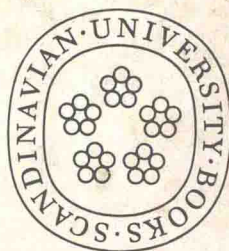


EIVIND MYHRE • KNUT BJÖRO

# Hormones and Cervical Cancer

Effect of Sex Hormones and  
Antifertility Compounds on  
Chemically Induced Carcinoma of the  
Mouse Uterine Cervix  
Comparative Morphological  
and Histochemical Studies



**Universitetsforlaget**

# Hormones and Cervical Cancer

Effect of Sex Hormones  
and Antifertility Compounds  
on Chemically Induced Carcinoma  
of the Mouse Uterine Cervix  
Comparative Morphological  
and Histochemical Studies

By

EIVIND MYHRE

Professor of Pathology  
University of Oslo

in cooperation with

KNUT BJÖRO

Assistant Professor of Gynecology  
University of Oslo



UNIVERSITETSFORLAGET 1971  
OSLO - BERGEN - TROMSÖ

© Universitetsforlaget 1971

Printed in Norway

AAS & WAHLS BOKTRYKKERI, OSLO

# Contents

## PART 1

### CHAPTER I. THE CERVICAL CANCER PROBLEM: A CLINICAL REVIEW 11

- A. General considerations 11
- B. Epidemiology 12
- C. Factors influencing frequency 13
  - 1. Age 13
  - 2. Marital status 14
  - 3. Parity 15
  - 4. Age at first coitus 15
  - 5. Heredity 15
  - 6. Social and environmental factors 16
- D. Nature of the agent 17
  - 1. Venereal infections 17
  - 2. Chronic cervicitis 17
  - 3. Viruses 17
  - 4. Carcinogens 17
  - 5. Immunological factors 18
  - 6. Hormones 18

### CHAPTER II. EXPERIMENTAL CARCINOMA OF THE CERVIX UTERI IN MICE 19

- A. General considerations 19
  - 1. Spontaneous tumours 19
  - 2. Experimental tumours 19
  - 3. Important factors in experimental carcinogenesis 19
- B. Hormone induced carcinoma 20
  - 1. Survey of literature 20
  - 2. Comments on endocrine carcinogenesis 21
- C. Chemically induced carcinoma 22

- 1. Survey of literature 22
- 2. Comments on chemical carcinogenesis 25
- D. Hormonal influence on chemically induced carcinoma 25
  - 1. Survey of literature 25
  - 2. Comment on the effect of hormonal imbalance on chemical carcinogenesis 28
- E. Summary and conclusions of hormonal effects on chemical carcinogenesis 29

### CHAPTER III. HISTOPATHOLOGY AND HISTOCHEMISTRY OF CARCINOMA OF THE CERVIX UTERI 30

- A. Histopathology 30
- B. Histochemistry 33

### CHAPTER IV. CHEMISTRY AND BIOLOGY OF HORMONES, ANTIFERTILITY DRUGS AND CHEMICAL CARCINOGENS 38

- A. Hormones and antifertility drugs 38
    - 1. History of the hormonal control of fertility 38
    - 2. Chemical constitution of antifertility steroids 39
    - 3. Mode of action of steroid hormones 42
  - B. Chemical carcinogens 43
    - 1. Polycyclic hydrocarbons 43
    - 2. Mechanism of action 44
    - 3. Interaction with tissue 45
- References to Part I 46

## PART 2

### CHAPTER V. INTRODUCTION TO OWN INVESTIGATIONS 59

### CHAPTER VI. OWN INVESTIGATIONS 61

- A. Preliminary experiments 61
- B. Main experimental series 62
  - Material and methods

- 1. Strain of mice 62
- 2. Dietary and environmental conditions 62
- 3. Painting of the cervix 62
- 4. Treatment 62
- 5. Size of groups 63
- 6. Observation time 64
- 7. Histological procedure 64
- 8. Histochemical procedure 64

9. Anatomy and histology of the genital tract in mice 65

## CHAPTER VII. RESULTS 67

- A. Survival 67
  - 1. Total material 67
  - 2. Long-term observation 67
  - 3. Short-term observation 68
- B. Yield of carcinomas 68
  - 1. Long-term observation 68
  - 2. Short-term observation 69
- C. Morphology 72
  - 1. Long-term observation 72
  - 2. Short-term observation 73
- D. Histochemistry 73

## CHAPTER VIII. DISCUSSION 80

- 1. The background for the experiments 80
- 2. Administration of pellets 81
- 3. Survival 81
- 4. Yield of carcinomas 81
- 5. Morphology 82
- 6. Conclusions 82
- 7. Histochemistry 83

