate.) During the operation the surgeons re-evaluate the patient's condition to determine whether unsuspected findings mitigate against including the patient in the