## CHAPTER IX. SUMMARY AND CONCLUSIONS 85

- References to Part 2 85

HORMONES AND  
CERVICAL CANCER

SCANDINAVIAN UNIVERSITY BOOKS

*Universitetsforlaget, Oslo/Bergen/Tromsø*

*Munksgaard, Copenhagen*

*Läromedelsförlagen, Stockholm/Göteborg/Lund*

UNIVERSITETSFÖRLAGET

*Distribution offices*

*NORWAY*

BLINDERN, OSLO 3

*UNITED KINGDOM*

CANNON HOUSE, PARK FARM ROAD,  
FOLKESTONE, KENT

*UNITED STATES*

BOX 142  
BOSTON, MASS. 02113

# Hormones and Cervical Cancer

Effect of Sex Hormones  
and Antifertility Compounds  
on Chemically Induced Carcinoma  
of the Mouse Uterine Cervix  
Comparative Morphological  
and Histochemical Studies

By

EIVIND MYHRE

Professor of Pathology  
University of Oslo

in cooperation with

KNUT BJÖRO

Assistant Professor of Gynecology  
University of Oslo



UNIVERSITETSFORLAGET 1971

OSLO - BERGEN - TROMSÖ



© Universitetsforlaget 1971

Printed in Norway

AAS & WAHLS BOKTRYKKERI, OSLO

## Preface

This book is primarily concerned with the effect of sex hormones and anti-fertility compounds on a predictable, chemically induced carcinomatous development of the cervix uteri in mice. The preliminary experiments began in autumn 1966, while the main experiments started one year later and were brought to an end early in 1970. The work has been carried out at the Institute for General and Experimental Pathology, University of Oslo, Rikshospitalet.

The cervical cancer problem in women, briefly reviewed from epidemiological and pathogenetical points of view, is intended to give a complete literature review of the effect of chemical carcinogens and hormonal influences on neoplastic processes in the mouse uterine cervix. The histopathology and histochemistry of these lesions are discussed, as well as the chemistry and biology of hormones, antifertility drugs, and chemical carcinogens.

My most sincere gratitude goes to my fellow worker and friend, associate professor Knut Bjøro, M.D., Department of Obstetrics and Gynaecology, University of Oslo, Rikshospitalet. Dr. Bjøro is responsible primarily for Chapters I and IV; the remainder were written by me, but all chapters have been thoroughly discussed between us.

This work would not have been possible without the great support and supervision given by the chief technician of our histochemical division, Miss Marianne Malmstrøm. She has been responsible for all technical procedures as well as the manuscript typing. I hereby wish to express my deepest gratitude to her for the fine cooperation and great loyalty throughout these years. Further, I wish to thank Miss Jorun Vik, a skilled technician, secretary, and a very good collaborator. These two have prepared all the Tables and Figures. Their never failing interest and enthusiasm are greatly appreciated.

I also wish to express hearty thanks to Mrs. Lillian Olderskog, who has been the careful keeper of the animals – a very important task which has been in the best of hands. During the preliminary experiments Miss Gulli Kneisel took care of the animals, for which I wish to thank her.

Most pellets containing the hormones and the antifertility compounds were prepared by A/S Apothekernes Laboratorium for Specialpræparater, Oslo,

except the pellets containing chlormadinone acetate, which were prepared by A/S Farmaceutisk Industri, Oslo. I hereby extend my sincerest thanks to these pharmaceutical firms for their kind cooperation. I also wish to thank the Phototechnical Department, Rikshospitalet, for their invaluable photographic assistance.

Norsk forening til Kreftens Bekjempelse and The Norwegian Research Council for Science and the Humanities have paid the technical assistants. Furthermore, financial support has been given by Anders Jahres fond til vitenskapens fremme, Elisabeth og Knut Knutsens O.A.s fond for Kreftforskning, Doktor Alexander Malthes Legat, and Direktør Gotfred Lie og hustru Marie Lies fond. Without help from these institutions the work could not have been carried out. I thank them all.

Last, but not least, I wish to thank Universitetsforlaget, Oslo, for excellent cooperation.

Oslo, 1971

*Eivind Myhre*

# Contents

## PART 1

### CHAPTER I. THE CERVICAL CANCER PROBLEM: A CLINICAL REVIEW 11

- A. General considerations 11
- B. Epidemiology 12
- C. Factors influencing frequency 13
  - 1. Age 13
  - 2. Marital status 14
  - 3. Parity 15
  - 4. Age at first coitus 15
  - 5. Heredity 15
  - 6. Social and environmental factors 16
- D. Nature of the agent 17
  - 1. Venereal infections 17
  - 2. Chronic cervicitis 17
  - 3. Viruses 17
  - 4. Carcinogens 17
  - 5. Immunological factors 18
  - 6. Hormones 18

### CHAPTER II. EXPERIMENTAL CARCINOMA OF THE CERVIX UTERI IN MICE 19

- A. General considerations 19
  - 1. Spontaneous tumours 19
  - 2. Experimental tumours 19
  - 3. Important factors in experimental carcinogenesis 19
- B. Hormone induced carcinoma 20
  - 1. Survey of literature 20
  - 2. Comments on endocrine carcinogenesis 21
- C. Chemically induced carcinoma 22

## PART 2

### CHAPTER V. INTRODUCTION TO OWN INVESTIGATIONS 59

### CHAPTER VI. OWN INVESTIGATIONS 61

- A. Preliminary experiments 61
- B. Main experimental series 62
  - Material and methods

- 1. Survey of literature 22
- 2. Comments on chemical carcinogenesis 25
- D. Hormonal influence on chemically induced carcinoma 25
  - 1. Survey of literature 25
  - 2. Comment on the effect of hormonal imbalance on chemical carcinogenesis 28
- E. Summary and conclusions of hormonal effects on chemical carcinogenesis 29

### CHAPTER III. HISTOPATHOLOGY AND HISTOCHEMISTRY OF CARCINOMA OF THE CERVIX UTERI 30

- A. Histopathology 30
- B. Histochemistry 33

### CHAPTER IV. CHEMISTRY AND BIOLOGY OF HORMONES, ANTIFERTILITY DRUGS AND CHEMICAL CARCINOGENS 38

- A. Hormones and antifertility drugs 38
    - 1. History of the hormonal control of fertility 38
    - 2. Chemical constitution of antifertility steroids 39
    - 3. Mode of action of steroid hormones 42
  - B. Chemical carcinogens 43
    - 1. Polycyclic hydrocarbons 43
    - 2. Mechanism of action 44
    - 3. Interaction with tissue 45
- References to Part 1 46

- 1. Strain of mice 62
- 2. Dietary and environmental conditions 62
- 3. Painting of the cervix 62
- 4. Treatment 62
- 5. Size of groups 63
- 6. Observation time 64
- 7. Histological procedure 64
- 8. Histochemical procedure 64

9. Anatomy and histology of the genital tract in mice 65

## CHAPTER VII. RESULTS 67

- A. Survival 67
  1. Total material 67
  2. Long-term observation 67
  3. Short-term observation 68
- B. Yield of carcinomas 68
  1. Long-term observation 68
  2. Short-term observation 69
- C. Morphology 72
  1. Long-term observation 72
  2. Short-term observation 73
- D. Histochemistry 73

## CHAPTER VIII. DISCUSSION 80

1. The background for the experiments 80
2. Administration of pellets 81
3. Survival 81
4. Yield of carcinomas 81
5. Morphology 82
6. Conclusions 82
7. Histochemistry 83

## CHAPTER IX. SUMMARY AND CONCLUSIONS 85

References to Part 2 85

# PART 1



# Chapter I. The Cervical Cancer Problem: A Clinical Review

## A. GENERAL CONSIDERATIONS

One of the earliest evaluations of the basic problems of cervical carcinoma was stated by James Henry Bennett in 1850 (22):

Cancerous growths rarely commence in the body of the uterus or at least are rarely there first recognized, the neck of the organ being the region in which they are usually first observed. According to my experience, cancer in the neck of the uterus is almost invariably found in the advanced or ulcerated stage of its development before the female applies for relief. It would seem as if cancerous growths in this region give such slight indications of their presence during the first period of their formation, and progress so insidiously, that the attention of the patient and of her medical attendant is scarcely ever directed to the uterus.

Although it seems at present that Bennett's ideas were basically quite correct, this was not the general impression of the day. Much of the confusion concerning the malignant disorders of the cervix has been resolved as a result of increased knowledge of cancerous growths in general. Newer methods for gross and microscopic evaluation of tissue have added much to this knowledge. One difficulty that has hampered the study of cervical carcinoma has been the inclusion of cancer arising in both uterine corpus and cervix in a single category. These two lesions, however, behave quite differently clinically and are found in different kinds of people. Although they were differentiated in the latter part of the last century, they still are not always separated. For example, official mortality statistics based on death certificates often list cancer of the uterus in which corpus and cervix are combined.

Carcinoma of the cervix exhibits in its clinical course all signs of malignancy. The tumour infiltrates and destroys neighbouring organs; it metastasizes and kills the host within a certain time. Carcinoma in situ of the cervix shows all

the cytological signs of malignancy but lacks one of the most essential histological characteristics of malignant growth, invasion. The first description and illustration of a symptomless early carcinoma of the cervix uteri is that of Sir J. Williams in 1886 (292), but the term 'carcinoma in situ' was only definitely introduced in 1932 by Broders (38).

There have been three highlights of histological and clinical importance which have produced new concepts. In chronological order the first was the recognition by Schauenstein in 1908 (259) of the epithelial aberration, now termed 'carcinoma in situ'. Slowly over the next four decades this brought with it a vindication of his conclusion that carcinoma in situ may be an antecedent condition to invasive carcinoma. The second was the invention of the colposcope by Hinselmann in 1925 (143). His discovery, that there was no nodule such as may arise from a single cell but only 'atypical epithelium', marks a critical point in the development of the conceptual approach to the origin of the disease. Thirdly, more than 25 years have passed since Papanicolaou & Traut in 1943 (223) developed a reliable cytological technique that makes possible the detection of premalignant and very early malignant lesions of the cervix. Although of great value in the detection of carcinoma, exfoliative cytology has been of limited conceptual value in regard to the cause of the disease.

Many factors have been implicated in human cervical carcinogenesis. This study is an attempt to bridge the gap between the human and the experimental animal, integrating pertinent, fundamental observations with our current knowledge of human carcinogenesis in the uterine cervix. However, despite the accumulated epidemiological evidence, the ultimate causes of cervical cancer are still unknown.



## B. EPIDEMIOLOGY

In the development of knowledge about the cause of a disease, the first stage is the search for clues on which hypotheses can be based. Knowledge of where cervical cancer is most common, how much of it there is, and which women are at most risk have a practical bearing on aetiology, prevention, and control. Epidemiological studies of the incidence of cervical cancer are becoming easier to undertake as cancer registration coverage improves.

Age-adjusted mortality rates per 100,000 females are available from many areas and range from 3 to 64 per year. The highest recorded rates are in Latin America, parts of Africa, and Asia. The lowest recorded rates are in Israel, North American whites, New Zealand and Europe. The frequency of cervical carcinoma in relation to all cancer in females ranges from 5 to 60 per cent in different population groups. Both the mortality rates and the numbers of one type of cancer expressed as a pro-

portion of all cancers, provide only an indirect estimate of incidence. Table I shows the incidence of malignant neoplasm of the cervix uteri in some countries and regions as recorded by the International Union Against Cancer (47).

The Cancer Registry of Norway has been in operation since 1952. Based on compulsory notification, the registration scheme covers the whole of Norway and aims at a complete registration of all recognized cases of cancer among the total population of the country. The average annual number of new cases of cancer of the cervix (invasive cancer only) in the period 1953—1967 was 348. The average figure in the first seven-year period 1953—1959 was 332, compared to 363 over the eight years 1960—1967 (Fig. 1) (48, 49, 228).

The most valuable data are undoubtedly the rates obtained by recording the occurrence of every case of cancer in a defined community during a specific period. Since incidence varies with time, it is clearly desirable to compare data for different communities over the same period. A difficult problem was introduced by the recognition and treatment of pre-invasive lesions of cervix uteri. The relationship between carcinoma in situ and truly invasive cancer has not been established with complete clarity yet, and the question arises whether pre-invasive lesions should be included in the figures for cancer incidence or not. Most commonly only the figures of the truly invasive lesions are used for study of aetiology.

Routine mass screening by cervical smears are now being carried out on a large scale in some countries. Cytological examinations make it possible to identify, almost without failure, women with dysplasia and in situ lesions, and since these conditions can be eliminated completely, usually by limited surgery, the incidence of invasive cancer could be appreciably reduced. It is extremely important to know the probability of progression of the in situ lesions.

In one county (Østfold) of Norway a population screening was started in 1959, the aim being to examine as many as possible of the 46,500 resident women then aged 25—59 and to repeat the screening of this group after two years and again after another three years.

Table I. Cervical cancer incidence in various countries and regions

	Incidence rate per 100,000 per year, standardized for age		
	Standard population		
	African	World	European
Denmark	25.7	28.3	35.6
England and Wales (4 regions)	10.0	12.5	16.2
Finland	13.0	16.0	20.8
Germany F. R. (Hamburg)	31.7	36.5	45.6
Iceland	12.6	16.2	20.6
Netherlands (3 provinces)	16.6	18.9	24.2
Norway	13.5	15.3	19.5
Sweden	15.6	17.2	21.6
Connecticut	10.7	13.6	17.7
New York State	11.6	14.6	19.2
Canada (5 provinces)	24.0	25.3	31.6
South Africa	35.6	52.0	69.9
Colombia	77.0	100.6	129.9
Japan	15.4	22.1	29.2
New Zealand	11.1	14.1	18.4
Israel	4.4	5.9	7.7